

# Journal of Undergraduate Research



*Spring 2024, Volume Twenty-two  
Issue Two*



UNIVERSITY of  
ROCHESTER

*The Journal of Undergraduate Research (JUR) is dedicated to providing the student body with intellectual perspectives from various academic disciplines. JUR serves as a forum for the presentation of original research, thereby encouraging the pursuit of significant scholarly endeavors.*

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## Letter from the Editors

To our readers, faculty, and amazing staff, we are excited to present the Spring 2024 issue of the Journal of Undergraduate Research. Research is an endeavor into discovering the unknown. The Journal of Undergraduate Research not only seeks to promote that discovery, but also to act as a forum for undergraduates from all fields to learn more about the research on campus. In this issue of JUR, a wide range of scholarly work is highlighted, all of which explore interesting and unique questions.

In this issue, we highlight seven articles that represent the wide breadth and diversity of research being conducted at the University. Bianca Santonastaso '24 presents an interesting case study on the pathology of acquired FXII inhibitors. Xupei Ou '24 investigated the role of Macrophages in Erythropoiesis, and Lilli Tamm '25 evaluated NMDA receptors as a potential target to treat stroke. Michelle Bischoff '24 brings important insight to the career of Martha Chase at Rochester, and Rose Frank '24 explores the life of Fredrick Douglass through his photographs. Daniel Heberle '24 discusses the historical narratives underlying voyeuristic art in Early Modern Italy, and Adam Kolers '27 examines the sociopolitical subtext of *The Golden Girls*. We also present an interview with Professor Welte, giving insight into his research in biology and career, and select abstracts from the 2024 Lightning Talks and Speaker's Symposium.

We would be remiss if we did not express our gratitude to our outstanding editorial board, including our managing editors and content editors for their hard work ensuring our articles were of the highest quality, as well as our layout chair, Catherine Lan, and layout editors for assembling the journal design. As well, we are ever grateful for the support of the Office of Undergraduate Research, Ms. Emily Rendek and Ms. Ann Robinson, without whom this publication would not be possible.

To our readers, we hope you enjoy and draw inspiration from the research presented in this issue.

Sincerely,

*Michael Christof & Wimeth Dissanayake*

Editors-in-Chief



# Journal of Undergraduate Research

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This issue of the Journal of Undergraduate Research was assembled on macOS Big Sur using Affinity Publisher. Microsoft Word and Google Docs were used for text editing and review. Fonts used include Minion Pro, the main font for body text, and Myriad Pro, the main font for headings and decorative text. This physical version of this journal was bound by Emerald Print Management of Rochester, NY.

# Professor Interview

## Michael Welte, PhD

*Professor*

*Department of Biology*

*University of Rochester*



*Dr. Michael Welte is a Professor of Biology at the University of Rochester, USA. Dr. Welte received his Ph.D. in Molecular Genetics and Cell Biology at the University of Chicago in 1994. He was a post-doctoral researcher in the lab of Dr. Eric Wieschaus at Princeton. In 2000, Dr. Welte served as an assistant professor of biology at Brandeis University. He moved to the department of biology at the University of Rochester in July 2007, where he has served as a Professor and Chair of the department. At Rochester, he leads a premier cell biology lab that pioneered the study of lipid droplets (fat storage organelles). Using cell biology and imaging techniques in drosophila models, he has studied the formation, trafficking, and function of these novel organelles. He also enjoys mentoring the many undergraduates in his lab, is a prominent member of 500 queer scientists, and teaches Advanced Cell Biology in the Fall.*

**JUR:** Could you give us a quick background on your research?

**Dr. Welte:** I am a cell and developmental biologist using fruit flies (*Drosophila*). Most of my work these days is on the role of lipid droplets in animal development. Lipid droplets are cellular organelles used for fat storage – discovered by the same person who discovered mitochondria in the late 1800s. However, there were no serious cell-biological or molecular studies on them for the next 100 years and work really started in the 1990s. [Lipid droplets] turn out to affect lots of cellular processes, and there's still lots being discovered.

**JUR:** How did you first get interested in lipid droplets?

**Dr. Welte:** I had been a graduate student working on *Drosophila* development, and then I did a postdoc with Professor Eric Wieschaus at Princeton, who happened to have a side project on lipid droplets. Very few people were studying them at the time, but I was fascinated. What he had found is that they relocalize during certain stages of embryo development in flies, slowly drifting inward from the periphery towards the center. Using real-time imaging, I discovered that they rapidly move back-and-forth along microtubules and that this motion is temporally regulated. In collaboration with biophysicist Steven Gross, we also figured out how to measure the force with which they move. I knew very little about lipid droplets themselves, in part because hardly anybody did. It was the time when the field as a whole got started.

I was lucky to get into the field early, and the field sort of grew up around me, so that I could witness and participate in major breakthroughs.

**JUR:** In such exploratory work, how did you decide what projects to pursue and what strings to pull on?

**Dr. Welte:** Many of the directions I went into over the years were simply because there was a mystery to be solved, and I'm attracted to solving puzzles – especially if it's a very open puzzle where we don't know much yet. Along the way, we made chance observations that turned the research in a whole new direction. So, when we were studying how lipid droplets move inside cells, one approach we decided on was to purify the lipid droplets biochemically – to see what proteins are on them. I had expected we may find around 12 proteins, some of them would be microtubule motors and the others would be the motor regulators that we were interested in. But instead, there were hundreds of proteins on there. Interestingly, some of the most common proteins were histones, the proteins that package DNA in the cell nucleus, which didn't make any sense at the time, but that puzzle really interested me. So, we started doing research where we first figured out how the histones are attached to the lipid droplets, and then that led us into what the function of the histones were.

**JUR:** Over the past few years, publishing has gotten more important, and a publish-or-perish mentality has become dominant in the scientific field. How has this

changed the way you do research, and how do you balance this against exploring what you find exciting?

**Dr. Welte:** I do feel the need to publish, so that I can get grants and keep my research program going, in part because it's not just about me, it's about my students and other lab personnel that need to be paid. It is certainly true that I go after the things that I find exciting. When you write grants, as long as you can convince other people that it's exciting, and that you can do the work, you will be able to get the funding. Some of those bigger ideas excite people, so to get funding you need to show that things are feasible, but also that they are interesting. I go after some things that are more mysterious, and that excites people more, so that's just one way to run a research program.

**JUR:** In the research process, when do you decide that your research has reached an appropriate conclusion to publish, given that each new finding opens up so many more questions?

**Dr. Welte:** That is always a difficult question to answer for any project, and certainly for graduate students, since they need have published to advance in their career, to get postdoctoral positions and their own grants. There is certainly a pressure to conform to the schedule of normal graduation, so students don't spend too much time in the lab, but I generally want my students to have complete stories that they publish. There are two general ways to do that – just putting more work into answering the original question, or to step back and see if the data already tell us something that's worth reporting. Often, especially with students, I found when they have a project outlined, they at least initially stick to the original idea we have and try to fill that in. I think that it's an easy frame of mind to fall into, and I do that too, but especially when you're forced by external circumstances, you can step back, look at the same data and find – oh – actually this tells us something new that we just hadn't appreciated. And then, you can wrap up a story in a completely different way than you had initially intended. So, stepping back occasionally from your data, and reimagining what they already tell you, I think is really important. In the natural life cycle of science, that happens whenever you have to present your work, as a poster, as a talk. You are forced to think about it more deeply, and as a result you often find new ideas that you simply didn't take the time to develop without the external pressure to present.

**JUR:** How do you prepare your students to present their work, and how do you promote them to see it as an opportunity to refine their work?

**Dr. Welte:** In our weekly lab meetings, people present all the time, and so then there is less pressure because it's a friendly group – so there will be less anxiety involved in the presentation. Whenever somebody gives a

presentation outside the lab, we do practice talks in the lab, and then everybody gives feedback on the talk, which is helpful for the presenter, but also just for the listener, because they learn how people give feedback and what sort of things they focus on. Often in these practice talks, we come up with ideas as a group that completely restructure the original talk, and so people get used to the idea that you have to revise your thinking all the time.

**JUR:** How do you manage to take your research and bundle it into a clear and coherent narrative?

**Dr. Welte:** One of the most impactful courses I took as a graduate student was a scientific writing course. I still use examples or guidelines I learned there to tell my students how to structure papers; having a good understanding of how English writing works in general, has been really beneficial for me for knowing how to structure arguments. I guess the other important thing is ... that you have to be able to step back from what you've done, and potentially rearrange it. The first time or maybe the 10th time you develop an argument, it may not be the best, and many times when I've written papers the order of paragraphs has changed many times. When I look at the draft with fresh eyes, there are lots of new thoughts emerging, and I realize telling the story going from C to A to B is better than A to B to C. Thus, being willing to go over what you've already done and look at it from a new perspective, that is very important. However, it takes lots and lots of time, but it is time well spent.

**JUR:** When do you decide to stop revising and be happy with the writing you have produced?

**Dr. Welte:** I think that's a difficult step for me to take. Often when you write papers you also give talks about them, and you see or experience how the audience reacts: what they understand, what messages they take away, and once you feel like it comes across the way you planned, then you realize you can probably stop.

**JUR:** How do you promote collaboration in your lab?

**Dr. Welte:** In my lab, projects typically do not overlap very much, which helps prevent competition. That also means that people typically are completely in charge of their projects and develop them on their own. So, it's not that we all work on one big project together – it's more a number of individuals that are sort of in parallel finding their own path, but then we talk to each other. Often techniques or ideas that somebody else has turn out to be applicable for another person, and in part because there is no competition people feel freer to share their ideas.

**JUR:** What roles do undergraduates play in these research projects?

**Dr. Welte:** Over the years, so many different undergrad-



uates were involved in research projects, by now over sixty. Often, they start out with very simple tasks in the lab: they learn techniques, they learn how to perform, think about, and plan experiments. I often let them explore their own ideas – one undergraduate started centrifuging embryos, that is one of the things we do to separate lipid droplets from the rest of the cell. He discovered something in one of the mutants that we had never seen before. Then, [he] worked with a graduate student that led to an entirely new project, and he sort of developed part of that project on its own. His findings became part of a published paper, with him as a co-author.

**JUR:** Undergraduates face greater constraints in terms of the time they can commit to research, what kinds of questions do you recommend undergraduates to pursue to maximize their time?

**Dr. Welte:** Try to find a question that excites you, but that's feasible to do; I guess that's true at every level. There are really big questions that might take decades to answer; I'm not sure that can motivate you day-to-day. In conversation with the people in the lab or with your PI, find a question that has a bigger implication but that seems like it could potentially make a dent, but also something which you can enjoy day-to-day.

**JUR:** Who were some of your mentors, and how did they support your exploration of science?

**Dr. Welte:** The two mentors that affected me the most were my graduate (Ph.D.) advisor, and my postdoc advisor. My graduate advisor was Professor Susan Lindquist, and I think I learned from her very rigorous thinking and careful systematic analysis. She also, once I got started in the lab, gave me lots of intellectual freedom. She would set me up with a postdoc, to learn the techniques. Then once I could start exploring, she let me explore and try out my own science, while giving advice to steer me in certain directions when needed, but otherwise let me run with my ideas which was very freeing. My postdoc mentor Eric Wieschaus was similar in this regard, but one of the things that is especially important for me is that he is just extremely excited about science. He will talk to you as a peer if you're an undergraduate or a full professor, and even though he won the Nobel Prize it didn't seem to affect how he relates to people – he still talks to everybody. Really, as long as the science is exciting, he will be excited. I guess both of these approaches I have tried to bring to the mentoring of my undergraduates – to get them excited about science and be excited about it when they bring thoughts to me.

**JUR:** Can you talk more on your experience as a PhD student, and what do you think could have improved?

**Dr. Welte:** It's been a while, and I'm sure I have romanticized it in my mind. I really enjoyed being a graduate student; I liked the freedom to explore without any real

responsibilities besides my own science, and I had very supportive lab mates. What I would change ... I've been really anxious all my life, and I wish I had reached out for mental health support earlier in my career. Especially at the time, it was still looked down upon; people didn't really talk about mental health issues very much, and it was pushed under the rug. So, if I knew about therapy and that it was accessible early on, I could have dealt with some of my anxieties much earlier, anxieties that I just have from how my personality is set up, but that also got amplified with a high stress job.

**JUR:** Do you have any advice on how to deal with experiments failing, how do you mentally cope with that?

**Dr. Welte:** There are two things there. First, especially once you've had your first success after a few failed experiments, you might feel ... that success outweighs all the failures before. You learn, that's just part of how science works – that you have to go through many failures to get occasional success, and if that success ... is enjoyable, that can make up for the many failures. The other thing is, if you enjoy the process itself – in my case the fact that I love looking at fruit fly embryos – that can make up for a lot. Thus, even if a particular experiment didn't give you the embryo staining pattern you hoped for, you still get to do something that you enjoy: seeing these beautiful embryos under the microscope. So psychological you still get something out of it, which can help you deal ... with that disappointment.

**JUR:** What was your path to PhD, what were the factors contributing to your decision to pursue one?

**Dr. Welte:** It is probably a very unusual usual path. I was an undergraduate in Germany, which at the time was a very rigid system, and at the university I was in, there wasn't a clear path for undergraduates to participate in research. There were a few that somehow got involved, but I had no idea how you could manage that. I ended up spending a year abroad during university in Seattle, Washington, and I didn't need the courses that I was taking there for my degree. So, it was a year for me to explore. I took a few courses that I was interested in, but I also got to work in a lab, and that changed my trajectory. Until that time, I had an equal interest in biology and mathematics, I was a double major, and to some extent I liked math more because I got to apply it to solving problems on my own, while in biology you just did pre-cooked experiments in lab courses and it didn't really matter if they worked or not. So, for nine months I got to do research at the bench and actually made a little bit of progress – after that I took one more math class and decided, although I still liked it, it was too far from things that mattered to me, and at that point I knew I wanted to go to graduate school in biology.

**JUR:** From your Ph.D. to your current work, you have been working extensively with the *Drosophila* (fruit fly)



model. How do you keep staying interested in this model organism?

**Dr. Welte:** There are so many questions that you can address, and there are very strong tools to address basic biological questions with this model. Over the years I've broadened my perspective from the embryo to other life stages, so that keeps things fresh. But in part what makes this model organism so great is that we already know so much about its biology. For example, when it comes to early *Drosophila* development, there's so much understood already; when you add a new component, like lipid droplets, there is a huge context for figuring out what it might mean when you observe something new, like the effect of mutations in a lipid-droplet protein. Also, I just love looking at fly embryos. I used to say ... and it is still true ... when I have a bad day in the office, I go to the lab and look at embryos and I find my happy place. There are just certain things that appeal to one in one's research because of their aesthetics or that you like not for any particular scientific reason, but just because it appeals to you in some other way. I'm very visual, I like looking at images, and embryos in my mind are simple enough that I can fool myself into thinking I could completely understand them eventually, but complex enough that there are lots of interesting things going on there that can still be explored.

**JUR:** Do you believe that your publication of many thorough literature reviews in your early career helped you get a better sense of the field?

**Dr. Welte:** It definitely helped me to wrap my head around the big picture – what sort of patterns I see in the literature, and how to articulate them. Mainly because I had to read a lot of original articles that broadened my own view, but also because I did it mostly on my own. I could develop my own thoughts and ideas – some a little bit speculative and maybe a little bit out there – but I could because I was the only author. I could take the risk and had the freedom, and some of them (those ideas), I think, inspired the field.

**JUR:** Your work is quite interdisciplinary, when you talk with other scientists in these fields, in conferences and such, how do you not feel like the odd man out?

**Dr. Welte:** There are lots of cell development biologists that study flies, there are some conferences where I fit right in, and for other conferences ... like for lipid droplet conferences, I definitely felt like an odd man out for a while, but that means I can bring a new perspective, and people tend to be interested in something not completely mainstream. You also then learn a lot about the rest of the field that might inspire your own research. For example, even though I've studied lipid droplets since 1994, I didn't focus on the lipid aspect of the lipid droplets until maybe starting ten years ago. I had studied the motion of lipid droplets and how they interact with proteins. But

I've gone to lipid droplet conferences since 2007 and learned a lot about lipid metabolism from just listening to talks and looking at posters. Now I'm applying that knowledge to ovary development, and we are finding new aspects of lipid metabolism in ovaries, an area that got very little attention previously, especially not when it comes to a role for lipid droplets.

**JUR:** You also teach a course, Advanced Cell Biology, how do you improve the research abilities of the students in your course?

**Dr. Welte:** So, in this course I teach ... my goal is for the students to learn professional skills in science and learn how to analyze papers critically. One big aspect of the course is that the students write a research proposal by the end of the semester, and because most students have never done that before, we do that in steps. They first write an outline, just one or two paragraphs, and I give them feedback on that. Then they write the long outline of that draft, and after that multiple additional drafts. After each step, they get feedback either from me or from a peer, so students gradually get used to all the elements that go into writing a proposal.

**JUR:** How has being a member of the LGBTQ community affected your scientific career?

**Dr. Welte:** I came out to myself as a gay man in my twenties and was lucky that the Ph.D. and postdoc labs I worked in were supportive and accepting. Over time, I met a few members of the LGBTQ community that were at the same career stage. Unfortunately, that was not always under positive circumstances: One of my gay classmates in graduate school was assaulted and beaten up on his way home from the lab one night, with the attackers shouting homophobic slurs. But for a long time, I never knew of a member of the community that was further ahead in their career and who could serve as a role model. Clearly, there must have been queer faculty out there, but I didn't know how to recognize them. Without such representation, it is easy to feel alone and to question one's ability to succeed in science. One of the things I now spend a lot of time thinking about is how to increase the visibility of LGBTQ folks in science, at UR and beyond. Such visibility is not only important for other members of the LGBTQ community; role models and feeling represented can make a huge difference. But it is also necessary for science and society at large, so that they realize that LGBTQ scientists are everywhere and make important contributions to research and teaching.

### Lightning Round

**JUR:** What is your favorite color?

**Dr. Welte:** I don't know; I like a lot of colors. I wouldn't want to choose [one of] them – I like the rainbow.

**JUR:** What is your favorite kind of microscopy, to look at,

and/or to use?

**Dr. Welte:** We use scanning confocal microscopy for many of the things that we are studying, as it is a good workhorse. I'm involved with a group that is developing a new light sheet microscope, working with optics, data science, and BME, so that will open many new avenues of looking at embryos and other things. So, in some way I like the microscopy that answers the questions I have at the moment.

**JUR:** What is your favorite type of cell?

**Dr. Welte:** Another question where I would rather not have to choose. Well, technically, early embryos are a single cell, and I love studying this stage of embryogenesis. Other than that, maybe nurse cells during oogenesis; those are the cells that help the oocyte grow. They make thousands of lipid droplets, and then transfer them to the oocyte. They are big and have a lot going on, and we have many tools to visualize the processes in them.

**JUR:** If you could give immortality to three living scientists, who would you give immortality to?

**Dr. Welte:** I don't know. Probably at least one of them would be my postdoc advisor, because he's just a fun person to talk to. He is brilliant, he's also a little crazy, but he's also a wonderful person. He is a lateral thinker; he thinks differently than other people and sees connections where nobody else has previously noticed them.

**JUR:** What books are you currently reading?

**Dr. Welte:** I'm currently reading three different books and listening to a podcast. I'm reading a book entitled "Human Errors" which talks about the ways in which the human body is not well designed. This idea that evolution did just enough to make an organism work, but that is doesn't come up with a perfect solution. In biology, we typically view organisms and cells as being really well adapted and perfect. That is true in many ways, but evolution doesn't necessarily select for perfection, it just has to be good enough. One favorite example is that rabbits eat their own feces to ... get Vitamin B12. It doesn't seem like a well-designed setup, but that's how it works since the bacteria that produced the B12 live in a region of the gut after where the transport proteins for the B12 vitamin are. So, rabbits have evolved this behavior that they eat their own feces, so that they can get the B12; it works, it's probably not an elegant solution, but it works. The book mostly focuses on the human body, but then also talks about some other mammals' evolutionary solutions that seem really inelegant, or don't really work that well, ... it's a refreshing perspective to entertain.

I'm also listening to an audio book on the history of Christianity. I'm really interested in ... the history of philosophical thought, and because of that in the development of religious thought. I'm also reading a book called

"Global Crisis"; it's the history of the 17th century, when there was a lot of climate change, including the little Ice Age, which affected temperatures globally. So, this historian looks at the collapse of various societies around the world from China to Europe to Africa; lots of societies had major wars or major upheavals that he tries to tie to climate change ... I find it really fascinating.

**JUR:** Why did you grow out the beard?

**Dr. Welte:** I started to grow it out when I became department chair, and I haven't shaved it since. I was department chair for two three-year terms, and that's when I started growing it. I'm not quite sure how it's connected, but in time it's connected, and ... I've grown fond of it.

**JUR:** Do you have any final thoughts or messages?

**Dr. Welte:** I guess what I tell many of my students: As you think about your career, you have to find something that you like to do, where you have fun doing it. That will bring you back to the lab even if there are failures, even if there is no progress and many setbacks.

# Selected Abstracts from the Annual Undergraduate Speakers Symposium 2024

University of Rochester, Rochester, NY

April 5<sup>th</sup>, 2024

## Mediated Memory and Propaganda in an Analysis of the United States Embargo on Cuba

**Author:** Dariel Guerra '25

**Mentor:** Molly Ball

This study examines how United States propaganda, Cuban state-run media, and mediated memories have shaped the understanding around the United States embargo on Cuba. The argument I make is that the United States embargo on Cuba is the culmination of Cold War misleading narratives and unproductive politics disseminated through various media. This examination of the embargo relies heavily on print sources (newspapers, advertisements, posters) and digital media sources (blogs and newspapers). I analyze existing historical, political, and legal literature through an investigative lens of mediated memory and propaganda. How have hegemonic reconstructions of Cuba in memory manifested into hard-line policies against the Cuban government? How does propaganda play into the reconfigured, imagined Cuba in the minds of both Americans and Cuban-Americans? Utilizing the methodological framework from *Aggressive and Passive Propaganda: Cuba and the United States* by Eugene H. Pons I rhetorically investigate trends, views, and sentiments emerging from the media covering the embargo. In recent years, discourse surrounding Cuba has significantly diminished despite the ongoing continuation of the embargo and the strengthening of sanctions in 2017. The two nations continue to antagonize one another through media, disseminating narratives of half truths through newspapers, online independent news outlets, and blogs. My research concludes that the United States embargo on Cuba is a culmination of Cold War media warfare and aggressive propaganda. As a result of this media warfare, communities in the U.S. have distorted perceptions of Cuba, therefore leading to the continuation of the United States embargo. These modern representations of Cuba reinforce false narratives that ultimately fall on the backs of the Cuban people. Future work will explore the historical significance of the decades in Cuba preceding the Revolution, specifically examining case studies of United States involvement under the Platt Amendment (1901-1934). These case studies underlie greater trends that have provided subtext for the Revolution and the embargo, as well as ensuing media wars between Cuba and the United States.

## Coarse-Grained Strategy for Modeling the Self-Assembly of Janus Nucleobases

**Author:** Derek Chien '25

**Mentor:** Ignacio Franco

Janus nucleobases are two-faced hydrogen-bonding heterocycles that undergo hierarchical and programmable self-assembly to form biomimetic supramolecular polymer networks—such as nanotube structures composed of stacked hexameric rosettes—with extensive biomedical applications. However, the mechanism and driving forces that dictate the non-covalent self-assembly of these molecules remain poorly understood, preventing the rational design of Janus nucleobases to guide the self-assembly into specific outcomes. Here we develop a computational strategy to investigate the thermodynamic driving forces directing the hierarchical self-assembly of hybrid guanine-cytosine (G/C) Janus nucleobases. We favor a coarse-grained strategy, as opposed to fully atomistic modeling, in order to computationally access the experimentally relevant system size and time scales that lead to self-assembly. In the model, the G/C nucleotide is represented as a patchy isosceles trapezoidal prism in which three types of facets allow for selectively directional interactions: two complementary Watson-Crick pairing edges and the aromatic planar face responsible for stacking. MP2 first-principle electronic structure computations are carried out on G/C module pairs in relative positions and orientations that exemplify hydrogen bonding or stacking; the resultant energies are used to parameterize the patchy particle model and accurately replicate the pairwise interactions between the coarse-grained facets. In turn, the self-assembly process is modeled through Monte Carlo simulations using the coarse-grained model. The strategy is able to reproduce the self-organization of G/C modules into rosette nanotubes and thereby informs about the importance of hydrogen-bonding and stacking interactions on modulating the hierarchical self-assembly.

## Enhanced Reliability Predictions: An AI-based Framework for Performing Failure Modes, Effects, and Criticality Analysis in Industrial Environments

**Author:** Stephanie Wang '25

**Mentor:** Frank Zou

Reliability engineering grapples with the imperative task of predicting and comprehending product failures across an array of sectors. Although traditional approaches to ensure product safety in reliability engineering like Failure Mode Effects and Analysis (FMEA) and Failure Mode, Effects, and Criticality Analysis (FMECA) are successful, their inherent manual intensity and reliance on expert insights introduce limitations. As today's systems surge in complexity, a more holistic approach becomes paramount. In response, we introduce an AI-driven risk assessment tool that guides the user to a host of failure modes and their effects for each component contained in a bigger system. Through a user-friendly graphical interface and a robust statistical modeling backend, the AI-driven tool streamlines the risk assessment process by prompting users to input a system's name and subsequently generating an extensive array of failure modes and associated effects for each constituent component, including Weibull, Rayleigh, and Bathtub distribution curves. By automating this aspect of FMEA/FMECA, the AI-based solution seeks to not only enhance reliability analyses but also optimize development timelines, improve resource allocation, and provide valuable educational avenues for junior across sectors, including chemical, automotive, aerospace, and beyond.

### **Sensory Stimuli: An Examination of Light, Sound, and Movement**

**Author:** Glory Linebach '24

**Mentor:** Anne Wilcox

Sound and light are utilized to create atmosphere, mood, and tone by artists. When generating a work of art, the creator takes into account what colors or sounds they should incorporate to evoke the emotion of their work. In dance specifically, choreographers use light and sound to create an atmosphere around the choreography. That said, how does the choreographer know what specific colors and sounds will go with specific movements to create the mood they are looking for? Current research within the field of color theory has shown strong connections linking light to specific emotional responses. In other words, specific colors represent emotions (MSEd, K). Similarly, in the field of brain-and-cognitive science, different pitches have also been correlated to cause emotional responses; however, the range of emotions that connect to a sound frequency span a more basic spectrum (happy to angry) compared to the emotions related to a color of light (Vos S). In recent research, movement analysts have found that specific movement vocabulary and qualities of movement are connected with the expression of emotions: specific movements correspond with the emotion a person feels in that moment (Tracy). My research proposes that we can connect the emotion felt with the color of light, the

pitch of sound, and the subject's movement qualities. Multiple workshops were conducted to collect data and investigate this correlation. A dance was then created to highlight the results of the relationship between light, sound, emotion, and movement.

