**Nicole Putnam, Ph.D., of Vanderbilt University**   
[**“The impact of innate immune recognition of Staphylococcus aureus on bone homeostasis and skeletal immunity”**](https://www.niaid.nih.gov/sites/default/files/nicoleputnamapplicationF31.pdf)

**Applicants Background and Goals for Fellowship Training:**

###### BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING

**Doctoral Dissertation and Research Experience** **Undergraduate Research**

I began to participate in independent research during my sophomore year at the University of Wisconsin-La Crosse, while pursuing degrees in Biochemistry and Psychology. I served as a research assistant and laboratory manager in the Visual Sciences Research Laboratory for Drs. Alex O’Brien and Bart Van Voorhis for two and a half years, during which time my critical thinking and scientific interpretation skills were greatly enhanced. Our main areas of study were object recognition and synesthesia, which we tested through the creation of programs to measure reaction time from our participants. Also during my sophomore year, I began to pursue research in organic chemistry with the chair of the Chemistry Department, Dr. Aaron Monte, in the synthesis of the beta-alkaloid compound tetrahydroharmine. These research experiences had a profound impact on my desire to pursue research as acareer.

###### Research and Development Internship

The summer before my December 2010 graduation, I attained a position as a cancer research and development intern with the global health care company, Covidien. My research skills matured significantly under the mentorship of Dr. Raghavan Rajagopalan. In this internship, I focused on the creation and therapeutic application of photosynthetic compounds targeted for use in colon and ovarian cancers. The premise of this research was to localize a compound that alone is nontoxic, but can be subsequently activated by light to form radicals, inducing death of surrounding cancerous cells. I designed and synthesized a novel photosensitive compound, conjugated this compound to carrier molecules specific for ovarian and colon cancer cells, and performed cytotoxicity assays to determine its efficacy. In this internship, I further developed skills in cellular physiology and organic chemistry. I was able to demonstrate that the compounds in conjunction with light were cytotoxic and could selectively target the desired cancer cells, and was awarded the Best Poster Award among summer interns at the *Covidien Intern Poster Symposium*. These data were presented at the 2010 *SPiE BiOS: Biomedical Optics Symposium* and the 2011 *World Congress of the International Photodynamic Association*, and were published in *Photodiagnosis and Photodynamic Therapy* in 2011.

###### Interest in Microbiology and Immunology

As I prepared to embark upon training towards an advanced degree, I felt called to explore other scientific disciplines. I first became drawn to the field of microbiology and immunology during the Capstone Seminar of my final semester at University of Wisconsin-La Crosse. The seminar I gave focused on the emergence of infectious diseases into new populations and spread to previously uninfected areas. I became captivated with the study of infectious diseases while preparing this presentation. So much so, that I chose to apply for and complete a microbiology course after graduating with my degrees in Biochemistry and Psychology. After these experiences I was committed to applying to graduate degree programs in Microbiology and Immunology.

###### Master’s Degree Thesis Research

I went on to complete a Master of Science in Molecular Microbiology and Immunology at the Johns Hopkins Bloomberg School of Public Health. My Master’s degree thesis work involved the study of immune responses to measles virus in rhesus macaques in the laboratory of Dr. Diane Griffin, a world-renowned virologist. During this time, I developed expertise in immunologic techniques and experimental design, critical review of the primary literature, and scientific writing. My projects in the Griffin laboratory focused on (1) the characterization of Th17 (CD4) and Tc17 (CD8) T cell responses to wild-type measles in a rhesus macaque model, (2) monitoring viral RNA persistence, and (3) determination of the immunological effects of the measles virus C and V proteins between wild-type and vaccine strains. I became proficient in the analysis of primary samples, including peripheral blood mononuclear cells (PBMCs) from human and non-human primates, as well as bone marrow, skin, and lymph node biopsies. My research found three distinct activation periods of Th17 and Tc17 cells over the course of six months post-infection, a consequence of persistent, noninfectious measles virus RNA. Additionally, I found that the C and V proteins limited the human type I interferon response and were necessary for optimal viral replication. My research in the Griffin laboratory resulted in a middle authorship publication in the *Journal of Virology* in 2014, presentation of my research at the 2015 *International Conference on Negative Strand Viruses*, and the recent resubmission of a first author paper to *The Journal of Infectious Diseases* in December 2016.

###### Ph.D. Laboratory Rotations

The skills obtained from my undergraduate, internship, and master’s degree research allowed me to be extremely productive in my four laboratory rotations and as an incoming graduate student at Vanderbilt University. In these rotations, I was able to explore cell death mechanisms induced in human osteoblasts in Dr. Jim Cassat’s laboratory, design a mass cytometry (CyTOF) panel to phenotype T lymphocyte populations in the laboratory of Dr. Marco Davila, optimize an *ex vivo* T regulatory cell suppression assay and contribute to analyses focusing on immune tolerance in Type 1 Diabetes and Systemic Lupus Erythematosus (SLE) with Dr. Dan Moore’s research group, and provide preliminary data to support *Helicobacter pylori* toxin-mediated T cell suppression in conjunction with Drs. Tim Cover and Spyros Kalams. My rotation in the Moore laboratory resulted in a middle author publication in the *American Journal of Transplantation* in 2015.

