NIH NRSA F31 Predoctoral Fellowship Workshop

Dr. Miguel Garcia-Diaz

Stony Brook University

Supported by:

- The University Faculty Senate at SUNY
- The SUNY Office of Research and Economic Development
- The Research Foundation for SUNY
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Prof. Miguel Garcia-Diaz

- Faculty, Pharmacological Sciences, Stony Brook University
- Member, NIH Training Workforce and Development (TWD) Study Section
- Director, SBU's Molecular & Cellular Pharmacology Graduate Program
- PI for an NIH T32 Training Grant

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Outline for NIH NRSA F31 Fellowship Workshop

Part 1:

- Introduction to NIH and to F31 fellowships
- The NIH Grant review process

Part 2:

- Preparing a successful application
- Do's and don'ts
- The submission process

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NIH National Institutes of Health
Turning Discovery Into Health

NIH's mission is to seek fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to enhance health, lengthen life, and reduce illness and disability

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NIH Focus Areas

To realize these goals, the NIH will support research in:

- The causes, diagnosis, prevention, and cure of human diseases
- The processes of human growth and development
- The biological effects of environmental contaminants
- The understanding of mental, addictive and physical disorders

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NIH is made up of 27 Institutes and Centers

- National Cancer Institute (NCI)
- National Eye Institute (NEI)
- National Heart, Lung, and Blood Institute (NHLBI)
- National Human Genome Research Institute (NHGRI)
- National Institute on Aging (NIA)
- National Institute on Alcohol Abuse and Alcoholism (NIAA)
- National Institute of Allergy and Infectious Diseases (NIAID)
- National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
- Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
- Office of Research Infrastructure Programs (ORIP)
- National Institute on Deafness and Other Communication Disorders (NIDCD)
- National Institute of Dental and Craniofacial Research (NIDCR)
- National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
- National Institute on Drug Abuse (NIDA)
- National Institute of Environmental Health Sciences (NIEHS)
- National Institute of General Medical Sciences (NIGMS)
- National Institute of Mental Health (NIMH)
- National Institute on Minority Health and Health Disparities (NIMHD)
- National Institute of Neurological Disorders and Stroke (NINDS)
- National Institute of Nursing Research (NINR)
- National Library of Medicine (NLM)

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NIH GRANTS & FUNDING
NIH Central Resource for Grants and Funding Information

Your application needs to target one specific Institute or Center

Make sure to check their specific instructions

Consult the funding opportunity announcement

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NIH Institute/Center Information

July 8, 2020
Expiration Date: May 8, 2023

NIH Institute or Center	Institute Center or Specific Information
National Cancer Institute Scientific Program Contact Michael Schmidt, Ph.D. Email: korczakj@mail.nih.gov Jeannette Korczak, Ph.D. Email: korczakj@mail.nih.gov Sergey Radaev, PH.D. Email: sradaev@mail.nih.gov **Grants Management Contacts:** Nicole Jones Phone: (240) 276-7156 Email:jonesni@mail.nih.gov	**NCI Specific Information** NCI requires a clear cancer focus in the research training and gives funding to applications for which the primary sponsor has cancer related R01 (or equivalent) research funding. For the F31, R01-equivalent research funding includes peer-reviewed grants with a minimum of 3 years in duration and $150,000 in annual direct costs. Grants under a no-cost extension do not qualify.

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Purpose of NIH's F31 NRSA (National Research Service Award)

The purpose of the Kirschstein-NRSA predoctoral fellowship (F31) award is to enable promising predoctoral students to obtain individualized, mentored research training from outstanding faculty sponsors while conducting dissertation research in scientific health-related fields relevant to the missions of the participating NIH Institutes and Centers. The proposed mentored research training must reflect the applicant's dissertation research project and is expected to clearly enhance the individual's potential to develop into a productive, independent research scientist.

The training plan should document the need for, and the anticipated value of, the proposed mentored research and training in relationship to the individual's research career goals. The training plan should also facilitate the fellow's transition to the next stage of his/her research career.

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Eligibility

- Any candidate with the skills, knowledge, and resources necessary to carry out the proposed research.
- By the time of award, the individual must be a citizen or permanent resident.
- The candidate must be at the dissertation research stage of training at the time of award and must show evidence of high academic performance in the sciences, and commitment to a career as an independent research scientist.
- Individuals from underrepresented racial and ethnic groups as well as individuals with disabilities are always encouraged to apply for NIH support.

Commitment to Diversity

Ruth L. Kirschstein National Research Service Award (NRSA) Individual Predoctoral Fellowship to Promote Diversity in Health-Related Research (Parent F31 -Diversity)

- Individuals from racial and ethnic groups that have been shown by the National Science Foundation to be underrepresented in health-related sciences on a national basis
- Individuals with disabilities
- Individuals from disadvantaged backgrounds

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The Role of a Principal Investigator

The Research Foundation will apply for and administer the award

You, as the PI will:

- Write the proposal
- Coordinate with the Research Foundation
- Contact with the sponsor
- Be responsible for overseeing the project and reporting, if funded

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The NIH Peer-Review Process

- Upon submission, applications are assigned to a specific Institute or Center.
- The application is then assigned to a study section
- Study sections are composed of a diverse array of scientists who are often not experts in the field
- Applications are typically assigned three primary reviewers who conduct a pre-meeting review of the application and give preliminary scores

NIH Scoring

Impact	Impact Score	Interpretation
High		
1	Exceptional	Exceptionally strong with essentially no weaknesses
2	Outstanding	Extremely strong with negligible weaknesses
3	Excellent	Very strong with only some minor weaknesses
Moderate		
4	Very Good	Strong but with numerous minor weaknesses
5	Good	Strong but with at least one moderate weakness
6	Satisfactory	Some strengths but also some moderate weaknesses
Low		
7	Fair	Some strengths but with at least one major weakness
8	Marginal	A few strengths and a few major weaknesses
9	Poor	Very few strengths and numerous major weaknesses

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The Study Section Meeting

- At the review meeting, applications are reviewed by the entire group.
- The primary reviewer summarizes the application. Secondary and tertiary reviewers add to the discussion.
- After the reviewers finish their statements the discussion is open to the entire panel.
- At the end of the discussion, primary reviewers restate their scores, and then every member of the study section scores the application.
- Sample NIH panel video.