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### **From Belief to Retreat: Prophetic Failure in the Fifth Crusade**

**Author:** Clarice Speight McKee '24

**Mentor:** Laura Smoller

Paul Veyne famously asked the question "Did the Greeks believe in their myths?" I want to ask a version of this question about medieval Europe. Chronicles and letters from medieval Europe abound in mythical tales and prophetic texts, often conveniently aligning with the author's own polemical goals. Were these just cynical manipulations, a literary trope, or, to echo Veyne, did medieval Europeans believe their prophecies? I argue that, during the Fifth Crusade (1217-1221), they did. Sent to reconquer Egypt from the Ayyubid Caliphate, the Fifth Crusade ended in miserable failure. Arriving in Egypt in 1218, the Crusaders were a ready audience for tales about an eastern Christian king named Prester John, whose kingdom was wealthy and pious beyond the European imagination. They received prophecies, both Christian and Islamic in origin, that appeared to promise that Prester John was only weeks away, ready to save the Crusaders from the Ayyubid Caliphate's forces and liberate the holy city of Jerusalem from the "infidels." Simultaneously, a text known as the *Relatio de Davide* began circulating among the Crusaders, describing the central Asian conquests of a descendant of Prester John, King David, which sounded quite like the real conquests of the Mongol empire. This only reinforced their belief that Prester John was coming to rescue them. I argue that their belief in these tales explains one of the puzzles of the Fifth Crusade, the Crusaders' seemingly odd choice to advance into Egypt in the summer of 1221, just before the annual Nile flood would cut off any means of escape. Why retreat when reinforce-



ments were on their way? Of course, instead of receiving aid from the mythical Prester John or King David, crusading armies were trapped by the flooded river and soundly defeated. Chronicles produced after the Fifth Crusade repudiated their eager belief in the tales about Prester John, now arguing that the prophecies may have been wrong and that Prester John may not even have been Christian after all. That chroniclers felt the need to 'correct' the Prester John myth rather than ignore it shows the continuing importance of the myth. The continuing search to understand who Prester John was shows that yes, medieval Europeans really did believe in their myths.

### **Evaluating Interventions to Increase Cancer Screening Follow-Up Rates in High-Risk Emergency Department Patients**

**Author:** Trisha Mondal '27

**Mentor:** Beau Abar

**Background and Objectives:** Emergency departments (ED) play a vital role in addressing community health needs, serving as a unique gateway to preventive services like cervical, colorectal, and lung cancer screening. Interventions involving research associates/volunteers can be used to promote screening in the ED, though differing patient populations across screenings may necessitate differential efforts at intervention and retention. Our study compares follow-up rates to three preceding clinical trial pilots focused on cancer screening for cervical (females ages 21-65), colorectal (adults ages 45-75), and lung cancer (adults ages 50-80 with a 20 pack-year smoking history who currently smoke or quit smoking within the last 15 years). We aim to assess the extent to which retention efforts might require adaptation across cancer screening populations.

**Methods:** Separate clinical trial pilot studies were conducted for the three cancer screening types, evaluating the effectiveness of a text-based behavioral intervention versus a verbal referral with written materials through the ED. In the current analysis examining study retention, participants' outcomes were classified as successfully reached or lost to follow-up based on response to a phone call to self-report their screening data 150 days after their initial ED visit. Up to 5 call attempts were made for each participant. Data were compared across studies using chi-square tests for independence.

**Results:** In the colorectal and cervical cancer pilots, 61% (69/114) and 57% (54/95) of participants, respectively, were successfully reached at follow-up. Despite using the same methods, only 44% (88/198) of participants in the lung cancer pilot were reached via phone ( $p < 0.001$ ). No significant difference in reachability was observed between colorectal and cervical cancer screening. These disparities cannot be attributed to sex, given nearly identical response rates in colorectal (men and women) and

cervical (women) cancers, or COVID (timelines of the colorectal and lung cancer studies largely overlapped).

**Conclusion:** Patients requiring lung cancer screening exhibited the lowest likelihood of answering follow-up calls, posing a challenge in evaluating interventions in this specific population. Our findings highlight the need for more targeted efforts in retaining lung cancer screening patients to enhance the efficacy of preventive health care measures.

### **How parasites manipulate neural gene expression of developing hosts**

**Author:** Natasha Vacca '25

**Mentor:** Floria Mora-Kepfer Uy

Across animal systems, hosts and their parasites display a strong arms-race where parasites are incredibly successful pathogens that exploit the host via less understood mechanisms. To study these mechanisms, our lab recently found gene expression patterns in the brain of adult social wasp hosts, *Polistes fuscatus*, that reflect parasitic manipulation via their insect parasite *Xenos peckii*. Parasites infect female workers destined to be short-lived and expand host life span 9-13 times longer while creating a neural gene expression profile that is similar to long-lived queens. However, it has been unclear if these parasites begin manipulation as their hosts develop, or instead they only manipulate at the adult level. I developed a project to determine when parasitic manipulation commenced in host neuronal pathways using neuronal RNA extraction from infected worker larvae, uninfected worker larvae, and uninfected future queen larvae. My first results show differential gene expression between infected and uninfected larvae that mirrors the altered expression of future queens compared to workers during development (Fig. 1). In early stages of development, the parasite manipulates key genes associated with immune regulation, aging, and metabolism to alter the host's overall neural gene expression to match long lived future queens in its last instar. Additionally, the patterns of development from these hosts show an asynchronous development within hosts until the last parasite larval stage also supporting this novel finding in gene expression modulation. These findings shed light on how this emerging aging model system develops and subsequently reprograms hosts via parasitic neuro-modulation over time.

### **Neural Mechanisms Contributing to Auditory Processing Disorders in Developmental Disabilities**

**Author:** Sarah Mehta '25

**Mentor:** Emily Knight

Children with ADHD (attention-deficit/hyperactivity disorder) and ASD (autism spectrum disorder) struggle in understanding speech in noisy settings, causing difficulty in academic and social scenarios. Understanding the neural mechanisms causing these auditory processing disorders may inform future interventions in this area. Using simultaneous 3D virtual reality (VR) and electroencephalogram (EEG) integration, we have developed a novel experimental paradigm allowing the naturalistic study of how two proposed neural mechanisms (audiovisual integration and selective visuospatial attention) may influence auditory processing in children with ASD, ADHD, and typical development (TD) ages 7-12 years. The stimuli consists of three virtual characters: a center, target character and two flanking distractor characters presented in VR. The participant is told to press a button every time the center person says a target word, ignoring any words spoken by the distractor characters. We manipulate audiovisual integration by including randomly intermixed trials that are visual-only (character lip movements only), audio-only (character speech sounds only) and audiovisual. Spatial attention demands are manipulated with non-interference trials (where only the center character is speaking) and interference trials (where all three characters are simultaneously speaking). We measure steady state visual evoked potentials (SSVEPs) through high density EEG by tagging the target and distracting speakers with visual frequency oscillations (23 hz and 15 hz respectively). If the strength of corresponding SSVEP bands correlate to 23 Hz and/or 15 Hz, we are able to determine where visual attention was located. Preliminary behavioral results in children with TD (n=7), ADHD (n=4), ASD (n=1) and ASD+ADHD (n=1) reveal highest accuracy across groups in the non-interference condition. Relative to TD children, children with ASD or ADHD may show smaller performance gains in the audiovisual compared to audio-only condition. Preliminary EEG data suggests there may be a trend emerging toward potential group differences in allocation of visuospatial attention. Children with TD tend to show robust SSVEP primarily in the 23 Hz (target) band while those with ASD or ADHD tend to demonstrate SSVEP in both the 15 Hz (distractor) and 23 Hz (target) frequency band. Taken together, if these trends persist with continued data collection, this could imply that audiovisual integration and visuospatial deficits may contribute to auditory processing disorders in children with ASD and/or ADHD.

### **Unveiling the Pixels: A Critical Analysis of Black Representation in Action Video Games**

**Author:** Kendal Jordan '25

**Mentor:** Kristana Textor

The depiction of black and African identities in video games has been neglected due to societal perceptions of the 'main character' - white, male, muscular, and reli-

gious. This content analysis explores the representation of the Black experience in AAA action video games, addressing the evolution of stereotypes and their influence on player perceptions. Drawing on historical context, from Jim Crow Era stereotypes to contemporary portrayals, the study reveals a transition from overt to insidious forms of racism within gaming narratives. Previous literature findings indicate a systematic overrepresentation of white, male protagonists, with Black characters often relegated to stereotypical roles, props for abuse, or sidelined entirely. This study aims to highlight the perpetuation of learned biases and stereotypes through gaming experiences (e.g., affecting adolescents' perceptions of race and gender). Through a sample of 10 prominent titles, characters were selected and assessed based on various criteria, including education, gender, and positive role portrayal. To get a better grasp of the overall opinions of the game, 4 comments from each game were collected from Steam - a digital storefront. 48 participants completed a survey that collected their general views on the character's design. We found that certain characters (e.g., Sheva, Everett, and Marcus) had the most similar survey responses to the Steam comments of their respective games. The main takeaway of this research is to explain the importance of diverse representation both in character portrayal and within the gaming industry, through advocating for increased inclusion and empowerment of marginalized voices. This is a preliminary analysis so a bigger sample size and more varied games are needed to delve deeper into the complexities of intersecting representations.

### **Challenging the Stereotype of the Witch: Medea in 15th Century Burgundy**

**Author:** Kirsten Bell '24

**Mentor:** Laura Smoller

One specific trend that occurred in fourteenth and fifteenth century Europe was a shift in attitude towards witchcraft and magic, in that it hardened and eventually resulted in the multitude of witch trials of the sixteenth and seventeenth centuries. This shift also included the formation of the diabolical witch stereotype, where ideas about dangerous witches were no longer isolated to clerical scholarly circles but spread to the public. One way that this seems to be shown is through the literature published by some members of the Church discussing how dangerous witches are to society. In regards to literature not published by the Church, I found that stories featuring Medea, one of the most famous mythological ancient enchantresses, can indicate how these societies viewed magic and witchcraft. In the ancient world, Medea was a fictional princess of Colchis who used magic to assist the hero Jason in obtaining the Golden Fleece. In this myth, Jason later



betrays her by marrying another woman. When conducting research on the medieval literature where Medea appears, one book stood out, titled *L'Histoire de Jason*. This was written in 1460 by Raoul Lefèvre and commissioned by Duke Philippe le Bon of Burgundy. At the same time that this book was written, an incident occurred that can be considered one of the first institutionalized witch trials. These trials occurred in the city of Arras in the region of Burgundy between the autumn of 1459 and 1460. They were led by Dominican friars who attempted to equate witchcraft with apostasy, which is when one renounces Christianity and either cannot repent for their sins or only do so with great difficulty. In this thesis, I argue that Medea's specific portrayal in Lefèvre's *L' Histoire de Jason* reflects the attitudes around witchcraft and magic in Burgundy in the late 15th century, in that she is a redeemable magic user but harmful magic is still a problem. It indicates echoes of ancient and earlier medieval conceptions of magic, where magic is a tool that can be used for both good and evil. Medea is not inherently evil for using magic because she is always driven by her immense love for Jason, and reconciles with Jason at the end of the story after she becomes a penitent. Since Dominican friars attempted to equate witchcraft with apostasy through the Arras incidents, the fact that Medea is able to repent for her sins shows how 15th century Burgundians did not completely buy into this new conception of demonic and non-repentable witchcraft.

### **Atomic-scale Simulation of Nanowires Under Bending Deformation**

**Author:** Sicheng(JD) Qian '25

**Mentor:** Niaz Abdulrahim

Body-centered-cubic (bcc) metallic materials, especially Molybdenum (Mo), are used in a significant number of applications due to their excellent mechanical properties. Components made by nanomaterials for various uses usually encounter both simple uniaxial stress and complex stress conditions. Current studies have focused on material properties, such as phase transformation and twin boundary, in Mo metallic nanowires (NWs) under uniaxial tensile and compressive loading. However, only a limited amount of research has focused on bending deformation in BCC material due to the technical difficulty of the experiment. This research utilizes LAMMPS to compute the MD simulation of bending deformation on the BCC Mo NWs. A complete phase transformation path from bcc1 to face-centered-cubic (fcc) to bcc2 has been observed in the  $\langle 1\ 0\ 0 \rangle$  and  $\langle 1\ 1\ 0 \rangle$  Mo NWs. Tetra-twin boundary (TTB) forms in the structure along  $\{1\ 1\ 2\}$  slip system under bi-directional bending. In contrast, only  $\{1\ 1\ 0\}$  slip system has been activated under mono-directional bending. As a result, nucleation of TTB enables second elasticity and large uniform plastic deformation in the structure. This study reveals a new type of twining struc-

ture under non-uniaxial loading and largely fulfills the understanding of Mo and BCC material mechanical behaviors.

### **Testing the Effects of Artificial Intelligence Disclaimers in Political Advertising on Viewer Perceptions**

**Author:** Garrett Briggs '24

Recent polls demonstrate that American voters are distrusting of artificial intelligence. Simultaneously, lawmakers have started to regulate artificial intelligence (AI) in political advertising, with states passing legislation requiring the disclosure of AI assistance through a visual disclaimer. Surprisingly, no research explores whether attributions to artificial intelligence in campaign material affects viewers' reactions. My research investigates this gap through a video experiment testing the effects of partisan border wall ads with and without AI disclaimers. I find that advertisements are more trusted in the presence of AI disclaimers, with the differences being significant across four outcome measures. Additionally, I find that the results are heavily influenced by partisanship, with Democrats being substantially more likely to reward AI disclaimers than Republicans. These results provide valuable information to policymakers and candidates surrounding the implications of AI-assistance disclaimers in political advertising.

### **Identifying Early Biomarkers in Sclerotic Vulvar Disease**

**Author:** Ashley Updike '24

**Mentor:** Megan Falsetta

Lichen sclerosus (LS) is a chronic inflammatory and scarring disease commonly found in the anogenital region. LS affects male and female anatomy, but 85% of cases are identified on the vulva (external part of the female genitalia). The current estimated prevalence is as great as 3% in women. At least 5 million US women live with LS. The most common symptoms in are pain, vulvar pruritus (itching), dysuria (difficulty or pain while urinating), and dyspareunia (pain during intercourse). Over time, this leads to severe changes in the vulvar architecture that can only be altered by physically removing parts of the vulva, which may be necessary to restore voiding function (ability to urinate) and relieve other symptoms. The exact prevalence of LS is unknown and underreported. It is imperative that we find disease biomarkers that could be used to identify LS before these irreversible and potentially life-threatening architectural changes occur. Our hypothesis is there are genes that are highly expressed in the regions adjacent to the visibly scarred regions in LS patients that could serve as unique biomarkers for early disease

diagnosis. To look at the temporal effects of gene expression in perilesional areas, a time course experiment was performed and the preliminary data is consistent with my hypothesis that there are early disease markers. The genes we have identified could serve as biomarkers for disease to prevent permanent architectural changes and ultimately cancer. Steroids are effective for most patients, but they are ineffective if delivered after permanent architectural changes occur. These findings could be directly translated to clinical applications to improve quality of life for many patients.

### Detecting AI-Generated Essays: Unveiling Bias Against AI In Residency Application Evaluations

**Author:** Loralai Crawford '24 / Take 5 '25

**Mentor:** Nicole Wilson

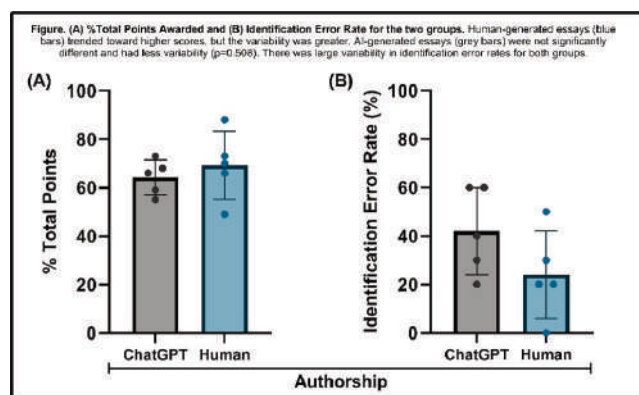
**Introduction:** Chat Generative Pre-Trained Transformer (ChatGPT) is one of many currently available large language models (LLMs) that can generate responses to user input. Applicants are increasingly using these tools to build their residency applications. However, based on internal discussions, program directors and faculty may consider use of artificial intelligence (AI)-generated responses a form of cheating. As LLMs improve, the ability of faculty to detect AI-generated text is questionable. Our purpose was to determine whether faculty of a general surgery residency program can detect AI vs. human-written responses to a text prompt and determine how assumed essay source affects reviewers' ratings. We hypothesized that faculty would not be able to reliably differentiate AI vs. human-written responses and that bias against presumed AI-generated essays would exist.

**Methods:** 10 faculty reviewers were recruited from the general surgery residency program. A standard text prompt, "Please tell us in 1-2 paragraphs why you are considering [our institution] for General Surgery residency" was used to prompt 10 essays, 5 from current trainees and 5 from GPT3.5 or GPT4. AI responses were selected as is and included in review packets after de-identification. Blinded reviewers rated essays (10-point Likert scale) for: desire to interview the applicant, relevance to general surgery residency, and overall impression; and indicated whether each essay was AI- or human-generated. Scores and identification error rates were compared between the two AI and human written essay groups using Mann-Whitney (median±interquartile range) and logistic regression (AUC±std error). Essays were redivided based on how they were identified by reviewers and total points awarded were compared between groups using Mann-Whitney (median±interquartile range) and 2-way analysis of variance (ANOVA).

**Results:** There was no difference between groups for % total points awarded (ChatGPT 66.0±13.5%, Human 70.0±23.0%,  $p=0.508$ , Figure 1). Higher overall impres-

sion scores were associated with reviewer identification of an essay as human-generated (AUC 0.82±0.04,  $p<0.001$ ). Except for one essay, all essays were identified incorrectly by at least 2 reviewers. There was no difference between groups for identification error rates (ChatGPT 40.0±35.0%, Human 20.0±30.0%,  $p=0.175$ , Figure B). Essays thought to be AI-generated (57.0±13.8%) scored lower in % total points awarded than those perceived to be human-written (71.5±9.3%,  $p=0.014$ )

**Conclusions:** Applicants use AI tools in all academic settings, but controversy exists regarding whether this should constitute a form of academic dishonesty. Our results demonstrate that while humans and AI tools generated essays of similar quality, bias exists against presumed AI-generated essays. Interestingly, faculty were not able to reliably differentiate human from AI-generated essays, suggesting the bias may be misdirected. AI-tools are becoming ubiquitous and their use is not easily detected. Therefore, faculty must expect these tools to begin to play larger roles in medical education.



# Selected Abstracts from the Inaugural Undergraduate Lightning Talks 2024

University of Rochester, Rochester, NY

April 10<sup>th</sup>, 2024

## Measuring Data Access Latency in Large CPU Caches

**Author:** Shaotong Sun '24

**Mentor:** Chen Ding

This talk describes a new, multi-locality benchmark program for testing memory access latency and using it to study recent AMD machines equipped with 3D vertical cache (V-Cache) that can be over 1 GiB in total size on a single node. The latency study shows that these large caches differ from traditional LLCs in two aspects: the V-Cache is partitioned rather than shared, and the cache replacement policy is more similar to random than it is to LRU.

## Bridging Accessibility Gaps: Developing The First COVID-19 Health Chatbot Tailored to the Deaf

**Author:** Akber Ahmed '24

**Mentor:** Jose Perez Ramos

In order to bridge the gap in creating more accessible technologies, my research project pioneers the development of the first COVID-19 health information chatbot tailored specifically to the deaf community. This utilizes a comprehensive approach that integrates UX Research methods and Human-Centered Design principles. Through different phases of group testing of the prototype, the chatbot is carefully developed to meet the needs of deaf users, ensuring accessibility and usability. Guided by a mission to blend technological innovations, user experience research, and translational science seamlessly, we aim to tackle health disparities and meet community needs through innovative Information Communication Technology (ICTs) solutions. By integrating expertise in UX research, translational science, and ICT innovation, our goal is to close technological access gaps for underserved communities, driving more impactful technological advancements.

## Serglycin Regulates Azurophilic Granule Formation in Neutrophils

**Author:** Wimeth Dissanayake

**Mentor:** Julia von Blume

Neutrophil granules are membrane-bound compart-

ments that release their contents upon a stimulus in a process that is called regulated secretion. Neutrophils contain three prevalent granule types, characterized by different protein compositions and capacity to fuse with the cell membrane: primary (azurophilic), secondary (specific), and tertiary (gelatinase) granules. The proper secretion and formation of Neutrophil granule proteins protect the body against infections, inflammation, and malignancies. However, the underlying molecular mechanisms of granule biogenesis in Neutrophils are poorly understood, limiting the treatment of neutropenic disorders. Granule proteins are synthesized, folded, and quality-checked before their translocation to the Golgi apparatus, with subsequent sorting to granules occurring in the trans-Golgi Network (TGN). The process by which granule-destined proteins are sorted and subsequently packaged into nascent secretory granules has remained a scientific enigma. Azurophilic granules (primary granules) are the first granules formed during Neutrophil differentiation and contain serine proteases, neutrophil elastase (Elane), and myeloperoxidases (Mpo). Previous studies suggested that Serglycin, Cd63, and Rab27a are required for the trafficking and function of azurophilic granules. Our unpublished work confirmed the increased production of Serglycin during the initial stages of neutrophil differentiation, corresponding with the initial expression of Elane, indicating that Serglycin may play a role in Azurophilic granule formation. To test if Serglycin is a granule-forming protein, we ectopically expressed serglycin in U2OS cells naturally deficient of secretory granules. Our data showed that Serglycin induces Elane, Rab27a, and Cd63 expression. Furthermore, immunofluorescence microscopy of Granulocyte-monocyte progenitors (GMPs), imaged at different time points of differentiation, showed increased expression and colocalization of Serglycin, Elane, Cd63, and Mpo during azurophilic granule formation. Our findings support the theory that Serglycin regulates azurophilic granule biogenesis by promoting the upregulation of cargo proteins (Elane) and trafficking machinery, including Cd63 and Rab27a. With this work, we have provided a first mechanistic insight into the biogenesis of azurophilic granules, setting the base for future investigations.

## Towards Deciphering the Mechanism of Microglia-Driven Remodeling in Brain Extracellular Space Using SFA-FRAP Imaging



**Author:** Showmick Ranjan Paul '25

**Mentor:** Dr. Edward Brown

Learning and memory require dynamic movement of different diffusible molecules and structural changes of dendritic spines, crucial for synaptic plasticity and the formation of new neural connections. However, being a physical barrier, the extracellular matrix (ECM) of the brain can affect this movement. We hypothesize microglia as a master manipulator of the ECM due to the remarkable motility of microglial processes, and the ECM-degrading enzymes that they produce. We utilize acute brain slices from mice and apply a quantitative optical method, Fluorescence Recovery After Photobleaching with Spatial Fourier Analysis (SFA-FRAP), which we have been developing and optimizing specifically for this investigation, to measure diffusive transport within ECM across various microglial motility states, including activated, deactivated, and absent conditions. Our objective is to assess whether microglia actively modulate diffusive transport and, if so, to elucidate the precise mechanism through which they accomplish such modulation. Results from this study are anticipated to shed light on the mechanisms underlying microglia-ECM interactions and provide insights into potential therapeutic strategies for neurological disorders associated with aberrant synaptic plasticity.

## The Democratization of Art through Technology: A Viennese Case Study

**Author:** Robert Marcinauskis '24

This project examines the democratization of the art market through digital instruments that unlock new revenue sources and investors. Technologies, such as NFTs and art shares, have brought a closer financial analysis of art as an asset. Comparatively, the blue-chip market has been subject to the historical arbitrary valuation of physical art pieces. Democratization occurs through the institutional validation of these instruments; such is the case of the Belvedere Museum. Pieces of art that have not been on the market for centuries are now accessible assets. In addition, the careful tracking of painting prices allows for stable, anticipated returns. The research conducted is through case studies focusing on the modern technologies explored. The primary case focuses on the partnership of artèQ and the Belvedere Museum in Vienna, Austria. This partnership created NFT's tiles of Gustav Klimt's *The Kiss*, which have unlocked new revenues for the museum and more investment opportunities for the art piece. This project challenges the uncertainty of digital instruments with the practical exposure of new investors to an asset class that had limited itself to an elite class. The cultural center of Vienna provides a backdrop to this revolution of Art, with its monarchical roots limiting artistic exposure of poorer classes. With the validation received

by the Belvedere, digital instruments become an important asset class that expands exposure to the returns of the art world.

## Tagmentation-based genomic mapping of Tol2 transgene insertion sites in zebrafish

**Author:** Paige Schneider '26

**Mentor:** Patrick Murphy

Transgenesis is a pivotal technique for unraveling gene regulatory networks and developmental processes. While the Tol2 transposon-based transgenesis system stands as the predominant method for generating transgenic lines in zebrafish, exact insertion sites for the majority of those generated lines remain unidentified. The lack of insertion site information raises concerns about potential local influences on transgene expression, off-target effects at protein-coding genes, and complications arising from copy number variations. To address these issues, we introduce Tol2Mapping, a novel method utilizing Tn5 transposase-mediated tagmentation for streamlined identification of genomic insertion sites for zebrafish Tol2 transgenic lines. Tol2Mapping is not only straightforward to perform but also allows for the identification of insertion sites from heterozygous transgenic lines and the detection of multiple insertion events. We also provide a detailed protocol for Tol2Mapping library generation, step-by-step sequencing analysis, and a user-friendly Shiny app, making this method accessible to researchers without programming expertise. Altogether, Tol2Mapping emerges as a valuable tool to enhance the precision and utility of zebrafish transgenesis studies, addressing critical challenges when using Tol2 transgenic lines.