###### Ph.D. Thesis Laboratory

My interests in the host responses to human pathogens led me to join Dr. Cassat’s laboratory to study osteoimmunologic responses to bacterial pathogens. Under Dr. Cassat’s guidance, I have become well-trained in microbiology and have developed a project that bridges multiple scientific disciplines with the long-term goal of determining the innate sensing capabilities of bone cells and how microbial pathogens impact skeletal remodeling. This project will guide my scientific training, both at the bench and professionally, under the sponsorship of Dr. Cassat and Dr. Julie Sterling of the Vanderbilt Center for Bone Biology. In the proposed research, I will explore how skeletal cells sense and respond to the human bacterial pathogen *Staphylococcus aureus*, the most common cause of osteomyelitis, and how these responses disrupt normal bone remodeling processes.

###### Training Goals and Objectives

My research ambitions are focused on studying infectious diseases that have a substantial public health burden. I am seeking a Ph.D. in Microbiology and Immunology within the Department of Pathology, Microbiology and Immunology (PMI) with the goal of establishing my own laboratory as an independent scientist studying translational research. Specifically, I hope to investigate critical immune responses and biological changes induced by pathogens, and contribute to how this knowledge can be leveraged to alleviate the morbidity and mortality associated with infection. In this Ph.D. program I aim to develop independent thinking and the experimental skills necessary to investigate translational research problems. I will continue to become proficient in project development and hypothesis-driven research. I hope to expand my scientific skills in critical thinking, experimental design, data interpretation, and statistical analysis.

Vanderbilt University is an exceptional environment to gain a comprehensive knowledge of host- pathogen interactions. To expand on previous didactic coursework in bacteriology, virology, parasitology, and immunology at JHSPH and VU, there are several ongoing seminars and journal clubs to fortify knowledge in microbiology and immunology in order to pursue a scientific career in the field of infectious disease. I have carefully chosen seminar series to enhance my didactic coursework in conjunction with my mentor and thesis committee. Specifically, I will attend weekly seminars from PMI and the Vanderbilt Center for Bone Biology (VCBB), a weekly Research in Progress seminar for student presentations, a monthly Infection, Inflammation, and Immunity Frontiers seminar featuring luminaries in microbiology and immunology, a bi-monthly Microbial- Host Interaction meeting, a bi-monthly Host-Pathogen Interaction journal club, and the annual Vanderbilt Symposium on Infection and Immunity.

In keeping with my desire to conduct translational research, I applied to and was subsequently selected to join the Vanderbilt Program in Molecular Medicine (VPMM). The VPMM is a unique training program established in 2010 with the goal to integrate thesis work with clinical experiences, didactic courses, and seminars under the guidance of my primary mentor, Dr. Cassat, and a clinical mentor, Dr. Isaac Thomsen in Pediatric Infectious Diseases. The VPMM provides valuable clinical experience that ensures that my research project will reflect the most important challenges posed by bone infections. In addition to observational experiences in clinic, the VPMM provides essential information on translational research through an Introduction to Clinical and Translational Research and VPMM Rounds, which brings a panel of clinicians, basic scientists, and patients into a classroom to discuss the different perspectives on human disease. Additionally, the VPMM supports attendance at Pediatric Infectious Diseases research conferences, rounds, and clinical case conferences, as well as the bi-monthly seminar series, and an annual VPMM Spring Retreat to share our thesis research. I expect to complete the VPMM training program by the summer of 2018.

Vanderbilt University also offers elective Modules through the Augmenting Scholar Preparation and Integration with Research-Related Endeavors (ASPIRE) Program, founded in 2013 under support of the NIH-

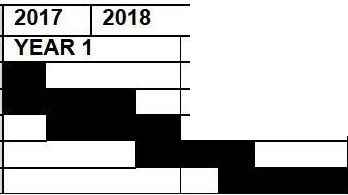
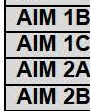
funded BEST grant. I have recently been accepted into the ASPIRE Module for Clinical Laboratory Medicine, which is a training experience to provide exposure to clinical research and laboratories. Due to the small number of graduate students and post-doctoral fellows accepted into the Clinical Laboratory Medicine Module each year, training can be personailzed to emphasize a particular clinical sub-discipline. The 2017 Clinical Laboratory Medicine Module will tailor my experiences to highlight training in Clinical Microbiology with Dr. Jonathan Schmitz, Associate Director of the Fellowship Training Program in Medical and Public Health Laboratory Microbiology and Instructor in PMI. This experience will provide meaningful preparation for the continued study of human infectious pathogens.