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What are Reviewers Looking For?

Scored Review Criteria

- Fellowship applicant
- Sponsors, collaborators and consultants
- Research Training plan
- Training potential
- Institutional environment and commitment to training

The good news:

"An application does not need to be strong in all categories to be judged likely to have major scientific impact."

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Fellowship Applicant

- **Candidate's academic record and research experience**
- **Potential to develop into an independent and productive researcher**
- **Commitment to a research career.**

Sponsors Collaborators and Consultants

- **Are the sponsor(s)' research qualifications (including recent publications) and track record of mentoring appropriate?**
- **Do the sponsors understand the candidate's training needs, ability and commitment?**
- **Are there adequate research funds?**
- **Does the sponsor's research and training record, as well as mentoring statement, indicate that the candidate will receive outstanding training … publish high quality papers and present research data at national meetings?**

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Research Training Plan

Essential Components:

- High scientific quality and well integrated with the training plan?
- Sufficiently distinct from the sponsor's funded research for the candidate's career stage?
- Consistent with the candidate's stage of research development?
- Is the proposed time frame feasible to accomplish the proposed training?
- Opportunities to present and publish research findings and meet with scientists in the community at national meetings, provide the professional skills needed to transition to the next career stage.

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Training Potential

- **Are the proposed research project and training plan likely to provide the candidate with the requisite individualized and mentored experiences in order to obtain appropriate skills for a research career?**
- **Does the training plan take advantage of the candidate's strengths and address gaps in needed skills? Does the training plan document a clear need for, and value of, the proposed training?**
- **Does the proposed training have the potential to serve as a sound foundation that will clearly enhance the candidate's ability to develop into a productive researcher?**

Institutional Environment & Commitment to Training

- Are the research facilities, resources (e.g., equipment, laboratory space, computer time, subject populations, clinical training settings) and training opportunities (e.g., seminars, workshops, professional development opportunities) adequate and appropriate?
- Is the institutional environment for the candidate's scientific development of high quality?
- Is there appropriate institutional commitment to fostering the candidate's mentored training?

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Sample Application from NIH NIAID

Fellowship Applicant

1. Fellowship Applicant:

Strengths

- B.S. in Physiology (2012) and M.S. in Professional Sciences (2014) at the University of Arizona.
- Active in student advocacy programs.
- Student representative on the Department's Diversity Committee.
- Recipient of many scholarships and awards: Wildcat Excellence Award 2008-2012 (Tuition Scholarship); Roman DeSanctis Scholarship 2010-2011; University of Arizona Graduate College Dean's Tuition Award 2014.
- Candidate has very strong supportive letters: very hard-working, highly motivated, highly dedicated to his research; the best graduate student in the department; enormous potential as an independent investigator in the future.

Weaknesses

- None noted.

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Fellowship Applicant

1. Fellowship Applicant:

Strengths

1 F31 AI131622-01 6 ZRG1 F07-T (20)
CONTRERAS, N

- The scholastic performance of the applicant is very good in grad school, all As.
- The applicant earned a Professional Science Master's degree with a project developing assays detecting blood borne pathogens.
- He has received NIH funding through the Initiative to Maximize Student Diversity Award and currently sit on the Department of Immunobiology Diversity Committees.
- The applicant is first in his family to complete a Bachelor's degree and go to graduate school.
- He has done his course work, passed his qualifying exam and completed successful lab rotations.
- His recommendation letters are outstanding.

Weaknesses

- None noted.

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Sponsors Collaborators and Consultants

2. Sponsors, Collaborators, and Consultants:

Strengths

- Dr. Graeme L. Conn (Associate Prof., Department of Biochemistry) and Dr. Anice Lowen (Assistant Prof., Department of Microbiology & Immunology) will serve as co-mentors.
- Dr. Conn has many years of mentoring experience and 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 provide career guidance as well as outstanding expertise in molecular virology and the cell culture systems.

Weaknesses

- None noted.

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Sponsors Collaborators and Consultants

2. Sponsors, Collaborators, and Consultants:

Strengths

- The sponsor has a good mentoring track record, having trained eight PhD students that have ultimately gone on to academic and industry positions.
- A co-sponsor is included. Having a co-sponsor is excellent since the applicant is remaining in the same lab in which she was previously a technician. The applicant rotated in the co-sponsor's lab and developed a good working relationship with her already. The co-sponsor will provide expertise in a cell culture model for some of the activity assays. She is well funded.
- The sponsor and co-sponsor both have very strong graduate training track records.

Weaknesses

- The sponsor's current funding is a R01 for RNA modification and antibiotic resistance. This will be offset if the pending R01 that is directly related to the proposed research is funded.

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Research Training Plan

3. Research Training Plan:

Strengths

1 F31 AI131622-01 4 ZRG1 F07-T (20)
CONTRERAS, N

- The proposed research is a largely unexplored area- important and clinically relevant data could come from these studies.
- On the whole the experimental plan is highly feasible and well within the expertise of the PI.
- Preliminary data are strong and intriguing.
- All the required expertise is readily available.
- Expected outcomes and alternatives for each aim are well described.
- The sponsor indicates a clear expectation for research productivity including the number of papers and the frequency of data presentation at the regional and national level.
- The applicant will participate in formalized professional lectures and workshops that are highly relevant to his future development.
- Will be applying to attend the NIA Advanced Course on the Biology of Aging.
- Involved in outreach programs to promote STEM careers.
- The descriptions of the exact scientific meetings to be attended, the frequency of meetings is well described.

Weaknesses

- None noted.

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Research Training Plan

3. Research Training Plan:

Strengths

- The research training plan is well written with a central hypothesis and path to test the hypothesis. Moreover, the outcomes, pitfalls have been presented and while early in the research provide a vision of the road ahead.

Weaknesses

- None noted.