## Synergistic Effects of an HDAC Inhibitor, a Therapeutic Cancer Vaccine, and an Immunocytokine in an HPV Tumor Model

**Author:** Sree Chatterjee '26

**Mentor:** Jeffery Schlom

There are over 630,000 new cases of HPV-related cancers worldwide each year. Despite the increasing prevalence of prophylactic vaccines as therapeutic treatment options, their impact on cancer incidence will be minimal for several decades. The only currently approved immunotherapy is anti-PD-1/PD- L1 in cervical and head and neck squamous cell carcinoma (HNSCC), whose response rate is only 10-20%. This project aimed to determine if three anti-cancer agents, PDS0101 (a prophylactic cancer vaccine), NHS-IL12 (histone targeting antibody), and Entinostat (HDAC inhibitor) used in combination have potential as a thera-

peutic treatment option for HPV-related cancer patients. We specifically looked at effects of the triple combination therapy on chemokine & cytokine levels within the tumor microenvironment & periphery, as well as links between chemokine/cytokine levels and anti- tumor response & immune cell populations in the tumor. We found that the triple combination therapy yielded the strongest anti-tumor effect, and that NHS-IL12 was a driving factor for increasing chemokine/cytokine levels. We also found consistency between MIG levels in tumor supernatant and CD8 levels in tumor, as well as KC levels and G-MDSC levels. Finally, high levels of immune stimulating cytokines were present within groups treated with some combination of agents (double & triple). However, the chemokine/cytokine data alone did not explain the level of anti-tumor response within triple therapy, so continued study is needed to determine synergistic effects of each agent.

### **The Effect of Language Framing on Climate Change Perception**

**Author:** Hanna Felber '24

**Mentor:** Karen Berger

Climate change is an increasingly pressing issue, but, despite its urgency, there is difficulty in garnering public support to address it. Many people still don't care, or worse, oppose the concept entirely. Because of this, it is crucial to understand how to communicate climate change information in a way that encourages people to reduce their climate footprints and support large scale efforts to decrease emissions. Previous research has revealed that the use of framing and metaphorical language can be extremely effective in shifting opinion, especially when used in media. This study applies these findings to climate change language, discovering just how influential framing is when discussing this topic. The participants, all US citizens between the ages of 18-35, first completed a pre-survey regarding their belief of climate change and willingness to act on it. They then read a paragraph on climate change that either used metaphorical language, non metaphorical language, accountability-based language, or non-accountability-based language. Lastly, they completed a post-survey with the same questions as the pre-survey. To measure the impact of framing, participants' pre- and post-survey responses were compared between the four conditions. Changes in response between these two surveys indicates an effect of language framing on climate change belief. The findings reveal that when discussing the harms of climate change and individual responsibility, non-metaphorical non-accountability language is more effective to use than metaphorical non-accountability language. Additionally, individuals were more likely to believe that they made effective actions to address climate change when presented with metaphorical non-accountability language. While further research is

needed, these findings support the notion that framing does impact climate change perception, and therefore must be utilized to garner support.

### **The Effect of Modifications to DNA Structure on the Architecture of Condensed Bacterial DNA with a Nucleoid-associated Protein**

**Author:** Kevin Zheng '24

**Mentor:** Anne Meyer

Escherichia coli increases the abundance of DNA-binding protein from starved cells (Dps) in response to various stressors bacteria encounter when exposed to a variety of environments. One of the primary functions of Dps is to bind and condense DNA. In vitro systems with different buffer conditions, different structures of Dps:DNA condensate have been observed. Our lab has termed these condensates spongiform, globular, and liquid-like. My research focuses on the contribution of different features of DNA to the morphologies of Dps: DNA condensates. DNA behavior and interactions differ over different length scales, supercoiling, and methylation states. Modifying these features of the DNA could alter the accessibility of the negatively charged DNA backbone and positively charged tail of Dps or alter the typical DNA structure, causing changes to the later formed condensates. To test the effect of varying DNA lengths, we incubated PCR products of different lengths (linear DNA fragments) with Dps to form condensates. The DNA in these condensates was mixed with fluorescently tagged DNA hairpins and Dps was mixed with Dps variants chemically labeled with Alexa fluor 647, enabling both to be visualized simultaneously. We used fluorescence microscopy to analyze the resulting morphologies. Our findings show that while changing the length of the DNA at the concentration we use does affect the size and number of condensates, the overall structure didn't vary. This suggests that shorter DNA may condense more effectively, while longer DNA creates larger structures. Further studies regarding the effects of DNA supercoiling and methylation states will provide a better understanding of the major factors that play into condensate formation.

### **Losing Diversity Through Hybridization? Distinct Populations of the Flame-Rumped Tanager Vanishing by Homogenization**

**Author:** Elizabeth Croyle '24

**Mentor:** J. Albert Uy and Maria Castaño

Incipient species boundaries can change over time due to gene flow and geographic isolation. These distinctions can blur, collapsing back into one population, or be reinforced, finishing the speciation process. Using

this framework, I am exploring the consequences of gene flow and isolation between two subspecies of a South American songbird. Throughout its range in Colombia, *Ramphocelus flammigerus flammigerus* and *R. f. icteronotus* are separated by the Andes but come into contact (sympatry) in at least three independent transects, where they produce a range of hybrids in areas of low-elevation mountain passes. To investigate the demographic history of the two subspecies and the effects of gene flow on divergence, I am using two coalescent, computational methods: 1) popsizeABC and 2) fastsimcoal2. First, I am using popsizeABC to estimate changes in effective population sizes up to 1 million years in the past, and investigating any historical decreases or increases. Second, I am testing different demographic history scenarios using fastsimcoal modeling to determine the most likely scenario that matches our observed transect 2 genomic data. My results show that the demographic model of divergence in sympatry (subspecies living in the same area) with increasing gene flow between subspecies is the most likely scenario for transect 2. Therefore, I predict that the subspecies will continue to become more similar, and eventually collapse back into a single population. Populations share a similar demographic history, with a prominent bottleneck around 6500 years ago, followed by rapid expansions. The timing of the bottleneck coincides with dramatic decreases in population sizes found in pre-Colombian human populations, most likely caused by unstable climatic conditions in the region. Next, I will run fastsimcoal on the remaining two transects and assess if sympatric homogenization is supported and if the demographic parameters (time of divergence, magnitude and directionality of gene flow) are consistent.

### Legal Accessibility for Domestic Violence Survivors

**Author:** Carly Zubrzycki '26

**Mentor:** Rachel O'Donnell

Willow Domestic Violence Center offers a variety of services to domestic violence survivors such as counseling, shelter, and legal advocacy. For the purposes of my project, I focused on increasing capacity for their legal advocacy program as there is limited capacity to keep community members informed of legal processes and resources by meeting with them one on one as the program typically entails. Likewise, there is a lack of resources in the local Rochester area that accept pro-bono or sliding scale domestic violence cases, particularly ones without a stringent income cutoff or enough variety to prevent conflict of interest. Thus, there is a need to create resource guides for common legal processes and establish pro-bono and sliding scale connections. The objective of the project was to establish 1-3 legal guides and develop at least one pro-bono or sliding scale connection with a local lawyer. In addition, the aim was to create a transition guide as well as a resource document so these

resources can exist beyond my work. In order to create the legal guides, I completed training days at Willow to observe what types of legal guides are needed, researched legal processes, and consulted my supervisors for review. To facilitate pro-bono and sliding scale connections, I conducted a literature review for best practices of contact and then I reached out to lawyers via email and phone to inquire about logistics and the extent of the partnership. For the resources document, I researched local area resources and use cases. Ultimately, these capacity building measures serve to increase legal accessibility by providing resources and services for those in need.



# NMDA Receptors in Stroke: Pathways and Potential Treatments

Lilli Noelle Tamm '25

Advised by Renée Miller, *Department of Neuroscience*

## Abstract

Ischemic strokes are the second-most common cause of death worldwide, with cerebral excitotoxicity following the cessation of oxygen and glucose delivery to the brain inducing confusion, changes in vision, sensation, and language capabilities. Poststroke brain injuries can persist for years, making post-ischemic recovery a crucial target for therapeutic treatments. NMDAR, a glutamate receptor, has been implicated in stroke pathology, with subunit GluN2B having been shown to promote cell death signal cascades. Stroke treatments must prevent apoptotic signals without impeding normal glutamatergic cellular function, propelling the search for treatments beyond mere GluN2B antagonists. This review examines pathways for treatment of ischemic stroke, beginning with low affinity extrasynaptic NMDAR antagonist memantine, then moving to the use of GlyT1 antagonist NFPS to increase glycine-induced NMDAR internalization and decrease excitotoxicity, and finally examining recent debates on CaMKII versus DAPK1 binding to GluN2B in post-ischemic cell death signaling.

**Keywords:** ischemia, excitotoxicity, NMDA receptors.

## I. Introduction

Strokes represent the second-most common cause of death worldwide according to Wu & Tymianski (2018), with ischemic strokes comprising 87% of all strokes (Donnan et al., 2008). The pathology of a stroke lies within the brain and involves cessation of blood flow to the brain resulting in the disruption of a variety of cognitively mediated functions such as speech, physical sensation, vision, and understanding. Beyond stroke mortality, cognitive damage induced by ischemic stroke can take years to heal, making stroke the third most common cause of neural disabilities (Wu & Tymianski, 2018). Treatments for ischemic stroke are extremely limited, as only a single FDA-approved treatment currently exists: recombinant tissue plasminogen activator (rtPA). Furthermore, most stroke patients are disqualified from rtPA due to the associated cerebral hemorrhage risks (Wahlgren et al. 2007), or delays in seeking medical care, as the drug must be administered intravenously within 4.5 hours of a stroke.

In addition to cognitive impairment resulting from immediate post stroke brain injury, recent developments have

indicated that ischemic stroke might increase risk for vascular dementia and Alzheimer's disease (Vijayan et al., 2016), although the exact molecular mechanisms linking these pathologies are not yet understood. There is thus an urgent need for better stroke treatments in order to combat stroke mortality as well as to improve recovery rates and limit cognitive damage caused by ischemic stroke. This damage can persist for years, severely impact patient quality of life, and potentially increase risk for the development of other neurological diseases.

One potential target in stroke pathology is N-methyl-D-aspartate receptors (NMDAR). Following the onset of stroke, when blood delivery to the brain has ceased, energy resources in the brain become depleted, leading to ionic dysregulation—as ATP-dependent ion exchange is no longer possible—and to subsequent uncontrolled glutamate release (Gasull et al., 2022). Excitotoxicity ensues, with incessant glutamate activation of NMDA and AMPA receptors prompting increased calcium release within neuronal membranes (Mao et al., 2022), triggering Ca<sup>2+</sup> dependent cell death pathways and resulting in neuronal death. Research into the mechanisms of NMDAR excitotoxicity and the discovery of molecular pathways specific to ischemic stroke can inform crucial targets for treatments that decrease excitotoxicity following stroke onset, as well as potentially decrease the spread of post-ischemic necrosis and improve the rate of repair for stroke-induced brain injury.

## II. NMDAR implication in stroke pathology

NMDAR plays a dual role in cell survival and death. A receptor localization hypothesis put forth by Sattler et al. (2000) theorizes that synaptic NMDAR contribute to cell survival through the activation of CREB, which suppresses pro-cell death genes (Hardingham, 2009) and promotes the expression of growth factor signal BTG2 found in BDNF and anti-apoptotic. In addition, synaptic NMDAR activation promotes calcium signaling which leads to the phosphorylation of Akt. Akt suppresses the apoptotic signaling protein BAD (Kim et al., 2001). In contrast, extrasynaptic NMDARs are believed to play the opposite role and promote cell death signals, including CREB dephosphorylation and inactivation. They also suppress environmental signals to the nu-

cleus via interference with the ERK pathway, which results in decreased gene expression and the promotion of cellular death (Hardingham, 2009). The role of extrasynaptic NMDARs in pro-cell death pathways may be rationalized by considering how tightly glutamate signaling is regulated in the brain and considering that extrasynaptic NMDAR activation would be indicative of excessive glutamate release and excitotoxicity.

Another theory for the role of NMDAR in ischemic stroke is the “NMDAR subtype” hypothesis, which claims that differences in the NMDA-binding subunits that make up a receptor determine the receptor’s role in promoting either survival or cell death (Liu et al., 2007). NMDAR is usually comprised of four subunits: two GluN1 and two GluN2. The relevant differentiation for excitotoxicity is between GluN2A and GluN2B, as it has been demonstrated that activation of NMDAR with the GluN2A subunit promotes cell survival, whereas activation of receptors containing GluN2B promotes cell death (Liu et al., 2007). To test this hypothesis, selective antagonists for each subunit were applied to cultures of cortical neurons, which were then bathed in glutamate. Inhibition of GluN2B activity resulted in reduced infarct volume when the antagonist was applied before the simulated stroke, demonstrating the implication of GluN2B in excitotoxicity. Moreover, activation of GluN2A via glycine agonist, as well as normal GluN2A activity in the presence of the GluN2B antagonist, resulted in decreased brain damage after stroke onset, evidencing the role of GluN2A in promoting cell survival and post-ischemic repair.

Subtype distribution shows trends of GluN2B subunits belonging primarily to extrasynaptic NMDAR, whereas GluN2A is more prevalent in synaptic NMDAR (Tymianski, 2011). This distribution is not absolute; therefore hypothetical treatments using GluN2B antagonists to target ischemic overexcitation might end up harming synaptic NMDA activity towards promoting cell survival. However, GluN2B has been shown to induce excitotoxicity at both synaptic and extrasynaptic NMDAR sites (Liu et al., 2007), a result that prompted research efforts to understand the role of GluN2B in mediating and prolonging excitotoxicity, and to develop treatments to suppress its interaction with downstream death-signaling pathways.

### III. Mediating consequences of GluN2B excitotoxicity

One possible research avenue for inhibiting the spread of GluN2B excitotoxicity is finding antagonists that possess enough strength to block the death signals without impeding NMDAR function at the synapse. Difficulties in examining the specificity of antagonists for differentiating between GluN2 subtypes have resulted in few viable treatment options, none of which have been FDA-approved. However, Wang et al. (2017) recently proposed the use of GluN2B antagonist memantine as a potential therapeutic for increasing post-ischemic recovery. Me-

mantine has been used to treat Alzheimer’s disease and exhibits a lower affinity for NMDAR compared to other antagonists. It was shown to preferentially bind extrasynaptic NMDARs, as well as to reduce brain injury in mice in the acute stroke phase (Lipton, 2004). Memantine binds to excessively open NMDARs, which explains its greater affinity for high-activity extrasynaptic NMDARs during stroke. Memantine also dissociates rapidly, meaning it will not interfere significantly with normal signaling (Lipton, 2004).

Wang et al. (2017) used memantine to treat mice for 4 weeks, beginning 3 days poststroke. Memantine was administered via pump directly to the cortex, in doses of either 4 mg/kg of mouse body weight or 20 mg/kg daily, with a control population receiving saline. Motor coordination tests (tight rope test and rotarod) were administered and evidenced significant improvements for the 20 mg/kg/day memantine group from the first data points onwards, while the 4 mg/kg/day group remained at the same performance level as the control group of mice (Wang et al., 2017, figure 2). This result suggested that direct administration of memantine can improve motor coordination recovery time following ischemic stroke. Because the progression of the control mice’s recovery over time was not followed, it cannot be concluded whether memantine increases the overall post-ischemic recovery from brain injury or if it solely increases the rate of recovery. The researchers also tested the different treatment groups for spatial memory using the Barnes maze test, through which they observed that the 20 mg/kg/day group showed decreases in total errors and latency during both the learning and retention phases. This result displays evidence related to the impact of memantine on cognitive repair (Wang et al., 2017, figure 3). Again, results were comparable for the 4 mg/kg/day group and the control group, and the lack of longitudinal study raises the question of the potential effects of memantine on the overall volume of recovery.

The researchers then euthanized the mice to observe the effects of memantine on synaptic plasticity and determine the molecular changes that resulted in greater behavioral improvements. Cresyl violet staining showed that the 20 mg/kg/day group displayed greater striatal volume 7 weeks post-stroke than the other two groups, as well as lower astrogliogenesis as determined through GFAP immunohistochemistry (Wang et al., 2017, figure 4, a–b). Researchers also found that memantine increases growth factor concentrations and regulates GluN2B, GluN2A, and PSD95 abundance in the contralesional motor cortex. Following a stroke, these proteins decreased in abundance, but memantine 20 mg/kg/day administration decreased GluN2B concentration after 14 days and partially restored GluN2A and PSD95 abundance after 28 days (Wang et al., 2017, figure 7, a–c). Decreased GluN2B reduced

NMDAR death signaling, while increased GluN2A increased cell survival. Furthermore, these results indicate that recovery improvements are still possible with later treatment administration. Memantine is thus a promising potential treatment for ischemic stroke patients. Future research should examine the effect of memantine on other brain areas and potentially other model organisms before attempting to administer it to human patients and should determine more precise dosages.

#### **IV. Receptor endocytosis: a novel indirect mediator of excitotoxicity**

NMDAR activation requires not only glutamate binding to the GluN2 subunit but also glycine as a co-agonist binding to the GluN1 at the glycine binding site (Rosenmund et al., 1998). Furthermore, Nong et al. (2004) demonstrated that glycine “priming” on NMDAR induces endocytosis through a clathrin- and dynamin-dependent pathway, with increased glycine signaling resulting in greater NMDAR internalization in hippocampal neurons, evidenced through weaker signals in response to NMDA application. This phenomenon, which requires the presence of both glutamate and glycine in the synaptic cleft, is called glycine-induced NMDAR internalization (GINI) and shows promise as a target for indirect mediation of excitotoxicity.

Cappelli et al. (2021) investigated GINI as a means of reducing NMDAR toxicity in ischemic stroke. First, they used cultures of CA1 pyramidal hippocampal neurons exposed to high concentrations of glycine to confirm that NMDAR-EPSC amplitude reduction was due to glycine-induced internalization. This hypothesis was verified when the effect of GINI was blocked by clathrin and dynamin inhibitors, preventing the endocytosis mechanism from operating. (Cappelli et al., 2021, figure 1). Cappelli et al. (2021) then demonstrated that  $\text{Ca}^{2+}$  influx is required for GINI, as NMDAR-EPSC increased with low extracellular calcium compared to normal amounts, indicating that a certain concentration of calcium is required. Application of nimodipine—a  $\text{Ca}^{2+}$  channel antagonist—to these cell cultures also decreased GINI amplitude, further confirming the need for calcium influx (Cappelli et al., 2021, figure 1). Further investigations into the mechanisms of glycine release into the synaptic cleft found that glycine is released during oxygen-glucose deprivation (Muller et al., 2013), conditions that correspond to the ischemic stroke environment.

GINI is countered by glycine uptake performed by glycine transporters like GlyT, which remove GINI from the synapse and therefore from glycine binding sites on NMDAR. Cappelli et al. (2021) hypothesized that GlyT +/- mice would experience greater GINI, as lower glycine concentrations would provoke the same amount of NMDAR signaling. Furthermore, glycine is neuroprotective (Chen et al., 2015), and it increases CREB phosphorylation and gene expression. Cappelli et al. (2021) addition-

ally hypothesized that the GlyT +/- mice would experience reduced stroke volume, owing to the neuroprotective effects of increased GINI, which endocytoses NMDAR and prevents GluN2B cell death signaling pathways. TTC staining of cortex slices poststroke resulted in smaller infarct volume for the GlyT +/- mice, demonstrating the role of GINI in stroke mediation (Cappelli et al., 2021, figure 2).

Following the confirmation of GINI as a potential therapeutic target, Cappelli et al. (2021) tested the use of NFPS, a selective glycine transporter antagonist, as a means of increasing GINI. They found that mice pretreated with NFPS had decreased stroke volume, evaluating this decrease through TTC cortex staining for the infarct volume. Pretreated mice also experienced improved motor behavioral deficits compared to controls. Motor behavior was evaluated through a horizontal ladder task requiring dexterity. Although recovery was not extensively studied, these results were extremely promising for GINI as a stroke therapy. The mechanism of protection was further tested by transfecting HEK293 cells with a mutated GluN1 subunit which could not bind glycine. The mutant GluN1 cells treated with NFPS showed no difference in stroke volume compared to the control mice transfected with WT GluN1, proving that glycine binding to NMDAR was crucial to the neuroprotection mechanism (Cappelli et al., 2021, figure 3). This experiment served to show the possibility of GlyT antagonists as promoters of ischemic recovery, and provided possible directions for future experiments to examine cerebral glycine concentration determination in excitotoxicity in human patients to ascertain how GlyT antagonism might be targeted to reduce stroke pathology.

#### **V. GluN2B cell death signaling**

Precise mechanisms of GluN2B apoptotic signaling remain unclear. The prevailing hypothesis within the field of NMDAR ischemic pathology has been that GluN2B interacts directly with death-associated protein kinase 1 (DAPK1) at extrasynaptic sites, prompting downstream signaling cascades that lead to apoptosis (Tang et al., 2018). However, a recent paper from Buonarati et al. (2020) rejects this long-standing claim and instead proposes a GluN2B-CaMKII interaction as the mediator of the death signal, challenging previously held findings.

CaMKII and DAPK1 bind GluN2B at the same location and their interactions are mutually exclusive (Goodell et al., 2017). CaMKII-GluN2B binding is a feature of long-term potentiation, and displacement of this interaction by DAPK1 competition is a feature of long-term depression. However, there is confusion surrounding the pathways of both enzymes: species designed to displace one must also displace the other, owing to overlap in binding sites, yet the resulting effects of



DAPK1 displacement (promotion of cellular survival) do not appear to correlate with the effects of CaMKII binding inhibition (promotion of cellular death). Further research is needed to elucidate the exact interactions of both species with GluN2B.

Buonarati et al. (2020) blocked the respective effects of CaMKII and DAPK1 to examine subsequent effects on GluN2B excitotoxicity. They created a GluN2B variant with two point mutations which prevented binding of either enzyme to the subunit. They then induced cardiac arrest in mice and stopped the arrest using epinephrine injections and increased oxygen supply. They then used H&E staining to examine the extent of cortical damage, which was found to be extremely limited compared to the damage inflicted on controls with wild-type GluN2B. This supported the involvement of either CaMKII or DAPK1 in GluN2B cell death signaling, and binding assays were subsequently performed to determine which one was implicated.

In vitro, pull-down assays were performed for both enzymes. CaMKII binding to GluN2B was significantly impaired for the mutant subunits compared to the wild-type, while DAPK1 binding was not impaired. These results surprisingly point towards CaMKII as the signal mediator. Moreover, immunofluorescence colocalization tests showed that DAPK1 colocalized to the cell membrane near NMDAR that contains mutant GluN2B. CaMKII must be activated by Ca<sup>2+</sup> pathways to bind GluN2B, so no colocalization was observed initially, but CaMKII remained separate from mutant GluN2B following stimulation to induce Ca<sup>2+</sup> influx, even though it was shown to colocalize with the wild-type subunit (Buonarati et al., 2020, figure 1). This shows that mutation of the GluN2B binding region and disruption of cell death signaling prevents CaMKII mediation of NMDAR apoptotic signaling, and not DAPK1 as previously thought. If these findings are correct, this may signify incredibly important progress towards our understanding of NMDAR-mediated ischemic stroke, and open up new avenues for treatment exploration.

However, it is difficult to discredit decades of research proclaiming DAPK1 signaling as the pathway associated with NMDAR cell death. As recently as 2018, publications have evidenced this pathway, with Tang et al. (2018) showing that different mutations of GluN2B blocked DAPK1 pathways. The actual cell death cascade is likely more complex than previously thought, with new research perhaps implicating DAPK1 signaling downstream of the CaMKII-GluN2B interaction. Further research is needed to determine the veracity of either of the two proposed pathways, with possible directions including knockout or attenuation studies of CaMKII to determine whether DAPK1 might take over as a secondary signaler, or perhaps knocking out DAPK1 to see whether it is required for NMDAR cell death signals to progress.

## VI. Conclusions

While the full extent of the molecular mechanisms mediating ischemic stroke pathology in NMDAR are not yet entirely understood, many recent advances have suggested new avenues for research and treatment development. Memantine and other low-affinity NMDAR antagonists show great promise for improving post-ischemic recovery for both motor and cognitive function, while pre-stroke treatment with GlyT antagonists reduces stroke volume, pointing towards GINI as a target pathway to weaponize for therapeutic treatment. Finally, the implication of CaMKII as the secondary messenger in NMDAR cell death signaling contradicts many previous findings which attribute this role to DAPK1, but this evidences greater complexity than previously understood in the GluN2B excitotoxic pathway. Future research will hopefully elucidate further details of this pathway and the interactions between these two opposing enzymes, and allow for more targeted treatments for NMDAR-induced ischemic stroke.

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## About the Author

I am a junior majoring in Biochemistry and Philosophy with a minor in chemistry. My research interests include protein-folding, biosynthesis, drug delivery vectors and metabolism. After graduating, I hope to pursue a PhD. in Pharmacology and contribute to improving existing therapeutics treatments and developing novel pathways for drug delivery, particularly in the field of neuropharmacology.

# Severe Bleeding Due to an Acquired FXIII Inhibitor in an Otherwise Healthy Patient

Bianca Santonastaso '24

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## Background

Factor XIII (FXIII), a key clotting factor in the coagulation cascade, reinforces the covalent bonds between fibrin monomers, stabilizes fibrin clots, and protects fibrins from premature fibrinolysis, a process that breaks down fibrin in blood clots. FXIII deficiency, a rare condition that is either congenital or acquired, results in a number of complications, such as impairing clot stability, increasing bleeding tendency, and delaying bleeding episodes.

## Case

A 76-year-old male with a history of Graves' disease, asthma, hyperlipidemia, and hypertension was presented to the emergency department with exertional fatigue, pain across his left flank, and ecchymosis over his right lateral abdominal wall. The patient reported prior bruising on the left lateral abdominal wall and denied any trauma, new medical conditions, or medications.

Initial laboratory work-up displayed normal basic coagulation studies and liver function, but decreased kidney function and acute anemia. However, a CT scan of the chest and abdominal region revealed extensive left flank hematoma, fluid attenuation across the right scapula, and hydronephrosis in the kidneys. Further tests observed enlargement of the right paraspinal muscles, evidence for a hematoma.

After being admitted to the hospital, the patient received one unit of packed red blood cells (pRBC). Further laboratory results the next morning showed significantly low FXIII levels (Table 1), and the patient continued to develop new and expanding ecchymosis and hematoma on his right back side over the next two days. Spontaneous bruising also appeared along his belt line and right groin, with mild tenderness on these areas (Figure 2). His FXIII levels continued to drop (from 37 to 4%) along with hemoglobin (Hgb) levels (from 7.5 to 6.7g/dL), but no other significant changes were observed. On the fourth and sixth day of the patient's stay in the hospital, two more pRBC units and three doses of cryoprecipitate were administered to mitigate his symptoms. Moreover, the lack of bleeding history in this patient and the critically low FXIII levels raised concern for presence of an acquired inhibitor; however, an FXIII inhibitor failed to be detected despite laboratory diagnoses using two different FXIII functional assays and a modified mixing study. Bleeding greatly improved after administration of prednisone and FXIII concentrate drugs (Corifact, CSL Behring, King of Prussia, PA). On the sixth day, the patient was discharged, and prescribed aminocaproic acid and prednisone for presumed FXIII inhibitor.

▲: High; ▼: Low; !: Abnormal.