Over the course of my Ph.D. training program, I will gain experience in mentoring and teaching. For example, in just the last year and a half, I have had the opportunity to train 4 rotation students coming in through the Medical Scientist Training Program and the InterdisciplinaryGraduate Program for Ph.D. students. In the future, I hope to foster the ongoing training of an undergraduate student over the course of a few years to understand cellular differentiation and/or advanced imaging and quantification techniques. Furthermore, I have facilitated communication with the School for Science and Math at Vanderbilt to teach periodic guest lectures to high school students, of which I have taught a "Microbes 101" course in the fall of 2016. Further teaching experience is incorporated into the Microbiologyand Immunology Graduate Program curriculum, in which graduate students serve as teaching assistants (TAs) to the first year medical students for the laboratory component of the Medical School Microbiologyand Immunology course.

I will continue to hone my skills in scientific communication through presentation of my research at laboratory meetings every one to two months, to my thesis committee twice a year, and to other graduate students, post-doctoral fellows, and faculty at the Research in Progress, Microbial-Host Interaction, VCBB, and VPMM seminars. Additionally, I will present my research at national and international meetings. To this end, I have presented my research at the 2016 *Vanderbilt Symposium on Infection and Immunity* and the 2016 *International Conference on Gram Positive Pathogens.* Next year, I plan to attend the St. *Jude's Invited Graduate Student Symposium* and the *Southeastern Immunology Symposium.* My written communication will be enhanced by assisting Dr. Cassat in the peer review of manuscripts, by the review of other manuscripts from the Cassat laboratory, and through submission of my own manuscripts and grantapplications.

**Act;yjtjes Planned under this Award**

I have applied for two years of NRSA support. I will dedicate 100% of my effort to research, training, and career development activities detailed below.



**CALENDAR YEAR**

**F31 AWARD YEAR AIM 1A**

**2019**

**YEAR2**

**Year 1****; July** **2011-Jun****e 201s**

**Research: 80%**

* + Meetings with Dr. Cassat (weekly)
  + Meetings with Thesis Committee (bi-annually)
  + Submit first lead-author publication, detailing the impact of staphylococcal coagulases and S. *aureus*

strain differences in osteomyelitis

* + Submit second first-author publication, detailing findings in Aim 1

**Seminars and Presentations: 10%**

* + Present research and participate at events at Vanderblit University Medical Center (VUMC):
    - Pathology, Microbiology,and Immunology seminar (weekly)
    - Microbiologyand Immunology Research in Progress seminar (weekly)
    - Microbial-HostInteraciton seminar (bi-monthly)
    - Host-Pathogen Interaction journal club (bi-monthly)
    - Infection, Inflammation & Immunity Frontiers seminar (monthly)
    - Vanderblit Symposium of Infection and Immunity (annually)
    - Bone Center Seminar Series (weekly)
  + Organize/Attend Southeastern Immunology Symposium 2017
  + Attend St. Jude's Invited Graduate Student Symposium 2017

###### Career Development/Mentorship: 10%

* Mentor Vanderbilt undergraduate (1) and graduate students (3) rotating through the laboratory
* Participate in Vanderbilt Program in Molecular Medicine Training Program Activities:
  + Vanderbilt Program in Molecular Medicine seminar (bi-monthly)
  + Vanderbilt Program in Molecular Medicine Retreat (annually)
  + Observational experiences
  + Clinical Rounds, Case Conferences, and Seminars
* Participate in activities sponsored by the VUMC Office of Career Development
  + ASPIRE Program, *Explore Phase*: Designed to highlight career options and networking
    - ASPIRE Module Clinical Laboratory Sciences
    - BRET Career Connections (monthly)
    - BRET Career Symposium (annual)
    - Complete Individual Development Plan (IDP) with Dr. Cassat
* Laboratory teaching assistant (TA) for the Medical School Microbiology and Immunology course

###### Year 2: July 2018- June 2019

**R****esearch: 80%**

* Meetings with Dr. Cassat (weekly)
* Meetings with Thesis Committee (bi-annually)
* Submit third first-author publication, detailing findings from Aim 2

###### Seminars and Presentations: 10%

* Present and participate in VUMC seminar series (see above)
* Present research at national and/or international meeting
* Mentor Vanderbilt graduate students rotating through the laboratory/undergraduate

###### Career Development/Mentorship: 10%

* Mentor Vanderbilt undergraduate (1) and graduate students (3) rotating through the laboratory
* Participate in activities sponsored by VUMC Office of Career Development
  + ASPIRE Program, *Enhance Phase*: Communication module designed to improve oral communication skills
    - BRET Career Connections (monthly)
    - BRET Career Symposium (annual)
    - Complete Individual Development Plan (IDP) with Dr. Cassat
* Interview for a postdoctoral fellowship
* Write thesis and defend PhD dissertation

