Date: April 17, 2024

Cc: Nathan Blouin, Director of the Office of Research Development

From: Rachelle Davenport, Limited Submissions Coordinator

Re: Pew Biomedical Scholars Program/Pew-Stewart Scholars for Cancer Research Program

Thank you for agreeing to participate on the selection committee for this year's internal candidates for the **Pew Biomedical Scholars Program** and the **Pew-Stewart Scholars for Cancer Research Program.** A meeting will be held **Thursday, April 25th, 2024, from 3:00pm-4:30pm** to select **one** institutional Pew Biomedical candidate from the seven internal submissions, and one Pew-Stewart candidate from the two internal submissions. Nathan Blouin, Director of the Office of Research Development, will chair the internal review.

Zoom Link: https://unc.zoom.us/j/91636047086

Meeting ID: 916 3604 7086

In the following pages you will find guidelines for presenting candidates, the internal call including background information on the program, and nomination materials for each internal candidate.

At the meeting, each reviewer will first briefly discuss (3-5 minutes) his or her candidate's potential for this award. Final candidate selection will follow.

If you have questions or need additional information before the meeting, please email limited_submission@unc.edu. **Thank you** in advance for your contributions to the review!

Rachelle Davenport

University Nominated Proposals
Internal Review Committee Guidelines

Thank you for taking the time to participate in this very important process. The following information is to help you both in presenting a proposal for discussion at the review meeting and in choosing the proposal(s) that best represents UNC Chapel Hill for the program under review.

Pew-Stewart Scholars for Cancer Research Program Review Guidelines:

Based on their performance during their education and training, candidates should demonstrate outstanding promise as contributors in science relevant to the field of cancer. This program does not fund clinical trials research. Strong proposals will incorporate particularly creative and pioneering approaches to basic, translational, and applied cancer research. Candidates whose work is based on biomedical principles but who bring in concepts and theories from more diverse fields are encouraged to apply.

Ideas with the potential to produce an unusually high impact are encouraged. Selection of the successful candidates will be based on a detailed description of the work that the applicant proposes to undertake, evaluations of the candidate's performance, and notable past accomplishments, including honors, awards, and publications. In evaluating the candidates, the National Advisory Committee gives considerable weight to both the project proposal and the researcher, including evidence that the candidate is a successful independent investigator and has the skill set needed to carry out their high-impact proposal.

Pew Biomedical Scholars Program Review Guidelines:

Based on their performance during their education and training, ideal candidates should demonstrate outstanding promise as contributors in science relevant to human health. This program does not fund clinical trials research. Strong proposals will incorporate particularly creative and pioneering approaches to basic, translational, and applied biomedical research. Candidates whose work is based on biomedical principles but who bring in concepts and theories from more diverse fields are encouraged to apply.

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The following factors will be taken into consideration when evaluating proposals:

WHEN PRESENTING A PROPOSAL TO THE COMMITTEE:

Please limit your remarks to 5 minutes and cover the most relevant information below:

1. Proposal's responsiveness to program criteria: How well the proposal addresses the program's stated goals? How well does the nominee meet the foundation's criteria?
2. Presentation of the proposal: Is the proposal well-written and easily understood? Does it anticipate, and address potential areas of weakness?
3. Nominee: Address the qualifications and research experience of the nominee. What is their stature in the academic community? Are they ideal for the developmental stage the foundation is looking for (early vs. tenured, etc.)? Are they well-qualified based on the criteria? Share what is most significant (based on the foundation's criteria) about the nominee's publication experience, funding history, how they have made a significant contribution to science/scholarship.
4. Impact of the proposal and potential benefit to the university: Will the proposal make a significant impact on the academic fields involved? To society? Will it advance the state-of-the-art in its field? How will the university community benefit if the proposal were funded?

Please ensure comments summarize and enhance information already distributed to review committee members.

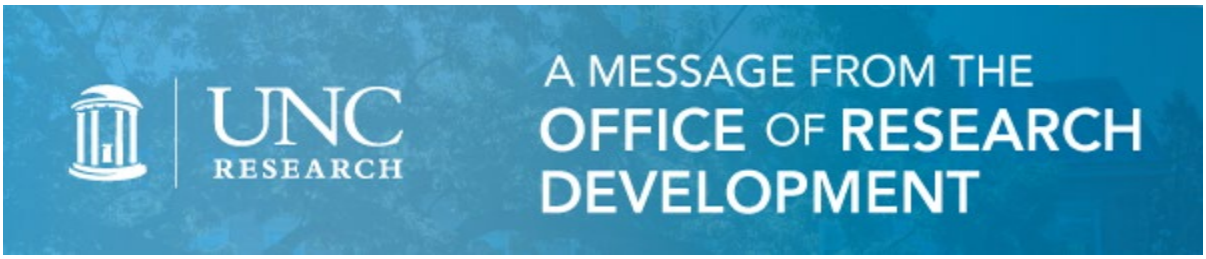
WHEN RECOMMENDING A PROPOSAL:

At this point of the review, you are being asked to represent the University in recommending the proposal(s) that will be the most competitive for the award. Please be candid in your assessment.

All comments discussed in the review are confidential with the exception of feedback.

Again, thank you for the tremendous effort you have made in participating in this review!

If you have additional comments regarding information that would be helpful to you or have questions regarding any of the above, do not hesitate to contact the Limited Submissions Team at Limited_Submissions@unc.edu.



Limited Submissions: Internal Call for Proposals

Pew-Stewart Scholars for Cancer Research 2025

UNC Internal Deadline: 11:59PM, Monday, April 1, 2024

Please distribute to relevant faculty

Key Dates

UNC Internal Deadline: 11:59pm, Monday, April 1, 2024
Institutional Nomination Deadline: May 15, 2024
Application Deadline: August 28, 2024

Important Information

Number of Applications per Institution: One nomination will be invited from the University of North Carolina's Lineberger Comprehensive Cancer Center.

Investigator Effort: It is expected that Pew-Stewart scholars will spend at least 80 percent of their time in work or activities related to the accomplishment of their overall research goals (which are not restricted to the specific aims proposed for this award). However, Pew provides flexible support to the general research aims of the Pew-Stewart scholar and does not require effort reporting.

Award Information

An award of $75,000 per year for four years will be provided to the sponsoring institution for use by the Pew-Stewart scholar, subject to an annual review of his or her progress. Grant agreements will be issued in August of the award year. The awarded funds may be used at the discretion of the Pew-Stewart scholar, for personnel, equipment, supplies, or travel directly related to the Pew-Stewart scholar's research and as to best advance his or her research and career.

- The amount of the award that may be used for the principal investigator's salary is limited to $12,500 per year (including benefits) or $50,000 over the duration of the grant. There are no limits on student or postdoctoral salaries.
- Not more than 8 percent ($24,000) of the total award value may be allocated for facilities and administration (F&A) charges or indirect costs (IDCs).

- Should the funds not be immediately required, they may be accumulated and carried over through the grant period and, with written approval of the program office, the grant may receive a no-cost extension for one additional year (without additional funds).
- Subawards are allowed.

To Apply

Submit the following as attachments via LAMSeS by 11:59PM, Monday, April 1, 2024.

- PI's full CV, including publications and research support.
- Project Summary (two-page maximum) **including statement confirming eligibility requirements (date of first Assistant Professor appointment and confirmation of independent lab).**
- Letter of nomination from department chair.
- List of three individuals to provide external letters of recommendation: the candidate's thesis advisor, postdoctoral advisor, and a scientific reference from an individual external to UNC who is not a collaborator or mentor. For each recommender, include titles, institutional affiliations, and a two-three sentence overview of their area of expertise.
- Names of three internal (to UNC) experts who could speak knowledgeably about the candidate's research and who could potentially serve on an internal review panel to evaluate nominees.
    - **Please do not include the names of faculty named on the project, chairs, deans, directors, direct reports, or others who have a conflict of interest.**
    - **Please notify all potential internal reviewers before submitting the pre-proposal packet to ORD.**

Program Overview

The Pew-Stewart Scholars Program for Cancer Research is a national initiative designed to support promising early career scientists whose research will accelerate discovery and advance progress to a cure for cancer. The program is funded by The Alexander and Margaret Stewart Trust and administered by Pew. The Stewart Trust has invested in innovative, cutting-edge cancer research and scientists for over 15 years. Through this partnership, The Pew-Stewart Scholars for Cancer Research have tremendous potential to solve some of cancer's weightiest challenges.

In line with The Alexander and Margaret Stewart Trust's mission to invest in innovative, cutting-edge cancer research that may accelerate and advance progress toward a cure for cancer, applications are invited from nominees conducting cancer research. This program is distinct from the Pew Scholars Program, and it follows a different, but parallel set of guidelines and procedures for nominating an applicant whose research is related to cancer.

Eligibility

Candidates must meet all of the following eligibility requirements:

- Affiliated with the University of North Carolina's Lineberger Comprehensive Cancer Center.
- Hold a doctorate in biomedical sciences, medicine, or a related field, including engineering or the physical sciences.

- **As of Aug. 28, 2024, run an independent lab and hold a full-time appointment at the rank of assistant professor**. (Appointments such as research assistant professor, adjunct assistant professor, assistant professor research track, visiting professor, or instructor are not eligible.)
- **Must not have been appointed as an assistant professor at any institution prior to June 10, 2020**, whether or not such an appointment was on a tenure track. Time spent in clinical internships, residencies, in work toward board certification, or on parental leave does not count as part of this four-year limit. Candidates who need an exception on the four-year limit should contact Pew's program office to ensure that application reviewers are aware an exception has been given.
    - Please note that eligibility criteria have been temporarily expanded to account for COVID-related lab shutdowns and research disruptions.
- **May apply to the program a maximum of two times.** All applicants must be nominated by their institution and must complete the 2025 online application.
- If applicants have appointments at more than one eligible nominating institution or affiliate, they may not reapply in a subsequent year from a different nominating entity.
- This program does not fund clinical trials research.
- **Funding from the NIH, other government sources, and project grants from nonprofit associations do not pose a conflict with the Pew-Stewart program.**
- **May not be nominated for the Pew Scholars Program and the Pew-Stewart Scholars Program for Cancer Research in the same year.**

Review Criteria

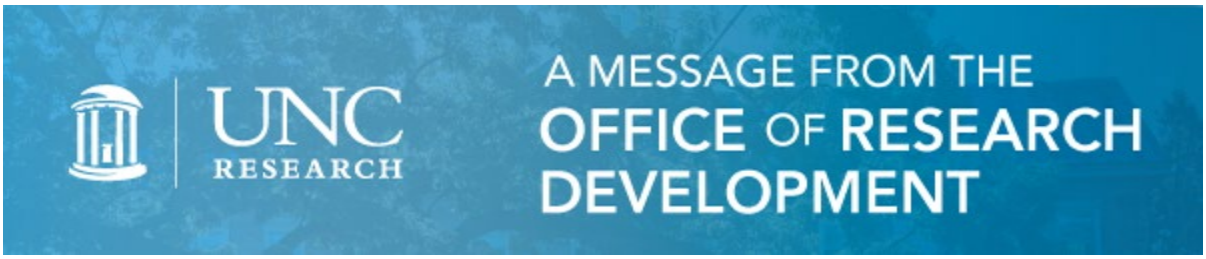
Based on their performance during their education and training, **candidates should demonstrate outstanding promise as contributors in science relevant to the field of cancer.** This program does not fund clinical trials research. Strong proposals will incorporate particularly **creative and pioneering approaches to basic, translational, and applied cancer research**. Candidates whose work is **based on biomedical principles but who bring in concepts and theories from more diverse fields are encouraged to apply.**

Ideas with the potential to produce an unusually high impact are encouraged. Selection of the successful candidates will be based on a detailed description of the work that the applicant proposes to undertake, evaluations of the candidate's performance, and notable past accomplishments, including honors, awards, and publications. In evaluating the candidates, the National Advisory Committee gives **considerable weight to both the project proposal and the researcher, including evidence that the candidate is a successful independent investigator and has the skill set needed to carry out their high-impact proposal.**

Additional Information

Pew-Stewart Scholars for Cancer Research Webpage

Pew-Stewart Scholars Recent Class: The Scholars Directory.



Limited Submissions: Internal Call for Proposals

Pew Biomedical Scholars 2025

UNC Internal Deadline: 11:59PM, Monday, April 1, 2024

Please distribute to relevant faculty

Key Dates

UNC Internal Deadline: 11:59pm, Monday, April 1, 2024
Institutional Nomination Deadline: May 15, 2024
Pew Biomedical Scholars Application Deadline: September 5, 2024

Important Information

- One nomination will be invited from each of the participating institutions.
- **Investigator effort:** It is expected that Pew scholars will spend at least 80 percent of their time in work or activities related to the accomplishment of their overall research goals (which are not restricted to the specific aims proposed for this award). However, Pew provides flexible support to the general research aims of the scholar and does not require effort reporting.

Award Information

- **An award of $75,000 per year for four years** will be provided to the sponsoring institution for use by the scholar, subject to annual review of the scholar's progress. Grant agreements will be issued in August of the award year. The awarded funds may be used at the discretion of the Pew scholar, for personnel, equipment, supplies, or travel directly related to the scholar's research and as to best advance his or her research and career.

- The amount of the award that may be used for the principal investigator's salary is limited to $12,500 per year (including benefits) or $50,000 over the duration of the grant. There are no limits on student or postdoctoral salaries.
- **Not more than 8 percent ($24,000) of the total award value may be allocated for facilities and administration (F&A) charges or indirect costs (IDCs).**
- Should the funds not be immediately required, they may be accumulated and carried over through the four years of the grant period and, with written approval of the program office, the grant may receive a no-cost extension for one additional (fifth) year (without additional funds).

- Subawards are allowed.

To Apply

Submit the following as attachments via LAMSeS by 11:59PM, Monday, April 1, 2024.

1. PI's full CV, including publications and research support.
2. Project Summary (two-page maximum) **including statement confirming eligibility requirements (date of first Assistant Professor appointment and confirmation of independent lab) .**
3. Letter of nomination from department chair.
4. List of three individuals to provide external letters of recommendation: the candidate's thesis advisor, postdoctoral advisor, and a scientific reference from an individual external to UNC who is not a collaborator or mentor. For each recommender, include titles, institutional affiliations, and a two-three sentence overview of their area of expertise.
5. Names of three internal (to UNC) experts who could speak knowledgeably about the candidate's research and who could potentially serve on an internal review panel to evaluate nominees.
    - **Please do not include the names of faculty named on the project, chairs, deans, directors, direct reports, or others who have a conflict of interest.**
    - **Please notify all potential internal reviewers before submitting the pre-proposal packet to ORD.**

Program Overview

The Pew Scholars Program in the Biomedical Sciences provides funding to young investigators of outstanding promise in science relevant to the advancement of human health. The program makes grants to selected academic institutions to support the independent research of outstanding individuals who are in their first few years of their appointment at the assistant professor level.

Based on their performance during their education and training, **ideal candidates should demonstrate outstanding promise as contributors in science relevant to human health. This program does not fund clinical trials research. Strong proposals will incorporate particularly creative and pioneering approaches to basic, translational, and applied biomedical research. Candidates whose work is based on biomedical principles but who bring in concepts and theories from more diverse fields are encouraged to apply.**

Ideas with the potential to produce an unusually high impact are encouraged. Selection of the successful candidates will be based on a detailed description of the work that the applicant proposes to undertake, evaluations of the candidate's performance, and notable past accomplishments, including honors, awards, and publications. In evaluating the candidates, the National Advisory Committee gives considerable weight to evidence that the candidate is a successful independent investigator and has the skill set needed to carry out their high-impact proposal.

Funding from the NIH, other government sources, and project grants from nonprofit associations do not pose a conflict with the Pew Scholars' program.

Eligibility

Candidates must meet all of the following eligibility requirements:

- Hold a doctorate in biomedical sciences, medicine, or a related field including engineering or the physical sciences.
- As of Sept. 5, 2024, run an independent lab and hold a full-time appointment at the rank of assistant professor. (Appointments such as research assistant professor, adjunct assistant professor, assistant professor research track, visiting professor, or instructor are not eligible).
- Must *not* have been appointed as an assistant professor at any institution prior to June 10, 2020, whether or not such an appointment was on a tenure track. Time spent in clinical internships, residencies, in work toward board certification, or on parental leave does not count as part of this four-year limit.
- May apply to the program a maximum of two times. All applicants must be nominated by their institution and must complete the 2025 online application.
- If applicants have appointments at more than one eligible nominating institution or affiliate, they may not reapply in a subsequent year from a different nominating entity.
- May not be nominated for the Pew Scholars Program and the Pew-Stewart Scholars Program for Cancer Research in the same year.

Additional Information

Pew Biomedical Scholars Program Announcement

Directory of Scholars

Prior UNC Scholars

For additional information, please contact the Limited Submissions Team with questions at Limited_Submission@unc.edu.

Internal Candidates: Pew-Biomedical Scholars Program/Pew-Stewart Scholars for Cancer Research Program

Ranking Sheet

Pew Biomedical Scholars Program

Only ONE nominee may be selected for the institution.

Nominee	Department	Nominee's Reviewer	Reviewer's Department	Ranking
Bai, Wubin	Applied Physical Sciences	Stein, Jason	Genetics	
Berlow, Rebecca	Biochemistry and Biophysics	Pielak, Gary	Chemistry	
Chen, Jiakun	Biology	Hige, Toshihide	Biology	
Kratochvil, Huong	Chemistry	Pielak, Gary	Chemistry	
McCauley, Heather	Cell Biology and Physiology	TBD	TBD	
Walsh, Jessica	Pharmacology	Stein, Jason	Genetics	
Yang, En	Biology	Philpot, Ben	Cell Biology and Physiology	

Rank your top choice, using through 7, with 1 representing the best score.

Ranking Sheet

Pew-Stewart Scholars for Cancer Research Program

Only ONE nominee may be selected for the institution.

Nominee	Department	Nominee's Reviewer	Reviewer's Department	Ranking
Brunk, Elizabeth	Pharmacology	Emanuele, Michael	Pharmacology	
Shelton, Sarah	Biomedical Engineering	TBD	TBD	

Rank your top choice, using through 2, with 1 representing the best score.

UNC | COLLEGE OF ARTS AND SCIENCES

WEI YOU
PROFESSOR AND DEPARTMENT CHAIR

wyou@unc.edu
919-962-4358

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
COLLEGE OF ARTS AND SCIENCES
Department of Chemistry

UNC | SCHOOL OF MEDICINE

HENRIK DOHLMAN
PROFESSOR AND DEPARTMENT CHAIR

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THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
SCHOOL OF MEDICINE
Department of Pharmacology

March 26, 2024

Dear Members of the Review Committee:

It gives us great pleasure to provide this letter of departmental support on behalf of Dr. Elizabeth Brunk, PhD, who is applying for the Pew-Stewart Scholars Award.

Dr Brunk is an exceptionally talented scientist, working at the interface of chemistry, genome sciences and computational sciences. She holds a doctoral degree in Computational Chemistry and Chemical Engineering. She is tenure track Assistant Professor in the Integrated Program in Biological Genome Sciences (iBGS), jointly appointed in the Departments of Chemistry and Pharmacology. Her faculty appointment started July 1, 2021. She has a dedicated laboratory (with both wet and dry lab space) and an office in the Genome Sciences Building. She has 60% protected time for research and her teaching activities take part one semester per year. In addition to salary support, Dr Brunk received a $1.1 million startup package. Her current research is focused on elucidating the biological impacts of structural variation, such as extrachromosomal DNA (ecDNA). As described in this letter, Dr Brunk's training, experience and research vision makes her an ideal fit for the Pew-Stewart Scholars Award.

Dr. Brunk, originally trained as a computational chemist, **transitioned into genomics with a deep fascination for extrachromosomal DNA (ecDNA)** and its impact on cell functioning. Intrigued by the existence of independent, autonomously replicating, protein-coding DNA molecules, Dr. Brunk started to look for clues of how frequently these DNAs occur in commonly used model systems, such as human cancer cell lines. Her computational expertise enabled her to analyze vast, population-scale multi-omics datasets to make predictions about which model systems express ecDNA and which genes are localized to ecDNA. **Despite having only computational training, Dr Brunk undertook a wet lab molecular biology postdoc**, showcasing her determination to experimentally test ecDNA hypotheses. Unfazed by the obstacles of acquiring new skills and transitioning into a new field of research, **her lab has made several significant discoveries** that underscore fundamental principles of DNA segregation during cell division (discussed in more detail below). Now leading an independent hybrid lab, blending wet and dry techniques, Dr. Brunk focuses on unraveling the functional effects of ecDNA in cells, marking her journey from computational chemist to fundamental cytogenomics researcher.

Dr. Brunk's diverse journey since her PhD and experience in the pharmaceutical industry uniquely position her as a candidate capable of tackling **interdisciplinary projects** that **span multiple fields** to provide comprehensive, **"wide-angle" insights**. Her independent work on ecDNA harnesses skills in analysis of large datasets, systems-level biology, molecular biology, and functional biology. Leveraging her computational background, she created a database for cell line models with ecDNA annotations, uncovering distinct properties and vulnerabilities in ecDNA-expressing cells (*manuscript in review*). Her molecular biology expertise aids in visualizing ecDNA and determining its structure, leading to another manuscript *(in revision)*. Currently, she studies the uneven segregation of ecDNA during cell division using a mix of experimental

techniques and computational (stochastic and physics-based) approaches, drawn from her graduate background. These specific examples showcase the **fusion of her varied skill set**, which **sets her apart in her ability to advance the field**'s understanding of ecDNA and its impact on cellular functioning.

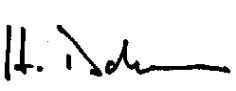
Dr. Brunk's recent discoveries in ecDNA research are already making waves. Leveraging her computational expertise, she found hundreds of cell lines with ecDNA that no one knew about before, increasing the number of model systems that express ecDNA by 200%. Dr. Brunk found that **cells with ecDNA act differently** than cells without ecDNA. They have special molecular features (e.g. activated pathways) and respond uniquely to drugs and genetic perturbations. She also found that the number of ecDNA copies per cell links with cellular RNA and protein levels, showing how **copy numbers influence cell traits** and fitness. Dr. Brunk created a clever method using Fluorescence Activated Cell Sorting (FACS) to sort cells by ecDNA levels, uncovering that **low ecDNA stops cell growth**, while **more ecDNA boosts replication and speeds up growth**. Her exciting work makes her a standout leader in this field, and has set her on a research trajectory that is abundant, diverse and open-ended.

Despite transitioning across fields and between academia and industry, Dr. Brunk demonstrates unwavering productivity and boasts a remarkable track record, reflecting her resolute commitment as a researcher. With **31 publications, 2779 citations**, and an impressive h-index of 22, she ranks in the **top 2% of junior faculty at her career level**. Currently preparing her lab's 5th manuscript, Dr. Brunk leads her lab with determination. Amid hiring freezes, equipment shortages, and operational constraints over the 2.5 years of her experience as a junior faculty, her research has flourished: she is sole senior and corresponding author on 4 manuscripts under review, highlighting her exceptional achievements in a relatively short time frame. In addition to her research productivity, she has established **a vibrant, full lab of 12 people**, including 4 graduate and 5 undergraduate students. Two undergrads are conducting an honors thesis in her lab and 5 have been involved as first authors or contributing authors in her lab's recent manuscript submissions. **Two grad students have submitted first author publications** for peer review, and her first graduate student is scheduled for a thesis defense in 2025. Her research has received several awards and recognitions, including UNC IDEA award, IBM Junior Faculty Development Award and the Computational Medicine Pilot Award.

Dr Brunk not only conducts research but also teaches an **undergraduate biochemistry course** for the College of Arts and Sciences. In 2022, she transformed the class by incorporating data exploration exercises, introducing students to Python programming, omics data, and biological data science. To support this initiative, she secured $70K in seed funding for the development of computational resources. Professor Brunk developed python-based Jupyter notebook tutorials that guide interactive learning in the analysis of protein crystal structures, genomics, transcriptomics, metabolomics, and proteomics data. **Through this course, Dr Brunk has introduced more than 1,500 undergraduate students (mostly non-computer science majors) to Python and biological data exploration.** Her curriculum changes have already demonstrated significant impacts, documented in her senior author publication on *chemrxiv* and in a manuscript pending review in *ACS Chemical Education*.

In summary, the Departments of Chemistry and Pharmacology at the University of North Carolina Chapel Hill strongly support Professor Brunk as a candidate for the Pew-Stewart Scholars Award. Please do not hesitate to contact us should you have further questions,

Sincerely,



In the complex world of cancer, some genes get extra copies, a phenomenon known as DNA amplification. These extra gene copies can act like fuel, driving the growth of cancer cells. Importantly, they play a crucial role in how patients respond to treatments and influence their overall prognosis, essentially predicting the course of their disease. For certain genes, like MYC and ERBB2, amplifications signal more aggressive cancers with a less favorable outcome, impacting the patient's survival. Treatments have been designed to precisely target the products of these extra gene copies, taking advantage of cancer cells' dependence on the amplified genes. Such targeted therapies can prove highly effective when cancer cells lack ways to quickly adapt their systems. However, in 15% of all cancers, cells are equipped with a *different* kind of DNA that confers new capabilities, enabling cancer cells to fast-track adaptation. This different DNA, called extrachromosomal DNA (ecDNA; *Figure 1A*), amplifies DNA on flexible, mobile elements that change the way DNA is divided and manipulated in response to drug treatment. In contrast to normal human chromosomes, where DNA divides evenly into daughter cells, ecDNAs divide unevenly, leaving one cell with different amounts than the other[1–4]. Cells with ecDNAs also manipulate DNA in novel ways to evade drug treatment; cells rapidly eliminate[5–7], multimerize or "hide" ecDNAs inside chromosomes, temporarily until drug treatment is ended[7] (*Figure 1B-1C)*.

These unique division and drug response capabilities provide cancer cells with a powerful toolkit for adaptation[5,8], preservation and drug evasion[7,9]. Currently, we face a formidable challenge, as we lack effective strategies to counteract this toolkit because we don't have a clear understanding of how these ecDNA-mediated adaptation mechanisms work. In my lab, we focus on addressing this critical gap by systematically and globally studying how cells change their ecDNAs in response to drugs. This past year, we have started to create a large-scale cytogenetic atlas that profiles the dynamic responses of ecDNAs across cell lines for different small molecule inhibitors. This atlas includes thousands of Fluorescence in situ Hybridization (FISH) images that allow us to look inside a single cell's nucleus and study individual ecDNA molecules. Analysis of thousands of FISH images requires automation in the form of Artificial Intelligence based algorithms, which can detect, count and characterize each ecDNA molecule during drug treatment. Our preliminary imaging data confirms a large dynamic range of responses in eight different established cancer cell lines. In addition, we collect other data, such as qPCR and real-time growth monitoring assays, which provides strong evidence that the changes we see in ecDNAs during drug treatment go hand in hand with changes in molecular pathways and cell functioning. This atlas will serve as a starting point for understanding the mechanisms underlying ecDNA-mediated drug evasion and drug resistance. In the next four years, my lab will expand this atlas to include 25 additional cell lines and integrate novel live imaging data that can help us to understand *how* ecDNAs reintegrate into chromosomes. This rich data collection and detailed analysis will enhance our understanding of cancer biology and pave the way for the development of targeted therapies, capable of disrupting the cancer cells' adaptive toolkit and overcoming ecDNA-mediated drug resistance. We will focus on two main project areas:

Project 1: Quantify Changes in ecDNA using Artificial Intelligence

Artificial intelligence approaches, like convolutional neural networks, increase the throughput and accuracy of ecDNA detection[10]. We will build models to identify ecDNA and chromosomes in our cytogenetic data (*Fig. 1D*).

Project 2: Create an Atlas of ecDNA-Mediated Drug Response Mechanisms

Understanding the role of ecDNA in drug resistance requires global profiling across cell lines and drugs. We will create a rich dataset that systematically identifies response patterns in cells with ecDNA.

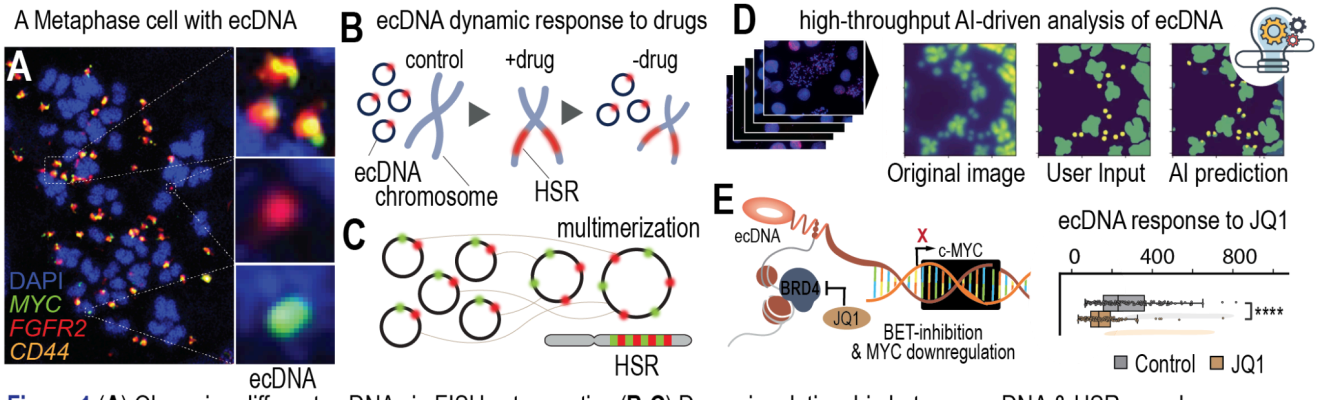


Figure 1 (**A**) Observing different ecDNA via FISH cytogenetics (**B-C**) Dynamic relationship between ecDNA & HSRs on chromosomes during drug treatment. (**D**) AI algorithm to predict ecDNA and HSRs. (**E**) JQ1 drug downregulates MYC and ecDNAs are eliminated.

SUITABILITY FOR AWARD As an assistant professor (since July 2021) with an independent research lab, I am eligible to receive this award. Research described in this proposal is highly suited for the Pew-Stewart Scholars award for several reasons. First, it will foster the development of a new research project and collaboration between my lab, the Niethammer lab RENCI, and AI@UNC, an undergraduate-led computer science machine learning club. This research integrates computational approaches, such as computer vision and neural networks, to enhance biomedical cancer research and pharmacology by establishing automated methods for detecting highly aberrant oncogene amplifications. Second, it will enable my lab to generate sufficient preliminary data required to submit my first extramural grant. The preliminary data generated in this project includes a novel algorithm for automating the counting of ecDNA as well as a large-scale cytogenetic imaging dataset that will be complete in one year.

RATIONALE, PRELIMINARY DATA, METHODOLOGY, AND APPROACH

Over the past year, my lab has generated a global cytogenetics dataset (8 cancer cell lines, 4 treatment conditions, two time points) to characterize the dynamic changes in ecDNAs during drug treatment. The main challenge has been scaling up our analysis of hundreds to thousands of cytogenetic images. Using Fluorescence in situ Hybridization (FISH), we image cells arrested in metaphase to precisely count each ecDNA molecule within the nucleus of a single cell. Each treatment condition requires 150-200 different images of single cell nuclei, which will produce over 6,000 total FISH images for this preliminary dataset. I am applying to the Pew-Stewart Scholars Award to help fund research that will develop a computer vision algorithm that we will apply to our developing drug profiling dataset to accurately count ecDNAs in my lab's thousands of FISH images. Development of an automated counting method will enable my group to process thousands of images in a fraction of the time that has been spent manually counting. For example, manual counting of 200 images takes one person an entire week for cell lines with high amounts of ecDNA. I expect that this algorithm will enable the future development of a high-throughput platform to systematically track dynamic changes in ecDNA during the evolution of drug resistance.

This project fosters a new collaboration between the Brunk lab, RENCI, an undergraduate-led machine learning club, AI@UNC, and the Niethammer lab. Together, we are developing computational tools using convolutional neural networks that are trained to detect ecDNA in images of cell nuclei. Our focus begins with studying dynamic changes during drug treatments, starting with a BET inhibitor, JQ1, known for ecDNA elimination, multimerization, and reintegration into chromosomes (*Fig. 1E*). JQ1 has been shown to disrupt not only transcription of key oncogenes on ecDNA (e.g. MYC) but also to break apart ecDNA hubs[11], which form when multiple ecDNAs cluster together. These hubs co-recruit transcriptional machinery, serving as superior enhancers for oncogene expression[11]. Notably, the presence of MYC on ecDNA is observed in numerous cancer cell lines. This allows for a comprehensive evaluation of cancer cell responses to the drug JQ1 in relation to ecDNA. Using real-time-continuous growth monitoring, we have derived IC50s at 24, 48 and 72 hours for eight different cancer cell lines. For each cell line, we have collected RNA data and FISH data in different drug treatment conditions.

Over the past two years, my lab has generated over 3,800 high resolution FISH images, of which over 2,000 have been manually annotated. We will use these annotations as training data to develop our counting algorithm. Using these data, we will implement supervised learning using a training dataset that consists of manually derived coordinates of all ecDNAs. We will test several approaches, including resampling, using SMOTE (Synthetic Minority Over-sampling Technique), Gradient Boosting, customized loss functions, and One-Class Classification.

STATISTICAL ANALYSIS PLAN To assure the rigor and robustness of our assays and computational methods, the experiments are blinded and performed in biological and technical replicates. When possible, we verify the results using orthogonal assays. All statistical results are carefully scrutinized by multiple experts for reliability and accuracy. Specifically, we work with the UNC Department of Biostatistics, which provides free consulting and statistical modeling.

STATEMENT OF RELEVANCE DNA amplifications are relevant and pervasive in most cancer types. Whether these amplifications occur in chromosomes or on extrachromosomal DNA dramatically changes how patients respond to therapies and could determine prognosis and treatment course of action. Understanding how cells modulate ecDNAs during drug treatment would address an urgent, unmet need, offering critical insights into how cells manipulate ecDNAs to evade drug therapy. Understanding the mechanisms underlying this drug evasion will present new opportunities for targeted treatments for ecDNA-driven cancers (e.g. 14% of all cancers[12]).

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6 Von Hoff DD, McGill JR, Forseth BJ, Davidson KK, Bradley TP, Van Devanter DR, *et al.* Elimination of extrachromosomally amplified MYC genes from human tumor cells reduces their tumorigenicity. *Proc Natl Acad Sci U S A* 1992;**89**:8165–9.

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11 Hung KL, Yost KE, Xie L, Shi Q, Helmsauer K, Luebeck J, *et al.* ecDNA hubs drive cooperative intermolecular oncogene expression. *Nature* 2021;**600**:731–6.

12 Kim H, Nguyen N-P, Turner K, Wu S, Gujar AD, Luebeck J, *et al.* Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers. *Nat Genet* 2020;**52**:891–7.

Three potential external letters writers:

Ivet Bahar (Distinguished Professor, Louis and Beatrice Laufer Chair and Director, Stony Brook University)

Before joining Stony Brook University, she served as Distinguished Professor, John K. Vries Chair and Founder of the Department of Computational and Systems Biology at the University of Pittsburgh School of Medicine. Dr. Bahar adapted fundamental theories and methods of polymer statistical mechanics to biomolecular structure and dynamics. She pioneered a modified version of the classical Rouse model, to examine the collective dynamics of proteins modeled as elastic network models (ENMs). She is an elected member of the European Molecular Biology Organization (EMBO) since 2000. She was elected to the National Academy of Sciences in 2020 and the Biophysical Society.

Bernhard Palsson (Distinguished Professor, University of California, San Diego)

Bernhard Palsson is the Galetti Professor of Bioengineering and the Principal Investigator of the Systems Biology Research Group in the Department of Bioengineering at the University of California, San Diego. Dr. Palsson has co-authored more than 340 peer-reviewed research articles and has authored three textbooks, with one more in preparation. His research includes the development of methods to analyze metabolic dynamics (flux-balance analysis, and modal analysis), and the formulation of complete models of selected cells (the red blood cell, E. coli, hybridoma, and several human pathogens). Professor Palsson is Professor Brunk's postdoc supervisor.

Phil Bourne (Distinguished Professor and Dean, University of Virginia)

Philip E. Bourne leads a range of initiatives to encourage and facilitate the use of big data in large-scale research across the scientific and technological disciplines, with special emphasis on structural bioinformatics and systems pharmacology. He is the Founding Dean of the School of Data Science and Professor of Biomedical Engineering. From 2014-2017, Phil was the Associate Director for Data Science at the National Institutes of Health. In this role he led the Big Data to Knowledge Program, coordinating access to and analyzing biomedical research from across the globe and making it available to scientists and researchers. Professor Bourne is not a mentor or a collaborator.

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EDUCATION

Doctorate of Philosophy in Chemistry, Institute of Chemical Sciences and Engineering,
École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; August 2013

Masters of Science in Chemistry, École Normale Supérieure, Lyon, France; July 2008

Bachelor of Science in Biochemistry,
University of Michigan, Ann Arbor, Michigan; June 2007

PROFESSIONAL EXPERIENCE

July 2021– Present	**Assistant Professor**, **Department of Chemistry**, College of Arts and Sciences, University of North Carolina at Chapel Hill **Assistant Professor**, **Department of Pharmacology**, School of Medicine, University of North Carolina at Chapel Hill **Assistant Professor**, **Integrative Program for Biological and Genome Sciences**, School of Medicine, University of North Carolina at Chapel Hill **Member,** Lineberger Comprehensive Cancer Center, Computational Medicine Program, Carolina Center for Interdisciplinary Applied Mathematics
Sept. 2019 – Present	**Adjunct Teaching Faculty**, Altman Translational Clinical Research Institute, University of California San Diego
Aug. 2020 – April 2021	**Research Data Analyst,** laboratory of Pablo Tamayo, Division of Genomics and Precision Medicine, Moores Cancer Center, University of California San Diego
Aug. 2018 – July 2020	**Postdoctoral Fellow,** laboratories of Paul Mischel and Pablo Tamayo, Division of Genomics and Precision Medicine, Moores Cancer Center, University of California, San Diego. Computational genomics and extrachromosomal DNA (ecDNA)
Sept. 2016 – Aug. 2018	**Industry Consultant,** Celgene (now Bristol Myers Squibb), San Diego. Computational genomics and proteomics of protein degradation for drug discovery.
Sept. 2015 – Aug. 2016	**Industry Consultant,** Center for Biosustainability, Coppenhagen, Denmark. Molecular Dynamics simulations of novel protein designs for drug targeting.

May 2014 – May 2016 **Postdoctoral Fellow,** laboratory of Bernhard Palsson, Department of Bioengineering, University of California San Diego. Systems biology of genome variation.

Nov. 2013 – May. 2014 **Visiting Scholar,** laboratory of Jay Keasling, Joint Bioenergy Institute (JBEI), University of California, Berkeley. Systems biology of engineered *E. coli.*

Aug. 2008 – Aug. 2013 **Graduate Student** laboratory of Ursula Roethlisberger, Institute of Chemical Sciences and Engineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland. Computational biology and QM/MM Molecular Dynamics simulations of enzyme catalytic mechanisms. Dissertation Title: "Understanding Evolutionary Mechanisms of Enzymes and Exploiting Them For Biomimetic Purposes."

SELECT AWARDS AND HONORS

Computational Medicine Pilot Award, UNC Chapel Hill, Dec. 2023–Dec 2024

IBM Junior Faculty Development Award, Office of the Provost, UNC Chapel Hill, Jan. 2023–Jan. 2024

IDEA Award, Office of the Vice Chancellor of Research, UNC Chapel Hill, Aug. 2021

NIH Kirschstein-NRSA F32 Postdoctoral Fellowship, University of California San Diego, 2018–2020

Swiss National Science Foundation Early Postdoc Mobility Award, University of California Berkeley, 2013–2015

Swiss Academy of Sciences Chemistry Travel Award, 2013

National Research Foundation of Singapore, Global Young Scientist Summit Award, 2013

Erasmus Mundus AtoSim Masters Fellowship, École Normale Supérieure, Lyon, France, 2007–2008

SELECT UNIVERSITY SERVICE

Faculty Mentor for EDGE Genomics Undergraduate Research Program, 2024
Faculty Mentor for SUROC Undergraduate Research Program, 2024
Diversity Equity and Inclusion Committee, UNC Chemistry Department, 2023–Present

Faculty Mentor for Carolina Summer Fellows Program, UNC Chapel Hill, 2022–Present

Faculty Mentor for WinSPIRE Program, UNC Chapel Hill, 2022

Faculty Search Committees, UNC Chapel Hill: Department of Pharmacology, Department of Genetics, Computational Medicine Program, 2021–2023

Graduate Student Recruiting Committee, UNC Chemistry Department, 2021–2022

SELECT PROFESSIONAL SOCIETIES AND ACTIVITIES

ACS Conference Presider, SERMACS, 2023, ACS National conference, 2011

Ad Hoc Grant Reviewer: Congressionally Directed Medical Research Programs (CDMRP) Breast Cancer Research Program (BCRP), Department of Defense, 2022

Faculty Mentor for Carolina Summer Fellows Program, UNC Chapel Hill, 2022–Present

Member, American Chemical Society (since 2010), AACR (since 2022)

Ad Hoc Reviewer: *Science, Nature, Cell, Cell Reports Methods, Nature Methods, Nature Communications, Bioinformatics, Biotechnology and Bioengineering , Plos Computational Biology, Antibody Therap.*

Advisor to the Program Director of the Translational Science Certificate, Altman Clinical and Translational Research Institute, University of California San Diego, 2019

Scientific Consultant, Celgene, 2016–2018; Novo Nordisk and the Center for Biosustainability, 2015–2016

PUBLICATIONS

1 **Brunk E**, Ashari N, Athri P, Campomanes P, de Carvalho FF, Curchod BF, Diamantis P, Doemer M, Garrec J, Laktionov A, Micciarelli M, Neri M, Palermo G, Penfold TJ, Vanni S, Tavernelli I, Rothlisberger U. Pushing the Frontiers of First-Principles Based Computer Simulations of Chemical and Biological Systems. *Chimia (Aarau)*. 2011;65(9):667-71.

2 **Brunk E**, Neri M, Tavernelli I, Hatzimanikatis V, Rothlisberger U.
Integrating computational methods to retrofit enzymes to synthetic pathways.
Biotechnol Bioeng. 2012 Feb;109(2):572-82.

3 **Brunk, E**; Arey, JS; Rothlisberger, U.
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Directed Evolution of the Suicide Protein O-6-Alkylguanine-DNA Alkyltransferase for Increased Reactivity Results in an Alkylated Protein with Exceptional Stability.
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5 **Brunk, E**; Mollwitz, B; Rothlisberger, U.
Mechanism for Triggering Unfolding in O6-Alkylguanine DNA Alkyltransferase.
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Computational, Structural and Kinetic Evidence that Vibrio vulnicus FrsA is not a Cofactor- Independent Pyruvate Decarboxylase.
Biochemistry. 2013 Mar 19;52(11):1842-4.

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Biochemistry. 2014 Jun 17;53(23):3830-8.

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Lessons from Nature: Computational Design of Biomimetic Compounds and Processes.
Chimia (Aarau). 2014 Sep;68(9):642-7.

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Proc Natl Acad Sci U S A. 2015 Jan 20;112(3):929-34.

