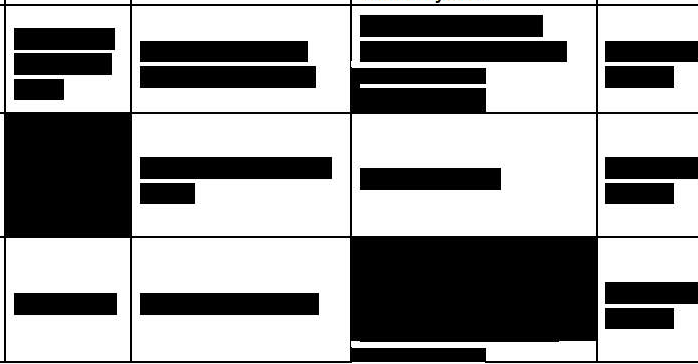
**Nicole Putnam, Ph.D., of Vanderbilt University**   
[**“The impact of innate immune recognition of Staphylococcus aureus on bone homeostasis and skeletal immunity”**](https://www.niaid.nih.gov/sites/default/files/nicoleputnamapplicationF31.pdf)

**Sponsor and Co-Sponsor Statements:**

**SPONSOR AND CO-SPONSOR STATEMENTS**

## 1. sponsor statement

1. **Research Support Available**



Dates

Award Amount annual

-

-

NIH/NIAID

Host-pathogen

6 K08 Al113107- 03 interactions during

osteomyelitis

James **E.**

Cassat

6/1/2014- $172,098

5/31/2019

Active

Principal Investigator

Title of Research

Identifying Number

Source

1. **Sponsor's Previous Fellows and Trainees**

After completing a residency in Pediatrics and a clinical fellowship in Pediatric Infectious Diseases in 2014, I joined faculty at Vanderbilt as an Assistant Professor on the physician-scientist tenure track in the Departments of Pediatrics and Pathology, Microbiology, and Immunology. My laboratory currently consists of two graduate students in the Microbiology and Immunology program, an MSTP student in the Biomedical Engineering program, and a co-sponsoredgraduate student in the Chemical and Biomolecular Engineering program. As a junior faculty member, I have no previously sponsored trainees. Therefore, to support Nicole Putnam during her graduate fellowship, two senior mentors will play key roles in the Training Plan. Dr. Eric Skaar serves as the mentor on my K08 award and will serve as the chair of Nicole's thesis committee. Dr. Skaar has trained 11 graduate students and 13 postdoctoral fellows, 6 of which now hold tenure track positions. Dr. Julie Sterling, a primary facultymember in the Vanderbilt Center for Bone Biology, will serve as Co-Sponsor (see below).

1. **Tra****ining Plan, Environment, Research Facilities**

**Icainio0 e1ao**

N icole joined the Vanderbilt Interdisciplinary Graduate Program in 2014 after completing undergraduate

studies at the University of Wisconsin-La Crosse and a Master of Science Degree in Molecular Microbiology and Immunology from the Johns Hopkins Bloomberg School of Public Health. Her master's thesis work was performed in the laboratory of Dr. Diane Griffin, an internationalleader in the fields of viral pathogenesis and T­ cell responses to viral pathogens. Nicole's project in the Griffin lab focused on characterzi ation of Th17 responses to measles infectionin non-humanprimates. This training experience provided strong foundational knowledge in innate and adaptive responses to human pathogens, and led to Nicole's desire to study microbial-host interactions during her doctoral training at Vanderbilt. I was thrilled when Nicole picked my laboratory for one of her rotations during the first year of graduate school. It was immediately clear that she was a talented and hard-working student, and that her prior research experiences positioned her to address fundamental questions in the pathogenesis of invasive infections. My laboratory studies host-pathogen interactions during osteomyelitis, a common and debilitating infection of bone most commonly caused by the human bacterial pathogen *Staphylococcus aureus.* Given her prior training in T-cell biology, Nicole immediately noted the field-changingobservation from the early 2000's that the canonical differentiaiton factor for bone­ resorbing cells, or osteoclasts, was a T-cell cytokine called RANK-ligand. She began to ask broad questions about how inflammation from invasive bacterial infections might perturb osteoclast biology, and the potential for osteo-immunologic crosstalk. She envisioned a project focused on understanding how S. *aureus* triggers

changes in osteoclast differentiation and behavior, and how innate and adaptive immune responses protect from, or contribute to, the pathogenesis of osteomyelitis. After completing her rotations, Nicole agreed to join my lab in 2015, where she has already had an enormous impact on my research program and on our understanding of how infectious and inflammatory stimuli impact bone homeostasis. We now propose a focused line of inquiry that will align her research and career goals as she completes doctoral training.

Although osteomyelitis is one of the most common manifestations of invasive bacterial infection, the pathogenesis remains poorly understood. Given the extreme morbidity and treatment recalcitrance associated with bone infections, there is an urgent need for new diagnostic approaches, antimicrobial treatments, and adjunctive therapies. The mechanisms by which bacterial pathogens invade, survive within, and ultimately trigger alterations in bone homeostasis are poorly understood. Moreover, very little is known about the immune responses to bone pathogens, largely do to the lack of genetically tractable animal models. Nicole therefore recognized the opportunity to accomplish two of her major career goals by training in my laboratory: to study an infectious disease with a drastic public health burden, and to conduct translational research on host- pathogen interactions. Shortly after joining my lab full time, Nicole made the exciting, and potentially groundbreaking, observation that *S. aureus* and other bacterial pathogens can trigger osteoclastogenesis independently of the canonical differentiation factor RANK-ligand. This finding suggests that bacterial factors can be sensed by skeletal progenitors, and that subsequent cellular responses can fundamentally alter bone cell behavior. In order to further elucidate the underlying mechanisms for bacterial-induced osteoclastogenesis, Nicole needed to incorporate a number of new assays into our lab. Of her many contributions to our research program, perhaps the most important was adopting all of the reagents and protocols necessary to study skeletal cell ontogeny and behavior in our lab. Since my training has been primarily in microbial pathogenesis, Nicole took the initiative to become the lab’s expert in bone biology. In order to gain the necessary expertise, she reached out to Dr. Julie Sterling, a founding member of the Vanderbilt Center for Bone Biology and co- sponsor for Nicole’s fellowship project (see co-sponsor statement below). Nicole will now unite her interests in host-pathogen interactions and skeletal cell biology to discover the microbial factors and host-pathways involved in altered osteoclastogenesis.

Nicole’s project has the potential to significantly impact human health by uncovering pathways that lead to bone destruction, thereby contributing to the morbidity of osteomyelitis and facilitating treatment failure. Importantly, in research occurring parallel with Nicole’s dissertation project, we are exploring new local drug delivery strategies to facilitate targeted therapy for bone diseases. This work will ensure that we are in a position to immediately capitalize on Nicole’s basic science discoveries, thereby fulfilling her career goal to participate and direct translational research efforts. Additionally, by studying the host signaling pathways that govern skeletal homeostasis in response to infectious and inflammatory cues, Nicole’s work is poised to reveal biologic responses that may impact other human diseases, such as rheumatologic, auto-inflammatory, and oncologic disease of bone.

I believe that my laboratory, the Department of Pathology, Microbiology, and Immunology, the Center for Bone Biology, and the Vanderbilt academic community as a whole offer the ideal environment for Nicole to complete her doctoral training and achieve her career goal of becoming a translational scientist in the field of host- pathogen interactions. Of particular importance to Nicole’s project are the outstanding core facilities at Vanderbilt, including a world-renowned imaging center – the Vanderbilt University Institute for Imaging Sciences (VUIIS). Our lab is uniquely positioned to study how bacterial pathogens impact bone remodeling in part because we have created a new genetically tractable animal model of osteomyelitis, and subsequently have adopted some of the outstanding imaging technologies available in the VUIIS. During her doctoral training, Nicole will learn these advanced imaging techniques for skeletal tissues, while also becoming proficient in a host of new skills that will empower her research on bone biology and ensure that she is well positioned for the next stage in her career. The Vanderbilt Center for Bone Biology provides exemplary resources for the study of bone pathology, and the inclusion of Dr. Sterling as a co-sponsor will allow Nicole to effectively bridge the fields of bone biology and infectious diseases to be positioned at the forefront of osteoimmunology research. Finally, as a new faculty member who has benefited enormously from outstanding mentoring, I recognize the paramount importance of establishing effective mentoring for Nicole. We have therefore carefully selected her thesis committee with experts in host-pathogen interactions, bone biology, immunology, and translational research. Nicole and I meet formally each week, and have daily discussions on her research and career development activities. The entire lab meets once weekly as a group, at which time we discuss research in progress, the responsible conduct of research, and essentials in public speaking and written science communication. To ensure that Nicole learns the skills necessary for critical review of

manuscripts and grant applications, she reviews 3-4 manuscripts per year under my guidance, and is expected to provide peer review of all funding applications submitted from the lab. Nicole also participates in a variety of seminars, interest groups, and career development programming across the institution. Collectively, these activities ensure that Nicole will have institutional support and mentoring to complete her research training.