**Nico Contreras, University of Arizona**

[**“The Immunological Consequences of Mouse Cytomegalovirus on Adipose Tissue”**](https://www.niaid.nih.gov/sites/default/files/F31-sample-application_nico_contreras.pdf)

**Applicants Background and Goals for Fellowship Training:**

**GOALS FOR FELLOWSHIP AND TRAINING CAREER**

### To this point of my training I have fulfilled all required coursework as well as passed my qualifying exams to continue to PhD Candidacy. However, I will continue to be an active participant in journal clubs to discuss primary research, our research seminars to present my own research, as well as attend invited speakers from other institutions to continue to broaden my knowledge of my scientific field. In addition to these activities I will also attend domestic and international conferences, as will be outlined in the timeline below, and participate our department’s local Frontiers in Immunobiology Symposium as well as my laboratories own sponsored International Cytomegalovirus Meeting. The main focus of my training will be the further development of the project herein described under the supervision of Dr. Janko Nikolich-Zugich, MD, PhD. In conjunction with the scientific training provided by Dr. Nikolich-Zugich, his network of colleagues and collaborators provides an unparalleled level of interaction with leaders of my field. To date I have been involved in several aspects of Program Projects Grant meetings, development of a second Program Project, and one-on- one interaction with Vishwa Deep Dixit, Charlie Surh, Michael Diamond, Richard A. Miller, and others. The beneficial impact that these interactions have to my career are immeasurable, as they provide interactions that will undoubtedly help me reach my primary goal of a post-doctoral training position following my PhD completion. After obtaining my PhD in Immunobiology, I aim to continue a career in biomedical and scientific research focused on the interface of infectious disease and adipose tissue immunology.

Post-doctoral training following completion of my PhD studies will help me to achieve the penultimate goal of an academic position where I am capable of having an impact not only in the biomedical research community and to also be involved in the development and training of students who are under represented minorities, such as myself. In line with the latter goal, I sit as a student- member of the Department of Immunobiology Diversity Committee and contribute to increasing visibility of a career in scientific research to non-traditional and under represented students. To this point in my career I have been given numerous opportunities to develop my grant writing skills, through a grant writing course proctored by several faculty members, I have been provided ample opportunity to speak in both scientific and non-scientific settings, I have been provided leadership positions as a planning member of a joint biological sciences program retreat at the University of Arizona. I will continue developing these skills during my remaining years as a PhD candidate in Dr. Nikolich-Zugich’s laboratory. Finally, I will continue developing increased independence and autonomy as a researcher. Adipose tissue immunology in Dr. Nikolich-Zugich’s laboratory was a completely unexplored field prior to my arrival and as such it has provided myself a level of independence and freedom that I believe would be difficult to come by in other laboratories. All of these various opportunities, I believe, will allow for my continued success in the sciences as my training progresses.

The Ruth L. Kirschstein NRSA Diversity Fellowship presents a unique opportunity to bolster many of the skills that I have been developing during my training. The identification of a specific unanswered and unexplored question in the field of immunobiology, and the generation of ways to address the question through the development and drafting of this proposal will pay dividends in my ability to apply for and obtain grant funding. Continuation of the funding will require the development, execution, and analysis of experiments and data. These activities will expand upon my critical thinking skills, the drafting of reports for both a scientific and non-scientific community, and ability to defend my work and thought process. As this is a diversity fellowship, I certainly understand and appreciate what that means for not only myself but also those who have applied before and will apply after me. The development of under represented minority students is crucially important to me, as it has provided a world that otherwise I would never have experienced. I have been funded by the NIH Initiative to Maximize Student Development and now am currently funded by an R01 Diversity Supplement. During my career I hope to continue to be provided by these programs and encourage my trainees to apply as well. Finally, the funding provided by this fellowship would help accomplish my research projects in a timely fashion whereby I can continue onto the future stages of my training.

**ACTIVITIES PLANNED UNDER THIS AWARD**

**Year 1 (2017): Nikolich-Zugich Laboratory (The University of Arizona)**

**Research: 80%, Seminars/Journal Club: 10%, Conferences/Career Development: 10% Research:** Complete Aim 1.

**Conferences/Career Development:** Attend and present at the 6th International Workshop on CMV and Immunosenescence in Tucson, AZ. Attend and present at The American Association of Immunologists in Washington, DC.

**Year 2 (2018): Nikolich-Zugich Laboratory (The University of Arizona)**

**Research: 80%, Seminars/Journal Club: 10%, Conferences/Career Development: 10% Research:** Submit manuscript on Aim 1. Complete Aim 2. Being writing thesis/dissertation.

### **Conferences/Career Development:** Attend and present at the Keystone Symposium Integrating Metabolism and Immunity in Dublin, Ireland. Attend and present at The American Association of Immunologists in Austin, Texas.