Test	12/3/22 (1 <sup>st</sup> Admission)	12/12/22 (2 <sup>nd</sup> Admission)	Reference Range	Units															
WBC	5.7	11.9 ▲	4.2-9.1	THOU/microl.															
RBC	2.3 ▼	2.5 ▼	4.6-6.1	MIL/ $\mu$ L															
Hemoglobin	7.7 ▼	8.1 ▼	13.7-17.5	g/dL															
Hematocrit	23 ▼	25 ▼	40-51	%															
Platelet	240	253	150-330	THOU/microl.															
PT	12.3	13.7	10-12.9	seconds															
INR	1.1	1.2	0.9-1.1	N/A															
PTT	25.1	22.9	25.8-37.9	seconds															
Fibrinogen	227	N/A	172-409	mg/dL															
D-Dimer	0.32	N/A	0-0.5	$\mu$ g/dL															
FVIII	189 ▲	203 ▲	59-163	%															
vW Activity	211 ▲	N/A	55-167	%															
vW Antigen	230 ▲	N/A	56-166	%															
FXIII	24 ▼	46 ▼	75-155	%															
Lupus Anticoagulant	Negative	N/A	N/A	N/A															
Thrombin Time	12.3	N/A	13.0-17.7	seconds															
UN	37 ▲	33 ▲	6-20	mg/dL															
Creatinine	1.35 ▲	1.00	0.67-1.17	mg/dL															
eGFR BY CREAT	55 !	78 !	>60	mL/min/1.73 m <sup>2</sup>															
Calcium	8.6	8.5	8.6-10.2	mg/dL															
AST	29	10	0-50	U/L															
ALT	9	35	0-50	U/L															
Total Protein	6.5	7.2	6.3-7.7	g/dL															
Albumin	3.5	3.8	3.5-5.2	g/dL															
Mixing Study	<div>Chromogenic Assay</div> <table><thead><tr><th>Mins</th><th>FXIII</th><th>PTT</th></tr></thead><tbody><tr><td>0</td><td>53.9</td><td>28.8</td></tr><tr><td>30</td><td>51.7</td><td>27.8</td></tr><tr><td>60</td><td>52.0</td><td>27.5</td></tr><tr><td>120</td><td>53.5</td><td>29.3</td></tr></tbody></table>		Mins	FXIII	PTT	0	53.9	28.8	30	51.7	27.8	60	52.0	27.5	120	53.5	29.3	<div>Functional Assay</div> Results suggest severe FXIII deficiency (<1% of normal activity); No inhibitor was identified.	
Mins	FXIII	PTT																	
0	53.9	28.8																	
30	51.7	27.8																	
60	52.0	27.5																	
120	53.5	29.3																	

Table 1. Laboratory work-up for both admissions.



Figure 2. Right flank hematoma (bruising) present upon first admission.

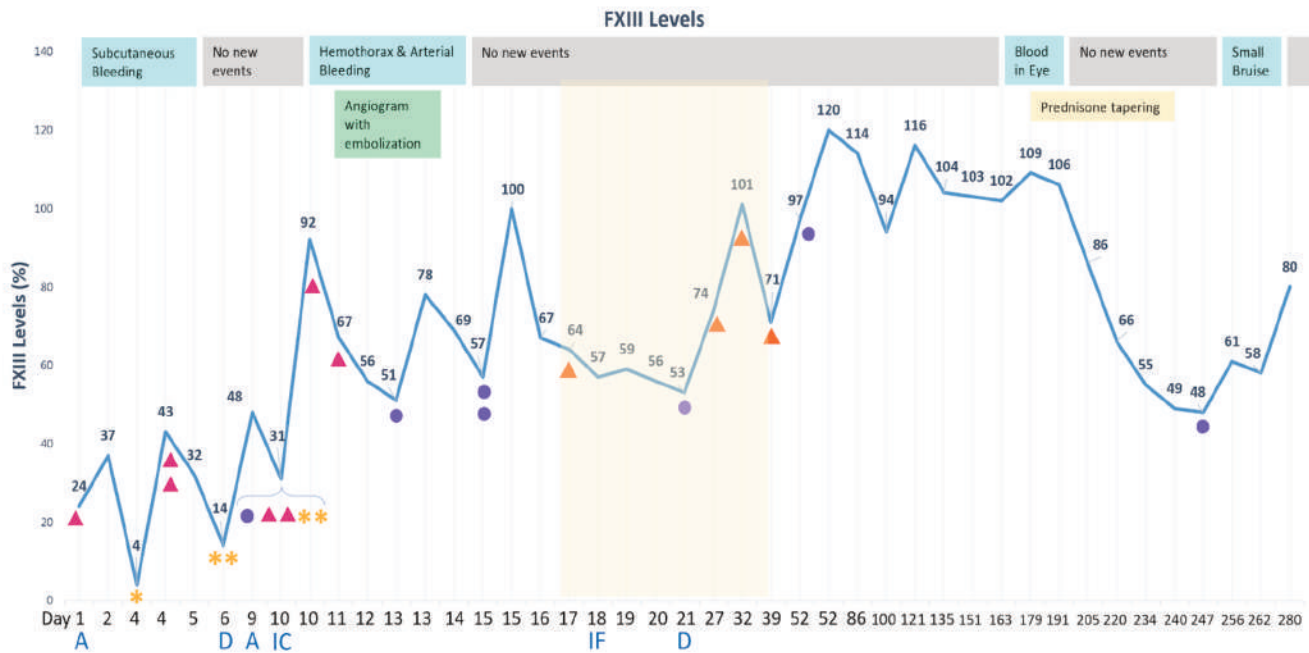
Three days later, he was readmitted with weakness, fatigue, dizziness, nausea, diffuse ecchymosis bruising, and swelling and numbness in his latissimus dorsi and lateral right thigh. He also reported right-sided leg pain and numbness in the distal right thigh. Lab tests revealed a FXIII level of 46% and a significantly low Hgb level of 8.1 g/dL. Further FXIII tests performed at a reference laboratory (ARUP) revealed <1% of normal activity, consistent with severe FXIII deficiency (Table 1).

An abdominal CT scan revealed hematoma along the abdomi-



▲: Red Blood Cells; \*: Cryoprecipitate; •: FXIII Concentrate; ▲: Rituximab (shaded box);

A: Admission; D: Discharge; IC: FXIII Inhibitor Assay (chromogenic); IF: FXIII Inhibitor Assay (functional).



**Figure 1.** FXIII levels (chromogenic) of a patient over the duration of two hospital admissions.

nal wall musculature and the same swelling seen in the scapula upon his first admission. Throughout the following days in the hospital, the patient developed bilateral hemothorax (bleeding into the chest) and arterial bleeding in his thigh. To prevent further bleeding and injury, he underwent an angiogram with embolization procedure, receiving 2 pRBCs, 3 doses of cryoprecipitate, tranexamic acid (TXA), and multiple FXIII concentrate (40U/Kg) infusions (Figure 1).

Over the next few days, intermittent FXIII concentrate infusions controlled the patient's bleeding and maintained FXIII levels between 51-69%. Weekly Rituximab antibody medication, prednisone, and concentrated FXIII replacement therapy was prescribed, and the patient was discharged four days later once bleeding was maintained.

Four weeks after discharge, the patient's aminocaproic acid treatment was discontinued. The first cycle of Rituximab was completed four months after discharge, while the prednisone was reduced from 40mg daily to 5mg biweekly. For the following six months, his FXIII levels maintained within normal limits; however, these changes in FXIII levels over the course of the treatment were directly correlated with immunotherapy and FXIII replacement administration prescribed (Figure 1). Although the patient tolerated a decrease in prednisone dosage to 5mg daily, he experienced a vast drop in FXIII levels once prednisone was reduced to biweekly doses. Once the steroid dosage was increased to 10mg daily and supplemented with additional FXIII concentrate, his FXIII levels immediately returned to baseline (Figure 1).

## Conclusion

The half-life of FXIII is approximately 11 to 14 days, which is useful in determining administration of specific drugs. It is supported in literature that FXIII levels above 5% are hemostatically adequate, but anything below 5%, especially at levels less than 1-3%, can result in severe bleedings. Although not identified serologically, the inhibitor was nonetheless present in this patient, indicated by significant bleeding, and clinical tests that showed persistently low FXIII levels, suboptimal response to cryoprecipitate and FXIII concentrate replacement therapy, and the favorable response to immunotherapy.

## Background

The fibrin-stabilizing effect of Factor XIII (FXIII) was initially reported in 1923 by Barkin and Gasper, who demonstrated that fibrin clots were insoluble in weak bases [1]. In 1948, Laki and Lorand identified a "protein fibrin stabilizing factor" that was responsible for the formation of the fibrin clot [1]. A decade later, Lowey et al. purified and determined the mechanism of this factor [2]. In 1961, the first pediatric patient with impaired wound healing, abnormal scarring, severe bleeding, and decreased "protein fibrin stabilizing factor" was reported[1]. The role of this clot stabilizing factor was then recognized and designated by the International Committee on Blood Clotting Factors as "Factor XIII" [3].

In plasma, FXIII circulates as a heterotetramer composed of two catalytic A-subunits and two carrier B-subunits [4]. It is a

multifunctional molecule known for its role in hemostasis, wound healing, angiogenesis, pregnancy maintenance, bone metabolism, and cardio protection [4,5]. FXIII deficiency can be either congenital or acquired, and may result in a number of complications such as delayed bleeding episodes, as it plays a critical role in stabilizing fibrin monomers in the final step of clot formation.

Congenital FXIII deficiency is a rare autosomal recessive disorder, estimated to affect one in two million people, with activity levels between 5-20% [6,7]. Heterozygous FXIII deficiency is usually asymptomatic, with an activity range of 50-70% [8]. In newborns, umbilical cord bleeding is a strong indication for diagnosis of congenital FXIII deficiency, and the most common presentation of the disorder. Other signs of FXIII deficiency in heterozygous cases include subcutaneous bleeding, intracranial hemorrhages, muscle hematomas, and severe bleeding [4,9].

Acquired FXIII deficiency is much less common than congenital deficiencies, and onset may be caused by increased consumption, either from bleeding, antibodies, disseminated intravascular coagulation (DIC), or decreased synthesis from liver failure or antibodies. Immune-related disorders such as leukemia, liver disease, rheumatoid arthritis, and systemic lupus erythematosus are correlated with decreased FXIII activity [4,10]. Furthermore, the acquired deficiency could be attributed to long-term treatment and utilization of drugs such as isoniazid penicillin, phenytoin, practolol, and amiodarone [4,11]. The manifestation of these anti-FXIII antibodies may neutralize activated FXIII, increase FXIII clearance, or interfere with FXIII-fibrin interaction [7].

## Diagnosis

FXIII deficiency is characterized by delayed bleeding episodes and impaired wound healing in soft tissues [12]. When diagnosing a bleeding disorder such as FXIII deficiency, it is important to collect the patient's personal and family history. This will provide a means to ascertain whether the disorder is acquired or congenital in nature. Acquired deficiency may be suspected if there is no relevant bleeding history [4].

Patients with congenital FXIII deficiencies typically present with umbilical cord bleeding [4]. Due to the role FXIII plays in pregnancy, recurrent spontaneous miscarriage and postpartum hemorrhage is relatively common [4,6]. Although incredibly rare, central nervous system bleeding is the leading cause of death in these patients [6]. Severe bleeding may also occur in patients who become resistant to FXIII replacement therapy [12]. As a whole, most patients with congenital deficiency range between 5-20% FXIII activity and have a variety of bleeding symptoms [7].

Up to 70% of patients with acquired FXIII deficiencies present with soft tissue hematomas [4]. With newly developed antibodies, many patients experience a drastic plummet in FXIII activity and consequently, life-threatening intracranial, intrathoracic, or intraperitoneal hemorrhages [4,12]. These

bleeding episodes may also present as mucocutaneous and intramuscular bleeding [12]. Nevertheless some reports note that symptoms of acquired FXIII deficiency have no correlation with residual FXIII activities [7].

Due to the rarity of both congenital and acquired FXIII deficiency, other bleeding disorders, such as von Willebrand disease (vWD), must be ruled out to ensure proper diagnosis. In one study, disseminated intravascular coagulation was most commonly confused with FXIII deficiency; however, DIC normally presents as a decrease in more than one coagulation factor from increased consumption [11].

## Laboratory Evaluation

Most routine coagulation testing, including prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and platelet count show normal levels, making FXIII deficiency difficult to diagnose [13,14]. Moreover, since FXIII is responsible for stabilizing the clot formed throughout the coagulation cascade, neither the PT or aPTT monitor the clot beyond initial formation and both appear within normal limits even in FXIII deficiency cases. In the past, clot solubility tests were utilized to screen for FXIII deficiency, but have since been discontinued due to the high rates of false negatives in milder forms of the deficiency [4,7].

Since FXIII deficiency is difficult to detect, other testing options include a variety of assays: photometric, incorporation, fluorometric, Bethesda, clot-based inhibitor assays, as well as antigen immunoassays and mixing studies [4]. Some studies propose that thrombin generation and thromboelastometry studies could be beneficial in monitoring hemostasis and clot strength [12]. Other studies suggest utilizing FXIII antigen assays to quantify the amount of FXIII in circulation based on subunit A and B, as well as the AB complex; however, there is no consensus on what level of FXIII indicates FXIII deficiency [4,14].

## Treatment / Management

Treatments for FXIII disorders are difficult to standardize given the rarity of FXIII deficiency [4], but many patients with acquired deficiencies are treated with recombinant FXIII concentrates and immunosuppressants. However, congenital FXIII deficiency may also develop inhibitors in response to FXIII concentrate doses.

Once FXIII deficiency has been established, it is crucial to begin preventative prophylactic treatment due to the variable nature of the associated symptoms [4]. Though clinical guidelines are limited, many utilize a combination of fresh frozen plasma (FFP), cryoprecipitate, FXIII concentrates, and/or antifibrinolytic agents to restore FXIII levels [10,14]. Some argue FXIII concentrate should not be used in perioperative care due to insufficient evidence of benefits, and recommend only using it for life-threatening cases of bleeding [7,14]. Alternatively, antifibrinolytics, tranexamic acid, and aminocaproic acid treatments are relatively commonly used when treating

FXIII deficiency [11]. Tranexamic acid (TXA) is capable of facilitating antifibrinolytic mechanisms, mimicking FXIII, which is capable of both antifibrinolytic properties and fibrin stabilization [11]. In cases of immune-acquired inhibitors, immunosuppressants are a necessity in preventing recurrent bleeding events and eliminating antibodies [10,12]. This may include steroids, cyclophosphamide, or Rituximab, which have all been shown to eradicate FXIII inhibitors [4,10]. In particular, Rituximab is an anti-CD20 monoclonal antibody used in approximately 24% of acquired FXIII deficiency cases since 2005 [11]. An alternative option is plasma exchange therapy, which removes inhibitors from circulation [11]. However, even with therapy, both patients with congenital or acquired deficiencies can experience refractory bleeding [7].

With no consensus on a standard FXIII level, there are various recommendations ranging from levels above 0.5%, 2%, 5%, 10%, to even as high as 30% to maintain hemostasis [4,14,15]. Hemostatic levels can also be achieved with FXIII concentrate infusions, which are recommended at the same dosage as FFP, every 4-6 weeks [4,16]. It is recommended to maintain elevated hemostatic levels in severe acute bleeding cases, and for major surgeries at levels of 50% or higher [4]. Historically, greater than 5% of FXIII was adequate for preventative prophylaxis, though newer case reports are finding that these levels are not always satisfactory to prevent spontaneous bleeding, as levels can present in a number of ways.

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# Investigating the Interaction between Macrophages and Erythroid Cells in Bone Marrow: Implications for Erythropoiesis and Anemia

Xupei Ou '24

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## Abstract

Macrophages play a pivotal role in erythropoiesis, the process of red blood cell (RBC) formation, through their involvement in various stages of erythrocyte development. Located in the bone marrow, these macrophages create specialized microenvironments called erythroblastic islands (EBIs) by associating with erythroid cells.<sup>1</sup> Additionally, macrophages may perform different functions in the bone marrow, either by interacting with erythroid cells or with other cell types. Identifying specific markers to classify macrophage subsets could enhance our understanding of their roles in erythropoiesis and blood disorders. This work applied different molecular markers to study the interactions between macrophages and erythroid cells in murine bone marrow using imaging flow cytometry. The results suggest a higher number of erythroid-associated macrophages compared to single macrophages in the bone marrow as well as the potential tendency for macrophages to become “nurse” cells to support the development of erythroid cells in anemia.

## Background

Erythropoiesis is a complex and strictly controlled biological process that begins in the bone marrow with a multipotent stem cell and ends with mature erythroid cells, or RBCs.<sup>2</sup> In mammals, erythropoiesis occurs within specialized microenvironments in the bone marrow, known as erythroblastic islands. These islands are composed of maturing erythroblasts closely associated with a central macrophage.<sup>3,4</sup> Macrophages and erythroblasts exhibit adhesive interactions essential for maintaining the structural integrity of erythroblastic islands.<sup>5</sup> During erythroid maturation, erythroblastic island macrophages play an important role by supplying essential nutrients and signals that promote the proliferation and survival of erythroblasts.<sup>5</sup> They are also responsible for phagocytosis of the extruded nuclei from erythroblasts, a critical step in erythroid maturation.<sup>5</sup>

Adhesive interactions between macrophages and erythroblasts within the erythroblastic island are critical for RBC development under both physiological and pathological conditions.<sup>6</sup> Anemia, characterized by reduced hemoglobin concentration, arises either from an increased rate of RBC destruction in the periphery, a decreased production rate of these cells in the bone marrow, or a combination of both processes.<sup>4</sup> Mutations that impede the capability of macrophages to congregate within erythroblastic islands or to effectively digest nuclei of erythro-

blasts can lead to the development of anemia.<sup>7</sup> Additionally, it has been demonstrated that macrophages directly regulate the proliferation and maturation of erythroid progenitors in both normal and erythroleukemic mice models.<sup>8</sup> However, relatively little is known about the heterogeneity of macrophages in the bone marrow. Our lab has provided evidence that bone marrow macrophages are heterogeneous and can be categorized into three superclusters: A, B, and C, based on Uniform Manifold Approximation and Projection (UMAP) plots of single-cell RNA expression (scRNA) in bone marrow macrophages, with proportions in each cluster significantly changed in anemia compared to steady-state.

The exact mechanisms governing the interactions between erythroid cells and macrophages, and their roles in response to anemia, have not been clearly elucidated. In this project, we mostly applied imaging flow cytometry to obtain high resolution image data for the expression level of different protein markers from macrophages in both steady-state and anemic mice. By comparing the expression level between erythroid-associate macrophages (EA-mac) and total macrophages (Single-mac), we hope to identify, study, and isolate subpopulations of macrophages that physically associate with erythroid precursors in the bone marrow of mice. This will allow us to study the role of molecular markers in macrophages during steady-state and in response to acute anemia or other blood disorders using imaging flow cytometry assays.

## Materials and Methods

**Steady-state and anemic mice preparation:** ICR mice aged between 8 and 18 weeks were used for all studies. Anemia was induced by phlebotomy. Mice were treated with two retro-orbital bleeds over 4 hours and around 1200ul (50%) of blood was removed about 72 hours prior to the experiment.

**Single-mac isolation:** Femoral marrow was flushed with 1ml solution of ethylenediaminetetraacetic acid (EDTA), PBS, and 25 µg/ml heparin into a tube. The isolated marrow was allowed to settle for 5 minutes, and then all but 200 µl of supernatant was removed and transferred into a new tube. The solution in the new tube was centrifuged (200g, 5 minutes). 100 µl of supernatant was discarded, and the remaining solution was resuspended and transferred back into the original tube. 200 µl StemCell Collagenase and 1.5 µl DNase I were added, and the tube was incubated at 37°C for 30 minutes. Afterwards, the solution was pipetting in the middle of

the 30 minutes. The solution was then passed through a polystyrene test tube with a cell strainer snap cap. 270 µl of 16% formaldehyde was added. Then the tube was inverted a few times and left to settle at room temperature for 10 minutes. After centrifugation (200g, 5 minutes), Single-mac sample was resuspended in 1ml of PB2 for each sample.

**EA-mac isolation:** Femoral marrow was flushed into PB2 with 25 µg/ml heparin. The solution in the tube was pipetted approximately 10 times to dissociate EA-mac from the bone marrow. 270 µl of 16% formaldehyde was added, the tube was inverted a few times and left to settle at room temperature for 10 minutes. After centrifugation (200g, 5 minutes), EA-mac was resuspended in 1 ml of PB2 for each sample.

**Cell staining:** The tubes containing the samples were centrifuged (200g, 5 minutes), and the samples were resuspended in about 90 µl of PB2. Then, 10 µl of Normal Rat Serum was added, and the tubes were incubated for 15 minutes on ice. The samples were then stained with a 1:100 dilution of antibodies for 20 minutes on ice without light exposure. The antibodies used in different panels, as shown in the figures, included PE (Phycoerythrin) MHCII, PE Cx3cr1, PE CD74, PE CD9, PE CD14, PE CD16, PE LGALS3, AF488 (Alexa Fluor 488) F4/80, PEcy7 (PE-Cyanine7) Ter119, BV421 (Brilliant Violet 421) CD3, BV421 CD19, BV421 Ly6G, BV421 CD170, and BV421 CD335.

**Acquisition of imaging flow cytometry data:** Image data for cells were acquired on an imaging flow cytometer named ImageStreamX Mark II. Events were read in the following channels: Brightfield channels 1 (488 nm laser – 467.5/75 nm) and 9 (594 nm laser – 577.5/35 nm), AF488 channel 2 (488 nm laser – 532.5/55 nm), PE channel 3 (561 nm laser – 577.5/35 nm), PEcy7 channel 6 (561 nm laser – 627.5/65 nm), BV421 channel 7 (405 nm laser – 467.5/75 nm), and APC channel 11 (643 nm laser – 700/80 nm). Compensation for channel 3 was collected using PE IgG, channel 2 using AF488 F4/80, channel 6 using PEcy7 Ter119, channel 7 using BV421 CD3, and channel 11 using APC Ly6C. Imaging flow cytometry collected image data from bone marrow cells stained with the macrophage marker F4/80 and erythroid cell marker Ter119. Further purification of F4/80+ macrophages from contaminants was accomplished by eliminating cells that were CD3e+, CD19+, Ly6G+, CD170+, CD335+, Ly6C+, and possibly Ter119+.

**Analysis of imaging flow cytometry data:** Image data was analyzed using IDEAS (version 6.2, Amnis/EMDmillipore) software and its compensation wizard and gating tools. The data of image features was then extracted and used to compose graphs for further analysis using FlowJo v10 software.

## Results

### *Identification of candidate molecular markers in bone marrow macrophages*

To begin determining if specific protein markers in bone marrow macrophages were associated with EBI macrophages, we analyzed the expression of candidate markers on EA-mac ver-

sus Single-mac using imaging flow cytometry. We selected candidate markers based on their differential expression between steady-state and anemia in single-cell RNA-Seq datasets for macrophages (Fig. 1). These included: major histocompatibility complex class II (MHCII), CX3C motif chemokine receptor 1 (Cx3cr1), CD74, CD14, CD9, CD16, and Galectin-3 (LGALS3). Based on the UMAP plots of scRNA analysis, our lab grouped macrophages into three superclusters, A, B, and C (Figure 1). We observed more macrophages expressing MHCII and Cx3cr1 in cluster A in anemic mice than in steady-state mice and fewer macrophages in cluster C (Fig. 1). This is also true for other markers (Fig. 1). Therefore, our lab hypothesized that the cells in cluster A are EA-mac, which tend to serve as “nurse” cells and support erythropoiesis by interacting with erythroid cells for the recovery from anemia.

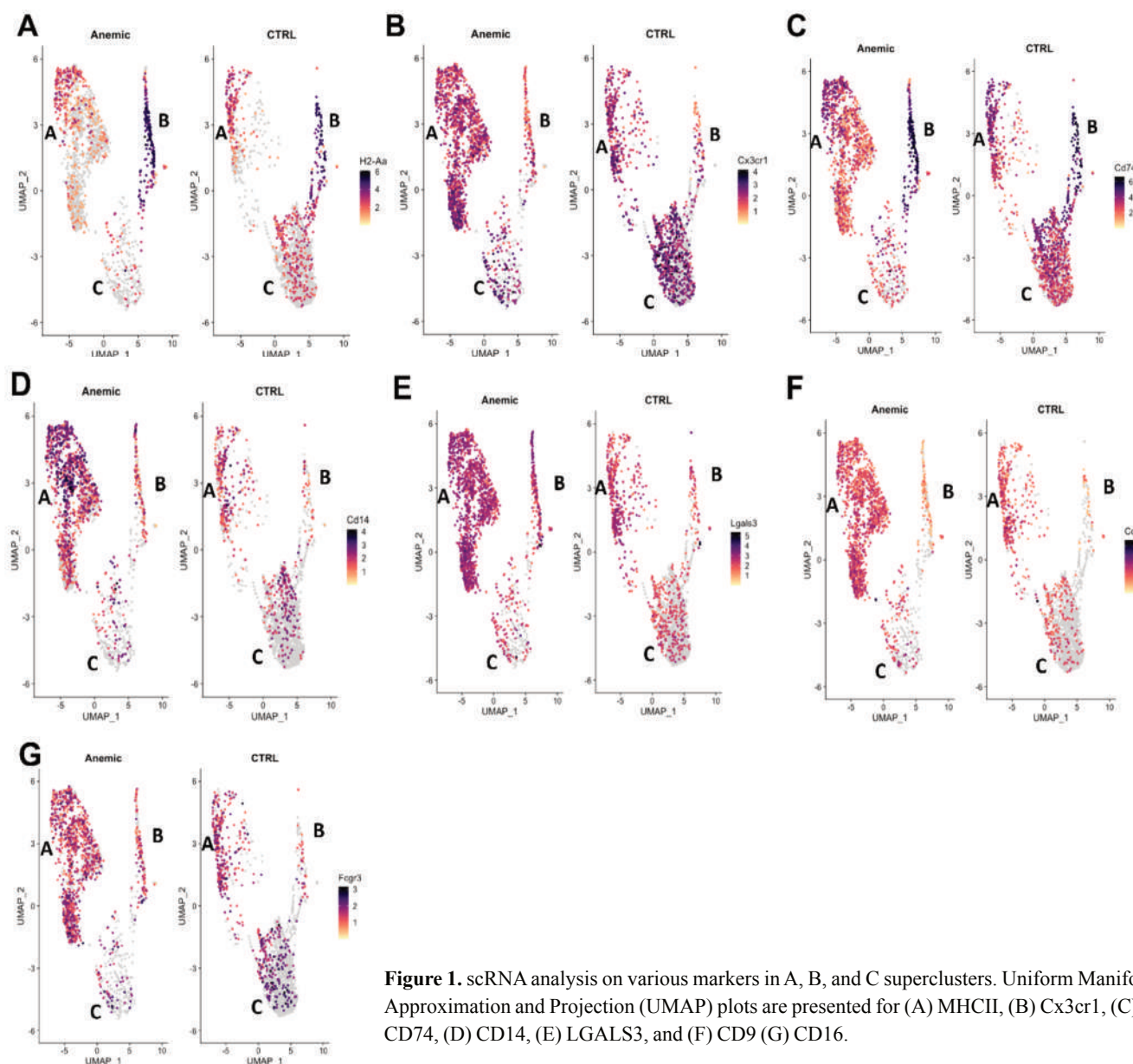
### *Analysis of macrophages in murine bone marrow samples*

In preparing Single-mac and EA-mac, we applied StemCell Collagenase and DNase I to dissociate and separate Single-mac from other cells in the bone marrow samples from femurs of steady-state and anemic mice. As a result, we captured more clumps in EA-mac samples than Single-mac samples because EA-mac did not receive the treatments and did not dissociate as strongly. This was intentional, as we aimed to acquire macrophages associated with erythroid cells or other cells for EA-mac samples, and our strong dissociation treatments would break the adhesion between them in Single-mac samples. To study all the markers indicated by scRNA analysis, we stained the cells with specific antibodies that exhibit fluorescence and ran them through an imaging flow cytometer to prospectively look at their expression in Single-mac and EA-mac. We collected 250,000 cell events for each sample within an experiment, either for Single-mac or EA-mac, and the image data was exported into IDEAS software.