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Model-driven discovery of synergistic inhibitors against E. coli and S. enterica serovar Typhimurium targeting a novel synthetic lethal pair, aldA and prpC.
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11 Latif H, Szubin R, Tan J, **Brunk E**, Lechner A, Zengler K, Palsson BO.
A streamlined ribosome profiling protocol for the characterization of microorganisms.
Biotechniques. 2015 Jun;58(6):329-32.

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Biophys J. 2015 Apr 7;108(7):1727-1738.

13 **Brunk E**, Rothlisberger U.
Mixed Quantum Mechanical/Molecular Mechanical Molecular Dynamics Simulations of Biological Systems in Ground and Electronically Excited States.
Chemical Reviews. 2015 Jun 24;115(12):6217-63.

14 Lechner A, **Brunk E**, Keasling JD.
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15 **Brunk E**, Mih N, Monk J, Zhang Z, O'Brien EJ, Bliven SE, Chen K, Chang RL, Bourne PE, Palsson BO.
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16 **Brunk E*,** George KW*, Alonso-Gutierrez J, Thompson M, Baidoo E, Wang G, Petzold CJ, McCloskey D, Monk J, Yang L, O'Brien EJ, Batth TS, Martin HG, Feist A, Adams PD, Keasling JD, Palsson BO, Lee TS.
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23 **Brunk E**, Chang RL, Xia J, Hefzi H, Yurkovich JT, Kim D, Buckmiller E, Wang HH, Cho BK, Yang C, Palsson BO, Church GM, Lewis NE
Characterizing post-translational modifications in prokaryote metabolism using a multi-scale workflow.
Proc Natl Acad Sci U S A. 2018 Oct 23;115(43):11096-11101.

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Nature Biotechnology. 2018 Mar;36(3):272-281.

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Bioinformatics. 2018 Jun 15;34(12):2155-2157.

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in Escherichia coli strains lacking the Phosphotransferase System.
Metabolic Engineering. 2018 Jul;48:233-242.

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Nature Communications. 2018 Sep 18;9(1):3796.

30 McCloskey D, Xu S, Sandberg TE, Brunk E, Hefner Y, Szubin R, Feist AM, Palsson BO
Multiple Optimal Phenotypes Overcome Redox and Glycolytic Intermediate Metabolite Imbalances in Escherichia coli pgi Knockout Evolutions.
Appl Environ Microbiol. 2018 Oct 1;84(19)

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NAD metabolic dependency in cancer is shaped by gene amplification and enhancer remodeling.
Nature. 2019 May;569(7757):570-575.

32 Browning, NJ; Perez, MAS; **Brunk, E**; Rothlisberger, U. EVOLVE:
An Evolutionary Toolbox for the Design of Peptides and Proteins.
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Advances in the Management of Early-Stage Triple-Negative Breast Cancer
International Journal of Molecular Sciences. 2023 24 (15), 12478

34 Shukla K, Idanwekhai K, Naradikian M, Ting S, Schoenberger SP, **Brunk E**
Machine learning of three-dimensional protein structures to predict the functional impacts of genome variation.
ACS J. Chem. Inf and Mod. 2024 (Just accepted)
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CytoCellDB: A Resource Database For Classification and Analysis of Extrachromosomal DNA in Cancer
NAR Cancer (*in review*) 2024
biorxiv [Preprint]. Available from doi.org/10.1101/2023.12.18.572197

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A Standardized Protocol for Sample Preparation for Scanning Electron Microscopy (SEM) to Visualize Extrachromosomal DNA (ecDNA).
Biotechniques (*minor revisions*) 2024
biorxiv [Preprint]. Available from doi.org/10.1101/2023.08.24.554665

37 Brunk, R, Shukla, K, Hutson, B, Wang, Y, Verber, M, Gutierrez Ford, C, Dennis, W, Mehta, A, Hogan, B, Swetnam, T, **Brunk, E**.
Data science for Chemists: Integrating and evaluating the use of interactive digital python notebooks in a large enrollment undergraduate biochemistry course
ACS Chem Education (*in review*) 2024
biorxiv [Preprint]. Available at doi.org/10.26434/chemrxiv-2023-qqcs9

PATENTS ISSUED

1 Voldborg, B; Kol, S; Arnsdorf, J; Joseph, S; **Brunk E**.
Neurotrophic factor protein conjugates and related embodiments.
US Patent 17601061 2022

FUNDING

Current

Using Convolutional Neural Networks to Detect Extrachromosomal DNA and Track its Dynamics during Drug Treatment
PI: Elizabeth Brunk
0% Effort; 1/1/2024–1/1/2025
Agency: *UNC Computational Medicine;* Type: Pilot Award
Total Direct to Brunk: $50,000

Development of Computational Workflows for Students
PI: Elizabeth Brunk
0% Effort; 8/1/2021–8/1/2024
Agency: *Department of Pharmacology, Integrated Program in Biological Genome Sciences, RENCI, Computational Medicine Program*
Type: Internal Funding
Total Direct to Brunk: $65,000

UNC Startup Funds
PI: Elizabeth Brunk
7/1/2021–7/1/2026
Agency: *UNC Chapel Hill;* Type: Start-up funding
Total Direct to Brunk: $1.1M

Completed

Classifying Extrachromosomal Dna Presence Across Cancer Cell Lines
PI: Elizabeth Brunk
0% Effort; 1/1/2023–1/1/2024
Agency: *IBM Junior Faculty Development Award;* Type: Development Award
Total Direct to Brunk: $10,000

Development of Computational Workflows for Students
PI: Elizabeth Brunk
0% Effort; 8/01/2021–12/31/2021
Agency: *Office of the Provost*, University of North Carolina at Chapel Hill
Type: Internal Funding
Total Direct to Brunk: $5,000

INVITED ACADEMIC LECTURES

1 “Modeling at the intersection of structural and systems biology”, Gene Variation in 3D Workshop, University of Washington, Seattle, 2017

2 “Computational chemistry and machine learning to leverage systems biology”, SAGIM & Vertex Pharmaceuticals, University of California San Diego, 2018

3 “Integrating omics data using systems biology, machine learning and constraint based modeling”, Integrative Omics, NCRG & NM-INBRE, University of New Mexico, 2019

4 "Advanced Analytics, Big Omics Data and Visualization", Bioengineering Grad Student Practicum, Northwestern University, 2019

5 "Big Data in 3 Dimensions: Integrating omics data with systems and structural biology", Department of Molecular & Computational Biology, University of Southern California, 2019

6 "Big Data in 3 Dimensions: Integrating omics data with systems and structural biology", Department of Biostatistics, University of Colorado, Anschutz Medical Campus, 2020

7 "Big Data in 3 Dimensions: Integrating omics data with systems and structural biology", Department of Pharmacology, University of North Carolina at Chapel Hill, 2020

8 "Big Data in 3 Dimensions: Integrating omics data with systems and structural biology", Department of Bioinformatics and Computational Biology, MD Anderson, 2020

9 "Elucidating Mechanisms of Uneven DNA Segregation and its Impact on Cellular Processes", Department of Mathematics, North Carolina State University, 2024

INVITED CONFERENCE LECTURES

1 "Elucidating functions of bacterial post-translational modifications through genome editing, pathway modeling, and molecular dynamics", Zing Conference on Computational Chemical Biology, Cairns, Australia, 2015

2 "Machine learning to leverage Omics data integration and Systems Biology approaches", Protein Engineering Global Summit (PEGS), Boston, 2019

3 "Systematic Characterization of Functionally-Relevant Mutational Landscapes", ASPET, Cancer Systems Pharmacology Panel, 2021

4 *TBD*, ACS 2024, Data Analytics and AI for Manufacturing and Healthcare, Denver Colorado, 2024

INVITED INDUSTRY LECTURES

1 "Modeling at the intersection of molecular and systems biology in the era of big data", Celgene (now Bristol Myers Squibb), San Diego, 2016

2 "Hacking Multi-omic data integration with Machine Learning", MLxBio4 Machine Learning and Biology Conference, San Francisco, 2019

INVITED GOVERNMENT LECTURES

1 Systematic Characterization of Functionally Relevant Mutational Landscapes, National Cancer Institute's Multidisciplinary Approaches to Understand Cancer Treatment Resistance, 2020

UNIVERSITY TEACHING

CHEM 430, "Introduction to Biological Chemistry." 3 hours. Introduction to biochemistry, large-enrollment (all majors) undergraduate course. Implemented Flipped Classroom Approach and designed curriculum to include 4 python-based data explorations that accompanied lecture materials. Students were provided access to University of Arizona's Cyverse supercomputer to perform analyses and were tested via concept check quizzes. I also designed literature review activities for students to gain hands-on-experience with scientific paper comprehension.

Fall 2021: 165 students
Fall 2022: 140 students
Fall 2023: 85 students

CHEM 731, "Seminars in Biological Chemistry." 2 hours. Weekly seminars on biological chemistry from national and international speakers.

Fall 2022: 20 students

PHCO 731, "Introduction to Molecular Pharmacology." 2 hours. Weekly seminars on biological chemistry from various UNC faculty in the Pharmacology Department.

Fall 2022: 20 students

BCB 899, "Student Seminar Series." 1 hour. Weekly seminars on bioinformatics and computational biology from 2nd and 3rd year graduate students.

Spring 2023: 20 students

GRADUATE STUDENTS MENTORED

1 **Yue Wang.** Department of Pharmacology. June 2021– Present. Expected graduation: December 2024

2 **Jingting Chen.** Department of Biochemistry and Biophysics. June 2022– Present. Expected graduation: June 2027

3 **Kriti Shukla.** Department of Chemistry. Nov 2022– Present. Expected graduation: June 2027

HONORS UNDERGRADUATE THESES MENTORED

1 **Kohen Goble.** Chemistry. Spring 2024

2 **Danielle Cannon.** Biology. Spring 2024

UNDERGRADUATE STUDENTS MENTORED

1 **Jasmine Jehad.** B.S. in Biology, Minor in Chemistry. Dec. 2022–June 2023. *Attending Dental School*

2 **Kohen Goble.** B.S. in Chemistry with Honors. Mar. 2023–June 2024. *Accepted to NCSU graduate school*

3 **William Dennis.** B.S. in Chemistry. Sept. 2023–June 2024. *Plans to apply to Medical School*

4 **Danielle Cannon.** B.S. in Biology. Sept. 2023–June 2024. *Applying to Medical School*

5 **Nithya Gurumurthy.** B.S. in Nutrition. Sept. 2023–June 2024. *Applying to Medical School*

6 **Jacob Fessler.** B.S. in Computer Science. May. 2023–June 2024. *Graduating 2025*

7 **Aarav Mehta.** B.S. in Computer Science. June. 2023–June 2024. *Graduating 2025*

PI: Shelton, Sarah (Pages 27-38)

Joint Department of
BIOMEDICAL ENGINEERING

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March 29, 2024

To the Pew-Stewart Cancer Research Scholars internal selection committee:

On behalf of the Joint Department of Biomedical Engineering at the University of North Carolina at Chapel Hill and North Carolina State University, it is with great enthusiasm that I support Dr. Sarah Shelton's nomination for the Pew-Stewart Cancer Research Scholars program. Sarah meets all the eligibility criteria for this award; she began her independent research lab on August 16, 2023 when she was appointed as an Assistant Professor, and she joined the UNC Lineberger Comprehensive Cancer Center shortly after.

Sarah has been associated with the Joint BME department since she began her Ph.D. in 2012 under my supervision. Sarah was one of the top students in our graduate program and proved herself as an exceptional scientist during her time in my group. Her thesis was focused on understanding the process of angiogenesis and how it is related to tumor growth, and more specifically, what biomarkers associated with tumor angiogenesis we can detect non-invasively with imaging. Sarah used a new microvascular imaging tool, acoustic angiography, to observe the development of tumor neovasculature, with the intent of using this knowledge to improve specificity and sensitivity in ultrasound cancer imaging. Beyond this primary project, Sarah developed and led a collaboration between our group and Professor Drew Dudley. Drew's lab had uncovered a subpopulation of melanoma cells with vascular-like properties. The question was whether these vascular-like melanoma cells would coalesce with host blood vessels and form more-perfused tumors relative to their non-vascular-like counterparts, and Sarah independently designed studies showing that there was indeed a substantial difference in perfusion between these two different subpopulations. Sarah's impactful research led to her publication of seven papers as first author and five additional papers as co-author during her 5 years of Ph.D. studies. Her work was part of a UNC press release and recognized as one of the top stories on the UNC Lineberger Comprehensive Cancer Center website. Sarah's abstracts were selected multiple years in a row for oral presentation at the IEEE Ultrasonics Symposium, one of the top meetings in the ultrasound imaging field, where less than 20% of submitted abstracts are accepted for podium presentation. Her thesis work was the foundation of a successful F99/K00 Predoctoral to Postdoctoral Transition Award from the NCI that supported her postdoctoral work as well. Before becoming the first UNC recipient of the F99/K00 award, Sarah was also a trainee on an institutional T32 training grant, the "Integrative Vascular Biology Training Program" and was awarded the best oral presentation at their yearly symposium in 2014.

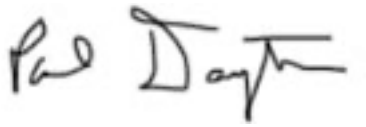
For her postdoctoral studies, Sarah joined Dr. Roger Kamm at Massachusetts Institute of Technology and Dr. David Barbie at the Dana-Farber Cancer Institute. She recognized the opportunity to combine the translational patient-oriented approach of DFCI with the world class bioengineering capabilities at MIT. She immediately made a key contribution to one study developing vascularized models of KRAS mutant lung cancer, demonstrating that the cGAS-STING pathway plays an important role in priming the tumor vasculature, a highly collaborative project. Sarah developed and performed the critical assays proving that transfer of the second messenger 2'3'-cGAMP, in addition to type I IFN, induces vascular permeability, upregulates T cell adhesion molecules, and functionally induces T cell adhesion. She also continued her relationship with the Dudley lab (now at UVA) and performed T cell adhesion assays in vascular microfluidics to support their manuscript describing epigenetic control of endothelial activation through the DNMT1 pathway. Sarah received impressively positive

feedback from faculty at conferences in which she presented her postdoctoral work and earned awards at the North American Vascular Biology Organization conference, at a Cancer Tissue Engineering Consortium meeting (NCI), as well as a "Rising Star" award at MIT. Since then, Sarah has taken on the most challenging research direction, which is developing fully vascularized, autologous patient-derived models. It is rare to have a scientist with her engineering expertise who is working with primary human tumor specimens. Given the emerging importance of cell therapies and the understudied role of tumor vasculature as a key barrier to their efficacy, the biology she is uncovering along with her innovative model systems will have a major impact.

In 2023, we recruited Sarah back to the Joint BME Department as an Assistant Professor. She joined the "Translational Predictive Biology" faculty excellence cluster to work across multiple departments and colleges on multidisciplinary studies to improve the predictive capabilities of preclinical research. Her innovative organ-on-chip research enables novel hypothesis testing for both basic science and translational objectives. Sarah uses engineering tools including microfluidics and imaging to dissect the tumor microenvironment for biomarker discovery and to predict and enhance immunotherapy efficacy. Her work designing functional, 3D, *in vitro* vascular networks and using high resolution functional imaging to assess endothelial phenotypes associated with therapeutic response is both highly innovative and likely to provide substantial knowledge to the field. The combination of tightly controlled microfluidic models with live imaging, patient tissue sources, and validation in animal models will result in highly innovative and translational discoveries. Furthermore, her mission to use these methods to examine biological mechanisms of disparities in cancer survival will increase equity and representation in the scientific literature through the use precision medicine tools to examine ways in which genetic sex and ancestry drive pathology and treatment response.

In summary, the Joint Department of Biomedical Engineering and I strongly support Sarah's nomination for the Pew-Stewart Cancer Research Scholars opportunity. She excelled without question during her Ph.D. studies in my group, her next steps into postdoctoral work in Roger Kamm's lab at MIT and Dave Barbie's lab at DFCI were made with equal achievement, and now she is pioneering novel organ-on-chip oncology research in her own lab. She is exactly the kind of scientist that will make a significant impact in the biomedical field.

Sincerely,



Paul Dayton, PhD, FAIMBE, FIEEE
William R. Kenan, Jr. Professor and Chair
Joint Department of Biomedical Engineering
University of North Carolina at Chapel Hill
North Carolina State University
Professor, UNC Eshelman School of Pharmacy
Adjunct Professor, UNC Department of Radiology
9018A Mary Ellen Jones Building, CB 7575
Chapel Hill, NC, 27599
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UNC LCCC Internal Nomination Proposal for Sarah Shelton, Ph.D.
Pew-Stewart Cancer Research Scholars

I've always been fascinated by uncovering what drives complex systems. Early in my training, I was an environmental engineer studying climate change, ecosystems, and adapting a mathematical framework developed for tracing groundwater contaminants to study nutrient transport through tumors. I then joined the UNC-NC State Joint Department of Biomedical Engineering for my Ph.D. to develop novel vascular imaging techniques. I was the LCCC's sole nominee for a Predoctoral to Postdoctoral Transition Award from the NCI, which I was granted in 2016. I then joined Roger Kamm at Massachusetts Institute of Technology and David Barbie at Dana-Farber Cancer Institute for my postdoctoral studies. Since 2018, I have focused on developing MPS models for oncology research, publishing 13 papers in the field (4 reviews) and 21 abstracts. In August of 2023, I joined the faculty of the Joint BME Department as an Assistant Professor and part of an interdisciplinary faculty cluster called "Translational Predictive Biology". I fully meet the eligibility criteria, including date of initial faculty appointment (Aug. 16, 2023) with an independent lab and Lineberger affiliation (Associate Member, Immunology Program, Nov. 10, 2023), and I am a strong candidate for the Pew-Stewart Cancer Research Scholars Award.

My lab's mission is to develop new tools for biomedical research that will improve and extend lives. We're pursuing this goal by building microphysiological systems (MPS), also known as "organ-on-chip" devices, that enable us to model tissue with increasing complexity and biological fidelity. My lab has defined a unique niche; we specialize in building vascularized, immunocompetent models made possible by both cellular engineering and through the incorporation of patient-derived tissue in custom microfluidics. These MPS move us closer to replicating *in vivo* conditions, yet with the ability maintain control over the system (physical conditions, etc.), to isolate and test individual variables, and to observe emergent behaviors of the system. The platform can be used to test novel therapeutics, to dissect the tumor microenvironment, and for personalized medicine. Furthermore, the long-term goal of my lab is to use patient-specific models to discover biological mechanisms underlying health disparities. With patient-on-chip models derived from a single individual, we can explore disparate disease outcomes that are linked to biological sex or ancestry and uncouple genetics from environmental factors.

My work building patient-derived, immunocompetent vascular models is an important advance to improve the translational capacity of the MPS field, and we're poised to revolutionize how clinicians and basic scientists can use *in vitro* models to unravel biological process at the tissue level and for predicting disease outcomes and drug response. **Fig. 1** illustrates the basic methodology for MPS generation. Each of the requisite cell types are combined in precise conditions within a microfluidic device to generate a vascular bed surrounding tumor spheroids, and immune cells are perfused through the vasculature to mimic circulation through the body. Aim 1 of this project develops a platform that can be applied widely across oncology, and in Aim 2 we validate these tools for predicting therapeutic efficacy. The FDA recently empowered researchers to expand the repertoire of preclinical assays, and our technology will play a key role as a testing platform for novel immunotherapies and cell therapies such as CAR-T/NK or autologous T cells. Furthermore, with the ability to design and control the

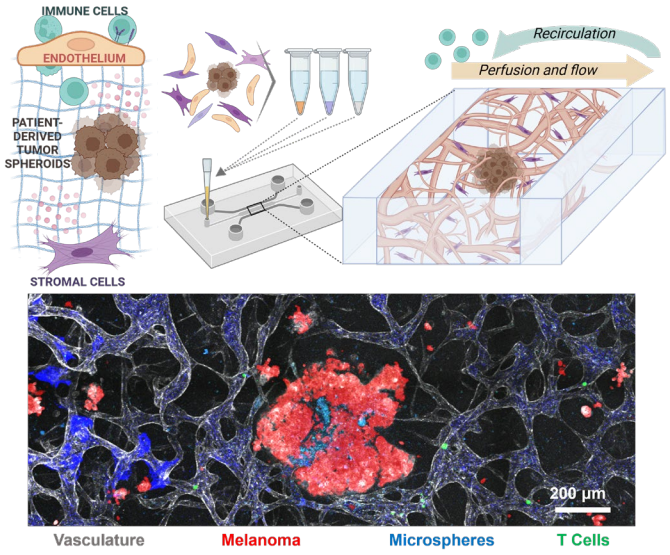

Fig. 1: Overview of vascular MPS models of the tumor microenvironment with immune cell perfusion (top) and an example of a perfused melanoma spheroid with limited T cell recruitment (bottom).

components of the model, we can simultaneously explore the mechanistic underpinnings driving response or resistance to therapy, and my goal is to dissect biological drivers of disease disparities. As my lab grows and flourishes, successfully competing for the Pew-Stewart Cancer Research Scholars award will give my team the freedom to explore risky, exciting research with the potential to positively impact the lives of cancer patients in NC and beyond.

Across organoid models and MPS, there are several approaches for studying cancer spheroids in 3D. However, most of these models can't replicate a functional immune response and lack a vascular system, which is critical to understanding drug delivery and cellular transport at the tissue level. Therefore, my lab specializes in immunocompetent, vascularized tumor-on-chip models, and we will expand and optimize these methods in the following aims.

AIM 1: Building patient-derived, immunocompetent, vascular MPS. The first approach I developed for avoiding allogeneic T cell responses in mixed-donor experiments is using endothelial and stromal cells engineered to eliminate β2-microglobulin expression, resulting in the loss of MHC I presentation (unpublished data). Thus, we can generate "immune invisible" microvascular networks compatible with T cells from any donor. We will establish additional endothelial sources in aim 1, including differentiating induced pluripotent stem cells with the ETV2 transcription factor, isolating primary tissue endothelial cells via magnetic/fluorescent sorting for lineage-specific surface markers, and expanding endothelial colony forming cells from peripheral blood. Endothelial colony forming cells are a rare type of circulating endothelial cell that can be isolated from the mononuclear fraction of peripheral blood and proliferate through several passages. Organotypic tumor spheroids, immune cells, and stromal cells are collected from a resection sample or biopsy to incorporate into the microfluidic devices, generating a model of the tumor microenvironment that is amenable to multiplexed drug treatments in parallel, real-time microscopy, sampling, and endpoint molecular biology assays. These approaches will enable us to move beyond the engineered immunocompetent models I've already developed to fully autologous, single-patient, tumor-on-chip models.

AIM2: Validating translational predictivity of patient-derived, immunocompetent, vascular MPS. We will test standard-of care treatment in these models to validate the ability of the model to replicate clinical response across various tumor types. In a pilot study, I evaluated 2 specimens from head and neck squamous cell carcinomas (HNSCC). First, the "immune-invisible" microvasculature was formed around a circular port within microfluidic devices. Then, tissue specimens were processed to obtain organotypic tumor spheroids (40-70 μm) and loaded into the chamber surrounded by vasculature. Patient-matched T cells were activated (CD3/CD28) and perfused through the vasculature. Half of the devices from each donor were treated with pembrolizumab, and confocal imaging was used to quantify the degree of T cell infiltration towards the tumor spheroids, which was increased in devices treated with immunotherapy (**fig. 2**). The results we obtain from MPS models will be compared to clinical outcomes using RECIST criteria to quantify the degree of correlation between on-chip and *in vivo* disease trajectories. Ultimately, we strive to use these models to determine the biological drivers of disparities in disease progression and response to therapy-- questions that can't be answered in other model systems. These MPS devices could also replace some animal studies and enable co-clinical trials (on-chip/*in vivo*), capturing more heterogeneity across populations and within tumor tissue than ever possible before.

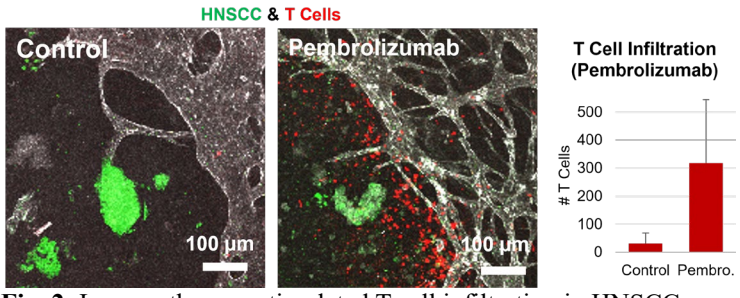

Fig. 2: Immunotherapy-stimulated T cell infiltration in HNSCC.

UNC LCCC Internal Nomination Proposal for Sarah Shelton, Ph.D.
External Letters of Recommendation: candidate's thesis advisor, postdoctoral advisor, and a scientific reference from an individual external to UNC who is not a collaborator or mentor.

Paul Dayton, Ph.D.
Professor, UNC-NC State Joint Department of Biomedical Engineering
UNC-Chapel Hill & NC State University
Ph.D. thesis advisor (internal)
Dr. Dayton develops contrast-enhanced ultrasound imaging technology for functional and anatomical imaging of vasculature.

David Barbie, M.D.
Professor, Division of Medical Oncology
Dana-Farber Cancer Institute & Harvard Medical School
Postdoctoral advisor (external)
Dr. Barbie is a thoracic oncologist and researcher specializing in lung cancer. His research describes linkages between innate and adaptive immunity in cancer.

Roger Kamm, Ph.D.
Professor, Department of Biological Engineering
Massachusetts Institute of Technology
Postdoctoral advisor (external)
Dr. Kamm is an engineer with expertise in mechanobiology- how cells sense and react to physical stimuli. His work in the cancer field primarily focuses on the physical forces involved in the metastatic cascade.

Shreyas Rao, Ph.D.
Associate Professor, Department of Chemical and Biological Engineering
University of Alabama
Reference, non-mentor, non-collaborator (external)
Dr. Rao designs biomimetic materials to improve 3D tissue models. His work confronts cancer drug resistance from a biomaterials approach for sensing and intervention.

CURRICULUM VITAE: SARAH E. SHELTON

EMPLOYMENT

Assistant Professor — **Aug. 2023-present**
Joint Department of Biomedical Engineering:
University of North Carolina at Chapel Hill, School of Medicine
North Carolina State University, College of Engineering
Associate Member, UNC Lineberger Comprehensive Cancer Center, Immunology Division

Research Associate — **Jan. 2023 – Aug. 2023**
Senior Postdoctoral Fellow — **Jan. – Dec. 2022**
Postdoctoral Fellow — **Jan. 2018 – Dec. 2021**
Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA
Advisor: Roger D Kamm, PhD
Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA
Advisor: David A. Barbie, MD

EDUCATION

Ph.D. in Biomedical Engineering — **Dec. 2017**
Joint Department of Biomedical Engineering:
University of North Carolina at Chapel Hill, School of Medicine
North Carolina State University, College of Engineering
Advisor: Paul A. Dayton, PhD
Thesis: “Analysis of Early Angiogenesis Using Acoustic Angiography Ultrasound”

M.S. in Environmental Sciences and Engineering — **May 2011**
University of North Carolina at Chapel Hill, Gillings School of Global Public Health
Advisor: William G. Gray**,** PhD
Thesis: "Mechanistic Modeling of Cancer Tumor Growth Using a Porous Media Approach"

B.S. in Environmental Science, Highest Honors — **Aug. 2006**
University of North Carolina at Chapel Hill
Biology Minor, Concentration in Modeling
Study Abroad: “College Year in Athens”. Spring 2005, Athens, Greece
Honors Thesis: *A Heat Budget for University Lake, Carrboro NC*

RESEARCH SUPPORT

NCI Predoctoral to Postdoctoral Fellow Transition Award (PI, F99K00CA212227, ~$460,000) — **2016 – 2023**
Integrative Vascular Biology Training Program (Trainee, T32HL069768, stipend/tuition/fees) — **2013 – 2016**

AWARDS

Rising Stars in Mechanical Engineering, Massachusetts Institute of Technology — **October 2021**
IEEE International Ultrasonics Symposium Student Travel Award — **September 2014**
NCSU College of Engineering Dean’s Doctoral Fellowship — **2012 – 2013**
NCSU College of Engineering Graduate Merit Award — **2012 – 2013**
NSF Fellowship for Cancer Nanotechnology Summer Institute — **June 2011**
Bachelor of Science awarded with Highest Honors, UNC-Chapel Hill — **August 2006**
Dean’s List: 5 semesters, UNC-Chapel Hill Undergraduate — **2002 – 2006**

PEER-REVIEWED PUBLICATIONS

SE Shelton. *Vascular Microphysiological Systems.* Current Opinion in Hematology. (Jan. 19, 2024)

E Cambria, M Coughlin, M Floryan, G Offeddu, **SE Shelton**, RD Kamm. *Linking mechanical cell memory and cancer metastasis.* Nature Reviews Cancer. Pages 1-13 (Jan. 18, 2024).

S Chen, RD Kamm, **SE Shelton***, G Offeddu* (*Equal contributions). *Development of a Three-Compartment Microvascular Model-on-a-Chip*. Lab on a Chip. (Sep. 29, 2023).

E Angelidakis, S Chen, S Zhang, Z Wan, RD Kamm, **SE Shelton**. *Impact of fibrinogen, fibrin thrombi and thrombin on cancer cell extravasation using in vitro microvascular networks*. Advanced Healthcare Materials. (Apr. 29, 2023).

Z Wan, M Floryan, M Coughlin, S Zhang, A Zhong, **SE Shelton**, X Wang, C Xu, DA Barbie, RD Kamm. *New strategy for promoting vascularization in tumor spheroids in a microfluidic assay*. Advanced Healthcare Materials. Vol. 12, No. 14 (2023)

DJ Kim, S Anandh, J Null, P Przanowski, S Bhatnagar, **SE Shelton**, RD Kamm, DA Barbie, AC Dudley. *Priming a vascular-selective cytokine response permits CD8+ T-cell entry into tumors*. Nature Communications, Vol. 14, No. 1 (2023).

Y Wang*, **SE Shelton*** (* Equal contributions), G Kastrunes, DA Barbie, G Freeman, W Marasco. *Preclinical models for immune-oncology therapy development*. Immuno-Oncology Insights. Vol. 3, No. 8, p.396-398 (2022).

M Campisi*, **SE Shelton*** (*Equal contributions), M Chen, RD Kamm, DA Barbie, E Knelson. *Engineered microphysiological systems for testing effectiveness of cell-based cancer immunotherapies*. Cancers. Vol 14. No. 15. (2022).

Z Wan, A Zhong, S Zhang, G Pavlou, M Coughlin, **SE Shelton**, HT Nguyen, J Lorch, DA Barbie, RD Kamm. *A robust method for perfusable microvascular network formation in vitro.* Small Methods, 2200143 (2022).

Z Wan, S Zhang, A Zhong, **SE Shelton**, M Campisi, S Sundararaman, G Offeddu, E Ko, L Ibrahim, M Coughlin, T Liu, J Bai, DA Barbie, RD Kamm. *A robust vasculogenic microfluidic model using human immortalized endothelial cells and Thy1 positive fibroblasts*. Biomaterials. Jul. 16, (2021).

G Offeddu, JC Serrano, SW Chen, **SE Shelton**, Y Shin, RD Kamm. *MicroHeart: A Microfluidic Pump for Functional Vascular Culture in Microphysiological Systems.* Journal of Biomechanics. Vol 119. (2021).

SE Shelton*, HT Nguyen* (*Equal contributions), DA Barbie, RD Kamm. *Engineering Approaches for Studying Immune-Tumor Cell Interactions and Immunotherapy.* iScience. Vol. 24, No. 1, (2021).

M Campisi*, SK Sundararaman*, **SE Shelton*** (*Equal contributions), E Knelson, E Ivanova, I Cañadas, R Yoshida, T Osaki, SWL Lee, T Thai, S Han, B Piel, S Gilhooley, CP Paweletz, V Chiono, RD Kamm, S Kitajima, DA Barbie. *Tumor-Derived cGAMP Regulates Activation of the Vasculature.* Frontiers in Immunology. Vol. 11 (2020).

SE Shelton, RD Kamm. *In Vitro, Primarily Microfluidic Models for Atherosclerosis.* Chapter 13, "Biomechanics of Coronary Atherosclerotic Plaque", p. 303-319 (2020).

SE Shelton, J Stone, F Gao, D Zeng, PA Dayton. *Microvascular Ultrasonic Imaging of Angiogenesis Identifies Tumors in a Murine Spontaneous Breast Cancer Model.* International Journal of Biomedical Imaging. Feb 6 (2020).

K Mohanty, V Papadopoulou, I Newsome, **SE Shelton**, PA Dayton, M Muller. *Ultrasound Multiple Scattering with Microbubbles Can Differentiate between Tumor and Healthy Tissue In Vivo*. Physics in Medicine and Biology. Vol. 64, No. 11 (2019).

A Panfilova, **SE Shelton**, C Caresio, RJG van Sloun, F Molinari, H Wijkstra, PA Dayton, M Mischi. *On the Relationship between Dynamic Contrast-Enhanced Ultrasound Parameters and the Underlying Vascular Architecture Extracted from Acoustic Angiography.* Ultrasound in Medicine and Biology. Vol. 45, No. 2, p. 539-548 (2019).

SE Shelton, BD Lindsey, PA Dayton, YZ Lee. *First-In-Human Study of Acoustic Angiography in the Breast and Peripheral Vasculature.* Ultrasound in Medicine and Biology. Vol. 43, No. 12, p. 2939-2946 (2017)

BD Lindsey*, **SE Shelton*** (*Equal contributions), FS Foster, PA Dayton. *Assessment of Molecular Acoustic Angiography for Combined Microvascular and Molecular Imaging in Preclinical Tumor Models*. Molecular Imaging and Biology, Vol. 19, No. 2, p. 194-202 (2017)

F Lin, **SE Shelton**, D Espíndola, JD Rojas, G Pinton, PA Dayton. *3-D Ultrasound Localization Microscopy for Identifying Morphology Features of Tumor Angiogenesis at a Resolution Beyond the Diffraction Limit of Conventional Ultrasound.* Theranostics, Vol. 7, No. 1, p. 196-204 (2017)

BD Lindsey, **SE Shelton**, KH Martin, KA Ozgun, JD Rojas, FS Foster, PA Dayton. *High Resolution Ultrasound Superharmonic Perfusion Imaging: In Vivo Feasibility and Quantification of Dynamic Contrast-Enhanced Acoustic Angiography.* Annals of Biomedical Engineering, Vol. 45, No. 4, p. 939-948 (2017)

SE Shelton *, BD Lindsey * (*Equal contributions), JK Tsuruta, FS Foster, PA Dayton. *Molecular Acoustic Angiography: A New Technique for High Resolution Superharmonic Ultrasound Molecular Imaging.* Ultrasound in Medicine and Biology, Vol. 42, No. 3, p. 679-81 (2016)

SR Rao*, **SE Shelton*** (*Equal contributions), PA Dayton. *The 'Fingerprint' of Cancer Extends Beyond Solid Tumor Boundaries: Assessment with a Novel Ultrasound Imaging Approach*. IEEE Transactions on Biomedical Engineering, Vol. 63, No. 5, p. 1082-1086 (2016)

SE Shelton, YZ Lee, SR Aylward, M Lee, FS Foster, PA Dayton. *Quantification of Microvascular Tortuosity During Tumor Evolution Utilizing Acoustic Angiography*. Ultrasound in Medicine and Biology, Vol. 41, No. 7, p. 1896-1904 (2015)

BD Lindsey*, **SE Shelton*** (*Equal contributions), PA Dayton. *Optimization of Contrast-to-Tissue Ratio Through Pulse Windowing in Dual-Frequency "Acoustic Angiography" Imaging*. Ultrasound in Medicine and Biology, Vol. 41, No. 7, p. 1884-1895 (2015)

JM Dunleavey, L Xiao, J Thompson, MM Kim, JM Shields, **SE Shelton**, DM Irvin, VE Brings, DW Ollila, RA Brekken, PA Dayton, JM Melero-Martin, AC Dudley. *Vascular Channels Formed by Subpopulations of PECAM1+ Melanoma Cells.* Nature Communications, Vol. 5 (2014)

BD Lindsey, JD Rojas, KH Martin, **SE Shelton**, PA Dayton. *Acoustic Characterization of Contrast-to-Tissue Ratio and Axial Resolution for Dual-Frequency Contrast-Specific "Acoustic Angiography" Imaging.* IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control, Vol. 61, No. 10, p.1668-1687 (2014)

G Sciumè, **SE Shelton,** WG Gray, CT Miller, F Hussain, M Ferrari, P Decuzzi, BA Schrefler. *A Multiphase Model for Three-Dimensional Tumor Growth*. New Journal of Physics, Vol. 15, p. 1-35 (2013)

G Sciumè, **SE Shelton,** WG Gray, CT Miller, F Hussain, M Ferrari, P Decuzzi, BA Schrefler. *Tumor Growth Modeling from the Perspective of Multiphase Porous Media Mechanics*. Molecular and Cellular Biomechanics, Vol. 9, No. 3, p. 193-212 (2012)

Full bibliography: https://www.ncbi.nlm.nih.gov/myncbi/sarah.shelton.2/bibliography/public/

ORAL AND POSTER PRESENTATIONS

Oral: E Angelidakis, RD Kamm **SE Shelton**. *Modeling Concurrent Metastasis and Thrombosis.* Microphysiological Systems World Sumit. Jun. 10-14, 2024. Seattle, WA.

Oral: E Cambria*, **SE Shelton***, A Blazeski, E Ko, T Thai, DA Barbie, RD Kamm. *Lung Microvasculature-on-chip to Study the Effect of Myofibroblasts on Vasculogenesis and Angiogenesis in Fibrosis.* Microphysiological Systems World Sumit. Jun. 10-14, 2024. Seattle, WA.

Oral: **SE Shelton**, DA Barbie, RD Kamm. *Patient-Specific Models of the Immune-Competent Tumor and its Microenvironment with Physiological Perfusion.* Biomedical Engineering Society Annual Meeting. Oct. 11-14, 2023. Seattle, WA.

Poster: L Durland, Y Michaels, **SE Shelton**, RD Kamm, P Zandstra. *A 3D Vascularized Microfluidic Model of the Tumor Microenvironment for Assaying PSC Derived CAR-T Cell Function.* International Society of Cell & Gene Therapy Annual Meeting. May 31-Jun. 3, 2023. Paris, France.

Poster: **SE Shelton**, DA Barbie, RD Kamm. *Patient-Derived Models of the Tumor Microenvironment for Immunotherapy Studies*. SEXX and Immunity: Exploring the impact of female (and male) biology on normal and pathological immune responses to infection and other immune triggers. Massachusetts Institute of Technology. Nov. 2, 2022.

Poster: **SE Shelton**, DA Barbie, RD Kamm. *Cancer-Associated Fibroblasts Produce a Pro-Angiogenic Environment in Microvasculature-on-a-Chip*. Biomedical Engineering Society Annual Meeting. Oct. 12-15, 2022. San Antonio, TX.

Oral: S Chen, RD Kamm, G Offeddu*, **SE Shelton***. *Development of a Three-Compartment Microvascular Model-On-A-Chip*. Biomedical Engineering Society Annual Meeting. Oct. 12-15, 2022. San Antonio, TX.

Oral: E Cambria, G Offeddu, **SE Shelton**, M Floryan, M Coughlin, M Humayun, RD Kamm. *Influence of Extracellular Matrix Stiffness on Tumor Cell Extravasation via Cell Mechanical Memory*. Biomedical Engineering Society Annual Meeting. Oct. 12-15, 2022. San Antonio, TX.

Oral: N Asquith, D Freire, **SE Shelton**, K Machlus, RD Kamm, J Italiano. *The Development of a 3D Microvasculature Assay to Study the Sub-Cellular Dynamics of Megakaryocyte Endothelial Barrier Interactions and Pro (Platelet) Formation and Release.* Congress of the International Society on Thrombosis and Haemostasis. July 9-13, 2022. London, U.K.

Poster: **SE Shelton**, Z Wan, S Zhang, M Campisi, DA Barbie, RD Kamm. *Generating Perfusable Microvasculature-On-A-Chip Models for Lymphocyte Perfusion and Cancer Studies*. NCI Tissue Engineering Consortium Annual Meeting. July 6-7, 2022. Madison, WI. **Outstanding Poster Award**

Oral: **SE Shelton**, B Piel, T Thai, H Nguyen, J Lorch, K. Sehgal, DA Barbie, RD Kamm. *An In Vitro Model of The Tumor Microenvironment Shaped by Cancer-Associated Fibroblasts.* Summer Biomechanics, Bioengineering, and Biotransport Conference. Jun. 20-23, 2022. Eastern Shore, MD.

Oral: **SE Shelton**, B Piel, T Thai, J Lorch, K Sehgal, DA Barbie, RD Kamm, *Microvascular Model Incorporating Cancer-Associated Fibroblasts and Immune Cell Perfusion*. Microphysiological Systems World Summit, May 30-June 3, 2022. New Orleans, LA.

Poster: **SE Shelton**, B Piel, J Lorch, K Sehgal, DA Barbie, RD Kamm. *Engineered Microvascular Models Incorporating Cancer-Associated Fibroblasts for Immune Cell Perfusion.* Vascular Biology Meeting, North American Vascular Biology Association. Oct. 25-29, 2021.

Oral: **SE Shelton**, B Piel, J Lorch, K Sehgal, DA Barbie, RD Kamm. *Patient-Derived Microvascular Models for Immuno-Oncology Studies*. Biomedical Engineering Society Annual Meeting. Oct. 6-9, 2021. Orlando, FL.

Poster: M Campisi, N Mahadevan, E Knelson, **SE Shelton**, I Ozgenc, J Miret, C Pawaletz, RD Kamm, DA Barbie. *Engineered Microphysiological systems for testing effectiveness of cell-based cancer immunotherapies.* NCI Physical Sciences-Oncology Network Annual Meeting. Aug. 16-18, 2021.

Poster: **SE Shelton**, DA Barbie, RD Kamm. *Patient-derived microvascular models for immunotherapy studies.* Tissue Engineering Consortium Annual Meeting. July 19-20, 2021.

Oral & Poster: **SE Shelton**, S Sundararaman, M Campisi, N Mahadevan, E Knelson, S Kitajima, DA Barbie, RD Kamm. *Activation of Endothelial STING by Tumor-Derived cGAMP*. Vascular Biology Meeting, North American Vascular Biology Organization. Oct. 26-29, 2020. **Outstanding Poster Award**

Oral: **SE Shelton**, D Barbie, RD Kamm. *Visualizing the Response to Activation of the STING Pathway in a Microfluidic Vascular Model*. Biomedical Engineering Society Annual Meeting. Oct. 14-17, 2020.

Oral: P Balogh, D Pulieri, **SE Shelton**, K Haase, RD Kamm, A Randles. *A Framework to Quantify 3D Microvascular Remodeling and Correlate with Hemodynamic Forces.* Biomedical Engineering Society Annual Meeting. Oct. 14-17, 2020.

Oral: M Campisi, T Osaki, **SE Shelton**, S Sundararaman, SW Lee, C Mattu, G Adriani, C Voena, I Mota, E Patrucco, S Kitajima, R Chiarle, DA Barbie, RD Kamm, V Chiono. *Microphysiological Systems for Modelling Microvasculature And Multicellular-Vascular Interactions using Microfluidic Technology*. 2020 TERMIS SYIS AM & EU Webinar Series. Sept. 24, 2020.

