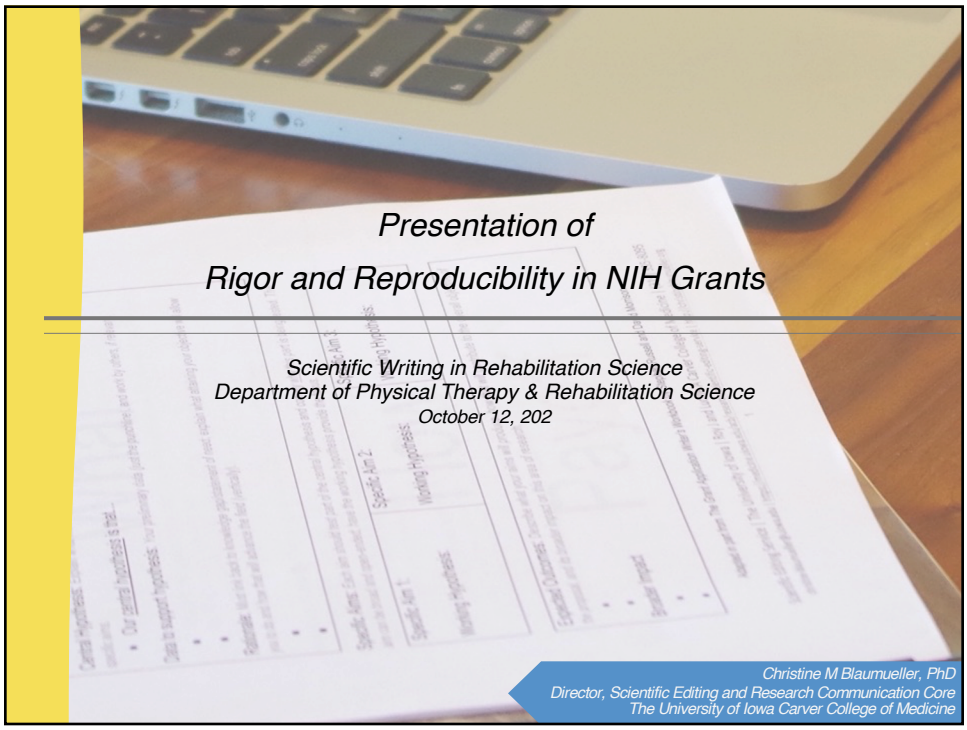
Presentation of
Rigor and Reproducibility in NIH Grants
Scientific Writing in Rehabilitation Science
Department of Physical Therapy & Rehabilitation Science
October 12, 202
Christine M Blaumueller, PhD
Director, Scientific Editing and Research Communication Core
The University of Iowa Carver College of Medicine

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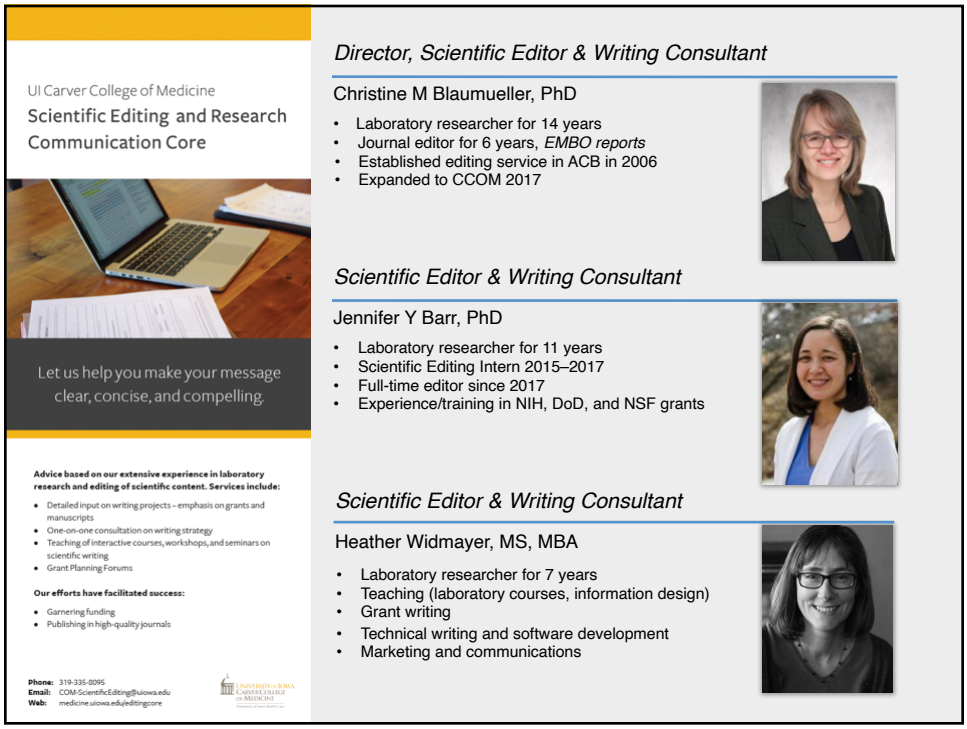
UI Carver College of Medicine
Scientific Editing and Research Communication Core
Let us help you make your message clear, concise, and compelling.
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Director, Scientific Editor & Writing Consultant
Christine M Blaumueller, PhD
Laboratory researcher for 14 years
Journal editor for 6 years, EMBO reports
Established editing service in ACB in 2006
Expanded to CCOM 2017
Scientific Editor & Writing Consultant
Jennifer Y Barr, PhD
Laboratory researcher for 11 years
Scientific Editing Intern 2015–2017
Full-time editor since 2017
Experience/training in NIH, DoD, and NSF grants
Scientific Editor & Writing Consultant
Heather Widmayer, MS, MBA
Laboratory researcher for 7 years
Teaching (laboratory courses, information design)
Grant writing
Technical writing and software development
Marketing and communications

2

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Let us help you make your message clear, concise, and compelling.

