**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)

**Facilities and Other Resources:**

###### FACILITIES AND OTHER RESOURCES

The Cassat laboratory studies host-pathogen interactions during invasive bacterial infection, with a special emphasis on understanding how infection and inflammation perturb musculoskeletal cell biology. We have developed a variety of tools to understand how bacteria and inflammation trigger changes in bone biology, including a murine model of *Staphylococcus aureus* osteomyelitis, high-resolution 3D imaging of bone remodeling, and cell culture models of osteoblast and osteoclast differentiation and function. Institutionally, Vanderbilt has supported the acquisition of preliminary data for my research proposal with small pilot funds from the Vanderbilt Center for Microbial Pathogenesis (VCMP) and the Vanderbilt Institute for Clinical and Translational Research (VICTR). Physical resources have been provided primarily from the Vanderbilt Center for Bone Biology (VCBB), Vanderbilt University Institute for Imaging Sciences (VUIIS), and the Vanderbilt Program in Molecular Medicine (VPMM). At the juxtaposition of my project are the fields of bone biology, microbial pathogenesis, infectious diseases, and immunology. Intellectual rapport has been provided by experts in each of these areas for the development on my research proposal, with the following members serving on my thesis committee: Dr. Eric Skaar, Professor of Pathology, Microbiology, and Immunology (PMI) and Director of the Division of Molecular Pathogenesis, serves as the chair to my thesis committee and provides insight on the microbial pathogenesis of *S. aureus*. Expertise in bone biology continues to be provided through close communication with Dr. Julie Sterling, Assistant Professor in Medicine, Clinical Pharmacology, and Cancer Biology, who is co-sponsoring this application. Dr. Isaac Thomsen, Assistant Professor in Pediatric Infectious Diseases, is my clinical mentor through VPMM and he continues to provide advice on the clinical ramifications of invasive musculoskeletal diseases, ensuring that my research questions are both important and relevant. And finally, scientific counsel regarding immunology has been guided by the expertise of Drs. Dan Moore, Assistant Professor in Pediatrics and PMI, and Jeff Rathmell, Professor of Cancer Biology and PMI and Director of the Center for Immunobiology. *In sum, Vanderbilt University has been the ideal environment to study a unique host-pathogen interaction in an invasive infection model, with access to core facilities for state-of-the-art technology that provide assistance in data acquisition and analysis, and successful collaborations between experts in departments and centers at Vanderbilt University Medical Center and Vanderbilt University.*

###### Institution

Vanderbilt University Medical Center is located between the undergraduate and Peabody campuses and has approximately 7.2 million square feet of building space. The close proximity of Vanderbilt University and Vanderbilt Medical Center promotes interactions, sharing of resources, and collaboration. Biomedical research at Vanderbilt has long been recognized for its contributions to the advancement of medicine. The School of Medicine claims two Nobel Laureates, Earl W. Sutherland Jr., in 1971, for his discover y of the metabolic regulating compound cyclic AMP, and Stanley Cohen, in 1986, for his discovery (with a colleague) of epidermal growth factor. The Medical School’s reputation for outstanding research is reflected in the amount of federal and private support it receives. Because of the creativity of the faculty, the School of Medicine ranks among the nation’s top 10 medical schools for NIH funding, with VUMC research funding from all sources having more than doubled since 2001. Support for competitive research grants from all external sources has grown continually to more than $675 million as of fiscal year 2015.

###### Biomedical Research Education and Training (BRET) Office at Vanderbilt

The BRET Office is located on the 3rd floor of Light Hall, just seven floors below the Cassat lab space. Established in 1999, the BRET office coordinates graduate education, postdoctoral training, career development, and training programs. In addition, the BRET Office manages the NIH-funded Broadening Experiences in Scientific Training (BEST) grant awarded in 2013 to fund the Vanderbilt Augmenting Scholar Preparation and Integration with Research-Related Endeavors (ASPIRE) program. The ASPIRE program provides resources to trainees in the form of yearly progress reports through an Individual Development Plan (IDP), “Career Connections” seminar series, an annual career symposium, externship programs, and didactic modules. The BRET Office has been beneficial to me personally by providing the IDP resource to outline my scientific progress and goals as a graduate student through yearly iterations, by providing training through the annual Responsible Conduct of Research (RCR) sessions and the Vanderbilt Program in Molecular Medicine (see description below), and by sponsoring the annual career symposium which I attended in 2016.