Oral and Poster: **SE Shelton**, D Barbie, RD Kamm. *T Cell Behavior in Engineered Microvascular Networks.* National Cancer Institute F99/K00 Fellows Meeting. Jan. 16-17, 2020. Bethesda, MD.

Poster: **SE Shelton**, D Barbie, RD Kamm. *An In Vitro Model of T Cell Retention in Cancer-Associated Vasculature*. Vascular Biology Meeting, North American Vascular Biology Association. Oct. 29, 2019. Monterey, CA.

Poster: **SE Shelton**, D Barbie, RD Kamm. Biomedical Engineering Society Annual Meeting. *A 3D Model of T Cell Interactions with Perfusable Vasculature In Vitro*. Oct. 18, 2019. Philadelphia, PA.

Oral: PA Dayton, **SE Shelton**, F Lin, D Espindola, R Gessner, S Aylward, FS Foster, G Pinton. *Next-Generation Ultrasound Imaging for Assessing the Microvascular Fingerprint of Cancer.* IEEE International Symposium on Biomedical Imaging. April 6, 2018. Washington, D.C. *Invited lecture*.

Oral: A Panfilova, **SE Shelton**, C Caresio, RJG van Sloun, F Molinari, H Wijkstra, PA Dayton, M Mischi. *On What Dynamic Contrast-Enhanced Ultrasound Tells Us About the Underlying Vascular Architecture.* European Symposium on Ultrasound Contrast Imaging. January 18-19, 2018. Rotterdam, Netherlands.

Oral: IG Newsome, BD Lindsey, **SE Shelton**, E Cherin, J Yin, FS Foster, PA Dayton. *Characterization of a Prototype Transmit 2 MHz Receive 21 MHz Array for Superharmonic Imaging.* IEEE International Ultrasound Symposium. September 6-9, 2017. Washington, D.C.

Oral: J Aditya, **SE Shelton**, V Papadopoulou, BD Lindsey, G Pinton, PA Dayton, M Muller. *In-Vivo Characterization of Angiogenesis in Tumor-Bearing Rats using Multiple Scattering of Ultrasound.* IEEE International Ultrasound Symposium. September 6-9, 2017. Washington, D.C.

Poster: A Panfilova, **SE Shelton**, RJG van Sloun, C Caresio, H Wijkstra, PA Dayton, M Mischi. *Which properties of the vascular architecture are reflected by dynamic contrast-enhanced ultrasound imaging of dispersion and wash-in rate? A comparison with acoustic angiography*. IEEE International Ultrasound Symposium. September 6-9, 2017. Washington, D.C.

Poster: **SE Shelton**, BD Lindsey, YZ Lee, SR Aylward, PA Dayton. *Using High-Resolution Ultrasound Imaging to Visualize Cancer Angiogenesis*. Gordon Research Conference and Gordon Research Seminar- Angiogenesis. August 5-11, 2013. Newport, RI.

Oral: **SE Shelton**, BD Lindsey, FS Foster, PA Dayton. *Molecular acoustic angiography: comparison of contrast-to-tissue ratio with multi-pulse techniques and imaging multiple targeted microbubbles*. IEEE International Ultrasound Symposium. September 20, 2016. Tours, France.

Oral: **SE Shelton**, SR Aylward, FS Foster, PA Dayton. *The application of acoustic angiography to assess the progression of angiogenesis in a spontaneous mouse model of breast cancer.* IEEE International Ultrasound Symposium. September 19, 2016. Tours, France.

Poster: A Panfilova, **SE Shelton**, RJG van Sloun, L Demi, H Wijkstra, P Dayton, M Mischi. *Does contrast ultrasound dispersion imaging reveal changes in tortuosity? A comparison with acoustic angiography.* IEEE International Ultrasound Symposium. September 18-21, 2016. Tours, France.

Oral: **SE Shelton**, BD Lindsey, FS Foster, SR Aylward, PA Dayton. *Structural and molecular imaging of tumor vasculature using dual-frequency acoustic angiography*. Ultrasonic Biomedical Microscanning International Conference, May 2, 2016. Kralendijk, Bonaire.

Oral: **SE Shelton**, B Lindsey, R Gessner, Y Lee, S Aylward, H Lee, E Cherin, FS Foster, PA Dayton. *Acoustic angiography: a new high frequency contrast ultrasound technique for biomedical imaging*. (Invited). SPIE Commercial + Scientific Sensing and Imaging 2016. April 17-21, 2016. Baltimore, MD.

Oral: BD Lindsey, **SE Shelton**, JK Tsuruta, FS Foster, PA Dayton. *Molecular acoustic angiography: Demonstration of in vivo feasibility for high resolution superharmonic ultrasound molecular imaging*. IEEE International Ultrasound Symposium. October 22-24, 2015. Taipei, Taiwan.

Oral: **SE Shelton**, SR Rao, YZ Lee, M Lee, E Cherin, FS Foster, SR Aylward, PA Dayton. *Ultrasound acoustic angiography imaging of angiogenesis as a cancer biomarker*. Biomedical Engineering Society Annual Meeting. October 9, 2015. Tampa, FL.

Poster: **SE Shelton**, SR Rao, BD Lindsey, M Lee, E Cherin, FS Foster, SR Aylward, PA Dayton. *Vascular tortuosity in acoustic angiography images of cancer angiogenesis.* Gordon Research Conference- Angiogenesis. August 2-7, 2015. Newport, RI.

Poster: **SE Shelton**, SR Rao, PA Dayton. *Ultrasound acoustic angiography imaging of tumor angiogenesis in a mouse model*. Integrative Vascular Biology and McAllister Heart Institute Research Symposium. March 10, 2015. Chapel Hill, NC.

Oral: **SE Shelton**, BD Lindsey, RC Gessner, M Lee, SR Aylward, FS Foster, PA Dayton. *Acoustic angiography structural imaging of tumor angiogenesis*. Ultrasonic Biomedical Microscanning International Conference. October 1, 2014. Eddleston, Scotland.

Poster: SR Rao, **SE Shelton**, PA Dayton. *Analysis of spatial heterogeneity in acoustic angiography images of tumor vasculature*. UNC-NCSU BME Research Retreat. September 26, 2014. Raleigh, NC.

Poster: BD Lindsey, JD Rojas, **SE Shelton**, KH Martin, PA Dayton. *Optimization of contrast-to-tissue ratio and role of bubble destruction in dual-frequency contrast-specific "acoustic angiography" Imaging*. IEEE International Ultrasonics Symposium. September 5, 2014. Chicago, IL.

Poster: **SE Shelton**, JM Dunleavey, AC Dudley, M Lee, FS Foster, PA Dayton. *Quantification of tumor vasculature using acoustic angiography ultrasound*. IEEE International Ultrasonics Symposium. September 4, 2014. Chicago, IL.

Oral: PA Dayton, RC Gessner, L Phillips, **SE Shelton**, KH Martin, M Lee, FS Foster. *The implementation of acoustic angiography for microvascular and angiogenesis imaging*. IEEE Engineering in Medicine and Biology Conference. August 29, 2014. Chicago, IL.

Oral: **SE Shelton**, JM Dunleavey, AC Dudley, PA Dayton. *Quantification of tumor vasculature using Acoustic Angiography ultrasound imaging*. Integrative Vascular Biology and McAllister Heart Institute Research Symposium. March 11, 2014. Chapel Hill, NC. **Outstanding Oral Presentation Award**

Oral: **SE Shelton**, JM Dunleavey, AC Dudley, PA Dayton. *Acoustic angiography ultrasound imaging can distinguish vascular differences in tumor cell lines*. Acoustical Society of America. December 2-6, 2013. San Francisco, CA.

Poster: **SE Shelton**, RC Gessner, PA Dayton. *Novel "acoustic angiography" imaging creates 3-d, high-resolution images of microvasculature*. Gordon Research Conference- Angiogenesis. August 4-9, 2013. Newport, RI.

Poster: JM Dunleavey, L Xiao, J Thompson, M Kim, J Shields, **SE Shelton**, D Irvin, D Ollila, R Brekken, PA Dayton, AC Dudley. *Vascular channel formation by a novel subpopulation of CD31+ melanoma cells*. Gordon Research Conference- Angiogenesis. August 4-9, 2013. Newport, RI. **Outstanding Poster Award**

Poster: **SE Shelton**, MR Scola, CM Gallippi. *Viscoelastic Strain Response (ViSR) ultrasound as a potential new approach for imaging cancer*. UNC Radiology- Biomedical Research Imaging Center Research Symposium. March 21, 2013. Chapel Hill, NC

Oral: BA Schrefler, G Sciume, **SE Shelton**, CT Miller, WG Gray. *Mechanics of porous media: from geomaterial to tumor growth modelling.* International Conference on Computational Plasticity Fundamentals and Applications. 2011. September 7-9. Barcelona, Spain. *Invited Lecture.*

CONFERENCE PUBLICATIONS

IG Newsome, BD Lindsey, **SE Shelton**, E Cherin, J Yin, FS Foster, PA Dayton. *Characterization of a prototype transmit 2 MHz receive 21 MHz Array for Superharmonic Imaging.* IEEE International Ultrasound Symposium. September 6-9, 2017. Washington, D.C.

A Panfilova, **SE Shelton**, RJG van Sloun, C Caresio, H Wijkstra, PA Dayton, M Mischi. *Which properties of the vascular architecture are reflected by dynamic contrast-enhanced ultrasound imaging of dispersion and wash-in rate? A comparison with acoustic angiography.* IEEE International Ultrasound Symposium. September 6-9, 2017. Washington, D.C.

A Joshi, M Muller, **SE Shelton**, V Papadopoulou, B Lindsey, PA Dayton. *In-vivo quantitative analysis of the angiogenic microvasculature in tumor-bearing rats using multiple scattering: A preliminary study.* The Journal of the Acoustical Society of America 140 (4), 3187-3187 (2016)

SE Shelton, PA Dayton, SR Aylward, FS Foster. *The application of acoustic angiography to assess the progression of angiogenesis in a spontaneous mouse model of breast cancer.* IEEE International Ultrasound Symposium. September 18-21, 2016. Tours, France.

SE Shelton, BD Lindsey, PA Dayton, FS Foster. *Molecular acoustic angiography: comparison of contrast-to-tissue ratio with multi-pulse techniques and imaging multiple targeted microbubbles.* IEEE International Ultrasound Symposium. September 18-21, 2016. Tours, France.

A Panfilova, **SE Shelton**, RJG van Sloun, L Demi, H Wijkstra, P Dayton, M Mischi. *Does contrast ultrasound dispersion imaging reveal changes in tortuosity? A comparison with acoustic angiography.* IEEE International Ultrasound Symposium. September 18-21, 2016. Tours, France.

SE Shelton, B Lindsey, R Gessner, Y Lee, S Aylward, H Lee, E Cherin, FS Foster, PA Dayton. *Acoustic angiography: a new high frequency contrast ultrasound technique for biomedical imaging*. Proceedings volume 9871, Sensing and Analysis Technologies for Biomedical and Cognitive Applications 2016. SPIE Commercial + Scientific Sensing and Imaging. 2016.

BD Lindsey, **SE Shelton**, JK Tsuruta, FS Foster PA Dayton. *Molecular acoustic angiography: Demonstration of in vivo feasibility for high resolution superharmonic ultrasound molecular imaging*. IEEE International Ultrasound Symposium. October 22-24, 2015. Taipei, Taiwan.

PA Dayton, RC Gessner, L Phillips, **SE Shelton**, KH Martin, M Lee, FS Foster. *The implementation of acoustic angiography for microvascular and angiogenesis imaging*. Engineering in Medicine and Biology Society. p. 4283-4285, August 26-30, 2014. Chicago, IL.

SEMINARS

College of Veterinary Medicine Cancer Research Seminar Series, North Carolina State University **Mar. 5, 2024**
Bioinformatics Research Center Seminar Series, North Carolina State University **Feb. 8, 2024**
Frontiers in Quantitative BioDesign Seminar Series, Duke University **Oct. 20, 2023**
Quantitative and Computational Biology Retreat, North Carolina State University **Sep. 26, 2023**
Blood Research Center Lab Meeting, University of North Carolina at Chapel Hill **Sep. 19, 2023**

PRESS RELEASES

http://news.unchealthcare.org/news/2015/june/imagining-a-new-kind-of-image
https://unclineberger.org/news/ultrasond-studies
http://news.unchealthcare.org/news/2014/october/unc-scientists-discover-hidden-subpopulation-of-melanoma-cells

OTHER EDUCATION AND TRAINING

Funding Your Research: NIH **Feb. – Apr., 2020**
Harvard Catalyst: The Harvard Clinical and Translational Science Center. Cambridge, MA
Online course (8 weeks) about strategically crafting your first R01. Consisted of lectures, readings, and assignments.

Critical Issues in Tumor Microenvironment: Angiogenesis, Metastasis, and Immunology **Sep. 16-19, 2019**
Harvard Medical School. Boston, MA

A short course covering the latest research in the tumor microenvironment. Presented by leaders of the field and organized by Dr. Rakesh Jain.

Kaufman Teaching Certificate **May – Jun., 2019**
Massachusetts Institute of Technology. Cambridge, MA
Earned certificate of teaching in higher education, including 16 in-class hours, readings, assignments, and a teaching session.

Introduction to Translational Medicine **Apr. 8-9, 2019**
Harvard Catalyst: The Harvard Clinical and Translational Science Center. Cambridge, MA
Didactic short-course detailing every aspect of performing first-in-human studies and how to maximize the translational impact of basic science research.

Environmental Health and Safety Representative **Nov. 2018 – Jul. 2023**
Massachusetts Institute of Technology, Cambridge, MA
Completed EHS representative training to manage lab safety, chemical storage, biological hazards, and inspections for the Mechanobiology lab at MIT. Served as the COVID protocol liaison for the lab during 2020-2023.

Integrative Vascular Biology Training Program **Jul. 2013 – Aug. 2016**
University of North Carolina at Chapel Hill, NIH funded training program T32HL069768
Graduate student fellow in a multidisciplinary training group that held bi-weekly seminars, grant writing workshops, and an annual student-run symposium.

Cancer Nanotechnology: Analysis, Imaging, and Treatment over Multiple Scales **Jun. 7-9, 2011**
National Science Foundation Summer Institute - Houston, TX
An interdisciplinary meeting analyzing new nanotechnology approaches for diagnosing and treating cancer.

Multiscale Modeling of Porous Medium Systems **Mar. 7-11, 2011**
"International Training Group" short course. University of Stuttgart, Germany
Served as a teaching assistant for an advanced graduate-level, short-course on modeling theory for porous media.

ACTIVITIES AND EXPERIENCE

Leadership Team of **Flamenco Carolina-** Durham, NC **2005 – 2018**
- Founding member and treasurer of a semi-professional flamenco performance group.
- Duties included accounting, outreach, teaching, performing, and organizing workshops by visiting artists.

Math Instructor at **Jordan Lake School of the Arts-** Apex, NC **Jan. – May 2012**
- Provided one-on-one and small group instruction in elementary through high school mathematics.
- Performed initial testing and maintained progress reports for each student.
- Selected instructional materials and designed individualized lesson plans for students with special needs.

Center Director and *Tutor* at **The Tutoring Center**- Morrisville, NC **Mar. 2009 – Jan. 2010**
- Evaluated students, presented findings and recommendations to parents, and designed personalized tutoring programs.
- Interviewed, hired, and scheduled staff members.
- Guided day-to-day operations and tutoring activities including monthly payroll and bills.

Flutist for the **Really Terrible Orchestra of the Triangle**- NC **Jun. 2009 – Jan. 2010**
- Performed with the zany and informative community orchestra committed to making classical music fun for everyone.

Special Exhibit Staff at **North Carolina Museum of Natural Sciences**- Raleigh, NC **Aug. 2008 – Jan. 2009**
- Engaged visitors in educational activities and answered questions about the history and archaeology of the Dead Sea Scrolls.
- Performed all 6 operational roles involved in visitor service, exhibit maintenance, and security.

Volunteer at **Carrboro Elementary School**- Carrboro, NC **2004 – 2006**
- Mentored students in kindergarten through grade 5 in the science lab.
- Assisted in preparing weekly science lessons and experiments.

THEO DINGEMANS
CHAIR, DEPARTMENT OF APPLIED PHYSICAL SCIENCES

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March 17, 2024

RE: Pew Biomedical Scholarship Statement of support for Wubin Bai

Dear Selection Committee,

I am delighted to support the application of Prof. Wubin Bai for the Pew Biomedical Scholarship. We hired Dr. Bai in January of 2021 based on his remarkable record of interdisciplinary accomplishments at Massachusetts Institute of Technology (MIT) and at Northwestern University. While this record secured Dr. Bai an interview at UNC, we hired him for his passion, for his science, the breadth of his creativity, and his innate ability to lead effective teams. For myself, I am an interdisciplinary polymer scientist who designs novel polymers for both functional and structural applications and I have experience in polymer physics, polymer chemistry, polymer characterization, and materials science. Therefore, I have a deep appreciation of Bai's brilliance and creativity. Since his arrival at UNC, Prof. Bai has impressed me with the rapid launch of his lab (in the middle of the corona pandemic!), his ambitious research plans, his recent independent scholarly impact as a junior faculty and his contributions to building a new scholarly community at UNC Chapel Hill.

Prof. Bai's broad background in materials science and biomedical engineering is allowing him to establish diverse classes of bio-integrated technology with capabilities for improving health and increasing our understanding of living systems. This makes him truly unusual amongst his peers spanning basic material science with the entrepreneurial spirit to work in teams to move technology quickly to animal models for testing. His goal is as profound as it is audacious: to create new technologies for wearable or implantable devices that can sense physiological quantities to deliver drugs or direct tissue growth and disappear when no longer needed. His record of scholarly accomplishment with over sixty five publications to date quantifies his productivity and training to be an international star. This productivity results from the combination of his own initiative and his training in extraordinary labs. Amongst his portfolio are seventeen first-authored papers (combining postdoctoral and Ph.D. periods), including many high impact works published in family journal of Nature and Science. Since he started his independent research lab, his lab has produced six high-level papers and four patents where Wubin is the leading senior corresponding author/inventor. This is a tremendously stunning record and has prepared him for a very high standard of accomplishment, an international perspective on his science and engineering, and the skill sets needed to lead large groups to accomplish the span of science-to-engineering.

His research as a graduate student at MIT led to a number of notable basic material science accomplishments, ranging from advances in lithographic capabilities based on co-polymer templates to fundamental insights into the phase separation process, some obtained through impressive use of 3D imaging methodologies. Those advances developed by Prof. Bai led to the development of a self-assembling system that can potentially enable low-cost, scalable nanofabrication for making sensors and electronic devices. This background in the basic science of materials prepared him for his postdoctoral fellowship in one of the most productive academic laboratories in the world. During this period at Northwestern University in the lab of John Rogers, his work focused on bio-integrated electronics for advanced healthcare. Here he took his instinct for basic science and turned it to engineering outcomes resulting in 27 publications in top journals and

a provisional patent that focuses on wireless probes for monitoring blood flow in biological tissue.

This range of expertise combined with a perspective on international science and engineering was exactly what we need at UNC when we hired him. His intelligence and creativity crackle with electricity, and his passion for science and engineering is infectious in his developing research group and throughout the Department of Applied Physical Sciences. Here he is a founding member of a young department devoted to convergent engineering. His track record attests to his continued success with the ambitious research he proposes to pursue with the support of the Pew Biomedical Scholarship.

Prof. Bai is establishing himself quickly as a world-class leader in bioelectronic technology applied to implantable and wearable devices. He has won numerous awards for his pre-faculty scholarship including awards for his dissertation, his inventions, and his entrepreneurship. Notably, he received the *Querrey Simpson Institute for Bioelectronics (QSIB) award* in 2020. This highly competitive award supports his innovation in a novel wearable device that provides personalized phototherapy at deep tissue. He also received the *Center for Advanced Molecular Imaging (CAMI) award* in 2019 to support his aspiration to design a wireless and battery-free transient device for discovery in neuroscience. During his PhD study, he assembled a team of chemists and materials scientists that led to the development of stretchable solar cells based on a novel conjugated polymer. This went on an entrepreneurial journey with support from an *MIT MADMEC award* in 2014. Those awards recognized Dr. Bai's extraordinary promise, early record of accomplishments, and innovation creativity. Prof. Bai has already had a strong impact through his national leadership in his scholarly community. He chaired a session at the Materials Research Society 2014 Fall Meeting and at a Gordon Research Conference (focusing on nanostructure fabrication), and was elected to be a topic editor in the journal Micromachine in 2020, a guest editor in the journal Frontiers in Bioelectronics in 2021, Editorial Board Member in Discover Electronics (Springer Nature) in 2023. Most recently, as a rising leader in the field of bioelectronics, Prof. Bai has been invited to present his research at the American Chemistry Society Fall meeting, NC State Electrical and Computer Engineering Colloquia, Electrochemical Society meetings, and FastTraC Clinical Advisory Meeting, which sparked local collaborations and built a dynamic community for advancing biomedical technology. Furthermore, Wubin has been actively contributing to community service for academia by serving as the reviewer for NIH study sections (three times), NSF review panel, and Samsung SRFIC review panel.

Turning to his science and the lab that Prof. Bai is establishing at UNC, he aims to design and develop next-generation bioelectronic systems that harness the unique properties of soft nanomaterials, to integrate them into living organisms, and/or to be resorbable so that they can disappear without additional surgery for extraction. He combines the materials science of soft conducting materials, biochemical sensing, and dosing, with the polymer chemistry of resorption, all within a system engineering perspective to make complete solutions. Within the last two years at UNC, his lab has invented a multi-modal deep-tissue sensing patch for proactive monitoring, which yielded a provisional patent and a publication in *Advanced Materials Technology*. His lab also developed 3D multiscale electronic scaffolds for studying organoids, which forged strong collaboration with a group of neuroscientists at UNC to secure funding from multiple sources and aim to develop precision medicine for neurological disorders. His lab also developed a microscale kirigami strategy for making reconfiguration sensing probes and published the work in *Science Advances* along with a provisional patent. Recently, Wubin developed a wireless patch that offers on-demand transdermal drug delivery with digital automation. His development enables dozens of microgram-dose drugs to be actively delivered with high precision in time and space, serving as a promising solution to improve patient compliance and/or adherence to a pharmaceutical plan, increase treatment precision, and realize pharmaceutical intelligence. His approach demonstrates a simple yet effective way for on-demand transdermal drug release via a thin gold coating onto drug-loaded microneedles to realize electrically triggered crevice corrosion. The additional demonstration via intracranial injection in mice highlights its high spatiotemporal resolution and on-demand drug release, making it an advanced tool for brain research with model animals, especially in studying neural circuit mapping and cause-and-effect relationships between neural activity and neurological disorders. This work has been published in *Nature Communications* along with a provisional patent.

Another system Wubin and his team have developed is a multimodal sensing mask that leverages

AI analysis to improve the sensing accuracy of biomarkers, especially ammonia. The system relies on a thermally cleaved conjugated polymer to enable ultrahigh sensitivity and selectivity of breath ammonia. The simultaneous capturing of breath dynamics and humidity feeds into an AI algorithm based on K-means clustering, further decoupling confounding effects to improve the sensing accuracy. This work is published in *Advanced Materials Technology*. Beyond this, Wubin and his team have been developing a wearable multi-wavelength optical patch, capable of continuous monitoring of pulse oximetry with high precision that eliminates confounding effects from skin tone. The wearable spectrometer coupling with a regression-based AI model demonstrates an enhanced precision in pulse oximetry, thus improving clinical screening and decision-making ubiquitously in surgical, post-operative, emergency medicine, inpatient hospital, and outpatient ambulatory healthcare settings. Along with a provisional patent, they have obtained funding from NC Biotech Center to further this translational research into clinical trials. The training opportunities in his laboratory are as exciting as the engineering itself, inspiring our undergraduate and graduate students to combine basic scientific studies with inventive engineering efforts, to rapidly launch practical technologies. I see both the societal benefits and commercial opportunities associated with these innovations in his lab, which defines an important set of broader impacts in novel clinical insights and in medical technology.

Finally, Prof. Bai has had remarkable success in developing his lab. He recruited students and post-docs from across other disciplines, bringing them together to quickly earn accolades including seven Abrams Scholars, a Sheerer Scholar, two UNC NeuroSpark awards, an NC TraCS award, a UNC AGILE award, as well as being among finalists of the UNC Creativity Hubs. Most striking, Wubin has launched a daring program of involving undergraduates in research. While most junior faculty members focus on recruiting a few effective postdoctoral fellows and graduate students to build their research portfolio, Wubin has embraced the ethos of being a university faculty member. Within twenty months since he started his lab, going beyond his cadre of mature researchers, his group has attracted more than 55 undergraduate researchers, with diverse backgrounds, to develop their research, management, and leadership skills necessary for careers in science and engineering. His infectious spirit inspires his students and recruits them from his classroom, and he has created an organizational structure to mentor these student teams so that they are productive. I am fascinated to see the success of his endeavors and, indeed, he has already launched students to top medical schools and graduate programs.

In summary, Professor Bai's combination of research accomplishments, leadership, and transformative impact in materials engineering and biotechnology positions him for a unique career of innovation and inventorship. Positioned as a founding member of a highly interdisciplinary materials science and engineering department, he has already established collaboration ns within the UNC Departments of Biomedical Engineering, Pharmacology, Chemistry, and Neuroscience and demonstrated early success as an independent faculty member. He is certain to have a deep and broad impact across the applied sciences and biomedical engineering, and he would be a fantastic representative of the Pew Biomedical Scholarship.

Sincerely,

Theo Dingemans
Chair, Department of Applied Physical Sciences

I started my first assistant professor appointment on January 1st, 2021. Since then, I have been leading my lab independently.

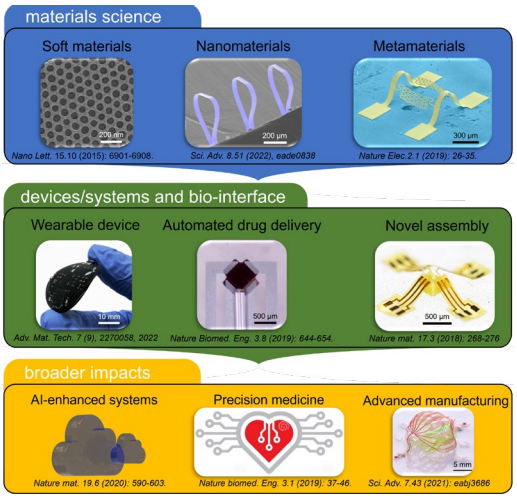

Figure 1. Overview of my research focus, starting from the design and fabrication of flexible multisystem platforms and bioresorbable electronics to the realization of targeted applications in intelligent systems, precision medicine, and advanced manufacturing.

Overview. DNA and proteins fold in three dimensions (3D) to enable functions that sustain life. Emulation of such structuring schemes for functional materials can unleash enormous potential in advancing a wide range of technologies, especially in medical technology. By harnessing the unique properties of soft materials and nanomaterials, the next-generation electronic systems will integrate intelligently with living organisms, medical robotics, and other dynamic physical systems. However, the intrinsic mismatches between soft materials and nanomaterials in their electrical, optical, and mechanical properties often challenge their effective integration into application-specific systems. Such multi-material integration is otherwise ready to drive rapidly expanding research in bioelectronics within applied science and engineering. Our new microfolding technology in advanced manufacturing facilitates multiscale, multidimensional integration of soft conformal electronics with living tissues to understand, with a high spatiotemporal resolution, how brains are developed, how therapeutic stimulation affects recovery, and how biology evolves with exposomes. The Pew Biomedical scholarship will enable me to further address the mismatches among functionally polarized materials and lead to real-world applications via inventive engineering efforts that grow the foundational knowledge for practical NexGen technologies in medicine (**Fig. 1**). We will deepen our understanding of the interface between heterogeneous materials — soft matter and electronic interfaces — for creating unconventional integrations of devices and biology.

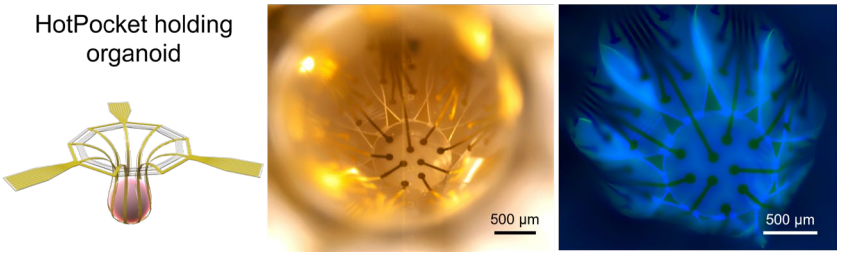

Figure 2. Bioelectronically networked organoids for bi-directional communication at biotic-abiotic interface for fundamental study of organ developments and drug screening.

Bioelectronically networked organoids. Medications to treat psychiatric and neurological disorders have limited efficacy, significant adverse effects, and highly variable responses. Testing of novel therapeutics for neuropsychiatric disorders has largely relied on animal models, which do not adequately capture human cellular complexity and human-specific genetic variation found in patients with these disorders. Personalized therapeutics for prevalent disorders are greatly needed to individually optimize treatment efficacy. To address the great need, We plan to develop a technology platform that allows for nondestructive, long-term, bidirectional communication with brain organoids, providing a promising approach to understanding neuronal response to external stimuli and validating therapeutics that alleviate pathologically relevant neuronal activity (**Fig. 2**). Organoids, 3D *in vitro* systems that recapitulate aspects of human development, cytoarchitecture, and function, are promising models for screening medications for neuropsychiatric disorders. We will develop a morphable, biocompatible, microelectronic, 3D network that can conformally hold a brain organoid for real-time tracking and manipulation of cellular electrophysiology (e.g. electrical stimulation and optogenetics). Derived from induced pluripotent stem cells, organoids can generate cell types with the same genetic background as that of specific individuals and can be scaled for screening against a large number of pharmaceutical compounds to effectively study targeted therapeutics for a range of disorders. However, they face several hurdles before approval for their broad application in drug discovery and screening. Current approaches to monitoring organoids require: i) attachment of a spherical organoid to two-dimensional multi-electrode arrays using extracellular matrix proteins, which can only record from neurons near the electrodes; or ii) electrodes to be inserted into the organoid, which damage the cells around the insertion path. We will develop a soft, biocompatible, microelectronic, 3D network to serve as a bidirectional, closed-loop interface that enables long-term, real-time tracking of cellular

electrophysiology and optogenetic stimulation. Our study explores heterogeneous integration between soft polymeric membranes and metallic nanoribbons, to form a 3D electronic scaffold that gently engages entire organoids for bidirectional communications. The high biocompatibility, soft mechanics, and shape morphability allow long-term interfaces with organoids as they grow in size, paving the way for studying brain development and neurological diseases.

Digitally automated drug delivery coupled with microsensors. Biological systems governing the behavior of individual cells, organs, or even entire organisms, perceive and generate a diverse collection of chemical, electrical, mechanical, thermal, and optical stimuli. Exchange of ions, electrons, and molecules appearing across the lipid bilayer of cellular membranes, the organelles, and most of the complex and large biomolecules such as proteins and DNA, establish the foundations of inter-and intracellular communications that support essential functions of all living biological systems. Probing ionic and/or electronic exchange and delivering drug molecules at the biotic-abiotic interface with clinical safety, digital precision, and dynamic accuracy can provide promising opportunities for furthering precision medicine in treating neurological disorders and cancers. We seek to develop digitally controlled drug delivery devices that are integrated with microsensors to form a closed-loop systems capable of automating dosing schedule and quantity. The digital control relies on a thin gold coating onto drug-loaded reservoir to realize electrically triggered crevice corrosion, that exposes the drug for releasing. Such on-demand automatable drug delivery holds great promise to improve patient compliance and/or adherence to a pharmaceutical plan, increase treatment precision, and realize pharmaceutical intelligence. Configuring the closed-loop systems into thin needles facilitates intracranial injection, which open up new opportunities for targeted drug treatment on neurological diseases and for advancing fundamental brain research in understanding cause-and-effect relationships between neural activity and neurological disorders.

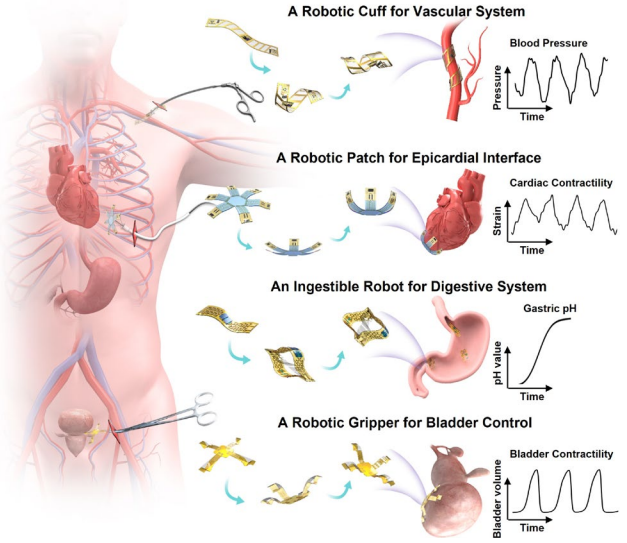

Figure 3. Bio-inspired, sensory robots as minimally invasive smart implants for diagnosis, stimulation, and drug delivery.

Actuating, sensory soft implants. We will explore hybrid integrations between responsive materials and sensing materials to realize intelligent materials and self-autonomy, thereby forming robotic systems for unmet clinical needs in human augmentation and post-operative recovery. Our study is designed to aggregate data that will underpin more advanced "interfaces" such as soft robots as electronic implants (**Fig. 3**). Creating bioinspired somatosensory soft robots as implants holds the promising potential to innovate medical technology, especially in surgery, diagnosis, drug delivery, prostheses, artificial organs, and tissue-mimicking active simulators for rehabilitation. To that end, we will explore the utility of soft robots by utilizing a stimuli-responsive hydrogel, which will not only serve as a primary actuation base but also form a highly biocompatible interface without inducing an immune response. Such motor-sensor integration enables intelligent robotic systems with softness mimicking skin to safely explore and interact with dynamic, unstructured, and often uncertain environments, particularly when robots interface with biological tissues and organs to enable precision therapeutics. The robotic systems will allow the device to be folded inside a thin tube to feature minimally invasive implantation and then deploy actively onto tissue surface to form a conformal interface for optimum sensing and treatment. Our long-term goal is to endow physical intelligence in medical implants to realize precision medicine.

Outlook. The research areas outlined above will expand our understanding of how interfacial integration can leverage collective properties and functionalities from multiple heterogeneous materials involved. Successful outcomes will enable unique device applications in smart healthcare platforms and precision medicine. Funding from the Pew Biomedical Scholarship will enable new equipment purchases and support of graduate students during the scholarship period, letting us launch new efforts to develop next-generation devices that can offer unprecedented healthcare monitoring, tissue/cell diagnosis, and pharmaceutical functionalities. These technologies promise to transform the landscape of medical technology.

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Independent work as PI at UNC Chapel Hill:

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Publication prior to work at UNC Chapel Hill:

30. Di Lu, Shupeng Li, Quansan Yang, Hany M Arafa, Yameng Xu, Ying Yan, Diana Ostojich, **Wubin Bai**, et al, “Implantable, wireless, self-fixing thermal sensors for continuous measurements of

microvascular blood flow in flaps and organ grafts", *Biosensors and Bioelectronics,* **2022**, 206, 114145, **p. 1-11**.

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43. Yoonseok Park, Haiwen Luan, Kyeongha Kwon, Shiwei Zhao, Daniel Franklin, Heling Wang, Hangbo Zhao, **Wubin Bai**, Jong Uk Kim, Wei Lu, Jae-Hwan Kim, Yonggang Huang, Yihui Zhang, John A. Rogers, “Transformable, freestanding 3D mesostructures based on transient materials and mechanical interlocking”, *Advanced Functional Materials*, **2019**, 1903181, , selected as Frontspiece.
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45. Mengdi Han, Heling Wang, Yiyuan Yang, Cunman Liang, **Wubin Bai**, Zheng Yan, Haibo Li, Yeguang Xue, Xinlong Wang, Banu Akar, Hangbo Zhao, Haiwen Luan, Jaeman Lim, Irawati Kandela, Guillermo A. Ameer, Yihui Zhang, Yonggang Huang, John A. Rogers, “Three-dimensional piezoelectric polymer microsystems for multifunctional multistimuli-responsive and in vivo biomechanical energy harvesting”, *Nature Electronics*, **2019**, 2 (1), **p. 26-35**, selected as front cover, highlighted in News & Views, Nature Electronics 2, 15–16 (2019), highlighted by News&Views (*Nature Electronics* 2, 15-16 (2019))
46. Hao Zhang†, Philipp Gutruf†, Kathleen Meacham, Michael C. Montana, Xingyue Zhao, Antonio M. Chiarelli, Abraham Vázquez-Guardado, Aaron Norris, Luyao Lu, Qinglei Guo, Chenkai Xu, Yixin Wu, Hangbo Zhao, Xin Ning, **Wubin Bai**, Irawati Kandela, Chad R. Haney, Debashis Chanda, Robert W. Gereau IV, John A. Rogers, “Wireless, Battery-free Optoelectronic Systems as Subdermal Implants for Tissue Oximetry”, *Science Advances*, **2019**, 5 (3), eaaw0873, **p. 1-13**.
47. Xueju Wang, Xiaogang Guo, Jilong Ye, Ning Zheng, Punit Kohli, Yi Zhang, Zhaoqian Xie, Haiwen Luan, Dongwhi Choi, Kewang Nan, Qihui Zhang, Yameng Xu, Xiwei Shan, **Wubin Bai**, Zizheng Wang, Bong Hoon Kim, Hokyung Jang, Fan Zhang, Yinji Ma, Zheng Xu, Xue Feng, Tao Xie, Yonggang Huang, Yihui Zhang, and John A. Rogers, “Freestanding 3D Mesostructures, Functional Devices and Shape-Programmable Systems based on Mechanically Induced Assembly with Shape Memory Polymers”, *Advanced Materials*, **2019**, 31 (2), 1805615, **p. 1-9**.
48. **Wubin Bai**†, Hongjun Yang†, Yinji Ma, Hao Chen, Jiho Shin, Yonghao Liu, Quansan Yang, Irawati Kandela, Zhonghe Liu, Seung-Kyun Kang, Chen Wei, Chad R. Haney, Anlil Brikha, Xiaochen Ge, Xue Feng, Paul Braun, Yonggang Huang, Weidong Zhou, John A. Rogers, “Flexible Silicon-based Transient Optical Waveguide and Surface-wave Biosensor”, *Advanced Materials*, **2018**, 30 (32), 1801584, **p. 1-12**. selected as Frontspiece.
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51. Li-Chen Cheng, **Wubin Bai**, Eduardo Martin, Kun-Hua Tu, Konstantinos Ntetsikas, George Liontos, Apostolos Avgeropoulos, Caroline Ross, “Morphology, directed self-assembly and pattern transfer

from a high molecular weight polystyrene-block-poly(dimethylsiloxane) block copolymer film", *Nanotechnology*, **2017**, 28 (14), 145301, **p. 1-11**.

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53. **Wubin Bai**, Caroline A. Ross, "Functional Nanostructured Materials Based on Self-assembly of Block Copolymers", *MRS Bulletin*, **2016**, 41 (02), **p. 100-107**,
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56. **Wubin Bai**†, Karim Gadelrab†, Alfredo Alexander-Katz, Caroline A. Ross, "Perpendicular Block Copolymer Microdomains in High Aspect-Ratio Templates", *Nano Letters*, **2015**, 15 (10), **p. 6901–6908**.
57. **Wubin Bai**†, Kevin Yager†, Caroline A. Ross, "In Situ Real-time Characterization of PS-PDMS block copolymer self-assembly during solvent vapor annealing", *Macromolecules,* **2015**, 48 (23), **p. 8574–8584**.
58. **Wubin Bai**, Adam Hannon, Kevin Gotrik, Hong Kyoon Choi, Karim Aissou, George Liontos, Konstantinos Ntetsikas, Alfredo Alexander-Katz, Apostolos Avgeropoulos, Caroline A. Ross, "Thin Film Morphology of Bulk-Gyroid Polystyrene-block-polydimethylsiloxane under Solvent Vapor Annealing", *Macromolecules*, **2014**, 47 (17), **p. 6000–6008**
59. Christine C. Kathrein, **Wubin Bai**, Jean Anne Currivan Incorvia, George Liontos, Konstantinos Ntetsikas, Apostolos Avgeropoulos, Alexander Böker, Larisa Tsarkova, and Caroline A. Ross, "Combining Graphoepitaxy and Electric Fields towards Uniaxial Alignment of Solvent-annealed Poly(styrene)-b-Poly(dimethylsiloxane) Block Copolymer", *Chemistry of Materials*, **2015**, 27 (19), **p. 6890–6898**
60. Kun-Hua Tu, **Wubin Bai**, George Liontos, Konstantinos Ntetsikas, Apostolos Avgeropoulos, Caroline Ross, "Universal Pattern Transfer Methods for Metal Nanostructures by Block Copolymer Lithography", *Nanotechnology*, **2015**, 26 (37), 375301, **p. 1-12**.
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62. Adam F. Hannon, **Wubin Bai**, Alfredo Alexander-Katz and Caroline A. Ross, "Simulation Methods for Solvent Vapor Annealing of Block Copolymer Thin Films", *Soft Matter*, **2015**, 11 (19), **p. 3794-3805**
63. Kevin Gotrik, Thomas Lam, Adam Hannon, **Wubin Bai**, Yi Ding, Jonathan Winterstein, Alfredo Alexander-Katz, J. Alexander Liddle, Caroline A. Ross, "3D TEM Tomography of Templated Bilayer Films of Block Copolymers", *Advanced Functional Materials*, **2014**, 24 (48), **p. 7689-7697**
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65. Caroline A. Ross, Kevin Gotrik, Hong Kyoon Choi, Karim Aissou, Adam Hannon and **Wubin Bai**, "Self-assembling polymer patterns could shrink lithographic limits", *SPIE Newsroom*, 30, January 2013, **p. 1-2**.
66. **Wubin Bai**, Yen-Chih Lin, Tzon-Kun Hou, and Tzay-Ming Hong, "Scaling Relation for a compact crumpled thin sheet", *Phys. Rev. E.* **2010**, 82.066112, **p. 1-5**.

Patent

Produced at UNC Chapel Hill:

1. **Wubin Bai, Lin Zhang,** "Skin-inspired, sensory robots for electronic implants", provisional patent, No. 035052/602726: filed on Oct. 13, 2023.

2. **Wubin Bai, Yihang Wang,** “Wearable Apparatus for Deep Tissue Sensing and Digital Automation of Drug Delivery”, provisional patent, No. 63/343,888, filed on May 19, 2022.
3. **Wubin Bai**, **Yihan Liu,** “Wearable Apparatus for Deep Tissue Sensing and Drug Delivery”, provisional patent, No. 63/343,888; filed on May 19, 2022.
4. **Wubin Bai, Lin Zhang,** “3D Morphable Systems via Deterministic Microfolding for Vibrational Sensing, Robotic Implants, and Reconfigurable Telecommunication”, provisional patent, No. 63/380,355; filed on Oct. 20, 2022.
5. **Arjun Putcha, Gage Ellis, Kevin Schichlein, Bryson Wicker, Wubin Bai**, Andrea Giovannucci, “A Pulse Oximeter with Increased Accuracy for All Skin Pigmentations”, provisional patent, No. 63/394,941; filed on Aug. 3, 2022.