Advice based on our extensive experience in laboratory research and editing of scientific content. Services include:

- Detailed input on writing projects – emphasis on grants and manuscripts
- One-on-one consultation on writing strategy
- Teaching of interactive courses, workshops, and seminars on scientific writing
- Grant Planning Forums

Our efforts have facilitated success:

- Garnering funding
- Publishing in high-quality journals

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Email: COM-ScientificEditing@uiowa.edu
Web: medicine.uiowa.edu/editingcore

Activities:

- Provide input on drafts of writing projects
- Provide consultation on writing strategy
- Teach scientific writing
- Brainstorm with authors on projects
- Collect and generate resources
  - Changes in funding agency requirements
  - Grant writing templates (NIH "R" and "F" grants)
- Liaise with other RD professionals

3

Strategy for writing the Specific Aims page…

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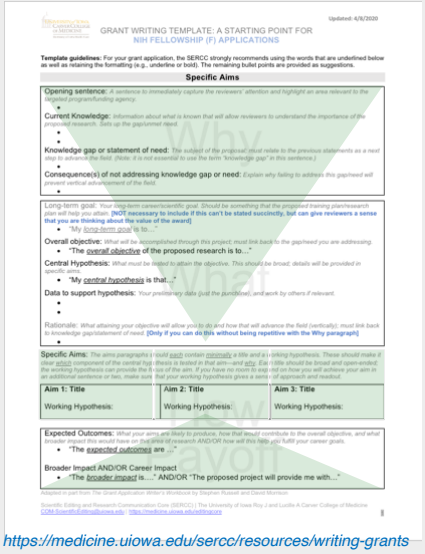


GRANT WRITING TEMPLATE: A STARTING POINT FOR NIH FELLOWSHIP (F) APPLICATIONS

Updated: 4/8/2020

Template guidelines: For your grant application, the SERCC strongly recommends using the words that are underlined below as well as retaining the formatting (e.g., underline or bold). The remaining bullet points are provided as suggestions.

Specific Aims

Opening sentence: *A sentence to immediately capture the reviewers' attention and highlight an area relevant to the targeted program/funding agency.*

Current Knowledge: *Information about what is known that will allow reviewers to understand the importance of the proposed research. Sets up the gap/unmet need.*

Knowledge gap or statement of need: *The subject of the proposal must relate to the previous statements as a next step to advance the field. (Note: it is not essential to use the term "knowledge gap" in this sentence.)*

Consequence(s) of not addressing knowledge gap or need: *Explain why failing to address this gap/need will prevent vertical advancement of the field.*

Long-term goal: *Your long-term career/scientific goal. (Should be something that the proposed training plan/research plan will help you attain.* **[NOT necessary to include if this can't be stated succinctly, but can give reviewers a sense that you are thinking about the value of the award]**

- "My long-term goal is to…"

Overall objective: *What will be accomplished through this project; must link back to the gap/need you are addressing.*

- "The overall objective of the proposed research is to…"

Central Hypothesis: *What must be tested to attain the objective. This should be broad; details will be provided in specific aims.*

- "My central hypothesis is that…"

Data to support hypothesis: *Your preliminary data (just the punchline), and work by others if relevant.*

Rationale: *What attaining your objective will allow you to do and how that will advance the field (vertically); must link back to knowledge gap/statement of need.* **[Only if you can do this without being repetitive with the Why paragraph]**

Specific Aims: *The aims paragraphs could each contain minimally a title and a working hypothesis. These should make it clear which component of the central hypothesis is tested in that aim—and why. Each title should be broad and open-ended; the working hypothesis can provide the fi ne-tuning of the aim. If you have no room to explain how you will achieve your aim in an additional sentence or two, make sure that your working hypothesis gives a sense of approach and readout.*

Aim 1: Title	Aim 2: Title	Aim 3: Title
Working Hypothesis:	Working Hypothesis:	Working Hypothesis:

Expected Outcomes: *What your aims are likely to produce, how that would contribute to the overall objective, and what broader impact this would have on the field of research AND/OR how will this help you fulfill your career goals.*

- "The expected outcomes are …"

Broader Impact AND/OR Career Impact

- "The broader impact is…" AND/OR "The proposed project will provide me with…"

Adapted in part from The Grant Application Writer's Workbook by Stephen Russell and David Morrison.

Scientific Editing and Research Communication Core (SERCC) | The University of Iowa Roy J and Lucille A Carver College of Medicine
COM-ScientificEditing@uiowa.edu | https://medicine.uiowa.edu/sercc/resources

https://medicine.uiowa.edu/sercc/resources/writing-grants

Opening sentence
Current knowledge
Gap in knowledge/why it matters

Long-term goal
Objective (proposed research)
Central hypothesis (or urgent need)
Rationale for study

Aims Title
Hypothesis of Aim

Expected outcomes
Broader impact
Impact on career goals

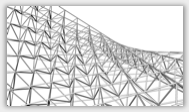
4

Rigor and Reproducibility in NIH Grants

NIH Instructions

Fellowship Research Strategy

Rigor and Reproducibility



How to Structure the Research Strategy

Examples of Wording

Rigor & Reproducibility

Other Aspects

Questions?

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Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

Research Training Plan:

1) Specific Aims page, no > 1 page (include 2–4 aims)

2) Research Strategy, no > 6 pages

   a. Significance

   b. Approach

SF424 (R&R) APPLICATION PACKAGES
FELLOWSHIP INSTRUCTIONS FOR NIH AND OTHER PHS AGENCIES
Forms F series

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Changes in NIH Requirements Since 2010

- 2010 – for all grant mechanisms
  - Shortened Research Strategy by 50%
  - "Background and Significance" –> "Significance"
  - "Innovation" section added (for "K" and "R" mechanisms)
- 2016 – for K and R mechanisms
  - Required evidence of rigor and reproducibility, including:
    - Discussion of ***scientific premise*** within Significance section
    - Discussion of ***rigor of proposed research*** in Approach
    - Discussion of ***biological variables*** in Approach
    - Explanation of ***how key resources will be authenticated*** (attachment)

For Fellowship (F) mechanisms summer 2020

- 2019 – for K and R mechanisms
  - Changed ***scientific premise*** to ***weaknesses in rigor of prior research***
  - Requires discussion of ***how weaknesses in rigor of prior research will be addressed*** in Approach

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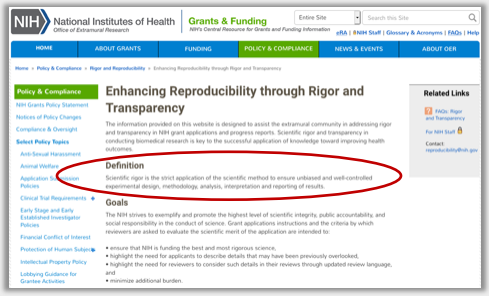
NIH definition of Scientific Rigor (2018)…