###### Facilities

* 1. **Research Laboratory Space**

The Cassat Laboratory (Room 1035) in Medical Research Building IV consists of 850 square feet of laboratory space that encompasses three bays and two tissue culture hood rooms, additional hall space where freezers for biologic and chemical storage and large equipment are located, and adjacent facilities that include access to a cold room, autoclave, and dish washing amenities. The main laboratory contains equipment necessary to conduct all standard cell biology, microbiology, and molecular biology techniques. The Cassat lab has cell culture hoods and incubators that are individually dedicated for primary cell use or bacterial use. There are 4 members in the laboratory (2 Ph.D. students, 1 M.D./Ph.D. student, and 1 research technician), each with expertise in molecular biology of microbial pathogens and host-pathogen interactions.

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 agents described in this application. Furthermore, all members of the Cassat laboratory undergo extensive training prior to working with biohazardous material. I have completed all the necessary training to work in a Biosafety Level 2 laboratory. *These facilities were specifically designed and equipped to support research at the crossroads of microbiology and bone biology, which is the cornerstone of the proposed research.*

###### Individual Space

I have an individual desk and bench space located within the main laboratory in 1035 MRB IV. Dr. Cassat’s office is just off of the larger lab area and this close vicinity permits significant daily interactions between Dr. Cassat and myself. My personal bench space is furnished with equipment that allows me to complete experiments efficiently. This includes pipettes, reagents, and tools. The desk space is directly adjacent to my bench and includes a computer, office supplies, and filing cabinets. *These facilities ensure that I will have the necessary space in which to formulate experiments, analyze results, and prepare manuscripts for publication.*

###### Computer

My desk space is equipped with an iMac powered by Mac OS X Yosemite, high-speed internet access, software for image editing and analysis (FIJI), word processing and data analysis (Microsoft Office, GraphPad Prism 6.0), a reference and citation manager (ReadCube), and access to networked printers and a shared server. The shared server allows data generated or edited on any computer within the lab to be immediately accessible with this personal computer. There are two additional shared computers in the laboratory, with one connected to a BioTek plate reader with Gen5.0 software, a UVP GelDoc-It2 imager with UVP software, and an Olympus microscope and Q-imaging camera with Q-Capture Pro 7 software. *The combination of these information technologies contributes to the potential for success by assuring efficient data management and processing, and optimal communication among members of the research team.*

###### Animal Facility

All proposed activities using vertebrate animals at VUMC and VU are reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and the U.S. Department of Agriculture. The facility has a standing Animal Care Committee, which must review and approve all animal research protocols prior to their initialization. In addition to providing continuing protocol review in accordance with the Animal Welfare Act Regulations, the IACUC also establishes policies (e.g., acceptable mouse cage density, food/water regulation) and reviews, semi-annually, the animal care program and all animal housing and study areas. The Office of Animal Welfare Association (OAWA) provides administrative support to the IACUC with a staff of three Protocol Analysts, two Post Approval Monitors, a Veterinarian Reviewer, and an OAWA Training Coordinator.

All personnel listed on IACUC protocols must complete required training modules and pass the corresponding tests contained within each module. I have completed Animal Biohazard Safety Level 2 (ABSL-

1. training, meaning that all equipment, resources, and training are in place to work with biohazardous agents, while protecting yourself and others. To ensure that I remain compliant with any new ABSL-2 or IACUC regulations, I will continue to attend ABSL-2 refresher training courses annually.

