**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)

**Equipment:**

###### EQUIPMENT

**Cassat Laboratory (1035 MRB IV)**

The Cassat laboratory houses all of the necessary equipment to study the molecular biology of microbial pathogens and skeletal cell biology. The equipment that may be utilized in this proposal includes the following: a Thermo Sorvall Lynx 6000 superspeed centrifuge with general purpose and ultraspeed rotors, Thermo Sorvall Legend XTR benchtop centrifuge with 2 rotors; Thermo Legend Micro21 and 21R benchtop microcentrifuges, Locator Jr. Plus Cryo Vessel for liquid nitrogen cell storage, Nuaire Class II Type A2 Biosafety Cabinets (2), Olympus inverted microscope CKX53 with QImaging OQCLR5 digital camera, an AquaSolutions Water Purification System, Eppendorf Nexus Mastercycler Gradient Thermocycler, Mettler Toledo Excellence balance, Thermo Forma series CO2 incubators (2), New Brunswick Innova Model 44 stackable incubator shaker, Thermo MaxQ4450 tabletop shaking incubator, Fisher Isotemp General Microbiologic Incubators (3), Isotemp bath incubators (3), variable speed rotating shakers (3), hypoxia chambers with ProOx Oxygen controller modules (3) and ProC02 Carbon Dioxide controller modules (2), a BioTek Hybrid Synergy microplate reader, GeneSys 10S UV-VIS spectrophotometer, Next Advance Bullet Blender for bone homogenization, UVP GelDoc-It2 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, Thermo Scientific EC 300 XL power supply for gel electrophoresis, BioRad polyacrylamide gel casting equipment and Western transfer apparatus, and a BioRad GenePulser Xcell electroporation apparatus.

###### Vanderbilt University Institute of Imaging Sciences (VUIIS)

The Center for Small Animal Imaging (CSAI) in the VUIIS contains advanced 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 microCT scanners for *ex vivo* imaging, Siemens MicroCAT II X-ray microCT 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.

###### Vanderbilt Center for Bone Biology (VCBB) (1215 MRB IV, Olin Hall)

Shared laboratory space in the VCBB is located two floors above the Cassat laboratory and available for use by our team. The VCBB shared laboratoryincludes the following equipment, General: 4°C refrigerators,

-20°C and -80°C freezers, analytical balances, pH meters, laminar-flow hoods, mammalian and bacterial incubators/shakers, gamma-counters, hybridization ovens, ultraturax homogenizer, Bio-Rad DNA gel visualization and documentation system, Beckman and IEC centrifuges, cell culture hoods and incubators, automated Bio-Rad cell counter, luminometer, spectrophotometers, a BioTek plate reader, sonicators (2), FPLC instruments, and cryotanks. Molecular biology: Real-time qPCR instruments (2: ABI and Bio-Rad), Bio- Rad thermal cyclers (2), a Bio-Rad CF x 96 qRT-PCR thermal cycler, DNA and protein gel migration apparatus. Bone histomorphometry: Equipment for soft and calcified tissue histology, a dissecting microscope, an automated paraffin processor, a paraffin embedding station, an explosion proof -20°C freezer for MMA polymerization, a Wehmer plastic embedding grinder, Leica RM2255 microtome (3), analytical balances, pH meters, water baths, chemical hoods dedicated for tissue staining (2), an upright Olympus microscope with fluorescence, an inverted Olympus microscope, and a high-resolution Olympus DP70 camera connected to a computer with BIOQUANT and OsteoMeasure histomorphometry software. Shared laboratory space for VCBB also extends into the Olin Engineering Hall, which is located just across 25th street. This space may be used for alternative approaches necessitating the lyophilization of proteins using the Labconco FreeZone 4.5 benchtop freeze dry system and the loading of compounds into polymer scaffolds with the SeedMixer DAC 150 FVZ.

In sum, access to equipment within our laboratory, the VUIIS, and VCBB will ensure that I am uniquely positioned to study fundamental processes in skeletal cell biology and host-pathogen interactions.

**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)

**Equipment:**

**[Included in Facilities section]**

**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)

**Equipment:**

### EQUIPMENT

As described in the sections below, collectively the Sponsor and Co-sponsor laboratories, the Departments of Biochemistry and Microbiology & Immunology as well as the Emory core facilities can provide me with access to all of the equipment I will need to complete my proposed research.

**Conn Laboratory:** All necessary equipment for RNA *in vitro* transcription and protein synthesis, purification, analysis, and crystallization is already available in the Conn laboratory on the fourth floor of the Rollins Research Center. We have Infors Multitron dual and Ecotron orbital shaker incubators (both with cooling module for low temperature expression) for bacterial culture for protein expression; a Beckman Allegra 25R table top centrifuge with swinging bucket and fixed angle rotors; two ÄKTA Purifier10 (FPLC) systems each housed in a dedicated 4 ˚C chromatography refrigerator and equipped with column selection kit for protein and protein-RNA complex purification; a BioTek Synergy 4 multimode (absorbance, luminescence, fluorescence) plate reader on which we have optimized our 96-well plate chromogenic assay of OAS activation; a Cary400 UV/Vis spectrophotomer with 6x6 cell multichanger (used for RNA UV thermal melting analysis); a Nanodrop 2000 UV/Vis spectrophotomer; three incubators for macromolecular crystallization and Leica optical microscope with digital camera for viewing and recording crystallization experiments; electrophoresis equipment for horizontal agarose, vertical mini/ preparative PAGE (the latter custom designed for large scale purification of *in vitro* transcribed RNA), three BioRad Sequi-Gen GT sequencing gel systems, and power supplies; all other essential small equipment, *e.g.* pH meter, balances, PCR thermocycler, microcentrifuges; and, low temperature storage units, *e.g.* chiller cabinets and refrigerator (4 ˚C) and freezers (-20 and -80 ˚C).