- *the strict application of the scientific method*
- *to ensure unbiased and well-controlled*
  - *— experimental design*
  - *— methodology*
  - *— analysis*
  - *— interpretation and*
  - *— reporting*
- *of results*



https://grants.nih.gov/policy/reproducibility/index.htm

Posted 11/27/18

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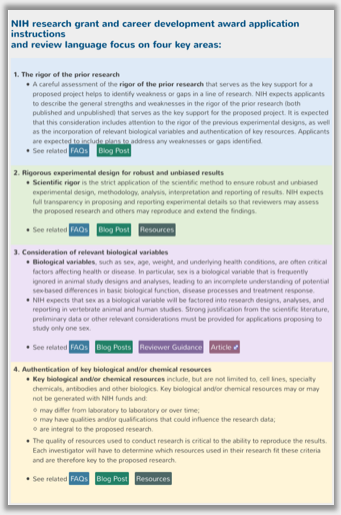
Current NIH Instructions



NIH research grant and career development award application instructions and review language focus on four key areas:

1. **The rigor of the prior research**
   - A careful assessment of the **rigor of the prior research** that serves as the key support for a proposed project helps to identify weakness or gaps in a line of research. NIH expects applicants to describe the general strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project. It is expected that this consideration includes attention to the rigor of the previous experimental designs, as well as the incorporation of relevant biological variables and authentication of key resources. Applicants are expected to include plans to address any weaknesses or gaps identified.
   - See related FAQs Blog Post
2. **Rigorous experimental design for robust and unbiased results**
   - **Scientific rigor** is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results. NIH expects full transparency in proposing and reporting experimental details so that reviewers may assess the proposed research and others may reproduce and extend the findings.
   - See related FAQs Blog Post Resources
3. **Consideration of relevant biological variables**
   - **Biological variables**, such as sex, age, weight, and underlying health conditions, are often critical factors affecting health or disease. In particular, sex is a biological variable that is frequently ignored in animal study designs and analyses, leading to an incomplete understanding of potential sex-based differences in basic biological function, disease processes and treatment response.
   - NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data or other relevant considerations must be provided for applications proposing to study only one sex.
   - See related FAQs Blog Posts Reviewer Guidance Article
4. **Authentication of key biological and/or chemical resources**
   - **Key biological and/or chemical resources** include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics. Key biological and/or chemical resources may or may not be generated with NIH funds and:
     - may differ from laboratory to laboratory or over time;
     - may have qualities and/or qualifications that could influence the research data;
     - are integral to the proposed research.
   - The quality of resources used to conduct research is critical to the ability to reproduce the results. Each investigator will have to determine which resources used in their research fit these criteria and are therefore key to the proposed research.
   - See related FAQs Blog Post Resources

Rigor: prior research

Rigor: proposed research

Relevant biological variables

Authentication of biol/chem resources

https://grants.nih.gov/policy/reproducibility/guidance.htm
Updated November 26, 2018

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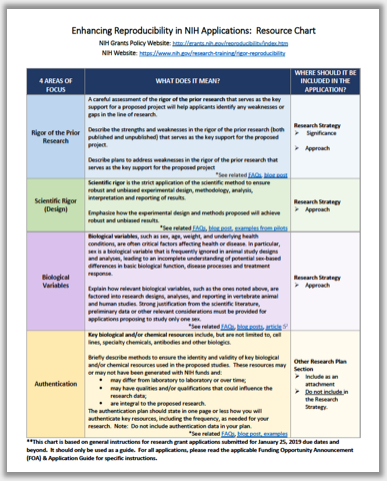
Rigor of prior research – Additional clarification



Enhancing Reproducibility in NIH Applications: Resource Chart

NIH Grants Policy Website: http://grants.nih.gov/reproducibility/index.htm
NIH Website: https://www.nih.gov/research-training/rigor-reproducibility

4 AREAS OF FOCUS	WHAT DOES IT MEAN?	WHERE SHOULD IT BE INCLUDED IN THE APPLICATION?
Rigor of the Prior Research	A careful assessment of the rigor of the prior research that serves as the key support for a proposed project will help applicants identify any weaknesses or gaps in the line of research. 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. Describe plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project. *See related FAQs, blog post	Research Strategy ➢ Significance ➢ Approach
Scientific Rigor (Design)	Scientific rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results. Emphasize how the experimental design and methods proposed will achieve robust and unbiased results. *See related FAQs, blog post, examples from pilots	Research Strategy ➢ Approach
Biological Variables	Biological variables, such as sex, age, weight, and underlying health conditions, are often critical factors affecting health or disease. In particular, sex is a biological variable that is frequently ignored in animal study designs and analyses, leading to an incomplete understanding of potential sex-based differences in basic biological function, disease processes and treatment response. Explain how relevant biological variables, such as the ones noted above, are factored into research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data or other relevant considerations must be provided for applications proposing to study only one sex. *See related FAQs, blog posts, article	Research Strategy ➢ Approach
Authentication	Key biological and/or chemical resources include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics. Briefly describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies. These resources may or may not have been generated with NIH funds and: • may differ from laboratory to laboratory or over time; • may have qualities and/or qualifications that could influence the research data; • are integral to the proposed research. The authentication plan should state in one page or less how you will authenticate key resources, including the frequency, as needed for your research. Note: Do not include authentication data in your plan. *See related FAQs, blog post, examples	Other Research Plan Section ➢ Include as an attachment ➢ Do not include in the Research Strategy.

**This chart is based on general instructions for research grant applications submitted for January 25, 2019 due dates and beyond. It should only be used as a guide. For all applications, please read the applicable Funding Opportunity Announcement (FOA) & Application Guide for specific instructions.

*A careful assessment of the **rigor of the prior research** that serves as the key support for a proposed project will help applicants identify any weaknesses or gaps in the line of research.*

- *In Significance section: 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.*
- *In Approach section: Describe plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project.*

https://grants.nih.gov/policy/reproducibility/guidance.htm
Updated November 26, 2018

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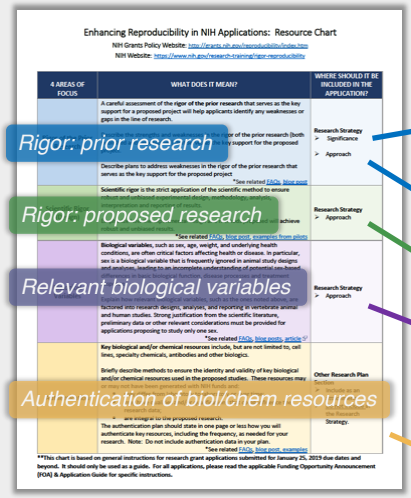
Adapting to change



https://grants.nih.gov/policy/reproducibility/guidance.htm; Updated November 26, 2018

- SERCC: Are new requirements addressed in the *right places*?

