F32 BOOT CAMP

From start to submission (grants due December 8, 2019)

Significance

Presented 23Sept2019

Science sections of the grant

- **Narrative**
- **Summary/abstract**
- **Aims**
- **Research Strategy**
  - **Significance**
  - **Innovation (none for F32**
  - **Approach**

page length varies

- **Other sections**
  - **Fellowship specific (sponsor, training activities, contributions, etc)**
  - **Human subjects**
  - **Vertebrate animals**
  - **Responsible conduct**
  - **Resources/Environment**

SF424 instructions for the significance section

- Explain the importance of the problem or critical barrier to progress that the proposed project addresses.
- Describe the strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project.
- Explain how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
- Describe how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.

SF424 instructions for the significance section

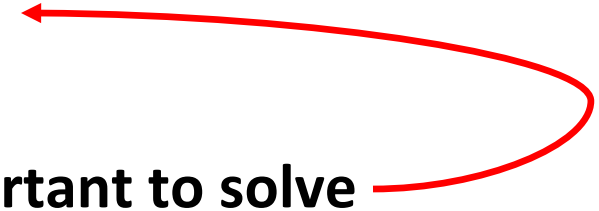
- Importance of the problem or critical barrier to progress that the project addresses
- Strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project.
- Explain how project will improve knowledge, technical capacity and/or clinical practice
- ~~How concepts, methods, technologies, treatments,~~ services, preventative interventions that drive the field will be changed

SF424 instructions for the significance section

- Importance of the problem or critical barrier to progress that the project addresses
- Explain how project will improve knowledge, technical capacity and/or clinical practice
- How concepts, methods, technologies, treatments, services, preventative interventions that drive the field will be changed

The significance section describes:

- What has been done, but with emphasis on:
  - The problem
  - Gap
  - Barrier
  - Weaknesses
- Why it's important to solve
- What this proposal will do
- How the results will move the field forward



Tips:

- **Make it easy for the reviewer**
  - **Don't give facts without indicating why the fact is important**
- **Engage the reviewer**

In comparing previous EMG channels for muscle activity analysis, visual chin EMG has provided the highest performance rating for RBD identification (94.4% identification rate)[8]. However, this approach is labor intensive and is plagued by inherent biases, which include low intra- and inter-rater reliability in both video and EMG activity interpretation for RBD identification. SA1 of this proposal is significant because it addresses labor intensiveness and inter-rater reliability concerns by offering an efficient computerized processing scheme, which will seamlessly fit within pre-existing clinical procedures, to automatically identify patients with RBD when they are evaluated with overnight EMG.

Tips:

- **FACT**
- **GAP/PROBLEM**
- **WHAT PROPOSAL WILL DO**

In comparing previous EMG channels for muscle activity analysis, visual chin EMG has provided the highest performance rating for RBD identification (94.4% identification rate)[8]. However, this approach is labor intensive and is plagued by inherent biases, which include low intra- and inter-rater reliability in both video and EMG activity interpretation for RBD identification. SA1 of this proposal is significant because it addresses labor intensiveness and inter-rater reliability concerns by offering an efficient computerized processing scheme, which will seamlessly fit within pre-existing clinical procedures, to automatically identify patients with RBD when they are evaluated with overnight EMG.

Why is it important to engage the reviewer?

Good science

Good science

Good science

Good science

Good science

Good science

Good science

Good science

Good science

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Critical need/gap

Good science
Good science
Good science
Good science

Innovative Rigorous

Good science
Good science
Good science

Move the field forward

Good science

The reviewer may start with 10 grants that all have good science – they have to narrow the field down and rank the grants based on specific factors

Why is it important to engage the reviewer?

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Critical need/gap

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Move the field forward

Good science

Your job in the Significance, then, is to build desire for your project. It is not there to show how smart or well-read you are. It is not there to add even more details to how you're going to carry out the project. It is there to give a compelling reason for the question: why does this project deserve scarce funding dollars?

https://morganonscience.com/grantwriting/nih-grant-writing-tips-the-significance-of-significance/

The Significance section is about the problem(s) –

- Why are they important?
- Whom do they impact?
- Why is a solution currently missing?
- What have people tried? (be brief with this, don't do a literature review)
- What are the strengths/weakness of the prior research?
- Why is a solution needed *now*?
- What has happened that makes you think you and your team have a solution? (in broad terms – leave the specifics for later)
- In which aim will you solve which problem?
- Why is your team qualified to solve the problem(s)?

https://morganonscience.com/grantwriting/nih-grant-writing-tips-the-significance-of-significance/

Tips:

- Approximately 1 page in an F32
- Mirror the content of the abstract and aims
- Clearly state public health implications
- Make it easy
  - Use headers
  - ‘This is significant…’, ‘This is innovative…’, ‘This will address the gap…’
- Reference
  - Avoid outdated research
  - Use enough references to support what the gap/barrier is now
  - There are no limits on the number of references, but don’t over reference (eg 8 to support a fact)

(Example of funded R21 provided by NIH – PI Petrie)

https://deainfo.nci.nih.gov/extra/extdocs/gntapp.pdf

Notably, the signals that lymphoid cells provide to stromal cells to induce their proliferation, differentiation, and/or survival are completely unknown. As mentioned above, the historical perspective of the thymus is lympho-centric, a view that is understandable since production of lymphoid cells is the main function of the thymus. This perspective is further exacerbated by the ease with which lymphoid and other hematopoietic cells are manipulated in experimental systems; in contrast, isolation and manipulation of thymic stromal cells is quite challenging, particularly in the hypotrophic state that accompanies lymphoid immunodeficiencies. To meet this challenge, we have devised a computational method for global identification of stromal gene expression *in situ* (Griffith et al., 2009). In brief, RNA isolated from microdissected tissue (cortex, medulla, or any other region of interest) is used to measure gene expression using cDNA microarrays. Simultaneously, gene expression in the lymphocytes that correspond to that region (isolated from other thymuses) is also measured by microarray. Stromal gene expression can be defined as gene expression in the tissue that is not attributable to gene expression by the corresponding lymphoid cells. The validity of this approach has been demonstrated by its ability to capture virtually all of the genes that are known to characterize stromal cells in the (young) thymus, including known stromal signals for developing lymphoid cells (e.g., Notch ligands, IL7, kit ligand, Cxcl12, MHC proteins, etc.), as well as genes known to be intrinsically required for stromal development or function (e.g., Foxn1, Pax1, Egfrs, etc.). This approach has several advantages over conventional approaches to studying thymic stromal cells. For one, there are no changes in gene expression caused by disruption of the 3D context of the thymus, or by enzymatic digestions or lengthy incubations at 37°C. This approach is also non-biased, and returns information on all of the stromal cells in a given region (the exact identify can be established later, using immunohistochemistry, RNA in situ hybridization, etc.). Most importantly for the current proposal, this approach requires very little tissue, and thus is amenable to the study of stromal cells in hypotrophic or atrophied tissues, such as the one proposed here (see Fig.1).

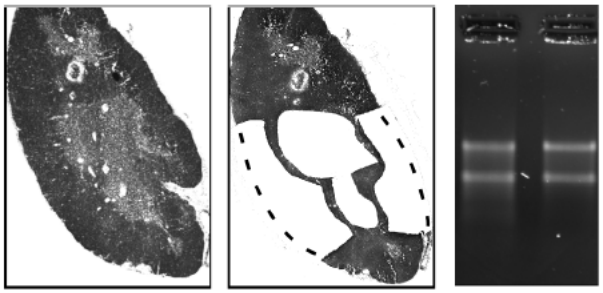

Fig.1. Isolation of RNA by microdissection of an atrophic thymus. The left image shows a transverse section (20μm thickness) from the the thymus a 12 month-old mouse; this thymus is only slightly larger than that of a 4 week-old $IL7R^{-/-}$ mouse. The middle panel shows the same section after microdissection of cortical and medullary regions. The right panel shows a gel image of mRNA isolated from these tissue

(Example of funded R21 provided by NIH –PI Petrie)

3(a). Significance. Intracellular bacterial pathogens have evolved to exploit host cells in order to survive within and spread from mammalian hosts. Many of these bacterial pathogens reside in vacuoles and translocate virulence factors into the host cell cytosol through specialized secretion systems in order to avoid destruction by the endocytic pathway and allow replication within the cell (12). The activities of secreted effectors and their impact on host cell functions is an area of intense investigation. To completely understand how bacteria impact, exploit, or affect host cell functions during infection, one must study multiple aspects of host cell protein regulation during infection, including transcriptional regulation, regulation of translation, as well as protein modification, localization and turnover. Much research activity over the past decade has focused on the opportunities available to study transcriptional regulation of host cell functions during infection. The ability to screen arrays of host cell genes during bacterial infection has led to an explosion in the available data showing which mammalian genes are induced and repressed as a result of infection. However, bacterial manipulations of host cell proteins (for example the activity of bacterial proteases) (18) cannot be discovered by monitoring transcription. It simply has not been previously possible to examine how bacterial infection directly affects the stability of individual host proteins at the scale afforded by transcriptional arrays. *L. pneumophila* has served as an exceptional model pathogen for dissecting complex changes in host cell biology that occur during infection. Especially well-characterized are the protein effectors secreted by the *L. pneumophila* type IV secretion system. Here we propose to conduct a "Global Protein Stability" (GPS) screen to determine the impact of *Legionella* type IV secretion on the stability of >12,000 individual host cell proteins. To accomplish this, we will identify proteins whose stability differs when cells are infected with wild-type *L. pneumophila* vs. a mutant strain (*ΔdotA*) that lacks a functional type IV secretion system. This approach will allow us to identify a significant subset of host cell proteins whose stability is altered by the *L. pneumophila* type IV effectors. Once we have identified proteins whose stability is altered during infection, we will conduct experiments to determine whether some of these host protein alterations are necessary for intracellular growth of *L. pneumophila*. At the same time, we will also work to identify which *L. pneumophila* effector proteins are responsible for individual protein changes in the host. As a longer-term goal, we would like to target with experimental therapeutics the host proteins that are required for successful bacterial replication – developing novel classes of "host-based" antibiotics.