**Lowen Laboratory:** Dr. Lowen’s laboratory is located on the third floor of the Rollins Research Center and is fully equipped for molecular and cellular biology experiments. Major equipment includes two laminar flow hoods, CO2 incubators, an inverted phase contract microscope, microcentrifuges, a table-top centrifuge, several electrophoresis systems, water baths, a bacterial culture shaker/incubator, liquid nitrogen tank, and a - 80 ˚C freezer. Additionally, a BioRad ChemiDoc imager, two DNA thermal cyclers, two BioRad real-time PCR instruments with 384-well capability, and a new droplet digital PCR instrument (BioRad QX200) are located in Dr. Lowen’s laboratory. BioRad GenePulser Xcell and Lonza Nucleofector devices for electroporation of cells are available in an adjacent lab. A Zeiss fluorescent microscope with digital recording system is available in the department, and the lab has full access to a Becton Dickenson FACS Calibur flow cytometer that is located down the hall. An ultracentrifuge and darkroom facilities are located within 100 feet of thelab.

**Departmental Shared Equipment:** To support X-ray crystallographic studies, the Biochemistry Department provides a state-of-the-art facility for automated crystallization and ‘in-house’ X-ray data collection to support the research programs of three structural biology groups (led by Drs. Conn, Eric Ortlund, and Christine Dunham). This equipment is housed in dedicated laboratory space within the Rollins Research Center and includes a Phoenix nanoliter dispensing crystallization robot (Art Robbins Instruments), a Formulator liquid handling robot (Formulatrix) for crystal optimization, and a Rigaku MicroMax007HF generator with an ACTOR automated crystal mounting robot, Varimax-HF optics, a Saturn 944+ CCD detector, and Oxford Cryosystem X- Stream system. Adjacent to these facilities is an extensive molecular graphics area with dedicated space for computers for X-ray data processing, model building, and refinement. Emory is also 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.

For recording and quantitation of OAS assays using 32P or fluorescently labeled RNAs, we have access to two imaging systems: a GE **Typhoon FLA9000** (for 32P only) in the Department of Biochemistry and a GE Typhoon Trio variable mode imager system (32P and multi-color fluorescence) located in the Department of Microbiology and Immunology on the third floor of the Rollins Research Center building (adjacent to Dr. Lowen’s laboratory).

Additional shared laboratory areas immediately adjacent to the Conn Lab house equipment available as needed for this project, including preparative and ultracentrifuges, autoclaves, DNA/protein gel-imaging system, scintillation counter, and X-ray film developer. Another shared gel imaging system, scintillation counter, and X-ray developer are available as in the Department, adjacent to the X-ray crystallography suite on the ground floor of the Rollins Research Center building.

### Emory Core Facilities:

The following core facilities house equipment that I will use during the course of my project:

***Emory Chemical Biology Discovery Center (ECBDC)*:** For analysis of OAS1 interaction with dsRNAs, we have access to a high-throughput bio-layer interferometry (BLI) instrument, the **FortéBIO OctetRED384**. The ECBDC is located in the adjacent building to the Rollins Research Center (<1 minute walk via an internal connecting bridge). Further info: <http://www.pharm.emory.edu/ECBDC/>.

***Emory HDX-MS Core*:** This newly established **HDX-MS facility** is located in the Department of Pediatrics in the Emory Childrens’ Center (~5 minute walk from the Rollins Research Center) on the Emory Clifton Road Campus. The instrument consists of an ACQUITY UPLC System with HDX Manager and automatic sampler paired with a Waters Q-TOF Premier mass spectrometer capable of a maximum resolution of 1/17,500 Da. Masslynx 4.1, ProteinLynx Global SERVER™ (PLGS 3.0), and Dynamix 3.2 software packages control the instrument and HDX-MS analysis. The instrument collects an H/D exchange data profile after each sample injection, with as many as 50 sequential injections setup in one experiment using the autosampler program.

***Emory Integrated Genomics Core (EIGC):*** For analysis of RNase L activation in cell assays the **Agilent 2100 Bioanalyzer** on a chip platform is available through the EIGC. This system allows for rapid quantification of nucleic samples (here, ribosomal RNA using the RNA nano chip) and information about the size distribution of the fragments. Further info: [*http://cores.emory.edu/eigc/*](http://cores.emory.edu/eigc/)*.*

***Emory Comprehensive Glycomics Core (ECGC)*:** The ECGC houses additional equipment potentially relevant to this proposal in a dedicated laboratory in the same hall as the Conn lab on the fourth floor of the Rollins Research Center building. Specifically, for measurement of molecular interactions, we have available a shared a GE Biacore X-100 surface plasmon resonance (SPR) instrument and also an automated small cell isothermal titration calorimeter (Malvern/ MicroCal Auto-iTC200). These instruments are maintained under service agreements by the ECGC to ensure instrument availability.

Further info: [*http://www.cores.emory.edu/ecgc/*](http://www.cores.emory.edu/ecgc/).