We applied IDEAS software to exclude unwanted images or cells and selected the proper images that contained our macrophages of interest for further analysis. This required applying a gating strategy as follows: first, a common gating for Single-mac and EA-mac selected cells of certain sizes and with certain signals of the F4/80 macrophage marker (Figure 2A). Then, for Single-mac gating, the first specific gate after the common gating excluded cells containing high levels of the Ter119 erythroid cell marker to ensure were single macrophages without attaching to erythroid cells and cells that have low signals for other immune cells, such as CD3 (T cells), CD19 (B cells), Ly6G (neutrophils), CD170 (eosinophils) (Figure 2B). The next gate excluded macrophage images containing high levels of immune cell signals Ly6C and low levels of F4/80 for further purification (Figure 2C). Finally, we selected cells with a certain level of F4/80 signal covering a specific area, indicating Single-mac (Figure 2D).

For the selection of EA-mac, the first specific gate for EA-



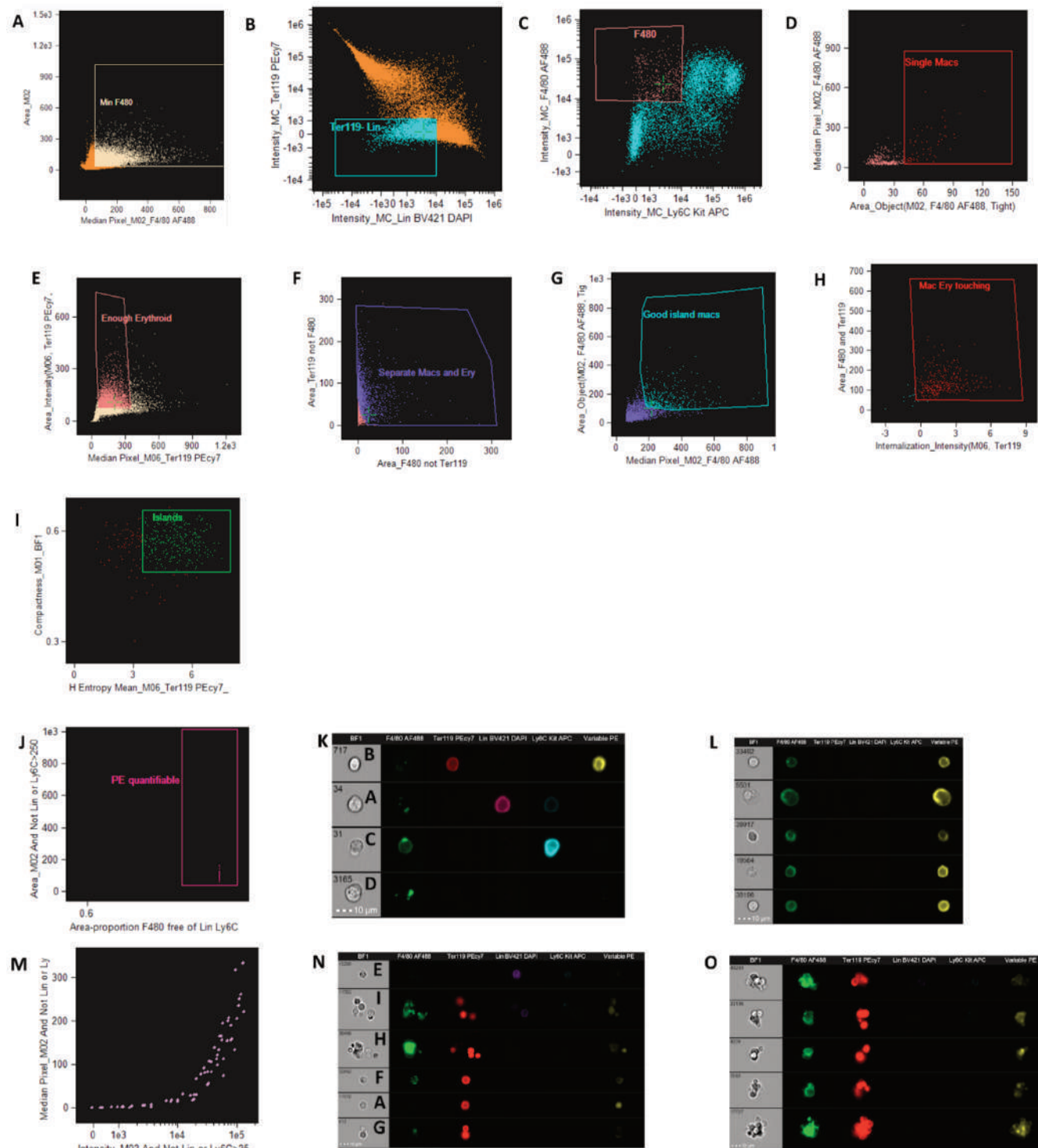


**Figure 1.** scRNA analysis on various markers in A, B, and C superclusters. Uniform Manifold Approximation and Projection (UMAP) plots are presented for (A) MHCII, (B) Cx3cr1, (C) CD74, (D) CD14, (E) LGALS3, and (F) CD9 (G) CD16.

mac samples after the common gating selects macrophages with a sufficient amount of erythroid cells by excluding cells with a low level of the Ter119 erythroid cell marker (Figure 2E). The next gate excludes events where the Ter119 signal overlaps excessively with the F4/80 signal, which might interfere with the analysis (Figure 2F). Then the next gating selects the cells with a certain level F4/80 signal which cover a certain amount of area, indicating good island macrophages (Figure 2G). The next gate excludes events that contain erythroid cells not touching macrophages because they were not good erythroblastic islands (Figure 2H). The gating shown in Figure 2I excludes events that contain cells diffuse next to macrophages and erythroid-associated islands by looking at entropy and compactness features. We utilized various mathematical methods to quantify variance in pixel intensity patterns and contrasts, known as texture features. The feature finder program

identified compactness and entropy as the most effective features for distinguishing islands from events with diffused cells.

After the Single-mac or EA-mac's specific gating, another common gating for both samples selects the macrophage cells that are good for the quantification of PE signals, which are the signals for markers, by excluding signals that are out of focus (Figure 2J). For the ultimate feature to measure expression levels of different markers with PE signals, we had two options – intensity or median pixel. The value of the “intensity” feature represents the total average fluorescence intensity of the area, but the cells would have different sizes and the size of the cells can determine the signal value by applying the “intensity” feature. In contrast, the “median pixel” will give us the average intensity within any specific

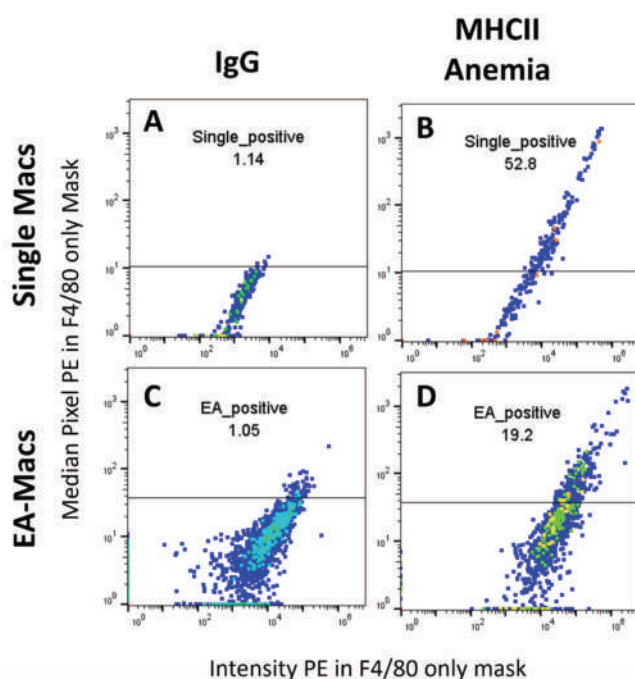


**Figure 2.** Gating strategy for imaging cytometry analysis of macrophage cells in murine bone marrow in IDEAS software. (A) Common gating for Single-mac and EA-mac. (B-D) Gating specific for Single-mac (E-I) Gating specific for EA-mac. (J&M) Common gating and analysis for the cells with quantifiable PE signals. (K) Example cells excluded by Single-mac gating. (L) Cells ultimately selected by Single-mac gating strategy. (N) Example cells excluded by EA-mac gating. (O) Cells ultimately selected by EA-mac gating strategy.

area, and we can normalize the intensity for different sizes of cells. Thus, the median pixel is a better choice. Finally, PE signal quantification was applied to PE quantifiable populations by looking at the “intensity” feature at the X-axis and “median pixel” feature at the Y-axis (Figure 2M). Good examples in PE quantifiable populations are demonstrated in Figure 2L for Single-mac and Figure 2O for EA-mac.

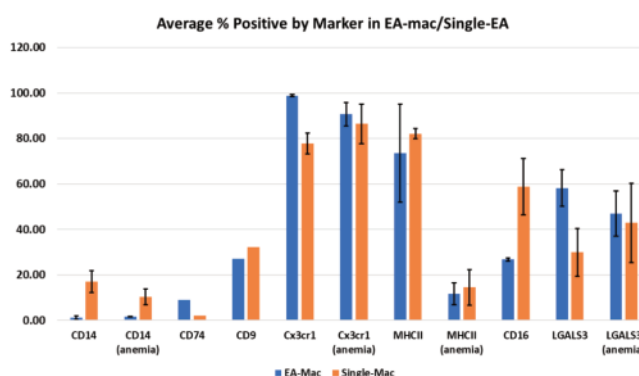
### Expression analysis in steady-state and anemic mice

To present the complex data more clearly, we extracted the “intensity” and “median pixel” feature values of channel 2 from the PE quantifiable population to determine the expression of protein markers to create FSC files. These feature values were collected on the mask of F4/80, which is the macrophage marker, and this means the feature values were only collected from macrophages and not from other cells beside them. We then analyzed these FSC files using FlowJo v10 software by plotting, with “intensity” as the X-axis and “median pixel” as the Y-axis. By using the signals from IgG samples as a negative signal reference, we established gating for positive signals of each sample (Figure 3). Since the cells with signals above the IgG gating should express positive signals, we then applied the gating to samples with marker expression to determine the proportion of cells with positive signals (% Positive) (Fig. 3).



**Figure 3.** Expression analysis and gating strategy in FlowJo v10 software. In each plot, the X-axis represents the “intensity” feature values, while the Y-axis represents the “median pixel” feature values. (A) Expression of IgG in Single-mac for anemic mice. (B) Expression of MHCII in Single-mac for anemic mice. (C) Expression of IgG in EA-mac for anemic mice. (D) Expression of MHC II in EA-mac for anemic mice.

We analyzed all the “intensity” and “median pixel” feature values from the results of every experiment to all of the protein markers by composing the graphs shown in Figure 3. For a better demonstration, we utilized the values from the graphs to create a bar chart showing the average proportion of cells expressing positive signals of a certain marker with the standard error of the mean (SEM) values as error bars (Figure 4). The results for the steady-state mice showed that CD9 has similar expression levels in Single-mac and EA-mac, while CD74 had higher expression levels in EA-mac than Single-mac (Fig. 4). CD14 displayed higher expression levels in Single-mac than in EA-mac for both steady-state and anemic mice.



**Figure 4.** Bar chart which shows the average proportion of Single-mac or EA-mac with positive signals for expression analysis of various markers in macrophages from either steady-state or anemic mice. The markers include CD14, CD16, CD74, CD9, Cx3cr1, LGALS3 (steady-state), Cx3cr1 (anemia), MHCII (steady-state), and MHCII (anemia). The markers without specifying “anemic” were for steady-state mice. Error bars are indicated by the standard error of the mean (SEM) values.

For steady-state mice, Cx3cr1 showed more cells with positive signals in EA-mac than in Single-mac (Fig. 4). Compared to anemic mice, the proportion of Single-mac with Cx3cr1 expression increased (Fig. 4). For steady-state mice, average MHCII expression was higher in Single-mac than in EA-mac, but the wide error bars indicate an insignificant difference (Fig. 4). MHCII expression levels in both Single-mac and EA-mac become lower for anemic mice, with no significant difference observed between the two cell types (Fig. 4). The cells demonstrated higher expression levels of CD16 in Single-mac than EA-mac in steady-state mice. For LGALS3, there was no significant difference in expression between Single-mac and EA-mac for anemic mice, but the expression levels are higher in EA-mac than Single-mac for steady-state mice.

### Discussion

scRNA analysis of macrophage cells in the bone marrow for both steady-state and anemic mice suggests that the macrophages can be grouped into three superclusters of macrophages. Our lab generated a hypothesis that the



macrophages in cluster A are EA-mac that can support erythropoiesis better than the overall macrophages. The main purpose of this project is to explore various markers and determine if these markers can help classify macrophages between these superclusters and hope to study the role of molecular markers in macrophages during steady-state and with blood disorders, such as anemia. As the examples of cells excluded and selected by the gating strategy in IDEAS shown in Figure 2, our gating strategy successfully identified Single-mac and EA-mac with quantifiable PE signals that indicate the expression of these markers. Therefore, our data analysis based on the “median pixel” feature values of PE signals from the selected cells should be valid.

MHCII plays a crucial role in the immune functions of macrophages, particularly in the context of antigen presentation and the activation of adaptive immune responses.<sup>9</sup> Lower expression levels of MHCII expression were observed in anemic mice compared to steady-state mice (Fig. 4). This suggests that macrophages in anemia might tend to reduce some immune function and participate more in other roles, such as supporting erythropoiesis to produce more RBC for the recovery of anemia.

CX3CR1, a receptor for the chemoattractant cytokine CX3CL1, is integral in modulating inflammatory responses, encompassing the phenotype and functionality of macrophages.<sup>10</sup> However, while detailed information specifically relating to erythropoiesis was not immediately evident, the general understanding is that Cx3cr1-expressing macrophages have diverse functions in the immune system and may contribute to hematopoietic processes in the bone marrow.<sup>11</sup> Given the higher expression of Cx3cr1 in EA-mac compared to Single-mac in steady-state mice (Fig. 4), Cx3cr1 has the potential to serve as a good candidate for classifying cells between Single-mac and EA-mac. Since there are significantly more cells expressing Cx3cr1 in cluster A of anemic mice compared to those in steady-state mice, it might also help classify macrophages among the three superclusters as well (Fig. 1).

More cells demonstrated positive signals of CD14 in Single-mac than EA-mac for both steady-state and anemic mice (Fig. 4). Because a larger number of cells express CD14 in cluster A of anemic mice compared to steady-state mice, CD14 could be a suitable marker for classifying macrophages among the three superclusters (Fig. 1). A greater proportion of cells expressed positive signals of CD16 in steady-state mice. However, because we didn't access its expression for anemic mice, we could not conclude its potential roles in macrophage classification and erythropoiesis promotion. LGALS3 demonstrated similar expression levels for anemic mice since the error bars overlap, but higher levels in EA-mac for steady-state mice (Fig. 4). This result interestingly contradicts our hypothesis that the cells in cluster A are EA-mac as scRNA data also shows a higher expression in cluster A for anemic mice but the results showed a lower expression (Fig. 1). We plan to test the interior expression of LGALS3 using cell fixation and cell permeabilization kit in the future to verify consistency of the results. Moreover, since

CD9 failed to show a significant difference in expression levels for both Single-mac and EA-mac (Fig. 4), no significant conclusions can be drawn from it.

For limitations, we did not have enough valid, repeated trials for CD74, even though it actually demonstrates a difference in expression levels in Single-mac and EA-macs. Therefore, it is not possible to draw reliable conclusions from CD74 with only one trial for steady-state mice, but it would be interesting to investigate CD74 since its expression levels in EA-mac were much higher than Single-mac (Fig. 4). We also lacked experiments to test CD74 and CD16 markers for anemic mice. In addition, the strand errors for the expression signals of small markers are large. Therefore, we plan to conduct more experiments with CD74 and CD16 and other markers for both steady-state and anemic mice. Additionally, we set the IgG gating to subjectively select cells with positive expression signals in our graphs composed using Flowjo. The gating was not set above all signals in IgG samples but actually included a very small proportion of cells, around 1-2%, with IgG signals (Fig. 3). These cells are outliers that separate from the general IgG population with relatively high signals, so we decided to not consider them when creating the IgG gating. However, it may be biased to draw conclusions based on our subjective IgG gating.

Even though the data on expression levels can help us suggest the potential role of these factors in anemia and in the classification of macrophages, further cellular experiments are essential for more comprehensive conclusions. These could include immunohistochemistry to confirm the expression of genes by macrophages physically associated with erythroblasts and functional tests of macrophages in tissue culture. Furthermore, we plan to isolate macrophages with high expression of certain protein markers by using cell sorting and coculture them with erythroid progenitors to test the potential roles of markers in the promotion of erythropoiesis. By conducting cellular experiments with isolated macrophages and erythroid cells in the future, we hope to gain insights into their interaction and its impact on erythropoiesis or recovery from blood disorders such as anemia.

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## About the Author

My name is Xupei Ou, and you can also call me Prince. I am a Molecular Genetics major from the class of 2024. Currently, I am working in Dr. James Palis's lab on the interaction between macrophages and erythroid cells, and I also have long-term research experience in microbiology and genetics. My current interest mainly focuses on the molecular basis of diseases, such as blood disorders. I believe that by elucidating the mechanisms governing disease occurrences at cellular and molecular levels, we will gain a better understanding of the diseases and be able to develop proper medical strategies against them.

# Aging Well: Politics of Aging, Sexuality, and Womanhood in *The Golden Girls*

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Advised by Jean Pedersen, *Department of Art and Politics*

DOROTHY:

Oh c'mon, Blanche. Age is just a state of mind.

BLANCHE:

Tell that to my thighs.

From its pilot on September 14, 1985, to its finale on May 9, 1992, *The Golden Girls* was one of the most beloved sitcoms on television. The seven-season, 180-episode series follows the lives of four women: Blanche, Rose, Dorothy, and Dorothy's mother Sophia. All four women, age fifty and older live together as friends in a house in Miami, Florida. Not only was the show very well received at the time, winning eleven total Emmy Awards<sup>1</sup>, but *The Golden Girls* continues to delight its audience today and even find new viewers three decades after the show stopped airing. Sure, the show is full of hilarious jokes and delivery, and that's a large part of its popularity, but *The Golden Girls* isn't all fun and games. The show's creator, Susan Harris, once told the Chicago Tribune, "I don't like lighter shows. I like substance."<sup>2</sup> The "substance" of *The Golden Girls* lies in the complex social issues that the series deals with in an on-the-mark and thoughtful way. In this essay, I assert that *The Golden Girls* has aged so well because of its progressive depictions of social and cultural issues including aging, sexuality, and womanhood, which have, in turn, altered our society's perception of those topics.

## I. Is *The Golden Girls* Aging Well?

*The Golden Girls* is not only aging well, but it is also a fantastic example of media created decades ago that still holds up today. The series is still critically acclaimed - in 2013, almost thirty years after the show first aired, TV Guide ranked it as one of the top sixty television shows of any genre ever made.<sup>3</sup> However, it is still gaining popularity in the general public. In 2019, Terry Tang of the Associated Press wrote in an article that in recent years, there has been a new "wave of merchandising" featuring shows, including Funko figures, PEZ dispensers, and even a Golden Girls themed cruise,<sup>4</sup> which continues to operate for an exciting upcoming 2024 season touring Sicily.<sup>5</sup>

While some of this renewed interest can be attributed to nostalgia, seeing as many of those fans who watched the show as children are now nearing the age of the main characters, much of it is fueled by new fans with whom the show resonates. It would be easy to assume that the reason for its popularity is just its brilliant humor. However, as I am sure we have all experienced, a show that was initially considered hilarious can often be cringe-inducing today. As Jeremy Urquhart of Collider puts it, "The humor and messages could be out of touch with current social mores," which ends up "souring" the experience.<sup>6</sup> Thus, a crucial component of a show's longevity is how its humor and themes deal with social and cultural topics, and as we will soon discuss, *The Golden Girls* does so through a progressive and forward-thinking lens, allowing it to continue to hold up to this day.

In a panel with the rest of the cast in 2006, Betty White, who played Rose, noted that whenever the cast got fan mail, spanning from right when the show started up until at least 2006 when the panel was held, 70 percent of it was from people under the age of twenty-five.<sup>7</sup> Incredibly, that is a similar age demographic that the show attracts to this day, generations later. In 2019, Marc Berman of Forbes wrote that the current obsession with the show is particularly among the millennial generation,<sup>8</sup> many of whom were either infants or not even born when the show started running.<sup>9</sup> Thus, whatever appealed to the young generation when the show aired continues to appeal to young people today.

## II. Were *The Golden Girls* Aging Well?

A large part of this appeal is the series' depiction of aging. Perhaps the most important aspect of *The Golden Girls* is that all four of its stars are women over fifty years old and either widowed or divorced. However, the four women aren't held back by their pasts with their husbands, instead leading exciting and happy lives full of friendship. *The Golden Girls* is a paragon of depicting modern healthy aging for older people.

In her 2022 book *Marginal People in Deviant Places*, Janice M. Irvine notes, "The story of old age told by *The Golden*

<sup>1</sup>"The Golden Girls" Television Academy, n.d., <https://www.emmys.com/shows/golden-girls>.

<sup>2</sup>Frank Sanello, "From 'Soap' Madness to 'Empty Nest' Sadness," *Chicago Tribune*, October 20, 1988, <https://www.chicagotribune.com/news/ct-xpm-1988-10-20-8802090551-story.html>.

<sup>3</sup>Bruce Fretts and Matt Roush, "TV Guide Magazine's 60 Best Series of All Time," *TVGuide.Com*, December 23, 2013, <https://www.tvguide.com/news/tv-guide-magazine-60-best-series-1074962/>.

<sup>4</sup>Terry Tang, "'Golden Girls' Appears to Get Better with Pop Culture Age," *AP News*, April 21, 2021, <https://apnews.com/article/803d42ed224049ca9747001fe3e43032>.

<sup>5</sup>"2024 Rooms / Pricing — Golden Fans at Sea," *Golden Fans at Sea*, n.d., <https://www.goldenfansatsea.com/rooms-pricing-2024-sicily>.

<sup>6</sup>Jeremy Urquhart, "10 Classic Movies With Content That Hasn't Aged Well," *Collider*, March 18, 2023, <https://collider.com/movies-that-havent-aged-well/#39-around-the-world-in-80-days-39-1956>.

<sup>7</sup>Justin Ullmann, uploader, "The Golden Girls at PaleyFest LA 2006: Full Conversation," January 14, 2022, <https://www.youtube.com/watch?v=p4cghCwwlbw>.

<sup>8</sup>Marc Berman, "Age Is Just a Number...And the 50+ Category Matters," *Forbes*, May 9, 2019, <https://www.forbes.com/sites/marcberman/2019/05/09/age-is-just-a-number-and-the-50-category-matters/?sh=3b1c39fa665b>.

<sup>9</sup>Alicja Zelazko, "Millennial I Definition, Characteristics, Age Range, & Birth Years," *Encyclopedia Britannica*, November 12, 2023, <https://www.britannica.com/topic/millennial>.

*Girls*...emphasized that aging is a culture as much as it is a biological experience.”<sup>10</sup> In today’s society, the culture of aging is shifting in a world with increased technology, a longer average lifespan (especially among women), and higher senior divorce rates.<sup>11</sup> In line with that, the qualities of healthy aging have changed dramatically in the past century. Furthermore, not only does *The Golden Girls* reflect those qualities of healthy aging, but it also does so in a way that subverts stereotypes about its main characters’ age group. What comes to mind when you think of middle-aged to elderly women? None of the four main characters fixate their entire lives on their family or their children, or show signs of mental regression. Most importantly, they simply all seem to be happy in their own lives. To that point, in a 2014 presentation, Drs. Satinder K. Mahal and Pei Huey Nie noted that the way *The Golden Girls* portrayed independence, activity, and even sexiness in old age not only broke the mold for television at the time but also aligned with qualities that scientific research has found to reflect healthy and happy aging. They concluded that the portrayal of successful aging in media such as *The Golden Girls* can both “reduce ageism and sexism against older generations while promoting healthier behaviors in this age group.”<sup>12</sup>

As such, just as television and popular culture are affected by the society in which they are created, they can have tangible effects on society itself. It is not surprising that many people saw the four women of *The Golden Girls* and wanted to follow in their footsteps. Thus, the “Golden Girls Model” was born, in which senior citizens, often without romantic partners, live together as housemates.<sup>13</sup> One might think that while it sounds appealing in theory, it would be much more challenging. After all, buying a house is a huge monetary commitment, and housemates with different desires could make for an unsustainable and harmful situation. However, in a recent survey by LendingTree, 29 percent of Americans said they would be open to the idea of living as roommates in a Golden Girls Model home,<sup>14</sup> and it’s not altogether surprising that the idea is becoming popular. After all, it could solve many of the most difficult problems for single older people: cost of living, household work, and being part of a community with no assisted living.<sup>15</sup> Therefore, with the right housemates, *The Golden Girls* Model can serve as a style of living that promotes healthy aging, beautifully exemplifying how a great piece of art can influence the society in which it was created.