**Year 3 (2019): Nikolich-Zugich Laboratory (The University of Arizona)**

**Research: 80%, Seminars/Journal Club: 10%, Conferences/Career Development: 10%**

The beginning of year 3 is the proposed year of thesis/dissertation defense.

**Research:** Submit manuscript for Aim 2. Defend thesis/dissertation.

**Conferences/Career Development:** Attend and present at relevant international meeting. Attend and present at The American Association of Immunologists in San Diego, California.

Attend relevant international conference.

**RESEARCH EXPERIENCE**

My research experience was delayed compared to most of my peers. I was the first person in my family to attend and graduate from university and as can be imagined I didn’t really have much of a plan or understanding of what volunteering and academic research even was. I worked for most of my undergrad until I was fortunate to get a scholarship

The only laboratory experience that I had during my undergraduate studies had been the obligatory curriculum associated labs of chemistry, organic chemistry, physiology, biology, and organic chemistry. These were obviously not focused on answering unsolved questions but more focused on immersion of students into basic laboratory techniques. It was not until after I graduated that I first experienced basic scientific research.

I was accepted into a Professional Science Masters program, which, in my mind, can best be described as a mixture of MBA level business courses and first year PhD sciences courses. It was here that I first began conducting scientific research. As part of my Master’s thesis I worked in Dr.

Linda Powers’ laboratory where the primary task was the development of a lateral flow assay for the detection of blood borne pathogens. This device was funded by a Office of Naval Research contract, and the assay was intended be used as a point of care device to determine if blood was suitable for transfusions. Specifically, my objectives were to use phage display peptide library to determine peptide interactions between Hepatitis B and C viruses, as well as HIV. The fundamental concept behind this project was that peptides identified to interact with these viruses would be conjugated to fluorescent markers and these markers would be detected and inform the operator whether blood was infected or not. During my time in the Powers lab I learned chemical techniques such as the sanitation of glassware by ‘piranha etch,’ which is a mixture of sulfuric acid and hydrogen peroxide. I learned how to perform peptide library biopanning with phage display technology and to crosslink peptides to glass surfaces. I learned proper techniques in handling select-agents. I successfully defended my Master’s thesis after 1.5 years in the program.

Shortly before completing my Master’s I was accepted into the University of Arizona biological sciences umbrella program. At the beginning of my studies I initially identified Dr. Nikolich-Zugich’s laboratory as where I wished to conduct my thesis research, but as required by the program I had to perform research rotations. Fortunately for me I was funded by the NIHs Initiative to Maximize Student Development program and began research earlier than my cohort in the summer. I began my first rotation in the laboratory of Dr. Kirstian Doyle who investigates the long-term cognitive effects of stroke, and the immune response within stroke lesions. In this laboratory I was involved in setting up the workflow of a Magpix Luminex Assay, which allows for the detection of up to 25 different cytokines. We used this assay to investigate the inflammatory environment of the stroke lesions in the brain following stroke. From this work I gave an oral presentation to my cohort and faculty members.

My second research rotation was in the laboratory of Dr. Anita Koshy. Her laboratory focuses in the lifelong chronic infection of a parasite named *Toxoplasma gondii*. My responsibilities were two- fold, I helped quantify the number of macrophages and T cells in the brain following infection by using immunohistochemistry and I was responsible for engineering a mutant *Toxoplasma* with Crispr/Cas9 technology. Once again I presented my resulting data in an oral presentation to faculty and students of my cohort.

My third and final rotation was in the Nikolich-Zugich laboratory, where I currently am working on several projects. I am investigating if caloric restriction can be used to rejuvenate the aged immune system and, as seen in this research proposal, the contribution of adipose tissue immune cells to lifelong infection of cytomegalovirus. Since joining the lab I have given several oral and poster presentation on my work. Most, notably I was awarded an under represented minority travel award to attend the International ThymUS Meeting in Maui, Hawaii to present my work on calorie restriction. It is these experiences that I believe will allow me success in completing the goals stated within this proposal.

**Samantha Lynne Schwartz, Emory University**

[**“Regulation of 2'-5'-Oligoadenylate Synthetase 1 (OAS1) by dsRNA”**](http://www.niaid.nih.gov/sites/default/files/F31-Sample-Application_Samantha-Schwartz.pdf)

**Applicants Background and Goals for Fellowship Training:**

### APPLICANT'S BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING

1. **Doctoral Dissertation and Research Experience**

My previous research experiences have all revolved around microbiology, biochemistry, or a combination of the two. I had the opportunity to try two very different research experiences before entering graduate school at Emory University. I began my research career as an undergraduate at Armstrong State University and then worked for three years as a research technician at Emory University. Upon joining Emory University’s Biochemistry, Cell, and Developmental Biology (BCDB) Program, I completed three different laboratory rotations prior to joining the Conn lab in May 2016.