Significance

- Weaknesses in rigor of *prior research*

Approach

- How weaknesses in rigor of *prior research* will be addressed
- How rigor of *proposed research* will be ensured
- Consideration of biological variables, including sex, in the proposed research

- Divison of Sponsored Programs:
  - Are requirements addressed? (Will return application if Authentication page is missing)

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Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

Research Training Plan:

1) Specific Aims page, no > 1 page (include 2–4 aims)
2) Research Strategy, no > 6 pages
   a) Significance: *Describe…*
      - *the **importance of the problem or critical barrier** to progress that the proposed project addresses.*
      - *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.*
      - ***how the proposed project will improve** scientific knowledge, technical capability, and/or clinical practice in one or more **broad fields**.*
      - ***how** the concepts, methods, technologies, treatments, services, or preventative interventions that drive **this field will be changed** if the proposed aims are achieved.*
   b) Approach

Green text: relevant to Rigor & Reproducibility

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Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

a) Significance

b) Approach: *Describe…*

- *The overall strategy, methodology, and analyses to be used…*
- *Potential problems, alternative strategies, and benchmarks for success*
- *If the project is in the early stages of development, any strategy to establish feasibility/address management of any high risk aspects*
- *How relevant biological variables, such as sex, are factored into research designs/analyses for studies in vertebrate animals/humans*
- *Any procedures/situations/materials that may be hazardous to personnel and the precautions to be exercised*
- *If research on Human Embryonic Stem Cells (hESCs) is proposed but an approved cell line from the NIH hESC Registry cannot be chosen, strong justification for why*
- *If you are proposing to gain clinical trial research experience (i.e., you will not be leading an independent clinical trial), your role on the clinical trial*

Blue text: relevant to Rigor & Reproducibility

13

Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

a) Significance

b) Approach: *Describe…*

- *The overall strategy, methodology, and analyses to be used, including:*
  - *plans to address weaknesses in rigor of prior research that serves as the key support for the proposed project*
  - *experimental design and methods proposed; how they will achieve robust and unbiased results*
  - *how the data will be collected, analyzed, and interpreted, and any resource sharing plans, as appropriate.*
  - *methods for analysis and sample size determination, as appropriate*
- *Potential problems, alternative strategies, and benchmarks for success*
- *If the project is in the early stages of development, any strategy to establish feasibility/address management of any high risk aspects*
- *How relevant biological variables, such as sex, are factored into research designs/analyses for studies in vertebrate animals/humans.*

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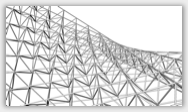
Topics

NIH Instructions

Fellowship Research Strategy

Rigor and Reproducibility



How to Structure the Research Strategy

Examples of Wording

Rigor & Reproducibility

Other Aspects

Questions?

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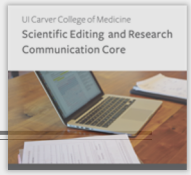
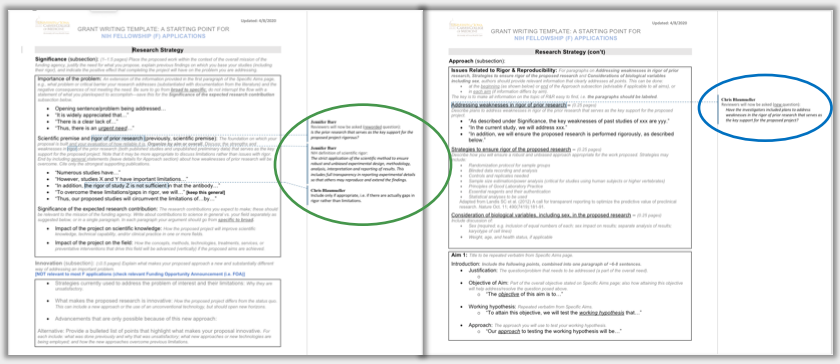
Our grant writing templates…





https://medicine.uiowa.edu/sercc/resources/writing-grants

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Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

Research Training Plan:

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2) Research Strategy, no > 6 pages

a) Significance: *Describe…*

- *the **importance of the problem or critical barrier** to progress that the proposed project addresses.*
- *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.*
- ***how the proposed project will improve** scientific knowledge, technical capability, and/or clinical practice in one or more **broad fields**.*
- ***how** the concepts, methods, technologies, treatments, services, or preventative interventions that drive **this field will be changed** if the proposed aims are achieved.*

b) Approach

Green text: relevant to Rigor & Reproducibility

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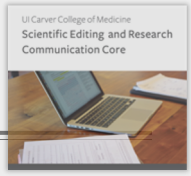
Significance – Our Recommendations 2019



Importance of the problem and/or critical barriers to progress

Scientific premise *and rigor of the prior research* *

Significance of the expected research contribution

- Impact of the project on scientific knowledge / technical capability / clinical practice
- Impact of the project on the field

* The relevant literature: Strengths and weaknesses

- Rigor of study design (e.g. statistical power, blinded analysis)
- Incorporation of relevant biological variables (e.g. detail regarding sex)

Your preliminary data that contribute to scientific foundation of proposal.

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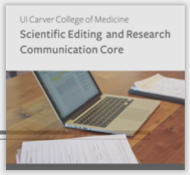
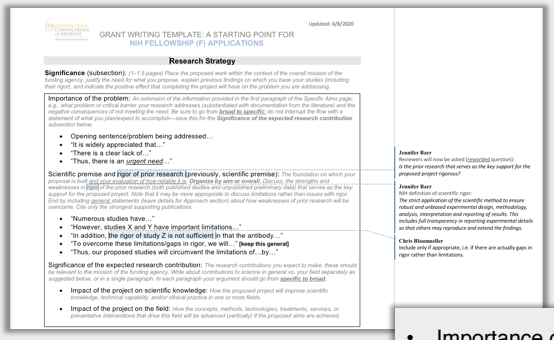
Significance Section…





GRANT WRITING TEMPLATE: A STARTING POINT FOR NIH FELLOWSHIP (F) APPLICATIONS

Updated: 4/8/2020

Research Strategy

Significance (subsection): *(1–1.5 pages) Place the proposed work within the context of the overall mission of the funding agency, justify the need for what you propose, explain previous findings on which you base your studies (including their rigor), and indicate the positive effect that completing the project will have on the problem you are addressing.*