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Training Potential

4. Training Potential:

Strengths

- The applicant has identified a novel area in immunology and virology to pursue his training—the studies have a good chance of having an impact on the field.
- There are outstanding training opportunities (e.g. seminars, workshops, professional development opportunities) available for the applicant's development.
- The descriptions of the exact scientific conferences to be attended, the frequency of the group meetings and the regular communication of research results to the scientific community are well described.

Weaknesses

- Lack of plans to acquire new skills in cutting-edge cellular immunology techniques important for the career path he hopes to follow.

Institutional Environment & Commitment to Training

5. Institutional Environment & Commitment to Training:

Strengths

- Outstanding resources are available for the proposed research; and an extremely well detailed overview of all areas of training over the course of graduate school were provided; really a truly wonderful and carefully crafted description. Everything seems to be in place for solid training.

Weaknesses

- None noted.

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Summary Statement

2. Sponsors, Collaborators, and Consultants:

Strengths

•Excellent mentor, Dr. Moon Nahm.

Weaknesses

•It would be better to have a mentoring team to monitor the progress of Mr. Juan.

3. Research Training Plan:

Strengths

Weaknesses

•Ambitious proposal.

•No preliminary data for Aims 2 and 3.

4. Training Potential:

Strengths

•Outstanding applicant.

•Accomplished mentor(s).

Weaknesses

•The training plan/proposal needs to be revised.

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Understanding Your Summary Statement

- Excellent pre-doctoral applicant
- First-generation college student
- Very good undergraduate academic record
- Undergraduate research experience that resulted in one first-author and several co-author publications
- She is viewed as very strong

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Resume and Summary of Discussion

An **excellent pre-doctoral applicant**, in this application, seeks training in virus-host interactions with a project that focuses on studying the regulation of the 2'-5' oligoadenylate synthetase (OAS) by cytosolic double-stranded RNA (dsRNA). The applicant is a **first-generation college student** who achieved **a very good undergraduate academic record** and gained undergraduate research experience that resulted in one first-author and several co-author publications. She is now a graduate student in the Biochemistry, Cell and Development Biology (BCDB) program at Emory. **She is viewed as very strong.** The sponsor and co-sponsor are reviewed as very strong with complementary research expertise and experience in mentoring graduate students. The **research training plan is well articulated**, although **some review it as somewhat risky (high risk, high reward).** The applicant will **need to learn techniques** such as x-ray crystallography and mass spectrometry. Some reviewers view this as a potential weakness, whereas others view it as a strength of an overall excellent training plan. **The institutional environment is excellent.** Overall, there is a high enthusiasm for the applicant, outstanding sponsors, excellent institutional environmental, and **important potential impact on advancing our understanding of host-pathogen interactions**.

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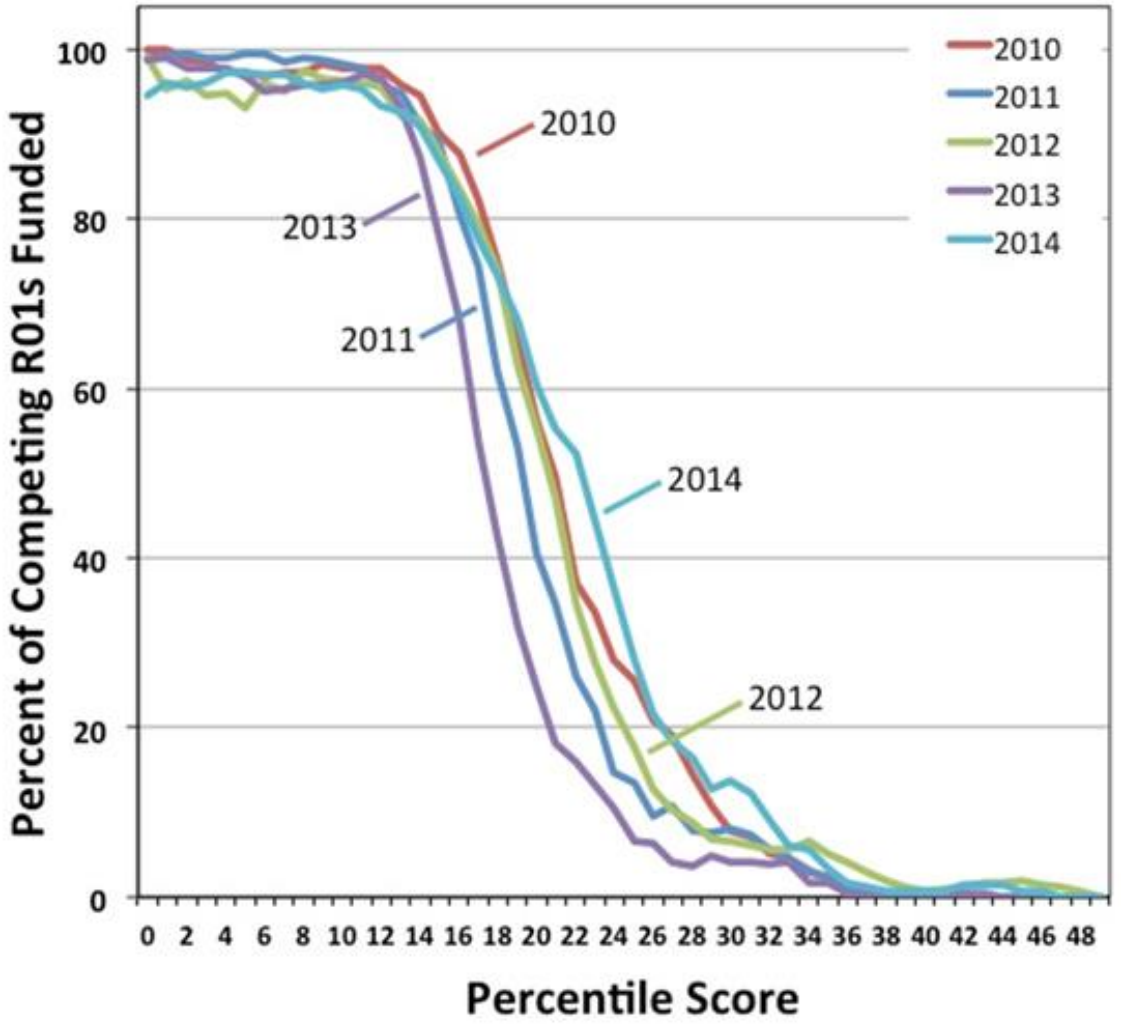
Post-meeting Review