As an undergraduate at Armstrong State University, I wholeheartedly applied myself to volunteering in Dr. Jennifer Brofft’s lab investigating microbes that penetrate sea turtle eggs and which are implicated with failed turtle embryo development. My research culminated in a poster presentation at two American Society for Microbiology meetings (Gainesville, Florida in 2011 and San Francisco, California in 2012), for which I was awarded travel funds via the National Science Foundation’s STEM program. I also gave a formal oral presentation at Armstrong State University's Student Scholars Symposium. Following this experience, I felt sure graduate school was in my future, but I wanted to explore additional areas of research before applying. While this research opportunity at my undergraduate institution was instrumental in my early development as a researcher, other options were limited in scope due to the small size of the institution. To broaden my horizons and develop an understanding of the rigors of a major research institution, I applied for a research specialist (technician) position at Emory University, an institute I had also identified as a potential top choice for graduate school. I took a leap and left for Atlanta for no other reason than to pursue academic research, despite my lack of exposure to graduate-level research and no family role model for highereducation.

In Dr. Graeme Conn's lab at Emory University, I developed technical and organizational skills to efficiently carry out full-time research. My work ranged from laboratory prep work to troubleshooting complex assays and processing resultant data. My three years of lab experience as a research specialist provided an important initial exposure to aspects of being a researcher, such as reading of primary research articles, attending research seminars, giving presentations, and contributing to manuscript preparation. From these initial insights, I gained a clear concept of the critical skills I would develop for success in graduate school. My research in the Conn lab focused on elucidating the molecular details of protein kinase R (PKR) regulation by viral non-coding RNAs (VA RNAI and HIV-1 TAR) as part of the host innate immune response. I gained skills in RNA *in vitro* transcription and purification, and developed a new high-throughput version of the radiometric assay used by the lab to measure PKR activity. Developing this assay in particular permitted me to evolve from becoming frustrated by experimental road blocks to a scientist who is excited and driven by the often subtle complexities that define a highly reproducible, invaluable experimental tool. What initially began as a test of perseverance grew into a love of experimental science. It was in this transformation that I knew I was ready to take the next step: graduate school. Upon joining Emory University’s Biochemistry, Cell, and Developmental Biology (BCDB) Program, I completed three laboratory rotations prior to joining my thesis lab.

My first rotation was in the research group of Dr. Christine Dunham. The Dunham lab uses X-ray crystallography to study ribosome structure and translational regulation. Here, my work focused on the toxin MazF-mt6 from *Mycobacterium tuberculosis* that inhibits protein synthesis during stress by cleaving 23S rRNA at Helix 70. The goal of my project was to determine MazF-mt6 residues important for cleavage of Helix 70 RNA. Using X-ray crystal structures of MazF-mt6 and *Bacillus subtilis* MazF-mRNA complex as a guide, I substituted predicted catalytic residues with alanine and tested the variant MazF proteins for ribonuclease activity using an *in vitro* cleavage assay. Dr. Dunham also entrusted me with an X-ray crystallography side project where I attempted to co-crystallize a novel inhibitor compound with the ribosome. I gained experience setting trays, optimizing conditions, and testing crystals remotely at the SER-CAT synchrotron beamline at the Advanced Photon Source.

My next rotation was with Dr. Daniel Reines’ research group. The Reines lab uses yeast as a model system to study RNA-binding proteins containing low complexity domains and their propensity to form amyloid filaments. Here, my work focused on the RNA-binding protein, Pcf11, a component of the cleavage and polyadenylation complex that is involved in orchestrating transcription termination in yeast. Computational analysis suggested that Pcf11 possesses a prion-like domain predicted to form amyloid filaments. The Reines lab has shown through biochemical data and electron microscopy imaging that the low complexity domain of Pcf11 alone was able to form amyloid filaments. The main focus of my project was to address whether or not the low complexity domain of Pcf11 could form filaments when linked to structure-containing domains, as it exists naturally in the full-length protein. During my rotation, I spent the majority of my time optimizing the expression and solubility of the full-length Pcf11 protein. I purified enough stable protein to perform a fluorescence assay with Thioflavin T as an indirect measurement of filament formation. My results were indicative of filament formation, but required confirmation by electron microscopy. A current graduate student is continuing my work on this project.

My last rotation was with the Lowen lab. Dr. Anice Lowen’s group uses molecular and classical virology techniques to study influenza A virus reassortment, the process by which two viruses that co-infect the same cell exchange intact gene segments. Here, my work focused on developing a novel technique using droplet digital PCR (ddPCR) as a method for detecting influenza virus defective interfering (DI) particles within a virus preparation. Three A/Panama/2007/99 (H3N2) virus stocks were used in these experiments that were either enriched in DI particles, purified to remove possible DI’s, or unknown. My results revealed that ddPCR can successfully detect DI particles within these virus stocks and confirmed the approach to be more sensitive and precise when compared to traditional quantitative PCR methods. My ten-week rotation proved to be very successful: I collected publishable data, and wrote and published a methods paper. Perhaps most importantly, I forged an excellent mentor-mentee relationship with Dr. Lowen who is now the Co-sponsor on this fellowship application. During my rotation, she also encouraged me to attend the 14th Annual Southeastern Regional Virology Conference at Emory University and that we present my work at the 9th Annual NIAID Centers of Excellence for Influenza Research and Surveillance (CEIRS) Network Meeting at St. Jude Children’s Research Hospital.