The Division of Animal Care (DAC) provides procurement, husbandry and veterinary care services in support of research at VUMC and VU. 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 staffs. Animals identified as ill or injured during daily rounds are given a thorough physical examination. Treatment is prescribed as deemed appropriate by a veterinarian in consultation with researc h personnel. Veterinarians and veterinary technicians communicate directly with each other and with investigative staff regarding diagnostic requests and initiation of, or changes in, treatment. In addition, all treatments are documented in the animal’s individual record. Records are maintained by the veterinary/research staff and "closed out" upon resolution.

Animals for this study will be procured through and housed in a BSL-2 Animal Facility located in Medical Center North. Our laboratory’s designated animal space in suite A-8223 includes a mouse breeding facility that remains free of infectious agents, a housing room and a procedure room, both for hazardous agent use, which each include two biohazard cabinets to ensure sterility during surgical procedures and are equipped with anesthesia machines and euthanasia systems. Each room provides sterile food, water and cages for the breeding and infections immunocompromised mice. All animal procedures and harvests will be conducted in the A-8223 suite to ensure safety and sterility. An online reservation system for biosafety cabinets confirms that equipment is available when necessary. *Success of the proposed research is critically dependent on the maintenance and husbandry of mice. ABSL-2 facilities as described above are critical for conducting infections and dissections with sterility and safety in mind.*

###### Institutional Resources and Cores

* 1. **The Vanderbilt University Institute of Imaging Science (VUIIS)**

VUIIS is a University-wide interdisciplinary research initiative to develop new imaging technology based on advances in physics, engineering, and computer science. In addition to high-field MRI and MR spectroscopy, ultrasound, optical and other modalities in human subjects, the VUIIS offers state-of-the-art options for small animal imaging in all modalities. In 2007 Vanderbilt completed a four-floor facility adjacent to Medical Center North to house the VUIIS. The $28 million project ($21 million for construction) provides a 42,000-square-foot facility to integrate current activities in imaging research and provide research space for 42 faculty members and more than 80 graduate students and postdoctoral fellows in biomedical science, engineering, and physics.

The Center for Small Animal Imaging (CSAI) within the VUIIS is dedicated to research studies of small animals for a variety of applications. The CSAI is a comprehensive resource for advanced biomedical imaging instruments spanning a wide range of modalities, including MRI, X-ray/X-ray CT, PET, SPECT, ultrasound, bioluminescence and fluorescence. The center provides training for users on optical imaging, and provides expert support/training for data/image analysis. In particular, I have been trained on the Scanco microCT50, for high resolution 3D X-ray computed tomography of bone, with training and assistance provided by Dr. Dan Perrien (see letter of support). *Support and expertise from VUIIS faculty have been valuable in the development of analysis programs for computed tomography of femurs from our model, providing an exceptional ability to precisely calculate changes in bone remodeling. VUIIS will continue to be essential as we incorporate new models and research strategies.*

###### The Vanderbilt Center for Bone Biology (VCBB)

The VCBB facility was created to investigate diseases of bone and mineral metabolism. Investigators associated with VCBB 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. Major goals of the VCBB are to unravel novel biological mechanisms and to develop new treatments and diagnostic tools that can eventually change the quality of life for patients with bone diseases. The VCBB sponsors a weekly seminar in which investigators present preliminary data, describe new techniques, and develop multi-disciplinary collaborations between bioengineering, cancer biology, endocrinology, rheumatology, and infectious disease researchers.