###### Environment

Members of my laboratory participate in a number of meetings and seminars. Given Nicole’s focus on host- pathogen interactions and bone biology, we have carefully selected institutional activities to ensure that she is surrounded by colleagues that will enrich her training experience and facilitate her career goals. Weekly seminars include the Pathology, Microbiology, and Immunology Departmental Seminar, in which local and invited scientists present topics across the spectrum of host-pathogen interactions, and the Microbiology and Immunology Research In Progress series, in which pre- and postdoctoral trainees present their own work and receive critical feedback on their project. Twice monthly, Nicole will participate in the Microbial-Host Interactions meeting, in which investigators across multiple departments and divisions at Vanderbilt come together to discuss microbial pathogenesis. Once monthly, Nicole will attend the new Frontiers in Infection, Inflammation, and Immunity seminar series, where field-leading scientists come to Vanderbilt to present cutting edge research. Since Nicole envisions a career in translational science, she applied, and was subsequently accepted to, the prestigious Vanderbilt Program in Molecular Medicine (VPMM). The VPMM was established through funding from the Howard Hughes Medical Institute in 2010, with the overarching goal of training the next generation of translational scientists. VPMM training seeks to align basic science trainees with a clinical mentor so that they can experience the clinical challenges associated with the pathologies that they study. This experience has allowed Nicole to see patients suffering from osteomyelitis and other invasive staphylococcal diseases, and also to gain an appreciation for the challenges in diagnosis and treatment of these infections. In addition to these transformative clinical experiences, Nicole attends a bi-monthly VPMM seminar series and an annual retreat. I therefore feel confident that Nicole is in an outstanding environment to support her growth as a translational scientist. Finally, Vanderbilt offers an exciting array of career development opportunities through the Office of Biomedical Research Education and Training. Notably, this includes a “Career Connections” seminar series and symposium aimed at exposing trainees to the diverse opportunities for careers in the biomedical sciences, rather than simply assuming that all students will run an independent, NIH-funded laboratory. Because of these outstanding research and career development activities, I feel strongly that Vanderbilt is the ideal environment for Nicole to complete her training and achieve her research and career objectives.

###### Research Facilities

**Laboratory**: The Cassat laboratory, located in 1035B Light Hall, consists of 850 square feet of space and is sufficient to accommodate a team of 8-10 people. The lab contains capital equipment, biologicals, two tissue culture hoods, and supplies required for standard molecular biology and biochemistry techniques (see Equipment). Adjacent facilities with immediate access include a cold room and equipment corridor. The Cassat laboratory is designated as a Biohazard Safety Level 2 facility meaning that all equipment and resources are in place to work with the biohazardous agent described in this application. Furthermore, all members of the Cassat laboratory undergo extensive training prior to working with biohazardous material. We have worked closely with the Institutional Biosafety Officer at Vanderbilt to ensure that BSL-2 practices are maintained during all facets of this proposal.

**Computer**: The Cassat lab is equipped with 4 PC and 3 iMac computers, each with ample video processing and memory capabilities to support advanced imaging analysis. Each computer is connected to the Vanderbilt network providing direct access to current versions of Genebank, EMBL, and other protein/nucleic acid sequence databases. Each computer includes software for imaging analysis, advanced image editing and graphics production (Adobe Professional Suite), word processing (Microsoft Office Suite), statistical software (Prism) and genomic analysis tools (CLC bio). All computers in the Cassat lab are linked to a shared server that can be accessed from each computer ensuring that all data are backed up on tape. A networked, color laser printer is housed in the Cassat laboratory space.

**Equipment**: The Cassat laboratory has all the requisite equipment to study the molecular biology of microbial pathogens and skeletal cell biology. In regards to this proposal, the equipment includes a Thermo Sorvall Lynx 6000 superspeed centrifuge with general purpose and ultraspeed rotors, Thermo Sorvall Legend XTR benchtop centrifuge; Thermo Micro21 and 21R benchtop microcentrifuges, Locator Jr. Plus Cryo Vessel for liquid nitrogen cell storage, Nuaire Class II Type A2 Biosafety Cabinet (2 ea), Olympus inverted microscope CKX53 with QImaging OQCLR5 digital camera, Eppendorf Nexus Mastercycler Gradient Thermocycler, Mettler

Toledo Excellence balance, Thermo Forma series CO2 incubators (2 ea), New Brunswick Innova Model 44 stackable incubator shaker, Thermo MaxQ4450 tabletop shaking incubator, Fisher Isotemp General Microbiologic Incubators (3 ea), Isotemp bath incubators (3 ea), three variable speed rotating shakers, a BioTek Hybrid Synergy microplate reader, GeneSys 10S UV-VIS spectrophotometer, Next Advance Bullet Blender for bone homogenization, UVP GelDocIT Gel Documentation system, Thermo TSU series 600 -80°C freezer, Thermo IsoTemp -20°C freezer, Thermo MR49PA 4°C refrigerator, Mettler Toledo S220 pH meter, protein purification columns and reagents, BioRad polyacrylamide gel casting equipment and Western transfer apparatus, and a BioRad GenePulser Xcell electroporation apparatus. The Center for Small Animal Imaging (CSAI) in the VUIIS contains biomedical imaging instruments spanning a wide range of modalities, including MRI, CT, PET, SPECT, ultrasound, bioluminescence (BLI), fluorescence, and optical imaging. Equipment includes a 4.7T Varian MRI, 9.4T Varian MRI, 15.2T Bruker Biospec MRI, Xenogen IVIS 200 bioluminescent and fluorescent imaging system, Scanco μCT40 and μCT50 micro-CT scanners for *ex vivo* imaging, Siemens MicroCAT II X-ray micro-CT and Scanco VivaCT for *in vivo* imaging, 400Mhz vertical Bruker Avance III spectrometer for small molecule NMR, Siemens MicroPET Focus 220, Bioscan NanoSPECT SPECT/CT, CRI Maestro optical imaging systems for *in vivo* fluorescence, Visen FMT for quantitative optical tomography, and a VisualSonics high-resolution ultrasound system.

**Animal Facility.** Animals for this study will be procured through and housed in an ABSL-2 Animal Facility located adjacent to Light Hall in Medical Center North. The animal facility includes an animal suite with anesthesia machines and biohazard cabinets equipped for surgical procedures. The DAC provides procurement, husbandry and veterinary care services in support of research and teaching at VUMC. The DAC’s comprehensive preventative medicine and veterinary care program includes daily observation of animals (including weekends and holidays) by animal care, veterinary, and research staff.

###### Number of Fellows/Trainees to be Supervised During the Fellowship

My laboratory currently consists of 5 individuals (two graduate students, one MSTP student, a co-sponsored student in Biomolecular and Chemical Engineering, and a lab manager). A second MSTP student will join the lab full time in August of 2017. The laboratory manager oversees day-to-day operations of the lab such as animal husbandry, autoclaving, media preparation, dishwashing, and ordering, ensuring that Nicole can focus on her research project without undue administrative tasks. The presence of two other graduate students with complementary projects will allow Nicole to have ongoing intellectual stimulation and camaraderie in the lab as she completes her doctoral research. Nicole will also benefit from a number of colleagues at different stages in training within the labs comprising the Center for Bone Biology (see co-sponsor statement below).

