BIOGRAPHICAL SKETCH

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NAME: Meredith Nicole Frazier

eRA COMMONS USER NAME (credential, e.g., agency login): mfrazier5

POSITION TITLE: Assistant Professor, Department of Chemistry and Biochemistry, College of Charleston

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Elon University, Elon, NC	BS	05/2013	Biochemistry
Vanderbilt University, Nashville, TN	PhD	05/2018	Biochemistry/Structural Biology
University of North Carolina at Chapel Hill, Chapel Hill, NC	N/A (postdoc)	09/2019	Biochemistry/Developmental Biology
National Institute of Environmental Health Sciences (NIEHS)/ NIH, Research Triangle Park, NC	N/A (postdoc)	07/2022	Biochemistry/Structural Biology

A. Personal Statement

My early research exposure and the close mentoring I experienced as an undergraduate at Elon University inspired me to pursue a career at a primarily undergraduate institution, which I have begun at the College of Charleston. I now mentor my own undergraduate research students to provide the experience I benefited from, including a long-term commitment to a research project that culminated in an undergraduate thesis and sparked my interest in pursuing a biochemical Ph.D.

I carried out my Ph.D. in Biological Sciences at Vanderbilt University where I used structural biology to study vesicle trafficking. At Vanderbilt, I joined Dr. Lauren Jackson's lab as her first graduate student, which allowed me the opportunity to play a key role in setting up the lab: establishing protocols, mentoring students, and initiating collaborations. In Dr. Jackson's lab, I expanded my knowledge of biochemistry to structural biology and biophysics, studying the molecular mechanisms of how coat machinery selects and regulates vesicle trafficking. Dr. Jackson encouraged me to integrate different structural and biophysical techniques (X-ray crystallography, NMR, ITC) in my research, because each technique can shed light on different aspects of protein interactions. This led me to develop a toolbox of techniques that I utilize in my independent research.

Following my Ph.D. at Vanderbilt, I pursued a short-term postdoc focused on integrating genetics and proteomics with structural biology in Dr. Frank Conlon’s lab at the University of North Carolina at Chapel Hill. I specifically wanted to learn how proteomics can be integrated with structural biology to provide insight into larger complexes or complexes that are difficult to produce and purify recombinantly. I spent a year in the Conlon lab working on a high-risk, high-reward project to uncover how a chromosome remodeling complex is recruited to specific DNA loci. Ultimately, I discovered that the complex was not suited to structural studies. Therefore, I decided to pursue another postdoctoral position to continue to develop my interests in the structural biology of protein machines.

Dr. Robin Stanley's lab at the National Institute of Environmental Health Sciences had a strong track record uncovering the mechanisms of ribosome biogenesis through the combination of structural biology and

biochemistry, so I began my immersion in the field of RNA biology. Five months later, non-essential research was shuttered by the emergence of the novel SARS-CoV-2 coronavirus. Wanting to contribute to our skills in studying RNA processing, we began reading the literature and discovered coronaviruses encode an endoribonuclease that shared many similarities with the endoribonucleases the Stanley lab studied in ribosomal biogenesis and tRNA processing. This endoribonuclease, EndoU/Nsp15, became the center of my current and future research.

In my research lab at the College of Charleston, I am building a robust program training undergraduates in cutting-edge techniques such as cryo-EM while expanding our knowledge of EndoU protein structure and function in diverse nidoviruses. This work will provide insight into how this conserved protein evolved, why oligomerization is important to its function, and how it processes RNA in other oligomeric states. The students in my lab use my established pipeline of protein purification and biochemical assays and apply it to studying related yet distinct enzymes. This program is designed to mentor students in cloning, expressing protein, analyzing enzymatic activity, and making evolutionary comparisons. These studies will yield data that can be used to design antiviral therapeutics against coronaviruses and related viruses with great zoonotic potential.