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Parts of a Fellowship (F Series) Application

- SF 424 Form
- Assignment request form
- Other Project information
- Project performance form
- Senior/Key person (with bio sketches)
- Fellowship supplemental form (the actual application)
- Sample applications links

Section of Application

Section of Application	Page Limits (if different from FOA, FOA supersedes)
Project Summary Abstract	30 lines of text
Project Narrative	Three sentences
Introduction to Resubmission or Revision Application (which applicable)	1
Applicant's Background and Goals for Fellowship Training	6
Specific Aims	1
Research Strategy	6
Respective Contributions	1
Selection of Sponsor and Institution	1
Training in Responsible Conduct of Research	1
Sponsor and Co-Sponsor Statements	6
Letters of Support from Collaborators, Contributors, and Consultants	6
Description of Institutional Environment and Commitment to Training Note: This page limit includes the Additional Educational Information required for F30 and F31 applications	2
Applications for Concurrent Support (when applicable)	1
Biographical Sketch	5

How to apply application guide link

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Assignment Request Form

- Request assignment of your application to a specific institute or center and/or to a specific study section
- You can also request a particular expertise in reviewers or declare conflicts of interest for review

Fellowship Study Section

Study Section ^	Study Section Description	Scientific Review Officer
F01A	Fellowships: Brain Disorders and Related Neurosciences	Dr. Vilen Movsesyan
F01B	Fellowships: Learning and Memory, Language, Communication and Related Neurosciences	Dr. Jyothi Arikkath
F02A	Fellowships: Behavioral Neuroscience	Dr. Mei Qin
F02B	Fellowships: Sensory and Motor Neurosciences, Cognition and Perception	Dr. Cibu Thomas
F03A	Fellowships: Neurodevelopment, Synaptic Plasticity and Neurodegeneration	Dr. Tze-Tsang (Tina) Tang
F03B	Fellowships: Biophysical, Physiological, Pharmacological and Bioengineering Neuroscience	Dr. Sussan Paydar
F04A	Fellowships: Chemistry, Biochemistry and Biophysics A	Dr. David Jollie
F04B	Fellowships: Chemistry, Biochemistry and Biophysics B	Dr. Sudha Veeraraghavan
F05-D	Fellowships: Cell Biology, Developmental Biology, and Bioengineering	Dr. Alexander Gubin
F05-U	Fellowships: Cell Biology, Developmental Biology, and Bioengineering	Dr. Raj Krishnaraju
F06	Fellowships: Endocrinology, Metabolism, Nutrition and Reproductive Sciences	Dr. Gregory Shelness
F07	Fellowships: Immunology and Area	Dr. Liying Guo
F08	Fellowships: Genes, Genomes and Genetics	Dr. Lystranne Maynard Smith
F09A	Fellowships: Oncological Sciences	Dr. Reigh-Yi Lin
F09B	Fellowships: Oncological Sciences	Dr. Jian Cao
F09C	Fellowships: Oncological Sciences	Dr. Sarita Sastry

Fellowship Study Section link

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Project Summary

PROJECT SUMMARY

Staphylococcus aureus is a ubiquitous human pathogen, resulting in superficial, invasive, and disseminated infections. One of the most common invasive manifestations of *S. aureus* disease is osteomyelitis, a frequently occurring and debilitating infection of bone. Osteomyelitis triggers dramatic alterations in bone architecture, leading to severe complications such as bone destruction, pathologic fractures, and growth defects. An emerging body of literature suggests that both local and systemic inflammation trigger altered interactions between bone-forming osteoblasts and bone-resorbing osteoclasts to impact bone homeostasis. Skeletal cells are known to express innate pattern recognition receptors (PRRs), but the contribution of innate sensing towards bone homeostasis and antibacterial immunity during *S. aureus* osteomyelitis has not yet been explored. The overarching objective of this proposal is to characterize how innate sensing of bacterial pathogens by skeletal cells triggers alterations in bone physiology. In order to define the impact of skeletal cell PRRs on bone homeostasis, we first focused on the critical signaling adaptor protein, MyD88, which is necessary to transduce signals through toll-like receptors (TLRs) and IL-1 receptors (IL-1R). Our preliminary data demonstrate that MyD88 is necessary to control *S. aureus* replication and dissemination *in vivo* and that osteoclast differentiation can be stimulated by bacterial components in a MyD88-dependent manner *in vitro*. Therefore, the central hypothesis of this proposal is that *S. aureus* modulates osteoclast precursor cell biology and bone remodeling through ligation of osteoclast PRRs and the induction of inflammation. To test this hypothesis, I will use a newly developed murine *S. aureus* osteomyelitis model from our laboratory. This model is advantageous compared to other osteomyelitis models because it allows us to utilize genetically modified animals, high-resolution quantitative imaging analysis, and unique histologic techniques for quantifying perturbations in bone remodeling. Experiments proposed in Aim 1 will investigate the roles of TLR and IL-1R signaling on osteoclast differentiation by monitoring osteoclastogenic signaling cascades, transcription factor activity, expression of mature osteoclast markers, and functionality of osteoclasts formed *in vitro*. Aim 2 will explore how MyD88 signaling in skeletal cells impacts clearance of *S. aureus* and bone remodeling. Collectively, these data will define signaling crosstalk between canonical osteoclast differentiation and innate immune pathways to activate osteoclast differentiation and maturation programs. Additionally, these findings will describe how MyD88 signaling in skeletal cells contributes to immune defenses and affects the kinetics of bone remodeling. This proposed work will have broad implications for how innate skeletal cell sensing contributes to the development of an effective immune response and influences bone homeostasis.

The Project Summary should not have more than 30 lines.

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Project Narrative

Project Narrative Sample

Normal bone remodeling is a tightly regulated process that is dramatically alerted by infection and both systemic and local inflammatory conditions. The proposed research will investigate how skeletal cells sense and respond to the human bacterial pathogen Staphylococcus aureus, the most common cause of bone infections, and how these cellular responses disrupt normal bone remodeling. This work will therefore describe how bone is altered by the presence of bacterial pathogens and resulting immune responses providing critical information for the development of therapeutics that may reduce bone pathology triggered by infections or inflammation.