The VCBB facility has multiple high-tech instruments to perform molecular, cellular and biochemical studies, and to precisely quantify changes in bone volume, architecture, biomechanical properties and histology upon gene alterations, growth, aging, disease or pharmacologic treatments. Given that Dr. Cassat is

a primary faculty member in the VCBB, I will have access to and training on this specialized instrumentation. Additionally, the VCBB recruited an expert in bone histology (Josh Johnson) in 2016 as the head of the newly formed Bone Histology core (see letter of support). *The VCBB facility has been a vital resource to our lab emerging in the bone biology field and will provide technical support for completion of studies in the proposal. Additionally, the expertise of Julie Sterling in VCBB and her co-sponsorship for this fellowship is important for our continued progress in acquisition of new skills in the study of bone biology and for accessing necessary equipment to conduct histological analyses.*

###### Vanderbilt Center for Microbial Pathogenesis

The Center for Microbial Pathogenesis within the Department of Pathology, Microbiology, and Immunology was created to address the growing need for new therapeutics to treat infectious diseases. Dr. Eric Skaar is the Director, and his goal for the center is bring together researchers in both basic and translational research to establish a community of investigators interested in discovery and therapeutic development. By combining Vanderbilt’s strengths in both basic and clinical science, the center is poised to make numerous exciting discoveries in infection biology, and leverage these advances to develop new drugs to treat a variety of infectious diseases.

The Center for Microbial Pathogenesis presents $2,000 Mini-Sabbatical awards to two Ph.D. students each year to establish collaboration with other Vanderbilt investigators. I was the recip ient for a 2015-2016 award to work with Dr. Julie Sterling in the Vanderbilt Center for Bone Biology and learn bone histology techniques. *The Vanderbilt Center for Microbial Pathogenesis will continue to facilitate our studies on the host- pathogen interface in bone, and we will continue to work closely with Dr. Eric Skaar, who is the head of my Ph.D. Thesis Committee (see letter of reference).*

###### Vanderbilt Institute for Clinical and Translational Research (VICTR)

VICTR provides a Voucher and Pilot Program that offers awards for the generation of preliminary data and pilot work for clinical and translational studies, allowing for rapid acquisition of proof-of-concept initiatives that might justify full-scale investigation. Applications are accepted on a rolling basis for review and all researchers are eligible to apply. Submitted projects are required to meet the following criteria: clinical and translational research, meaning it involves human subjects, human tissue, human cell lines, and/or human information; hypothesis driven with specific research question; and appropriately powered to obtain preliminary data. I received VICTR voucher funding in 2015 as the principal investigator to examine the role of myeloid cell recognition of TLR agonists and subsequent NFκB activation. *We have successfully utilized VICTR as a resource for the funding of preliminary data in experiments utilizing human cells. VICTR vouchers may be used to acquire additional pilot funds to bridge any discoveries made in a mouse model with this research proposal into human cells or samples.*

###### Vanderbilt Program in Molecular Medicine (VPMM)

VPMM is a unique training program available to graduate students and postdoctoral fellows in the Biomedical Sciences at Vanderbilt University, allowing for integration of thesis work with relevant clinical experiences. This program provides a supervised experience in clinical and translational research, including didactic and experiential courses, seminars and individual experiences conducted under the guidance of a clinical mentor and basic science mentor. My application into this program was accepted in 2015, when I was paired with a clinical mentor, Dr. Isaac Thomsen, Assistant Professor and Physician in Pediatric Infectious Diseases (see letter of reference). The VPMM has provided me with real-world observations of patients with severe musculoskeletal infections under supervision in clinic by Dr. Thomsen and observation of orthopedic surgical procedures with Dr. Jonathon Schoenecker at the Monroe Carell Jr. Children’s Hospital at Vanderbilt. I have used these experiences to reflect back on my basic science research on osteomyelitis in the Cassat lab. *VPMM has offered me opportunities and training to connect my work as a basic scientist to clinically relevant patient cases at Vanderbilt. My passion for scientific discovery lies in translational research, and this perspective will remain valuable during the research pursuits as outlined in my research strategy.*

###### Additional Core Services

Although I do not anticipate using other Core services for the studies outlined in this application, Vanderbilt is equipped with many additional core facilities. A complete listing can be found at the following link: <http://www.vanderbilt.edu/oor/cores/>