**Narrative of Doctoral Dissertation.** Since joining the Conn laboratory, I have focused my studies on the regulation of OAS1 by double-stranded RNA (dsRNA). Data from previous lab members showed that mutations to particular consensus sequence nucleotides cause stark differences in OAS1 activation. However, the molecular basis for these differences was not clear from available structural and functional data. The goal of my dissertation research is to understand how RNA features (sequences, structural motifs, etc.) contribute to OAS1 activation. Preliminary data suggests that the stark differences observed in OAS1 activity by these dsRNA are not due to differences in binding affinity. From these data, I hypothesize that the differences in OAS1 activity likely lie in differences in enzyme kinetics or structural rearrangements and will test these hypotheses as part of my current proposal.

1. **Training Goals and Objectives**

My first goal for the training period is to continue developing my skills in independent experimental design, execution, and interpretation of results obtained. To date, I have refined our expression and purification scheme for OAS1, resulting in a better yield of more active protein. As presented in the preliminary data in this proposal, I have also begun experiments using bio-layer interferometry (BLI) (using a new biotinylated OAS1 construct I generated) and produced the samples for the hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) peptide mapping study.

The proposed research will aid my scientific career by first allowing me to acquire many new technical skills

e.g. in performing and analyzing the results of BLI binding assays, HDX-MS analyses of protein dynamics, X- ray crystallographic structure determination, OAS enzyme assays, cell culture, and viral infection assays. These new skills will complement those from my time as a research specialist (e.g. in RNA synthesis and purification, and protein kinase assays) as well deepen my knowledge of critical practical skills for any biochemist, such as protein expression and purification. I will be supported by my Sponsor and Co-sponsor in many of these approaches but will also be able to call on other researchers and experts to develop new protocols and troubleshoot difficult problems in approaches, including BLI and HDX-MS. I am excited to be contributing to bringing these new approaches into our lab’s repertoire. With the help of Drs. Conn and Lowen, I have developed an integrated plan to assess OAS1 regulation by specific RNA features using the above broad range of approaches. In Specific Aim 1, I will measure OAS1 enzyme kinetics and correlate the differences *in vitro* with their impact on the OAS1/RNase L pathway in human cells by measuring ribosomal RNA (rRNA) cleavage and transcript level changes of known RNase L targets, all of which is new to me. In Specific Aim 2, I will measure dsRNA binding kinetics (on/off rates) and OAS1 structural dynamics using BLI and HDX-MS, respectively, both entirely new techniques to me. These experiments will allow us to gain insight into exactly how the RNA can mechanistically alter OAS1 structure to achieve different levels of activation. By building upon my current foundation of knowledge, I am setting myself up for the best possible post-doctoral experiences available.

I will also continue to develop my communication skills. Dr. Conn places a strong emphasis on perfecting presentation and writing skills, which is one of the main reasons I ultimately chose to join his lab for my thesis research. I practice my seminars in front of Dr. Conn and lab members to improve my style by becoming more clear and polished. The process of developing and writing this fellowship with Dr. Conn and others, and my manuscript with Dr. Lowen from my rotation in her lab, not only has been an amazing experience, but also it revealed I still have much to learn from them in this area. Developing both of these communication skills is an ongoing process as they are essential to my advancement in science. Throughout the training period, I will attend meetings to present my research and network with other scientists. These meetings will not only further my research through the critical feedback I will receive, but will also help me build connections in my chosen field. Additionally, I will take advantage of the many training opportunities provided by the BCDB graduate program (yearly student seminars, journal clubs, etc.) to refine and practice my communication skills. Finally, I will expand my writing skills to include the preparation and submission of my work for publication. My expectation is that, from the project I have developed, I will be able to publish at least two first-author peer- reviewed papers in leading journals in my new field.

My long-term career goal is to become an independent researcher using biochemical and virological approaches to study mechanisms underpinning cellular function and disease related to infection and immunity. Given my early stage in graduate school, I currently aim to keep an open mind about the ultimate setting in which I will build this career (e.g. academia, government lab, or industry). I plan to take advantage of opportunities to learn about all career options, including a career seminar series, “Pathways Beyond the Professoriate,” offered by the Laney Graduate School (LGS), and professionalization workshops offered by my program. One important strength of the BCDB Program is its requirement that students produce an individual development plan (IDP) in their first year and then update this plan on an annual basis, including short/ long term research goals and career plans. This process will help Dr. Conn and I ensure that my technical and other professional training continues to be well suited to my future career. Dr. Conn has been exceptionally supportive of my career goals to date, and I am optimistic that the training and mentorship I will receive from him and Dr. Lowen will allow me to reach my goal. My initial target following successful graduate work will be to secure a post-doctoral training position at a top research university in a lab working in the areas of innate immunity field but using approaches tangential from my current studies. Long term, I want to continue studying innate immunity with the goal that my research will lend insight into both basic cellular mechanisms and disease.