###### Applicant's Qualifications and Potential for a Research Career

I have had the pleasure of working closely with Nicole for over two years now, and I can confidently say that she possesses all of the characteristics that portend success for a career in biomedical sciences. She is incredibly hard working, bright, curious, and resilient. She has a technological courage that many trainees at her stage lack, and this has greatly facilitated her growth in the fields of skeletal cell biology and osteoimmunology. She is an extremely effective teacher, having trained four rotation students in our laboratory, one of which ultimately decided to join our group full time. Nicole’s greatest attribute is her tenacity in acquiring new skills, including many experiences and techniques that are outside of my area of expertise. This has greatly benefited our research program, and Nicole has played a key role in bridging our strengths in microbial pathogenesis with established and emerging technologies in the field of bone biology. She is the perfect applicant for a Ruth L. Kirschstein Individual National Research Service Award Fellowship.

Looking back on Nicole’s accomplishments prior to joining my lab, it was already evident that she had a bright future as a young scientist. In her Master’s thesis work, Nicole made fundamental observations regarding how Th17 and Tc17 cells respond to wildtype measles virus in nonhuman primates, and how measles vaccine impacts adaptive immune responses. This work is detailed in a first-author manuscript that is currently under revision, and a middle author paper in the *Journal of Virology*. Because of her outstanding undergraduate and Master’s thesis scholarship, Nicole was accepted into the Interdisciplinary Graduate Program at Vanderbilt in 2014. During her four research rotations, her incredible work ethic and productivity were readily apparent, and her work in one of these two-month rotations resulted in a manuscript in the *American Journal of Transplantation*. After joining my lab in 2015, Nicole quickly established several *in vitro* models of skeletal cell differentiation and function. Her preliminary data allowed for the successful publication of two additional manuscripts in the journals *PLoS Pathogens* and *Antimicrobial Agents and Chemotherapy*. In total, Nicole is

now included as an author on five publications, a remarkable accomplishment given that we have yet to publish the exciting preliminary data forming the foundation of this fellowship application.

At present, Nicole has completed all graduate coursework and successfully passed her dissertation candidacy exam. She has assembled an outstanding thesis committee, with experts from all facets of her project: microbiology, immunology, and skeletal cell biology. Her induction into the highly prestigious Vanderbilt Program in Molecular Medicine will ensure that her research project remains grounded in the most important clinically relevant questions, and that she is receiving targeted support towards her goal of a career in translational science. In the coming years Dr. Sterling and I will work closely together, and in concert with her thesis committee, to ensure that we continue to develop Nicole's mentoring skills, to fortify her written and oral scientific communication, and to nurture the leadership skills that she is already displaying. I anticipate that Nicole will write a first-authored paper describing her exciting preliminary data on bacterial-induced osteoclastogenesis in the next year. I envision that this work will lead to fundamental shifts in how we view bone homeostasis in the presence of both pathogenic and commensal organisms. As you will see from her enclosed letters of recommendation, Nicole is truly a special student. I am very fortunate to have her in my laboratory, and I support her NRSA fellowship application with the highest possible enthusiasm.

## co-sponsor statement

* 1. **Research Support Available**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Source | Identifying Number | Title of Research | Principal Investigator | Dates | Award  Amount (annual) |
| Active | Department  of Veterans Affairs | 1101BX001957-01 | Strategies for early  treatment of bone metastases | Julie A.  Sterling | 7/1/2013-  6/30/2017 | $237,399 |
| Active | NIH/NCI | 1R01CA163499-  01A1 | The Role of Mechanotransduction in Progression of  Tumor-induced Bone Disease | Julie A. Sterling, Scott A. Guelcher | 9/1/2012-  6/30/2017 | $205,424 |
| Active | NIH/NIAMS | 1R01 AR064772-  01A1 | Biofilm Dispersive Bone Grafts to Improve Healing of  Contaminated Fractures | Scott A.  Guelcher | 4/1/2014-  3/31/2019 | $315,539 |
| Active | Department of Defense  / CDMRP | BC141789 | Targeted Drug Nanocarriers for Inhibiting Bone  Metastatic Breast Cancer | Julie A.  Sterling | 9/30/2015-  9/16/2018 | $119,468 |
|  |  |  | |
|  | | | |

* 1. **Co-Sponsor's Previous Fellows and Trainees**



During my 8 years at Vanderbilt, I have had the opportunity to train 9 graduate students and one post-doctoral fellow. Many of these trainees are still in my laboratory, however, those that have completed training have secured advanced positions in the biomedical sciences. Five representative prior trainees are listed below:

|  |  |  |
| --- | --- | --- |
| **Trainee** | **Position and tenure in Sterling lab** | **Current institution and position** |
| Nazanin Ruppender | Graduate Student (2007-2011) | Teaching faculty, North Seattle College |
| Rachelle Johnson | Graduate Student (2008-2011) | Assistant Professor, Vanderbilt University |
| Sabrina Danilin | Postdoctoral Fellow (2009-2011) | Research Scientist, University of Strasbourg |
| Shellese Cannonier | Graduate Student (2012-2016) | Analyst at Proactive Worldwide |
| Ushashi Dadwal | Graduate Student (2012-2016) | Postdoctoral Fellow, IUPUI {Shankar Lab) |

###### Training Plan, Environment, Research Facilities Training Plan

I am thrilled to serve as a Co-Sponsor for Nicole Putnam in her application to the Ruth L. Kirschstein Individual National Research Service Award Fellowship Program. I first met Nicole about two years ago when she and Dr. Cassat approached my lab to learn new techniques in the study of skeletal cells. Our lab focuses on the skeletal and inflammatory events surrounding tumor-induced bone disease, with a specific interest in cancer metastases. Since the Cassat lab is interested in studying how inflammation during osteomyelitis triggers changes in bone biology, I felt that our labs could have considerable synergy moving forward. As someone who has trained in bone cell biology, I can say without hesitation that Nicole’s project is very unique and exciting, and is poised to move the emerging field of osteoimmunology forward. Nicole’s observation that bacterial factors may induce osteoclastogenesis independently of canonical differentiation factors is potentially groundbreaking, and could have enormous ramifications not only for bone infections, but also more generally for our understanding of how bone cells respond to inflammation and microbes.

Our lab has considerable expertise in the isolation, differentiation, and characterization of primary skeletal cells, and we are happy to share all relevant reagents and protocols with Nicole and the rest of the Cassat lab. Dr. Cassat and I will coordinate our mentorship of Nicole, meeting with her formally as a group at least once monthly, and individually at least once weekly. Nicole will present her data at our lab meetings and at research in progress meetings in the Vanderbilt Center for Bone Biology (VCBB). She will have ample interactions not only with members of my laboratory, but also with the numerous other trainees of primary investigators in the VCBB. It is my expectation that these experiences, coupled with the Training Plan that Dr. Cassat has outlined above, will allow Nicole to emerge from her dissertation training as an expert not only in host-pathogen interactions, but also in bone biology. Collectively, this skillset will position Nicole to achieve her career goal of becoming a translational scientist that bridges infectious diseases and cell biology.

###### Environment

My laboratory is part of the Vanderbilt Center for Bone Biology (VCBB), located two floors above the Cassat laboratory. The VCBB was created to investigate diseases of bone and mineral metabolism, and primary investigators associated with the Center study embryonic bone development, neuroskeletal biology, biomechanics, fracture repair, osteoporosis, bone infections, and cancers such as breast cancer and prostate cancer, which frequently affect the skeleton. Dr. Cassat and I are primary faculty members of the Center and expect that Nicole will benefit greatly from the collegial interactions with experts in skeletal cell biology. Additionally, the Center has a number of specialized resources for the study of skeletal biology, including the recent addition a full-time bone histotechnologist.

###### Equipment

In addition to standard equipment for molecular biology, the VCBB shared laboratory includes the following specialized equipment related to Nicole’s project – mammalian surgical area, ultraturax homogenizer, Miltenyi MidiMACS manual separator, cell culture hoods and incubators, automated Bio-Rad cell counter, sonicators, cryotanks, two real-time qPCR instruments (ABI), Bio-Rad CF x 96 qRT-PCR thermal cyclers, DNA and protein gel migration apparatus, equipment for soft and calcified tissue histology, a Wehmer plastic embedding grinder, 3 Leica RM2255 microtomes, an Olympus microscope with fluorescence and high-resolution camera, a computer with the histomorphometry analysis software inclucing Metamorph, Bioquant, andOsteomeasure.