The Project Narrative should not have more than 3 sentences.

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Fellowship Supplemental Form

- Introduction (resubmission only)
- Applicant's Background and Goals for Fellowship Training
- Research Training Plan
- Sponsor and co-sponsor statements
- Letters of support
- Institutional Environment and commitment to training
- Other research (human subjects, vertebrate animals, etc.)
- Resource sharing plan

Introduction (resubmissions only)

- One page max
- Acknowledge main reviewer criticisms
- Explain how you have addressed them

Training Plan

- A. Doctoral Dissertation and Research Experience

Training Plan

- A. Doctoral Dissertation and Research Experience
- B. Training Goals and Objectives

Training Plan

- A. Doctoral Dissertation and Research Experience
- B. Training Goals and Objectives
- C. Activities Planned Under this Award

Training Plan

Some Possible Goals

- Polish skills or improve expertise
- Acquire an entirely new technique or sub-discipline or method
- Acquire career development skills or fill in missing pieces (pubs, teaching, etc.)
- Identify career options
- Develop research independence

Ways to Achieve them

- Add a mentor or collaborator/consultant (but these must be clearly integrated into your career development plan!)
- Take courses or workshops outside of previous training focus or outside of university
- Arrange to teach a course or two; mentor others; etc.

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Training Plan

Key Things to Address

- Why you chose the mentor(s) you did
- Your training path
- Your training in communication
- Unique opportunities at your institution
- Professional training
- Soft skills training
  - Negotiation, persuasion, diplomacy, mentoring younger students
- Training in the responsible conduct of research (ethics) and rigorous and reproducible research

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The Specific Aims Page

One page, self-contained; will be read by the entire panel

- Introductory paragraph
- Second paragraph
- Aims (2-4)
- Final summary Impact

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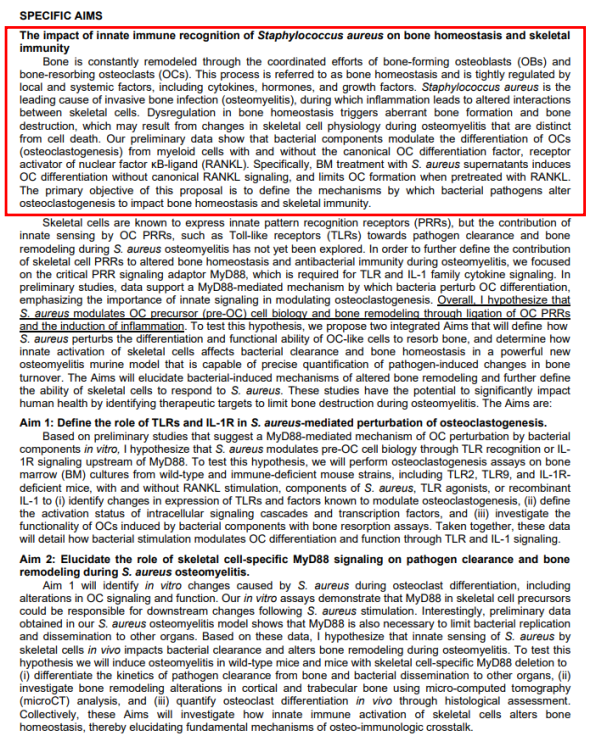
The Specific Aims Page

The impact of innate immune recognition of *Staphylococcus aureus* on bone homeostasis and skeletal immunity

Bone is constantly remodeled through the coordinated efforts of bone-forming osteoblasts (OBs) and bone-resorbing osteoclasts (OCs). This process is referred to as bone homeostasis and is tightly regulated by local and systemic factors, including cytokines, hormones, and growth factors. *Staphylococcus aureus* is the leading cause of invasive bone infection (osteomyelitis), during which inflammation leads to altered interactions between skeletal cells. Dysregulation in bone homeostasis triggers aberrant bone formation and bone destruction, which may result from changes in skeletal cell physiology during osteomyelitis that are distinct from cell death. Our preliminary data show that bacterial components modulate the differentiation of OCs (osteoclastogenesis) from myeloid cells with and without the canonical OC differentiation factor, receptor activator of nuclear factor κB-ligand (RANKL). Specifically, BM treatment with *S. aureus* supernatants induces OC differentiation without canonical RANKL signaling, and limits OC formation when pretreated with RANKL. The primary objective of this proposal is to define the mechanisms by which bacterial pathogens alter osteoclastogenesis to impact bone homeostasis and skeletal immunity.

Background



SPECIFIC AIMS

The impact of innate immune recognition of *Staphylococcus aureus* on bone homeostasis and skeletal immunity

Bone is constantly remodeled through the coordinated efforts of bone-forming osteoblasts (OBs) and bone-resorbing osteoclasts (OCs). This process is referred to as bone homeostasis and is tightly regulated by local and systemic factors, including cytokines, hormones, and growth factors. *Staphylococcus aureus* is the leading cause of invasive bone infection (osteomyelitis), during which inflammation leads to altered interactions between skeletal cells. Dysregulation in bone homeostasis triggers aberrant bone formation and bone destruction, which may result from changes in skeletal cell physiology during osteomyelitis that are distinct from cell death. Our preliminary data show that bacterial components modulate the differentiation of OCs (osteoclastogenesis) from myeloid cells with and without the canonical OC differentiation factor, receptor activator of nuclear factor κB-ligand (RANKL). Specifically, BM treatment with *S. aureus* supernatants induces OC differentiation without canonical RANKL signaling, and limits OC formation when pretreated with RANKL. The primary objective of this proposal is to define the mechanisms by which bacterial pathogens alter osteoclastogenesis to impact bone homeostasis and skeletal immunity.