Produced prior to arrival at UNC Chapel Hill:

6. John Rogers, Changsheng Wu, Di Lu, **Wubin Bai**, Hexia Guo, Shuo Li, Hany Arafa, "Wireless probes for monitoring blood flow in biological tissue", US Patent App. 18/249,880, 2023

Invited Lectures

1. **Wubin Bai**, “Unconventional 3D Systems for Neuron Communications”, Workshop: In Vitro Neural Platforms: Development and Integration into Health Care, Chapel Hill, NC, Oct. 2, 2023
2. **Wubin Bai**, “3D Morphable Electronic Systems via Deterministic Microfolding”, 243rd ECS meeting, Boston, MA, June 2023
3. **Wubin Bai**, “3D Morphable Electronic Systems via Deterministic Microfolding”, UNC-CMP Seminar, University of North Carolina at Chapel Hill, January 2023
4. **Wubin Bai**, “3D Morphable Electronic Systems via Deterministic Microfolding”, International Conference on Flexible Electronics (ICFE) 2022, Online webinar, December 2022
5. **Wubin Bai**, “3D Morphable Electronic Systems via Deterministic Microfolding”, IEEE Forum “Nanotechnology for Soft Electronics”, Raleigh, December 2022
6. **Wubin Bai**, “Novel Drug Delivery Devices and Sensors”, UNC Eshelman School of Pharmacy, Chapel Hill, November 2022
7. **Wubin Bai**, “3D Morphable Electronic Systems via Deterministic Microfolding”, Materials Research Society Fall Meeting, Boston, November 2022
8. **Wubin Bai**, “3D Morphable Electronic Systems via Deterministic Microfolding”, LabLinks, Cell Press, NC State University, October 2022
9. **Wubin Bai**, “Materials Integration in Bioelectronics for Smart Health”, NC State University, ASSIST Center, December 2021
10. **Wubin Bai**, “3D Electronic Sccafold for Nerual Interface”, Neuroscience center, UNC Chapel Hill, December 2021
11. **Wubin Bai**, “Heterogeneous integration of bioelectronic materials for smart health”, Fudan University, MSE, December 2021
12. **Wubin Bai**, “Heterogeneous integration of bioelectronic materials for smart health”, NC State ECE Colloquia, September 2021
13. **Wubin Bai**, “Bioresorbable materials and integration as implantable electronics for smart health”, American Chemistry Society, Fall meeting, 2021, Division of Analytical Chemistry, Session: Advancements in Implantable and Wearable Technologies
14. **Wubin Bai**, “Bioelectronics for Advanced Diagnostics and Therapeutics”, FastTraC Clinical Advisory Meeting, UNC Chapel Hill, 2021
15. **Wubin Bai**, “Transient Materials and Bioelectronics for Advanced Healthcare”, Seminar, February 2020, University of North Carolina at Chapel Hill
16. **Wubin Bai**, “Bioresorbable Electronic and Photonic Systems for Healthcare, Neurological Monitoring, and Metabolic Tracking”, Seminar, March 2019, Rice University
17. **Wubin Bai**, “Bioresorbable Electronic and Photonic Systems for Healthcare, Neurological Monitoring, and Metabolic Tracking”, Seminar, February 2019, UMass Amherst
18. **Wubin Bai**, “Block Copolymer Self-Assembly and Nanostructure fabrication”, Seminar, February 2016, UIUC

19. **Wubin Bai**, “Block Copolymer Self-Assembly and Nanostructure fabrication”, Seminar, January 2016, Applied Materials, Inc, Santa Clara, CA
20. **Wubin Bai**, “Block Copolymer Self-Assembly and Nanostructure fabrication”, Seminar, January 2016, UCSB
21. **Wubin Bai**, Kevin Yager, Caroline A. Ross, “Real-Time observation of PS-PDMS block copolymer self-assembly under solvent vapor annealing” APS March Meeting 2015, Volume 60, Number 1, highlighted in the APS March Meeting 2015 media gallery
22. Christine Kathrein, **Wubin Bai,** Larisa Tsarkova, Tao Huang, Apostolos Avgeropoulos, Alexander Boker, Caroline A. Ross, “Combining Graphoepitaxy and Electric Fields towards Uniaxial Alignment of Solvent-annealed Cylinder forming Poly(styrene)-b-poly(dimethylsiloxane) block copolymers” APS March Meeting 2015, Volume 60, Number 1
23. **Wubin Bai**, Kevin Yager, Caroline A. Ross, “Real-Time Observation of PS-PDMS Block Copolymer Self-Assembly under Solvent Vapor Annealing”, MRS Fall Meeting 2014, KK3.02: Directed Self Assembly for Nanopatterning- Simulations and Measurements
24. Melissa Kreider, **Wubin Bai**, George Liontos, Konstantinos Ntetsikas, Apostolos Avgeropoulos, Caroline Ross, “Use of UV-Solvent Annealing for Morphology and Orientation Control in Self-Assembled PS-PDMS Thin Films”, MRS Fall Meeting 2014, KK5: Directed Self-Assembly for Nanopatterning
25. **Wubin Bai**, Caroline A. Ross, Karl Berggren, Amir Tavakkoli, “Block Copolymer Nanolithography”, NMS Patterning Review, September 21st, 2014, University of Chicago
26. **Wubin Bai**, Adam Hannon, Kevin Gotrik, Karim Aissou, Hong Kyoon Choi, George Liontos, Konstantinos Ntetsikas, Alfredo Alexander-Katz, Apostolos Avgeropoulos, Caroline A. Ross, “Thin Film Morphology of a Bulk-Gyroid Block Copolymer”, Poster, Pall Corporation Poster Session, April 1st, 2014, MIT
27. **Wubin Bai**, Adam Hannon, Kevin Gotrik, Karim Aissou, Hong Kyoon Choi, George Liontos, Konstantinos Ntetsikas, Alfredo Alexander-Katz, Apostolos Avgeropoulos, Caroline Ross, “Thin Film Morphology of a Bulk-Gyroid Block Copolymer”, APS Spring Meeting 2014,
28. **Wubin Bai**, Adam Hannon, Kevin Gotrik, Karim Aissou, Hong Kyoon Choi, George Liontos, Konstantinos Ntetsikas, Alfredo Alexander-Katz, Apostolos Avgeropoulos, Caroline Ross, “Thin Film Morphology of a Bulk-Gyroid Block Copolymer”, MRS Fall Meeting 2013, WW7: Soft Materials III.
29. **Wubin Bai**, Kevin Gotrik, Adam Hannon, Alfredo Alexander-Katz, Apostolos Avgeropoulos, Caroline Ross, “Thickness and Confinement Effects on the Morphology of Gyroid PS-PDMS Thin Films”, APS March Meeting 2013, Volume 58, No. 1.
30. Kevin Gotrik, Thomas Lam, **Wubin Bai**, Adam Hannon, J. Alexander Liddle, Caroline Ross, “3D TEM Tomography of Bilayer Diblock Copolymer Thin Films”, APS March Meeting 2013 Volume58, No. 1

Teaching Activities

A. Courses

	Course name	Course #	# Students	Semester
1.	Advances in Drug Delivery (as guest lecturer)	DPMP 864	11	Fall 2023
2.	Advanced Materials Sciences	MTSC 780	7	Fall 2023
3.	Introduction to Design and Making: Developing Your Personal Design Potential	APPL 110	69	Spring 2023
4.	Advanced Materials Sciences	MTSC 780	10	Fall 2022
5.	Advances in Drug Delivery (as guest lecturer)	DPMP 864	12	Fall 2022
6.	Introduction to Design and Making: Developing Your Personal Design Potential	APPL 110	70	Spring 2022
7.	Introduction to Design and Making: Developing Your Personal Design Potential	APPL 110	49	Fall 2021
8.	Introduction to Design and Making: Developing Your Personal Design Potential (as assistant instructor)	APPL 110	57	Spring 2021

B. Graduate Students:

	Name	Education	Thesis title	Completion date
1.	Yihan Liu	B.S. Zhejiang University; M.S. Northwestern University	Flexible Wearable Near-Infrared Spectroscopy (NIRS) as a Monitor in Physiological Activities	2025 (anticipated)
2.	Yihang Wang	B.S. Nankai University	Interfacing strategies of neural electronics	2026 (anticipated)
3.	Arjun Putcha	B.S. UNC Chapel Hill	Intelligent Systems for Muscle Tracking	2027 (anticipated)
4.	Wanrong Xie	B.S. Beijing University of Chemical Technology; M.S. Duke University	Wearables for biosensing and Thermal modulation	2027 (anticipated)
5.	Anran Zhang	B.S., China University of Geosciences; M.S., South China University of Technology	Self-healing Bioelectronics	2028 (anticipated)

C. Postdoctoral Scholars:

1. Xun Xiao, Ph.D from UNC Chapel Hill w/Prof. Jinsong Huang (Jun. 2021 – Aug. 2021)
2. Lin Zhang, Ph.D from Villanova Univ., w/Prof. Gang Feng (Jan. 2021 – Present)
3. Yizhang Wu, Ph.D from Nanjing Univ., w/Prof. Xiaoshan Wu (Mar. 2023 – Present)
4. Hyeok-jin Kwon, Ph.D from Pohang Univ. of Sci. & Tech., w/Prof. Se Hyun Kim (Mar. 2023 – Present)

D. Undergraduate Researchers:

The undergraduate researchers represent a strong momentum for our research pursuit. So far, five of our undergraduate researchers have been awarded Abrams scholarships, one with Sheera Scholarship, one with Chancellor's Scholarship, and one with a summer scholarship at NIH. Five of our undergraduate researchers formed a team that has been enrolled in the i4 competition with an initial success being selected as a finalist. All of the undergraduate researchers have shown strong aspirations toward our research with different levels of engagements to fulfill their career explorations.

	Name	Major, Affliation, Class year, fellowship/credits	Duration (months)	Project	Talk/poster	Paper
1	Rahul Menon	UNC Chapel Hill, B.S. Biology, Class 2022, BIOL 395 credits	6	Wearable patch	Und. Res. Sym.	Adv. Mat. Tec, 2200468, 2022
2	Hannah Weisbecker	UNC Chapel Hill, B.S. Biology, Minors: Chemistry and Spanish, Class of 2022	13	3D systems	Und. Res. Sym.	Sci. Adv., 2022,
3	Michael DeMaesschalck	UNC Chapel Hill, B.S. Chemistry, Class 2023	6	Hydrogel sensor	Und. Res. Sym.	in preparation
4	Gage Ellis	UNC Chapel Hill, B.S. Biomedical Engineering, Class 2022	6	Pulse oximeter	i4 competition	Adv. Mat. Tec, 2200468, 2022
5	Junye Li	UNC Chapel Hill, B.S. Biomedical Engineering, Class 2023	12	Wearable patch	Und. Res. Sym.	Adv. Mat. Tec, 2200468, 2022

6	Rohan Vora	UNC Chapel Hill, B.S. Biomedical Engineering, Class 2024, Abrams scholar	8	Wearable patch	Abrams program	Adv. Mat. Tec, 2200468, 2022
7	Arjun Putcha	UNC Chapel Hill, B.S. Biomedical Engineering, Class 2022	14	Pulse oximeter	i4 competitio n	Adv. Fun. Mat., 2200468, 2022; Adv. Int. Sys, 2022, under review
8	Hiep Tran	UNC Chapel Hill, B.S. Biomedical Engineering, Class 2023, Sheerer Scholar	15	Hydrogel sensor	Sheerer program	in preparation
9	Terry Han	NC State, B.S. Biomedical Engineering, Class 2023, BME 495 credits	15	Hydrogel sensor	Und. Res. Sym.	Sci. Adv., 2022,
10	Lauren Fletcher	UNC Chapel Hill, B.S. Quantitative Biology, Class 2023	3	Wearable patch		Adv. Mat. Tec, 2200468, 2022
11	Litao Tu	UNC Chapel Hill, B.S. Biology and Chemistry, Class 2023, BIOL 395 credits	4	Hydrogel sensor	Und. Res. Sym.	in preparation
12	Jean Okonkwo	UNC Chapel Hill, B.S. Chemistry, Class 2021, CHEM 395 credits	5	Hydrogel sensor	Und. Res. Sym.	
13	Avanti Panajkar	North Carolina State University, B.S. Biomedical Engineering, Class 2024, Abrams scholar	5	Hydrogel robot	Abrams program	
14	Anna Lassiter	University of North Carolina at Chapel Hill, B.S., Applied Math, Class 2024	5	Wearable patch		Adv. Mat. Tec, 2200468, 2022
15	Ziheng Guo	UNC Chapel Hill, B.S., Biomedical Engineering, Class 2024	10	Hydrogel robot		Sci. Adv., 2022,
16	Amber Huang	UNC Chapel Hill, B.S., Biology, Class 2023, BIOL 395 credits	10	Breath sensor	Und. Res. Sym.	in preparation
17	Tian Wang	UNC Chapel Hill, B.S., Biomedical Engineering, Class 2024	11	Drug delivery patch		in preparation
18	Brayden Davis	UNC Chapel Hill, B.S., Biomedical Engineering, Class 2025	13	Drug delivery patch	Und. Res. Sym.	in preparation
19	Sicheng Xing	UNC Chapel Hill, B.S., Biomedical Engineering, Class 2025, Abrams scholar	13	Hydrogel robot	Abrams program	in preparation
20	Will Lipman	UNC Chapel Hill, B.S., Neuroscience, Class 2025, NSCI 395 credits	10	HotPocket		
21	Chris Nguyen	UNC Chapel Hill, B.S., Biomedical Engineering, Class of 2025	10	Wearable patch	Und. Res. Sym.	
22	Brynn Brusseau	UNC Chapel Hill, B.S., Biology and Global Studies, Class of 2024	4	Hydrogel robot		in preparation
23	Jennifer Sieredzki	UNC Chapel Hill, B.S., Biology, Class of 2023, BIOL 495 credits	6	Hydrogel robot	Und. Res. Sym.	

24	Beth Schalk	UNC Chapel Hill, B.S. Chemistry and Neuroscience, Class of 2024	3	Breath sensor		
25	Tien Anh Nguyen	UNC Chapel Hill, B.S., Biomedical Engineering, Class of 2023, Abrams scholar	5	Pulse oximeter	Abrams program	Adv. Int. Sys, 2023
26	Noah Lambert	UNC Chapel Hill, B.S., Biomedical Engineering, Class of 2026	3	Breath sensor		
27	Krystal Luo	UNC Chapel Hill, B.S., Biomedical Engineering, Class of 2025	3	Hydrogel robot		
28	Rachel Choffin	UNC Chapel Hill, B.S., Clinical Laboratory Science, Class of 2025	11	Wearable muscle tracking	Summer award for research	Adv. Int. Sys, 2023
29	Maya Yun	UNC Chapel Hill, B.S., Psychology, Class of 2025	4	Hydrogel patch for wound healing		
30	Morgan Gilbert	UNC Chapel Hill, B.S., Biomedical Engineering, Class of 2025	3	Wearable thermal regulator		
31	Jett Messenger	Purdue University, B.S., Biomedical Engineering, Class of 2025	3	Neural interface	Summer internship	
32	Nanqi Peng	UNC Chapel Hill, B.S., Biomedical Engineering, Class of 2025	3	Wearable oximeters	Und. Res. Sym.	
33	Renee (Yiyao) Wang	Mount Holyoke College, Class of 2024	3	Wearable thermal regulator	Summer internship	

E. Research Technicians

1.	Chuqi Huang	B.S. Huazhong Univ. of Sci. and Tech., M.S. Cornell University	2021-2022
2.	Arjun Putcha	B.S. UNC Chapel Hill	2021-2022
3.	Hannah Weisbecker	B.S. UNC Chapel Hill	2022-present
4	Tien Anh Nguyen	B.S. UNC Chapel Hill	2023-present

F. High School Researchers

1.	Chris You	Chapel Hill High School	2023-present
2.	Henry Wang	Chapel Hill High School	2023-present
3.	Michelle Lan	East Chapel Hill High School	2023-present
4.	Ruiyang Wu	Carry Academy	2023-present

Grants

Ongoing:

1. Funding source: Communications, Circuits, and Sensing-Systems (CCSS), National Science Foundation (NSF)
   Title: 3D bionic network as a closed-loop interface for bidirectional communication with cells and tissues
   Role: Principal Investigator
   Amount: $391,301.00
   Duration: 07/01/2022 – 06/30/2025
   Percentage of effort: 10%

Percentage of grant to my group: 100%

2. Funding source: National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institute of Health (NIH)
   Title: Wearable, Wireless Deep-tissue Sensing Patch for Continuous Monitoring of Recovery from Microsurgical tissue Transfer
   Role: Principal Investigator
   Amount: $1,741,404.00
   Duration: 07/01/2023 – 06/30/2028
   Percentage of effort: 30%
   Percentage of grant to my group: ~95%
3. Funding source: ICS AGILE Award, Institute for Convergent Science, UNC Chapel Hill
   Title: HotPockets: Novel devices for non-destructive, long-term electrophysiological recording and stimulation of entire human brain organoids
   Role: Principal Investigator
   Amount: $151,996.00
   Duration: 10/01/2022 – 10/01/2024
   Percentage of effort: 10%
   Percentage of grant to my group: ~70%
4. Funding source: North Carolina Biotechnology Center
   Title: HotPockets: Novel devices for non-destructive, long-term electrophysiological recording and stimulation of entire human brain organoids
   Role: Co-Principal Investigator
   Amount: $20,000.00
   Duration: 1/13/2024 – 1/12/2025
   Percentage of effort: 10%
   Percentage of grant to my group: ~50%
5. Funding source: North Carolina Biotechnology Center
   Title: Evaluation of MABOS: Melanin-Adjusted Blood Oxygen Sensor
   Role: Principal Investigator
   Amount: $110,000.00
   Duration: 1/13/2024 – 1/12/2026
   Percentage of effort: 10%
   Percentage of grant to my group: ~80%
6. Funding source: NeuroSpark pilot award, UNC Chapel Hill
   Title: Digital automation of synergistic, long-term drug delivery for treating Alzheimer’s disease
   Role: Principal Investigator
   Amount: $25,000.00
   Duration: 1/13/2024 – 1/12/2025
   Percentage of effort: 10%
   Percentage of grant to my group: ~80%

Finished:

1. Funding source: NC TraCS
   Title: Non-invasive optoelectronic systems for diabetes health monitoring at the skin interface
   Role: Principal Investigator
   Amount: $25,000.00
   Duration: 9/01/2022 – 9/01/2023
   Percentage of effort: 10%
   Percentage of grant to my group: ~90%
2. Funding source: Creativity Hubs, Finalist, UNC Chapel Hill
   Title: Enhancing brain organoids for high throughput screening of personalized therapeutics
   Role: Co - Principal Investigator
   Amount: $5,000
   Duration: 01/01/2022 – 04/08/2022

Percentage of effort: 5%
Percentage of grant to my group: ~30%

3. Funding source: NeuroSpark pilot award, UNC Chapel Hill
   Title: CLOZiPS: Mechanisms of clozapine response using iPSC-derived organoids
   Role: Co-Investigator
   Amount: $20,000
   Duration: 01/23/2022 – 01/23/2023
   Percentage of effort: 5%
   Percentage of grant to my group: ~20%
4. Funding source: NC TraCS
   Title: Non-invasive optoelectronic systems for diabetes health monitoring at the skin interface
   Role: Principal Investigator
   Amount: $50,000
   Duration: 01/17/2023 – 01/17/2024
   Percentage of effort: 5%
   Percentage of grant to my group: 100%
5. Funding source: QSIB funding
   Title: Wireless wearable patch for personalized phototherapy
   Role: Principal Investigator
   Amount: $15,000
   Duration: 06/07/2010 – 12/20/2020
   Percentage of effort: 10%
   Percentage of grant to my group: 100%
6. Funding source: Northwestern CAMI pilot funding
   Title: Wireless and Battery-free Transient Devices for Discovery in Neuroscience
   Role: Co-investigator
   Amount: $5,000
   Duration: 06/01/2019 – 12/15/2019
   Percentage of effort: 10%
   Percentage of grant to my group: 100%
7. Funding source: MIT Madmec
   Title: Block Copolymer as Flexible Photodetector
   Role: Principal investigator
   Amount: $2,000
   Duration: 03/01/2014 – 06/01/2014
   Percentage of effort: 10%
   Percentage of grant to my group: 100%

Pending:

1. Funding source: National Heart, Lung, and Blood Institute (NHLBI), National Institute of Health (NIH)
   Title: Soft, sensory robots as electronic implants for monitoring and treating cardiovascular diseases
   Role: Principal Investigator
   Amount: $1,907,887.00
   Duration: 04/01/2024 – 06/30/2028
   Percentage of effort: 30%
   Percentage of grant to my group: ~90%
2. Funding source: National Institute of Neurological Disorders (NINDS), National Institute of Health (NIH)
   Title: HotPockets: Novel devices for non-destructive, long-term electrophysiological recording and stimulation of entire human brain organoids
   Role: Principal Investigator
   Amount: $ 3,674,231.48
   Duration: 10/30/2024 – 10/30/2029
   Percentage of effort: 30%
   Percentage of grant to my group: ~55%

3. Funding source: National Heart, Lung, and Blood Institute (NHLBI), National Institute of Health (NIH)
   Title: Somatosensory Injectable Robot for Advanced Cardiac Monitoring and Therapy
   Role: Principal Investigator
   Amount: $ 2,476,810.000
   Duration: 7/1/2024 – 6/30/2029
   Percentage of effort: 30%
   Percentage of grant to my group: ~70%

4. Funding source: Department of Defense
   Title: Wearable, Wireless Deep-tissue Sensing Patch for Continuous Monitoring of Recovery from Microsurgical Free-tissue Transfer
   Role: Principal Investigator
   Amount: $ 995,228.000
   Duration: 7/1/2024 – 6/30/2027
   Percentage of effort: 30%
   Percentage of grant to my group: ~90%

5. Funding source: National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institute of Health (NIH)
   Title: A High-precision Wearable Multi-Wavelength Oximeter that Overcomes Confounding Effects from Skin tone
   Role: Principal Investigator
   Amount: $ 2,307,887.00
   Duration: 1/1/2025 – 1/1/2029
   Percentage of effort: 30%
   Percentage of grant to my group: ~70%

Professional Services

A. Departmental

Talent Search Committee	2021 - 2023
Graduate Recruiting Committee	2023 – Present

Preliminary Oral Exam Committees (excludes own Ph.D students):

Daixuan Zhang, member	PI: Sergei Sheiko	Nov., 2020
Haoyang Jiao, member	PI: Jinsong Huang	Mar., 2021
Xiaowei Zhong, member	PI: Wei You	Feb., 2022
Jordan Shanahan, member	PI: Wei You	Aug., 2022
Yuan Gao, Chair	PI: Ronit Freeman	Nov., 2022
Hossein Eybposh, member	PI: Nicolas Pégard	Dec., 2022
Xiaoqiang Shi, chair	PI: Jinsong Huang	April. 2024
Josiah H. Marshall, member	PI: Sergei Sheiki & Wei You	Apr. 2023
Vincent R. Curtis, member	PI: Nicolas Pégard	Nov. 2023
Mingze Li, chair	PI: Jinsong Huang	Feb. 2024
Kameryn Hinton, member	PI: Ronit Freeman	May 2023
Quincy Snyder, member	PI: Ronit Freeman	May 2024
Anzhou Wen, member	PI: Nicolas Pégard	May 2024
Hyunjung Lee, member	PI: James Cahoon	May 2024

Dissertation Defense Committees:

Daixuan Zhang, member	PI: Sergei Sheiko	Jun., 2021

Seminar hosting:

Dr. Guillermo A. Ameer	Northwestern University	Sep., 2022
Dr. John A. Rogers	Northwestern University	Dec. 2022

Dr. Michael Cima	Massachusetts Institute of Technology	April, 2023
Dr. Dae-Hyeong Kim	Seoul National University	July, 2023

B. University

Faculty Information Technology	2021 - Present
CHANL Advisory Board	2023 - Present
Sleight of Hand, Faculty Advisor	2023 - Present

C. National & International

- NSF Review Panel, Integrative Strategies for Understanding Neural and Cognitive Systems (NCS) program, 2023, invited reviewer.
- NIH Study Section ZRG1 ISB-S (11) B 2024/05 Council, invited reviewer.
- NIH Study Section ZRG1 ISB-S (11) B 2024/01 Council, invited reviewer.
- NIH Study Section BBBT-M (82) 2023/01 Council, invited reviewer.
- Samsung Electronics, SRFIC Review Panel, invited reviewers, 2023.
- Gordon Research Conferences, Nanostructure Fabrication, Student Organizer, Biddeford ME. July 2014.
- Special Issue Editor, "Advanced Technologies for Implantable Brain-Computer Interface", Cyborg and Bionic Systems, a Science Partner Journal, 2023
- Editorial Board Member, Discover Electronics, Springer Nature
- Associate editor, Cureus Journal of Engineering, Springer Nature
- Topic Editor, Micromachine, MDPI
- Special Issue Editor, "Bioelectronic Materials and Systems for Smart Healthcare", Micromachine, MDPI
- Special Issue Editor, " Advanced Biosensing Systems: Design, Fabrication and Application", Micromachine, MDPI
- Topic Editor, Body-interfaced Electronics for Noninvasive Monitoring and Stimulating, Frontiers in Bioelectronics
- Member, American Chemical Society
- Member, Biomedical Engineering Society
- Journal Reviewer (>70 papers as of Dec. 2021): Nature Nanotechnology, Nature Electronics, ACS Nano, Small, Advanced Materials, Nanotechnology, Progress in Polymer Science, Smart Materials and Structures, Materials Chemistry and Physics, Applied Surface Science, Optica, Soft Matter, Biosensors & Bioelectronics, Advanced Fiber Materials, Nano Select, Nature Communications, Science Advances, etc.

PI: Berlow, Rebecca (Pages 61-74)

UNC SCHOOL OF MEDICINE

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

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March 19, 2024

Office of Research Development Internal Selection Committee

Dear Colleagues,

It is my great pleasure to enthusiastically recommend **Rebecca B. Berlow**, PhD, for the 2025 Pew Scholars Program in the Biomedical Sciences. Dr. Berlow was recruited to the Department of Biochemistry and Biophysics as a tenure-track Assistant Professor in January 2022. After a national search that attracted 168 qualified candidates, she was the top candidate and was unanimously recommended for the position by the Full Professors of the Department. She was an outstanding graduate student at Yale University, and an exemplary scientist as a postdoctoral fellow and staff scientist at The Scripps Research Institute in La Jolla, California. Her work addresses *fundamental questions about the role of intrinsically disordered proteins (IDPs) in the regulation of cellular signaling pathways*. IDPs are the flexible and responsive components of biological systems that respond to internal and external signals, and they are critical to all aspects of molecular and cell biology, yet they arguably remain part of the "dark matter" of protein biophysics. Despite tremendous advances in structure-function studies of stable and ordered protein domains, intrinsic disorder in proteins is still poorly understood because these regions do not adopt a single shape amenable to current technology. Dr. Berlow's work is pioneering for understanding IDP structures and their biological functions. Achieving this understanding has broad implications for human health because a great many proteins involved in development, cell signaling, proliferation, homeostasis, and disease have large regions of disorder.

Dr. Berlow earned her PhD in 2011 from Yale University under the direction of J Patrick Loria, PhD, a biophysicist in the Chemistry Department. During her graduate studies, she addressed questions about the role of molecular motion in large enzyme systems. She developed methods and applied solution nuclear magnetic resonance (NMR) spectroscopy using a model enzyme to show how specific amino acids in the active site modulate enzyme function. She also studied DNA polymerase β (Pol β) enzyme-substrate recognition using NMR technology and showed that cancer-causing mutations lead to DNA copying errors – a finding relevant to cancer therapeutics. Her graduate school studies were published in high-quality peer-reviewed journals (*Biochemistry*, *J Molecular Biology*), and in a review article in *Accounts of Chemical Research*. Dr. Loria states in a letter on her behalf that she is "undaunted by the challenges that come from studying new systems" and that she is "smart, thorough, creative, hardworking and intelligent", and that "what sets her apart" is her "independence and her deep-thinking". He states she was "in the *top 1% of all graduate students* that I have encountered in nearly 21 years at Yale" (emphasis mine).

Dr. Berlow's post-doctoral research at the Scripps Research Institute is even more impressive than her graduate work. She worked under the mentorship of Peter E. Wright, a renowned NMR spectroscopist, who holds the Green Chair of Biomedical Research at Scripps and is a member of the National Academy of Sciences. Here Dr. Berlow built on her expertise in NMR spectroscopy and focused on intrinsically disordered proteins (IDPs) and allostery to uncover molecular mechanisms important in biological function. One of her most important contributions relates to the signaling pathway that downregulates the hypoxic response. By studying the interaction of several proteins (e.g., CITED2, HIF-1α, TAZ1) she discovered a unidirectional switch that attenuates the hypoxic response. Dr. Wright wrote in a letter on her behalf that "the work led to a *paradigm shift* in our understanding of the molecular mechanisms by which IDPs regulate cellular signaling". The work was published in *Nature*, *Biochemistry*, and *PNAS*, and has therapeutic implications because the hypoxic response is a distinguishing feature of cancer, blinding eye diseases, and other disorders. Moreover,

her studies of disorder in these particular disease-relevant proteins is *already establishing new principles for understanding IDPs in general*.

Her findings then led to an entirely independent collaboration with Dr. Martin Friedlander at Scripps who studies macular degeneration and other hypoxia-driven eye abnormalities. Dr. Berlow instigated the collaboration which led to her identification of a peptide that can modulate hypoxia signaling pathways in the eye (*in vivo*), and it has great potential to treat ischemic retinal diseases and other neovascular disorders. This work illustrates her unique strengths – to see broad implications of her work, initiate studies outside of her specific area of expertise, and collaborate with experts in totally different scientific areas.

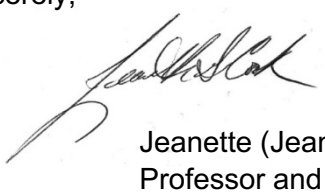
Dr. Berlow has now designed a *highly innovative and potentially transformational proposal*. She will leverage her expertise in analyzing the range of shapes and conformations intrinsically disordered protein domains can adopt to study human proteins that are fully disordered. She will define the various conformations these proteins take and, importantly, link those conformations and the dynamic changes among them to biological function. Not only will success provide key conformation-function information about entirely disordered proteins – about which we currently know almost nothing – but it will also *establish principles* that will be applicable to the great many intrinsically disordered domains that occur in all proteomes, including domains that are key to both normal and disease states. She is uniquely suited to carry out these fundamentally important studies.

Dr. Berlow is already a *leader in the protein science community*. She has had multiple speaking invitations, including at national and international meetings such as the 2024 Biophysical Society meeting and the 2024 Gordon Research Conference on Protein Folding Dynamics where she presented exciting new findings from her independent lab (several corresponding author papers already). She has been a session chair at Gordon Research Conferences, organized an EMBO lecture course in India, is an *ad hoc* reviewer for several high-quality scientific journals and funding agencies, and she is an Associate Editor of *Biophysical Reports*. She organized the 2023 Carolina Biophysics Symposium. She is keenly aware of the need for an inclusive research environment and the strength that diversity brings to our profession. Over her career thus far, she has been involved in programs for high school students, undergraduate students, and junior scientists that encourage students from historically excluded groups to pursue scientific careers. She has a *long-track record of teaching and service* and has sought and completed training workshops to best support trainees' well-being and professional development.

In addition to her intelligence and outstanding training, Dr. Berlow is creative, motivated, ambitious, and persistent. All indications are that she thinks outside of the box, integrates apparently disconnected ideas, and has outstanding communication skills. Since arriving 2 years ago, Dr. Berlow has wasted no time in establishing her lab in the Genetic Medicine Building. She has been popular with first-year graduate students and has already recruited two students for dissertation work. She has also demonstrated a strong commitment to teaching and service. She joined the umbrella first year biomedical PhD program, BBSP as a faculty mentor and instructor, serves on 15 graduate thesis committees, and teaches in three graduate classes.

I strongly recommend Dr. Berlow as the UNC nominee for the Pew Scholars Program in the Biomedical Sciences. The potential impact of her research is truly transformational. The combination of her sophisticated biophysical expertise, her strong background in tackling fundamental mechanistic problems, her ability to take risks, to recognize the broader health and disease implications of her work, and to collaborate bode well for great success in the future.

Sincerely,



Jeanette (Jean) G. Cook, Ph.D.
Professor and Chair of Biochemistry and Biophysics

Leveraging the Power of Protein Dynamics for Fine-Tuning Biology

Protein conformational changes over time (herein referred to as dynamics) challenge the traditional structure-function paradigm, as a snapshot of a protein's structure at any given time may not be representative of the molecule's true functional state(s). Functional protein dynamics underlie nearly every known biological process, but our knowledge of how functional dynamics are encoded by protein sequence is lacking. A better understanding of the relationships between protein sequences, conformational dynamics, and biological function would provide an avenue for viewing the many biological processes that rely on protein dynamics through a new lens, ultimately providing the inspiration for new therapeutic strategies for the many diseases associated with dynamic molecules, including cancer, cardiovascular disease, and neurodegenerative disorders.

Intrinsically disordered proteins (IDPs) are a particularly intriguing class of dynamic macromolecules that have challenged traditional models of protein structure and function due to their innate conformational flexibility and lack of stable, well-defined structure in isolation[1-3]. IDPs play crucial roles in cellular signaling, regulation, and organization and have been implicated in driving the progression of many diseases. Rather than existing in a single state, IDPs exist as an ensemble of available configurations in solution. IDPs function as modular scaffolds for binding and assembly of higher order structures and many IDPs possess unique material properties and can undergo liquid-liquid phase separation. Interestingly, recent studies by our lab and others have identified that the conformational ensembles, dynamics, and functions of IDPs are tunable by the environment.

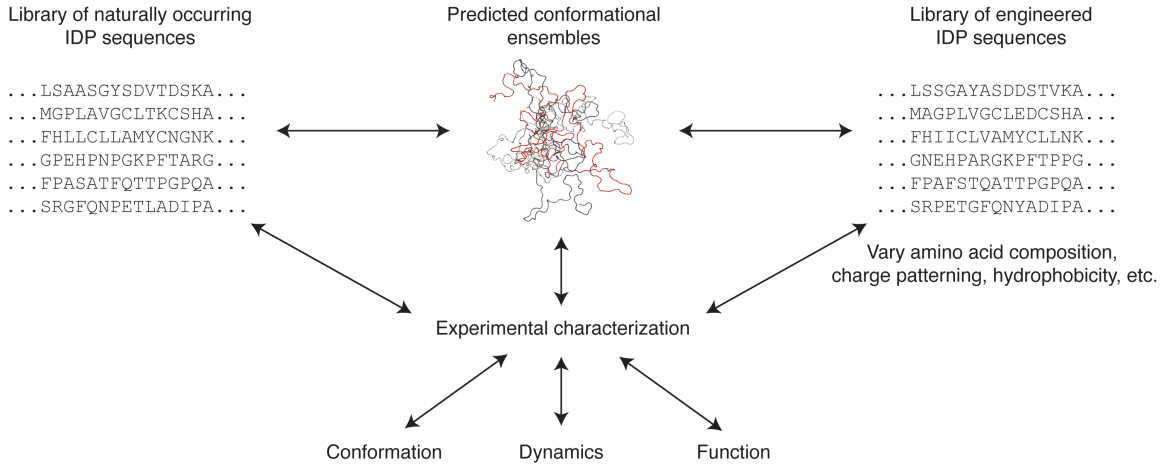
A recent survey identified that 35% of the human proteome is disordered[4]. Our current understanding of IDPs is largely influenced by extensive studies of a small set of model systems, which inherently limits our ability to identify generalizable features across all disordered sequences and biological functions. IDPs can be entirely disordered or can contain regions of disorder flanked by folded domains. In humans, approximately 5% of the proteome is entirely disordered, with ~1300 proteins ranging from 30 to nearly 500 amino acids in length that have known roles in processes central to all aspects of life. Despite the existence of a rich pool of highly dynamic molecules available for investigation, not a single fully disordered protein has been characterized with the goal of relating physicochemical properties to biological function. *I hypothesize that this understudied arm of the disordered proteome holds the key to unlocking the relationships between protein sequence, conformational dynamics, and biological function.* Nature has provided us with a roadmap for how to encode functional protein dynamics, but we have not yet followed it. With support from the Pew Biomedical Scholars program, my laboratory will pursue a new research direction aimed at decoding the molecular determinants for IDP functional dynamics. Our combined theoretical and experimental approach will allow for careful dissection of the mechanisms by which IDPs utilize dynamics to carry out their numerous functions in biology and disease. We will leverage detailed information about IDP functional dynamics to develop new methods for controlling biological outcomes, with an eye towards identifying strategies that harness the unique regulatory abilities of disordered proteins and address current gaps in our ability to modulate the behaviors of dynamic proteins.

Part 1: Building a Scalable Platform for Harnessing the Functional Dynamics of the Understudied Disordered Proteome

Our goal is to establish a scalable platform combining theory and experiment in an iterative process to gain new insights into the complex relationships between protein sequence and functional dynamics (Figure 1), with the ultimate goal of developing predictive models that will streamline processes for identifying and characterizing functional protein dynamics. Recently developed methods for simulating disordered proteins make it possible to simulate the conformational ensembles of large libraries of IDP sequences in a short period of time[4]. These tools, along with algorithms that allow for generation of novel sequences with predicted conformational properties, will allow us to generate a custom library of IDP sequences for experimental investigation. Proof of concept for this approach will be established using a curated library of naturally occurring and engineered IDP sequences that possess a wide range

of physicochemical properties and thus will serve as an outstanding test case for exploring the links between amino acid sequence, dynamics, and biological function on a broad scale. The test library will be constructed from a subset of IDP sequences that: (1) can be expressed and purified from standard culture systems and/or produced by synthetic methods; (2) are amenable to experimental characterization using biophysical techniques capable of reporting on molecular conformation and dynamics; and (3) have known functions that can be characterized through established biochemical assays or phenotypic screens. The experimental data will then be integrated with the results from the theoretical simulations to fully evaluate the relationships between conformational ensembles, dynamics, sequences, and function.

Figure 1. Schematic of proposed platform for decoding protein dynamics from IDP sequences.



Part 2: Developing New Approaches for Modulating the Interactions of Dynamic Molecules

IDPs involved in stress response pathways account for approximately 10% of the fully disordered proteome, but their dynamic interaction landscapes and mechanisms have been overlooked. Given the known roles of IDPs in cellular processes that require the coordinated actions of many macromolecules, it is anticipated that these highly dynamic molecules exist in complex interaction networks. My prior work has identified that disordered proteins utilize a range of cooperative and competitive interactions to regulate transcription and cellular signaling cascades. This behavior is completely reliant on the conformational flexibility of IDPs and dynamic linkage between modular interaction sites and confers IDPs with unique regulatory abilities[5-7]. Thus, *the very properties that confer unique functional roles to IDPs offer excellent inspiration for designing next-generation tools for controlling their behavior.* We will leverage detailed information about the dynamic conformations and interactions of stress-responsive IDPs to develop new methods and molecular tools for controlling biological outcomes. This work will address current gaps in our ability to target dynamic proteins and provide critical insights for designing new molecules that harness the distinct molecular features of disordered proteins.

My laboratory (established in January 2022 in the Department of Biochemistry and Biophysics) is uniquely suited to carry out this work. Our current research projects draw on my extensive training and expertise in experimental methods for characterizing protein dynamics in complex biological systems and proven track record of pursuing new applications for disordered proteins and dynamic polymers. I anticipate that this work will result in the development of new predictive tools and datasets that will benefit the entire scientific community and enable rapid development of new protein-inspired materials with bespoke physicochemical properties and tunable functions.

References: (1) Berlow, R. B., *et al. FEBS Letters*, 2015 (**2**) Holehouse, A. S. & Kragelund, B. B. *Nat Rev Mol Cell Biol,* 2024 (**3**) Moses, D., *et al. Trends in Biochemical Sciences*, 2023 (**4**) Tesei, G. *et al. Nature,* 2024 (**5**) Berlow, R. B., *et al. PNAS*, 2022 (**6**) Usui-Ouchi, A. *et al. PNAS,* 2020 (**7**) Berlow, R. B., *et al. Nature,* 2017.

Rebecca Berlow
External References for the 2024 Pew Biomedical Scholars Program

J. Patrick Loria, PhD
Professor of Chemistry and of Molecular Biophysics and Biochemistry
Yale University
patrick.loria@yale.edu
Thesis Advisor

Dr. Loria is an expert in the development and application of new NMR methods for characterizing protein dynamics and elucidating relationships between protein dynamics and function. His laboratory's research has provided key insights into the role of conformational fluctuations in enzyme catalysis.

Peter E. Wright, PhD
Professor and Cecil H. and Ida M. Green Investigator
Department of Integrative Structural and Computational Biology
Scripps Research
wright@scripps.edu
Postdoctoral Advisor

Dr. Wright is a leading expert in the field of intrinsically disordered proteins. Dr. Wright has been at the forefront of describing the functional roles of intrinsically disordered proteins in cellular signaling and regulation since the field's inception nearly 30 years ago and his laboratory has carried out pioneering work in the application of NMR spectroscopy and complementary biophysical methods for the characterization of intrinsically disordered proteins and their interactions.

Birthe Brandt Kragelund, PhD
Professor of Biology, University of Copenhagen
Head of the Structural Biology and NMR Laboratory and Integrative Structural Biology Cluster
bbk@bio.ku.dk
External Reference

Dr. Kragelund is a world leader in using NMR and other biophysical approaches to study dynamic and disordered proteins and their interactions. Her research group has made essential contributions to our understanding of the relationships between conformational ensembles of disordered proteins and biological function and how sequence composition of disordered regions can alter structural and binding properties.

Rebecca B. Berlow, Ph.D.

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Email: rberlow@med.unc.edu

EDUCATION:

Yale University **2005 – 2011**
Ph.D., Molecular Biophysics and Biochemistry, 2011
Dissertation: Dynamic and Functional Characterization of High Molecular Weight Enzymes
M. Phil., Molecular Biophysics and Biochemistry, 2007

Johns Hopkins University **2001 – 2005**
B.A., Chemistry, with university honors, 2005

PROFESSIONAL EXPERIENCE:

University of North Carolina at Chapel Hill **2022 – present**
Assistant Professor
Department of Biochemistry and Biophysics
UNC School of Medicine
Member, Lineberger Comprehensive Cancer Center
Position Start Date: January 24, 2022

The Scripps Research Institute **2018 – 2022**
Staff Scientist
Laboratory of Peter E. Wright
Department of Integrative Structural and Computational Biology

The Scripps Research Institute **2011 – 2018**
Postdoctoral Research Associate
Laboratory of Peter E. Wright
Department of Integrative Structural and Computational Biology
Funded by an American Cancer Society Postdoctoral Fellowship

Yale University **2005 – 2011**
Graduate Student
Laboratory of J. Patrick Loria
Department of Chemistry
Department of Molecular Biophysics and Biochemistry

Johns Hopkins University **2003 – 2005**
Undergraduate Research Assistant
Laboratory of Joel R. Tolman
Department of Chemistry

HONORS AND AWARDS:

TSRI Society of Fellows Conference Travel Award, 2018
Biophysical Society Education Committee Travel Award, 2018
American Cancer Society Postdoctoral Fellowship, 2013-2016
Excellence in Teaching Award, Yale University, 2009
National Education Association of New York Capital District Academic Scholarship, Johns Hopkins University, 2001-2005
Martin and Mary Kilpatrick Prize, Johns Hopkins University, May 2004
Dean's List, Johns Hopkins University, Fall 2001, Fall 2002, Spring 2004

BIBLIOGRAPHY AND PRODUCTS OF SCHOLARSHIP:

Peer-Reviewed Publications:

Sipko, E.L., Chappell, G.F., and Berlow, R.B*. 2024. Multivalency emerges as a common feature of intrinsically disordered protein interactions. *Current Opinion in Structural Biology* **84**: 102742.