I am also building collaborations with colleagues internally and externally to determine cryo-EM structures of protein complexes. With the Forconi (CofC) and Harris (University of Florida) groups, we are studying another hexameric nuclease, YloC, which is found in bacteria and belongs to the YicC family, which processes small RNA and mediates mRNA decay. Unlike EndoU, Yloc cleaves RNA in a metal-dependent mechanism, although the specific mechanistic details are unknown. Furthermore, it undergoes a large conformational change from an open to closed clamshell, with the RNA binding in the center. The Forconi and Harris groups have provided kinetic analyses while my lab tackles structural studies to help uncover the mechanism for this novel enzyme.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2010 – 2013	General Chemistry Tutor, Elon University, Elon, NC
2013 – 2018	PhD Candidate, Vanderbilt University, Nashville, TN
2016 – 2016	Biochemistry (BSCI2520) Teaching Assistant, Vanderbilt University, Nashville, TN
2018 – 2018	Postdoctoral research associate, Vanderbilt University, Nashville, TN
2018 – 2019	Postdoctoral research associate, University of North Carolina at Chapel Hill, Chapel Hill, NC
2019 – 2022	IRTA postdoctoral fellow, National Institute of Environmental Health Sciences, Durham, NC
2022 – 2022	Chemistry Department Instructor (CHEM 430), University of North Carolina at Chapel Hill, Chapel Hill, NC
2022 –	Assistant professor, Department of Chemistry and Biochemistry, College of Charleston

Other Experience and Professional Memberships

2010 –	Member, American Chemical Society
2015 – 2016	Member, American Society for Cell Biology
2018 – 2022	Member, Women in Bio, RTP chapter
2022 –	Member, Biophysical Society

Honors

Pre-tenure

2024	Biophysical Society Meeting Travel Award for Research Excellence
2023	Sigma Xi inductee (*scientific research honor society)*

Postdoctoral

2021	NIEHS Signal Transduction Laboratory Rodbell Presentation Award (best departmental trainee talk)
2021	NIEHS Paper of the Month (*Nucleic Acids Research*)
2021	NIH Fellows Award for Research Excellence
2021	Cannon School Distinguished Alumni Service Award
2021	Professional Development Award, NIEHS Fellows 3 Minute Communication Challenge Winner
2021	NIEHS Paper of the Month (*Nature Communications*)
2020	NIEHS Summer Research Mentor Award (declined to mentor via NIH's Summer Intern Program)

Predoctoral
2018 Graduate Student Research Excellence Award, Department of Biological Sciences
2018 Anne Karpay Award, Center for Structural Biology
2017 Biochemistry Departmental Retreat Honorable Mention Graduate Student Poster Award
2015 Ann Bernard Martin Award for Excellence in Graduate Research, Dept. of Biological Sciences
2014-2016 Molecular Biophysics Training Grant (T32) appointee
2014 Biological Sciences Departmental Retreat Best Poster Award
Undergraduate
2013 Phi Beta Kappa inductee
2009-2013 Elon University Honors Fellow
2012 Phi Lambda Upsilon inductee (*national chemistry honor society*)
2011-2013 Cheek Scholarship, Department of Chemistry
2011 Phi Kappa Phi inductee
2010-2011 JM & KS McAdams Scholarship, Department of Chemistry
2010 A.L. Hook Scholarship for Summer Research
2010 CRC Press Freshman Chemistry Achievement Award
2010 Phi Eta Sigma inductee
2009-2013 Elon Presidential Scholarship

C. Contributions to Science

1. Independent Research: Viruses infect all kingdoms of life and can significantly impact human health and agriculture. Many RNA viruses that infect humans are zoonotic, meaning they can also infect other vertebrate hosts and initially emerged from a non-human source. In addition to the structural proteins that support the virion structure, RNA viruses encode many non-structural proteins (nsps)—often enzymes and their accessory factors—that aid in viral replication. Many of these nsps remain understudied and elucidating their roles in the viral life cycle may yield new antiviral therapeutic targets. This is critically important considering the major human health crises caused by novel coronaviruses SARS-1, MERS, and especially SARS-2, responsible for the COVID19 pandemic. Coronaviruses are members of the nidovirus order, which contains many viruses that infect animals and cause global economic impacts. Interestingly, nidoviruses encode a conserved uridine-specific endoribonuclease (EndoU) that targets viral RNA as a way of avoiding host innate immune sensors. *In vivo* studies have demonstrated that an EndoU deficient virus is less pathogenic and cleared more easily by the host immune system. *In vitro* studies show oligomerization of EndoU proteins is necessary for catalytic activity. My lab investigates the structure and function of different classes of nidoviral EndoU nucleases in order to better understand how these nucleases could be therapeutically targeted through oligomer disruption. This work will define molecular mechanisms of EndoU nucleases through classic structure-function approaches, with a focus on cryo-electron microscopy and nuclease cleavage assays. Thus far we have determined the first gamma-CoV EndoU structure and characterized how it cleaves ssRNA.