Skeletal cells are known to express innate pattern recognition receptors (PRRs), but the contribution of innate sensing by OC PRRs, such as Toll-like receptors (TLRs) towards pathogen clearance and bone remodeling during *S. aureus* osteomyelitis has not yet been explored. In order to further define the contribution of skeletal cell PRRs to altered bone homeostasis and antibacterial immunity during osteomyelitis, we focused on the critical PRR signaling adaptor MyD88, which is required for TLR and IL-1 family cytokine signaling. In preliminary studies, data support a MyD88-mediated mechanism by which bacteria perturb OC differentiation, emphasizing the importance of innate signaling in modulating osteoclastogenesis. Overall, I hypothesize that *S. aureus* modulates OC precursor (pre-OC) cell biology and bone remodeling through ligation of OC PRRs and the induction of inflammation. To test this hypothesis, we propose two integrated Aims that will define how *S. aureus* perturbs the differentiation and functional ability of OC-like cells to resorb bone, and determine how innate activation of skeletal cells affects bacterial clearance and bone homeostasis in a powerful new osteomyelitis murine model that is capable of precise quantification of pathogen-induced changes in bone turnover. The Aims will elucidate bacterial-induced mechanisms of altered bone remodeling and further define the ability of skeletal cells to respond to *S. aureus*. These studies have the potential to significantly impact human health by identifying therapeutic targets to limit bone destruction during osteomyelitis. The Aims are:

Aim 1: Define the role of TLRs and IL-1R in *S. aureus*-mediated perturbation of osteoclastogenesis.

Based on preliminary studies that suggest a MyD88-mediated mechanism of OC perturbation by bacterial components *in vitro*, I hypothesize that *S. aureus* modulates pre-OC cell biology through TLR recognition or IL-1R signaling upstream of MyD88. To test this hypothesis, we will perform osteoclastogenesis assays on bone marrow (BM) cultures from wild-type and immune-deficient mouse strains, including TLR2, TLR9, and IL-1R-deficient mice, with and without RANKL stimulation, components of *S. aureus*, TLR agonists, or recombinant IL-1 to (i) identify changes in expression of TLRs and factors known to modulate osteoclastogenesis, (ii) define the activation status of intracellular signaling cascades and transcription factors, and (iii) investigate the functionality of OCs induced by bacterial components with bone resorption assays. Taken together, these data will detail how bacterial stimulation modulates OC differentiation and function through TLR and IL-1 signaling.

Aim 2: Elucidate the role of skeletal cell-specific MyD88 signaling on pathogen clearance and bone remodeling during *S. aureus* osteomyelitis.

Aim 1 will identify *in vitro* changes caused by *S. aureus* during osteoclast differentiation, including alterations in OC signaling and function. Our *in vitro* assays demonstrate that MyD88 in skeletal cell precursors could be responsible for downstream changes following *S. aureus* stimulation. Interestingly, preliminary data obtained in our *S. aureus* osteomyelitis model shows that MyD88 is also necessary to limit bacterial replication and dissemination to other organs. Based on these data, I hypothesize that innate sensing of *S. aureus* by skeletal cells *in vivo* impacts bacterial clearance and alters bone remodeling during osteomyelitis. To test this hypothesis we will induce osteomyelitis in wild-type mice and mice with skeletal cell-specific MyD88 deletion to (i) differentiate the kinetics of pathogen clearance from bone and bacterial dissemination to other organs, (ii) investigate bone remodeling alterations in cortical and trabecular bone using micro-computed tomography (microCT) analysis, and (iii) quantify osteoclast differentiation *in vivo* through histological assessment. Collectively, these Aims will investigate how innate immune activation of skeletal cells alters bone homeostasis, thereby elucidating fundamental mechanisms of osteo-immunologic crosstalk.

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The Specific Aims Page

The impact of innate immune recognition of *Staphylococcus aureus* on bone homeostasis and skeletal immunity

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Novel observation/preliminary data

SPECIFIC AIMS

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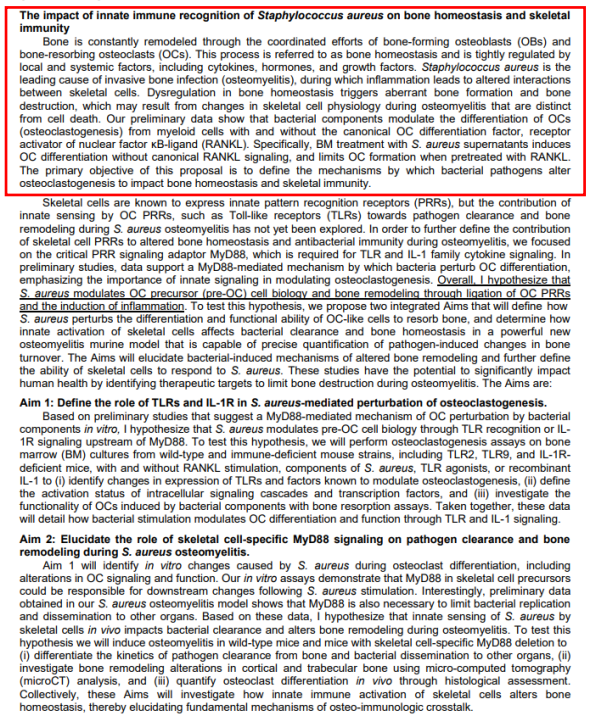
Novel observation/preliminary data

Goal/Gap in knowledge



SPECIFIC AIMS

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The Specific Aims Page

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Background

Gap in knowledge

Approach/feasibility

Hypothesis

SPECIFIC AIMS

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Background

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Approach/feasibility

Hypothesis

Approach/goals

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Background

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Title

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SPECIFIC AIMS

The impact of innate immune recognition of *Staphylococcus aureus* on bone homeostasis and skeletal immunity

Bone is constantly remodeled through the coordinated efforts of bone-forming osteoblasts (OBs) and bone-resorbing osteoclasts (OCs). This process is referred to as bone homeostasis and is tightly regulated by local and systemic factors, including cytokines, hormones, and growth factors. *Staphylococcus aureus* is the leading cause of invasive bone infection (osteomyelitis), during which inflammation leads to altered interactions between skeletal cells. Dysregulation in bone homeostasis triggers aberrant bone formation and bone destruction, which may result from changes in skeletal cell physiology during osteomyelitis that are distinct from cell death. Our preliminary data show that bacterial components modulate the differentiation of OCs (osteoclastogenesis) from myeloid cells with and without the canonical OC differentiation factor, receptor activator of nuclear factor κB-ligand (RANKL). Specifically, BM treatment with *S. aureus* supernatants induces OC differentiation without canonical RANKL signaling, and limits OC formation when pretreated with RANKL. The primary objective of this proposal is to define the mechanisms by which bacterial pathogens alter osteoclastogenesis to impact bone homeostasis and skeletal immunity.