Martinez-Yamout, M.A., Nasir, I., Shnitkind, S., Ellis, J.P., Berlow, R.B., Kroon, G., Deniz, A.A., Dyson, H.J., and Wright, P.E. 2023. Glutamine-rich Regions of the Disordered CREB Transactivation Domain Mediate Dynamic Intra- and Intermolecular Interactions. *PNAS* **120**: e2313835120.

Usui-Ouchi, A., Eade, K., Giles, S., Ideguchi, Y., Ouchi, Y., Aguilar, E., Wei, G., Marra, K.V., Berlow, R.B., and Friedlander, M. 2022. Deletion of Tgfβ signal in activated microglia prolongs hypoxia-induced retinal neovascularization enhancing Igf1 expression and retinal leukostasis. *GLIA* **70**: 1762-1776.

Skeens, E., Gadzuk-Shea, M., Shah, D., Bhandari, V., Schweppe, D.K., Berlow, R.B.*, and Lisi, G.P.* 2022. Redox-Dependent Structure and Dynamics of Macrophage Migration Inhibitory Factor Reveal Sites of Latent Allostery. *Structure* **30**: 840-850. (***corresponding author**)

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2022. Multivalency enables unidirectional switch-like competition between intrinsically disordered proteins. *PNAS* **119**: e2117338119.

Appling F.D., Berlow, R.B., Stanfield, R.L., Dyson, H.J., and Wright, P.E. 2021. The molecular basis of allostery in a facilitated dissociation process. *Structure* **29**: 1327-1338.

Eade, K., Gantner, M.L., Hostyk, J.A., Nagasaki, T., Giles, S., Fallon, R., Harkins-Perry, S., Baldini, M., Lim, E.W., Scheppke, L., Dorrell, M.I., Cai, C., Baugh, E.H., Wolock, C.J., Wallace, M., Berlow, R.B., Goldstein, D.B., Metallo, C.M., Friedlander, M., and Allikmets, R. 2021. Serine biosynthesis defect due to haploinsufficiency of phosphoglycerate dehydrogenase (PHGDH) causes retinal disease. *Nature Metabolism* **3**: 366-377.

Usui-Ouchi, A., Aguilar, E., Murinello, S., Prins, M., Gantner, M.L., Wright, P.E., Berlow, R.B.* and Friedlander, M*. 2020. An allosteric peptide inhibitor of HIF-1α regulates hypoxia-induced retinal neovascularization. *PNAS* **117**: 28297-28306. (***corresponding author**)

Berlow, R.B., Martinez-Yamout, M.A., Dyson, H.J., and Wright, P.E. 2019. Role of Backbone Dynamics in Modulating the Interactions of Disordered Ligands with the TAZ1 Domain of the CREB-Binding Protein. *Biochemistry* **58**: 1354-1362.

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2018. Expanding the Paradigm: Intrinsically Disordered Proteins and Allosteric Regulation. *Journal of Molecular Biology* **430**: 2309-2320.

Berlow, R.B.* 2018. A dual regulatory role for the disordered C-terminus of protein kinase Cα. *Biophysical Journal* **114**: 1513-1514. (***corresponding author**)

Berlow, R.B. and Wright, P.E. 2018. Tight complexes from disordered proteins. *Nature* **555**: 37-38.

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2017. Hypersensitive termination of the hypoxic response by a disordered protein switch. *Nature* **543**: 447-451.

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2015. Functional advantages of dynamic protein disorder. *FEBS Letters* **589**: 2433-2440.

Berlow, R.B., Swain, M., Dalal, S., Sweasy, J.B., and Loria, J.P. 2012. Substrate-Dependent Millisecond Domain Motions in DNA Polymerase β. *Journal of Molecular Biology* **419**: 171-182.

Wang, Y., Berlow, R.B., and Loria, J.P. 2009. Role of Loop-Loop Interactions in Coordinating Motions and Enzymatic Function in Triosephosphate Isomerase. *Biochemistry* **48**: 4548-4556.

Loria, J.P., Berlow, R.B., and Watt, E.D. 2008. Characterization of Enzyme Motions by Solution NMR Relaxation Dispersion. *Accounts of Chemical Research* **41**: 214-221.

Berlow, R.B., Igumenova, T.I., and Loria, J.P. 2007. Value of a Hydrogen Bond in Triosephosphate Isomerase Loop Motion. *Biochemistry* **46**: 6001-6010.

Submitted Manuscripts:

Day, E.C., Chittari, S.S., Cunha, K.C., Zhao, J., Dodds, J.N., Davis, D.C., Baker, E.S., Berlow, R.B., Shea, J-E., Kulkarni, R.U., and Knight, A.S. A High-Throughput Workflow to Analyze Sequence-Conformation Relationships and Explore Hydrophobic Patterning in Disordered Peptoids. Posted on *chemRxiv* 10/4/23. DOI: 10.26434/chemrxiv-2023-b84wl.

Day, E.C., Cunha, K.C., Zhao, J., DeStefano, A.J., Dodds, J.N., Yu, M.A., Han, S., Baker, E.S., Shea, J-E., Berlow, R.B., and Knight, A.S. Insights into Conformational Ensembles of Compositionally Identical Disordered Peptidomimetics. Posted on *chemRxiv* 8/28/23. DOI: 10.26434/chemrxiv-2023-6z1xd.

Refereed Conference Abstracts (Published):

Berlow, R.B., Appling, F.D., Dyson, H.J, and Wright, P.E. 2022. Rewriting the Rules of Molecular Competition: Transcriptional Regulation by Intrinsically Disordered Proteins**.** *Protein Science* **32.** Oral and poster presentation at the Protein Society Annual Symposium, San Francisco, CA.

Usui-Ouchi, A., Berlow, R.B., Aguilar, E., Ideguchi, Y., Wei, G., Marra, K.V., Wright, P.E., and Friedlander, M. 2020. CITED2 regulates hypoxia-induced retinal neovascularization in a mouse model of oxygen-induced retinopathy. *Investigative Ophthalmology and Visual Science* **61**: 4930. Virtual poster presentation at the Annual Meeting of the Association for Research in Vision and Ophthalmology.

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2020. Backbone Dynamics of the TAZ1 Domain of the CREB-Binding Protein Modulate Competition Between Disordered Ligands. *Biophysical Journal* **118**: 492a. Oral presentation at the Biophysical Society Annual Meeting, San Diego, CA.

Fallon, R., Berlow, R.B., Zernant, J., Nagasaki, T., Gantner, M.L., Harkins-Perry, S., Eade, K., Allikmets, R., and Friedlander, M. 2019. MacTel patients carry rare phosphoglycerate dehydrogenase (PHGDH) variants with reduced enzymatic activity. *Investigative Ophthalmology and Visual Science* **60**: 5994. Poster presentation at the Annual Meeting of the Association for Research in Vision and Ophthalmology, Vancouver, Canada.

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2018. Hypersensitive Termination of the Hypoxic Response by a Disordered Protein Switch. *Biophysical Journal* **114**: 560a. Oral presentation at the Biophysical Society Annual Meeting, San Francisco, CA.

Refereed Conference Abstracts (Unpublished):

Berlow, R.B., Appling, F.D., Dyson, H.J, and Wright, P.E. 2022. Rewriting the Rules of Molecular Competition: Transcriptional Regulation by Intrinsically Disordered Proteins. Poster presentation at the Gordon Research Seminar on Intrinsically Disordered Proteins, Les Diablerets, Switzerland.

Berlow, R.B., Appling, F.D., Dyson, H.J., and Wright, P.E. 2020. The Role of Backbone Dynamics in Modulating Competition Between Disordered Ligands. Oral presentation at the European Congress on Magnetic Resonance (EUROMAR), Online.

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2018. Deciphering the structural and molecular determinants for competition between the intrinsically disordered proteins HIF-1α and CITED2. Oral presentation at the Gordon Research Seminar on Intrinsically Disordered Proteins, Les Diablerets, Switzerland.

Berlow, R.B., Dyson, H.J, and Wright, P.E. 2018. Deciphering the structural and molecular determinants for competition between the intrinsically disordered proteins HIF-1α and CITED2. Poster presentation at the Gordon Research Conference on Intrinsically Disordered Proteins, Les Diablerets, Switzerland. *Prize awarded for Best Poster.*

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2017. Hypersensitive termination of the hypoxic response by a disordered protein switch. Oral presentation at Scripps Florida Research Fest, The Scripps Research Institute, Jupiter, FL. *Prize awarded for Best Oral Presentation.*

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2016. Regulation of the Hypoxic Response by Intrinsically Disordered Proteins. Poster presentation at the American Cancer Society Jiler Professors & Fellows Conference, Salt Lake City, UT.

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2016. Regulation of the Hypoxic Response by Intrinsically Disordered Proteins. Poster presentation at the Gordon Research Conference and Seminar on Intrinsically Disordered Proteins, Les Diablerets, Switzerland.

Berlow, R.B., Dyson, H.J., and Wright, P.E. 2014. Regulation of the Hypoxic Response by Intrinsically Disordered Proteins. Poster presentation at the Gordon Research Conference and Seminar on Intrinsically Disordered Proteins, Stonehill College, MA.

Berlow, R.B., Kempf, J.G., Igumenova, T.I., Sampson, N.S., and Loria, J.P. 2007. Dynamic requirements for a functional protein hinge. Poster presentation at the Frontiers of NMR in Molecular Biology Keystone Symposium, Snowbird, UT.

Invited Oral Presentations:

National Institute of Environmental Health Sciences, NIH, Signal Transduction Laboratory Seminar Series, Research Triangle Park, NC, scheduled for April 2024
Biophysical Society 68th Annual Meeting, Intrinsically Disordered Proteins Subgroup Symposium, Philadelphia, PA, February 2024
Gordon Research Conference on Protein Folding Dynamics, Galveston, TX, January 2024
Telluride Science Workshop on Intrinsically Disordered Proteins, Telluride, CO, July 2023
Point Loma Nazarene University, Departments of Chemistry and Biology, San Diego, CA, June 2023
University of North Carolina at Chapel Hill, Department of Chemistry, Chapel Hill, NC, January 2023
Lowy Medical Research Institute Annual Meeting, Tel Aviv, Israel, October 2022
University of North Carolina School of Medicine, Department of Biochemistry and Biophysics, Chapel Hill, NC, September 2022
Molecular Bases of Proteinopathies Webinar Series, Online, July 2022
36th Annual Symposium of the Protein Society, Session on Protein Phase Separation in Biomolecular Condensates, San Francisco, CA, July 2022
IDP Seminars, Online, December 2021
University of North Carolina School of Medicine, Department of Biochemistry and Biophysics, Online, March 2021
UC Merced Biophysics Interest Group, Online, March 2021
Biophysical Society 65th Annual Meeting, Intrinsically Disordered Proteins Subgroup Symposium, Online, February 2021
Korean Society of Biochemistry and Molecular Biology Conference, Online, September 2020
The Scripps Research Institute, Department of Integrative Structural and Computational Biology, La Jolla, CA, January 2020
14th Annual Peptide Therapeutics Symposium, La Jolla, CA, October 2019
Telluride Science Research Center Workshop on Intrinsically Disordered Proteins, Telluride, CO, July 2019
Yale University Biophysics and Structural Biology Symposium, New Haven, CT, April 2019 (**Student Invited Seminar**)
Southern California Users of Magnets Symposium, University of Southern California Medical School, Los Angeles, CA, April 2017

TEACHING, MENTORING, AND OUTREACH:

Courses Taught (UNC):

Advanced NMR Spectroscopy, BIOC 665, Spring 2024
BBSP First Year Group, BBSP 902, Spring 2024
Macromolecular Dynamics, BIOC 652, Fall 2023
Scientific Communication, BIOC 710, Fall 2023
BBSP First Year Group, BBSP 902, Fall 2023
BBSP First Year Group, BBSP 902, Spring 2023
BBSP First Year Group, BBSP 902, Fall 2022

Graduate Students Mentored:

Jake Simmons (Biochemistry and Biophysics Graduate Student, November 2023 – present)
Emily Sipko (Biochemistry and Biophysics Graduate Student, April 2023 – present)

BBSP Rotation Students Mentored:

Luca Montore (Spring 2024); Alli Jimenez (Winter 2023); Jake Simmons (Winter 2023); Matthew Binder (Fall 2023); Jamie Do (Fall 2023), Emily Sipko (Winter 2022), Gabrielle Puller (Fall 2022), Victoria Adler (Fall 2022)

Undergraduate Students Mentored:

Roshni Arun, UNC, January – December 2023

Research Staff Mentored:

Garrett Chappell, Post-Baccalaureate Research Technician, July 2022 – present

Graduate Thesis Committees:

Meghan Ricciardi (Chemical Biology and Medicinal Chemistry, Lee Lab)
Quentin Hahn (Biochemistry and Biophysics, Zhang and Liu Labs)
Tomiris Mulikova (Biochemistry and Biophysics, Kuhlman Lab)
Emily Grace Clark (Biochemistry and Biophysics, Flick Lab)
Iman El-Shiekh (Genetics and Molecular Biology, Ramsden and Gupta Labs)
Olivia Maurer (Biochemistry and Biophysics, Redinbo Lab)
Jordyn Markle (Chemistry, Pielak Lab)
Gabriel Arias (Biochemistry and Biophysics, Dittmer Lab, *Committee Chair*)
Maria Al-Haddad (Biochemistry and Biophysics, Campbell Lab, *Committee Chair*)
Matthew Hvasta (Biochemistry and Biophysics, Kuhlman Lab, *Committee Chair*)
Alex Neary (Biochemistry and Biophysics, Zhang Lab)
Amrita Nallathambi (Biochemistry and Biophysics, Kuhlman Lab)
Chelsea Yang (Biochemistry and Biophysics, Legant Lab, *Committee Chair*)
Brent Oskar Hutcheson (Chemistry, Pielak Lab)
Darex Vera Rodriguez (Biochemistry and Biophysics, Lee Lab)
Erin Day (Chemistry, Knight Lab, Dissertation Title: *Sequence-Structure Relationships in Disordered Peptoids: A Toolkit for Design and Discovery*, 2024)
I-Te Chu (Chemistry, Pielak Lab, Dissertation Title: *Protein Stability in Cells and Under Crowded Conditions in vitro*, 2023)
Joseph Thole (Chemistry, Pielak Lab, Dissertation Title: *Disordered Proteins in vitro and In Cells,* 2022)

Other activities:

Moderator, Biophysics Week Discussion with Kate Zernike, March 2024
Panelist, UNC Center for Faculty Excellence New Faculty Orientation, August 2023
Presenter, BCBP Summer Undergraduate Research Seminar Series, June 2023
Reviewer, UNC Biology Undergraduate Research Honors Thesis Program, Spring 2023
Mental Health First Aid Certificate Program, completed March 2023
Biophysical Society One-on-One Mentoring Program, February 2023 – present

Psychological Safety and Inclusion Workshop, UNC Office of Diversity and Inclusion, completed February 2023
Cultivating Mentors Workshop, UNC School of Medicine Office of Graduate Education, completed Fall 2022
Faculty Mentor and Instructor, UNC BBSP First Year Group and Responsible Conduct of Research, University of North Carolina at Chapel Hill, Fall 2022-Spring 2023
Guest Instructor, Macromolecular Dynamics, University of North Carolina at Chapel Hill, Fall 2022
Faculty Mentor Training for Biomedical Researchers, UNC School of Medicine Office of Graduate Education, completed May 2022
UNC LGBTQ+ Safe Zone Training, completed May 2022
Panelist, Academic Careers Roundtable, The Scripps Research Institute, November 2021
Instructor, Structural Biology and Biophysics, The Scripps Research Institute, Fall 2020
Co-Organizer and Scientific Communication Mentor, Scripps Research Community Outreach Showcase, The Scripps Research Institute, October 2020
Course Director and Instructor, Writing and Speaking About Science, The Scripps Research Institute, Fall 2019
Instructor, Structural Biology and Biophysics, The Scripps Research Institute, Fall 2019
Mentor, Junior Scientist Training Program, The Scripps Research Institute, Summer 2019
Course Director and Instructor, Writing and Speaking About Science, The Scripps Research Institute, Spring 2019
American Cancer Society Cancer Action Network Research Ambassador, 2013-2019
Course Director and Instructor, Essentials of Scientific Communication, The Scripps Research Institute PREP Program, Fall 2018
Instructor, Structural Biology and Biophysics, The Scripps Research Institute, Fall 2018
Instructor, Biophysics, The Scripps Research Institute, Spring 2018
Speaker and Discussion Leader, Career Compass STEM Careers Symposium, La Costa Canyon High School, March 2018
Institutional Host, Scripps College Career Exploration and Networking Trek, The Scripps Research Institute, October 2017
Mentor, UCSD Academic Internship Program, The Scripps Research Institute, Fall 2017
Instructor, Structural Biology, The Scripps Research Institute, Fall 2017
Mentor, Life Sciences Summer Institute High School Internship Program, The Scripps Research Institute, Summer 2013
Mentor, Undergraduate Research Program, Yale University, 2006-2010
Guest Lecturer, Physical Chemistry with Applications in Biological Sciences, Yale University, Fall 2010
Teaching Fellow and Lecturer, Principles of Biophysics, Yale University, Spring 2009
Teaching Fellow, Principles of Biophysics, Yale University, Spring 2008
Teaching Fellow, Laboratory of Biochemistry and Biophysics, Yale University, Spring 2007
Peer Tutor, Physical Chemistry, Johns Hopkins University, 2004-2005

GRANTS:

American Cancer Society Postdoctoral Research Fellowship
Title: Regulation of the Hypoxic Response by Intrinsically Disordered Proteins
Grant Number: 125343-PF-13-202-01-DMC
Dates: July 1, 2013 – June 30, 2016
Role: PI
Total Award: $150,000 (100% effort)

National Institutes of Health (NIGMS) Training Grant
Title: Predoctoral Program in Biophysics
Grant Number: 5T32GM008283
Dates: September 2005 – August 2008
Role: Trainee

PATENT APPLICATIONS:

Friedlander, M., Wright, P.E., Berlow, R.B., Usui-Ouchi, A. Methods and Compositions for Treating Ocular Vascular Disorders, PCT Patent Application No. 2020/056469, filed on October 20, 2020, published on April 28, 2022.

PROFESSIONAL SERVICE AND LEADERSHIP:

Ad Hoc Reviewer for *Science Advances, Cell, Current Opinion in Structural Biology, Structure, Protein Science, Biochemistry, PNAS, Nature Chemical Biology, Cell Reports, Biophysical Journal, Nature Communications, Journal of Molecular Biology, Journal of Biological Chemistry, Biochimie, Scientific Reports, Journal of Medicinal Chemistry*, *Methods, Trends in Pharmacological Sciences,* and *Nucleic Acids Research*
Ad Hoc Grant Reviewer, National Science Foundation, Molecular Biophysics, Spring 2024
Member, Amgen Scholars Review Committee, UNC Chapel Hill, February 2024
Session Chair, Intrinsically Disordered Proteins, Phase Separation and Aging, Gordon Research Conference on Protein Folding Dynamics, Galveston, TX, January 2024
Member, Professional Opportunities for Women Committee, Biophysical Society, 2023-present
Organizer, Carolina Biophysics Symposium, November 2023
Reviewer, Travel Grants for the Biophysical Society Annual Meeting, October 2023
Reviewer, US Army Corps of Engineers Engineer Research and Development Center (ERDC) FY2024 Proposal Program, Spring 2023
Organizer, India | EMBO Lecture Course on Structure, Dynamics, and Interactions in Biomolecular Systems using NMR Spectroscopy, December 2022
Member, UNC Biological and Biomedical Sciences Program Graduate Admissions Committee, 2022-present
Member, UNC Biochemistry and Biophysics Department Seminar Committee, 2022-present
Member, UNC Biochemistry and Biophysics Department Equipment Committee, 2022-present
Observer, UNC School of Medicine Appointments, Promotion, and Tenure Committee, Fall 2022
Poster Judge, UNC Biochemistry and Biophysics Department Annual Retreat, October 2022
Poster Judge, Graduate Student Poster Competition, 36th Annual Symposium of the Protein Society, San Francisco, CA, July 2022
Session Chair, Functional Mechanisms of IDPs, Gordon Research Conference on Intrinsically Disordered Proteins, Les Diablerets, Switzerland, June 2022
Poster Judge, Graduate Student Poster Competition, Gordon Research Conference on Intrinsically Disordered Proteins, Les Diablerets, Switzerland, June 2022
Associate Editor, *Biophysical Reports*, 2022-present
Session Chair, 16th Annual Peptide Therapeutics Symposium, La Jolla, CA, October 2021
Social Media Editor, *Journal of Molecular Biology*, 2021-present
Member, Diversity, Equity, and Inclusion Task Force and Committee for Recruitment, Retention, and Support, The Scripps Research Institute, 2020-2021
Member, Professional Skills Development Committee, Skaggs Graduate Program at The Scripps Research Institute, 2019-2021
Reviewer, Scripps Research Translational Institute Pilot Study Award Program, Spring 2021
Reviewer, French National Research Agency Generic Proposal Program, Biochemistry of Living

Organisms Section, Spring 2020
Reviewer, Scripps Research Translational Institute Pilot Study Award Program, Spring 2020
Executive Committee Member, Society of Fellows, The Scripps Research Institute, 2015-2019
Contributing Editor, The IDP State Letter, 2016-2018
Session Co-Chair, Intrinsically Disordered Proteins and Aggregates II Platform Session, Biophysical Society 62nd Annual Meeting, San Francisco, CA, February 2018
Vice President, Society of Fellows, The Scripps Research Institute, 2016-2017
Postdoctoral Representative, IDP Subgroup of the Biophysical Society, 2016-2017
Chair, Gordon Research Seminar on Intrinsically Disordered Proteins, Les Diablerets, Switzerland, June 2016
Chair, Society of Fellows Distinguished Lecture Series, The Scripps Research Institute, 2016
Organizer and Moderator, Career Panel at the Gordon Research Seminar on Intrinsically Disordered Proteins, Stonehill College, MA, July 2014
Organizer, Biophysics Training Grant Lecture Series, Yale University, 2010
Student Organizer, MB&B Departmental Retreat, Yale University, 2006 and 2007

PROFESSIONAL AFFILIATIONS:

Member, Association for Research in Vision and Ophthalmology, 2023-present
Member, Protein Society, 2022-present
Member, American Peptide Society, 2021-present
Member, Biophysical Society, 2016-present
Associate Faculty Member, Faculty of 1000 and Faculty Opinions, 2012-present
Member, American Association for the Advancement of Science, 2005-present
Member, New York Academy of Sciences, 2005-present
Member, National Society of Collegiate Scholars, 2001-present

PI: Chen, Jiakun (Pages 75-84)

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THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

March 25, 2024

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To Whom It May Concern:

I am writing with enthusiasm to recommend **Dr. Jiakun Chen** for a Pew Scholars Award. Dr. Chen was chosen for an Assistant Professor position in the Department of Biology at UNC-Chapel Hill from a highly competitive national search by distinguishing himself with the rigor of his science. We are delighted to have recruited him as a new member of our faculty and are committed to ensuring his future success as an independent investigator. He will be an outstanding recipient of this prestigious junior faculty award.

Dr. Chen completed his Ph.D. in 2017 in Molecular Cell Biology at Washington University and was Postdoctoral Fellow at the Vollum Institute at Oregon Health & Science University training with Drs. Marc Freeman and Kelly Monk, experts in *Drosophila* and zebrafish neurobiology, respectively. He has a remarkably productive publication record from his training, with papers in high profile journals like *Developmental Cell*, *Developmental Biology*, *Development*, *Neuron*, and *Molecular Cellular Biology*. In 2020 Dr. Chen published a landmark paper in *Nature Neuroscience* describing his discovery in zebrafish of neuronal astrocytes—a remarkable achievement for any scientist let alone a postdoctoral fellow.

Dr. Chen's research focuses on advancing our understanding about the fundamental mechanisms of how astrocytes contribute to nervous system formation and function. This important neuronal cell type was thought to be absent from zebrafish, but Dr. Chen's exciting discovery of *bona fide* astrocytes in this model genetic organism with molecular and functional features similar to the mammalian counterparts open up exciting new avenues of research. He can now deploy the myriad genetic tools in zebrafish to manipulate and visualize dynamic cellular behavior and activity of astrocytes *in vivo*. His research has resulted in two NIH grants (R21 and R01) in his advisors' labs, along with the *Nature Neuroscience* paper mentioned above. Dr. Chen is a rather unique scientist in that he employs a powerful combination of two model organisms, zebrafish and the fruit fly *Drosophila melanogaster*. He uses flies for rapid genetic identification of genes involved in astrocyte development and function and fish for live imaging of a vertebrate nervous system. Thus, he is combining the best aspects of two powerful, genetically tractable experimental organisms and I have no doubt that he will be successful in funding his independent work with extramural grants from a variety of agencies.

To help Dr. Chen's research program flourish upon his arrival at UNC, we provided him with **$1,043,801** in start up funds that he can use for personnel, equipment, and operation costs during the first 3 years of his appointment. These funds include support for a new Zeiss confocal microscope that will be housed in the department's imaging core facility. This arrangement relieves Dr. Chen of the burden of operations and maintenance for the microscope while still providing his laboratory members guaranteed full time access. UNC also has a world class zebrafish facilty that is managed by the Division of Comparative Medicine

and reports in to the University's Office of the Vice Chancellor for Research. Dr. Chen has guaranteed access to this facility, which will care for his zebrafish colonies.

Excitingly, Dr. Chen's laboratory will be housed in the Genome Sciences Building adjacent to three outstanding young scientists in the department, Celia Shia who uses zebrafish to study macrophages in the nervous sytem and En Yang and Toshi Hige who use zebrafish and *Drosophila*, respectively, to study how neuronal circuits drive behavior. I am anticipating great synergy among these labs and others within our neuroscience and cell biology communities. Further support for Dr. Chen will come from a mentoring committee composed of senior faculty with whom he meets at least once per year, followed by submission of a summary report to the chair.

Dr. Chen is an outstanding choice for this award, and I am eager to help him begin his independent research career as a faculty member in the Department of Biology at UNC-Chapel Hill. Thank you for considering his application.

Sincerely,

Robert J. Duronio
Professor & Chair, Department of Biology

Project Title: Astrocytes in neural circuit assembly and function

Background: As the structural and functional unit of nervous system circuitry, neurons wire with each other in development and undergo dramatic remodeling to shape the functional neural connections that ultimately control animal behavior. Astrocytes, the most common type of glial cells in the brain, have only recently been appreciated as integral components of neural circuits and are capable of directly regulating neuronal activity and behavior[1]. Astrocyte dysfunction is associated with numerous neurodevelopmental and cognitive disorders[2], and many human neurological disabilities have a primary basis of astrocyte-enriched genes[1], such as autism, epilepsy, schizophrenia, and Alzheimer's disease. However, there is little known about the molecular mechanisms that control astrocyte development and function, and how astrocytes modulate neural circuits. The goal of my research program is to understand the mechanisms by which astrocytes develop and interact with neural elements and to define the astrocytic basis of brain disorders.

We use *Drosophila* and zebrafish as model organisms to explore fundamental aspects of astrocyte biology. We leverage the unparalleled molecular and genetic toolkits in flies to uncover gene function in astrocytes, and we exploit the advanced live-imaging techniques in zebrafish to study astrocyte-neuron interactions in intact neural circuits. During my postdoc studies, I discovered and characterized astrocytes in zebrafish that closely resemble mammalian astrocytes by developmental, molecular, cellular, and functional criteria (Chen et al., 2020, *Nature Neuroscience*)[3]. Building on this key foundational work, I have developed and assembled a battery of tools to label and manipulate astrocytes with single-cell precision to visualize their cellular dynamics and activity changes in response to neuromodulation. In combination with *Drosophila* model, I recently identified an evolutionarily conserved Tre1/S1pr1 phospholipid-binding GPCR signaling in astrocyte morphogenesis (Chen et al., 2024, *Neuron*)[4], a developmental process we know surprisingly little about at the mechanistic levels. I found that loss of *Drosophila Tre1* or its vertebrate counterpart *s1pr1* in zebrafish resulted in markedly decreased astrocytic associations with synapses (Figure 1) and led to locomotion deficits[4]. This work offers exciting new entry points to study molecular underpinnings of astrocyte morphology and to explore how astrocytes affect neuronal wiring and remodeling at the molecular, cellular, and circuit levels.

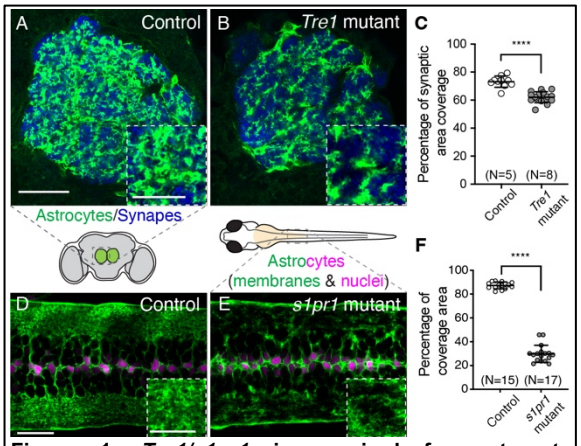

Figure 1. *Tre1/s1pr1* is required for astrocyte morphogenesis in *Drosophila* and zebrafish. (**A-C**) *Tre1* mutants show decreased astrocytic coverage of synapses in *Drosophila* brain. (**D-F**) Zebrafish *s1pr1* mutants show simplified astrocyte fine processes in the CNS. Scale bars, 20 μm; 10 μm for insets. (Chen et al., 2024, *Neuron*.)

Future Research: By deploying the complementary experimental strengths from *Drosophila* and zebrafish model systems, my lab is unique and well-positioned to identify molecules required for astrocyte development and to unravel the roles astrocytes play in neural circuit formation, function, and maintenance. In this proposal, I will work on the following projects:

Project 1. How is astrocyte morphogenesis regulated? Astrocytes exhibit a remarkably complex morphology with a myriad of extending fine processes that closely associate with synapses and perhaps all central nervous system (CNS) cell types. Recent studies suggest that astrocyte morphological changes are highly correlated with risk genes associated with common neurological disorders, including Alzheimer's disease, bipolar disorder, multiple sclerosis, and schizophrenia[5]. However, the molecular basis of how astrocytes acquire their sophisticated morphology remains elusive. My recent work demonstrated that lipid-binding GPCR Tre1/S1pr1 acts cell-autonomously to regulate astrocyte morphological elaboration from flies to fish[4], yet the underlying signaling mechanisms need to be explored further. Moreover, both secreted factors and contact-mediated molecules likely contribute to the establishment of mature astrocyte morphology[6], but genes related to this process await to be identified to achieve a deep mechanistic understanding of astrocyte morphogenesis. In this project, we will first investigate what phospholipid (PL) ligands activate Tre1/S1pr1 GPCR, and how the ligand activity is regulated in astrocytes and/or by surrounding cells. For instance, do neurons provide the instructive ligand cue for astrocytic process infiltration to enwrap synapses, and if so is neuronal activity required in controlling astrocyte morphology? Our preliminary data suggest that sphingosine kinases sphk1/2, which are lipid kinases that produce PL ligand sphingosine-1-phosphate (S1P), are necessary for normal astrocyte morphological growth. We will conduct cell-specific manipulations and live-imaging experiments to elucidate how Sphk1/2 function to generate S1P in astrocytes versus neurons to sculpt astrocyte morphology. In addition, we will begin to explore how Tre1/S1pr1 GPCR directs astrocytic fine processes to ensure intimate interactions with synapses. We found that Tre1/S1pr1 is required to balance small GTPase Rac1-dependent actin cytoskeleton dynamics[4], but the molecular linkage remains unclear. Here we will test two hypotheses that are non-mutually

exclusive in 1) promoting directional outgrowth of astrocyte processes by cell polarity regulators, and/or 2) establishing astrocyte-synapse associations via adhesion molecules. Furthermore, we will utilize the unparalleled genetic screening approaches in *Drosophila* to identify novel genes involved in astrocyte development, followed by deeper functional exploration in zebrafish.

Project 2. How do astrocytes modulate neural circuit assembly and activity? Astrocytes actively participate in the formation, pruning, and remodeling of synapses and neurons in the developing brain[7,8]. Altered number of synapses and miswiring between neurons are common in many neurological diseases[2]. We know that astrocyte ablation leads to devastating effects on brain development[9], and recent data demonstrate that mis-regulated astrocyte activity can result in defects at circuit and behavior levels[10,11].However, how genetic mutations in astrocytes lead to circuit disorders need to be directly addressed. Here we pose that genes (such as *Tre1*/*s1pr1*) required for astrocyte morphological complexity offer excellent opportunities to study how changes in astrocyte morphology (*e.g*. their close associations with synapses and interactions with other CNS cell types) affect neural circuit formation and function. With genetic tools we have established in the lab, we will determine how astrocytic depletion of *Tre1* in flies impacts circuit assembly. In particular, *Drosophila* have relatively simple but well characterized and tractable neural circuits that can be labeled and visualized across the lifespan at single cell resolution[12,13]. Using astrocyte-specific *Tre1* knockout flies[4], we will examine neuronal phenotypes in the stereotyped olfactory circuit, where glial cells have been suggested to participate in refining the circuit specificity[14]. By confocal imaging, we will assess the wiring specificity of this circuit (*e.g*. synapse number, neurite patterning) when astrocyte morphology is disrupted. In zebrafish, we will take advantage of the live-imaging techniques to explore the roles of astrocytes in neural circuit activity modulation under an intact animal brain. Similar to the relatively simple circuit we use in flies, we will begin by examining the norepinephrine (NE) neural circuit that astrocytes are actively engaged in[15] to assay for phenotypes in fish. Combining chemical and genetic manipulation approaches, we will determine how astrocyte-neuron and neuron-neuron communications are altered in the NE neural network with simplified astrocyte morphology that show drastically decreased contacts with neighboring neural elements. We expect these results will provide key insights into whether astrocyte morphology is crucial for proper neural connectivity. By further extensively characterizing astrocyte morphology-related genes identified in Project 1, our goal is to understand the correlation between the severity of astrocyte morphological defects and neural circuit aberrancy. Importantly, such circuit phenotypes will expand our future directions to probe the precise molecular mechanisms mediating astrocyte-neuron interactions. In addition, by collaborating with my neighboring lab Dr. En Yang at UNC, we will employ whole-brain imaging in zebrafish to investigate how astrocytes contribute to neuronal activity modulation across the entire brain.

Project 3. How is astrocyte plasticity controlled and its role in neural circuits? Astrocytes show striking structural plasticity in the developing brain across species[16,17], whereas it is unclear whether such robust astrocyte plasticity maintains in the adult brain. In our preliminary experiments, we found that adult astrocytes prominently decrease their morphological plasticity in comparison with developing astrocytes in *Drosophila*. This is an exciting phenomenon warranting further exploration. What are the molecular mechanisms controlling the markedly diminished astrocyte plasticity from development to adulthood? Do other CNS cell types play a role in regulating astrocyte plasticity? How do neural circuits respond to altered plasticity of astrocytes during development or in homeostasis. Addressing these unanswered questions is critical to reveal the role of astrocyte plasticity in health and disease. By performing TRAP-seq experiments in *Drosophila* astrocytes, we observed unique transcriptional signatures in developmental versus adult stage astrocytes. These differentially expressed genes serve as excellent candidates to unravel molecular mechanisms governing astrocyte plasticity control. For instance, we found that *Tre1* transcription is specifically switched off in adult astrocytes[4], correlating with the maturation of astrocyte morphology. Interestingly, altered S1PR1 (mammalian counterpart of Tre1) level or activity is associated with many brain disorders, including multiple sclerosis, schizophrenia, and stroke[18-20]. We hypothesize that dysregulation of Tre1/S1pr1 signaling in the mature CNS leads to astrocyte plasticity changes and thereby disturbing proper circuit function and maintenance, which we will begin to investigate in this proposal. In addition, we will explore other candidate genes from our TRAP-seq resource to identify additional molecules responsible for astrocyte plasticity regulation and function.

Significance: Recent studies have demonstrated that astrocyte dysfunction has profound cell non-autonomous effects on surrounding neurons; a glio-centric perspective of neurodevelopmental and psychiatric diseases has the potential to incisively transform our knowledge about disease pathogenesis and future therapeutic treatment in humans[21]. Our goal is to gain a fundamental understanding of astrocyte biology *in vivo*, and we hope this information will help to determine the mechanistic roles astrocytes play in disease.

Eligibility: I run an independent lab as an Assistant Professor in the Department of Biology at UNC-Chapel Hill since January 1st, 2024. I confirm that I meet all the eligibility requirements to apply to the Pew Scholars Program.

References:

1. Nagai, J., Yu, X., Papouin, T., Cheong, E., Freeman, M.R., Monk, K.R., Hastings, M.H., Haydon, P.G., Rowitch, D., Shaham, S., and Khakh, B.S. (2021). Behaviorally consequential astrocytic regulation of neural circuits. Neuron *109*, 576-596. 10.1016/j.neuron.2020.12.008.
2. Lee, H.G., Wheeler, M.A., and Quintana, F.J. (2022). Function and therapeutic value of astrocytes in neurological diseases. Nat Rev Drug Discov *21*, 339-358. 10.1038/s41573-022-00390-x.
3. **Chen, J.**, Poskanzer, K.E., Freeman, M.R., and Monk, K.R. (2020). Live-imaging of astrocyte morphogenesis and function in zebrafish neural circuits. Nat Neurosci *23*, 1297-1306. 10.1038/s41593-020-0703-x.
4. **Chen, J.**, Stork, T., Kang, Y., Nardone, K.A.M., Auer, F., Farrell, R.J., Jay, T.R., Heo, D., Sheehan, A., Paton, C., et al. (2024). Astrocyte growth is driven by the Tre1/S1pr1 phospholipid-binding G protein-coupled receptor. Neuron *112*, 93-112 e110. 10.1016/j.neuron.2023.11.008.
5. Endo, F., Kasai, A., Soto, J.S., Yu, X., Qu, Z., Hashimoto, H., Gradinaru, V., Kawaguchi, R., and Khakh, B.S. (2022). Molecular basis of astrocyte diversity and morphology across the CNS in health and disease. Science *378*, eadc9020. 10.1126/science.adc9020.
6. Baldwin, K.T., Murai, K.K., and Khakh, B.S. (2023). Astrocyte morphology. Trends Cell Biol. 10.1016/j.tcb.2023.09.006.
7. Stogsdill, J.A., Ramirez, J., Liu, D., Kim, Y.H., Baldwin, K.T., Enustun, E., Ejikeme, T., Ji, R.R., and Eroglu, C. (2017). Astrocytic neuroligins control astrocyte morphogenesis and synaptogenesis. Nature *551*, 192-197. 10.1038/nature24638.
8. Chung, W.S., Clarke, L.E., Wang, G.X., Stafford, B.K., Sher, A., Chakraborty, C., Joung, J., Foo, L.C., Thompson, A., Chen, C., et al. (2013). Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. Nature *504*, 394-400. 10.1038/nature12776.
9. Delaney, C.L., Brenner, M., and Messing, A. (1996). Conditional ablation of cerebellar astrocytes in postnatal transgenic mice. J Neurosci *16*, 6908-6918.
10. Ma, Z., Stork, T., Bergles, D.E., and Freeman, M.R. (2016). Neuromodulators signal through astrocytes to alter neural circuit activity and behaviour. Nature *539*, 428-432. 10.1038/nature20145.
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References

- Lilianna Solnica-Krezel, Ph.D. (Thesis advisor)
  Professor and Head, Department of Developmental Biology, Washington University School of Medicine

  Lila Solnica-Krezel's lab studies early zebrafish development, focusing on the roles of signaling pathways that regulate cell polarity and gastrulation. She is one of the pioneers in the zebrafish model.

- Marc Freeman, Ph.D. (Postdoctoral co-advisor)
  Senior Scientist and Director, Vollum Institute, Oregon Health & Science University

  Marc Freeman's lab uses fruit fly *Drosophila* as a model to understand glial biology and neuron-glia interactions. He is a leader in the study of glial cells.

- Cagla Eroglu, Ph.D. (External recommender)
  Professor and HHMI, Departments of Cell Biology and Neurobiology, Duke University

  Cagla Eroglu's lab studies the role of astrocytes in control of synaptic circuit development and cognition. She is a leader in the field of astrocyte biology.

Jiakun Chen, Ph.D.

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Education

2011 – 2017	Washington University in St. Louis, St. Louis, MO Ph.D. in Molecular Cell Biology Advisor: Lilianna Solnica-Krezel, Ph.D.
2008 – 2011	Xiamen University, Xiamen, Fujian, China M.S. in Biochemistry and Molecular Biology Advisor: Bo-An Li, Ph.D.
2004 – 2008	Xiamen University, Xiamen, Fujian, China B.S. in Biotechnology Advisor: Bo-An Li, Ph.D.

Professional Experience

2024 – present	Assistant Professor, Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC
2018 – 2023	Postdoctoral Fellow, Marc Freeman Lab & Kelly Monk Lab, Vollum Institute, Oregon Health & Science University, Portland, OR
2011 – 2017	Graduate Student, Lilianna Solnica-Krezel Lab, Department of Developmental Biology, Washington University in St. Louis, St. Louis, MO
2008 – 2011	Master's Student, Bo-An Li Lab, School of Life Sciences, Xiamen University, Xiamen, Fujian, China
2007 – 2008	Undergraduate Student, Bo-An Li Lab, School of Life Sciences, Xiamen University, Xiamen, Fujian, China

Honors and Awards

2020	Best Postdoc Paper Award, Vollum Institute, OHSU
2019	LSRF Fellowship committee selected finalist
2018	Irving Boime Publication Award, Department of Developmental Biology, Washington University in St. Louis

2017	Developmental Biology Outstanding Paper Award Runners-up, *Developmental Biology*
2011	XiangYu Outstanding Graduate Student Award, Xiamen University, China
2008	Excellent Undergraduate Thesis Award, Xiamen University, China
2006 & 2007	Undergraduate Scholarship of Xiamen University, China

Peer-reviewed Articles (#corresponding; *equal contribution)

1. **Chen, J.**#, Stork, T., Kang, Y., Nardone, K. A. M., Auer, F., Farrell, R. J., Jay, T. R., Heo, D., Sheehan, A., Paton, C., Nagel, K. I., Schoppik, D., Monk, K. R.#, & Freeman, M. R.# (2023). "Astrocyte growth is driven by the Tre1/S1pr1 phospholipid-binding G protein-coupled receptor." Neuron, 2024 Jan 3;112(1):93-112.e10.

2. **Chen, J.**#, K. E. Poskanzer, M. R. Freeman and K. R. Monk# (2020). "Live-imaging of astrocyte morphogenesis and function in zebrafish neural circuits." Nat Neurosci 23(10): 1297-1306.