Importance of the problem: *An extension of the information provided in the first paragraph of the Specific Aims page, e.g., what problem or critical barrier your research addresses (substantiated with documentation from the literature) and the negative consequences of not meeting the need. Be sure to go from broad to specific; do not interrupt the flow with a statement of what you plan/expect to accomplish—save this for the* ***Significance of the expected research contribution*** *subsection below.*

- Opening sentence/problem being addressed…
- "It is widely appreciated that…"
- "There is a clear lack of…"
- "Thus, there is an urgent need…"

Scientific premise and rigor of prior research (previously, scientific premise): *The foundation on which your proposal is built and your evaluation of their validity, e.g., Organize by aim or overall. Discuss the strengths and weaknesses in rigor of the prior research (both published studies and unpublished preliminary data) that serves as the key support for the proposed project. Note that it may be more appropriate to discuss limitations rather than issues with rigor. End by including general statements (leave details for Approach section) about how weaknesses of prior research will be overcome. Cite only the strongest supporting publications.*

- "Numerous studies have…"
- "However, studies X and Y have important limitations…"
- "In addition, the rigor of study Z is not sufficient in that the antibody…"
- "To overcome these limitations/gaps in rigor, we will…" **[keep this general]**
- "Thus, our proposed studies will circumvent the limitations of…by…"

Significance of the expected research contribution: *The research contributions you expect to make; these should be relevant to the mission of the funding agency. Write about contributions to science in general vs. your field separately as suggested below, or in a single paragraph. In each paragraph your argument should go from specific to broad.*

- **Impact of the project on scientific knowledge:** *How the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more fields.*
- **Impact of the project on the field:** *How the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be advanced (vertically) if the proposed aims are achieved.*

Jennifer Barr
Reviewers will now be asked (rewarded question):
Is the prior research that serves as the key support for the proposed project rigorous?

Jennifer Barr
NIH definition of scientific rigor:
The strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results. This includes full transparency in reporting experimental details so that others may reproduce and extend the findings.

Chris Bloomwriter
Include only if appropriate, i.e. if there are actually gaps in rigor rather than limitations.

Limit to 1–1.5 pages

- Importance of Problem
- Scientific Premise and Rigor of Prior Research
- Significance of Expected Research Contribution
  - impact on scientific knowledge
  - impact on the field

https://medicine.uiowa.edu/sercc/resources/writing-grants

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Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

a) Significance

b) Approach: *Describe…*

- *The overall strategy, methodology, and analyses to be used, including…*
- *Potential problems, alternative strategies, and benchmarks for success*
- *If the project is in the early stages of development, any strategy to establish feasibility/address management of any high risk aspects*
- *How relevant biological variables, such as sex, are factored into research designs/analyses for studies in vertebrate animals/humans*
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- *If you are proposing to gain clinical trial research experience (i.e., you will not be leading an independent clinical trial), your role on the clinical trial*

Blue text: relevant to Rigor & Reproducibility

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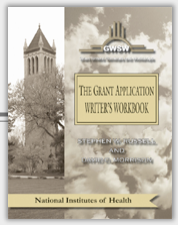
Structure for ***Approach*** section – previously



Approach

- For each aim
  - Title of Specific Aim
  - Introduction/rationale paragraph
  - Justification and Feasibility paragraph (including background and preliminary data)
  - Research Design paragraphs
  - Expected Outcomes paragraph
  - Potential Problems and Alternative Strategies paragraph
- Timeline and Benchmarks for success
- Future Directions

Research Design Paragraphs:
- Approach to be used
- Overview of methods used
- Essential minor/major equipment
- Detailed expectations
- How results will be interpreted

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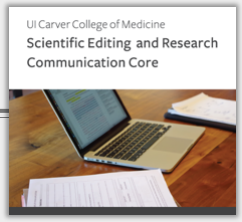
Structure for ***Approach*** section – new



Approach

- Issues related to rigor and reproducibility
  - Addressing weaknesses in rigor of prior research
  - Strategies to ensure rigor of proposed research
  - Consideration of biological variables including sex
- Aim x (for each aim)
  - Title of Specific Aim
  - Introduction/rationale paragraph
  - Justification and Feasibility paragraph (including background and preliminary data)
  - Research Design paragraphs
  - Expected Outcomes paragraph
  - Potential Problems and Alternative Strategies paragraph
- Timeline and Benchmarks for success
- Future Directions

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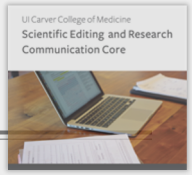
Approach – Our Recommendations 2019

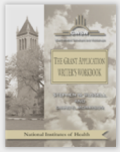


Approach

- Issues related to rigor and reproducibility
  - Addressing weaknesses in rigor of prior research
  - Strategies to ensure rigor of proposed research
  - Consideration of biological variables including sex

Separate paragraphs or combined

- Aim x (for each aim)
  - Title of Specific Aim
  - Introduction/rationale paragraph
  - Justification and Feasibility paragraph (including background and preliminary data)
  - Research Design paragraphs
  - Expected Outcomes paragraph
  - Potential Problems and Alternative Strategies par
- Timeline and Benchmarks for success
- Future Directions



Stephen W. Russell & David C. Morrison
Grant Writers' Seminars and Workshops, LLC
http://www.grantcentral.com

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Structure for ***Approach*** section — new

Approach

- Issues related to rigor and reproducibility
  - Addressing weaknesses in rigor of prior research
  - Strategies to ensure rigor of proposed research
  - Consideration of biological variables including sex
- Aim x (for each aim)
  - Title of Specific Aim
  - Introduction/rationale paragraph
  - Justification and Feasibility paragraph
  - Research Design paragraphs
  - Expected Outcomes paragraph
  - Potential Problems and Alternative Strategies paragraph
- Timeline and Benchmarks for success
- Future Directions

1. ***Rigor of proposed research*** *–> robust, unbiased results (discuss any of the categories below that apply)*
   - *Randomization protocol for sample groups, inclusion/exclusion criteria*
   - *Blinded data recording and analysis*
   - *Controls and replicates needed*
   - *Sample-size estimation/power analysis (critical for studies using human subjects and higher vertebrates)*
   - *Principles of good laboratory practice*
   - *Essential reagents and their authentication*
   - *Statistical analyses to be used*
   - *Controls and replicates needed*
2. ***Relevant biological variables including sex***
   - *Sex (equal numbers of each; impact on results; separate analysis of effects; karyotype of cell lines)*
   - *Weight, age, health status, body mas index, underlying comorbid conditions…*