###### Number of Fellows/Trainees to be Supervised During the Fellowship

My laboratory currently consists of three graduate students and a lab coordinator. The graduate students are working on projects in cancer biology, focusing on the molecular events and inflammatory pathways that promote bony metastasis. The presence of three graduate students at different stages of training and all working on bone cell biology will offer Nicole a unique perspective as she continues her work on osteomyelitis.

###### Applicant's Qualifications and Potential for a Research Career

To echo Dr. Cassat’s statements above, Nicole is an outstanding student and has a very bright future as a translational scientist. I have been very impressed with her work ethic, her creative approaches to defining host responses to bone infection, and her ability to articulate both her research findings and her career goals. She has a very unique, interdisciplinary project, and I commend her for her efforts to bridge two diverse fields to answer fundamental questions about osteo-immunologic crosstalk. I am confident that the training plan we have outlined, the resources from my lab and the VCBB, and her thesis committee will serve as strong foundation for her continued growth as a scientist. I am absolutely thrilled to support her NRSA application.

**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)

**Sponsor and Co-Sponsor Statements:**

#### Sponsor Section and Statement

* 1. **Research Support Available**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Source | Application ID | Project Number | Title of Program | Principal Investigator | FY  Start | FY  End | Amount |
| NIA | 8842072 | 5R21AG045734-  02 | LONGEVITY EXTENSION AND IMMUNE FUNCTION  IN AGING (R21) | Nikolich-Zugich, Janko | 2015 | 2017 | 177,449 |
| NIA | 9060874 | 5R01AG048021-  03 | IMPACT OF CMV UPON T-CELL AGING AND  IMMUNE DEFENSE | Nikolich-Zugich, Janko | 2016 | 2019 | 456,185 |

* 1. **Sponsor’s Previous Fellows/Trainees**

With regard to this I have trained and mentored 11 doctoral students (10 graduated with Ph.D., one with M.Sc) and 20 postdoctoral trainees, and am currently training/mentoring one doctoral student, 2 postdoctoral trainees and 5 junior faculty/clinical fellows with K or R awards. Of these, 11 former postdoctoral trainees hold faculty or senior industry positions, and of 10 former Ph.D. students, two are in faculty positions and 6 have continued postdoctoral education. Below is a sample of five representative former trainees:

|  |  |  |
| --- | --- | --- |
| Name | Position in Nikolich Laboratory | Current Position and Institution |
| Kristin Renkema | Predoctoral | Postdoctoral Fellow, University of Minnesota |
| Nicholas Fox, M.Sc. | Predoctoral | Research Associate,  Columbia University, New York, NY |
| Jason L. Pugh | Predoctoral | Postdoctoral Fellow, Stanford University |
| Emily Goldberg | Predoctoral | Postdoctoral Fellow, Yale University |
| Vesna Pulko, Ph.D. | Postdoctoral | Senior Scientist, Roche, Zurich, Switzerland |

* 1. **Training Plan, Environment, Research Facilities.**

**Training Plan**

Formal coursework at the University of Arizona for Nico will encompass his required and elective courses towards his Ph.D. in Immunobiology. These courses include general cell and molecular biology courses: MCB 595E Topics in Research, IMSD (2 units); MCB 528L Microbial Genetics Lab (2 units); MCB 528R Microbial Genetics (3 units); MCB 568 Nucleic Acids (4 units); MCB 516A Statistics Bioinformatics and Genomic Analysis (3 units), and IMB 565 Principles and Molecular Mechanisms of Microbial Diseases (3 units), all of which are in progress and will be over by the end of Spring, 2015; and IMB 564 Advanced Topics (3 units); IMB 521 Scientific Writing (2 units); MCB 695E Science, Society and Ethics (1 unit) that he will take in

the Fall 2015; and the Seminar Series Course IMB 595A (1 unit) / IMB 695A (1 unit) that run throughout his studies until graduation. Nico has already completed with high marks two courses: MCB 577 Principles of Cell Biology (4 units) and IMB 548 Basic Immunological Concepts (3 units),

To complement this with specific knowledge in the Biology of Aging, he will be attending the ArizonaMed (Medical Student Curriculum at the University of Arizona) classes devoted to aging within the Life Cycle block, including the Biology of Aging; Aging and Cancer; and a series of translational and clinical lectures on delirium, dementia and other pertinent issues in gerontology and geriatrics.

Nico participates in the Seminar Series course (IMB595/695), which, amongst other activities, requires him to present his work once a year to an audience composed of faculty and trainees from the Department of Immunobiology, the Arizona Center on Aging and other departments and centers with interest in immunobiology and aging, including, but not limited to, Departments of Molecular & Cellular Biology, Cellular & Molecular Medicine, Pharmacology, Physiology, Bioengineering and others. Critical thinking, clarity of presentation, presentation style, content and density are all evaluated by his peers and teachers. Moreover, that same venue brings about top immunologists, many of whom are interested in aging. In the past year alone, we have had Drs. Charles Surh (Immune homeostasis in aging); Michael Diamond (Immunity to flaviviruses with aging), Elias Haddad (Innate immune defects with aging). This past spring semester, we also focused on proteins in the aging process and together with Departments of Molecular and Cell Biology and Cellular and Molecular Medicine, we hosted Drs. Ana Maria Cuervo (proteostasis and aging) and Rick Morimoto (Protein integrity during the aging process). We plan to continue this in the upcoming season with visits by Dr Vojo Deretic (Autophagy in immunity and aging); Shannon Turley (Genentech, Inc.; aging of secondary lymphoid organs) and Derek Huffman (Einstein; aging and metabolism).

#### Environment.

The “Advances in Aging” lecture series at the University of Arizona, as well as the “Frontiers in Medical Research” are two of the main venues that bring extramural researchers working on biology of aging to Tucson. Nico will continue to be active in attending the seminars and meeting the speakers, which, over the past few years, included Drs David Hammerman, John Burton, Kevin High, Jeremy Walston, Daniel Goldstein, Randy Strong, Richard Besdine, Richard A. Miller, William Hall, Vishwa Deep Dixit and others.

Furthermore, Nico is now a member of the American Aging Association and will be attending the Annual Meeting in June 2017, and yearly thereafter; he will further be encouraged to attend the annual GSA meeting. He will be expected to present his work either in a Poster or Oral Presentation format for every meeting he attends.

Nico will be applying to attend the NIA Advanced Course on the Biology of Aging, held by the Nathan Shock Centers, probably slightly later in his graduate career (4th year). This course would be supremely useful to his development, albeit we realize that the preference for attendance is usually given to advanced postdoctoral fellows and junior faculty. In addition, if the Molecular Biology of Aging course is repeated during his Ph.D. training (MBL, Woods Hole, MA), he will apply to attend.

Nico is a graduate student in my laboratory and is part of our regular laboratory meetings that are entirely devoted to the immunology of aging, every Friday for 1.5h; moreover, we meet regularly to review his direct progress and results every other week for one hour at minimum. During the period of manuscript preparation, preparation for committee meetings or presentations (departmental Research-In-Progress, University, College or extra mural poster or oral presentation) that frequency is increased to up to several times a week, until we both feel that Nico is ready to present his work externally. Over and above that in Spring 2015 we established Nico’s Thesis Committee. There will be regular meetings (2-3/year) of Nico’s committee, which will oversee his progress toward the Ph.D. thesis.