Skeletal cells are known to express innate pattern recognition receptors (PRRs), but the contribution of innate sensing by OC PRRs, such as Toll-like receptors (TLRs) towards pathogen clearance and bone remodeling during *S. aureus* osteomyelitis has not yet been explored. In order to further define the contribution of skeletal cell PRRs to altered bone homeostasis and antibacterial immunity during osteomyelitis, we focused on the critical PRR signaling adaptor MyD88, which is required for TLR and IL-1 family cytokine signaling. In preliminary studies, data support a MyD88-mediated mechanism by which bacteria perturb OC differentiation, emphasizing the importance of innate signaling in modulating osteoclastogenesis. Overall, I hypothesize that *S. aureus* modulates OC precursor (pre-OC) cell biology and bone remodeling through ligation of OC PRRs and the induction of inflammation. To test this hypothesis, we propose two integrated Aims that will define how *S. aureus* perturbs the differentiation and functional ability of OC-like cells to resorb bone, and determine how innate activation of skeletal cells affects bacterial clearance and bone homeostasis in a powerful new osteomyelitis murine model that is capable of precise quantification of pathogen-induced changes in bone turnover. The Aims will elucidate bacterial-induced mechanisms of altered bone remodeling and further define the ability of skeletal cells to respond to *S. aureus*. These studies have the potential to significantly impact human health by identifying therapeutic targets to limit bone destruction during osteomyelitis. The Aims are:

Aim 1: Define the role of TLRs and IL-1R in *S. aureus*-mediated perturbation of osteoclastogenesis.

Based on preliminary studies that suggest a MyD88-mediated mechanism of OC perturbation by bacterial components *in vitro*, I hypothesize that *S. aureus* modulates pre-OC cell biology through TLR recognition or IL-1R signaling upstream of MyD88. To test this hypothesis, we will perform osteoclastogenesis assays on bone marrow (BM) cultures from wild-type and immune-deficient mouse strains, including TLR2, TLR9, and IL-1R-deficient mice, with and without RANKL stimulation, components of *S. aureus*, TLR agonists, or recombinant IL-1 to (i) identify changes in expression of TLRs and factors known to modulate osteoclastogenesis, (ii) define the activation status of intracellular signaling cascades and transcription factors, and (iii) investigate the functionality of OCs induced by bacterial components with bone resorption assays. Taken together, these data will detail how bacterial stimulation modulates OC differentiation and function through TLR and IL-1 signaling.

Aim 2: Elucidate the role of skeletal cell-specific MyD88 signaling on pathogen clearance and bone remodeling during *S. aureus* osteomyelitis.

Aim 1 will identify *in vitro* changes caused by *S. aureus* during osteoclast differentiation, including alterations in OC signaling and function. Our *in vitro* assays demonstrate that MyD88 in skeletal cell precursors could be responsible for downstream changes following *S. aureus* stimulation. Interestingly, preliminary data obtained in our *S. aureus* osteomyelitis model shows that MyD88 is also necessary to limit bacterial replication and dissemination to other organs. Based on these data, I hypothesize that innate sensing of *S. aureus* by skeletal cells *in vivo* impacts bacterial clearance and alters bone remodeling during osteomyelitis. To test this hypothesis we will induce osteomyelitis in wild-type mice and mice with skeletal cell-specific MyD88 deletion to (i) differentiate the kinetics of pathogen clearance from bone and bacterial dissemination to other organs, (ii) investigate bone remodeling alterations in cortical and trabecular bone using micro-computed tomography (microCT) analysis, and (iii) quantify osteoclast differentiation *in vivo* through histological assessment. Collectively, these Aims will investigate how innate immune activation of skeletal cells alters bone homeostasis, thereby elucidating fundamental mechanisms of osteo-immunologic crosstalk.

The Specific Aims Page

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Title

Background/hypothesis

Approach

Outcome

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Specific Aims

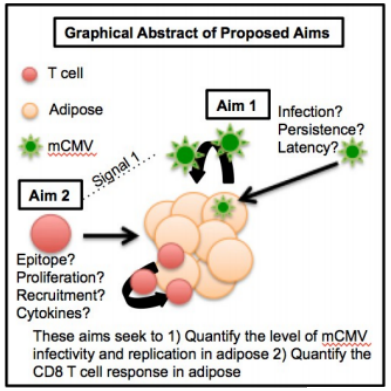
A. SPECIFIC AIMS

Adipose tissue consists of adipocytes that are crucial in lipid synthesis and energy storage, and a smaller population of cells of the stromal vascular fraction (SVF). The SVF represents a heterogeneous mixture of endothelial, stem, and immune cells, including T cells, macrophages, B cells, and NK cells. It has become increasingly clear that the immune responses within adipose tissue, such as cytokine secretion and tissue remodeling, influence host health and metabolism. Much emphasis has been placed on the activation of T cells and macrophages and their role in the chronic low-grade inflammation seen in obesity. However, inflammation in response to infections has been less thoroughly investigated. Adipocytes infected by adenovirus display an increased size and density, and *Trypanosoma cruzi* directly infects adipocytes resulting in adipose tissue inflammation. Recently, adipose CD4 T cells were shown to provide a site of latent viral infection in Human Immunodeficiency Virus (HIV) and Simian Immunodeficiency Virus (SIV).