### III. Sexuality and Womanhood in *The Golden Girls*

In a 2015 article, Tracey Ross stated, “The very premise of *The Golden Girls*... is feminist in nature.”<sup>16</sup> I see two ways to look at *The Golden Girls* in the historical context of the feminist movement. First, one could argue that because the show took place between the second and third waves of the feminist movement, the feminist ideology of the first and second waves was beginning to settle into the general public.<sup>17</sup> The premise of the show reflected the progress made by society toward accepting women as equals to men, in a way that would not have been possible ten to twenty years before.<sup>18</sup> While one could also argue that during this lull in the women’s movement—a lull reflected in the AIDS crisis and whispers that the movement had ended for good—the show was even braver and more radical than the first opinion suggests.<sup>19</sup> Perhaps its confidence in radicalism is what made it so successful, and possibly even contributed to the third wave of feminism, which began to surge in the final years of the show’s run.<sup>20</sup> Nevertheless, the women’s movement of the mid-to-late twentieth century was crucial to the writing and reception of *The Golden Girls*. This is particularly evident in the show’s depiction and empowerment of older women as the main characters, sexuality and queer representation, and the lack of male characters in the show.

Let us first consider the show’s empowerment of aging women. Paul Junger Witt, an Executive producer of *The Golden Girls*, explained that the writers “approached the series...with thought and a great deal of study.”<sup>21</sup> He further states, “[They] asked a number of mature people whom [they] knew about certain elements of their lives. [The writers] incorporated some of those philosophies into the show. The reaction from mature audiences over the age of 55, has been remarkably enthusiastic.”<sup>22</sup> Indeed, the empowerment of the middle-aged women viewers is evidence of the effects the show had on the general public’s perception of mature womanhood. In the 1980s, some women needed reassurance that “there’s life after 50,”<sup>23</sup> and *The Golden Girls* is famous for its unashamed portrayal of older women’s sex lives as free and natural. According to Ross, on a talk show in which Betty White and Bea Arthur, two of the stars of the show, were guests, a woman called in and thanked them for making

<sup>10</sup>Janice M. Irvine, “Conclusion,” In *Marginal People in Deviant Places: Ethnography, Difference, and the Challenge to Scientific Racism*, (University of Michigan Press, 2022), 257, <http://www.jstor.org/stable/10.3998/mpub.11519906.13>.

<sup>11</sup>Kristen Hicks, “Senior Living Options: The Golden Girls Model,” *SeniorAdvisor.com Blog*, October 14, 2020, <https://www.senioradvisor.com/blog/2015/12/senior-living-options-the-golden-girls-model/>.

<sup>12</sup>Satinder K. Mahal and Pei Huey Nie, “Thank You For Being a Friend. The ‘Golden Girls’ as a media model of successful aging,” *The American Journal of Geriatric Psychiatry*, 22(3), S97–S98. [https://www.ajgponline.org/article/S1064-7481\(13\)00545-9/pdf](https://www.ajgponline.org/article/S1064-7481(13)00545-9/pdf).

<sup>13</sup>Hicks, “Senior Living Options: The Golden Girls Model.”

<sup>14</sup>Taylor, Chris, “The Golden Girls approach: Buying homes together amid high prices,” *Reuters*, August 17, 2023, <https://www.reuters.com/lifestyle/golden-girls-approach-buying-homes-together-amid-high-prices-2023-08-17/>.

<sup>15</sup>Hicks, “Senior Living Options: The Golden Girls Model.”

<sup>16</sup>Tracey Ross, “30 Years Later, ‘The Golden Girls’ Is Still the Most Progressive Show on Television,” *Common Dreams*, September 9, 2015, <https://www.commondreams.org/views/2015/09/09/30-years-later-golden-girls-still-most-progressive-show-television>.

<sup>17</sup>Kerri Lee Alexander, “Feminism: The Second Wave,” *National Women’s History Museum*, June 18, 2020, accessed November 16, 2023, <https://www.womenshistory.org/exhibits/feminism-second-wave>.

<sup>18</sup>Datan, Nancy. “Aging Women: The Silent Majority.” *Women’s Studies Quarterly* 17, no. 1/2 (1989): 12–19. <http://www.jstor.org/stable/40003974>.

<sup>19</sup>Special thanks to Professor Jean Pedersen for her sharing personal experiences for this argument.

<sup>20</sup>Kerri Lee Alexander, “Feminism: The Third Wave,” *National Women’s History Museum*, June 23, 2020, accessed November 16, 2023, <https://www.womenshistory.org/exhibits/feminism-third-wave>.

<sup>21</sup>“The Golden Girls,” *Simply Put Blog*, January 14, 2018, <https://destinedtodenvr.blogspot.com/2018/01/the-golden-girls.html>.

<sup>22</sup>Simply Put Blog. “The Golden Girls.”

<sup>23</sup>Ross, “30 Years Later, ‘The Golden Girls’ Is Still the Most Progressive Show on Television.”



her “feel 52 and gorgeous.”<sup>24</sup> The emphasis that the writers put on mature sexuality cannot be understated. The blog *Refinery29* counted up all of the different men that *The Golden Girls* slept with throughout the show’s seven seasons, and Blanche came out on top with 165.<sup>25</sup> The show had 180 episodes.

Sex positivity for older women was not the only tough topic that the show delved into. For example, in just the third episode of the first season, Rose is romantically and sexually involved for the first time since her husband’s death, and instead of shying away from the topic or making a joke about it and moving on, the writers chose to base the arc of the episode around dealing with this very real problem. Similarly, the show portrayed issues of sexual harassment, infidelity, teenage pregnancy, AIDS, poverty, domestic violence, and much more. “All the issues are so real that [*The Golden Girls*] talk about, even though it was 35 years ago,” says Marsha Posner Williams, one of the show’s original producers.<sup>26</sup>

Notably, the show explored sexual orientation in a manner that was certainly ahead of its time. In his article “Staying Golden: The Politics of Gender, Sexuality, and Jazz in *The Golden Girls*,” Elliot H. Powell notes several queer characters in the show: Blanche’s gay brother, Dorothy’s lesbian best friend, and Sophia’s cross-dressing son. Yet, even more than that, Powell notes, the familial relationships of the four women reflect the idea of “‘queer kinship’ or ‘chosen family,’” important aspects of queer theory which can even be seen as a home life alternative to the confining structure of marriage.<sup>27</sup> This exemplifies just how radical *The Golden Girls* was.

Perhaps the most radical of all of the show’s qualities was the most simple: the comparative lack of men. Sure, there were often male love interests, especially for Blanche, but the reality is that almost all of the dialogue in the show occurred between and about women. One way to put into perspective how rare this is in Hollywood is the Bechdel Test, which is a quick process to measure how male-centric a show or movie is. The Bechdel Test asks three questions: Are there at least two women? Do they talk to each other? When they talk to each other is it ever about something other than a man? While it is a limited and surface-level test, seeing some pieces that are outwardly feminist still fail to pass this test, it can be useful as a baseline in many scenarios.<sup>28</sup> Instead of struggling to pass this test like so many movies and episodes of television do, *The Golden Girls* consistently passed. However, the show has trouble passing the “Bechdel Test,” seeing as very often, there is only one man in each episode, and his only role in the story is as a love interest for one of the women. To sum up, *The Golden Girls* was a show that had progressive ideas about feminism, aging, gender, and sexuality at its core. As Powell puts it, *The Golden Girls* “centered women as sexually desiring and desirable agents whose everyday lives challenged and did not de-

pend on the normative ideals of heteropatriarchy.”<sup>29</sup>

*The Golden Girls* continue to appeal to young people to this day due to its fearless and purposeful exploration of perpetually relevant social and cultural subjects. The series demonstrates that regardless of its premise, target audience, and generation, a truly great piece of art will endure through the decades and continue to touch the lives of those who experience it. *The Golden Girls* especially goes one step further - through its normalization of feminist ideas, breaking of boundaries in the perception of aging women, and creation of new social possibilities for people entering their older age, the show brings the flow of ideas full circle, from society affecting the art to the art leaving its permanent mark on society.

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<sup>24</sup>Ross, “30 Years Later, ‘The Golden Girls’ Is Still the Most Progressive Show on Television.”

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## About the Author

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# Voyeur & Violator: The Obscene Narrative in Early Modern Italy

Daniel Heberle '24

Advised by Christopher Heuer, *Department of Art and Art History*

I dedicate these lustful pieces to you, heedless of fake prudishness and asinine prejudices that forbid the eyes to gaze at the things they most delight to see. What harm is there in seeing a man mounting a woman? -Pietro Aretino, 1537<sup>1</sup>

Hands caress the curled, ribboned hair, grapple onto her arm, and search in between the thighs because the elders have finally coerced Joakim's chaste wife into having their way with her. She does not look frightened or scared, but rather optimistic. The woman gracefully holds onto the man's face and holds the crown of the man's head beneath her, continuing his search between laced fabric and flesh. Alessandro Allori's *Susanna and the Elders* (Fig. 1) reads closer to Pietro Aretino's obscene *Sonetti Lussuriosi* than a scene depicting feminine defiance against the elders who lust after her, canonically failing to violate her. Decades before the heroine Susanna of Artemisia Gentileschi, the painted and engraved Susanna sat in between the violators; the painted figures or internal viewers within the paintings of Renaissance Italy and well into the Baroque, and the voyeurs; the external viewers, who ogle before the work. Moreover, the relationship of viewing the female nude body from the perspective of the elders, and that of an awareness of the viewer's role in these acts of sexual violence, reflects a transgression in the conception of the obscene narrative, and in the reception through both painted figure and the early modern viewer in sixteenth century Italy. Outside these images lie the obscenities of Renaissance visual culture such as Marcantonio Raimondi's *I Modi*, engravings after drawings by Giulio Romano and Aretino's *Sonetti*.<sup>2</sup> During the period in which these works were created, the holy and the sinful were circumscribed by careful distinctions between the permissible erotic-nudity and the tasteful display of sexual intercourse, and the obscenely erotic-sexual content that threatened a centuries-long established decorum of how to depict nudity and intercourse in art. As a result of erotica in the Renaissance, and the connotations to antiquity and the pursuits of pleasure beyond the teachings of the Church, paintings and prints depicting the Book of Susanna fluctuates between the physical interaction and the purely visual observation.<sup>3</sup> This specific narrative scene reveals the troublesome relationship between a secularized erotic visual culture alongside the didactic artistic programs issued by the Church at the time of the Council of Trent and afterwards. Further, considering the history of critical approaches to the rational and functional roles of the painters, the viewer's position in the work becomes dependent on a retrospective understanding of the narrative and

whether or not to victimize Susanna, or to join the elders in objectifying the nude wife.



**Figure 1.** Alessandro Allori. *Susanna and the Elders*. 1561, oil on canvas, 202 x 117 cm, Musée Magnin, Dijon, France.

Alessandro Allori's approach to the scene radically alters perceptions of the beholder's innocence in the face of other, albeit later Italian examples such as Domenichino's *Susanna* or the Prado Guercino. Allori chooses the direct, confrontational, and hallucinatory route of perception via Mieke Bal's elucidations of the elder's internal intentions, and their actions actualized by their threats, whereas other artists opt for a passive, looking mode of contact between the elders and the unknowing Susanna. Additionally, the treatment of expression, gesture, and physical contact radically alters the passivity or active engagement, and perhaps indulgence, in the depiction of sexual violence. The obscenely erotic nature of the visualized Susanna narrative presents a unique problem in the realm of painted biblical narratives because of the extratextual nature of its depiction—an extratextual accessibility of this kind uncovers the severe extent to which narrative scenes can be

<sup>1</sup>Lawner, 9, 17.

<sup>2</sup>Nagel, 223.

<sup>3</sup>See (Olszewski, 44, 46). The Old Testament story appears either as Chapter 13 in the Book of Daniel, or within the Apocrypha. Though the history of the Book of Susanna is complex, we may assume that the painters were working off of the Hellenized Theodotion version of the story found in the Jewish text: the Old Greek Septuagint (Spolsky, *Satisfying Skepticism*, 111). By Theodotion's own time in Alexandria in the second century B.C.E., the pastoral was a popular approach to Greek romances (117-118).

lead astray from the safety of iconographic accuracy. The structural relationship between the male painter, the male gaze, and the reception of the decent and obscene erotica during the early modern period may also contribute to the nefarious appearance of Renaissance and Baroque renditions of this scene.<sup>4</sup>

It may seem strange to consider the sexual charge of the Susanna imagery without first mentioning the larger presence of the erotic within the early modern Christian world. Without delving into how the susceptibility of eroticizing the image functions, more on this later, there have been numerous instances where the religious image has inspired tremendous sensations within people. During the fourteenth century, one anonymous author encouraged Dominican nuns to "[i]magine the Lord [...] disrobed for your sake, so that he might rest beside you naked."<sup>5</sup> One century later in 1427, Saint Bernardino recounts that someone "defiled himself" while standing before a cross, contemplating Christ.<sup>6</sup> These among other instances show an unusual boundary between the devotional and the perverse. Christ can be imagined lying disrobed beside nuns and prayer can even be transformed into a moment of pleasuring the self. Moreover, the very visual representation of figures in paintings become objects, or sites, of inflamed desire. Vasari gives the potent example of Fra Bartolommeo's *St. Sebastian*, which "gave rise 'to light and evil thoughts' among female parishioners" when they were in confessions.<sup>7</sup> Centuries before the *Modi*, viewers did not need to be inundated with the severely erotic since patrons and worshippers understood how to exercise pleasure out of the unforeseen locations of the religious artwork.

When it comes to understanding what exactly is explicit and transgressive about the Susanna imagery, per Bette Talvacchia, we should hesitate to label these images "pornographic" due to anachronistic concerns with applying the term to early modern images.<sup>8</sup> With the nineteenth century lineage of the word coming into use during the excavations of Pompeii, Talvacchia looks instead to the binary of "*onesto/disonesto*" used by Vasari and his contemporaries pertaining to conventions of erotic imagery, explicitly regarding the *Modi* but which can extend to the Susanna imagery.<sup>9</sup> It is important to remember that while the *Modi* predates the Council of Trent and provides significant insight into the opinions of visual erotica, Allori's *Susanna* dates to the final years of sessions. This time period spanning between the *Modi* and Allori's *Susanna* is exceptionally peculiar in the treatment of visual erotica. In 1539, Giro-

lamo Romanino completed frescoes at the *Magno Palazzo* for the Tridentine Cardinal Bernardo Cles.<sup>10</sup> What was evidently treated as a comparison between Michelangelo's Sistine Ceiling painted some decades earlier, Romanino's *ignudi* exhibited their bodies, "with decency [...] in most cases the twisting postures are calculated to conceal the genitalia", which was received by the Sienese doctor Pietro Andrea Mattioli in a poem about the frescoes as Romanino's *ingegno*, and in turn his *onestà*.<sup>11</sup> Here, the erotic is balanced, and well received through Mattioli's poetry, and does not cause a stir. For the early moderns, visual erotica was not necessarily problematic since it was viewed as either decent, "*onesto*", or what could fall under obscenities, "*disonesto*".

Mentioned earlier, the most potent and infamous example of the obscene artwork in sixteenth century Italy: Marcantonio Raimondi's series of 16 engravings after Giulio Romano, *I Modi*, serves as a touchstone for printed visual representations of sex in papal Rome around 1524.<sup>12</sup> Moreover, the *Modi* remains the most thoroughly researched series of erotic imagery in sixteenth century Italy, in the present day, and concerning early modern criticism against obscene imagery. Through the *Modi*, among other examples covered herein, we can assimilate early modern reception onto Allori's creation. The series is unique for the complete deviation from a mythological or biblical narrative, it is rather a fixation on continuously changing positions, possible and impossible, in sexual intercourse.<sup>13</sup> This deviation from narrative may confuse the possible relationship to the Susanna imagery, but a closer look into the implications and experimentations of how to depict sex accomplished through *I Modi* may situate us in a position to understand Allori's ulterior motive in depicting *Susanna and the Elders*.

A few years after the scandal of Raimondi's jailing by Clement VII and the order to destroy all prints and plates to produce the series, a series of woodcuts after Raimondi's engravings, accompanied by poetry, were printed in 1527 by Pietro Aretino, called the *Sonetti Lussuriosi* (Salacious Sonnets).<sup>14</sup> The *Modi* fixates on the interaction between man and woman for the pure pursuit of pleasure, disregarding previous Christian discourse on the hierarchies of position and reasons for intercourse, "licit only under certain conditions and when done in certain ways."<sup>15</sup> The original positions may have been sketched out by Romano, "on the walls of the Sala di Costantino in a state of pique against Clement VII,"<sup>16</sup> uncov-

<sup>4</sup>Talvacchia, 103.

<sup>5</sup>Kren, 19.

<sup>6</sup>Kren, 25.

<sup>7</sup>Kren, 27. See also (Freedberg, 346) for a discussion on the removal of the painting.

<sup>8</sup>Talvacchia, 101.

<sup>9</sup>Talvacchia, 102, 104-05.

<sup>10</sup>Talvacchia, 111.

<sup>11</sup>Talvacchia, 112.

<sup>12</sup>Nagel, 223.

<sup>13</sup>Nagel, 230.

<sup>14</sup>Nagel, 223. See also (Lawner, 3) for the measures of restricting circulation of the *Modi* which were so severe that reprinting of the series was deemed "punishable by death".

<sup>15</sup>Nagel, 231. The history of positions that were acceptable and unacceptable is rather complex. Augustine argued for the overall blamelessness of sex for the purposes of procreation (231). More critical interpretations derive from Thomas Aquinas who categorized the "natural" and "unnatural" positions such as orifices not used for procreation (231).

<sup>16</sup>Lawner, 30.

ering the nefarious relationships between notable courtesans with members of the papacy.<sup>17,18</sup> The pope's government occasionally made rulings regarding printed images; one famous instance concerns a privilege obtained by Ugo da Carpi for a chiaroscuro woodcut, granted by Pope Leo X in 1518.<sup>19</sup> Printed text in books were subject to censorship as recalled by Lodovico Dolce in his *Dialogo della pittura*, "the law prohibits the printing of immoral books," however, the engraved or woodcut image was considered a separate and less important matter.<sup>20</sup> Thus, the censorship of the *Modi* is considered "the first time the Lateran ordinance was applied against printed images."<sup>21</sup> Although the censorship of Raimondi's *Modi* was evidently effective, Aretino's *Sonetti* containing the woodcuts would become famous for its brusqueness, the depiction of Raimondi's lost work, and the written dialogues Aretino constructs between the figures in the images.<sup>22</sup> The indulgence of the erotic printed on the pages of the *Sonetti* are at the same time the efforts of Aretino to tarnish the reputation of papal Rome after almost being fatally stabbed on July 28, 1525 by Achille della Volta, a member of Gian Matteo Giberti's household, the datary to the Pope.<sup>23</sup> Moreover, the *Sonetti* are caught between the tensile papal politics and an unusual attention to vocalizing the silent imagery of the *Modi*.

Although it may be tempting to fixate on the formal aspects of the *Modi*, the few plates revealed to us through Aretino's woodcuts in the *Sonetti* introduce an "old procuress" checking in on the courtesan and her client.<sup>24</sup> Aretino makes the old woman's intentions explicitly obvious, "Ah, shameless pair! I spy you / On that mattress pulled down to the floor."<sup>25</sup> The window itself is rendered as an opening to the outside world, albeit completely bare and left uncut save a single line to create the sill, and the old woman leans into the window with her hand gripping the ledge. A device not necessarily known to scenes of explicit content, besides the implications of voyeurism belonging to the iconography of Isaac and Rebekah and the spying Abimelech, or even Joseph and Potiphar's Wife, the window implies a distanced means of viewing erotic scenes as legitimated through the image itself. This addition to the engagements realizes the viewer's position in relation to the scene, and also the position of an unseeable viewer that may lie beyond the window, out of our sight, thus making our pres-

ence in the scene resemblant of the visible procuress without having an occupational relationship to the courtesan with legs lifted and stretched. We must consider that before the *Sonetti*, Raimondi's engravings were not ekphrastically rationalized or dramatized beyond their purely visual content. Before the *Sonetti*, the *Modi* were simply the "ways" or "positions." While Raimondi's series independently worked off of existent Venetian texts advertising and explicating the popular and eminent culture of courtesans in the sixteenth century,<sup>26</sup> Aretino gave a voice to the physically active, gesticulating and expressive figures.

The epigraph of this paper concerns a letter from Aretino to Battista Zatti, a surgeon from Brescia.<sup>27</sup> This remarkable document contains the first mention of the *Sonetti* by the author himself. Moreover, the Zatti letter contains highly intriguing elucidations about the importance of the phallus and its causal relationship to the creation of notable men from Italian history, along with the writer and the recipient.<sup>28</sup> After these pleasantries and praise of the phallus, Aretino engages in a fascinating exercise in the abject connotations of the hands and mouths of men:

Men's hands might well be hidden since they gamble money, swear oaths, practice usury, make obscene gestures, tear, pull, punch, wound, and kill. And what about the mouth that curses, spits in the face, devours, and makes you drunk and vomits?<sup>29</sup>

Aretino makes a stern argument for the appreciation of the phallus which is always "hidden" and compares the hidden nature of the human anatomy with seemingly innocent bystanders of the stigmatized phallus. For Aretino, the hands and the mouth are far more guilty of the potential for not only ugliness but the violence and obscenity, ostensibly the sinfulness, than the phallus can produce, or be capable of. With this letter, we should consider that the pornographer intends to write in support of the sexual organs and that the *Sonetti* are not merely a playful interpretation of Raimondi's engraved reproduction of Romano's *posizione*.

Returning to the elders, one commonality shared between almost every depiction is the hands and mouth. Il Guercino

<sup>17</sup>Lawner, 22-26.

<sup>18</sup>Giulio Romano's all'antica imagery, and the deliberate numbering of 16 positions, carefully references equally ancient traditions of Parrhasias' paintings for the emperor Tiberius on the island of Capri (Talvacchia, 57). Romano himself was a coin collector and likely owned *spintriae*, minted coins numbered 1-16 depicting various sexual positions to "commemorate such wicked compartment" of Tiberius. In the sixteenth century Sebastiano Erizzo's account, based heavily on Suetonius, furnished Tiberius's attachment to the *spintriae* although scholars now credit the coins to the emperor Domitian (56). Historical inaccuracies aside, Romano was acutely aware of the loaded connotations, concerning Art and Roman imperial debauchery, behind his positions.

<sup>19</sup>Talvacchia, 11.

<sup>20</sup>Talvacchia, 11.

<sup>21</sup>Talvacchia, 12.

<sup>22</sup>Talvacchia, 66-67. Aretino's name "became a password for obscene art and literature" as his work allowed him to be continentally well-known by the early seventeenth century.

<sup>23</sup>Lawner, 6.

<sup>24</sup>Lawner, 41. Posture 11 is the only woodcut out of the set that explicitly depicts another individual *viewing* the couple. The only other example of a third party is Posture 14 which includes another individual; directly referred to as "Cupid" by the male figure. In this scene, Cupid pulls a cart that the couple lays on in the middle of an enclosed room with one window revealing a simple nature scene of terrain and thin clouds, the male figure shouts "You little prick! Don't keep pulling the cart / Cupid, you bastard, stop it!" (86).

<sup>25</sup>Lawner, 80.

<sup>26</sup>Lawner, 30. Lawner argues that the *Modi* "represents a combination, and amplification of these various genres: the literary catalogs and tariffs, the lists of lovemaking positions, the albums and the galleries of courtesan portraiture".

<sup>27</sup>Lawner, 8-9. See also (Frantz, 50) for an alternate translation by Thomas C. Chubb from 1967. In comparison with Lawner's version, Chubb softens sentiments expressed by Aretino, especially in the final sentence of my epigraph; "What wrong is there in beholding a man possess a woman?" (50). See also (Talvacchia 85-86) for a third translation of Aretino's letter to Zatti.

<sup>28</sup>Lawner, 9. Aretino explicitly refers to "that thing nature gave us for the preservation of the species" (emphasis mine) and proceeds to fire off names such as Pietro Bembo, Titian, and Michelangelo, along with "Popes, emperors, and kings" (9). Talvacchia uses these names to support the letter's later date of 1537 "since various artists and writers named [...] became friends of Aretino in the 1530s" (233).

<sup>29</sup>Lawner, 9. Lawner, 9. See also (Frantz, 50) for the Chubb translation of this excerpt which reads more elegantly than my preferred choice herein. Lawner's translation emphasizes the jagged rhythms of the actions of hands. Whereas Chubb waxes that hands "gamble away money, sign false testimony, make lewd gestures, snatch, tug, rain down fisticuffs, wound and slay" (50).



deploys the gestural power of the hands to caution the viewer not to alert the bathing Susanna (Fig. 2). We are met with an elder's gaze and furrowed brow, he knows we are also present from behind the bushes and thus participants in their spying. He points with one hand and pulls back the bush with the other allowing the other elder to look at their target. With a foreshortened, splayed open hand that breaches deep into the foreground, the other elder exculpates the viewer, telling us "[d]on't disturb her" and "[d]on't be so quick to judge us - wouldn't you also be enchanted by her?"<sup>30</sup> From the letter to the Brescian surgeon, Aretino's words accompany Guercino's scene in a similar, appropriate manner as the *Sonetti* describes the *Modi*. Behind the bushes, hands "might well be hidden" from Susanna and these discreetly "obscene gestures,"<sup>31</sup> like the grasping of the erect and phallic staff, signal their nefarious intentions. Perhaps nowhere else in the history of biblical narrative scenes can the relationship between figures and viewer be so inherently problematic. While there are other examples such as depictions of Joseph and Potiphar's Wife, the viewer is the sole voyeur of the adulterous meeting. The story of Isaac and Rebekah spied on by Abimelech, famously painted by Raphael for the Vatican Loggias, contains another example of a third-party voyeur. However, Abimelech is always distanced from the viewer, looking down from a balcony. Though both parties view the adulterous pair, the complicity of the parties does not approach the ethically compromised criminal-accomplice relationship established and possibly encouraged by patron and painter.



**Figure 2.** Giovanni Francesco Barbieri, known as Guercino. 1617, oil on canvas, 176 x 208 cm, Museo Nacional del Prado, Madrid, Spain.