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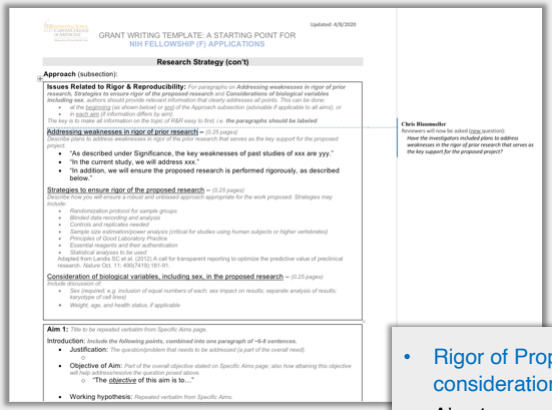
Approach Section…

UI Carver College of Medicine
Scientific Editing and Research Communication Core



GRANT WRITING TEMPLATE: A STARTING POINT FOR
NIH FELLOWSHIP (F) APPLICATIONS

Updated: 4/8/2020

Research Strategy (con't)

Approach (subsection):

Issues Related to Rigor & Reproducibility: *For paragraphs on Addressing weaknesses in rigor of prior research, Strategies to ensure rigor of the proposed research and Considerations of biological variables including sex, authors should provide relevant information that clearly addresses all points. This can be done:*

- *at the beginning (as shown below) or end of the Approach subsection (advisable if applicable to all aims), or*
- *in each aim (if information differs by aim).*

The key is to make all information on the topic of R&R easy to find, i.e. the paragraphs should be labeled

Addressing weaknesses in rigor of prior research – *(0.25 pages)*
Describe plans to address weaknesses in rigor of the prior research that serves as the key support for the proposed project.

- "As described under Significance, the key weaknesses of past studies of xxx are yyy."
- "In the current study, we will address xxx."
- "In addition, we will ensure the proposed research is performed rigorously, as described below."

Strategies to ensure rigor of the proposed research – *(0.25 pages)*
Describe how you will ensure a robust and unbiased approach appropriate for the work proposed. Strategies may include:

- *Randomization protocol for sample groups*
- *Blinded data recording and analysis*
- *Controls and replicates needed*
- *Sample size estimation/power analysis (critical for studies using human subjects or higher vertebrates)*
- *Principles of Good Laboratory Practice*
- *Essential reagents and their authentication*
- *Statistical analyses to be used*

Adapted from Landis SC et al. (2012) A call for transparent reporting to optimize the predictive value of preclinical research. Nature Oct 11; 490(7419):181-91.

Consideration of biological variables, including sex, in the proposed research – *(0.25 pages)*
Include discussion of:

- *Sex (required; e.g. inclusion of equal numbers of each; sex impact on results; separate analysis of results; karyotype of cell lines)*
- *Weight, age, and health status, if applicable*

Aim 1: *Title to be repeated verbatim from Specific Aims page.*

Introduction: *Include the following points, combined into one paragraph of ~6-8 sentences.*

- Justification: *The question/problem that needs to be addressed (a part of the overall need).*
- Objective of Aim: *Part of the overall objective stated on Specific Aims page; also how attaining this objective will help address/resolve the question posed above.*
  - "The objective of this aim is to…"
- Working hypothesis: *Repeated verbatim from Specific Aims.*

Chris Bloomseller
Reviewers will now be asked (new question):
How the investigators included plans to address weaknesses in the rigor of prior research that serves as the key support for the proposed project?

4–5 pages

- Rigor of Proposed Research (including consideration of Biological Variables)
- Aim 1
- Aim 2
- Timeline
- Future Directions

https://medicine.uiowa.edu/sercc/resources/writing-grants

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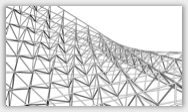
Topics

NIH instructions

Fellowship Research Strategy

Rigor and Reproducibility



How to Structure the Research Strategy

Examples of Wording

Rigor & Reproducibility

Other Aspects

Questions?

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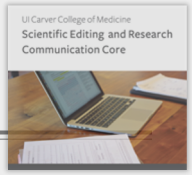
Significance – Our Recommendations 2019



1) Importance of the problem and/or critical barriers to progress
2) Scientific premise and rigor of the prior research (can organize overall or by aim)
   - Numerous studies have…
   - However, studies X and Y have important limitations…
   - In addition, the rigor of study Z was not sufficient in that…
   - To overcome these gaps in rigor, we will… [keep this general here]
   - Thus, our proposed studies will circumvent the limitations of… by …
3) Significance of the expected research contribution
   - Impact of the project on scientific knowledge / technical capability / clinical practice
   - Impact of the project on the field

If there was a lack of rigor and it's possible to discuss diplomatically…

Specifically mention limitations ... good lead-in for innovation

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Example of Strategies to Ensure Rigor (from our authors)

R37 Renewal, scored in 2nd percentile – New subsection (after Aim 3)

Research Rigor and Transparency: *Scientific rigor and reproducibility is maintained when opportunities for error are minimized through education of the team members about potential sources of error. To this end, the PI, staff, and students consult a Biostatistics and Research Design Core within the UI Institute for Clinical and Translational Sciences in the methodological planning of research protocols. This ensures robust statistical outcomes and post-experimental analysis of data. The PI and all associated personnel have also received NIH-mandated ethics training. All data will be reviewed by multiple team members to ensure its validity and to minimize operator biases; this occurs formally at twice weekly lab meetings, informally between trainees and the PI, and at the time of manuscript preparation, when the PI reviews all the raw data files. Morphometric analysis will be performed by blinded teams of students. Inbred C57BL6 strains will be used, with the exception of CF mice for which sibling CF and WT or heterozygous animals will be compared as previously described[78].*

Key points:
- *Multiple approaches used to test each hypothesis.*
- *Multiple steps in process of data review and analysis ensure validity and minimize author bias.*
- *The rigor of the scientific approach is outstanding.*

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Examples of Strategies to Ensure Rigor (posted by NIH)

- *Excerpts from awarded applications reviewed under a pilot FOA for rigorous experimental design … this is only one part of updated instruction and review language.*
- *Selected based on high overall impact scores and positive reviewer comments specific to rigor.*
- *Provided to show how elements of rigor and transparency have been succinctly provided in applications; they may not represent all of the aspects/may still have room for improvement.*
- *May be updated as applications are reviewed and awarded under the revised rigor and transparency review.*

Example 1:

Aim 3: Male and female mice will be randomly allocated to experimental groups at age 3 months. At this age the accumulation of CUG repeat RNA, sequestration of MBNL1, splicing defects, and myotonia are fully developed. The compound will be administered at 3 doses (25%, 50%, and 100% of the MTD) for 4 weeks, compared to vehicle-treated controls. IP administration will be used unless biodistribution studies indicate a clear preference for the IV route. A group size of n = 10 (5 males, 5 females) will provide 90% power to detect a 22% reduction of the CUG repeat RNA in quadriceps muscle by qRT-PCR (ANOVA, α set at 0.05). The treatment assignment will be blinded to investigators who participate in drug administration and endpoint analyses. This laboratory has previous experience with randomized allocation and blinded analysis using this mouse model [refs]. Their results showed good reproducibility when replicated by investigators in the pharmaceutical industry [ref].