To further illustrate the environment, I would like to provide a brief overview: I am Professor and Head, Department of Immunobiology, and co-Director of the Arizona Center on Aging. I am leading or am part several large collaborative studies on immune aging, using rodent, Rhesus macaques (RM) and/or human models –a N01 contract to evaluate vulnerability to the West Nile virus in elderly; a N01 contract, based in Hiroshima, Japan, involving five US groups and 12 Japanese groups to evaluate the impact of radiation upon immune aging; and an immediately past collaborative transatlantic grant to connect immune aging studies between human and rodent model (with Prof. Arne Akbar). Furthermore, I have just finished a collaborative grant to

investigate rebalancing of T-cell pools by immune modulation and rejuvenation in aging primates (with Prof. Louis Picker), and am awaiting review of a P01 application submitted to NIA, to understand and correct molecular basis of thymic involution and peripheral T cell maintenance failure in aging (with Profs. Ellen Richie

– MD Anderson; Lauren Ehrlich – Univ. of Texas, Austin; Nancy Manley – Univ. of Georgia; Marcel van den Brink – Sloan-Kettering and Charles Surh, Postech Institute). My entire group is heavily involved in studies of immune senescence, and we are engaged in several multidisciplinary studies with colleagues investigating cellular stress mTOR and aging (with Drs. Andrew Capaldi, UA Dept of. Molecular and Cellular Biology), nuclear 3-D organization in the course of aging and CMV infection (Drs Giovanni Bosco, Dartmouth Univ., and Felicia Goodrum, the UA BIO5 Institute), mTOR inhibition in metabolic, neurosensory and immunological aging (Drs. Theodore Price – UT Southwestern, Heddwen Brooks-UA, Sourav Ghosh - Yale, Kirsten Limesand – UA, John Konhilas – UA - Depts. Of Pharmacology, Physiology, Cell Biol. and Anatomy), and the ability of thermography to predict propensity for pressure ulcer in frail and resilient older adults (Drs. David Armstrong, Manish Bharara, Mindy Fain and Jane Mohler, Dept. of Surgery and the Arizona Center on Aging). Nico will be thoroughly exposed to all these scientific influences and contents. There are initiatives in microbiome and aging centered in our department and the Arizona Center on Aging, and we have a Biology of Aging Research Interest Group, encompassing about 35 scientists across campus. Nico will attend quarterly meetings of that group too.

#### Research Facilities

Laboratory space of 2,000 sq. ft. equipped with benches, desks, sinks, water, pressurized air, vacuum, etc. on the second floor of the Medical Research Building (MRB) of the University of Arizona College of Medicine. Additional dedicated tissue culture space of 300 sq. ft. is available adjacent to the laboratory space. Space for 3,500 mice and 30 rats is available to the investigator in the main vivarium (Building 201), as well as in the shared basement between the MRB and Keating buildings. That includes dedicated ABSL-2 and ABSL-3 areas for containment work with pathogens. All facilities are AALAAC accredited and staffed with full-time veterinarians and animal support staff. Core facilities: Transmission and scanning electron microscopes are available in the 4th floor of LSN building on a fee-for-service-basis. A facility for automated DNA sequencing and oligonucleotide synthesis is available on a fee-for-service-basis in the Keating building adjacent to MRB. Protein sequencing and mass spectrophotometry analysis are available at the College of Pharmacy.

Microarray and proteomic state-of-the-art facilities within the Arizona Research Labs are located in the adjacent Keating building and are available at a fee-for-service basis. Flow cytometry sorting core facilities are available both in the MRB building and at the Arizona Cancer Center across the street. Arizona Cancer Center also houses the Cobalt source animal and cell irradiator. Other state-of-the-art core facilities including, but not limited to, the Transgenic Animal Immunohistochemistry and Animal Health Core are available on campus. In addition, the following equipment is available directly in the laboratory: CO2 incubators, phase microscopes, inverted microscopes, centrifuges, PCR machines (including real time), a gel imaging system, tissue culture hoods and freezers (-80 C and -20 C) are present in the laboratory. Shared gamma and beta counters, DNA sequencers (ABI 3100 is available in our lab) and ultracentrifuges are on our floor. Available in this building are spectrophotometers, HPLC

#### Number of Fellows/Trainees to be Supervised During the Fellowship

Two, in addition to Nico:

Heather Thompson, Postdoctoral Fellow Mladen Jergovic, Postdoctoral Fellow

#### Applicant’s Qualifications and Potential for a Research Career

Nico Contreras is an exceptional candidate for this Fellowship. He came to my laboratory following successful undergraduate and masters studies, the latter accomplished in a bacteriology/engineering laboratory, under the tutelage of Dr Linda Powers. He was therefore more mature than an average, out-of- undergrad Ph.D. student. He distinguished himself quickly, both by the ability to assimilate complex concepts and experimental constructs and by the ability to put that knowledge to work when thinking about and designing experiments.

Moreover, he quickly came up with a well-defined set of experimental interests, that were unusually mature and shaped for an early-stage graduate student. He focused on the intersection of immunity, metabolism and aging, with a specific aim to address obesity in the context of these three themes. He started working on our collaborative projects with Drs Luigi Fontana (Washington University) and Valter Longo (University of Southern California) addressing effects of calorie restriction or its modifications upon the function of immune system and the overall physiology of the organism. He also bridged the mouse and human experimental models very nicely in the course of his initial training in my lab, providing him a perspective for translational work.

However, the most telling predictor of Nico’s potential and future success is the fact that he came up with a research project that required little to no input from me. His ideas were highly original and he synthesized them nicely into the present proposal. He assembled the reagents, methods and support to address a question whether and to what extent direct infection of the adipose tissue with a persistent virus (cytomegalovirus, CMV) can impact inflammation, immunity and metabolic dysfunction, particularly if evaluated over the lifespan and into aging. I have just presented his preliminary data at the FASEB Science Research Conference in Big Sky, Montana, on Aug. 3, 2016, and was greeted by significant and strong enthusiasm of the audience, that lauded this new line of investigation.

Nico is a good experimentalist already, and has already grown in that regard by leaps and bounds every semester. He is an adept organizer, highly resourceful and able to mobilize his laboratory colleagues to help him when needed with complex harvests and organ processing. He is also eager to lend a hand himself and help others. He covers the literature well and explores novel approaches appropriately. He is not shy about introducing new techniques, and already possesses an impressive skill set. Finally, he wrote the application with only cursory input from myself, demonstrating considerable thought and writing skills.

I therefore view Nico as a future leader in academic medicine and research, addressing highly pertinent health care issue (obesity, metabolic syndrome and type 2 diabetes) in the context of aging. This area remains woefully underexplored and poorly understood and his original ideas and the ability to perform well controlled experiments will undoubtedly be yielding high returns in the immediate, intermediate and long-term future. He is an exceptionally well qualified and well suited candidate for the individual predoctoral research fellowship (F31).

**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)

**Sponsor and Co-Sponsor Statements:**

# SPONSOR AND CO-SPONSOR STATEMENT

### RESEARCH SUPPORT AVAILABLE

|  |  |  |  |  |  |  |  |  |  |  |  |  |
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|  | **Source** |  |  | **Identifier** |  |  | **Title** | **PI** |  | **Dates** | **Direct $/ yr** |  |
|  |  |  |  |  |  |  | **Current-Conn (Sponsor)** |  |  |  |  |  |
|  | NIH/ NIAID |  |  | R01 AI088025-07 |  |  | RNA modification and antibiotic resistance | Conn |  | 6/1/10-  5/30/20 | $304,000 |  |
|  |  |  |  |  |  |  | **Pending-Conn (Sponsor)** |  |  |  |  |  |
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| **Current-Lowen (Co-sponsor)** | | | | | | | | | | | |  |
|  | NIH/ NIAID |  |  | R01 AI099000 |  |  | Reassortment of influenza viruses  in a co-infected host | Lowen |  | 9/1/12-  8/31/17 | $250,000 |  |
|  | NIH/ NIAID |  |  | R01 AI125268 |  |  | Impact of selective genome  packaging on influenza A virus  reassortment | Lowen |  | 8/4/16-  7/31/20 | $250,000 |  |
|  | NIH/ NIAID |  |  | R01 AI127799 |  |  | Host dependence of influenza A  virus reassortment | Lowen |  | 12/5/16-  11/30/21 | $493,000 |  |
|  | NIH |  |  | HHSN2722014  00004C |  |  | Host adaptation, reassortment  and transmission of influenza  viruses at the animal-human  interface | Orenstein  (Lowen,  Project 2  Co-leader) |  | 4/1/14-  3/31/21 | $259,462 |  |

* 1. **SPONSOR’S/ CO-SPONSOR’S PREVIOUS FELLOWS/ TRAINEES Conn (Sponsor)**
* **Eight (8) previous** and **three (3) current** pre-doctoral trainees, including the applicant Ms. Schwartz.
* **Six (6) previous** postdoctoral trainees.