3. **Chen, J.**, G. D. Castelvecchi, N. Li-Villarreal, B. Raught, A. M. Krezel, H. McNeill and L. Solnica-Krezel (2018). "Atypical Cadherin Dachsous1b Interacts with Ttc28 and Aurora B to Control Microtubule Dynamics in Embryonic Cleavages." Dev Cell 45(3): 376-391 e375.

4. **Chen, J.**, L. Xia, M. R. Bruchas and L. Solnica-Krezel (2017). "Imaging early embryonic calcium activity with GCaMP6s transgenic zebrafish." Dev Biol 430(2): 385-396.

5. Karner, C. M., E. Esen, **J. Chen**, F. F. Hsu, J. Turk and F. Long (2016). "Wnt Protein Signaling Reduces Nuclear Acetyl-CoA Levels to Suppress Gene Expression during Osteoblast Differentiation." J Biol Chem 291(25): 13028-13039.

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7. Shin, J., **Chen, J.**, and L. Solnica-Krezel (2014). "Efficient homologous recombination-mediated genome engineering in zebrafish using TALE nucleases." Development 141(19): 3807-3818.

8. Yang, M., S. N. Li, K. M. Anjum, L. X. Gui, S. S. Zhu, J. Liu, **J. K. Chen**, Q. F. Liu, G. D. Ye, W. J. Wang, J. F. Wu, W. Y. Cai, G. B. Sun, Y. J. Liu, R. F. Liu, Z. M. Zhang and B. A. Li (2013). "A

double-negative feedback loop between Wnt-beta-catenin signaling and HNF4alpha regulates epithelial-mesenchymal transition in hepatocellular carcinoma." J Cell Sci 126(Pt 24): 5692-5703.

9. **Chen, J.***, Q. Luo*, Y. Yuan*, X. Huang, W. Cai, C. Li, T. Wei, L. Zhang, M. Yang, Q. Liu, G. Ye, X. Dai and B. Li (2010). "Pygo2 associates with MLL2 histone methyltransferase and GCN5 histone acetyltransferase complexes to augment Wnt target gene expression and breast cancer stem-like cell expansion." Mol Cell Biol 30(24): 5621-5635.

Research Support

- How do astrocytes develop and function *in vivo*? (*UNC Biology Dept. Startup Funds*)

Presentations (selected talks)

2024	Glia-fari workshop, Genetics Society of America TAGC24, Washington, DC
2023	16th Zebrafish Disease Models (ZDM16) Conference, Durham, NC
2023	Neuroscience Institute SPiNES seminar series, NYU
2023	NIH Stadtman Investigator Candidate Seminar, CDBL, NIH
2022	Weill Institute for Neurosciences External Postdoc Seminar Program, UCSF
2022	Department of Cell Biology & Physiology, Washington University in St. Louis
2022	SDB Ethel Browne Harvey Postdoctoral Seminar Series (virtual)
2020	Glia Group Seminar (virtual), Washington University in St. Louis

Teaching & Mentoring

Teaching

2015	*Zebrafish Development and Genetics*, Marine Biological Laboratory (MBL), Woods Hole, MA (*Teaching Assistant*)
2013	*Principles of Biology*, Department of Biology, Washington University in St. Louis, MO (*Teaching Assistant*)
2006 – 2007	*JoyYoung Outreach Program*, Xiamen, China (*Volunteer*)

Mentoring

2024 – present	Akhil Malakapalli, UNC Undergraduate Student
	Mia Zhou, UNC Undergraduate Student
2021 – 2023	Emma Brennan, OHSU Research Assistant
2019 – 2021	Cameron Paton, OHSU Research Assistant
2019	Hannah Collins, OHSU Rotation Student

2018	Danielle Mathieson, OHSU Rotation Student
2017	Lenore Monterroza, WUSTL Summer Student
2015	Rebecca Cunningham, WUSTL Rotation Student

Service & Activities

Journal Peer Review

eLife, EMBO Reports, Journal of Neuroimmunology, JoVE, Molecular Neurobiology, Nature, STAR Protocols, Trends in Cell Biology

Student Advisory Committee

2024 – present	PhD advisory committee, Ethan Bedsole (*UNC Biology Dept. Q-Biol. Program*)

Other Activities

2024	Grant reviewer, Summer Undergraduate Research Fellowship (SURF) Program, UNC

Society Memberships

Society for Developmental Biology (SDB), Genetics Society of America (GSA)

Last updated on 3/31/2024

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April 1, 2024

RE: Huong Kratochvil, PhD

Internal Nomination for Pew Biomedical Scholar Program 2025

Dear Internal Selection Committee,

I write to you in strong support of Prof. Huong Kratochvil's application for the Pew Biomedical Scholars 2025 competition. As an Assistant Professor in the Department of Chemistry at UNC, Huong has already proven herself to be an exceptional and promising young scientist in the fields of protein design and membrane protein biophysics. As a department, we are thrilled to have Huong on our faculty and are committed to her success as an independent researcher.

Huong's productivity throughout her scientific career illustrates her ambition and initiative to address fundamental questions in biophysics. As a graduate student in the Department of Chemistry at the University of Wisconsin-Madison, she pushed the boundaries of ultrafast spectroscopy to elucidate a complex mechanism in ion channel biology. In this highly interdisciplinary and collaborative project, she used a combination of protein semisynthesis, ultrafast spectroscopy, and molecular dynamics simulations to distinguish between two competing models of ion translocation in potassium ion channels, offering the first direct experimental evidence for one mechanism over the other. This unparalleled work brings new approaches to the ion channel field and enables the study of ion channels in motion. Although her postdoctoral work at the University of California, San Francisco, was a swift departure from physical chemistry, Huong's research has made significant impact in the fields of membrane protein biophysics and protein design. Specifically, her use of membrane protein design as a tool to test key mechanisms in ion transport is innovative and allowed her to critically assess an experimentally elusive mechanism in proton-selective transport in membrane proteins. This hallmark study answered a long-standing question in proton channel function and extends computational and experimental approaches to design proton-selective proteins and materials for a range of applications from energy storage to water purification. Huong's contributions to the field have been recognized through the award of prestigious fellowships during her postdoc, including the National Institutes of Health F32 NRSA Postdoctoral Fellowships and the K99/R00 Pathway to Independence Award. Huong's multidisciplinary research has led her to become one of the prominent experts in membrane protein design and biophysics. To date, she has co-authored 19 publications in many high-impact journals including *Science*, *Nature Chemistry*, *PNAS*, etc.

Huong joined the department in January 2023 and is establishing a highly interdisciplinary, innovative research program that combines protein design and biophysical approaches to engineer functional proteins for sustainability and therapeutic applications. Her program pushes the boundaries of protein design to dissect the governing principles of membrane protein structure and function and to elucidate key protein-protein interactions in immunology. Of specific interest to her Pew Biomedical Scholars application, Huong's lab is working to define the roles of protein oligomerization in food allergy by engineering potentially hypoallergenic variants of common food allergens. While this research leverages her background in biophysics and protein design, it goes into the realms of immunology and synthetic biology, allowing Huong and her lab to explore new fields and directions. The work she is proposing not only combines computational and experimental biophysical approaches, but also spans fundamental and translational science. The use of molecular dynamics simulations will allow her group to screen designs to take forward to experimental validation. Additionally, what her group hopes to achieve will enable a deeper understanding of these protein-protein interactions at an atomic level, which answers fundamental questions about allergen oligomerization in allergenicity as well as open new avenues for therapeutic development.

The Department of Chemistry at UNC has provided Huong with the environment and resources necessary for her success as an independent researcher. Her lab is in the Genome Sciences Building, which boasts open-concept style laboratories where her lab can quickly integrate with other established labs in the department. This layout

facilitates the free flow of ideas, collaborations, and open communication between labs, which is important for the success of a young lab. In addition to her primary appointment in the Department of Chemistry, she is also associated with the Molecular and Cellular Biophysics, Biological and Biomedical Sciences, and Chemical Biology Interface Training Programs, which allows her to recruit and support talented students from many degree-granting programs from across campus.

As a faculty member at UNC, Huong and her lab will have access to over 50 core facilities on campus. These core facilities, which include facilities housed in the Department of Chemistry as well as the School of Medicine, will enable and expand Huong's research program. Specifically, her group will be able to access key instrumentation to the Chemistry Department Mass Spectrometry Facility, the Chemistry Department X-ray Facility, the Cryo-Electron Microscopy Core, and the Macromolecular Interactions Facility, to name a few. These facilities, along with many others on campus, will support Huong and her lab in many aspects of peptide/protein synthesis, sample purification, biophysical characterization, and structural determination. Additionally, UNC-Chapel Hill hosts two state-of-the-art computer clusters available to researchers across campus free of charge. These clusters, Longleaf and Dogwood, host over 15,000 computer cores combined. Longleaf also includes GPU resources on top of the 13,000 threads. Huong and her lab will have access to all these computational resources for her computational needs.

In summary, Huong's multidisciplinary background, impressive track record, and ambitious research vision make her an exemplary candidate for the Pew Biomedical Scholars Program. The department was thrilled when she accepted our offer to start her innovative and cutting-edge research program at UNC, and we look forward to her continued success as a young investigator. Without a doubt, I could not put forward another highly qualified candidate for the Pew Biomedical Scholars Program than Huong Kratochvil.

Sincerely,

Wei You
Professor
Chairperson, Department of Chemistry
University of North Carolina at Chapel Hill

Design of Hypoallergenic Proteins for Modulating Food Allergic Responses

Background: Food allergic reactions are adverse immunological responses that follow exposure to normally innocuous proteins found in food. Although food allergies are rapidly becoming a public health concern and an escalating economic burden[1], current standards for treatment and prevention of food allergic responses are inadequate. Questions of long-term efficacy and the protections these treatments offer patients during accidental exposures remain unanswered. Thus, there is a need to explore new preventative and therapeutic options.

Food allergic patients produce specific immunoglobulin E (IgE) that target food allergen proteins. Once bound to IgE, these allergens mediate the noncovalent crosslinking of the IgE-receptor complexes[2,3], inducing clustering on the surface of effector immune cells to initiate degranulation, or the release of pro-inflammatory mediators (Fig. 1A). **Our central hypothesis is that disruption of the key molecular interactions associated with allergen-IgE-receptor complexation would mitigate the downstream effects of IgE-receptor signaling pathways, thereby reducing the severity of allergic reactions.** Specifically, we propose to redesign the oligomerization interfaces of known oligomeric food allergens to generate hypoallergenic proteins with lowered allergenic potency.

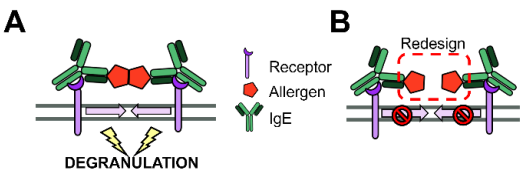

Fig. 1: Defining the role of oligomerization in food allergic responses. (A) Oligomeric food allergen enables the clustering of IgE-receptor complexes that initiate effector immune cell degranulation. (B) Redesign of allergen oligomerization interface has potential to prevent IgE-receptor complexation, thereby preventing cell degranulation.

Rationale: IgE-receptor crosslinking requires that at least two surface-bound IgE molecules bind to the allergen. However, if the allergen can form larger complexes, like dimers or higher order oligomers and aggregates, then this reduces the complexity and dimensionality of IgE binding such that cells only need to develop one IgE antibody to effectively crosslink the allergen[4-7]. Indeed, allergen oligomerization has been postulated to play a pivotal role in allergen stability and allergenic potential. Recent studies have found that 44 of the 55 documented allergen structures form transient dimers or higher order oligomers and that a number of these oligomers were significantly more allergenic than their monomers[7-9]. **If the oligomeric species is the allergenic species, we hypothesize that simply disrupting oligomerization would prevent crosslinking of IgE-receptor complexes, opening a new avenue for development of hypoallergenic proteins (Fig. 1B)**.

Approach: As a proof-of-principle, we propose to focus on bovine β-lactoglobulin (βLG), a known food allergen that accounts for 10% of proteins by weight in milk. In physiological conditions, βLG exists in a monomer-dimer equilibrium over a wide range of pH and temperatures[10-12]. This suggests that dimeric βLG (dβLG) is resistant to denaturation in conditions associated with heating and digestion. Taken together, this implies that dβLG is the allergenic species, and therefore, is a viable target. We will re-design the dimerization interface of βLG using computational protein design tools to test this hypothesis of allergen oligomerization in allergenicity. If βLG dimerization underlies its allergenic potential and its ability to crosslink IgE-receptor complexes, then designs that stabilize the βLG monomer (monomer-stabilizing βLG, msβLG) will be minimally allergenic, or hypoallergenic.

Aim 1: Computational design of msβLG and dimer-stabilizing βLG (dsβLG) mutants

We will build models of mβLG and dβLG to use as starting structures for ProteinMPNN[13], a deep-learning algorithm that can generate protein sequences from backbone structure inputs (Fig. 2A). Using the multistate and conditional design algorithms built into ProteinMPNN[13], we will determine key mutations along the dimerization interface of βLG to generate msβLG and dsβLG designs.

To investigate their potential for experimental validation, we will perform a series of biased molecular dynamics (MD) simulations (Fig. 2B). These MD simulations provide a physics-based estimate of the binding energies between the two dimers[14], which complements our data-driven ProteinMPNN design methodology and enables us to calculate the potential of mean force (PMF) and $\Delta G^{o'}$ for dimerization of our designs. msβLG designs would be expected to have a lower $\Delta G^{o'}$ for dimer formation than wild-type (WT), so designs with significantly lowered dimerization affinities will be tested. Likewise, dsβLG designs that have higher dimerization affinities will also move forward for experimental validation.

Aim 2: Biophysical characterization of msβLG and dsβLG mutants

Promising msβLG and dsβLG designs will be expressed and purified in *E. coli* using established protocols. Experimental K_d and $\Delta G^{o'}$ for purified samples of WT, single mutants, msβLG and dsβLG will be determined using an array of biophysical measurements, including analytical ultracentrifugation, SEC-MALS, and isothermal titration calorimetry. Results from this Aim will enable us to quantify the dimerization affinity of our designs relative to WT. Additionally, the biophysical data will be used to improve our computational modeling and design algorithms in Aim 1.

Aim 3: Quantification of allergenic potential of msβLG and dsβLG mutants

To directly gauge the allergenic potential of our designed proteins, we will run *in vitro* basophil and mast cell activation tests. Our collaboration with the UNC Food Allergy Initiative enables access to sera from de-identified milk-allergic patients from the NIH-sponsored Consortium of Food Allergy Research Central Biomarker Facility, which will be used to conduct the assay with our designed proteins and controls. Flow cytometry of CD63-stained mast and basophil cells will then allow us to determine the extent of degranulation of the patient-derived effector immune cells in the presence of the natural and designed food allergens[15,16]. If our msβLG are indeed hypoallergenic, we expect a lower degree of basophil and mast cell activation in patient samples challenged with our msβLG designed proteins.

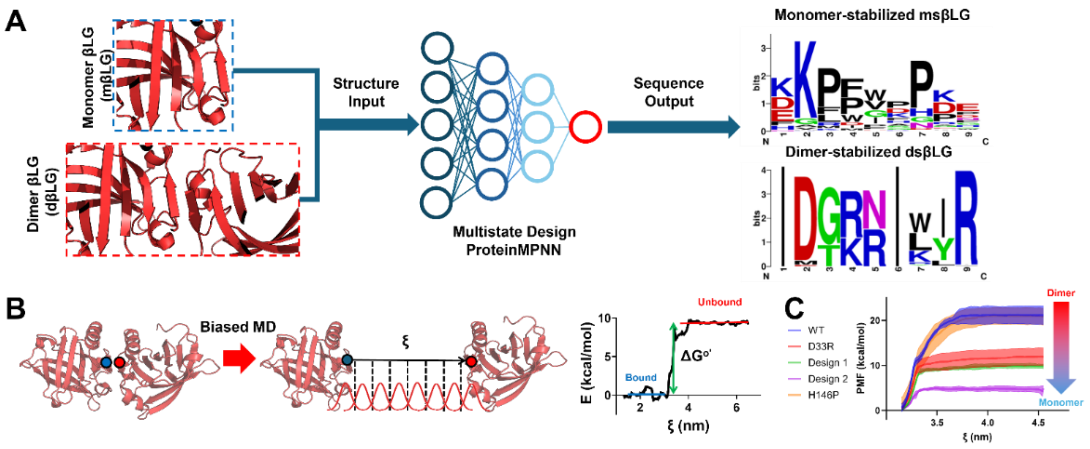

Fig. 2: Computational design and validation of βLG mutants. (A) Multistate design using ProteinMPNN[13] yield monomer- and dimer-stabilizing sequences from structure inputs. (B) Biased MD simulations of the designed dimers are run along the reaction coordinate (ξ) to generate a PMF plot, used to determine $\Delta G^{o'}$ for the designs. (D) Designs 1 and 2 show significantly decreased dimerization affinity compared to WT and are promising targets for experimental characterization.

Preliminary Results and Expected Outcomes: Models of mβLG and dβLG, from high-resolution crystallographic structures of WT βLG, were produced as structural inputs for ProteinMPNN to yield a series of msβLG and dimer-stabilizing βLG (dsβLG) designs that incorporate 5-9 unique mutations. AlphaFold2 and *ab initio* folding simulations of the sequence outputs of msβLG and dsβLG, mutants revealed that these designs maintain the desired tertiary structure, and thus, preserves the IgE binding sites. As illustrated by the PMFs and calculated $\Delta G^{o'}$ from our biased MD simulations (Fig. 2C), the mutations introduced to the dimerization interface of msβLG designs, Designs 1 and 2, ablate dimer formation. The support offered by the Pew Biomedical Scholars program will enable us to continue our research efforts to experimentally validate these designs as described in Aims 2 and 3. We can then use the biophysical and immunological data to determine the degree to which dimerization affinity impacts allergenicity.

Potential Impact: This work tests our fundamental understanding of protein-protein interactions in immunological signaling. Our approach yields new insights into the role protein oligomerization plays in allergenic potency and can pave the way for the design of new hypoallergenic proteins that may be incorporated into genetically modified organisms or in oral immunotherapies for food allergies. Even if this strategy does not produce the desired therapeutic effect, the designed proteins can serve as immunological probes to target specific interactions in the complex network of immunological factors.

This project's significance extends beyond food allergies as the ability to target specific protein-protein interactions has broad implications for the development of protein-based therapeutics. By deepening our understanding of molecular recognition at the chemical and structural levels, this research also has the potential to revolutionize protein engineering and contribute to advancements in the development of novel immunomodulatory biologics.

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4 Hasan-Abad, A. M. *et al. Clinical and molecular allergy : CMA* **20**, 5, doi:10.1186/s12948-022-00172-1 (2022).
5 Niemi, M. H. *et al. Scientific reports* **5**, 13841, doi:10.1038/srep13841 (2015).
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16 Kulis, M. D. *et al. The Journal of allergy and clinical immunology* **150**, 1144-1153, doi:10.1016/j.jaci.2022.05.020 (2022).

External References

Prof. Martin T. Zanni (PhD Advisor)
V.W. Meloche-Bascom Professor of Chemistry
Department of Chemistry, College of Letters and Science
University of Wisconsin-Madison
zanni@chem.wisc.edu
Expertise: Prof. Zanni's expertise lies in ultrafast spectroscopy and protein biophysics. He is known for his work in developing 2D-IR spectroscopy to probe protein structure and dynamics and has made contributions to the fields of amyloid aggregation and ion channel structure and function.

Prof. William F. DeGrado (Postdoc Advisor)
Professor of Pharmaceutical Chemistry
Department of Pharmaceutical Chemistry, School of Pharmacy
University of California, San Francisco
Bill.DeGrado@ucsf.edu or william.degrado@ucsf.edu
Expertise: Prof. DeGrado's expertise encompasses many fields, including protein design, membrane protein biophysics, peptide and protein chemistry, and protein structure and function. He is well-known for his work in protein design and has made many contributions to various fields like viral ion channel structure and function, protein-small ligand binding, biologics design, membrane protein design, and much more. He is also a member of the National Academy of Science.

Prof. James Fraser (Letter writer, former Pew Scholar)
Chair, Department of Bioengineering and Therapeutic Sciences
Ernest L. Prien Professor
University of California, San Francsico
James.fraser@ucsf.edu or jfraser@fraserlab.com
Expertise: Prof. Fraser's expertise lies in structural biology, specifically X-ray crystallography and cryo-EM technique development to understand protein structure, function, and dynamics. His broad interests include using these techniques to define functionally-relevant conformational states and understanding the transitions between such states.

Huong T. Kratochvil

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Education

2010-2016	**University of Wisconsin-Madison** (Madison, WI) Ph.D., Physical chemistry from Department of Chemistry Research advisor: Prof. Martin T. Zanni, Ph.D. *Thesis: Structure and Dynamics of Proteins and Peptides Revealed by Two-Dimensional Infrared Spectroscopy*
2006-2010	**The University of Texas at Austin** (Austin, TX) B.S., Chemistry from Department of Chemistry and Biochemistry Research advisor: Prof. Lauren J. Webb, Ph.D. *Thesis: Synthesis of a Non-Hydrolyzable GTP Analog for Vibrational Stark Effect Spectroscopy of the Ras Active Site*

Appointments

January 2023 -	**Assistant professor** Department of Chemistry, University of North Carolina at Chapel Hill
October 2016-December 2022	**NIH postdoctoral fellow** Department of Pharmaceutical Chemistry, University of California, San Francisco Advisor: Dr. William F. DeGrado, Ph.D.

Awards and Fellowships

2020-Present	**K99/R00 NIH Pathway to Independence Award**, NIGMS, NIH *Proton Conduction Pathways in Proton Channel Proteins*
2017-2020	**Ruth L. Kirschstein NRSA F32 Postdoctoral Fellowship**, NIGMS, NIH *A Structural and Biophysical Study of the Matrix Proteins in Influenza A/B Viruses: Mechanisms of Proton Conduction and Roles of Protein-Protein Interactions*
Fall 2021	**Intersections Science Fellow**
Fall 2021	**Stanford.Berkeley.UCSF Next Generation Faculty Symposium Honorable Mention**

PUBLICATIONS

Preprints/Submitted/Under Review

1. Celona, B.; Wu, H.; Dang, B.; **Kratochvil, H.T.**; DeGrado, W.F.; Black, B.L. "Zfp106 binds to G-quadruplex RNAs and inhibits RAN translation and formation of RNA foci caused by G4C2 repeats." *Under revision in PNAS*. Available on ***bioRxiv***, 2023: 10.1101/2023.03.05.531055

Peer-Reviewed Publications

2. Mravic, M.; He, L.; **Kratochvil, H.T.**; Hu, H.; Nick, S.E.; Bai, W.; Edwards, A.; Jo, H.; Wu, Y.; DiMaio, D.; DeGrado, W.F. "Designed transmembrane proteins inhibit the erythropoietin receptor in a custom binding topology." ***Nat. Chem. Biol.*** (2024).
3. **Kratochvil, H.T.**; Watkins, L.C.; Mravic, M.; Somberg, N.H.; Thomaston, J.L.; Nicoludis, J.M.; Liu, L.; Hong, M.; Voth, G.A.; DeGrado, W.F. "Transient water wires mediate selective proton transport in designed channel proteins." ***Nat. Chem.*** (2023). PMID: 37308712 PMCID: PMC10475958 DOI: 10.1038/s41557-023-01210-4
4. **Kratochvil, H.T.***; Newberry, R.W.*; Mensa, B.; Mravic, M.; DeGrado, W.F.* "Analysis and *de novo* design of membrane-interactive peptides." ***Faraday Discuss.***, 2021,232, 9-48. PMID: 34693965 PMCID: PMC8979563 DOI: 10.1039/d1fd00061f

***** denotes co-corresponding authors

5. Scott, A.J.; Niitsu, A.; **Kratochvil, H.T.**; Lang, E.J.M.; Sengel, J.T.; Dawson, W.M.; Mahendran, K.R.; Mravic, M.; Thomson, A.R.; Brady, R.L.; Liu, L.; Mulholland, A.J.; Bayley, H.; DeGrado, W.F.; Wallace, M.I.; Woolfson, D.N. "Constructing ion channels from water-soluble α-helical barrels." ***Nat. Chem.*** (2021). PMID: 33972753 PMCID: PMC7611114 DOI: 10.1038/s41557-021-00688-0
6. Schuller, M.; Correy, G.J.; Gahbaurer, S.; …**Kratochvil, H.T.**;…Shoichet, B.K.; Fraser, J.S.; Ahel, I. "Fragment Binding to the Nsp3 Macrodomain of SARS-CoV-2 Identified Through Crystallographic Screening and Computational Docking." ***Science Advances*** 7(16), 2021. PMID: 33853786 PMCID: PMC8046379 DOI: 10.1126/sciadv.abf8711
7. Schoof, M.; Faust, B.; Saunders, R. A.; Sangwan, S.; Rezelj, V.; Hoppe, N.; Boone, M.; Billesbølle, C. B.; Puchades, C.; Azumaya, C. M.; **Kratochvil, H. T.**; Zimanyi, M.; Deshpande, I.; Liang, J.; Dickinson, S.; Nguyen, H. C.; Chio, C. M.; Merz, G. E.; Thompson, M. C.; Diwanji, D.; Schaefer, K.; Anand, A. A.; Dobzinski, N.; Zha, B. S.; Simoneau, C. R.; Leon, K.; White, K. M.; Chio, U. S.; Gupta, M.; Jin, M.; Li, F.; Liu, Y.; Zhang, K.; Bulkley, D.; Sun, M.; Smith, A. M.; Rizo, A. N.; Moss, F.; Brilot, A. F.; Pourmal, S.; Trenker, R.; Pospiech, T.; Gupta, S.; Barsi-Rhyne, B.; Belyy, V.; Barile-Hill, A. W.; Nock, S.; Liu, Y.; Krogan, N. J.; Ralston, C. Y.; Swaney, D. L.; García-Sastre, A.; Ott, M.; Vignuzzi, M.; QCRG Structural Biology Consortium; Walter, P.; Manglik, A., "An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike." ***Science*** 370, (2020). PMID: 33154106 PMCID: PMC7857409 DOI: 10.1126/science.abe3255
8. Gordon, D.E.;Hiatt, J.; Bouhaddou, M.; Rezelj, V.V.; Ulferts, S.; Braberg, H.;…;**Kratochvil, H.T.**;…Vignuzzi, M.; Peden, A.A.; Beltrao, P.; Krogan, N.J. "Comparative host-coronavirus protein interaction networks reveal pan-viral disease mechanisms." ***Science*** 370, (2020). PMID: 33060197 PMCID: PMC7808408 DOI: 10.1126/science.abe9403
9. Bracken, C. J.; Lim, S. A.; Solomon, P.; Rettko, N. J.; Nguyen, D. P.; Zha, B. S.; Schaefer, K.; Byrnes, J. R.; Zhou, J.; Lui, I.; Liu, J.; Pance, K.; Zhou, X. X.; QCRG Structural Biology Consortium; Leung, K. K.; Wells, J. A., "Bi-paratopic and multivalent VH domains block ACE2 binding and neutralize SARS-CoV-2." ***Nat. Chem. Biol.*** 2021. PMID: 33082574 PMCID: PMC8356808 DOI: 10.1038/s41589-020-00679-1
   *Work done as part of the QCRG Structural Biology Consortium (see SI for contributions)
10. Wu, H.; Saltzberg, D.J.; **Kratochvil, H.T.**; Jo, H.; Sali, A.; DeGrado, W.F. "Glutamine side chain 13C=18O as a non-perturbative IR probe of amyloid fibril hydration and assembly." ***J. Am. Chem. Soc.*** 141 (18), 7320-7326 (2019). PMID: 30998340 PMCID: PMC6800148 DOI: 10.1021/jacs.9b00577
11. Zhang, K.; Ahmed, I.A.; **Kratochvil, H.T.**; DeGrado, W.F.; Gai, F.; Jo, H. "Synthesis and application of the blue fluorescent amino acid L-4-cyanotryptophan to assess peptide-membrane interactions." ***Chem. Comm.*** 55 (35), 5095-5098 (2019). PMID: 30957824 PMCID: PMC6508085 DOI: 10.1039/c9cc01152h
12. Zhang, S-Q.; Huang, H.; Yang, J.; **Kratochvil, H.T.**; Lolicato, M.; Liu, Y.; Shu, X.; Liu, L.; DeGrado, W.F. "Designed peptides that assemble into cross-α amyloid-like structures." ***Nat. Chem. Biol.*** 14, 870-875 (2018). PMID: 30061717 PMCID: PMC6497057 DOI: 10.1038/s41589-018-0105-5
13. **Kratochvil, H.T.**; Zanni, M.T. "Two-dimensional infrared (2D IR) spectroscopy for elucidating ion occupancies in the selectivity filter of ion channels." ***Biomed. Spectrosc. Imaging*** 7 (1-2), 3-15 (2018).
14. **Kratochvil, H.T.**; Maj, M.; Mutalef, K.; Annen, A;. Ostmeyer, J.; Perozo, E; Roux, B.; Valiyaveetil, F.; Zanni, M.T. "Probing the Effects of Gating on the Ion Occupancy of the K^+ Channel Selectivity Filter Using 2D IR Spectroscopy." ***J. Am. Chem. Soc.*** 139 (26), 8837-8845 (2017). PMID: 28472884 PMCID: PMC5544906 DOI: 10.1021/jacs.7b01594
15. Schneider, S.H.; **Kratochvil, H.T.**; Zanni, M.T.; Boxer, S.G. "Solvent Independent Anharmonicity for Carbonyl Oscillators." ***J. Chem. Phys. B.*** 121 (10), 2331-2338 (2017). PMID: 28225620 PMCID: PMC5453717 DOI: 10.1021/acs.jpcb.7b00537
16. **Kratochvil, H.T.**; Carr, J.K.; Mutalef, K.;Annen, A.W.;Li,H.; Maj, M.;Ostmeyer,J.; Raghuraman,H.; Moran, S.D.; Skinner, J.L.; Perozo, E.; Roux, B.; Valiyaveetil, F.; Zanni, M.T. "Instantaneous Ion Configurations in the K^+ Ion Channel Selectivity Filter Revealed by 2D IR Spectroscopy." ***Science*** 353, 1040 (2016). PMID: 27701114 PMCID: PMC5544905 DOI: 10.1126/science.aag1447
17. **Kratochvil, H.T.***; Ha, D.G.*, Zanni, M.T. "Counting Tagged Molecules One by One: Quantitative Photoactivation and Bleaching of Photoactivatable Fluorophores." ***J. Chem. Phys.*** 143, 104201 (2015). PMID: 26374025; DOI: 10.1063/1.4929991
18. Buchanan, L.E.; Dunkelberger, E.B.; **Tran, H.Q**.; Cheng, P.; Chiu, C.; Cao, P.; Raleigh, D.P.; de Pablo, J.J.; Nowick, J.S.; Zanni, M.T. "Mechanism of IAPP Amyloid Fibril Formation Involves an Intermediate with a Transient β-Sheet." ***Proc. Natl. Acad. Sci. U.S.A.*** 110(48):19285-19290, (2013). PMID: 24218609 PMCID: PMC3845187 DOI: 10.1073/pnas.1314481110
    * Published prior to name change in 2015

Teaching Experience

CHEM 101: General Descriptive Chemistry I (Spring 2023, Spring 2024)

CHEM 291: Academic Mentoring (Spring 2023, Spring 2024)
CHEM 395: Research in Chemistry for Undergraduates (Spring 2023, Fall 2024)
CHEM 731: Seminar Biological Chemistry (Spring 2024)

Graduate Students

Vincent Silverman (Chemical Biology Interface T32 Fellow), 01/2023 –
Nolan Jacob, 11/2023 -

Postdoctoral Scholars

Dr. Arash Firouzbakht, PhD, 10/2023 –

Undergraduate Students

Arora Rohrbach, 01/2023 –
Joshua Lopez, 01/2023 – 08/2023
Gautham Ravindran, 01/2023 -06/2023
Celeste Reeves (Packard Summer Research Fellow), 05/2023 -
Gavin Wiltshire, 05/2023 – 08/2023
Mia Brazeau, 05/2023 – 08/2023

Scientific Outreach and Communication

1. Quantitative Biosciences Institute TikTok Contest Winner, 2021: recorded TikTok to explain concepts of X-ray crystallography to a lay audience (Link here)
2. UCSF Postdoc Slam Finalist, September 2022. (Link here)

Invited Research Talks

1. Symposium of the Society of General Physiologists, Woods Hole, MA, September 2024.
2. American Chemical Society, Denver, CO, August 2024.
3. Telluride Workshop on Proton Transfers in Biology, Telluride, CO, July 2024.
4. University of North Carolina at Greensboro, Department of Chemistry, Greensboro, NC, January 2024.
5. Virginia Commonwealth University, Department of Structural Biology, Richmond, VA, November 2023.
6. University of North Carolina at Chapel Hill, Department of Biochemistry and Biophysics, Chapel Hill, NC, October 2023.
7. VIB-VUB Center for Structural Biology, Brussels, Belgium, June 2023.
8. Gordon Research Conference on Membrane Protein Folding, Barcelona, Spain, June 2023.
9. Sungkyunkwan University, Department of Nano Engineering, Suwon, South Korea, October 2022.
10. University of North Carolina at Chapel Hill, Joint Seminar in Chemical Biology and Bioorganic Chemistry, Chapel Hill, NC, August 2022.
11. Intersections Science Fellows Symposium, Virtual, November 2021.
12. University of Virginia Rising Stars Chemistry Symposium, Virtual, September 2021.
13. University of Chicago Department of Chemistry Future Faculty Conference, Virtual, May 2021.
14. American Society for Biochemistry and Molecular Biology, Virtual, April 2021.
15. American Chemical Society, Virtual, August 2020.
16. University of Utah Rising Stars Symposium, Salt Lake City, UT, September 2019.
17. Gordon Research Conference on Membrane Protein Folding, Easton, MA, July 2019.
18. FACSS/SCIX, Reno, NV, October 2017.
19. Pacifichem, Honolulu, HI, December 2015.

Conference Talks

1. Biophysical Society, Philadelphia, PA, February 2024.
2. Southwest Regional ACS Meeting, Durham, NC, October 2023.
3. Keystone Symposium: Computational Design and Modeling of Biomolecules, March 2023
4. Biophysical Society Molecular Biophysics of Membranes, June 2022.
5. American Chemical Society, San Diego, CA, March 2022
6. Biophysical Society, San Francisco, CA, February 2022
7. Pacifichem, Virtual, December 2021.
8. Telluride Workshop on Proton Transfer in Biology, Virtual, June 2021.
9. Biophysical Society, San Diego, CA, February 2020.

10. American Chemical Society, San Francisco, CA, April 2017

Diversity, Equity, and Inclusion Service Activities

Faculty representative, SWELL Committee, UNC-Chapel Hill	Spring 2023-Present
Representative on Committee on the Status of Women, UCSF: aided in the development and dissemination of TIPS sheets for reporting sexual harassment/assault, organized Women's Day at UCSF, provided guidelines for lactation room availability, made recommendations for the promotion and retention of women at all levels	Spring 2017-Fall 2019
Representative on the Campus Diversity and Climate Committee, UW-Madison: advocated for multilingual informational brochures for staff, worked with the university on building community with the greater Madison area	Fall 2015-Fall 2016
Representative on University Retention and Diversity Committee, UW-Madison: Organized meetings and led discussions on retention of diverse communities at the University of Wisconsin and diversity-related research. Developed strategic recommendations to enhance recruitment and retention for students, staff and faculty at the university.	Fall 2014-Spring 2015
Editor of the Chemistry Diversity Newsletter, UW Madison	Spring 2014-Fall 2016
Secretary, Chemistry Department Diversity Committee, UW-Madison: Recorded and distributed the meeting minutes, discussed measures to improve the climate and diversity of the department which led to the development of department resources list and monthly newsletters promoting diversity, recruited students for graduate program at ABRCMS and SACNAS	Spring 2013-Fall 2016

Service Activities

Biological Division Representative, Graduate Studies Committee, UNC-Chapel Hill	Fall 2022-Present
Graduate Admissions Committee, UNC-Chapel Hill	Fall 2022-Present
Conference Chair, GRS Membrane Protein Folding: organized and fundraised for inaugural GRS (postponed until 2023 due to COVID-19)	January 2020-June 2023
Team leader, QBI Structural Biology Consortium X-ray Crystallography Subgroup, UCSF**:** ran meetings and updated larger consortium on progress of subgroup; led prioritized crystallography experiments	April 2020-June 2021

Professional Memberships and Activities

Curator, Biophysics Colab	Spring 2023 – Present
Member, Triangle Protein Design	Spring 2023 - Present
Member, American Association for the Advancement of Science	Spring 2015-Present
Member, American Chemical Society	Fall 2009-Present
Ad Hoc Reviewer	Since 2016
Cell Systems, American Chemical Society, Scientific Reports	

Funding Sources

Current

NIH; NIGMS R00GM138753	Kratochvil, Huong T (PI)	09/01/2023-08/31/2026

Previous

NIH; NIGMS K99GM138753	Kratochvil, Huong T (PI)	09/01/2020-09/01/2022
NIH; NIGMS F32GM125217	Kratochvil, Huong T (PI)	09/01/2017-08/31/20204

UNC SCHOOL OF MEDICINE

Kathleen M. Caron, Ph.D.
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April 1, 2024

RE: Nomination of Heather McCauley, Ph.D. for the Pew Biomedical Scholars

To the Members of the Internal Selection Committee:

I am delighted to offer my enthusiastic recommendation to **Heather McCauley, Ph.D.** for the Pew Biomedical Scholars 2025 award. Dr. McCauley is a tenure-track Assistant Professor in the Department of Cell Biology and Physiology at the University of North Carolina at Chapel Hill. She began her appointment as an Assistant Professor on ***November 1, 2022***. Dr. McCauley was chosen as a top candidate from a highly-competitive national search of over 200 applicants for her creative and novel approaches to the study of enteroendocrine cells (EECs) in the intestine and for her expertise in using unique mouse and human pluripotent stem cell-derived organoid model systems to investigate intestinal development, physiology and pathology. The proposal that Dr. McCauley is submitting could result in fundamental breakthroughs in how the cellular responses to nutrients and the microbiome in the gut elicit local control of metabolic homeostasis and regulate endocrine processes that contribute to inflammatory bowel disease, obesity, and cancer. Her innovative ideas and cutting-edge techniques make her an excellent candidate for the Pew Biomedical Scholars Award as one of our top new independent investigators.

Dr. McCauley received her Bachelor of Arts in Biological Sciences and Bachelors of Science in Kinesiology from the University of Southern California in 2007, after which she spent two years at Medtronic Diabetes as a Therapy Associate obtaining valuable life experience in the field of her future work. Dr. McCauley earned her **Ph.D**. in 2015 as a graduate student in the lab of Dr. Geraldine Guasch at the University of Cincinnati. Heather's thesis work focused on the signaling mechanisms that maintain epithelial homeostasis and become dysregulated during transformation into invasive tumor cells and metastases. As a graduate student, Heather was highly independent, initiating projects primarily of her own design, making pioneering discoveries and developing novel techniques for the Guasch Lab. Heather's graduate work resulted in **5 first author manuscripts**, with two being ***co-corresponding author*** (*eLife*, 2017, *Bio-Protocol*, 2017). As a student, Dr. McCauley was already innovative, creative, and independent while obtaining broad knowledge and exceptional skills that predict a productive career as a faculty investigator.

Following her graduate school training, Dr. McCauley was recruited as a **postdoctoral** researcher to the laboratory of Dr. James Wells in the Department of Developmental Biology and Center for Stem Cell and Organoid Medicine at Cincinnati Children's Hospital Medical Center. Shortly after joining the Wells lab, Heather started a trend of obtaining independent support for her research by winning three postdoctoral fellowships for her projects on enteroendocrine cells, nutrition, and metabolism. Dr. McCauley was again highly independent, often **developing many of her own approaches** to answer fundamental questions about EEC function in nutrient sensing and their impact on neighboring cells. Her novel and pioneering work on EEC physiology and the intestinal microenvironment in dynamic systems communication has resulted in the publication of 14 peer reviewed manuscripts, including a **single-author**

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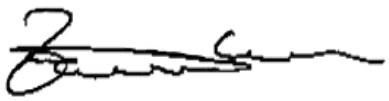
invited review (*Journal of Nutrition*, 2020) and a high-profile *Nature Communications* first-author paper (2020). Overall, her **5 first author papers** and numerous collaborative publications as a postdoc are an impressive achievement. Indeed, Dr. McCauley leveraged this success into a K01 Career Development Award from NIDDK entitled "*Enteroendocrine regulation of intestinal metabolism*" (K01DK125341).

Dr. McCauley holds a tenure-track Assistant Professor position within the Department of Cell Biology and Physiology in the School of Medicine. Dr. McCauley's independent research program focuses on how EECs affect the intestinal microenvironment (by regulating ion transport, cellular metabolism and mitochondrial function) via paracrine signals and how these functions impact the body as a whole (absorption of nutrients, metabolic disease, multi-organ communication) via endocrine signals. She employs modern techniques (e.g. single cell transcriptomics, metabolomics, organoids, and cutting-edge imaging) and has a proven track record of identifying interesting questions and turning them into productive hypothesis-driven research projects. Her proposal entitled "***Spatiotemporal Dynamics of Paracrine Signaling using Intestine as a Model System"*** is exciting and innovative. This project, which focuses on enteroendocrine cells (EECs) paracrine signaling in a complex 3D microenvironment that closely models *in vivo* intestinal architecture, tackles fundamental questions of gut cell biology and physiology with relevance to human health questions surrounding diet and gastrointestinal diseases, and also creates a framework for using a multiplexed approach to study other 3D biological contexts.

The McCauley Lab research program seeks to bridge the fields of digestive health and metabolism and are thus active members in both the UNC **Center for Gastrointestinal Biology and Disease (CGIBD)** and the **Nutrition Obesity Research Center (NORC)**. Dr. McCauley has already demonstrated outstanding promise due to the high quality of her novel research, her exceptional presentation skills, creative and innovative thinking, and collegial nature. Though she's been at UNC just over a year, Heather is already gaining a reputation as an excellent mentor. She has assembled a talented core team, attracted numerous undergraduate researchers and graduate rotation students, and has taken on her first graduate student, Jennifer Nwako. Her work fills a major gap in the basic science of gut physiology and is clinically relevant. I am confident Dr. McCauley will soon make significant contributions to her field and is an ideal candidate for the Pew Biomedical Scholars award.

In summary, Dr. McCauley is a fantastic junior faculty member in our department and I am enthusiastic that Heather's innovative questions has already infused new vitality and energy to our current research programs. She is sure to become an exceptional role model in the School of Medicine. Heather is a natural leader, collaborative and interactive, and I have found her to be extremely professional, mature, transparent and collegial. Most of all, she is genuinely compassionate, engaging, and full of vibrancy, qualities that will make her an excellent mentor and help her establish her lab as one of the best and most scientifically impactful in the country. I have complete confidence that Dr. McCauley has a spectacular career ahead of her and she is absolutely deserving of this prestigious award.