   External presentations:

   Daquilanea EJ, Fleming JM, Sharma K, Dutcher R, Borgnia MJ, Stanley RE, **Frazier MN**. *Structural and functional characterization of nidoviral endoribonucleases.* Symposium on RNA Biology XV: RNA Tool and Target, 2024. Durham, NC.

   Fleming JM, O'Reilly PE, Daquilanea EJ, O'Brien A, Morris K, **Frazier MN**. *Structural and functional characterization of viral ribonucleases.* South Carolina IDeA Networks of Biomedical Research Excellence (SC INBRE) Research Symposium 2024. Columbia, South Carolina.

2. Postdoctoral Work: In Dr. Robin Stanley's lab, I have gained expertise in cryo-EM structure determination and RNA biology, primarily through my work on the SARS-CoV-2 endoribonuclease. The ongoing Covid-19 pandemic caused by the novel coronavirus SARS-CoV-2 highlights the need to develop effective anti-viral treatments. Coronaviruses, a member of the nidovirus order, cause respiratory illnesses with a wide range of severity, from common colds to MERS, which had a >30% fatality rate. Coronaviruses have large ssRNA genomes that encode for 16 non-structural proteins (Nsps) which have various roles in viral replication and

transcription. Nsp15, also known is an endoribonuclease, encoded by the SARS-CoV-2 viral genome that cleaves viral RNA 3' of uridines (EndoU). It is primarily believed to cleave the polyU tail of the negative strand intermediate RNA in order to help the virus evade immune system detection. EndoU is found in all coronaviruses, as well as most nidoviruses; therefore it is a promising therapeutic target.

Although crystal structures of Nsp15 from other coronaviruses had previously been determined, none had nucleotide or RNA bound; states critical for understanding the mechanism of the enzyme and designing inhibitors.Thus, the specificity and molecular mechanism of RNA processing by Nsp15 was poorly understood. Nsp15 must assemble into a hexamer to cleave RNA, therefore we hypothesized that the architecture of complex is critical for regulating nuclease activity. Our goal was to determine structures of Nsp15 in pre- and post-cleavage states to better understand how it recognizes and cleaves RNA, ultimately providing insight into how it could be targeted.

We determined the first series of cryo-EM reconstructions of SARS2 Nsp15, in apo, UTP-bound, and pre- and post- cleavage states, at resolutions ranging from 2.2 to 3.3Å. Our seven cryo-EM reconstructions, combined with biochemistry, mass spectrometry, and molecular dynamics, exposed molecular details of how critical active site residues recognize uridine and facilitate catalysis of the phosphodiester bond (Fig. 1). A key serine residue forms hydrogen bonds with the uracil base, while a tyrosine in the active site forms van der waals interactions with the ribose. The RNA bound structures suggested that two conserved histidine residues support RNA cleavage through a transesterification reaction. This mechanism was confirmed by mass spectrometry, which revealed the accumulation of the cyclic phosphate transesterification product. Gel-based and FRET cleavage assays revealed additional sequence preferences beyond the uridine, specifically a preference for a purine in the position after uridine. Analysis of the apo and UTP-bound datasets revealed conformational dynamics that are likely important for substrate recognition and nuclease activity. Collectively, these findings advance our understanding of how Nsp15 processes viral RNA and provide a structural framework for the development of new therapeutics. These findings will also provide the conceptual and methodological bases for my independent research lab.

Research papers:

Wilson IM, Frazier MN*, Li JL, Randall TA, Stanley RE*. Biochemical Characterization of Emerging SARS-CoV-2 Nsp15 Endoribonuclease Variants. J Mol Biol. 2022 Oct 30;434(20):167796. doi: 10.1016/j.jmb.2022.167796. Epub 2022 Aug 19. PMID: 35995266; PMCID: PMC9389836. *Co-corresponding authors

Frazier MN, Wilson IM, Krahn JM, Butay KJ, Dillard LB, Borgnia MJ, Stanley RE. Flipped Over U: Structural Basis for dsRNA Cleavage by the SARS-CoV-2 Endoribonuclease. Nucleic Acids Research. 2022 Aug 12;50(14):8290-8301. doi: 10.1093/nar/gkac589. PMID: 35801916; PMCID: PMC9371922.