### Five representative previous trainees:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Name** | **Pre-/ Postdoctoral** | | **Current Position** | | | |
| Miloje Savic | Pre (Manchester)/ (Emory) | Post | Senior Advisor on Global Pharmaceutical Policy, Norwegian Institute of Public Health, Norway. | | | |
| Catherine Templeton | Pre (Manchester, | PhD) | Senior | Scientific Officer, Inst. for Cancer Research, | | UK |
| Ahmed Wahid | Pre (Manchester, | PhD) | Associate Prof., Biochemistry, Minia University, | | Egypt | |
| Emily Kuiper | Pre (Emory BCDB | program) | Postdoctoral Fellow, Dana Farber Cancer Institute | | | |
| Pooja M. Desai | Post (Emory) | | Research Scientist, Therapure Biopharma Inc., Canada | | | |

**Lowen (Co-sponsor)**

* **Two (2) previous** and **two (2) current** pre-doctoral trainees.
* **One (1) current** postdoctoral trainee.

### Previous trainees:

|  |  |  |
| --- | --- | --- |
| **Name** | **Pre-/ Postdoctoral** | **Current Position** |
| Jillian Seladi-Schulman | Pre (Emory MMG program) | Associate Lab Project Set-up Manager, Quintiles, Atlanta |
| Nicolle Marshall-Baird | Pre (Emory MMG program) | Postdoctoral Fellow, CDC Influenza Division |

* 1. **TRAINING PLAN, ENVIRONMENT AND RESEARCH FACILITIES**

**C1. TRAINING PLAN.** Samantha (Sam) Schwartz is a student in the Biochemistry, Cell & Developmental Biology (BCDB) Graduate Program at Emory University. Sam will be co-mentored by Dr. Graeme L. Conn (Associate Prof., Department of Biochemistry) and Dr. Anice Lowen (Assistant Prof., Department of Microbiology & Immunology). Dr. Conn has many years of mentoring experience and a research focus on understanding the structures, interactions and biological functions of RNA molecules and their protein binding partners. Relevant to Sam’s proposal, Dr. Conn’s group has a well-established record in the area of viral non-coding RNAs and their interactions with innate immune proteins, PKR and OAS1. Dr. Lowen will additionally bring a distinct perspective to Sam’s mentoring and career guidance as well as outstanding expertise in molecular virology and the cell culture systems Sam will use in her proposal.

**Sponsor Background.** I (Dr. Conn) place a great emphasis on student mentorship and development in my lab, and I have been actively engaged in graduate education since arriving at Emory in 2008 (please see my Biosketch Personal Statement for details). I am currently a member of two Emory Graduate programs, BCDB and Microbiology & Molecular Genetics (MMG), as well as the NIAID-funded T32 “*Antimicrobial Resistance and Therapeutic Discovery Training Program (ART-DTP).*” I currently serve on the Executive Committees of each of these programs, as the BCDB Program Director of Graduate Studies (DGS), and as one of four MMG Program student recruiters (along with Sam’s Co-sponsor Dr. Lowen). I am also actively involved in directing and teaching courses for each of these programs. In total, I have mentored 11 PhD students and served on the thesis committees of an additional 17 Emory graduate students. My lab currently consists of three graduate students (including Sam), and one senior (PhD level) Research Specialist Lead (technical staff). I am currently in the process of recruiting a postdoctoral fellow to my lab to work on our antibiotic-resistance rRNA methyltransferase project. In addition to the 8 PhD students who have graduated from my lab, I have mentored and trained 5 former postdoctoral researchers who have gone on to successful positions in academia and industry (e.g. see Table above). Through this experience I have developed, and try to continue to evolve, a broad perspective on training and mentorship. I recognize that each of my lab members may require a style of mentorship specifically tailored to them and I continually assess each individual’s strengths, weaknesses, expectations and ambitions.

**Co-Sponsor Background.** Dr. Lowen is similarly engaged in graduate education having been an active member of the MMG and Immunology and Molecular Pathogenesis (IMP) programs since joining Emory in 2011. Dr. Lowen teaches in “*Principles of Basic Biomedical and Biological Sciences*” and co-directs the “*Virology”* course required for students of both the MMG and IMP program, and which Sam will either audit or take as an elective to strengthen her fundamental virology knowledge. Dr. Lowen also serves on the MMG recruitment committee, regularly attends seminars hosted by both of her programs, and is currently a member of eight thesis committees. The Lowen lab currently comprises two PhD students, one post-doc, two senior technicians (Research Specialist Senior) and a more junior Research Specialist. The lab is expanding due to new funding and Dr. Lowen expects to take on an additional student and post-doc in the next year. Since starting her lab in 2011, Dr. Lowen has graduated two PhD students who are now employed in related fields (see Table above). She strives to provide constructive mentorship and scientific direction while leaving trainees enough independence to develop critical thinking and creativity. Dr. Lowen meets regularly with her trainees to discuss progress and plans, and is available for input as needed.

**Overview of Training Objectives.** My current trainees are engaged in projects investigating ribosomal RNA methyltransferase enzymes that confer bacterial resistance to antibiotics, or viral/ cellular non-coding RNA structure and activity against proteins of the human host cell antiviral response (PKR and OAS1). Sam’s project represents an important new direction in the latter area. My plans with Dr. Lowen to support Sam’s technical, professional and other training away from the bench are outlined in the following sections.

**Training in Diverse New Experimental Approaches.** When Sam began her thesis work my lab last summer she chose to develop a new direction in our studies on OAS1 which had begun a couple of years earlier (see Vachon, V.K. *et al. Nucleic Acids Res.* 2015). Specifically, Sam decided to devise a project to investigate the molecular basis for an unexpected observation, made by former graduate student Dr. Ginny Vachon, that very small changes in a model dsRNA used for prior structural studies of the OAS1-dsRNA complex could either substantially increase OAS1 activation or completely eliminate it. Sam’s project will employ diverse approaches that will provide new training in biochemical (OAS1 enzyme kinetics), structural (x-ray crystallography) and other biophysical approaches (BLI and HDX-MS) to tease apart new details of OAS1 regulation by RNA. The use of cell culture approaches in Dr. Lowen’s lab will further broaden Sam’s technical training while also providing greater biological context to increase the impact of her findings.

**Plan for Mentorship, Professional Development, & Opportunities for Scientific Networking.** Sam is part of a dynamic, interactive and collaborative group of researchers studying various aspects of RNA biology and translational control using many complementary biochemical, microbiological and biophysical techniques. Sam’s primary day-to-day interactions will be with me, Dr. Lowen, and the current members of our research groups. We also collaborate closely with Dr. Christine Dunham’s group in Biochemistry, with whom we have weekly joint lab meetings; Dr. Lowen’s group similarly works closely with that of Dr. John Steel in Microbiology & Immunology. In addition to the technical training and individual mentorship Sam will receive in my laboratory and that of her Co-sponsor Dr. Lowen, Sam’s project will bring a wealth of opportunities to interact with and learn from a diverse collection of scientists in the Emory University School of Medicine and more broadly across the Emory campus and its healthcare system.

*One-on-one meetings*: My office is located adjacent to the lab and one door down from our shared student/ postdoc office; as far as possible, my door is “open” for informal discussions. Sam is very proactive in seeking my input (and that of Dr. Lowen) on her experimental plans, the subsequent interpretation of her results, and decisions on the next priorities. We typically meet briefly in my office or in the lab to discuss the latest developments at least 3-4 times a week. Should I feel such interactions are too infrequent, during periods of particular challenge, or when greater input might be needed (e.g. finalizing a manuscript), I typically schedule individual meetings (at least once a week) to give these one-on-one discussions additional structure. In Sam’s case, our meetings have largely been informal but through the Fall semester regular scheduled meetings helped us keep on track with my feedback on her weekly assignments for the intensive IBS522r *Scientific Writing and Hypothesis Design* course. Sam was also a willing volunteer to pilot the use of an electronic notebook in my lab, using “Findings” (<http://findingsapp.com/>), allowing us to share protocols and experimental results via Emory Box (an Emory-wide implementation of the Box.net file sharing and collaboration tool). This approach has allowed me to track progress with experiments, offer immediate input on record keeping, analysis, interpretation and, most importantly, prepare for our regular discussions with greater depth of insight. Dr. Lowen will also continue to be available to address Sam’s questions and discuss her project as needed, and will also begin meeting with Sam regularly once she reaches the stage of her project that relies on use of cell culture systems. Though I expect Sam to coordinate these interactions with Dr. Lowen, I plan to participate in any regular scheduledmeetings.