Sincerely,



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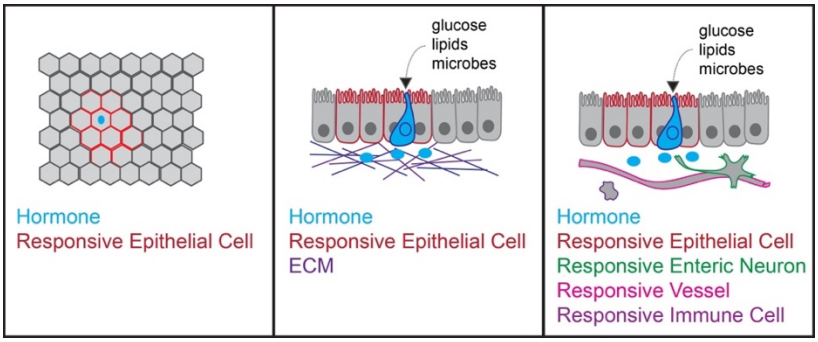
Spatiotemporal Dynamics of Paracrine Signaling using Intestine as a Model System

The gastrointestinal tract houses the largest endocrine system in the body, comprised of rare enteroendocrine cells (EECs) that secrete over 20 distinct hormones, neurotransmitters, and metabolites in response to environmental cues like nutrients and the microbiome[1]. Some of these secreted factors are well-known for their systemic effects on distant organs such as the pancreas and brain. In fact, new therapies for type 2 diabetes and, more recently, obesity, increase the action of one such hormone, GLP-1, to augment insulin secretion from the pancreas, slow gastric emptying, and send satiety signals to the brain. Other hormones also act systemically, such as cholecystokinin triggering bile and digestive enzyme release from the gallbladder and pancreas, and GIP influencing bone resorption and adipose deposition. While the roles of these circulating EEC-derived factors on whole-body physiology have been studied for decades, very little is known about how EECs and their secreted products impact their local environment within the intestine itself, largely due to a lack of tractable model systems.

To date, my team and I have discovered two important roles for EECs in intestinal epithelial homeostasis and function. First, we found that EECs are required to maintain the electrochemical gradients that support nutrient absorption in the small intestine[2]. For example, di- and tri-peptides are co-transported with H^+ ions and require a downward gradient for import into the enterocyte. We found that the intracellular pH of enterocytes was higher in mouse and human intestinal tissue without EECs, drastically diminishing protein absorption. This is significant as human patients born with mutations in *NEUROG3,* the transcription factor required for EEC differentiation, have no EECs and suffer from malabsorptive diarrhea, with approximately 90% of ingested protein being passed unabsorbed[3]. We administered exogenous peptide YY (PYY) to EEC-deficient mouse and human intestinal tissue resulting in restored intracellular pH and improved protein absorption. Intriguingly, through the course of our experiments, we observed that in mosaic regions of our animal model where EECs remained present, intracellular pH was normal. This suggested that EECs were regulating the electrochemistry and absorptive capacity of neighboring cells in a paracrine manner.

We next evaluated cellular metabolism and mitochondrial function in EEC-deficient intestine. Our results supported a model by which EECs are required to transmit nutritional cues to cells deep within the crypt that are spatially restricted from luminal contents, and that crypts, housing intestinal stem cells, phenocopied the metabolic and functional response to fasting when EECs were absent[4]. EECs are typically distributed along the crypt-villus axis, with some secreted products being more prevalent in the crypt and others enriched in the villus where they have ample access to luminal cues. An unanswered question remains: do the EECs at the villus tips, which sense nutrients apically and secrete basally, directly inform the crypt on the nutrient status of the gut, or do they act in a relay mechanism, by which a more distant EEC triggers a local crypt-based EEC to regulate crypt metabolism? Most EECs express the G-protein coupled receptors (GPCRs) for other EEC peptides, allowing the possibility of a relay mechanism.

My independent research program builds on these findings and seeks to uncover additional ways in which EECs transmit luminal cues to both epithelial and non-epithelial neighbors to regulate the function of the gut. The McCauley Lab opened its doors in November, 2022, at the University of North Carolina at Chapel Hill. As a new Assistant Professor, I am able to contribute 100% effort to building my research program. We are limited, however, by a simple question raised by both our previous studies: **What is the spatiotemporal range of EECs?** Upon stimulation, how many cells are exposed to EEC-derived products? Which epithelial and stromal cell types are impacted by EECs? Does stimulation of one EEC trigger the release of other hormones in local EECs? Addressing this gap in knowledge would provide empirical evidence for dosage and off-target effects in pharmaceutical studies; inform physicians on the development and severity of inflamed and non-inflamed tissue in patients with IBD; and, redefine our understanding of the gut-brain axis.



Building complexity to model the 3D spatiotemporal dynamics of enteroendocrine cell signaling in response to environmental cues.

EECs have been traditionally difficult to study for several reasons: 1) EECs only represent about 1% of the intestinal epithelium[1] ; 2) loss of all EECs in mouse results in impaired postnatal survival with severe malabsorptive diarrhea[5], consistent with the human phenotype[3]; 3) many EECs have overlapping hormone expression and function, such that loss of a single hormone in mouse results in only a mild phenotype[6]; and 4) the paracrine effects of EECs in an animal model can be obscured by endocrine effects of distant hormone-producing cells. To circumvent these obstacles, we use a human pluripotent stem cell (PSC)-derived intestinal organoid (HIO) model[7], in which we employed CRISPR/Cas9 gene editing to delete EECs[8], as a reductionist model system to isolate local effects of EECs on the gut. In this proposal, EEC-deficient HIOs will be used as a blank canvas for the expression of individual fluorescent hormone or receptor constructs without the confounding effects of endogenous secretion.

Recent advances in super-resolution microscopy now enable detection of single molecules as small as 20nm[9]. We will apply this technology to track individual EEC hormones as they are secreted by labeling peptides with a photoactivatable fluorescent protein, such as PA-GFP[10]. We have previously generated an E-cadherin-mRuby2 fusion construct using CRISPR/Cas9 and introduced it into human PSCs to enable live imaging of epithelial cells[4,11], and a doxycycline-inducible NEUROG3 construct that can be titrated to increase the proportion of rare EECs[8]. We will employ these constructs in the parental EEC-deficient PSC line, add our hormone-fluorophore fusions, and direct their differentiation into HIOs. Many mouse models employ hormone-fluorophore fusions that do not disturb the function of the hormone or its ability to bind to its cognate receptor, suggesting this strategy is feasible in our model system. As hormones are released quickly upon stimulation with nutrients, we will apply glucose to the apical surface while imaging using combined light sheet and single-molecule localization microscopy to capture the release of the photoactivatable hormones. Using techniques pioneered in calculating morphogen gradients in developmental biology[12], and evaluating cultures with varying matrix stiffness, we will calculate the diffusion coefficients for the EEC-secreted products PYY, somatostatin (SST), and neurotensin (NTS). We will begin with these hormones as their cognate receptors, NPY1R, SSTR5, and NTSR1, are abundant on neighboring intestinal epithelial cells.

In a complementary approach, we will use SNAP-tagged GPCR technology to visualize the spatial and temporal dynamics of these receptors at high resolution in PSC-derived HIOs. This method has been successfully used to visualize GLP1R localization and action in murine islets at homeostasis and receptor internalization in response to GLP1R agonists[13]. First, we will introduce each SNAP-tagged GPCR to the E-cadherin-mRuby2 labeled EEC-deficient HIO model and incubate intestinal monolayers with varying matrix stiffness and composition with beads soaked in PYY, SST, or NTS. Time-lapse live imaging at high resolution will be used to measure the number of cells surrounding a bead which internalize the peptide receptor upon ligand binding. Next, we will use each SNAP-tagged GPCR construct in the doxycycline-inducible EEC-increased model to evaluate receptor internalization by endogenous peptides after EECs are stimulated by glucose, lipids, and microbes. Together, these experiments lay the foundation for multiplexing several EEC-derived products and evaluating their range to signal to unique target types.

Intestinal epithelial cells are simple to identify using the E-cadherin-mRuby2 reporter and adapting a monolayer culture system. However, PSC-derived organoids are complex structures that co-develop with multiple germ layers, spontaneously develop additional stromal cell types[7], and are amenable to incorporation of enteric neurons[14] and immune cells (manuscript in prep) via tissue engineering. Stromal cells like fibroblasts, telocytes, smooth muscle cells, immune cells, lymphatic vessels, and enteric neurons express a much wider complement of EEC hormone receptors, such as GLP1R, GLP2R, GIPR, and others. Once we have established the constructs and experimental parameters as proposed here, we will expand the scope of this project to identify 3-dimensional and non-epithelial targets of EECs which more faithfully model the architecture and cellular milieu of native intestine. We will use 3D measurements and mathematical modeling to extrapolate our findings to the complex crypt-villus architecture of the *in vivo* small intestine.

While my primary interests lie in understanding the local regulation of nutrient sensing and intestinal functions by EECs, this project lays the framework for undertaking a multiplexed approach to paracrine signaling in other biological contexts, such as the pancreatic islet, the bone marrow niche, and angiogenic sprouting. The short-term impact of the experiments proposed here lies in fundamentally improving our knowledge of paracrine signaling in complex, human environments approaching *in vivo* structure. Furthermore, this work will improve understanding of GI disease pathology and facilitate the discovery of nutritional-based interventions and development of next-generation drugs which mitigate off-target effects. Together, the experiments proposed herein have the potential for great biological, physiological, and therapeutic impact.

REFERENCES

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6. McCauley HA. Enteroendocrine Regulation of Nutrient Absorption. *J Nutr*. Jan 1 2020;150(1):10-21. doi:10.1093/jn/nxz191
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REFEREES

James Wells, PhD
Professor
Center for Stem Cell and Organoid Medicine
Division of Developmental Biology
Division of Endocrinology
Cincinnati Children's Hospital Medical Center
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Postdoctoral Advisor. Dr. Wells pioneered the use of human pluripotent stem cell-derived organoids to study human gastrointestinal development and model rare diseases.

Geraldine Guasch, PhD
Principal Investigator
Centre de Recherche en Cancerologie de Marseille
geraldine.guasch-grangeon@inserm.fr

Thesis advisor. Dr. Guasch discovered that transition zones, where two distinct epithelia meet, function as a stem cell niche. Transition zones are highly susceptible to tumor development. The goal of her laboratory is to understand the molecular and cellular basis that permits transformation in these unique regions.

Nicholas Zachos, PhD
Associate Professor of Surgery
Associate Professor of Cell and Developmental Biology
Vanderbilt University
n.zachos@vumc.org

Scientific expert. Dr. Zachos was the first to develop 2D intestinal enteroid monolayer cell cultures which are now used widely in the field. This culture system allows for apical and basolateral access to epithelial cells, the coculture of other cell types like macrophages, and live imaging with high resolution. His laboratory uses this model and others to study the interactions between intestinal epithelia and immune cells.

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1) Education

- **Postdoctoral Fellowship,** 2015-2019
  Cincinnati Children's Hospital Medical Center
  Developmental Biology and the Center for Stem Cell and Organoid Medicine
  Laboratory of James M. Wells, Ph.D.

- **Ph.D. Molecular and Developmental Biology,** 2015
  University of Cincinnati and Cincinnati Children's Hospital Medical Center
  Laboratory of Geraldine Guasch, Ph.D.

- **B.A. Biological Sciences**, **B.S. Kinesiology,** 2007
  University of Southern California

2) Professional Experience

- **Assistant Professor,** 2022-present
  University of North Carolina at Chapel Hill School of Medicine
  Department of Cell Biology and Physiology
  Member, Center for Gastrointestinal Biology and Disease
  Member, Nutrition Obesity Research Center
  Member, Lineberger Comprehensive Cancer Center
  Trained Mentor, Office of Graduate Education

- **Instructor,** 2021-2022
  Cincinnati Children's Hospital Medical Center
  Developmental Biology and the Center for Stem Cell and Organoid Medicine

- **Research Associate,** 2019-2021
  Cincinnati Children's Hospital Medical Center
  Developmental Biology and the Center for Stem Cell and Organoid Medicine
  Laboratory of James M. Wells, Ph.D.

3) Honors

- **2023 Institutional Nominee for Pew Biomedical Scholars Program**
- **2022 Travel Award,** FASEB SRC "The Gastrointestinal Tract XX Conference"
- **2021 Trainee Award,** FASEB SRC "The Gastrointestinal Tract XIX Conference"
- **2019 Travel Award,** FASEB SRC "The Gastrointestinal Tract XIX Conference"
- **2018 Travel Award,** FASEB SRC "Nutrient Sensing and Metabolic Signaling"
- **2018 Poster Award,** CCHMC Developmental Mechanisms, Organogenesis and Stem Cells Annual Retreat
- **2018 Poster Award,** CCHMC Digestive Health Center Annual Scientific Symposium
- **2017 Poster Award,** CCHMC Developmental Mechanisms, Organogenesis and Stem Cells Annual Retreat
- **2013 Poster Award,** CCHMC Developmental Mechanisms, Organogenesis and Stem Cells Annual Retreat
- **2012 Outstanding Student Presentation,** CCHMC Molecular and Developmental Biology Student Symposium
- **2012 Akeson Award for Academic Excellence,** CCHMC Molecular and Developmental Biology Graduate Program
- **2012 Poster Award,** CCHMC Developmental Mechanisms, Organogenesis and Stem Cells Annual Retreat
- **2011 Richard A. Akeson Fellowship**
- **2011 Poster Award,** CCHMC Developmental Mechanisms, Organogenesis and Stem Cells Annual Retreat
- **2011 Outstanding Student Presentation,** CCHMC Molecular and Developmental Biology Student Symposium

4) Bibliography and products of scholarship

- **Peer-reviewed journal articles**

1. Sanchez, J.G., Rankin, S.A., Paul, E., **McCauley, H.A.**, Kechele, D.O., Enriquez, J.R., Jones, N.-H., Greeley, S.A.W., Letourneau-Friedberg, L., Zorn, A.M., Krishnamurthy, M., Wells, J.M. 2024. RFX6 Regulates Human Intestinal Patterning and Function Upstream of PDX1. *Development*, accepted.
2. Munera, J.O., Kechele, D.O., Bouffi, C., Qu, N., Jing, R., Maity, P., Enriquez, J.R., Han, L., Campbell, I., Mahe, M.M., **McCauley, H.A.,** Zhang, X., Sundaram, N., Hudson, J.R., Zarsozo-Lacoste, A., Pradhan, S., Tominaga, K., Sanchez, J.G., Weiss, A.A., Chatuvedi, P., Spence, J.R., Hachimi, M., North, T., Daley, G.Q., Mayhew, C.N., Hu, Y.-C., Takebe, T., Helmrath, M.A., Wells, J.M. 2023. Development of functional resident macrophages in human pluripotent stem cell-derived colonic organoids and human fetal colon. *Cell Stem Cell*, 30(11): 1434-1451. doi: 10.1016/j.stem.2023.10.002
3. Singh, A., Poling, H.M., Chaturvedi, P., Thorner, K., Sundaram, N., Kechele, D.O., Childs, C.J., **McCauley, H.A.,** Fisher, G.W., Brown, N.E., Spence, J.R., Wells, J.M., Helmrath, M.A. 2022. Transplanted human intestinal organoids: A resource for modeling human intestinal development. *Development,* 150 (9): dev201416. doi: 10.1242/dev.201416
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14. Lee, K.K., **McCauley, H.A.**, Broda, T.R., Kofron, M.J., Wells, J.M., Hong, C.I. 2018. Human stomach-on-a-chip with intraluminal flow and peristaltic-like motility. *Lab on a chip,* Oct 9;18(20):3079-3085. doi: 10.1039/c8lc00910d.
15. **McCauley, H.A.**, Wells, J.M. 2017. Pluripotent stem cell-derived organoids: Using principles of developmental biology to grow human tissues in a dish. *Development,* 144: 958-962. doi: 10.1242/dev.140731.
16. **McCauley, H.A.***, Guasch, G. 2017. Isolation of epithelial CD34+ cancer stem cells from Tgfbr2-deficient

squamous cell carcinoma. *Bio-protocol*, 7(17): e2524. doi: 10.21769/BioProtoc.2524. *co-corresponding author.

17. **McCauley, H.A.*** Chevrier V.C., Birnbaum D., Guasch, G. 2017. De-repression of ELMO1 in cancer stem cells drives progression of TGFβ-deficient squamous cell carcinoma from transition zones. *eLife,* 6:e22914. doi: 10.7554/eLife.22914. *co-corresponding author.
18. **McCauley, H.A.**, Wells, J.M. 2016. Sweet relief: Reprogramming gastric endocrine cells to make insulin. *Cell Stem Cell,* 18: 295-297. doi: 10.1016/j.stem.2016.02.009.
19. **McCauley, H.A.**, Guasch, G. 2015. Three cheers for the goblet cell: Maintaining homeostasis in mucosal epithelia. *Trends in Molecular Medicine*, 21(8): 492-503. doi: 10.1016/j.molmed.2015.06.003.
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21. **McCauley, H. A.**, Guasch, G. 2013. Serial orthotopic transplantation of epithelial tumors in single-cell suspension. *Stem Cell Niche: Methods and Protocols, Methods in Molecular Biology*, vol. 1035: 231-45. doi: 10.1007/978-1-62703-508-8_20.

- **Invited and refereed oral presentations (selected)**

1. 2024. *Enteroendocrine cells regulate intestinal physiology and function.* Duke University Division of Gastroenterology Research Conference. Virtual. Invited.
2. 2023. *Enteroendocrine cells regulate intestinal physiology and function*. UNC Center for Gastrointestinal Biology and Disease Seminar Series. Virtual. Invited.
3. 2023. *Enteroendocrine cells regulate intestinal physiology and function*. FASEB SRC: The Gastrointestinal Epithelial Conference: Interface with the Outside World, Steamboat Springs, CO. Invited.
4. 2023. *Enteroendocrine cells regulate intestinal physiology and function*. University of New Mexico. Virtual. Invited.
5. 2023. *Enteroendocrine cells protect the stem cell niche by regulating crypt metabolism in response to nutrients*. Digestive Disease Week, Research Symposium. Chicago, IL. Invited.
6. 2023. *Enteroendocrine cells regulate intestinal physiology and function.* UNC Marsico Lung Institute Seminar Series, Chapel Hill, NC. Invited.
7. 2023. *Enteroendocrine cells regulate intestinal physiology and function.* Cell Biology and Physiology Research Day, Raleigh, NC. Invited.
8. 2022. *Enteroendocrine cells protect the stem cell niche by regulating crypt metabolism*. FASEB SRC: The Gastrointestinal Tract XX Conference, Steamboat Springs, CO. Refereed.
9. 2022. *Enteroendocrine cells protect the stem cell niche by regulating crypt metabolism*. Digestive Disease Week, Research Symposium. San Diego, CA. Invited.
10. 2022. *Enteroendocrine cells regulate intestinal metabolism and function.* University of North Carolina at Chapel Hill Department of Cell Biology and Physiology. Chapel Hill, NC. Invited.
11. 2022. *Enteroendocrine cells regulate intestinal metabolism and function.* Emory University School of Medicine, Division of Digestive Diseases. Virtual. Invited.
12. 2021. *Enteroendocrine cells regulate intestinal metabolism and function.* Johns Hopkins University School of Medicine, Division of Gastroenterology and Hepatology. Virtual. Invited.
13. 2021. *Enteroendocrine cells regulate intestinal metabolism and function.* Baylor University School of Medicine, Department of Molecular Physiology and Biophysics. Virtual. Invited.
14. 2021. *Enteroendocrine cells regulate intestinal metabolism and function.* Boston University, Center for Regenerative Medicine. Virtual. Invited.
15. 2021. *Enteroendocrine cells regulate intestinal metabolism and function.* CCHMC Digestive Health Center Seminar Series. Virtual. Invited.
16. 2020. *Enteroendocrine cells regulate intestinal function.* EMBL Barcelona Postdoc Seminar Series. Virtual. Refereed.
17. 2020. *PSC-derived organoids in modeling endocrinopathies.* CCHMC Division of Endocrinology, External Scientific Advisory Council Review Board. Cincinnati, OH. Invited.
18. 2020. *Enteroendocrine cells couple nutrient sensing to nutrient absorption by regulating ion transport.* CCHMC Developmental Biology Division Meeting. Cincinnati, OH. Invited.
19. 2019. *Enteroendocrine cells couple an epithelial-neuronal signal to control nutrient absorption.* FASEB SRC: The Gastrointestinal Tract XVIII Conference. Steamboat Springs, CO. Refereed
20. 2019. *Enteroendocrine cells couple an epithelial-neuronal signal to control nutrient absorption.* CCHMC Center for Stem Cell and Organoid Medicine / RIKEN BDR Joint Workshop. Kobe, Japan. Invited.
21. 2019. *Enteroendocrine cells couple an epithelial-neuronal signal to control nutrient absorption.* CCHMC

Developmental Mechanisms, Organogenesis and Stem Cells Annual Retreat. Hueston Woods, OH. Invited.
22. 2018. *Enteroendocrine regulation of nutrient absorption.* Great Lakes International Imaging and Flow Cytometry 27th Annual Meeting. Covington, KY. Invited.
23. 2018. *Enteroendocrine regulation of nutrient absorption.* FASEB SRC: Nutrient Sensing and Metabolic Signaling. Snowmass, CO. Refereed.
24. 2018*. Using organoids to model regulatory mechanisms of absorption in the intestine.* ISSCR Annual Meeting: Innovation Showcase, hosted by STEMCELL Technologies. Melbourne, Australia. Invited.
25. 2017. *Enteroendocrine regulation of nutrient absorption.* CCHMC Endoderm Club. Cincinnati, OH. Invited.
26. 2017. *Enteroendocrine regulation of nutrient absorption.* CCHMC Developmental Mechanisms, Organogenesis and Stem Cells Annual Retreat. Hueston Woods, OH. Invited.
27. 2017. *Enteroendocrine cells, nutrients and metabolic disease.* CCHMC Postdoctoral and Research Associate Meeting. Cincinnati, OH. Invited.
28. 2015. *Loss of TGFβ signaling is required for Rac-mediated cancer stem cell invasion in transitional epithelial squamous cell carcinoma*. UCLA David Geffen School of Medicine, Department of Human Genetics. Los Angeles, CA. Invited.
29. 2015. *Loss of TGFβ signaling is required for Rac-mediated cancer stem cell invasion in transitional epithelial squamous cell carcinoma.* UC Irvine, Center for Epigenetics and Metabolism. Irvine, CA. Invited.

- **Refereed poster presentations (selected)**
  1. Dodson, H., Riedman, A.M., McCauley, H.A. 2023. Enteroendocrine cells regulate Wnt and BMP signaling in intestinal crypts. Silvio O. Conte Digestive Diseases Research Core Centers (DDRCC) 2023 Meeting. Cincinnati, OH.
  2. Riedman, A.M. and McCauley, H.A. 2023. Enteroendocrine cells regulate Wnt and BMP signaling in intestinal crypts. Digestive Disease Week. Chicago, IL.
  3. McCauley, H.A., Zhang, X., Enriquez, J.R., Sanchez, J.G., Wells, J.M. 2021. Enteroendocrine cells regulate intestinal metabolism and function. FASEB SRC: The Gastrointestinal Tract XIX Conference. Virtual.
  4. McCauley, H.A., Zhang, X., Enriquez, J.R., Nichol, J. Sanchez, J.G., Wells, J.M. 2021. Enteroendocrine cells regulate intestinal metabolism. Keystone Symposia: Metabolic Decisions in Development and Disease. Virtual.
  5. McCauley, H.A., Zhang, X., Enriquez, J.R., Nichol, J. Sanchez, J.G., Wells, J.M. 2020. Enteroendocrine cells regulate intestinal metabolism. FASEB SRC: Nutrient Sensing and Metabolic Signaling. Virtual.
  6. McCauley, H.A., Martini, C., Enriquez, J.R., Stone, W.J., Matthis, A.L., Montrose, M.H., Aihara, E., Wells, J.M. 2018. Enteroendocrine regulation of nutrient absorption. FASEB SRC: Nutrient Sensing and Metabolic Signaling. Snowmass, CO.
  7. McCauley, H.A., Martini, C., Enriquez, J.R., Stone, W.J., Matthis, A.L., Montrose, M.H., Aihara, E., Wells, J.M. 2018. Enteroendocrine regulation of nutrient absorption. International Society for Stem Cell Research Annual Meeting. Melbourne, Australia.
  8. McCauley, H.A., Aihara, E., Matthis, A.L., Martini, C.H., Enriquez, J.R., Stone, W.J., Montrose, M.H., Wells, J.M. 2018. Enteroendocrine regulation of nutrient absorption. CCHMC Developmental Mechanisms, Organogenesis and Stem Cells Annual Retreat. Hueston Woods, OH. *Poster prize.*
  9. McCauley, H.A., Aihara, E., Matthis, A.L., Martini, C.H., Enriquez, J.R., Stone, W.J., Montrose, M.H., Wells, J.M. 2018. Enteroendocrine regulation of nutrient absorption. CCHMC Digestive Health Center Annual Symposium. Cincinnati, OH. *Poster prize.*
  10. McCauley, H.A., Wells, J.M. 2017. Enteroendocrine regulation of nutrient absorption. Keystone Symposia: Gastrointestinal Control of Metabolism. Copenhagen, Denmark.
  11. McCauley, H.A., Wells, J.M. 2017. Enteroendocrine regulation of nutrient absorption. CCHMC Developmental Mechanisms, Organogenesis and Stem Cells Annual Retreat. Hueston Woods, OH. *Poster prize.*

5) Teaching Activities

- **Formal Teaching**
  - Lecturer, UNC-CH CBHP803: Cell Biology and Physiology in Health and Disease – Intro to the GI Tract, Spring 2023, Spring 2024
  - Lecturer, NCSU CBS764: Advances in Gastrointestinal Pathophysiology – Enteroendocrine Cells in GI Disease, Spring 2024

- **Lab/Research Mentorship**
  - Yunan Hu, BBSP rotation student, Spring 2024
  - Shubhi Singh, undergraduate student, 2024-present
  - Samara Williams, undergraduate student, 2024-present
  - Taevon Roach, undergraduate student, 2023-present
  - Meghan Anderman, BBSP rotation student, Fall 2023
  - Jennifer Nwako, CBP graduate student, 2023-present
  - Caden Sweet, BBSP rotation student, Spring 2023
  - Zachary Azevedo, undergraduate student, 2023-present
  - Hailey Dodson, undergraduate student, 2023
  - Anne Marie Riedman, research associate (McCauley Lab) 2021-present
  - Emily Paul, undergraduate student (Wells Lab) 2022
  - Kayle Riley, PREP scholar (McCauley Lab) 2021-2022
  - Vivien Sauer, MSTP student (Wells Lab) 2020-2022
  - J. Guillermo Sanchez, PhD candidate (Wells Lab) 2018-2022
  - Jacob R. Enriquez, PhD candidate (Wells Lab) 2016-2022
  - Jonah Nichol, undergraduate student (Wells Lab) 2018-2021
  - Catherine Martini, undergraduate student (Wells Lab) 2017-2018
  - William J. Stone, undergraduate student (Wells Lab) 2015-2018
  - Marion Brusadelli, Masters student (Guasch Lab) 2013-2015
  - Alyssa Gallas, Masters student (Guasch Lab) 2012-2014
  - Yanne Doucet, Masters student (Guasch Lab) 2011-2013
- **Thesis Committees**
  - Katie Clough, Toxicology, 2023-present
  - Savannah Wright, Pathobiology and Translation Science, 2023-present
  - Ismael Gomez-Martinez, Cell Biology and Physiology, UNC, 2022-2023
- **Other Supervision**
  - Anne Marie Riedman, Undergraduate Senior Thesis, Xavier University, 2022

6) Grants

- **ACTIVE**
  - **Cystic Fibrosis Foundation (006005G223)**
    "A novel human model to investigate GI manifestations of CF"
    PI: McCauley, H.A.
    01/01/2024-12/31/2026
    $501,747 ($350,000 direct)
    % Effort: 10

  - **UNC Center for Gastrointestinal Biology and Disease Pilot & Feasibility Award**
    "Enteroendocrine regulation of intestinal signaling pathways and tumorigenesis"
    PI: McCauley, H.A.
    07/01/2023-06/30/2024
    $35,000

  - **NIDDK Mentored Career Development Award (K01DK125341)**
    "Enteroendocrine regulation of intestinal metabolism"
    PI: McCauley, H.A.
    09/01/2021-07/31/2026
    $629,245
    % Effort: 90

- **COMPLETED**
  - **American Diabetes Association Postdoctoral Fellowship (1-17-PDF-102),** 2017-2019
    "Enteroendocrine cells, nutrients, and metabolic disease"
    PI: McCauley, H.A.
    01/01/2017-12/31/2019
    $166,356
    % Effort: 100

  - **Arnold W. Strauss Fellow Award**
    "Enteroendocrine cells, nutrients, and metabolic disease"
    PI: McCauley, H.A.
    07/01/2016-06/30/2017
    $10,000

7) Service

- **To discipline:**
  - Session organizer and co-chair, American Physiology Summit, 2024
  - Grant Review, Swiss National Science Foundation Spark, 2023
  - Peer review, 2016-present *(Acta Biochimica et Biophysica Sinica, Biomaterials, bioProtocol, Cell Metabolism, Cell Reports, Cell Stem Cell, Development, Disease Models and Mechanisms, iScience, Journal of Nutrition, Molecular Metabolism, Nature Communications, Nature Medicine, Nature Metabolism, Nutrients, Pharmaceutics, Science, Science Advances)*
  - Session co-chair, Digestive Disease Week, 2023
  - Panelist, "Career Development in the Time of COVID-19: Tools for Resilience," Association for Women in Science, Greater Cincinnati Chapter, 2022
  - Session co-chair, FASEB SRC: The Gastrointestinal Tract XIX, 2021
  - Network for Pancreatic Organ Donors with Diabetes, JRDF Type One Nation Summit, 2015
  - Member: American Gastroenterology Society, American Physiology Society, International Society for Stem Cell Research, 2016-present
- **Within UNC-Chapel Hill:**
  - Cell Biology and Physiology Preliminary Exam Committee, 2024-2025
  - BBSP Molecular and Basic Sciences Graduate Admissions Committee & Selection Subcommittee, 2023-present
  - BBSP First Year Group Faculty Mentor, 2023-present
  - BBSP Graduate Student Recruitment, Spring 2023, Spring 2024
- **At CCHMC:**
  - Poster judge, Ohio River Valley Imaging and Cytometry Day, 2018-2022
  - Poster judge, University of Cincinnati Medical Sciences Poster Fair, 2017-2022
  - Poster judge, CCHMC Summer Undergraduate Research Fellowship Capstone, 2017-2022
  - Poster judge, University of Cincinnati Graduate Student Research Symposium, 2017-2021
  - CCHMC Family Health and Science Expo, 2019
  - CCHMC High School Student Symposium, 2019

PI: Walsh, Jessica (Pages 107-117)

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April 1st, 2024

Dear Colleagues:

It is my pleasure to nominate Dr. **Jessica Walsh** for the 2024 Pew Biomedical Scholars program. Pew funds biomedical research in neuroscience, and supports investigators distinguished by their bold approaches to scientific questions with translational potential, with above-average risk and groundbreaking possibilities. Dr. Walsh has an exceptional track record of innovative and groundbreaking research, having garnered several scientific awards, 3 NIH grants, with her most recent R01 being funded with a 2% score. Additionally, she has authored 26 publications in top-tier journals, including five 1st author research manuscripts, two of which were published in *Nature*.

Dr. Walsh has developed a truly elegant research plan to investigate the molecular and neural circuit mechanisms underlying biased signaling in the serotonin system both *in vitro* and *in vivo*. Among its many strengths are employing cutting-edge approaches such as viral mediated gene transfer (developing and optimizing biased engineered receptors and selective G-protein knockdowns), combinatorial transgenic mice, *ex vivo* slice electrophysiology, and behavioral assays combined with exploratory and modulatory investigations of neural activity. Her efforts to develop and optimize novel genetic tools, as well as investigate the electrophysiological and behavioral effects of biased signaling in mice, has not been done with any G-protein coupled receptor. The results of these studies will provide novel information about the role of biased signaling by serotonin in the medial prefrontal cortex and set the stage for electrophysiological characterization of other neurotransmitters and their receptors in the brain. The ultimate goal of these efforts is to understand the mechanisms to screen and develop biased compounds for the treatment of specific endophenotypes present in psychiatric and neurodevelopmental disorders.

Dr. Walsh has already made significant advances in neuroscience and our understanding of the brain. She has spent her brief but meteoric career working towards understanding the neural circuits underlying motivated behaviors and how they go awry when behavioral deficits are present. Her early work pioneered the use of optogenetics to investigate social behaviors and the impact of stress on neural circuit activity involved in motivated behavior. These pivotal studies identified both a critical circuit as well as molecular mediators of social interactions. This laid the groundwork for pursuing the use of ezogabine, a potassium channel modulator, as a novel antidepressant treatment, which has recently shown promise in clinical trials.

Her most recent findings answered a longstanding question of in the field: can release of serotonin into the nucleus accumbens, a brain region implicated in motivated behavior, specifically regulate social behavior and reverse social deficits present in a mouse model for autism spectrum disorder (ASD). She not only showed that direct modulation of serotonin activity in discrete brain regions regulates social interactions in mice, but also isolated a unique serotonin receptor subtype as the molecular locus of action. These findings proved translationally

relevant as modulation of this receptor rescued social deficits present in several mouse models. As a consequence of these studies, subtype-selective serotonin receptor ligands are being pursued as novel therapeutics to enhance sociability. It is clear from these accomplishments that Dr. Walsh has great potential to remain at the forefront of her field and I have the utmost confidence that she will succeed in achieving the aims of this proposal.

Dr. Walsh takes a multi-disciplinary, leading-edge approach, one that integrates light-sheet microscopy, optogenetics, *in vivo* calcium imaging, slice electrophysiology and animal behavior. The University of North Carolina boasts an exceptional roster of core facilities, many of which the Walsh Lab already uses on a regular basis. The Colony Management Core, under the guidance of Dr. Natallia Riddick, has proved to be an invaluable resource, allowing lab members to focus their efforts directly on scientific experimentation. Additionally, the UNC Vector Core provides rapid access to the vectors needed by her research program.

Other critical resources include the Neuroscience Microscopy Core and Microscopy Services Laboratory, under the guidance of Drs. Michelle Itano and Pablo Ariel, which provide consultations and training in microscopy techniques and in methods needed to optimize imaging parameters. Dr. Ariel was a pioneer in the development of light-sheet microscopy for whole-organ imaging. Additionally, the Data Science Core will support the analysis of large data sets for both behavioral and imaging experiments. Finally, there are numerous faculty members in the Department of Pharmacology and Neuroscience Center whose expertise will help to ensure her successful completion of the proposed research. This includes established and emerging leaders in the field, such as fellow pharmacologists Bryan Roth, Tom Kash, and Juan Song.

On a personal note, Dr. Walsh exhibits all the characteristics we look for in a colleague. She is a trusted collaborator, an effective mentor and generous with her time. She is intellectually gifted and deeply motivated to push the boundaries of what is currently possible. She has unique skills and an eagerness to apply her talents to the advancement of scientific understanding and – ultimately – to improve the human condition. In sum, Dr. Walsh has the potential to emerge as an innovator and leading figure in the neurosciences.

Thank you for your consideration.

Respectfully,

Henrik G. Dohlman, Ph.D.
Sanford Steelman Distinguished Professor and Chair of Pharmacology

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Eligibility Requirements: Assistant Professor Appointment: 07/01/2021 with independent laboratory.

Molecular signaling bias and serotonergic modulation of motivated behavior.

G-protein coupled receptors (GPCRs) intracellular signaling pathways are a major regulator of cell physiology, neuronal activity, and ultimately behavior. GPCRs canonically interact with heterotrimeric Gαβγ proteins (G-proteins) as well as couple to the family of β-Arrestin (β-Arr1/2) proteins[1, 2]. Classically, GPCRs were thought to signal only through their specific G-proteins and the main function of β-Arr was receptor internalization to terminate signaling[3, 4]. However, recent work has demonstrated that ligands are able to stabilize specific GPCR conformations, which allows receptors to preferentially signal through only the G-protein or the β-Arr pathway, termed "biased signaling"[5]. The idea of biased signaling has been gaining interest in hopes of developing compounds that can preferentially target specific signaling pathways[6]. To date two approaches have predominantly been used to investigate biased signaling: 1) utilization of cell culture assays and 2) behavioral investigations systemically delivering biased compounds or globally knocking out key components of specific signaling pathways[7, 8]. Thus, there is a gap in understanding the effects of biased signaling in a cell-type or circuit specific manner.

The medial prefrontal cortex (mPFC) is an important brain region that regulates executive decision making, motivated behavior, and is thought to be the critical site of action underlying the psychedelic effects of hallucinogens[9]. Serotonin 2A receptors (5-HT2ARs) are expressed in the mPFC and have been gaining interest as the primary target for psychedelic compounds, as well as for their involvement in a myriad of physiological and behavioral phenotypes, including cognition, perception, and mood[10]. 5-HT2ARs signal through $G\alpha_q$ as well as β-Arr2 intracellular proteins, yet the consequences of each signaling pathway on neuronal activity and behavior is unclear[11]. There has been much debate about the mechanism of action underlying the therapeutic effects of activating these receptors. For example, evidence suggests that the therapeutic effects of 5-HTAR agonism are mediated by G-protein signaling, whereas the psychedelic effects occur via β-Arr2[12]. In a recent pre-clinical study, G-protein biased 5-HT2AR ligands were shown to have rapid anti-depressant, but not psychedelic, effects[13]. However, biased ligands may have opposite effects depending on the cell-type and brain region investigated[14], therefore, we propose to determine the electrophysiological and behavioral effects of biased signaling from 5-HT2ARs in mPFC pyramidal neurons in a circuit specific manner.

Aim 1. Investigate biased signaling from 5-HT2AR+ mPFC neurons in a circuit specific manner.

Aim 1.1: Development and optimization of tools to investigate GPCR biased signaling. Designer Receptors Activated Only by Designer Drugs (DREADDs) are bioengineered versions of endogenous receptors that signal through their canonical G-protein pathways via administration of an exogenous ligand, such as deschloroclozapine (DCZ)[15]. For example, the mutated human M3 muscarinic receptor DREADD (hM3Dq) activates $G\alpha_q$ signaling. However, they also engage non-canonical pathways such as β-Arr2[16]. While putative DREADDs biased to $G\alpha_q$ or β-Arr2 pathways have been developed, they have yet to be validated in intact neural systems and in contrast to the original DREADDs, were generated using the rat M_3 muscarinic receptor[16, 17]. Therefore, to determine whether selective activation of $G\alpha_q$ or β-Arr2 is sufficient to differentially alter neuronal physiology, we will develop and optimize new biased DREADDs. This is being done in collaboration with Dr. Bryan Roth based upon the previously described mutations, but utilizing the human M3 muscarinic receptor. To confirm $G\alpha_q$ or β-arr2 selectivity, we will conduct a transducerome screen, using bioluminescence resonance energy transfer (BRET) assays to assess receptor activation against all other Gα proteins[18]. Once optimized, we will package these DREADDs into adeno-associated viruses (AAVs) to enable precise manipulation of specific intracellular signaling pathways in neurons for *in vivo* investigations.

Next, to determine the necessity of $G\alpha_q$, or β-Arr2 signaling, we will utilize CRISPR/Cas9 gene editing to selectively delete $G\alpha_q$ or β-Arr2 from 5-HT2AR+ neurons. *5-HT2AR^{Cre}* knock-in mice (gifted from the Roth Lab[19]) will be crossed to *Cas9^{flx}* mice (*5-HT2AR^{Cre}*:*Cas9^{flx}*). Single guide RNAs will be packaged into Cre-dependent AAVs to mediate selective deletion of $G\alpha_q$ or β-Arr2 in 5-HT2AR+ mPFC neurons and generate the following experimental groups: 1) *5-HT2AR^{Cre}*:*Cas9^{flx}* 2) *5-HT2AR^{Cre}*:*Cas9^{flx}* $G\alpha_q$ deletion and 3) *5-HT2AR^{Cre}*:*Cas9^{flx}* β-Arr2 deletion mice.

Aim 1.2: Identify candidate brain regions involved in 5-HT2AR signaling. 25CN-NBOME, one of the most selective 5-HT2AR agonists, or vehicle will be administered systemically to each of the 3 experimental groups (see Aim 1.1) and the head-twitch response (HTR) assay will be performed. Brains will then be harvested for whole-brain clearing and c-Fos mapping. This will allow for identification of brain regions selectively activated by 5-HT2AR agonism in a biased and unbiased manner, as well as correlations between strength of behavioral responses and neural activity. To elucidate candidate circuits, *5-HT2AR^{Cre}*:*Cas9^{flx}* mice will receive viral injections of a Cre-dependent anterograde tracer into the mPFC and undergo whole-brain clearing to map 5-HT2AR+ mPFC afferents. These results, in combination with the c-Fos mapping experiments will generate high probability circuits involved in biased 5-HT2AR signaling.

Aim 2. Electrophysiological characterization of projection specific 5-HT2AR+ mPFC neurons.

Aim 2.1: Determine the physiological consequences of $G\alpha_q$ or β-Arr2 deletion in 5-HT2AR+ mPFC neurons.

While multiple candidate regions will be uncovered in Aim 1, the nucleus accumbens (NAc) is a likely target given its involvement in a multitude of behaviors, including natural and drug reward, social behavior, feeding and motivation,

as well as the presence of 5-HT2AR+ mPFC terminals in the NAc[20, 21]. Therefore, we will start by electrophysiologically characterizing 5-HT2AR+ mPFC neurons that project to the NAc, leveraging the same experimental groups from Aim 1.1. Acute coronal slices containing the mPFC will be prepared to perform perforated patch rather than whole-cell recordings to minimize dialysis of intracellular proteins[22, 23]. Mice will be injected with a retrograde AAV encoding a Cre-dependent fluorescent marker into the NAc to visually identify projection-specific 5-HT2AR+ mPFC neurons. The physiological effects of selective deletion of $G\alpha_q$ or β-arr2 will be assessed by measuring: spontaneous firing, excitability, AMPAR/NDMAR ratio, paired-pulse ratio (PPR) and the ability to induce long-term depression or potentiation (LTD/LTP). This will provide data regarding the role of intracellular proteins in regulating the physiology of mPFC 5-HT2AR+ neurons. By utilizing bath application of the selective 5-HT2AR agonist, 25CN-NBOME, we will repeat the above assays to interrogate the role of active biased signaling at this receptor.

Aim 2.2: Investigate synaptic adaptations in the 5-HT2R+ mPFC-to-NAc circuit due to $G\alpha_q$ or β-Arr2 deletion. We will employ optogenetic assisted circuit mapping by injecting a Cre-dependent channelrhodopsin into 5-HT2AR+ mPFC neurons, and record from the predominant cell-type in the NAc, medium spiny neurons, for each experimental group. We will assay synaptic properties by measuring the following: AMPAR/NMDAR ratios, PPR, and strontium-induced asynchronous release in drug naïve and 25CN-NBOME experienced mice. These experiments will provide us with thousands of data points, thus we will utilize dimensionality reduction via principal component analysis to explore how biased signaling affects different types of physiological parameters (i.e. synaptic plasticity vs intrinsic excitability of these neurons).

Aim 3: Assess biased signaling at 5-HT2AR+ mPFC neurons on behavior and neuronal activity.