Another virus capable of persistence and latency is cytomegalovirus (CMV), and it has been long implicated in low-level, systemic inflammation. The primary site of persistence for CMV is believed to be the salivary gland, but primary sites of latency have been difficult to conclusively identify. CMV infection results in a strong T cell response; in an acute infection ~5% of mouse, and up to 40% in some human patients, peripheral T cells are specific for CMV antigen. In our hands, we find that ~10% of adipose CD8 T cells in a mouse CMV (mCMV) infection are specific to mCMV at early comparable acute infection time points. This suggests that adipose tissue is an underappreciated site of viral infection and immune activity during CMV infection. **The primary hypothesis of this proposal is that mCMV disseminates to adipose tissue, replicates, establishes latency, leading to an lifelong CD8 T cell response.** Our long-term research objectives are to identify the lifelong immunological consequences of CMV infection. *The objective of this proposal* is to determine if mCMV establishes a productive infection within adipose tissue, and the consequences, if any, of that infection. As CMV is highly specific for host species, we will employ the mCMV model of infection to achieve the primary objective of this proposal through the following specific aims:

AIM 1: Evaluate adipose tissue as a reservoir for replicative and persistent virus. We hypothesize that adipose tissue is a location of active mCMV replication. To that end we will quantify viral load by plaque assay and qPCR in adipose compared to peripheral blood mononuclear cells (PBMCs) and salivary glands. We will determine the extent to which mCMV persists in adipose in a chronically infected mouse using multiple modes of reactivation. We will also identify infected cells within adipose. These experiments will determine what cells within adipose tissue can harbor replicative and persistent infection.

AIM 2: Determine the response of adipose tissue CD8 T cells during mCMV infection. We hypothesize that mCMV specific T cells expand in adipose tissue. We have observed a significant expansion of mCMV specific CD8 T cells following infection. As the immune response to mCMV in adipose tissue has never been fully characterized we will determine the kinetics of mCMV specific CD8 T cell expansion and proliferation. We will determine if local T cells clonally expand or naïve cells are constantly recruited to adipose. These experiments will provide, for the first time, an understanding of adipose tissue mCMV immune response.



See Graphical Abstract for Summary of Aims

IMPACT: Upon the completion of these studies, we will have significantly advanced the understanding of mCMV cell tropism. We will have identified adipose tissue as a site of replicative and persistent virus that is capable of reactivation. The functional response of CD8 T cells and their mechanism of recruitment to adipose will have been identified. We will also have revealed the mechanism of viral spread into adipose tissue. These findings will have far reaching implications on the consideration of adipose tissue during vaccine design.

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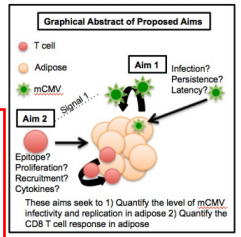

Selecting Your Aims

- Hypothesis driven vs exploratory
- Distinct, novel and related to the overall goal of the proposal
- Avoid
  - Unrealistic/overambitious aims
  - Poorly justified aims
  - Purely descriptive aims
  - Interdependent aims (or: have a plan B)

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Research Strategy

Subdivided into

- Significance (usually about 1 page)
- Approach

Organizational Method for Aims

A Possible Organization for the Approach

- Aims begin with an introductory paragraph
- Aims divided into Experiments or sub-aims
- Each experiment or sub-aim includes:
  - Hypothesis
  - Rationale
  - Approach
  - Expected results
  - Pitfalls and alternative approaches
- Overall significance for each Aim
- Overall significance for the proposal

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Data Elements

Preliminary Data!

- Feasibility
- Different options for presentation
- Be rigorous!

Respective Contributions

Respective Contributions

- Respective Contribution = one page in length
- Collaborative process crafting the Research Training Plan
- Discuss respective roles performing research

Sponsor Statement and Letters of Recommendation

Statement & Recommendations

- Sponsor's credentials and resources
- Support and commitment to mentoring
- Letters will establish your **research potential**
- Start early!

Resubmission Policy

Current NRSA Resubmission Policy

- Single resubmission (A1)
- Recycling ideas into a new (A0) application is ok
- Resubmissions (A1) must be submitted within 37 months of the (A0)
- For more details, visit the Resubmissions webpage and see NOT-OD-18-197.

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Resubmission

- Read your *Summary Statement*
- Add a 1-page *Introduction* detailing the changes
- You can talk to your Program Officer

How to Prepare a Successful Application

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- Start early!
- Have as many people as possible read your application!
- Be responsive to criticism
- Be aware of your weaknesses
- In a competitive environment, good grantsmanship can make all the difference
- Write clearly! Minimize jargon.
- Good figures/tables can help you
- Convey enthusiasm: make reviewers your advocates!
- A fellowship proposal should be an example of your very best work!

A Potential Method

- Write a checklist
- Think about your ultimate professional goal
- What are the “holes” in your training, courses, workshops?
- Do your research interests span disciplines, your co-mentor?
- Develop an outline. Think hard about how to formulate your hypothesis and specific aims. Think about your major weaknesses and how to overcome them.
- Develop a work plan (ask for letters early!
- Write
- Review and ask others to read it
- Revise

Be Mindful

- Don’t be overambitious!
- Don’t commit “NIH sins”: Interdependence of Aims, or things that appear incremental or confirmatory
- Don’t try to hide problems, but rather address them head-on and propose solutions
- Don’t fail to be scholarly in citing the literature
- Don’t forget to make everything explicit: significance, merits of your approach, preliminary data
- Don’t get discouraged!

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Campus Contacts

University at Albany

- Kevin Williams, Dean Graduate Studies: kwilliams@albany.edu
- Hui-Ching Chang, Dean of Honors College: hchang3@albany.edu

Binghamton University

- Jason Dearin, Grants and Contracts Administrator jdearin@binghamton.edu

University at Buffalo

- Kimberly Burns, Senior Program Manager: kshort@buffalo.edu

Downstate Health Sciences University

- Mark Stewart, Dean of School of Graduate Studies mark.stewart@downstate.edu

SUNY Polytechnic Institute

- Krista Thompson, Assistant Dean for Graduate Studies kthompson@sunypoly.edu

Stony Brook University

- Jen Green, Director for Graduate and Postdoctoral Professional Development: jennifer.green@stonybrook.edu

Upstate Medical University

- Jennifer Brennan, Executive Coordinator, Upstate College of Graduate Studies: brennanj@upstate.edu

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