Frazier MN, Dillard LB, Krahn JM, Perera L, Williams JG, Wilson IM, Stewart ZD, Pillon MC, Deterding LJ, Borgnia MJ, Stanley RE. Characterization of SARS2 Nsp15 nuclease activity reveals it's mad about U. Nucleic Acids Res. 2021 Sep 27;49(17):10136-10149. doi: 10.1093/nar/gkab719. PMID: 34403466; PMCID: PMC8385992.

Pillon MC*, Frazier MN*, Dillard LB*, Williams JG, Kocaman S, Krahn JM, Perera L, Hayne CK, Gordon J, Stewart ZD, Sobhany M, Deterding LJ, Hsu AL, Dandey VP, Borgnia MJ, Stanley RE. Cryo-EM structures of the SARS-CoV-2 endoribonuclease Nsp15 reveal insight into nuclease specificity and dynamics. Nat Commun. 2021 Jan 27;12(1):636. doi: 10.1038/s41467-020-20608-z. PMID: 33504779; PMCID: PMC7840905. *Authors contributed equally.

3. Dissertation Project 1: My first project as a graduate student in Dr. Lauren Jackson's lab was to characterize the interaction between the AP4 trafficking complex and its only known accessory protein at the time, tepsin. Mutations in AP4 cause hereditary spastic paraplegia in human patients. These novel structural investigations began to shed light on how AP4 trafficking functions, and subsequently what is being disrupted when AP4 is lost. I used isothermal calorimetry and NMR chemical shift perturbation experiments to identify, characterize, and map the interaction between a short hydrophobic motif in the C-terminus of tepsin and the β4 appendage domain of AP4. This was the first structural description of a motif that binds AP4. We also

collaborated with Dr. Scottie Robinson's lab at the Cambridge Institute for Medical Research (UK) to validate that the binding surfaces I had identified *in vitro* were important in cells as well. This project allowed me to develop competency and troubleshooting skills in protein expression and purification, ITC, and NMR. I presented this research in the form of a poster at the national American Society for Cell Biology, gaining experience in presenting my work externally.

Research papers:

Frazier MN, Davies AK, Voehler M, Kendall AK, Borner GH, Chazin WJ, Robinson MS, Jackson LP. Molecular Basis for the Interaction Between AP4 β4 and its Accessory Protein, Tepsin. Traffic. 2016 Apr;17(4):400-15. PubMed PMID: 26756312; PubMed Central PMCID: PMC4805503.

External poster presentations:

Frazier MN, Davies AK, Voehler M, Borner GH, Robinson MS, Jackson LP. *Molecular basis for the interaction between AP4 and its accessory protein tepsin.* American Society for Cell Biology, National Meeting 2015. San Diego, CA.

4. Dissertation Project 2: Following characterization of the C-terminal motif in tepsin that interacts with AP4, I turned my focus to the unique and uncharacterized ENTH/VHS-like domain in tepsin. Tepsin is the only epsin family member to have a second structured domain downstream of the epsin N-terminal homology (ENTH) domain, and this second domain was also predicted to be a member of the ENTH/ANTH/VHS family, which are commonly found in trafficking proteins, but always positioned at the very N-terminus. Thus, we thought determining the structure of this domain was important for understanding its function. I used x-ray crystallography to determine the tepsin VHS-like domain structure, and tested possible functions for the domain using ITC and NMR. We collaborated with Dr. Nicole Creanza at Vanderbilt to understand the evolution of tepsin and its ENTH and VHS-like domains. Together with Dr. Tara Archuleta, we published the first structural, functional, and evolutionary studies of the tepsin ENTH and VHS-like domains.

   Research papers:

   Archuleta TL*, Frazier MN*, Monken AE, Kendall AK, Harp J, McCoy AJ, Creanza N, Jackson LP. Structure and evolution of ENTH and VHS/ENTH-like domains in tepsin. Traffic. 2017 Sep;18(9):590-603. PubMed PMID: 28691777; PubMed Central PMCID: PMC5567745. *Authors contributed equally.

   External poster presentations:

   Frazier MN, Monken AE, Harp J, Jackson LP. *Structure and function studies of the VHS-like domain from the AP4 accessory protein tepsin.* International School of Crystallography, 50th course, 2017. Erice, Italy.

A complete list of my publications can be found at:
https://www.ncbi.nlm.nih.gov/myncbi/meredith.frazier.1/bibliography/public/