*Weekly laboratory meetings*: My lab holds weekly joint meetings with Dr. Christine Dunham’s group who have related research interests in translation control, including bacterial toxin-antitoxin systems, ribosomal frameshifting, and ribosome quality control. At these meetings, one member of each lab will typically present either their latest results or a relevant paper from the recent literature. We also use these meetings as a forum for students and postdocs to practice formal presentations of a more complete “story”,

e.g. prior to a conference talk or using the figures from anin prep manuscript to get group feedback. Dr. Lowen (and potentially members of her group) will attend the Conn/ Dunham lab meetings when Sam is presenting her work. Similarly, Dr. Lowen will include Sam on the regular presentation schedule for her group’s lab meetings which are held jointly with Dr. Steel’s group. Sam will be a regular participant in the Lowen/ Steel meetings and I will also attend when Sam is presenting.

*Presentations*: I encourage my lab members to present their research early and often in order to hone their presentation skills. Sam has already benefited from the BCDB Program’s focus on improving presentation skills through the Year 1 Introductory Seminar class, and is scheduled to present her first BCDB “Advanced Seminar” on Feb. 15th 2017. Ahead of this presentation, I will work closely with Sam, as will all my students and postdocs, to ensure her presentation is clear, polished and successful in making its main points. As noted above, Sam will also be scheduled to present her talk at least a week before in our weekly lab meeting. I also expect that Sam will present her work extensively at local journal clubs, the monthly Joint Structural Biology Groups Meeting and Emory RNA Club, and the annual Emory Graduate Symposium organized by the Graduate Division Advisory Council (DSAC). Sam will present a poster at the next DSAC Symposium in January 2017 and my expectation is that Sam submit an abstract for this event each year and will request a podium presentation in the second and/ or third year of this fellowship. Dr. Lowen and I will also strongly encourage Sam to attend and present her work at local, e.g. the Southeastern Regional Virology Conference (SERVC), and national scientific meetings (see below). Sam attended SERVC held near Emory in April 2016 while rotating in the Lowen lab and this is an excellent forum for students to present their research in a workshopsetting.

*Scientific Conferences and Meetings*: I will encourage Sam to attend at least one scientific meeting per year to present her results and to develop a network of interactions with senior colleagues and peers at other institutes. Specific conferences for Sam to target will be discussed ~6-9 months prior to planned attendance but over the course of her time in my lab will include both more focused meetings, like the GRC on Nucleic Acids or a Keystone meeting on Innate Immunity, and broader conferences such as the annual meetings of the RNA Society, American Society for Microbiology (ASM) or American Society for Biochemistry and Molecular Biology (Experimental Biology). A first priority for Sam will be attendance at a meeting of broader scope to immerse herself in her new field. As a goal of Sam’s technical training is to

learn x-ray crystallography, I will also encourage Sam to attend an intensive training course in macromolecular crystallography and at least one conference related to this technique. Several courses on x- ray data collection and/or structure determination are held annually at US synchrotron sources and elsewhere, and I will encourage Sam to apply once she has fully embarked on that aspect of her project. Upon completing the second aim of her proposal I would encourage Sam to present her structures and complementary binding and protein dynamics (HDX-MS) data at the national meeting of the American Crystallographic Association. In addition to lab funds, the Laney Graduate School provides “Professional Development Support (PDS)” funds (up to per student, potentially more on a competitive basis) to support conference attendance for students to present their work (poster or talk is required). Dr. Lowen and I will also guide Sam in the preparation of applications for travel awards and other meeting fellowships to maximize the range of her conference attendance and experiences throughout her graduatecareer.

*Thesis Committee*: Sam has assembled a thesis committee with diverse expertise to help guide her research and oversee her progress towards her career goals. In addition to Dr. Lowen and I, the committee comprises three Biochemistry Department faculty, Drs. Christine Dunham, Rick Kahn, and Daniel Reines. As noted above, Dr. Dunham brings relevant expertise in structural biology, protein synthesis and RNA- protein interactions. Dr. Reines was one of Sam’s BCDB rotation mentors and brings complementary expertise in RNA biology and protein structure related to transcriptional control. Dr. Kahn is a biochemist and cell biologist with expertise in cell signaling/ regulation by GTP-binding proteins, membrane traffic, cell division, and energy metabolism. Sam’s thesis committee will meet for 1-2 hours every six months as dictated by the BCDB program and she will meet individually with members of her committee as needed.

*Publications*: All students in my laboratory are required to complete an independent project leading to first author publications. When Sam is nearing the point of preparing her first manuscript, I will assist her in developing a framework for the paper as she prepares draft figures (a process that may identify any “gaps” in the data). Next, Sam will write each section in turn to prepare a complete first draft: Methods, Results, Discussion, Introduction and finally the Abstract. In my lab we typically share manuscripts via OneDrive (for sharing and team editing), which allows me to track progress and offer quick comments as each section develops. Once Sam completes a first draft I will provide detailed feedback-usually I do this as handwritten comments on a printed copy. I will then sit down with her and discuss my comments and explain why I made specific suggestions. My experience is that students may go through several drafts before a final product is reached; this can be a time-consuming process (at least for the first manuscript), but one that I believe is critical arming my students with effective skills for scientific writing.

*Mentoring as a component of training*: Later in her graduate career I plan to provide Sam with several opportunities to mentor undergraduates (we typically host one or two each summer), rotation or other junior graduate students, to gain skills as a mentor. My approach to this, as with previous trainees, will be to have an initial discussion with Sam and her mentee about the overall project goals and the timeframe, the general experimental plan, and the specific details of the first experiments. After this, I will allow Sam the freedom to train and mentor the student directly. I expect that Sam will be the first person her mentee will seek input from, e.g. on experimental details or later for reviews of abstracts, poster drafts, etc. However, I will schedule regular meetings with Sam and her mentee to ensure things are progressing well. At the end of the student’s time in the lab, I will discuss the outcomes with Sam and ask her to prepare a reference letter for the student on which I will provide feedback to help her develop this essentialskill.

### C2. ENVIRONMENT

**Biochemistry, Cell & Developmental Biology (BCDB) Program.** Sam is a member of the BCDB program and will continue to follow the innovative BCDB curriculum (please see *Institutional Environment and Commitment to Training*). Sam has completed most requirements for Years 1-2 (a Spring semester in biostatistics and teaching assistantship remain). Beyond Year 2, coursework centers mostly on honing presentation and other professional skills, through courses such as BCDB Advanced Seminar and Ethics in Research. Sam will also attend a twice-monthly BCDB journal club, monthly Professionalization Workshops covering topics such as Individual Development Plans (IDP) and time management, and an annual scientific and team building retreat with other students and many of the Program faculty.

**Biochemistry Department.** The Conn lab is part of the Biochemistry Department in the Emory University School of Medicine so Sam is also affiliated with the Department. Additional groups Sam will have opportunities to interact with (in addition to those already noted above) include those of Drs. Eric Ortlund (protein structure, protein-DNA/RNA interactions, X-ray crystallography), and Bo Liang (viral RNA polymerase structure, high-resolution cryo-EM). The Department hosts a weekly seminar series and students are invited to have lunch with the speakers. I encourage my students to participate in these

meetings and I expect that Sam will have opportunities to meet with a seminar speaker 2-3 times/ semester. **Microbiology & Immunology Department.** The Lowen lab is part of the Microbiology & Immunology Department, located one floor below Biochemistry. Virology is a strength at Emory and Sam will have the opportunity to interact with trainees and PIs from the Steel, Speck, Mocarski, Ahmed, Suthar, Hunter and Grakoui laboratories, to name a few. Dr. Lowen is a member of the Emory-UGA Center of Excellence for Influenza Research and Surveillance (CEIRS), which offers a number of opportunities for trainees to present their work, meet virologists, and travel to other institutions to learn pertinenttechniques.