What is particularly striking about Allori's Susanna is the openness of the figure for the elders. There are no necessary signs of resistance to what the elders are doing. This is what Freedberg refers to as a "particular kind of exposure of the body" wherein Susanna gives herself to the elders, and to the viewer as well.<sup>32</sup> The exposure is gained in two ways: namely the mannerist, nearly impossible contortion of the torso where Susanna's breasts are presented to the viewer, and the permission granted to the elder whose arm reaches in between her legs. Ultimately, this available bodily allows the "beholder"; whether the painted figure or the external viewer, to obtain a sensory-driven mode of exploration "in search of the sexual organs."<sup>33</sup> One may argue that the *Modi* allows the viewer to reach this heightened state of visual and perhaps visceral arousal, and in some measures the similarities between the two types of images begets this comparison. The distinction between the latent narrative content found in the *Modi*, contemporary courtesans in the Vatican and the clever allusions to antiquity, and the presence of a known narrative from the Book of Daniel is crucial if we understand the significance of Allori's rendering of the story. The openness of Allori's painting sets the elders to receive what they desire, and arguably this image grants the viewer an otherwise unavailable depiction of the violated Susanna. In a moment of unprecedented painterly access, Allori provides the viewer with an improbable outcome that has thus been realized and set into action. This rare moment in sixteenth century Italian painting, where the eroticism of textual narratives is not displaced by some abstraction and distancing akin to the *Modi*; of nameless figures in ambiguous all'antica spaces. The eroticism instead provides the extreme ends of potentiality in inaccurately depicting biblical narrative scenes near the end of the Tridentine meetings.

As *objet d'art*, "the painted woman is also an object, available to the viewer's gaze."<sup>34</sup> As a Derridean *parergon*, the female nude rests on the borders between the artistic and the obscene.<sup>35</sup> Where the female nude figure is placed before the elder's view, similar to how the viewer observes artwork in an exhibition space, a metaphysical condition is established between internal and external viewers. Guercino's *Susanna* gracefully captures this condition where Susanna is displayed for the painted figures of the elders in the same way that Susanna is painted for the external viewer. This condition gives rise to Nead's observation of a border. Addressing the border may clarify the objectifying depictions of Susanna in Allori's painting. Because the combination of interaction and observation forms the erotic structure of not only the Book of Susanna, but also the visualized, albeit improvised text. While the Prado Guercino carefully balances on that border between art and obscenity, Allori's radical approach topples over into

<sup>30</sup>Spolsky, *Judgment of Susanna*, 102.

<sup>31</sup>Lawner, 9.

<sup>32</sup>Freedberg, 324.

<sup>33</sup>Freedberg, 324.

<sup>34</sup>Spolsky, *Judgment of Susanna*, 102-103.

<sup>35</sup>Nead, 25.

the obscene. For not only do the elders get their way, but Susanna offers little resistance. Moreover, there also exists a border between looking and touching. When it comes to the voyeur, looking may be the primary and initial action, but looking semantically infers the desire of touching.<sup>36</sup> Allori boldly crosses this border; in a discussion of a Domenichino *Susanna* (Fig. 3), Ellen Spolsky also notices this apparent crossing between what she considers the jumping "into that space in both picture and story where he [the elder] doesn't belong."<sup>37</sup> However, this crossing concerns a balustrade, and not Allori's obscene direction of the elder's hand from outer to inner thigh. Domenichino's elder is making the attempt to reach the naked Susanna while Allori's elder has been more than successful at achieving this goal.



**Figure 3.** Domenico Zampieri, known as Domenichino. 1603, oil on canvas, 56.8 x 86.1 cm. Galleria Doria Phamij, Rome, Italy.

Within Domenichino's work, and specifically Susanna's upward gaze, Spolsky has argued that Susanna's plea for divine intervention provides the ultimate weapon against the frail elders.<sup>38</sup> With the long history of physical weakness resulting in spiritual strength, the "*imitatio Christi*," tracing back to Hildegard von Bingen,<sup>39</sup> the argument for Susanna's complete innocence and chastity is shattered in the consideration of Allori's perverse creation. Averse to the characteristic upward gaze towards God, Allori's Susanna locks eyes with the elder. In *Satisfying Skepticism*, Spolsky makes an even more ambitious claim that the painter and the viewer retrospectively acknowledge Susanna's innocence in an examination of works by Tintoretto and Anthony van Dyck:

By imagining and painting the moment of her instinctive resistance, the painter can both enjoy her unprotected powerlessness and can also attest her innocence as though he himself were the prophet Daniel [...] Thereby, [Tintoretto and van

Dyck] make themselves and the viewer into the Daniel who 'sees' the innocence of the woman and condemns the elders.<sup>40</sup>

Spolsky's argument implies that the painter, within an epistemology of the text now visualized through painting, actively understands Susanna's innocence at the level of judgment and justice already having been served through the text itself. She goes even further to state that the viewer also assumes the role of Daniel.<sup>41</sup> From the perspective of observation, Mieke Bal defines the situational relationship of the pornographer to the visual image as "not yet acting, they were 'hidden and spying'."<sup>42</sup> Whereas Spolsky appears to interpret the viewers' viewpoint as one of Daniel ready to interrupt the elders before anything physical were to happen, Bal carefully recognizes the voyeuristic conditions of an unaware, bathing Susanna, and lustful old men waiting for the right moment to act. Allori's approach is considerably pernicious to the argument Spolsky presents because it does not matter that Susanna is textually innocent when, in the moment pictorialized, her chastity may be breaking before the viewer's eyes. In the space between her covered thighs and the elders' "hidden" hands, Allori presents us with the ambiguity of Susanna defiled, or about to be. If the elders have gotten this far in this wildly extratextual depiction of the Book, then who is to say they will not carry out their rape after all? In a reading of the trial scene, Bal explicates that the elders' description of the young man and his actions are the self-identification of the elders: "lying down with her is what they saw, hallucinating the fulfillment of their desire."<sup>43</sup> This act of hallucination speaks to the potential not only of an imagined outcome of the interaction, but more importantly the visualized imagination brought forth by Allori, where their desire is becoming fulfilled.

When it comes to the potential for arousal with the Susanna depictions, we must consider the efficacy of erotic images and their shortcomings. David Freedberg contends that images showing "blatant sexual engagement" are most likely to fall short of arousing the viewer because they lack "the frisson, the tension, or the still stranger arousal that arises from the sense of" images that border between the artistic and the unartistic.<sup>44</sup> The *Modi* for example, although incredibly scandalous for its time, should not perform as well as the Susanna imagery because it simply shows too much. Though the engravings represent images as Nagel refers to them "without any cover,"<sup>45</sup> stripped of any censoring of perverse acts, what should we make of the aesthetic and sensual image concealed once more? What Freedberg understands is that tension, of the just yet unobtainable, is the driving force of arousal. Unlike the *Modi*, the Susanna imagery is exceptionally effective as a transmitter of arousal because the elders rarely physically

<sup>36</sup>Bal, 13.

<sup>37</sup>Spolsky, *Judgment of Susanna*, 106.

<sup>38</sup>Spolsky, *Judgment of Susanna*, 110.

<sup>39</sup>Spolsky, *Judgment of Susanna*, 53.

<sup>40</sup>Spolsky, *Satisfying Skepticism*, 127.

<sup>41</sup>Spolsky, *Satisfying Skepticism*, 127.

<sup>42</sup>Bal, 5.

<sup>43</sup>Bal, 8.

<sup>44</sup>Freedberg, 355.

<sup>45</sup>Nagel, 236. Nagel's discussion of the *Modi* in the context of serious religious art underpins the acknowledgment that the images "were inseparable from serious art" because "they emanated from the authoritative center. They had papal Rome written all over them" (236).

violate Susanna, save Allori's painting. Even then, there are no *cazzi* or *potta*, only arms and discreet hands. Although the viewer understands Susanna's ultimate innocence, the potential to hallucinate what "might've been" is evident with the presence of Allori. Susanna is not repelled by the elders' actions, instead, she is magnetized to them. Opposed to the rhetorical features or "pastoral conditions" of the Greek romances the Susanna story emanates out of, we may conclude that Allori contemplates the possibility that no *deus ex machina* is coming to save the endangered woman.<sup>46</sup> How could Allori assume the perspective of Daniel if he were already too late? Alessandro Allori's *Susanna* may perhaps be the most direct interpretation of this scenario, where there truly is no help on the way. These conditions strengthen the erotic tension possible in Susanna imagery because although other images are charged with sexual tension a la Domenichino's "tugging contest" between the elder and Susanna,<sup>47</sup> or Guercino's surveillant elders, they again do not cross the border between voyeur and violator.

Narrative scenes can inspire tremendous devotion from the viewer; the worshiper, and yet they can also inspire arousal from the same observer. In their potential for arousal, these paintings are threatening. The imagery of *Susanna and the Elders* creates unforeseen conditions of placing two viewers in contention with each other wherein the internal, painted viewers exculpate the external viewers standing before the painting. Though erotic art was understood and collected by the elite and literate nobles and intellectuals, an obscenely erotic art carries a volatility unlike other early modern pursuits. This volatility reached a breaking point in Rome emanating from Raimondi's printshop with the printing of *I Modi* in the 1520s. Though Clement VII censored Raimondi's reproduction of Giulio Romano's paintings, the thrice reproduced images in the *Sonetti* resurrect what the Vatican did not want in the hands of good Christians the exposure of the perversion and courtesans occurring behind the Vatican walls. Perhaps explored to a fuller extent in the literature of Aretino among other erotic authors, sexually explicit content in the time of the *Modi* was considered perverse and exceptionally sinful. In 1563, the final sessions would be held, the Book of Daniel would be considered canonical by the Church, and the treatment of images would be of utmost concern regarding textual and iconographic accuracy. In the undercurrent of the sessions, Alessandro Allori's *Susanna and the Elders*, and the Susanna imagery in general, marks perhaps the most important intersection between narrative scenes and a newfound, obscene narrative in sixteenth century Italian painting. While Guercino and Domenichino created unique renditions of the text some decades later, Allori's 1561 *Susanna and the Elders* surpasses most painted attempts at this scene because of its exceptionally graphic nature. Allori's elders are not necessarily aware of their external viewers, instead, they are beholden to Susanna's pictorial, hallucinatory, imagined consent.

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## About the Author

Daniel Heberle graduated from the University of Rochester in May 2024 with a B.A. in Art History and a minor in Spanish. The present article was from a Spring 2023 independent study. More recent research has included work on Goya's Black Paintings, Spanish encounters with Mexica featherwork, and workshop/legal practice related to the El Greco in the Memorial Art Gallery. With a focus on the early modern Hispanic world, Daniel is continuing his research in the Rhetorics of Art, Space and Culture: Ph.D. program in Art History at Southern Methodist University in Dallas, Texas.

<sup>46</sup>Spolsky, *Judgment of Susanna*, 113-114. There is a clear connection between Daniel and the "god in the machine" motif which would explain how the Theodotion version may have been a direct reference for early modern painters tasked with visualizing the garden scene.

<sup>47</sup>Spolsky, *Judgment of Susanna*, 106.



# Martha Chase at the University of Rochester: The Woman in STEM Who Was Forgotten

Michelle A. Bischoff '24, Xuke Wang '26, Erin Song '26,  
Gabrielle Wilson '25, Monica Brody, Suchi Iyer,  
Kirya Caine '26

Advised by Michael Clark, *Department of Biology*



**Figure 1.** Photograph of Martha Chase included in the 1953 University of Rochester employee file.

If you take a stroll through the fourth floor of Dewey Hall at the University of Rochester, you will pass a line of small offices. For students, these might house yet-to-be-graded papers, cramped office hours, and other mundane bits of everyday academia. However, if you pause in front of room 418, you stand near history. In 1953, this was not an office but the Doermann lab, and working inside was Martha Chase (Figure 1), one of the most notable names in this golden age of science: the infancy of molecular genetics.

This article aims to delve into Martha Chase's experience ing her time at the University of Rochester, encompassing her scientific contributions, academic environment, and place within the university community. Despite making a significant contribution early in her career, Martha Chase's personal and academic story is not a satisfying one to learn. She never secured a tenured position and was never given the chance to establish her own laboratory and research program. Because of a notable absence of scholarship regarding Martha Chase, we hope to rectify this gap with this work.

## Female ratio today vs. the 1950s

Over half of students in an introductory biology course are usually female (Sible et al., 2006). While this female-biased sex ratio is overlooked by students today, this was not the case when Martha Chase joined the University of Rochester in the 1950s. Using "The Interpres," the student yearbook of the University of Rochester, we approximate the sex ratio of faculty and students in the STEM field from 1952-1959 to be only 7% female, making up 31% of graduating STEM students being female. (*Interpres* | *Digital Collections*, n.d.). Imagine only a handful of female students in a packed biology classroom or having every chemistry class taught by male professors—that was the reality of the University of Rochester in the 1950s. Striving in an environment like this, female scientists collaborated and supported each other. In Chase's employment letter archived in the Department of Rare Books,

Special Collections, and Preservation at Rush Rhees Library, we found a trace. Dr. Barbara McClintock, who studies the transposable element at Cornell University and won the Nobel Prize in 1983, is among the people of reference for Chase (*The Nobel Prize in Physiology or Medicine* 1983, 2018; Figure 2).

The University of Rochester - Personal Data Record			
<b>ACADEMIC TRAINING</b>			
College or Institution	Degree	Year	Major Subject
College of Wooster, Wooster, Ohio	B.S.	1950	Biology
<b>ACADEMIC EXPERIENCE</b>			
Institution	Rank	Department	Inclusive Dates
<b>NON-ACADEMIC EXPERIENCE</b>			
Place	Position Held	Inclusive Dates	
Dept. of Zoology, Carnegie Institution, Cold Spring Harbor, N. Y.	Research assistant	August 1950 - January 1953	
Cold Spring Harbor National Lab., Cold Spring Harbor, New York	Research assistant	January 1953 - October 1953	
<b>RESEARCH AND PUBLICATION SUMMARY (A separate sheet may be attached if you prefer)</b>			
(attached sheet)			
<b>REFERENCES:</b>			
Dr. A. S. Hershey, Carnegie Institution, Cold Spring Harbor, N. Y., N. Y.			
Dr. Barbara McClintock, Carnegie Institution, Cold Spring Harbor, N. Y., N. Y.			
Dr. A. H. Doermann, Department of Biology, University of Rochester			
Dr. Warren F. Spencer, Biology Department, College of Wooster, Wooster, Ohio			
Signature: <i>Martha Chase</i>			
Date: October 18, 1953			

**Figure 2.** A set of references for Martha Chase. Chase's references were from her former boss and coauthor Arthur Hershey, her boss at the University of Rochester August Doermann, her former undergraduate professor Warren Spencer, and future Nobel laureate Barbara McClintock.

## Chase's work before the University of Rochester

Chase has never been as well-known as the "Hershey-Chase Experiment", unfortunately joining a long list of women in STEM who were denied the opportunities and accolades of their male counterparts. This article aims to remedy the latter, highlight her time at the University of Rochester, summarize her research, and begin to understand her place in the UR community.

In 1952, one year before joining the Biology Department at the University of Rochester, Martha Chase was working with

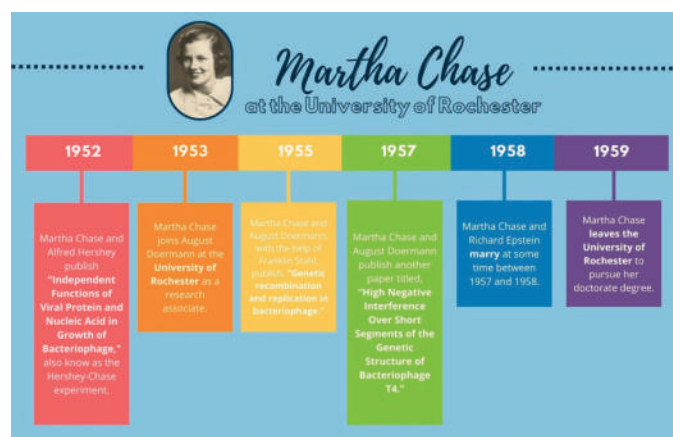


Alfred Hershey at Cold Spring Harbor Laboratory. They collaborated on an experiment that would earn them a place in most introductory biology and genetics textbooks (Hershey & Chase, 1952). In this work, they tagged proteins and DNA of bacteriophages (viruses that infect bacteria) radioactively, tracking what was transferred into the bacteria during the initial stages of viral infection. They hypothesized that the material transferred would be the material of heredity. The results of this experiment provided important evidence for one of biology's grandest conclusions: DNA is the material of heredity.

So how did a pivotal figure become effectively forgotten by the very institution she worked at, and what can we learn about her and the field of biology during the time of her contributions?

### Martha Chase at the University of Rochester

In 1953, Martha Chase moved to the University of Rochester and worked in the lab of biology professor August Doermann (Figure 3). Chase's work at the university revolved mostly around the budding field of molecular genetics with a focus on developing a greater understanding of the structure and function of phage and bacterial genetics, as well as how they could be used to understand eukaryotic organisms.



**Figure 3.** Timeline of Dr. Martha Chase's time at the University of Rochester.

While the "Hershey-Chase Experiment" is famous, the rest of Chase's scientific work from this period is far less known. Before moving to Rochester, Martha Chase worked in the Doermann lab along with graduate student Franklin Stahl at Oak Ridge National Laboratory. They focused on the genetic properties of bacteriophage, more specifically T2 and T4 (Hershey & Chase, 1952). The majority of new experiments in this research took place at Oak Ridge National Laboratory, with the remaining experiments and modeling done at the University of Rochester.

Many scientists were simultaneously exploring the nature of genetic material, and the scientific community had agreed on some key findings regarding the structure and function of

DNA. The close relation between T2 and T4 in structure allowed scientists to transfer a similar component of the bacteriophage, the loci (gene), from one to another. What scientists discovered after the displacement of loci was the presence of similar counterparts in T2 and T4.

Together, Chase and Doermann ran groundbreaking research in the field of phage genetics, the study of how phages, or viruses, pass down their traits to their offspring. The discoveries scientists made in phage genetics helped them understand how genetics worked in humans. One experiment that Chase participated in during her time in the Doermann Lab included using ultraviolet radiation to cause mutations in phage DNA. The resulting lethal mutations were then combined to investigate the effects of recombination between different phage genomes (Doermann et al., 1955). They found that ultraviolet light could damage a single, a pair, or all of the three loci in assumed genetic materials. When these damaged phage genes were combined within a single bacterium, they could survive and reproduce, revealing how different phages exchange genes to survive. As the duration of the dose increases, so does the number of effective compensations from wild-genotype bacteria. Chase's group stated that the ultraviolet irradiation targeted the genetic structure and genetic recombination with wild-type saved loci, protecting them from damage.

In addition, Martha Chase and August Doermann published a paper in phage genetics in 1958, titled "High Negative Interference Over Short Segments of the Genetic Structure of Bacteriophage T4" (Chase & Doermann, 1958). In this study, they aimed to establish map linearity in genes. A major conclusion of this work was that during gene mapping – the process of determining the locations of genes on chromosomes (condensed DNA) – genes are arranged in a specific order and displayed as a straight line. They also attempted to establish a relationship between the length of the gene and the length of the chromosome (Chase & Doermann, 1958).

After conducting their experiments and collecting their data, they discovered high negative interference over the genes in T4. This means that there is a higher rate of crossing over, or recombination, between two regions of chromosomes than normal. Recombination normally occurs when the chromosomes of two parents interact to create an offspring and gives the offspring a new and unique set of chromosomes and genes.

Although such findings are undoubtedly significant, Chase seems to have been overlooked when recognition was due. After the "Hershey-Chase Experiment," Hershey would go on to win the 1969 Nobel Prize in Physiology and Medicine (*The Nobel Prize in Physiology or Medicine 1969*, 2019). However, Chase and her scientific career are less well-known, largely ignored even by scholars of the history of science. In Horace Judson's exhaustive history of molecular biology, "The Eighth Day of Creation: The Makers of the Revolution in Biology," Martha Chase was never individually mentioned—just referenced three times for her work with Hershey (Horace Freeland Judson, 1980). Even at the University of Rochester, where Martha Chase came to continue her work on the phage DNA

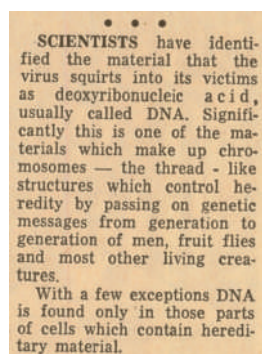
following the “Hershey-Chase experiment,” there is no mention of her University affiliation outside a contemporary year-book reference in 1958 (*Interpres* | *Digital Collections*, n.d.). Besides Chase, another notable member of the Doermann Lab was Franklin Stahl, best known for the famous “Meselson-Stahl Experiment,” where he and Matthew Meselson demonstrated that DNA is replicated by a semiconservative mechanism, meaning that each strand of DNA serves as a template for the production of a new strand (Meselson & Stahl, 1958). After receiving his bachelor’s degree at Harvard University, he matriculated to the University of Rochester to pursue his graduate studies (*Franklin Stahl*, 2022).

In 2015, Stahl published a memoir, “Serendipity and the Times,” where he reminisced about his time as a scientist (Stahl, 2015) and mentioned that he worked at the Doermann Lab. However, there is a notable absence of reference to Martha Chase, his coauthor and lab mate for several years.

Believing that Stahl might be our last connection to Chase and her life at the University of Rochester, we reached out to him through the alumni directory to gain more information, but he instead opted to not comment. This leaves us with no individuals left who remember Martha Chase and her legacy.

It appears that there is a testimony to Chase’s contribution in “The Interpres” of the year 1958, in which Chase is addressed as “Dr. Martha Chase,” although she did not receive her doctorate until 1964 (Dawson, 2003; *Interpres* | *Digital Collections*, n.d.). This inaccurate address, however, might indicate how Chase was perceived by others at the University of Rochester. While it may have been a simple mistake, it could also suggest a level of respect shown to her, at least by some members of the University of Rochester community.

Unfortunately, more evidence supports the idea that she was not given the respect or recognition that she deserved. A Rochester Times-Union article published in May 1956 describes some of the work that was conducted in the Doermann lab (Schmeck, 1956). One section of the article gives necessary background information about bacteriophage genetics in the “Hershey-Chase Experiment,” but simply describes the “scientists” findings, failing to explicitly mention Martha Chase by name (Figure 4).



**Figure 4.** Excerpt from the article “Mystery of Infections by “Killed” Viruses” as published in the Times Union.

Martha Chase’s time at the University of Rochester, although brief, was meaningful and left a lasting impact on the field of genetics. It was two years before women had arrived at the University’s River Campus when Martha Chase inadvertently demonstrated to women that they had the opportunity to live out their passions in STEM. Historically, STEM majors were always thought of as a male-dominated field whereas women gravitated towards the humanities and smaller helping roles adjacent to sciences. Martha Chase was a great role model for women on campus, proving that they were capable of engaging in research and carrying out their ideas. This reigns true today. Chase was a pioneer for women on River Campus, challenging the traditional career paths women were encouraged to take and making her name known in the ever-evolving world of genetics. Today, River Campus sees women stepping into the roles of STEM professors, teaching assistants, lab instructors, and heads of departments. While it may not be directly attributed to the increase of women in STEM at the University of Rochester, Chase’s presence has facilitated greater opportunities for women to be involved in research and continue pushing boundaries for future generations.

Throughout history, women’s autonomy has been intricately linked to societal norms and expectations, often subjecting their ability to engage in seemingly mundane activities to approval from male figures or authoritative sources. While progress has been achieved in certain areas, research indicates that women’s access to higher education has been characterized by limitations that reveal the pervasive influence of gender norms. Over time, women were gradually allowed to attend universities, signifying a notable step toward empowerment. However, a closer examination reveals that their academic pursuits were often circumscribed by societal expectations, restricting their freedom to choose certain fields of study or pursue careers of their preference. Supporting evidence suggests that even within educational institutions, gender bias, and restrictions continue to persist, reflecting deeply ingrained stereotypes about women’s roles and their capabilities.

In 2020, the University of Rochester published multiple short stories highlighting women affiliated with the university with achievements in STEM. Despite featuring individuals with lesser ties to the university, there was no mention of Martha Chase or her accomplishments before joining the University of Rochester, during her tenure there, or even after her departure (*Celebration 2020*, n.d.; *Celebration of Women 2020: Hajim School of Engineering and Applied Sciences: University of Rochester*, n.d.; STEM archives, n.d.).

This is also reflected in the personal files of Martha Chase (Figure 2,5). Something that is overlooked in the formal documents of female employees is their male-centered nature. In Chase’s personal data record, there is a section requiring her to provide her wife’s maiden name. While this practice helps trace lineage, the standardized question on a personal data record suggests that female employees at the University of Rochester were uncommon. In Chase’s paperwork, as she was unmarried at the beginning of her employment at the university, this section is simply crossed out.

THE UNIVERSITY OF ROCHESTER  
Personal Data Record

(To be filled in by all persons recommended for appointment to the University Faculty)

Name (Prior in full) Martha Cowles Chase Social Sec. No. 10-10-10

Address: Present Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Permanent 1550 Meadowbrook Blvd., Cleveland Heights 18, Ohio

Appointment Research Associate, Department of Biology

Birthplace Cleveland, Ohio Date of Birth November 30, 1907

Marital Status Single Wife's Maiden Name \_\_\_\_\_ Occupation \_\_\_\_\_

Number and Ages of Children None Other Dependents None

U.S. Citizen Yes If not U.S., when did you enter this country? \_\_\_\_\_

Military Service: Rank None Branch of Service \_\_\_\_\_ Length of Service 0 years

Special Training in Service \_\_\_\_\_

General Condition of Health Good

Height 5' 6" Weight 125 lbs. Physical Disabilities, if any None

Have you had any association in the past with The University of Rochester? No

If so, in what connection? \_\_\_\_\_

MEMBERSHIP in academic, professional, and fraternal organizations (Please state type of membership, offices held, and number of meetings of professional and scholarly societies attended in the last year):

American Genetic Association

**Figure 5.** Excerpts from Martha Chase's employment file at the University of Rochester.

OAK RIDGE NATIONAL LABORATORY  
OPERATED BY  
CARBIDE AND CARBON CHEMICALS COMPANY  
A DIVISION OF UNION CARBIDE AND CARBON CORPORATION

U4C  
POST OFFICE BOX 9  
OAK RIDGE, TENN.

Biology Division  
October 12, 1953

Dr. J. Edward Hoffmeister, Dean  
College of Arts and Science  
The University of Rochester  
Rochester 3, New York

Dear Dean Hoffmeister:

I am pleased to accept the position of Research Associate in the Department of Biology of the University of Rochester according to the terms of your letter of October 7, 1953.

Enclosed are the Treasury Form W-4, and the personal data form which you asked me to fill in. I shall submit a more recent photograph as soon as I obtain one.

Sincerely yours,  
*Martha Chase*  
Martha Cowles Chase

**Figure 6.** Martha Chase's acceptance letter for the research associate position at the University of Rochester.

Similarly, the endeavor for financial independence came with many challenges. Legalities and societal barriers impeded on women's ability to open bank accounts independently and research indicates instances where married women in particular faced restrictions in managing their finances or accessing credit without a male co-signer (Haughn, 2023). In the mid-20th century, legal reforms began to address these inequalities. These limitations underscored a broader pattern of women's economic dependency and highlighted the need for legal reforms to grant them equal financial agency. The *'Married Women's Property Acts'* enacted in the 19th century, gained momentum in the early 20th century and marked a significant shift by granting married women more control over their property and finances (*Married Women's Property Acts I United States*

[1839], n.d.). However, even after these legal changes, societal attitudes and practices persisted, perpetuating gendered expectations regarding financial roles within marriage.