Aim 3.1: Determine the effects of $G\alpha_q$ or β-Arr2 deletion on behaviorally relevant 5-HT2AR+ neuronal activity. Utilizing the three experimental groups from Aim 1.1, mice will additionally receive viral injections of genetically encoded calcium indicators (GECI) to assess neural activity in mPFC and NAc cell bodies, and mPFC terminals in the NAc with fiber photometry. Mice will be administered 25CN-NBOME or vehicle and undergo behavioral assays while simultaneously recording neuronal activity. Assays will include the HTR, open field test, 3-chamber sociability assay, juvenile interaction test, conditioned place preference, O-maze, and forced swim test.

While these behavioral assays are routinely used, there is a critical need to develop more accurate and precise methods to correlate real-time neural activity with discrete behavioral events. Manual behavioral analysis is laborious, challenging to standardize, and suffers from low inter-rater reliability[24]. Thus, using the open-source machine learning based programs DeepLabCut (DLC) and Simple Behavioral Analysis (SimBA) to analyze our assays is an innovative advance in studying the neural mechanisms underlying behavior. DLC allows us to precisely track mouse movement by generating pose estimation models of specific body parts with frame-by-frame precision. This provides us with detailed information concerning the mouse's orientation, direction, and magnitude of movement. Demonstrating the impact of this approach, a recent report identified discrete components of novelty seeking behavior that would have been missed by traditional analysis methods[25]. SimBA software allows us to combine tracking data from DLC with user guided video annotation to generate behavioral classifiers[26]. This allows for millisecond detection of specific components of behavior, such as social approach and active social interaction during a juvenile interaction test, far more precise and accurate than can be obtained by human scorers. Altogether these machine-learning based tools are a critical step in accurately analyzing behavior-related neural activity and providing reliable fine-grained analysis of multiple types of behavior within a single assay.

Aim 3.2: Determine the effects of $G\alpha_q$ or β-Arr2 activation on 5-HT2AR+ neuronal physiology in vivo. To determine if activation of $G\alpha_q$ or β-Arr2 signaling is sufficient to alter neuronal activity and/or behavior, we will infuse either $G\alpha_q$ or β-Arr2 biased DREADD in combination with a GECI in 5-HT2AR+ mPFC neurons. Mice will be administered DCZ or vehicle and undergo the same behavioral assays in Aim 3.1 while simultaneously recording neuronal activity via fiber photometry. These assays and recordings will allow us to study the consequences of biasing the intracellular signaling of 5-HT2ARs, providing invaluable information regarding the necessity and sufficiency of $G\alpha_q$ or β-Arr2 intracellular signaling to modulate neuronal physiology and ultimately behavior.

This project will develop and optimize novel tools to interrogate GPCR biased signaling in combination with cutting edge neural circuit technologies to understand how GPCRs modulate behavior in a receptor, cell-type and circuit specific manner. Furthermore, while this proposal is focused on GPCR signaling in neurons and the 5-HT2AR, these tools will be useful for investigating biased signaling outside of the field of neuroscience, such as understanding basic cell activity and its dysregulation in cancer. Finally, mapping the behavioral and physiological consequences of biased 5-HT2AR agonism will create a repository of data to build upon for future investigations. This proposal will generate critical knowledge about the molecular mechanisms of 5-HT2AR signaling and may aid in understanding the therapeutic effects of activating this receptor.

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Potential Recommendation Writers:

1. Robert C. Malenka, M.D., Ph.D. (post-doctoral fellow mentor)
   Pritzker Professor of Psychiatry and Behavioral Sciences, Director of the Nancy Pritzker Laboratory and Deputy Director of the Wu Tsai Neurosciences Institute, Stanford Univeristy

   Dr. Malenka is an expert on the molecular mechanisms and functions of a variety of forms of synaptic plasticity in numerous regions, including the hippocampus, nucleus accumbens, dorsal striatum and ventral tegmental area. His lab uses molecular manipulations in combination with cell biological, optogenetic, and electrophysiological assays in both *in vitro* and *in vivo* preparations. Ultimately, his lab also incorporates these forms of manipulations in defined neuronal populations with the goal of elucidating the functions of synapses, circuits, and behavior, with a focus on reward and aversion.

2. Ming-Hu Han, Ph.D. (Pre-doctoral mentor)
   Professor, The Brain Cognition and Brain Disease Institute of Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences

   Dr. Han is an expert neurophysiologist who developed a novel, powerful slice culture system to study neuronal intrinsic properties by use of viral-mediated gene transfer. His broad training across molecular, cellular and behavioral levels, has allowed his lab the unique ability to explore the cellular and neural circuit basis of depression using highly integrative research methodology.

3. Yasmin Hurd, Ph.D. (thesis committee member/general mentor)
   Ward-Coleman Chair of Translational Neuroscience and the Director of the Addiction Institute at Mount Sinai, Icahn School of Medicine at Mount Sinai

   Dr. Hurd's area of expertise is in understanding how genes and the environment play a role in the development of substance use disorder in the hopes of gaining insights that could aid in the advancement of treatments.

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Positions

UNC Chapel Hill School of Medicine, Chapel Hill NC, Assistant Professor 2021 – present
Stanford University School of Medicine, Palo Alto CA, Postdoctoral Fellow 2013 – 2021

Education

Icahn School of Medicine at Mount Sinai, New York, NY, Ph.D. in Neuroscience 2010 – 2013
Icahn School of Medicine at Mount Sinai, New York, NY, M.S. in Biomedical Sciences 2008 – 2010
Columbia University, Columbia College, New York, NY, B.A. in Neuroscience and Behavior 2004 – 2008

Research Experience

Stanford University School of Medicine, Palo Alto, CA 2013 – 2021
Postdoctoral Fellow, Advisor: Dr. Robert C. Malenka
• Investigating the role of serotonin in social impairments present in mouse models for ASD

Icahn School of Medicine at Mount Sinai (ISMMS), New York, NY
Predoctoral Fellow, Advisor: Dr. Ming-Hu Han 2010 – 2013
• Investigated the role of projection specific VTA dopamine neurons in a mouse model for depression
Master Student, Advisor: Dr. Patrick Hof 2008 – 2010
• Investigated the role of APP in dendritic and spine morphology in a mouse model for Alzheimer's disease

Marine Biological Laboratory, Woods Hole Research Institute, Woods Hole, MA Summer 2008
Laboratory Research Assistant, Advisor: Dr. Gerald Fischbach
• Examined the effects of neuregulin (NRG) on the expression of acetylcholine receptors in muscle fibers

Columbia University, New York, NY Summer 2006
*Scientific Undergraduate Research Fell*ow, Advisor: Dr. Gerald Fischbach
• Observed the effects of blocking the action of NRG using an inhibitor of NRG receptor signaling in co-cultures of mouse cells and spinal cord explants

New York Academy of Sciences Summer Research Training Program, New York, NY Summer 2003
High School Research Fellow, Advisor: Dr. Gerald Fischbach
• Examined the formation of the neuromuscular junction relative to the embryonic stages of chick development

Columbia University, College of Physicians and Surgeons, New York, NY 2002 – 2006
Laboratory Assistant, Advisor: Dr. Gerald Fischbach
• Created both a physical and digital archive of slides of slices of chicken embryos stained with TUJ-1 and BTX

Honors and Awards

NARSAD Young Investigator Award (Brain & Behavior Research Foundation) 2024 – 2026
Nature Research Award for Inspiring Science (Shortlist) 2019
Sammy Kuo Award in Neuroscience (best publication of the year by a Stanford postdoctoral fellow) 2018
11th Annual Autism Spectrum Disorders Update Scholarship (Stanford University) 2018
FENS/IBRO-PERC Travel Award (SfN) 2016
F32 Ruth L. Kirschstein National Research Service Award (NIMH) 2014 – 2016
A.P. Giannini Foundation Postdoctoral Research Fellowship (declined) 2014
T32 Training Fellow (NHLBI) 2013 – 2014

Hausfeld Award in Neuroscience for most outstanding student (ISMMS)	2012
F31 Ruth L. Kirschstein National Research Service Award (NIMH)	2012 – 2013
IBRO World Congress Travel Award (SfN)	2011
1st Place Poster Award at Mount Sinai Neuroscience Retreat (ISMMS)	2011
Travel Award to SfN Conference (ISMMS)	2010 – 2012

Publications

Research Manuscripts

All manuscripts here: https://www.ncbi.nlm.nih.gov/myncbi/1XoNrVnsRwPk6/bibliography/public/

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Walsh JJ*, AK Friedman*, Chaudhury D*, Juarez B, Ku SM, and Han MH. (2012) Injection of Retrograde Beads into the Nucleus Accumbens (NAc) and Medial Prefrontal Cortex (mPFC) to Isolate Projection-Specific Neurons in the Ventral Tegmental Area (VTA). ***Protocol Exchange***. DOI:10.1038/protex.2012.050.

Midthune B, Tyan SH, **Walsh JJ**, Sarsoza F, Eggert S, Dickstein DL, Hof PR, and Koo EH. (2012) Deletion of the amyloid precursor-like protein 2 (APLP2) does not affect hippocampal neuron morphology or function. ***Molecular and Cellular Neuroscience***. 49, 448-455.

Tyan SH, Shih AY, **Walsh JJ**, Murayama H, Sarsoza F, Ku L, Eggert S, Hof PR, Koo EH,and Dickstein DL. (2012) Amyloid precursor protein (APP) regulates synaptic structure and function. ***Molecular and Cellular Neuroscience***. 51, 43-52.

Choi KH, Edwards S, Graham DL, Larson EB, Whisler KN, Simmons D, Friedman AK, **Walsh JJ**, Rahman Z, Monteggia LM, Eisch AJ, Neve RL, Nestler EJ, Han MH, Self DW. (2011) Reinforcement-Related Regulation of AMPA Glutamate Receptor Subunits in the Ventral Tegmental Area Enhances Motivation for Cocaine. ***Journal of Neuroscience***. 31, 7927-7937.

Cao JL, Covington III HE, Friedman AK, Wilkinson MB, **Walsh JJ**, Cooper DC, Nestler EJ, and Han MH. (2010) Mesolimbic dopamine neurons in the brain reward circuit mediate susceptibility to social defeat and antidepressant action. ***Journal of Neuroscience***. 30, 16453-16458.

Walsh J, Mann MA, Fischbach G. (2007) The Effects of Neuregulin on Synapse Formation in Primary Muscle/Nerve Co-Cultures. ***Columbia University Undergraduate Science Journal***. 2, 46-51.

Review Articles

Walsh JJ[#], Christoffel DJ, Malenka RC[#]. Neural circuits regulating prosocial behaviors. (2022) ***Neuropsychopharmacology***. doi:10.1038/s41386-022-01348-8. [#]Co-corresponding authors.

Walsh JJ, Christoffel DJ, Wu X, Pomrenze M, Malenka RC. (2021) Dissecting neural mechanisms of prosocial behaviors. ***Current opinion in neurobiology***. 68, 9-14.

Walsh JJ, Han MH. (2014) The heterogeneity of ventral tegmental area neurons: projection functions in a mood-related context. ***Neuroscience***. 282C. 101-108.

Whittle AJ, **Walsh J**, de Lecea (2014) Light and chemical control of neuronal circuits: possible applications in neurotherapy. ***Expert Reviews in Neurotherapeutics***. 9. 1007-1017.

Dickstein DL, **Walsh JJ**, Brautigam H, Stockton SD, Gandy S, Hof PR. (2010) The Role of Vascular Risk Factors and Vascular Dysfunction in Alzheimer's disease. ***Mount Sinai Journal of Medicine***. 77, 82-102.

Invited Scientific Presentations

Stanford's 12th Annual Autism Spectrum Disorders Update 2019
Speaker, "The role of serotonin release on social behavior in a mouse model of autism"
Autism Working Group (Stanford University) 2018
Speaker, "The role of serotonin release on social behavior in a mouse model of autism"
Howard Fields Lab (UCSF) 2015
Speaker, "Functional contribution of projection – specific VTA dopamine neurons"
Society for Neuroscience 2013
Organizer, Chair and Speaker of the Mood Disorders: Animal Models Nanosymposium
Society for Neuroscience 2012
Organizer, Chair and Speaker of Nanosymposium
The New York Academy of Sciences 4th Annual President's Reception 2007
Speaker
Days on Campus (Columbia University) 2007
Science Research Fair – Presenter
Columbia University Spring Undergraduate Research Symposium 2007
Speaker
Columbia University SURF Symposium 2007
Speaker

Teaching and Outreach Experience

1000 Girls, 1000 Futures (New York Academy of Sciences) 2018 – present
Mentor
Brain Mind Summit (Stanford University) 2018 & 2019
Volunteer
Stanford ASD & Developmental Disabilities Clinic 2017 – 2019
Volunteer
Executive Function Coach (Dr. Caryn Kovar) 2016 – present

Work with children diagnosed with ASD and sensory processing deficits
Stanford Healthcare Consulting Group 2014 – 2015
Consultant
Introductory Neuroscience, Queens College (CUNY) 2012
Guest Lecturer
Department of Neurology, Icahn School of Medicine at Mount Sinai 2011
Teaching Assistant for Brain and Behavior Course
Department of Psychology, Columbia University 2007 – 2008
Laboratory Teaching Assistant

Research Support

Current Research Support:
R01MH136266-01 (PI: Walsh) 3/5/24 – 1/31/29
NIH/NIMH
Neural mechanisms underlying sustained enhancement of sociability

Brain & Behavior Research Foundation (PI: Walsh) 1/15/24 – 1/14/26
NARSAD Young Investigator Award
Investigation of serotonergic regulation of social behavior in multiple mouse models of autism

Completed:
F32MH103949 (PI: Walsh) 7/1/14 – 11/30/16
Ruth L. Kirschstein National Research Service Awards for Individual Postdoctoral Fellows
NIH/NIMH
Systems level investigation of di – synaptic circuit involved in panic disorder

T32HL110952 (MPI, Director: Allan Pack) 12/1/13 – 6/30/14
NIH/NHLBI
Multi – Institutional Training in Genetic/Genomic Approaches to Sleep Disorders

F31MH095425 (PI: Walsh) 8/1/12 – 10/14/13
Ruth L. Kirschstein National Research Service Awards for Individual Predoctoral Fellows
NIH/NIMH
Neural circuit basis of behavioral susceptibility and resilience to social defeat

Associations

Association for Women in Science (AWIS)
Society for Neuroscience (SfN)
Autism Society
New York Academy of Sciences

Ad Hoc Referee

Biological Psychiatry
Cell Reports
Neuropsychopharmacology
Journal of Neuroscience
Translational Psychiatry
Psychopharmacology
European Journal of Neuroscience
Medical Research Council

Editorial Boards

Frontiers in Neural Circuits

PI: Yang, En (Pages: 118-130)

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THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

March 31, 2024

Pew Charitable Trusts
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Philadelphia, PA 19103-7077

Dear Selection Committee Members:

I am writing with enthusiasm to nominate **Dr. En Yang** for our internal selection process for a Pew Scholars Award. Dr. Yang began her faculty position in the Department of Biology just this past January, and has already hit the ground running winning a competitive Chan Zuckerberg Initiative award for her groundbreaking neuroscience work (https://college.unc.edu/2024/02/chan-zuckerberg-grant/). She is a competitive and a most deserving applicant for the Packard award.

Dr. Yang has an interesting educational history for a biologist, and one that has allowed her to become an incredibly creative neuroscientist. She began as an engineer, earning a B.S. in Mechanical Engineering from Tsinghua University in Beijing and a PhD in Material and Science Engineering from Carnegie Mellon University in Pittsburgh. After working in industry and founding a biotech company, she decided to apply her computational and engineering skills to neuroscience and joined Misha Ahrens' laboratory at the Janelia Research Campus of the Howard Hughes Medical Institute for postdoctoral work. She made the transition to experimental neuroscience in spectacular fashion with publications in the highest profile journals, including *Cell*. She is undoubtedly a budding superstar, demonstrating uncommon intuition for animal behavior and neural dynamics.

Dr. Yang was chosen as the top candidate from our highly competitive 2023 national search of 122 applicants. We were searching for scholars who could provide a scientific and teaching foundation for our department in neuroscience that would fill university needs and goals in this important area in ways that other departments in the college or other schools were not. Dr. Yang generated the most excitement of any of our candidates in this search, and that excitement spread across the entire department from molecular biologists to behavioural neuroscientists. We are delighted to have selected her as a new member of our faculty and are committed to ensuring her future success as an independent investigator at UNC-Chapel Hill.

As noted above, Dr. Yang is both a neuroscientist and engineer, and during her time at HHMI she developed a virtual reality system for whole-brain neuronal imaging in larval zebrafish to understand neural dynamics at the global level. She identified a novel locus of memory in the fish brainstem and discovered that larval zebrafish can perform long term learning. In a matter of minutes, fish can learn to adapt to a new rule imposed on them to achieve a navigational goal. Her lab at UNC-CH will image, perturb and interpret, in real-time, the global functional reorganization process across the duration of learning, to study the spatial and temporal distribution of functional plasticity in the brain and central nervous system. She plans to

collaborate with other laboratories here to understand the general principles of neuronal circuit architecture and network dynamics underlying spatial navigation and learning across species.

Dr. Yang's work is a great fit for Biology as well as UNC-Chapel Hill because of her interactive nature and her desire to bridge biology, computer science and engineering. She will provide new classroom topics in neuroscience, with unique perspectives, for our students that will complement and/or could contribute to our relatively new neuroscience major. She has an ongoing interaction with Nico Pegard in applied physical sciences and likely will interact with other faculty in that department. In addition, and of great excitement for me and our department, Dr. Yang's laboratory will be housed in the Genome Sciences Building adjacent to three outstanding young scientists in the department, Dr. Celia Shia who uses zebrafish to study macrophages in the nervous sytem (with whom she won a CZI award as noted above), Dr. Toshi Hige who uses *Drosophila* to study how neuronal circuits drive behavior, and Dr. Jiakun Chen who uses both zebrafish and *Drosophila* to study how a specialized neuronal cell type called astrocytes contribute to brain and central nervous system function. I am anticipating great synergy among these labs and others within our neuroscience, computational, and applied sciences communities.

In summary, we are fortunate to have recruited such an outstanding scientist and educator with such a unique background to the Department of Biology and UNC-Chapel Hill. Dr. Yang will have a significant impact on our community. Should you have any questions or need additional information, please do not hesitate to contact me.

Sincerely,

Robert J. Duronio
Professor & Chair
Department of Biology

En Yang Ph.D

Department of Biology

Imagination, the ability to mentally envision scenarios, has traditionally been regarded as a hallmark of intelligence exclusive to humans. A recent study shows that rats can intentionally activate specific neural activities in their hippocampus to represent a distant location in a goal-directed manner[1], reminiscent of human's ability to imagine places. However, it remains unclear whether such ability existed in more ancient vertebrate brains, how it emerged from neural networks at the whole-brain level, and which other brain regions coordinate to support such mental representation. Understanding the circuits that enable such 'imagination' will shed light on theories of memory recall, mental simulation, planning, and the evolution of cognitive intelligence.

We address these questions using larval zebrafish navigating in virtual reality to perform goal-directed spatial learning. Fish have remarkable goal-directed navigational skills[2], and zebrafish larvae have displayed strong goal-directed special learning[3] and operant learning abilities[4]. A recent study further identifies place cells in the larval zebrafish telencephalon, analogous to the mammalian hippocampus[5]. By combining cellular resolution whole-brain imaging and brain-machine interface, we will be able to observe and manipulate the activities of every neuron across the entire brain throughout the spatial learning and goal-directed navigation process. First, we'll create a closed loop connecting fish behavior with virtual reality, enabling the animal to navigate and familiarize itself with the virtual world through swimming. Simultaneously, we'll conduct a comprehensive scan of the entire brain to identify neuronal activities that encode spatial locations. This will help us understand how different brain regions coordinate to form a mental spatial map. Next, we will pinpoint brain regions whose activity predicts the fish's location during new navigation trials. Finally, we will modify the closed loop between fish behavior and virtual reality, shifting from swimming-based navigation to utilizing the brain-machine interface (BMI) mode. This mode leverages neural activities encoding/predicting locations to guide virtual world movements based on the fish's thoughts. Consequently, fish can navigate the virtual world with their minds. Moreover, we'll implement a reward system when the neural activities align with the reward location, reinforcing desired spatial representation patterns. We plan to use this assay to target the following aims:

Aim 1: To identify the brain regions responsible for landmark encoding and spatial map formation, assessing their activity dynamics during spatial learning and the mental representation of specific locations. While the hippocampus is known to store spatial map information, recent studies indicate that multiple brain regions are essential for spatial navigation and goal-directed learning[6]. My previous work further demonstrated that a path integrator in the hindbrain enables animals to maintain positional memories and self-localization[7]. A recent study found that place cells in the fish forebrain integrate multiple sources of information, which untangles over time[5]. We hypothesize that during spatial learning, various brain regions within the fish brain extract different features from the external environment. Subsequently, these features are coordinated across the brain and collectively contribute to the formation of place cells and a spatial map within the forebrain. With whole-brain imaging technology, we aim to discover all brain regions encoding different aspects of the environment and their function roles in forming spatial maps. We will further determine temporal relations between their activities and how they change during spatial learning and BMI mode. These findings will deepen our understanding of the computations facilitated by communication among diverse brain regions, offering insights into global-level models and theories.

Aim 2: To unpack the circuit mechanism and establish a causal relationship between location encoding, spatial memory formation, and goal-directed navigation. Research indicates that damage to the hippocampus leads to deficits in forming new memories, while old memories may remain unaffected[8][9]. We hypothesize that memory formation and memory recall

may have distinct neural bases and involve different brain regions. By employing cellular resolution laser ablation, cell type-specific optogenetic activation or silencing, alongside chemogenetics and pharmacological manipulations, we can reversibly or irreversibly activate or silence any targeted neural activities in predefined temporal and spatial patterns. For example, following the completion of spatial learning and successful goal-directed navigation by the fish, we can perform ablation or silencing of neurons representing the spatial map. This process aims to determine: 1) whether the fish can still navigate through the virtual space in a goal-directed manner, and 2) if the brain will recruit new neurons to represent the spatial map. By employing single-cell resolution and cell type-specific manipulation, along with whole-brain imaging to observe network responses to manipulation, we aim to identify the necessary and the sufficient neural components for memory formation, consolidation, and recall. Additionally, we seek to elucidate the critical relationship between neural representation, imagination, memory recall, and motor planning.

Aim 3: To investigate whether larval zebrafish can deliberately activate neural patterns corresponding to distant locations, akin to human 'imagination.' During BMI mode, fish will receive visual feedback on locations tied to their neural activity, allowing them to learn to navigate to desired locations in the virtual world through trial and error. However, if fish possess true 'imagination,' they should either spontaneously or through learning activate neural patterns representing a specific remote location without traversing through the virtual space. Recent BMI research indicates that many neurons from different cortical regions can be trained to fire in a goal-directed manner[10]. Even neurons cultivated in a dish can be trained to play pong[11]. We hypothesize that neurons responsible for location/map encoding in certain fish brain regions can be trained to instantaneously activate a pattern representing a remote location, allowing the fish to mentally teleport itself to that location. By integrating virtual reality, Brain-Machine Interface (BMI) technology, and quantitative measurements of behavior and visual feedback, we will investigate the capability of fish to engage in mental teleportation. We aim to comprehend the essential conditions required for the emergence of this remote location representation from neural network dynamics, particularly with the assistance of other recurrently connected brain regions. Our investigation will encompass every brain region encoding and predicting fish locations, allowing us to explore and grasp the computational hierarchy within the fish brain.

In virtual reality (VR) environments and Brain-Machine Interface (BMI) mode, fish were empowered to mentally teleport to specified locations, achieving goals and receiving rewards without physical movement. This approach will enable us to distinguish cognitive learning and memory processes from motor output and its recurrent feedback. Combining whole-brain imaging and manipulation of network dynamics, we aim to dissect the distinct neural mechanisms for cognitive intelligence and kinesthetic intelligence. This will provide insights into how advanced cognitive functions emerge from relatively simple neural network dynamics and how physical and physiological constraints shape the evolution of cognitive intelligence. Additionally, this research may shed light on advancing engineering approaches for rescuing many learning disorders, aging-related cognitive decline, and modern prosthetics.

Reference

[1] Lai C, Tanaka S, Harris TD, Lee AK. *Volitional activation of remote place representations with a hippocampal brain-machine interface.* Science. 2023 Nov 3;382(6670):566-573. doi: 10.1126/science.adh5206. Epub 2023 Nov 2. PMID: 37917713; PMCID: PMC10683874.

[2] University of California - Santa Cruz. *"Fish uses tool to dig up and crush clams."* ScienceDaily. ScienceDaily, 29 September 2011

[3] Aletheia Lee, Ajay S. Mathuru, Cathleen Teh, Caroline Kibat, Vladimir Korzh, Trevor B. Penney, Suresh Jesuthasan,*The Habenula Prevents Helpless Behavior in Larval Zebrafish,* Current Biology,Volume 20, Issue 24,2010,Pages 2211-2216

[4] Li, Jennifer Mengbo. 2012. Identification of an Operant Learning Circuit by Whole Brain Functional Imaging in Larval Zebrafish. Doctoral dissertation, Harvard University.

[5] Chuyu Yang, Lorenz Mammen, Byoungsoo Kim, Meng Li, Drew N Robson, Jennifer M Li, A population code for spatial representation in the larval zebrafish telencephalon. bioRxiv 2023.11.12.566708; doi: https://doi.org/10.1101/2023.11.12.566708

[6] Fabian Chersi, Neil Burgess,The Cognitive Architecture of Spatial Navigation: Hippocampal and Striatal Contributions, Neuron,Volume 88, Issue 1,2015,

[7] Yang E, Zwart MF, James B, Rubinov M, Wei Z, Narayan S, Vladimirov N, Mensh BD, Fitzgerald JE, Ahrens MB. *A brainstem integrator for self-location memory and positional homeostasis in zebrafish*. Cell. 2022 Dec 22;185(26):5011-5027.e20.

[8] Knowlton BJ, Fanselow MS. *The hippocampus, consolidation and on-line memory*. Curr Opin Neurobiol. 1998;8:293–296

[9] Kim JJ, Clark RE, Thompson RF. *Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses*. Behav Neurosci. 1995;109:195–203.

[10] Lebedev, *M. Brain-machine interfaces: an overview*. Translat. Neurosci. 5, 99–110 (2014).

[11] Brett J. Kagan, Andy C. Kitchen, Nhi T. Tran, Forough Habibollahi, Moein Khajehnejad, Bradyn J. Parker, Anjali Bhat, Ben Rollo, Adeel Razi, Karl J. Friston, *In vitro neurons learn and exhibit sentience when embodied in a simulated game-world,* Neuron, Volume 110, Issue 23, 2022

Eligibility Statement

As a full-time Assistant Professor in the Department of Biology at the University of North Carolina at Chapel Hill since January 1, 2024, I meet the eligibility criteria outlined by the Pew Scholars Award. I have a master's degree in Instrument Science. My Ph.D. was in Material Science and Engineering. My postdoc work focused on system and computational neuroscience. My research spans the natural and physical sciences and engineering fields.

I'm deeply driven to delve into the neural underpinnings of cognitive behaviors often considered uniquely human. Bringing together the technical expertise I have as an engineer and the experimental and computational tools I developed as a system neuroscientist, I will invent creative approaches, develop innovative technologies, to unravel the fundamental scientific mysteries of the cognitive ability of the brain, and translate them into engineering solutions.

En Yang 03/31/2024

Application materials by En Yang

List of three individuals to provide external letters of recommendation: the candidate's thesis advisor, postdoctoral advisor, and a scientific reference from an individual external to UNC who is not a collaborator or mentor. For each recommender, include titles, institutional affiliations, and a two-three sentence overview of their area of expertise.

1, Thesis advisor: David Laughlin,
Professor Emeritus at Carnegie Mellon University, affiliated with the Department of Materials Science and Engineering and the Electrical and Computer Engineering Department of CMU.
David Laughlin specializes in materials science and engineering, focusing on structure-property relationships, the development of advanced nanomaterials, and researching microstructure evolution, phase transformations, and spin electronic materials and devices, establishing him as a prominent figure in this field.

2, Postdoctoral advisor: Misha Ahrens, Senior Group leader, Janelia Research Campus, Howard Huge Medical Institute.
Misha Ahren's expertise lies in developing cutting-edge imaging techniques to investigate the neural circuits underlying behavior in larval zebrafish, contributing significantly to our understanding of brain function and behavior at a cellular level.

3, Rainer W. Friedrich ,Senior Group Leader, Friedrich Miescher Institute for Biomedical Research, Basel
Rainer W. Friedrich specialized in the olfactory system of Zebrafish. Through his innovative approaches and meticulous studies, Friedrich's work advanced our understanding of how the brain processes and interprets smell.

Apr 1, 2024

En Yang

Department of Biology
University of North Carolina at Chapel Hill
RM 2151, 250 Bell Tower Dr., Chapel Hill, NC
Phone: (412) 759-0719
E-mail: enyang@unc.edu

WORK EXPERIENCES

University of North Carolina at Chapel Hill, Chapel Hill, NC
- Assistant Professor in the Department of Biology 1/1/2024-present

Institution for Convergent Science, Chapel Hill, NC 1/1/2024-present
- Faculty Fellow

Howard Hughes Medical Institute, Janelia Research Campus, Ashburn, VA
- Research Scientist in Systems and Computational Neuroscience 2018-2023

Western Digital, Advanced Materials, San Jose, CA
- Project Lead & Senior Staff Scientist 2014-2017

Hitachi Global Storage Technology, Advanced Materials, San Jose, CA
- Senior Research Staff Member 2013-2014

Avalanche technology, Spintronics, Fremont, CA
- Senior Design Engineer, R&D, 2012-2013

GRANT RECEIVED

- **Chan Zuckerberg Initiative Collaborative Pairs Pilot Project Awards (Cycle 2)** 3/1/2024-8/31/2025

EDUCATION AND TRAINING

Howard Hughes Medical Institute, Janelia Research Campus, Ashburn, VA
- Postdoctoral Associate in Systems and Computational Neuroscience 2017-2018

Carnegie Mellon University, Pittsburgh, PA
- Postdoctoral Associate in Electrical and Computer Engineering 2011-2012

Carnegie Mellon University, Pittsburgh, PA, USA
- Ph.D. in Material Science and Engineering 2008-2011

En Yang
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- M.S in Material Science and Engineering 2006-2008

Tsinghua University, Beijing, P. R. China
- M.S. in Instrument Science and Technology 2003-2006
- B.S. in Mechanical Engineering 1999-2003

HONORS AND AWARDS

• Paxton Award for Best Doctoral Dissertation	Carnegie Mellon University	2011
• Carnegie Institute of Technology Graduate Fellowship	Carnegie Mellon University	2007 - 2011
• Carnegie Institute of Technology Dean's Fellowship	Carnegie Mellon University	2006
• Best Master's Dissertation Award	Tsinghua University	2006
• Excellent Bachelor Dissertation Award	Tsinghua University	2003
• Excellent Student Scholarship (first prize)	Tsinghua University	1999 - 2003

PREPRINTS (bioRxiv)

- **E. Yang**, M. F. Zwart, M. Rubinov, B. James, Z. Wei, S. Narayan, N. Vladimirov, B. D. Mensh, J. E. Fitzgerald, M. B. Ahrens: A brainstem integrator for self-localization and positional homeostasis (2021), *bioRxiv* 2021.11.26.468907, *doi: https://doi.org/10.1101/2021.11.26.468907*

MANUSCRIPTS IN PREPARATION

- **E. Yang**, M. F. Zwart, J. E. Fitzgerald, M. B. Ahrens: Working memory modulates a behavioral strategy through neural rebound, *in preparation;*
- **E. Yang** , J. E. Fitzgerald, M. B. Ahrens: Brain-wide remapping of neuronal function during learning in zebrafish, *in preparation.*

JOURNAL PUBLICATIONS (PEER-REVIEWED)

- **E. Yang**, M. F. Zwart, M. Rubinov, B. James, Z. Wei, S. Narayan, N. Vladimirov, B. D. Mensh, J. E. Fitzgerald, M. B. Ahrens: A brainstem integrator for self-localization and positional homeostasis in zebrafish ***Cell*** 185 (26), 5011-5027, (2022);
- Montoya, E.A., et al., **E. Yang**: Immunity of nanoscale magnetic tunnel junctions with perpendicular magnetic anisotropy to ionizing radiation, *Sci Rep 10, 10220 (2020*);
- **E. Yang,** Z. Liu, H. Arora, T.-w. Wu, V. Ayanoor-Vitikkate, D. Spoddig, et al., B. Terris: Templated Assisted Direct growth of 1Td/in2 Bit patterned Media, *nano letters* 16 *(7), 4726- 4730(2016);*

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- T.R. Albrecht, H. Arora, et al., **E. Yang**: Bit-Patterned Magnetic Recording: Theory, Media Fabrication, and Recording Performance, *IEEE Transaction on Magnetics, Vol. 51, No. 5* (2015)*;*
- **E. Yang,** Y. Zhou, Y. Huai: Correlation of Magnetic thermal fluctuation induced noise and thermal stability factor for magnetic nano-devices, *Journal of Magnetism and Magnetic Materials (2014);*
- **E. Yang**, V. Sokalski, M. Moneck, D. Bromberg M, J.-G. Zhu, Annealing effect and under/capping layer study on Co/Ni multilayer thin films for domain wall motion, *Journal of Applied* Physics. 113, 17C116 (2013);
- V. Sokalski, M. T. Moneck, D. Bromberg; **E. Yang**, and J.-G. Zhu: FeCo-Oxide as a magnetic coupling layer for electrically isolated read/write paths in mLogic, *IEEE Transaction on Magnetics,49, 4351-4354* (2013);
- V. Sokalski, M. T. Moneck, D. BromberG; **E. Yang**, and J.-G. Zhu: Increased perpendicular TMR in FeCoB/MgO/FeCoB magnetic tunnel junctions by seedlayer modifications, *IEEE Transaction on Magnetics*,49, 4383 - 4385 (2013);
- H. Ho**, E. Yang**, D. E. Laughlin, and J.-G. Zhu: Multiple oxide content media for columnar grain growth in L10 FePt thin films, *Applied Physics Letters* 102, 112411 (2013);
- V. Sokalski, M. T. Moneck, **En Yang**, and J.-G. Zhu: Optimization of Ta thickness for perpendicular magnetic tunnel junction applications in the MgO-FeCoB-Ta system, *Applied Physics letters 101, 7*(2012);
- **E. Yang**, D. E. Laughlin, and J.-G. Zhu: Correction of order parameter calculations for FePt perpendicular thin films, *IEEE Transaction on Magnetics*, 48, 7-12 (2012);
- **E. Yang,** H. Ho, D. E. Laughlin, and J.-G. Zhu: Columnar grain growth of L1$_0$-FePt thin films, *Journal of Applied Physics* 111, 07B720 (2012);
- **E. Yang**, H. Ho, D. E. Laughlin, and J.-G. Zhu: Epitaxial growth of L1$_0$-FePt granular thin films on TiC/RuAl, *IEEE Transaction on Magnetics*, vol. 47, pp4077-4079(2011);
- **E. Yang***, S.* Ratanaphan, D. E. Laughlin, and J.-G. Zhu: Highly Ordered FePt L1(0) Thin Films With Small Grains on RuAl Seed (**Invited**), *IEEE Transaction on Magnetics,* 47, 81-86 (2011);
- **E. Yang,** Ratanaphan, S, D. E. Laughlin, and J.-G. Zhu: Structure and magnetic properties of L10-FePt thin films on TiN/RuAl underlayers, *Journal of Applied Physics*, vol.109 (2011);
- **E. Yang***,* D. E. Laughlin, and J.-G. Zhu: Buffer layers for highly ordered L1$_0$ FePt-Oxide thin film media at reduced processing temperature (**Invited**), *IEEE Transaction on Magnetics,* 46, 2446-2449 (2010);
- **E. Yang**, David E. Laughlin, L1$_0$ FePt-Oxide Multilayer perpendicular media with high coercivity and small grain size*, Journal of Applied Physics,* vol. 104*, 2008;*

- **E. Yang**, L. Li: Magnetic field aberration induced by cycle stress, *Journal of Magnetism and Magnetic Materials*, p312, 72-77 (2007);
- **E. Yang**, L. Li: Magnetization Changes Induced by Low Cycle Fatigue Both in the Geomagnetic Field and the Magnetic-Free Environment, *SPIE for Smart Materials*, San Diego, USA, March, 2005;
- X. Chen, L. Li., **E. Yang**: Effects of Stress Cycles on Surface Magnetic Field, *Materials Science Forum*, Vol. 490, pp317.

PATENTS (Refereed)

- **E. Yang,** Bruce Gurney (2014): Perpendicular magnetic recording disk with patterned servo regions and templated growth method for making the disk. US8824084 B1
- **E. Yang,** Frank Zhu, and Bruce Gurney (2013): Structure with seed layer for controlling grain growth and crystallographic orientation. US 20150248909 A1
- **E. Yang,** Yuchen Zhou (2013): Novel method for measuring thermal stability for magnetic devices. Provisional
- **E. Yang,** Jeff Childress (2016): Double reference layer enabled enhancement of perpendicular magnetic anisotropy in CoFeB\MgO tunnel junctions. Provisional
- **E. Yang**, D. E. Laughlin and Jian-Gang Zhu (2011): Buffer Layers for $L1_0$ thin film Perpendicular media US8449730 B2

BOOK CHAPTERS (Refereed)

- D. E. Laughlin, H. Yuan, **E. Yang,** and C. Wang: Control of Texture in Polycrystalline Thin Films Used as Data Storage Media, in *Applications of Texture Analysis* (ed A. Rollett), John Wiley & Sons, Inc., Hoboken, NJ, USA (2008)
- **E. Yang**, M. Moneck, and J.-G. Zhu, Technical Methods in Data Storage and Spin Electronics Research, **(Invited)** in *Review of Specific Applications of Technical Methods for Nanotechnology Research* (ed A. Eftekhari), John Wiley & Sons, Inc., Hoboken, NJ, USA (2012)

SESSION CHAIRS

- SESSION CHAIR and presentations at International Magnetics Conference (Intermag), Magnetic recording media, May 2015, Beijing, China

INVITED TALKS & PRESENTATIONS

En Yang
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- INVITED SEMINAR: Friedrich Miescher Institute for Biomedical Research (FMI), Brain-wide plasticity, a holistic understanding of memory and learning, Sep. 2022, Basel, Switzerland
- INVITED SEMINAR: Janelia Symposium 2020, Short-term memory modulated sensory-to-motor transformation, Jan. 2020, Janelia Research Campus, VA
- INVITED SEMINAR: Research Institute of Molecular Pathology (IMP), From transistors to neurons, my journey to the West, June 2018, Vienna, Austria
- INVITED TALK: The 25th Magnetic Recording Conference (TMRC 2014), Magnetic Materials for Bit Patterned Recording, Aug. 2014, Berkeley, CA, USA
- INVITED TALK: TMS 2012 (141st annual meeting and exhibition), Control of Texture and Morphology of Thin Films for Magnetic Recording Applications, Mar. 2012, Orlando, FL, USA
- INVITED TALK: The 21st Magnetic Recording Conference (TMRC 2010), Highly Ordered FePt L1(0) Thin Films with Small Grains on RuAl Seed, Aug. 2010, San Diego, CA, USA
- INVITED TALK: 11th Joint MMM-Intermag Conference, Buffer layers for highly ordered L10 FePt-Oxide thin film media at reduced processing temperature, Jan. 2010, Washington, D.C., USA

OTHER TALKS & PRESENTATIONS (Referred)

- POSTER: The Ascona 2022 Neuronal Circuits Meeting, A brainstem integrator for self-localization and positional homeostasis in larva zebrafish, 2022, Ascona, Switzerland
- TALK: 6th Imaging Structure and Function of the Zebrafish Brain Conference, A brainstem integrator for self-localization and positional homeostasis, 2022, Trondheim, Norway
- POSTER: FENS Forum 2022, A brainstem integrator for self-localization and positional homeostasis, 2022, Paris, France
- POSTER: International Congress of Neuroethology 2022, A brainstem integrator for self-localization and positional homeostasis, Lisbon, Portugal
- TALK: 4th Interdisciplinary Navigation Symposium, A brainstem integrator for self-localization and positional homeostasis, June, iNAV symposium, 2022
- TALK: Zebrafish Neural Circuits seminar, A brainstem integrator for self-localization and positional homeostasis, 2022
- TALK: HHMI Science Meeting: Cognitive and System Neuroscience, A brainstem positional integrator for self-localization, October, HHMI Science Meeting, 2021
- POSTER: HHMI Science Meeting: Cognitive and System Neuroscience, A brainstem positional integrator for self-localization, October, HHMI Science Meeting, 2021

- TALK: Zebrafish Neural Circuits & Behavior, Short-term memory modulated sensory-to-motor transformation, 2019, Cold Spring Harbor, NY, USA
- POSTER: Society for Neuroscience (SfN), Motor readiness through rebound in an identified sensory integrator, 2018, San Diego, USA
- TALK: Circuits and Behavior in Tuscany, Motor Readiness modulates reaction time through neural rebound, 2018, Montecastelli, Italy
- POSTER: Cold Spring Harbor meeting: Neuronal Circuits, Motor preparation through rebound in an identified sensory integrator, 2018, Cold Spring Harbor, NY, USA
- TALK: Computational and Systems Neuroscience (Cosyne), Motor preparation through rebound in an identified sensory integrator, 2018, Denver, USA
- MULTIPLE TALKS AND POSTERS: 59th MMM conference, Topics of nano-structured magnetic materials for data storage applications, Nov. 2014, Honolulu, HI, USA
- MULTIPLE TALKS AND POSTERS: 12th Joint MMM-Intermag Conference, Topics of domain wall motion and magnetic tunnel junctions for logic and memory applications. Jan. 2013, Chicago, IL, USA
- POSTER: International Magnetics Conference (Intermag), Correction of order parameter calculations for textured thin films, May 2012, Vancouver, BC, Canada
- POSTER: 56th Annual Conference on Magnetism & Magnetic Materials (MMM), Field annealing studies of Co/Pd multilayers, Oct. 2011, Scottsdale, AZ, USA
- TALK: International Magnetics Conference (Intermag), Epitaxial growth of L10 -FePt granular thin films on TiC/RuAl Apr. 2011, Taipei, Taiwan.
- TALK: 55th Annual Conference on Magnetism & Magnetic Materials (MMM), Structure and magnetic properties of L10-FePt thin films on TiN/RuAl underlayers, Nov.2010, Atlanta, GA, USA
- MULTIPLE TALKS AND POSTERS: Carnegie Mellon DSSC Annual Fall and Spring Technical Reviews, "Heat assisted magnetic recording media work update," from 2006 to 2010, Pittsburgh, PA, USA
- POSTER: SPIE Conference, Magnetization Changes Induced by Low Cycle Fatigue Both in the Geomagnetic Field and the Magnetic-Free Environment, Mar. 2005, San Diego, Ca, USA.

TEACHING AND MENTORING EXPERIENCES

Howard Hughes Medical Institute, Janelia Research Campus, Ashburn, VA

- Mentoring and teaching graduate students and post docs in the Ahrens lab 2019-2022

DawnSail Biotech, Jiaxing, China

En Yang
250 Bell Tower Dr. Chapel Hill, NC 27599 Phone: (412) 759-0719 E-mail: enyang@unc.edu

- Founder and COO 2011-2016

Western Digital, San Jose, CA
- Project lead & Mentor 2014-2017

Carnegie Mellon University, Pittsburgh, PA, USA
- Teaching Assistant 2008-2011

Tsinghua University, Beijing, P. R. China
- Teaching Assistant 2003-2006