**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)

**Facilities and Other Resources:**

**Facilities and Other Equipment.** During this proposed work I will have access to personal cubicle space in the Medical Research Building at the University of Arizona. In addition to the personal area I will also have access to the BSL-2 shared laboratory space (J. NIkolich-Zugich) 2,000 sq. ft. equipped with benches, sinks, water, pressurized air, vacuum, etc. in the Medical Research Building at the University of Arizona College of Medicine. Dedicated tissue culture space of 300 sq. ft. is adjacent to the lab. Equipment includes CO2 incubators, phase and inverted microscopes, spectrophotometers, centrifuges, PCR machines (including real time), a gel imaging system, a custom made 4-laser/26-color BD-Fortessa analyzer, DNA sequencers (ABI 3100), ultracentrifuges, tissue culture hoods, and freezers (-80 and -20 C).

**Flow cytometry analysis.** Data acquisition will be performed on a custom-made, four-laser BD Fortessa flow cytometer (Becton Dickinson, Sunnyvale, CA), and analyzed using FlowJo software (Tree Star, Inc., Ashland, OR). Further, rainbow calibration beads will be run within all experiments to allow for cross comparison of studies over time

**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)

**Facilities and Other Resources:**

### FACILITIES AND OTHER RESOURCES INTELLECTUAL ENVIRONMENT:

I am well positioned to take advantage of the many outstanding intellectual resources Emory has to offer. In the Conn and Lowen labs, I will work directly alongside graduate students from several programs (including BCDB, Immunology and Molecular Pathogenesis (IMP), and Microbiology and Molecular Genetics (MMG)) as well as postdocs and other lab personnel with diverse backgrounds. Lab meetings, BCDB program- sponsored events, research group meetings (e.g. Structural Biology and Emory RNA Club), and other Emory or local symposia will afford me many opportunities to seek important critical feedback on my work and enrich my intellectual and professional development.

### PHYSICAL RESOURCES:

As described below, my Sponsor’s and Co-sponsor’s labs have the physical resources necessary for me to complete my proposed research (please also see *Equipment* for description of Conn and Lowen lab equipment, as well as Departmental shared equipment and Emory core facilities). Additionally, both labs have sufficient funding to provide me with any additional reagents/small equipment I will need.

### Laboratory:

**Dr. Conn’s** research group occupies ~1250 sq. ft. of laboratory space on the fourth floor of the Rollins Research Center on the Emory University School of Medicine Clifton Road campus. I have designated bench space, complete with ample storage, and my own set of four pipetman and small equipment (microfuge, vortex, etc.). The laboratory is divided into four main rooms for organizational and containment purposes: the larger main lab space (where each lab member has their own bench), and three small labs for microbiology work (bacterial culture for protein expression, etc.), a hot lab for all radioisotope work, and crystallography area, including a stereo microscope and storage space for crystallization screens (three dedicated crystallization incubators). The laboratory is equipped with standard wet benches, certified fume hood, sinks, utilities, deionized water, etc. for experimental biochemistry, biophysics, and molecular and structural biology research. The Conn laboratory is **approved for Biosafety Level 2 (BSL-2)** work and has currently approved biosafety and radioisotope protocols. I also have completed all of the required safety training established by the Environmental Health and Safety Office, including research laboratory safety, biosafety, and radiation safety. Adjacent shared laboratory space is also available in common laboratories, computer rooms, darkrooms, temperature-controlled environments (-20, 4, 30 and 37 ˚C), libraries, and meeting areas. The Conn laboratory has (or has immediate access to) all of the essential equipment required for the proposed work (see *Equipment* for further details).

**Dr. Lowen’s** research group occupies 850 sq. ft. of recently renovated laboratory space on the third floor of the Rollins Research Center. The laboratory is equipped with standard wet benches, a certified fume hood, sink, utilities, deionized water, etc. and includes an adjoining room dedicated for cell culture. Additionally, Dr. Lowen has 50% use of a 100 sq. ft. cold room and 10% use of a freezer room. All equipment required is housed within the laboratory. The Lowen laboratory is **approved for Biosafety Level 2 (BSL-2)** work with current biosafety approvals.