**Emory as an Outstanding Training Environment.** Emory has an extremely collaborative research environment and many of our current studies rely on the expertise of colleagues. For our students, this collaborative environment creates a unique working environment where they can draw on the expertise of many individuals in the absence of formal training relationships. The interdisciplinary nature of the GDBBS graduate programs also enhances our research environment creating interactions and bonds between College departments (such as Biology and Chemistry) and those in the School of Medicine. Thus, students have the opportunity to broaden their training and learn new approaches from experienced experts.

As noted above, the Graduate Division holds an annual student symposium providing opportunities for poster and oral research presentations. I require that my students submit an abstract each year and Sam will begin participating in January 2017. Various additional seminar series are available that will provide Sam with opportunities to become familiar with diverse techniques, biological systems and biomedical problems. These include: 1) *Emory RNA Club*, a bimonthly seminar series that brings together diverse research groups using biochemical, cellular, genetic, developmental, chemical biological and structural biological techniques that focus on understanding the emerging world of RNA biology. More than 10 research groups at Emory are involved in this series that will provide an excellent forum for Sam to present her research and hone her presentation skills for a diverse audience. 2) *Structural Biology Joint Group Meeting*, a monthly meeting involving groups with expertise in x-ray crystallography (Drs. Conn, Dunham, and Ortlund), cryo-EM and tomography (Drs. Liang and Elizabeth Wright), and other biophysical approaches such as HDX-MS (Dr. Renhao Li). In both cases, students and postdocs give presentations on their work or recent literature in an informal setting where discussion is strongly encouraged.

**Relationship of Training to Applicant’s Career Goals.** Sam’s immediate goal after completing her PhD is to secure a postdoctoral position at a top research university to continue on the path to a career as an independent researcher. My goal in training Sam is to provide the laboratory setting, atmosphere and continuous mentorship and feedback that will allow her to develop the technical and professional skills necessary to accomplish this goal. Sam’s project will give her experience of experimental design and interpretation, and essential technical skills in protein expression/ purification of RNA, biochemical assays of enzyme activity, cell culture, and biophysical approaches to study protein structure, dynamics and interactions. I am also committed to ensuring Sam is trained as broadly as possible in all other essential professional skills, including scientific writing (e.g. manuscripts, grants), presentations (posters, talks) and in mentorship. Our efforts will be complemented by the comprehensive BCDB programmatic activities and the outstanding intellectual environment at Emory. Taken together, all the requisite resources are in place to promote Sam’s development as an independent research leader and an outstanding scientist.

### C3. RESEARCH FACILITIES

The Conn and Lowen laboratories, Departments of Biochemistry and Microbiology and Immunology, and Emory core facilities will provide everything Sam needs to complete the experiments described in her proposal (please see *Facilities and Resources* and *Equipment* sections for more detail). Briefly, the Conn lab has dedicated spaces for bacterial culture (for protein expression, etc), radioisotope use, and macromolecular crystallography; a main lab space where each member has their own dedicated bench; and, is well equipped for protein and RNA preparation/purification and *in vitro* biochemical/ structural studies of OAS1 regulation by dsRNA. Of particular relevance for Sam’s cell culture experiments, the Lowen lab has three Class II biosafety cabinets, four CO2 incubators and a light microscope for cell culture work, and is also well equipped with quantitative PCR instrumentation. The Department of Biochemistry has modern x- ray crystallography suite with crystallization robotics and x-ray generator with sample mounting robot, and also provides regular scheduled time at the SER-CAT beamline at the Advanced Photon Source. These outstanding resources and regular availability of synchrotron beamtime at APS will expedite Sam’s research program and enhance her training in x-ray crystallography. Finally, Sam’s project will make use of equipment in two Emory core facilities (for HDX-MS and BLI analyses); it is important to note that both facilities require thorough user training from the core but will subsequently allow Sam to independently design, run and analyze the results of her experiments under their expert guidance.

### NUMBER OF TRAINEES

In addition to Sam, I currently advise two other pre-doctoral trainees and a PhD-level technician. I expect one student to graduate by summer 2017 and my lab is open to a new student for their thesis research. I am also actively recruiting a new postdoctoral fellow. Over my time at Emory, my group has typically had 5- 8 members (graduate students, postdocs and technicians) plus 1-2 undergrads or other visiting scientists. Dr. Lowen currently advises two pre-doctoral trainees and one postdoctoral fellow. Her lab has consisted of

~4 members since its inception in 2011, but is currently transitioning to a larger group; Dr. Lowen plans to take one additional student and aims to recruit a postdoctoral fellow or staff scientist within the next year.

### APPLICANT’S QUALIFICATIONS AND POTENTIAL FOR A RESEARCHCAREER

**Sponsor (Conn)**: Sam is smart, driven, and a careful and already highly competent experimentalist. These qualities, among many others, point to her outstanding potential as a future research leader. Not all these abilities are innate, some were built on hard work over 3 years as a research technician; some of them she now continues to develop in grad school. As Sam describes elsewhere, being a first generation college student and completing her undergrad degree at an institute with limited research opportunities, she took the (very mature) decision to gain further research experience before committing to grad school. As a result, I have in effect recruited Sam to my lab twice, each time with very different goals. When Sam applied for the open Research Specialist position in my lab in 2012, her CV made my short list but I had reservations both about her limited research experience and some “features” of her undergrad transcript. Needless to say, the interview convinced me she was the right choice for my lab. Two main factors contributed to this decision. The first was Sam’s clear objective to gain research experience with graduate school the future goal (something I felt my lab and I were well placed to help her achieve). Second, was how Sam dealt with my list of standard “lab tech” questions, gleaned from many co-interviews with experienced HR professionals, as mandated in Manchester for all tech hires. While I had unknowingly always found myself going too deep into specific research experiences or future career goals, these questions were simple, direct, and, to my initial surprise, *highly* discriminating. These included asking about task prioritization or how conflicts should be handled, to the seeming simple question, “*If I asked you to make a 1M solution of NaCl, what would you need to know?*” which completely throws >90% of my interviewees. How about one who got an F grade in Organic Chemistry? As with everything else I threw at her from my repertoire, Sam aced the answers (I suppose I should have known, since she retook the class and got an A!). I was completely convinced and Sam was a stellar technician. What about her potential for grad school and beyond? As Sam began to develop her innate scientific curiosity to complement her excellent experimental hands, I knew it was time she left. I encouraged Sam to explore broadly and she did (choosing an interview at UTSW over waiting on an invitation to the Emory BCDB event the same weekend). Having seen the BCDB Program up close already, she chose to stay at Emory (I didn’t blame her). Next, I insisted she explore other labs during her rotations. While she ultimately chose to return to a new project in my lab, she did so with a new perspective on her abilities, interests, and future goals and an outstanding co-mentor in Dr. Lowen! I cannot over state how different I view Sam’s future grad experience and training in my lab (and my role in that training) from what she has already experienced and accomplished. What I know is that I am fortunate to have an amazingly talented student in my lab who is destined for great things in her future career in research.

**Co-Sponsor (Lowen)**: During her rotation in my lab, Sam demonstrated excellent technical ability, maturity, organizational skills and an ideal balance of independence and willingness to ask for help. Sam’s impressive productivity during her 10-week rotation is evidenced by her publication on the use of ddPCR to detect and quantify defective viral genomes. Sam is the only author on this paper with me because she did all the experiments, analyzed the data and, with my guidance, interpreted the data and wrote the first draft of the paper. She accomplished all of this because she was thoughtful in her experimental design, ensuring that all necessary pilot experiments had been run and all reagents were in good condition before starting a larger series of experiments. Sam had very few, if any, failed experiments. Sam is also an excellent communicator: she reported her results regularly without me having to prompt her, she came to my door or set up meetings as needed to discuss future plans, and she worked very well with other group members. Although in my lab only briefly, Sam became our expert on ddPCR, which was new to us at the time of Sam’s rotation. I therefore asked her to write up a protocol and to train others to use the instrumentation and software; she took these tasks on willingly and did a great job. Sam was also a lot of fun to have in the lab because she is very enthusiastic about her research and science in general. She is ambitious, willing to work hard to achieve her goals, and has excellent potential for a successful career in research.