1. **Activities Planned Under this Award**

I am currently a second year in the BCDB graduate program at Emory University. As such, I have completed the majority of the required BCDB coursework, including the intensive, literature- and discussion-based course Foundations in BCDB I and II, Beginning Seminar, and research rotations in Year 1. I have also completed the Year 2 course “Hypothesis Design and Scientific Writing” in which I developed this proposal (see *Respective Contributions* for details). I also passed my written qualifying exam in April 2016. My remaining coursework and program-related activities include a Year 2 spring course on statistics for biology research and a semester-long teaching assistantship (as part of the formal LGS TATTO program). I am therefore currently focusing the majority of my time between research and coursework. I spent the summer prior to the fall semester of my second year writing up and publishing my methods paper with Dr. Lowen, and I have spent the fall semester obtaining preliminary data presented here and writing this proposal. I have also submitted an abstract for a poster presentation at the 14th Annual DSAC Student Research Symposium at Emory in January 2017. Importantly, both of my remaining Program obligations (biostatistics course and teaching assistantship) will be completed in the Spring 2017 semester before this NRSA F31 fellowship would be awarded. This will allow me to devote my time primarily to research and other related activities, including mentoring undergraduates or other junior lab members, preparing manuscripts, and attending local, national, or international conferences to present my work, as outlined in the Table below.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Grad Year**  **3** | **F31**  **Year**  1 | **Research\***  90 | **Data collection/ analysis**  75 | **Mentoring**  5 | **Manuscript/ thesis prep**  10 | **BCDB**  **Program Activities**  3 | **Seminars, Conferences, & Symposia**  7 |
| **4** | 2 | 90 | 65 | 15 | 10 | 3 | 7 |
| **5** | 3 | 90 | 55 | 5 | 30 | 3 | 7 |

\*Research time includes three specific activities: Data collection/ analysis, Mentoring, and Manuscript/ thesis prep. Therefore, the sum of these three categories equal the total percentage indicated for Research.

At Emory, I have the opportunity to attend and participate in many excellent seminars. I attend a weekly Advanced Seminar through the BCDB graduate program. Here, two students give half hour presentations on their research, and I am required to present once a year. I also participate in two monthly special interest seminars. The first is a Joint Structural Biology Groups meeting where one or more lab members from one of the Emory structural biology research groups gives a one-hour presentation on their project(s) and any problems or difficulties encountered using biophysical methods, including X-ray crystallography. This meeting involves four groups from the Department of Biochemistry (Drs. Conn, Dunham, Bo Liang, and Eric Ortlund) and others from across campus (e.g. Drs. Renhao Li and Elizabeth Wright from the Department of Pediatrics). The other special interest seminar series is the ‘Emory RNA club,’ which brings together labs with varied interests in RNA biology from both the School of Medicine (e.g. Drs. Conn, Dunham, and Daniel Reines from Biochemistry, and Gary Bassell from Cell Biology) and Emory College (e.g. Drs. Khalid Salaita and Anita Corbett from Chemistry and Biology, respectively). At these meetings, two students or postdocs each give a 30 minute presentation on their research, with an emphasis on diverse methods used related to RNA biology (e.g. structural biology, cell biology, fluorescence microscopy, and RNA-seq/ bioinformatic methods). Here, I am exposed to the many techniques used to study RNA outside the mostly *in vitro* structure-function assays I perform. I will continue to participate in local scientific seminar series and plan to attend both national and international conferences. Future conferences I would like to attend include: RNA Society, American Society for Biochemistry and Molecular Biology, Cold Spring Harbor, Keystone, and a Gordon Conference.

I also enjoy volunteering and contributing to the BCDB program and plan to maintain this level of involvement throughout my time at Emory. Currently, I am a “Student Director” for the first year Foundations course, an instructor for the Methods workshop, an editor for The Leading Edge BCDB Newsletter, and an organizer for the program’s Professionalization Workshops. I also helped to lead the organization of this year’s BCDB retreat (August 2016), and was asked to help organize recruitment for the 2017 admissions cycle. Being a part of these non-research activities will help me gain important transferable skills, like communication, organization, and collaboration that are essential for my professional development and are not achievable by exclusively working at the bench. It is important to me to give back to the program. I find these experiences incredibly valuable, and I want to contribute directly to future BCDB student’s personal and professional development.