Key points:
- *Number of groups, allocation random, age, why that age.*
- *Dosage, number of doses administered*
- *Route of administration, contingency*
- *Group size, power*
- *Blinding, of whom*
- *Experience*

Rigor and Reproducibility
https://grants.nih.gov/reproducibility/index.htm

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Examples of Strategies to Ensure Rigor (posted by NIH)

Example 2:

Aim 1: Primary screen: In this high throughput screening assay, we combined the SMN promoter with exons 1-6 and an exon 7 splicing cassette in a single construct that should respond to compounds that increase SMN transcription, exon 7 inclusion, or potentially stabilize the SMN RNA or protein [refs]. The details of the assay and the SMN2-luciferase reporter HEK393 cell line have been extensively validated [refs]. Each point is run in triplicate, the compounds are tested on three separate occasions, and the results are averaged to give an EC50 with standard deviation. Secondary screen: …We analyze SMN protein levels by dose response in quantitative immunoblots with statistical analysis by one-way ANOVA with post-hoc analysis using Dunnett or Bonferroni, as appropriate.

Aim 2: Each set of compounds will include a blinded negative control compound that has been determined to be inactive and that is solubilized in the same manner as test compounds. Mice will be randomly assigned within a litter, and data will be collected and submitted to the PI. For compounds that demonstrate extended survival, the PI will be sure to have these tested in {the collaborators'} labs, and data will be merged and evaluated. To calculate the number of the experimental mice, we will perform an SSD sample size power analysis to ensure that the appropriately minimal number of mice is used in each experimental context. Typically for each compound in life span studies, we will need ~20 SMA animals in the treated group; ~20 SMA animals in the vehicle treated group; ~20 SMA animals in the untreated group. If we can administer the compound in aqueous solution without expedient, the vehicle and untreated groups might be combined, as these should have identical survival. Therefore, no more than 80 SMA animals will be needed per compound.

Key points:

Aim 1
- Brief summary of overall approach
- Number of replicates, same/ different dates, reporting of average with standard deviation
- Types of statistical analysis

Aim 2
- Blinding, solubilization of test and control compounds
- Random assignments
- Who will analyze
- Power analysis; number of animals per group
- Number of animals, contingency

Rigor and Reproducibility https://grants.nih.gov/reproducibility/index.htm

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Examples of Strategies to Ensure Rigor (posted by NIH)

Example 3:

Aim 2: Intensity signal data will be transformed into log values and then modeled by longitudinal methods (reference cited). Specifically, the composite difference in mean intensity signals over time between the bi-specific T cells vs. control groups is assumed to be 2.8 logs with a composite standard deviation of 2.2 logs. Furthermore, we will assume at least five repeated measurements per mouse after T cell infusion and a within-mouse intra-correlation coefficient equal to 0.50. Thus, a sample size of 10 mice per group will provide at least 80% power to detect the above difference between treated versus control group with a 5% significance level. Log-rank test will be used to compare the survival distribution between groups. VAS: Animal numbers are based on the requirement to perform each experiment (power and sample size calculations are described in the Research Strategy), which includes an independent experimental repeat.

Key points, Example 3:

- Methods for conversion of signal data and modeling
- Number of measurements and assumptions made for power analysis
- Statistical measures to be used
- Numbers of animals needed; to be determined independently for each experiment

Example 4:

Aim 1: Statistical considerations: In our preliminary studies consisting of this same cohort of DFUs (n=100) and utilizing 16S rRNA sequencing, we were able to detect dimensions of DFU microbiome, including microbial diversity, that were significantly associated with DFU outcomes. We therefore anticipate that the sample size will provide sufficient power to detect significant differences using metagenomic sequencing, as this is a more sensitive and less-biased assay of microbial identification and diversity.

Key points, Example 4:

- Statistical considerations based on preliminary data
- Anticipated power of sample size for new, more sensitive assay
- Statistical measures to be used

Rigor and Reproducibility
https://grants.nih.gov/reproducibility/index.htm

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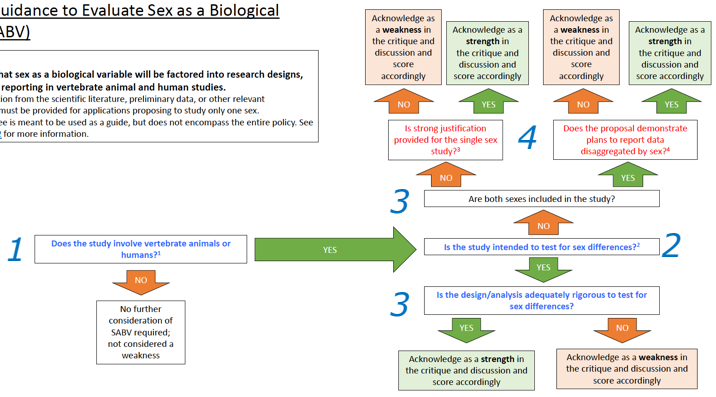
Consideration of Sex as a Biological Variable (SABV)

Reviewer Guidance to Evaluate Sex as a Biological Variable (SABV)

Main points

- **NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies.**
- Strong justification from the scientific literature, preliminary data, or other relevant considerations must be provided for applications proposing to study only one sex.
- This decision tree is meant to be used as a guide, but does not encompass the entire policy. See NOT-OD-15-102 for more information.