### Clinical:

N/A

### Animal:

N/A

### Computer:

I have an Apple MacBook Pro loaded with the Microsoft Office suite, GraphPad Prism, Adobe Illustrator and Photoshop, Endnote, and Findings electronic notebook software. I have wireless Internet access throughout the lab and office spaces, as well as access to a departmental server with a backup system. The laboratory and office are each supplied with networked computers and printers. In addition to my personal computer, a shared facility on the ground floor of the Rollins Research Center building adjacent to the X-ray laboratory has an extensive molecular graphics area in which the Conn Laboratory has dedicated space for their existing computer workstations (2 x Apple Mac OSX) for data processing, model building, and refinement.

### Office:

I have access to ~200 sq. ft. of shared office space for Conn lab postdocs, students, and staff. Drs. Conn and Lowen each have ~200 sq. ft. of office space with enough room to meet with students and staff located adjacent to their respective labs.

### INSTITUTIONAL SUPPORT:

**X-ray Crystallographic Facilities**. The Department of Biochemistry maintains an ~150 sq. ft. state-of-the- art automated crystallization and ‘in-house’ X-ray data collection facility to support the research programs of four structural biology groups (see *Equipment* for details).

***Synchrotron (Advanced Photon Source, APS)*:** Emory is a member of SER-CAT (Southeast Regional Collaborative Access Team) at the Advanced Photon Source (APS), providing 12 days ID (undulator insertion device) beamtime and 12 days of BM (bending magnet) beamtime per year to the Biochemistry X- ray crystallography groups. Further details are provided in the *Equipment* section.

**Emory Core Facilities**. This proposal will use facilities within several Emory centers and core facilities: ***(1)*** The *Emory Chemical Biology Discovery Center* (<http://www.pharm.emory.edu/ECBDC/capabilities.html>) houses ‘hands-on’ equipment for quantifying molecular interactions using label-free methods, including a FortéBIO OctetRED384 bio-layer interferometry instrument (see attached letters). The ECBDC is located on the fourth floor of the Whitehead Research building, which is connected directly by internal bridge walkway on each floor to the Rollins Research Center (<1 minute walk from our lab). ***(2)*** The *Emory Integrated Genomics Core* (EIGC, <http://cores.emory.edu/eigc/>) houses instrumentation we will use for analysis of extracted ribosomal RNA to assess RNase L activity in transfected and/ or virus infected cells. The EIGC is located in a Clinical Laboratory Improvement Amendments (CLIA) certified (CLIA ID:11D1086150) laboratory located on the seventh floor of the Woodruff Memorial Research Building on the Clifton Road campus (about a 5 minute walk from our lab in the Rollins Research Center), with 2400 sq. ft. of dedicated wet-lab space. The EIGC’s laboratory areas include dedicated pre- and post-PCR spaces. ***(3)*** *HDX-MS* is available on campus through a new core facility established by Dr. Renhao Li in the Department of Pediatrics at Emory (see attached letter and *Equipment* for further details on the facility). The facility is housed in ~250 sq. ft. of newly renovated space adjacent to Dr. Li’s lab in the Emory Childrens’ Center on the Clifton Road campus (about a 10 minute walk from our lab in the Rollins Research Center). ***(4)*** the *Emory Comprehensive Glycomics Core* (ECGC; <http://www.cores.emory.edu/ecgc/>) houses additional ‘hand-on’ instrumentation for quantifying molecular interactions using label-free methods (BiaCore X100 surface plasmon resonance and a Malvern/ MicroCal Auto-iTC200 isothermal titration calorimetry instrument) in a 250 sq. ft. laboratory on the fourth floor of the Rollins Research Center.