The intersection of gender, societal expectations, and legal frameworks has shaped a narrative wherein women navigated a landscape by partial freedoms, underscoring the complex and ongoing struggle for gender equality across various facets of life. Within academia, particularly in STEM, societal norms have suggested that men were inherently more suited for these disciplines. However, with women breaking these barriers, their achievements can be attributed to a combination of societal, educational, and organizational changes.

## Life after Rochester

After leaving the University of Rochester, Martha Chase attended the University of Southern California, where she received her doctoral degree in Microbiology in 1964. Following her PhD, Martha Chase's career did not follow the typical route expected of a scientist with similar qualifications, experience, and connections to the research community. Unlike her male peers with comparable credentials, she was never offered a faculty position, never had her own laboratory, and never had the opportunity to pursue her own research questions. Martha Chase was the archetype of what it meant to be a woman in STEM during that era – a dedicated and hard-working individual who was deprived of sufficient recognition and opportunity. Ultimately, she ultimately moved back to Lorian, Ohio where she spent the remainder of her life with her family, away from the scientific institutions where she could have contributed more to the field. Furthermore, she spent the last decades of her life suffering from dementia which greatly affected her short-term memory. Martha Chase died from pneumonia at the age of seventy-five on August 8, 2003 (Lavietes, 2003).

So, who was Martha Chase? She was a prominent figure within the genetics community, whose contributions were largely forgotten with time due to the lack of opportunities, except for her most notable experiment with Alfred Hershey. The project to rediscover her story began with the publishing of her obituary, which revealed her affiliation with the University of Rochester's biology department. At the time of her death, few, if any, at the University of Rochester knew that she had ever worked there. We hope this article will begin to revive interest in the life and work of Martha Chase and help inspire aspiring scientists from traditionally underrepresented groups. Her story serves as a reminder of what is lost when inequalities and unequal opportunities persist in the field of science.

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## About the Authors

Met in BIOL 110 Principles of Biology I, we are a group of students who are surprised to learn that Martha Chase worked here at the University of Rochester, yet almost nothing is known for her time at the University. We established the Martha Chase Club to further our interest in advocating for underrepresented groups in science. Our academic interests include genetics, biochemistry, public health, and business. We aspire to be the role models of women in STEM in academia.



# Frederick Douglass: Man of Pictures

Rose Frank '24

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## Introduction

History remembers Frederick Douglass as a man of words. He is recognized for his captivating speeches advocating for abolition, his three autobiographies, whose literary qualities belie the fact that Douglass taught himself to read while still enslaved, and his newspaper, *The North Star*, which circulated anti-slavery content in the northeast in the years leading up to the Civil War. Despite recognizing Douglass's masterful storytelling, we often overlook how he had extended this into the visual realm.

Douglass matured alongside the rise of photography in 1840s America, and the two intertwined throughout the 19th-century. Originating as a French invention, photography was swiftly integrated into the U.S.'s entrepreneurial culture. As photography studios began to open, Douglass traveled across the country delivering anti-slavery speeches, where he likely encountered this exciting new medium. Throughout his life, Douglass sat for numerous photographs and wrote extensively on the subject. Recognizing that photographs captured portraits more affordably compared to paintings, Douglass envisioned this technology as a democratizing tool, challenging the tradition of reserving portraits for wealthy elites. He took many photographs of himself and delivered multiple lectures, presenting his views on photography as a democratizing force capable of dismantling class and racial barriers within the U.S. Reevaluating Douglass as a photography pioneer highlights his profound understanding of the medium's potential to democratize portraiture and challenge societal barriers in the 19th-century United States.

## Part I: Daguerreotypes

The story of Frederick Douglass and his profound connection with photography begins in 1820's France. It was in this period that Louis Daguerre, a masterful theater set painter, dedicated himself to devising a method for permanently preserving images using sunlight. A seasoned artist, Daguerre was accustomed to using his camera obscura, which was a draftsman's aid consisting of a wooden box with a small aperture. This contraption allowed light to filter through, projecting a reversed image onto the surface inside. If Daguerre could only figure out how to preserve this image on the page, then it could open up a new realm of possibilities in the theater and art world, and would cement him in the annals of history as not only a great painter, but as a revolutionary inventor.

Daguerre took twenty years to accomplish his lofty goal. Over time, Daguerre realized he could permanently capture images on silver plated sheets of copper coated in silver iodide, a light sensitive chemical. By exposing these plates to light through a camera lens, he was able to fix the image onto the sheet using a mixture of mercury fumes and saline solution.<sup>2</sup> In 1839, the French Government purchased rights to the daguerreotype process and shared it with the public. In return, Daguerre received generous pensions and yearly payments throughout the rest of his life.<sup>3</sup>

Daguerre's efforts culminated in a landmark moment in the history of visual representation. Were it not for the invention of the daguerreotype, photography as we know it today would not exist. Throughout the rest of the 19th-century, individuals across the world improved upon Daguerre's foundations. They refined this technology to better suit the demands of the modern era, experimenting and adapting photography to cater to their specific needs. This dynamic process of experimentation and adaptation allowed photography to find a meaningful place within many cultures, as individuals and communities explored how to harness this exciting medium.<sup>4</sup>



**Figure 1.** Louis Daguerre posing for a daguerrotype in 1844 (International Photography Hall of Fame Museum).

<sup>1</sup>Vi Whitmire, *Louis Jacques Mandé Daguerre*, International Photography Hall of Fame, <https://iphf.org/inductees/louis-jacques-mande-daguerre/>.

<sup>2</sup>Ibid.

<sup>3</sup>Britannica, *Photography: Photography's Early Evolution*, <https://www.britannica.com/technology/photography/Photographys-early-evolution-c-1840-c-1900>.

<sup>4</sup>Britannica, *Photography's Early Evolution*.

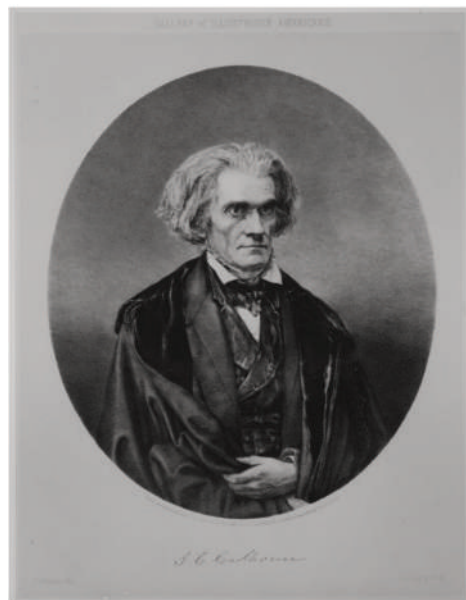
The news and use of Daguerre's invention quickly spread across the world, and the United States was particularly quick to adopt this new technology: the first daguerreotype produced in the United States was made just four weeks after the process was made public in France. Subsequently, the first photography studio opened in New York City in 1840, just a year after the initial announcement. By the end of the 1840's, daguerreian photography studios had opened in every major city in the country, and the United States became a world leader in daguerreotype production.<sup>5</sup>



**Figure 2.** Modern example of a daguerreotype plate with image transferred (The George Eastman Museum).

The U.S. quickly adopted daguerreotypes due to its enduring culture of improvement and commitment to experimentation, a legacy dating back to the nation's founding. European colonists had long viewed the United States as a place of opportunity, and many immigrated to this new land seeking to escape the rigid and oppressive hierarchical structures of their home nations.<sup>6</sup> Free from the shackles of rigid social hierarchy, American citizens believed they were uniquely positioned to achieve unprecedented heights. With inexhaustible land, energy, and, as Alexander Hamilton noted in 1791, "a peculiar aptitude for mechanic[al] improvements", Americans saw themselves as the natural inheritors of mankind's progressive march.<sup>7</sup> By the 1840s, Americans had already demonstrated an impressive repertoire of achievements including the Erie Canal and the invention of the steamboat and cotton gin.<sup>8</sup> Hence when daguerreotypes reached American soil, this medium was immediately absorbed by a culture that valued ambition, innovation, and improvement.

Americans sought to apply photography to portraiture<sup>9</sup>, however early exposure times for daguerreotypes took up to an hour, making it an impractical endeavor. Experiments in Europe and the U.S. soon resulted in more light-sensitive plates which cut exposure times to only a few minutes, making portraiture feasible. Wealthy families, important figures, and common people alike sat for self-portraits in these studios, and it quickly became the most popular genre of photography in the U.S.<sup>10</sup> As this technology spread throughout the country, people started to develop certain norms in portraying individuals. Some artists, like Mathew B. Brady of New York City, preferred to depict their sitters in serious, severe expressions in front of a plain backdrop. This image of John C. Calhoun is representative of Brady's preferred style:<sup>11</sup>



**Figure 3.** Print based on a daguerreotype of John C. Calhoun from "The Gallery of Illustrious Americans" (The Metropolitan Museum of Art).

Unlike Brady's formal and stern portrayals, other photographers, like Albert Sands Southworth and Josiah Johnson Hawes, took a more relaxed approach. In 1843, they established a daguerreotype studio in Boston and embraced a more informal style, presenting figures as they naturally appeared in everyday life, in contrast to capturing only the upper bodies of rigid, carefully groomed, and stern-looking subjects, as Brady did.<sup>12</sup>

<sup>5</sup>Ibid.

<sup>6</sup>Daniel Feller, *The Jacksonian Promise*, (Baltimore: The University of Johns Hopkins Press, 1995), 7.

<sup>7</sup>Feller, *The Jacksonian Promise*, 26-28.

<sup>8</sup>Ibid.

<sup>9</sup>Britannica, *Photography's Early Evolution*.

<sup>10</sup>Ibid.

<sup>11</sup>Ibid.

<sup>12</sup>Ibid.



**Figure 4.** A portrait of Lola Montez, famous Spanish dancer and mistress of King Louis I of Bavaria, taken in the Southworth and Hawes studio in 1850. Notice her relaxed and effortless gaze as she dangles a cigarette over an armchair (The Metropolitan Museum of Art).

As the 19th-century progressed, technological advancements in each decade made photography increasingly efficient, affordable, and accessible. The mid 19th-century marked an exciting period of exploration as individuals embraced and personalized this evolving process. It was into this dynamic era that Frederick Douglass entered adulthood.

## Part II: Frederick Douglass's Early Life

Frederick Douglass escaped from slavery in 1838,<sup>13</sup> just one year before the technology for the daguerreotype was released to the public. Douglass had been living in Baltimore working for his master's in-laws as a ship's caulker and tradesman. At first, Hugh and Sophia Auld had welcomed Douglass into their family, teaching him how to read and showing him a kindness no white people had ever granted Douglass before. Douglass was shocked by their generosity and felt himself to be a part of their family. Soon, however, their kindness turned into bitter resentment as the couple realized that slavery worked best when slave owners dehumanized their slaves. If the Aulds were to continue treating Douglass as the child he was, and not as the slave they wanted him to be, then Douglass might become rebellious against their authority. In response, the Aulds took away his reading materials and harshly watched over his

every move. Gone were the days of warmth and comfort; now Douglass was back to being treated like a slave.<sup>14</sup>

However, Douglass no longer felt like a slave. He noticed his reading lessons began to cause a shift in his masters' behavior. Recognizing that literacy could be a pathway to freedom, Douglass committed himself to secretly acquiring this skill—trading stolen bread from home with immigrant boys in the streets in exchange for reading lessons, and practicing writing when the Aulds were not watching.<sup>15</sup> Throughout the years, Douglass taught himself how to read and write—a remarkable achievement demonstrating his intelligence and determination. This early exposure to the transformative power of words stayed with Douglass throughout his life, ultimately shaping his future political persona.

When Douglass was about twenty years old, he escaped from Baltimore and headed North to establish his life as a free man. Dressed as a sailor and armed with fraudulent identification papers, he boarded trains, avoided eye contact with familiar faces, and eventually made his way to the free state of Massachusetts.<sup>16</sup> Despite the challenges of starting afresh, Douglass created a life for himself in New Bedford, where he worked odd jobs sawing wood, digging cellars, and moving heavy caskets of oil at an oil refinery.<sup>17</sup> He became an avid reader of *The Liberator*, an abolitionist newspaper run by the stoutly religious William Lloyd Garrison, and began attending meetings held by the local abolitionist movement.<sup>18</sup> Although Douglass had found a routine in the city, he was not destined to lead a life of manual labor. Within a few years out of slavery, he would soon find himself embarking on another adventure.

In 1841, Douglass attended an anti-slavery convention in Nantucket that changed his life forever. Douglass had demonstrated his talent as an orator in New Bedford by delivering sermons at a local black church, but did not expect to participate in the convention. Fortunately, a prominent abolitionist had witnessed his skills in New Bedford and was so impressed that when he recognized Douglass at the anti-slavery convention, he asked Douglass to give an impromptu speech.<sup>19</sup> At this early point in Douglass's young life, his appearance would have been striking to a mostly white audience. Shy in demeanor and strong in stature, Douglass had physical features that commanded attention—a strong chin, chiseled cheekbones, and well-dressed in his signature formal jacket and white button down shirt. Although Douglass could never recall exactly what he said that evening, it is evident to have been an incredible performance. A reporter for the *National Anti-Slavery Standard* remarked on the event, "Flinty hearts were pierced, and cold ones melted by his eloquence. Our best pleaders for the slave held their breath for fear of interrupting him."<sup>20</sup> It was clear to everyone in the audience

<sup>13</sup>Blight, *Prophet of Freedom*, 146.

<sup>14</sup>Frederick Douglass, *Narrative of the Life of Frederick Douglass*, Chapters 5-6.

<sup>15</sup>Douglass, *Narrative of the Life of Frederick Douglass*, Chapter 7.

<sup>16</sup>David Blight, *Prophet of Freedom* (New York: Simon & Schuster, 2018), 110-112.

<sup>17</sup>Frederick Douglass, *Life and the Times of Frederick Douglass*, <https://docsouth.unc.edu/neh/doug192/doug192.html>, 259.

<sup>18</sup>Douglass, *Life and the Times*, 263-265.

<sup>19</sup>Douglass, *Life and the Times*, 266-267.

<sup>20</sup>Linda Hirshman, *The Speech That Launched Frederick Douglass's Life as an Abolitionist*, <https://time.com/6148114/frederick-douglass-abolitionist-book/>.

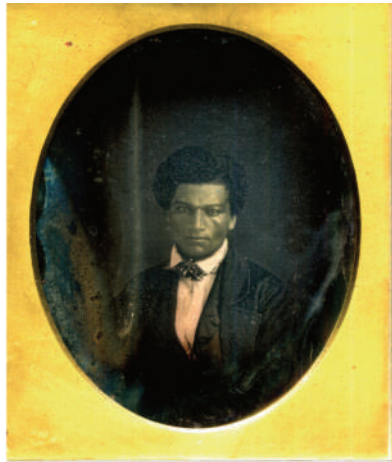


that Douglass was born for public speaking. As soon as the meeting ended, he was asked to join William Lloyd Garrison's Massachusetts Anti-Slavery Society as a traveling agent.<sup>21</sup> Over the following three years, he roamed across the northeast and midwest regions of the country with a team of orators, giving speeches in support of the abolitionist cause. This experience launched his rise to political and national fame.

### Part III: Frederick Douglass the Activist

From 1841 to 1845, Douglass traveled with Garrison's team and made use of the growing number of accessible daguerreotype studios, making it a point to have his portrait taken in nearly every town he visited.<sup>22</sup> The abundance of surviving images from this period depicts a progressive arc in which Douglass learned to navigate the medium, as he strategically adapted his posture to create images of himself that aligned with the message he wanted to project.

Douglass's journey in mastering his visual representation is evident when scrutinizing his earlier portraits. The earliest known daguerreotype of Douglass, taken in 1841, captures a sense of bewilderment or, in his own words, a "statue-like" demeanor. His demeanor can be attributed in part to the extended exposure times of 1841, lasting several minutes compared to the later, shorter durations of just a few seconds. It is also likely that Douglass had not yet determined his preferred composition; this image takes an oddly zoomed out perspective, placing Douglass in the center of the photograph and leaving much empty space above his head. The result (Figure 5) gives a sense that Douglass is placed far in the back of the image, as opposed to proudly facing the viewer from the front.



**Figure 5.** The earliest known portrait of Douglass (Courtesy of the Collection of Greg French).

In a subsequent 1843 daguerreotype (Figure 6), the camera is positioned at chest level, and his gaze is directed slightly upwards and to one side, adhering to the recommended pose outlined in photographic manuals.<sup>23</sup> This particular gaze was thought to convey confidence and intelligence, but Douglass had not yet mastered the pose. In this daguerreotype, his eyes appear partially open and veiled in shadows, indicating that he was still navigating the nuances of proper posing.



**Figure 6.** Douglass adapted to the photographic pose. Likely taken between 1841 and 1843 by an unknown photographer (Onondaga Historical Association).

A later daguerreotype taken in 1848 by the Edward White Gallery (Figure 7) shows Douglass' continued experimentation with the photography medium. Here, Douglass's disposition is noticeably guarded, with a sidelong glance and head slightly lowered, suggesting a reluctance to fully trust the camera or the photographer. These three images indicate Douglass's uncertainty in his appearance and the way he presented himself to the public, which is underscored in a letter he wrote in 1846 to another fugitive slave, admitting that, "I got real low spirits yesterday...I looked so ugly that I hated to see myself in the glass."<sup>24</sup>

Douglass's uncertainty in front of the camera did not last long. By 1850, Douglass adopted his distinctive look, evident in the daguerreotype below (Figure 8). He gazes sternly into the camera with a calculated pose, conveying a sense of deliberate defiance. The frontispiece of his popular second autobiography, "My Bondage and My Freedom", published in 1855, mirrors the daguerreotype, projecting a similar air of calculated defiance (Figure 9). Modeled on another lost photograph, it highlights his physical strength and confidence, depicting a sturdy young man with furrowed brows and firm

<sup>21</sup>Douglass, *Life and the Times*, 271.

<sup>22</sup>Stauffer, John, Zoe Trodd, and Celeste-Marie Bernier, *Picturing Frederick Douglass: An Illustrated Biography of the Nineteenth Century's Most Photographed American* (New York: Liveright Publishing, 2015).

<sup>23</sup>Snelling, Henry H, *The History and Practice of the Art of Photography; or, the Production of Pictures Through the Agency of Light* (New York: G. P. Putnam, 1849).

<sup>24</sup>Douglass to Ruth Cox, May 16, 1846.

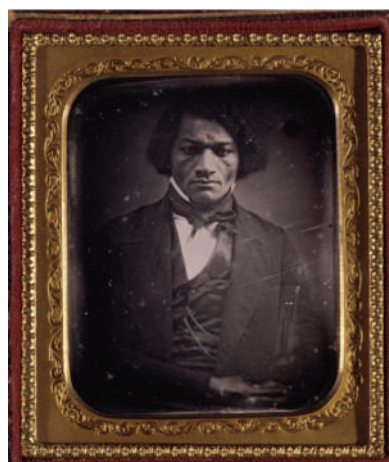


lips. This became Douglass's signature pose in the late 1850s and throughout the Civil War.<sup>25</sup>



**Figure 7.** This portrait conveys Douglass's insecurity in front of the camera (Albert Cook Myers Collection).

**Figure 8.** 1850, unknown photographer (National Portrait Gallery, Smithsonian Institution).



**Figure 9.** Engraving by John Chester Buttre based off of a lost daguerreotype. Frontispiece for "My Bondage My Freedom" (University of Rochester Rare Books Library).

In these images, we not only witness Douglass mastering the technical aspects of photography, but also his growing confidence in his own self-expression. As Douglass gained mastery of this visual medium, the images documented his journey from a determined yet uncertain political activist, to a more assured and self-confident leader.

The transformation evident in Douglass's self-portraits mirrors his broader journey—from a former slave determined to share his story into an intellectual activist challenging the very foundations of slavery and discrimination. The visual narrative encapsulates Douglass's evolution, illustrating the intersection of his political activism, intellectual endeavors, and mastery of the emerging art of photography.

## Part IV: From Enthusiast to Theorist

After two years speaking independently in the United Kingdom, Frederick Douglass returned to the U.S. in 1847 and split from the Garrisonian crusade due to differences in their approaches to abolition and William Lloyd Garrison's attempts to control and suppress Douglass's intellectual abilities.<sup>26</sup> From the beginning of their partnership, Garrison's troupe sought to hold back Douglass's talents. Douglass recalled that Garrison's associates urged him to speak with less eloquence so he sounded more like an uneducated former slave. They stated it was "better to have a little of the plantation speech than not" and cautioned against Douglass appearing "too learned".<sup>27</sup> Tension persisted throughout their relationship because Douglass aimed to go beyond, whereas Garrison preferred to keep him fixed within the role of an uneducated former slave to support the arguments made by white abolitionists. Their conflict led Douglass to part ways with the Garrisonian approach, marking a significant moment in his journey toward greater independence and intellectual freedom within the abolitionist movement.

After the split, Douglass decided to settle in Rochester, New York, where he established his own newspaper, *The North Star*. This platform allowed him to publish views and opinions that had been restricted under Garrison's oversight.<sup>28</sup> Douglass not only asserted his editorial independence but also began expressing his thoughts on photography in a more intellectual manner, moving beyond the realm of self-portraiture.

Douglass recognized the potential of photography to dismantle racial barriers within the United States. In 1854, he prominently featured an article on the accomplished black photographer James Presley Ball. Ball had opened a studio in Cincinnati that became known as the "Great Daguerreian Gallery of the West"<sup>29</sup> Impressed with Ball's gallery, Dou-

<sup>25</sup>Stauffer, *Picturing Frederick Douglass*, 50-54.

<sup>26</sup>Frederick Douglass, *Life and the Times of Frederick Douglass*, 268-269.

<sup>27</sup>Page 269 of *Life and the Times*. Douglass did not reproach these men for their comments as he understood that they "were actuated by the best of motives." Nevertheless, he emphasized his strong desire for the freedom to express his thoughts openly.

<sup>28</sup>Frederick Douglass, *Life and the Times of Frederick Douglass*, 317-322.

<sup>29</sup>Stauffer, *Picturing Frederick Douglass*, 36.

glass showcased it along with an engraving of the studio on the front page of his newspaper. For Douglass, Ball's studio symbolized a vision of the integrated nation he aspired to see. It served as a testament to the acknowledgment of African American artists' contributions to the nation's visual culture and envisioned a space where individuals of different races could come together, united by their shared passion for art. In this way, Ball's studio became a tangible representation of Douglass's vision for a more inclusive and integrated future.<sup>30</sup>

Douglass extensively explored photography in his later years, delivering four lectures on the subject.<sup>31</sup> In these lectures, he articulated a theory for the uses of photography that was both radical and unprecedented for its time, given that photography was still a developing medium. On December 3, 1861, at the Boston Tremont Temple, Douglass gave a lecture called "Lecture on Pictures" to a group of abolitionists, fellow activists, and community members. Departing from his usual presentation style (which included frequent improvisation and interaction with the crowd), Douglass took a more scholarly approach by reading from a stack of handwritten papers.<sup>32</sup> In this address, Douglass articulated his views on how photography had the potential to bridge racial and economic divides in the United States. He emphasized that the affordability and accessibility of photography allowed even the poorest individuals to experience a sense of importance, noting, "the humblest servant girl, whose income is but a few shillings per week, may now possess a more perfect likeness of herself than noble ladies and even royalty, with all its precious treasures, could purchase fifty years ago."<sup>33</sup>

He also lauded photography for presenting subjects as they truly appeared, immune to artificial improvements or distortions. Douglass's thoughts were undoubtedly shaped by the challenges he had faced in representing himself to the public as he desired.<sup>34</sup> From the constraints imposed by Garrison on Douglass's intellect to the manipulation of Douglass's engravings by white artists, photography held significant importance for him because it emphasized the truth of its subjects.

When Douglass delivered his "Lecture on Pictures", a reviewer praised him as a "genius", stating, "no one has crystallized [the theory behind photography] more clearly than Mr. Douglass; and this seemed to be the feeling of the highly educated and thinking audience."<sup>35</sup> By 1861, Douglass had evolved from an uncertain young man posing before a camera to a confident master of the craft, assigning philosophical meaning to creations from this transformative technology.

## Part V: Rethinking Frederick Douglass

Douglass's engagement with photography occurred during a Goldilocks period in the history of the medium. As technological advancements after Douglass's era led to reduced shutter

speeds, cameras became worse at capturing images of black individuals. Shorter shutter speeds made it more difficult to capture depth and detail in darker skin tones, and as W.E.B. Du Bois noted, few white photographers bothered to remedy this.<sup>36</sup> Since Douglass's many portraits were captured by daguerreotypes with longer shutter speeds, they yielded higher quality images than those captured in the later nineteenth century. Douglass's contributions to photography, therefore, occurred at the perfect moment in the medium's development to leave behind a collection of photographs that continue to stun viewers today.

Historians often emphasize that Douglass was the most photographed American in the nineteenth century to underscore the fame he achieved in his own time. While this is true, it oversimplifies his much more complex and intentional relationship with photography. It leaves out an entire portion of Douglass's intellectual life, his fascination with photography and its potential to generate social change. Beyond his success as an abolitionist who happened to sit for numerous portraits, Douglass warrants recognition as a pioneering photography theorist. His intellectual engagement with photography extended far beyond the lens, reflecting a profound understanding of its potential to drive social change.

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<sup>30</sup>Ibid.

<sup>31</sup>Stauffer, *Picturing Frederick Douglass*, 10.

<sup>32</sup>Stauffer, 338–339.

<sup>33</sup>Stauffer, 340.

<sup>34</sup>Stauffer, 27.

<sup>35</sup>Stauffer, 338.

<sup>36</sup>Deborah Willis, *The Sociologist's Eye: W. E. B. Du Bois and the Paris Exposition*, (New York, 2003), 51.

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## About the Author

While taking Professor Jarvis's Frederick Douglass's Rochester course I became very interested in Douglass's relationship to photography. Douglass was the most photographed American of the 19th century with over 160 surviving photos and portraits. While examining original photographs of Douglass housed in the Rare Books and Special Collections Library, I grew increasingly curious about what motivated Douglass to sit for so many portraits. It was fascinating to uncover Douglass's strategic approach to photography and to realize how he utilized this medium to advance the abolitionist movement. I am grateful to Professor Jarvis and the Rare Books team for providing the support and resources essential for conducting this research project.