The ability to identify, study and target host proteins altered by microbial infection will be compelling to most cellular microbiologists regardless of the microbe they study. Therefore we are confident that GPS will be widely adopted to study the pathogenesis of infectious disease caused by bacteria, viruses and parasites.

(Example of funded R21 provided by NIH –PI Stambach)

Notes on previous 2 examples

Additional background and rationale are provided under each aim in the approach section

Per the new instructions, you also need to address strengths and weaknesses in the prior research (published and unpublished) – If weaknesses, provide a plan to address in the strategy

Significance Vs. Impact -- How to Tell the Difference

- Significance is whether the project is important enough to carry out
- Impact is the value of what NIH gets in exchange for its grant funding at the project's completion
- Both Significance and Impact are crucial to your proposal's success, because reviewers look at the two as dependent upon one another

Problems with Significance

- Neither significant nor exciting new research (i.e., will not advance science)
- Lack of compelling rationale
- Incremental and low impact research
- Prior research that serves as the key support for the proposal is not sufficiently rigorous - AND - no plan to address the weakness

https://www.nimh.nih.gov/funding/grant-writing-and-application-process/common-mistakes-in-writing-applications.shtml

Example

Another area of important impact for automated detection of EMG signals involves monitoring treatment effects. The labor intensive nature of visually scoring muscle activity in sleep has precluded use of this important set of measures to monitor outcomes. Therefore, SA2 of my proposed research will be to apply our PEM detection system, from SA1, to demonstrate its **clinical application.** By developing better indicators of successful treatment, we will increase the quality of life and reduce bed partner injuries (lacerations, contusions, and fractures) for patients and their families[4]. Although **gender differences** in RBD diagnosis are not the primary focus for this research, the proposed methods will provide the tools to investigate these issues as well.

Example

Although it is clear that CFTR domains (and sub-domains within NBDs) assemble in an organized, co-translational manner (15, 21), and although there is increasing awareness of ways in which translational speed and mRNA codon composition (22) may impact CFTR-domain folding, no systematic studies for CF (or any other genetic disease) have been undertaken to test the influence of SNPs (both synonymous or non-synonymous) on translational velocity and consequently on protein conformation. This lack of information represents a significant gap in knowledge. With availability of full genomic sequence data for thousands of individuals with CF, large numbers of synonymous and non-synonymous sSNPs (not classically believed to influence clinical phenotype) have been identified, and the field has moved to a point where allelic heterogeneity has become increasingly invoked as the explanation for differences among individuals with the same causative variants (e.g., F508del homozygotes). This project is intended to furnish the first evidence that CF molecular defects can be profoundly impacted by sSNPs traditionally viewed as 'silent,' and that the mechanism by which this occurs is most likely attributable to effects on translational velocity. While conducting the first studies to directly test translational rate as a contributor to CF pathogenesis, we will use leading-edge ribosomal profiling and identify novel polymorphisms (including sSNPs) that alter CFTR trafficking and stability.

Summary

- Significance is about the ‘problem’
- Relate the problem to the proposal
- Describe the impact of the results on the field

- Special considerations for an F32
  - Innovation is not required
  - Proposal impact can be balanced by training impact

Resources

- **NIH grant writing tips**
- https://morganonscience.com/grantwriting/nih-grant-writing-tips-the-significance-of-significance/
- https://deainfo.nci.nih.gov/extra/extdocs/gntapp.pdf
- https://www.nimh.nih.gov/funding/grant-writing-and-application-process/common-mistakes-in-writing-applications.shtml
- https://www.nih.gov/institutes-nih/nih-office-director/office-communications-public-liaison/clear-communication/plain-language/plain-language-getting-started-or-brushing
- https://www.nih.gov/institutes-nih/nih-office-director/office-communications-public-liaison/clear-communication/plain-language