Notes
[1] See FAQs on inclusion, primary cells and tissues, and established cell lines.
[2] See FAQs on considering sex as a biological variable and use of males and females in basic research.
[3] See FAQ on justification of single sex studies.
[4] Based on the research question and availability of relevant data, statistically powered comparisons between the sexes may not be required. Analyzing and publishing sex-based data, even in the absence of powered sex differences analyses, would permit the consideration of the influence of sex in the interpretation of study results and the appropriate generalization of research findings.

Rigor and Reproducibility
https://grants.nih.gov/reproducibility/index.htm

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Example of Consideration of SABV

"Recent" (2016) example including SABV – New subsection (before Aim 1)

Methods to achieve robust and unbiased results:

… and WT littermate controls were generated as described in Fig. 1. These lines were genotyped and cataloged across 10 backcrosses into the C57BL/6J strain. Only animals that are of the same genetic background and handled in the same way will be compared. Congenic Xxxx KO mice (B6.129P2-Xxxx^{zzzz}/J; stock #xxxx) were obtained from Jackson Laboratories. These mice had been backcrossed with C57BL/6J animals >30 generations. For cultures of dissociated PFC cells obtained from neonates, there is no reason to think that gender differences exist; hence male and female pups will be randomly allocated to experimental groups at P1. For the experiments involving [brain] slices from P30 animals, samples will be prepared from equal numbers of age-matched male and female animals and results will be tracked by gender. Each experiment will be performed in triplicate and repeated at least three times. Dose-response and time-course analyses will be conducted for each compound to ensure that the responses are maximal. We have extensive experience with blinded analysis, treatment paradigms, and group analyses$^{e.g.50\text{-}55}$. The Co-Investigator has extensive experience in establishing LTP and LTP-D paradigms in both rats and mice^{44,45}. Experimental designs are rigorously vetted including, at a minimum, testing of only a priori hypotheses and blinding for subjective ratings. Except as noted, biological and chemical resources will be obtained from standard commercial suppliers; effects of novel agents are documented in the literature. Data will be analyzed using ANOVA followed by posthoc testing with Student's t-test.

NO

YES

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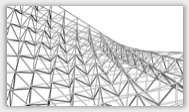
Topics

NIH instructions

Fellowship Research Strategy

Rigor and Reproducibility



How to Structure the Research Strategy

Examples of Wording

Rigor & Reproducibility

Other Aspects

Questions?

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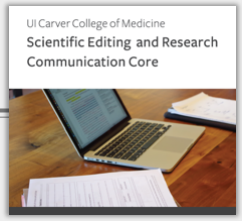
Structure for ***Approach*** section – new



Approach

- Issues related to rigor and reproducibility
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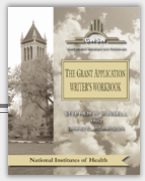
Suggested structure for Approach section



Introduction paragraph, formula:

- Justify why this aim needs to be performed / what aspect of the overall problem will be addressed (1–2 sentences only)
- Explicitly state the objective, e.g. "The *objective of this aim* is to…"
- Restate (verbatim) the working hypothesis from Specific Aims page, e.g.: "To attain the objective of this aim we will test the *working hypothesis* that …"
- State the overall strategy / approach for testing hypothesis (1–2 sentences)
- Provide rationale for work under this aim (i.e. what will become possible after this work is carried out)
- Summarize overall outcome and positive impact of this aim, at a general level

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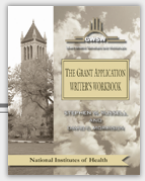
Suggested structure for Approach section



Justification (& Feasibility) paragraph:

- Will potentially include two kinds of information
  - Justification of need — based on the literature
  - Evidence that you can do the work necessary to solve the problems you have framed — preliminary data
- If so, you need a good transition that bridges justification of need to evidence of feasibility, e.g.:

These findings illustrate that … identifying x will be necessary to understand … , which will require knowledge of … . The following preliminary data support the feasibility of this approach in our hands.

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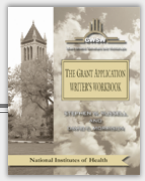
Suggested structure for Approach section



Research design paragraphs:

- Write paragraphs related to ***research activities*** that will be undertaken to accomplish the objectives of that aim.
- In each paragraph, make a single conceptual point.
- Start each research activity off with an interest-grabbing headline.

Aim 2.1. Determine which cells require the Mmd protein
Approach / methods overview / essential reagents / critical equipment / numbers of subjects/animals and how numbers were derived

statistical analysis to be used / controls / replicates / detailed expectations / how results will be interpreted / any major anticipated problems / time to complete

Aim 2.2. Identify the mechanism whereby TXS activity repositions Mmd
Approach / methods overview / essential reagents / critical equipment …

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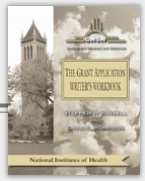
Suggested structure for Approach section



In writing ***research activities*** for each aim:

- Emphasize concepts
- Avoid anything tangential

For methodologies:
[as a student, you may need to include more detail than more senior researchers]

- reference any appropriate papers by your research team;
- if nobody on team has published with a certain methodology, then include preliminary data on this;
- refer to earlier descriptions of protocols where they are repeated.

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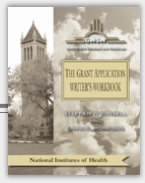
Suggested structure for Approach section



Expected Outcomes paragraph (very important!):

- Purpose – Highlight expected return on the agency's investment more explicitly than in summary or in introductory paragraph for the aim.
- In this paragraph you should:
  - summarize expected outcomes for this aim (one per activity)
  - convey how outcomes collectively achieve the objective of the aim
  - underscore importance of this activity to:
    - the field, of its own accord
    - the overall objective of this aim
  - mention any important caveats

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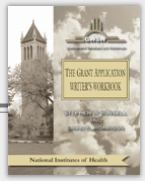
Suggested structure for Approach section



Expected Outcomes paragraph (very important!):

- Example language for this —

These experiments will provide the first analyses of …. Biochemical analyses will provide …, yielding a level of knowledge that has not been achieved in other systems. Combining this information with pharmacologic perturbations … will yield insight into the function of …, will also provide insight into …. Thus, the results will provide a foundation for attaining the overall objective of the proposal, i.e …

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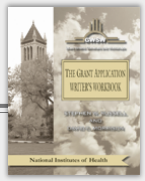
Suggested structure for Approach section



Potential Problems and Alternative Strategies paragraph:

- Identify problems that could arise but probably won't; only the most important and probable, e.g.:
  - if assays are not sufficiently discriminating
  - if critical reagents/patient samples are not available
  - if your working hypothesis is proven invalid
- For each, identify:
  - nature of the problem
  - reasons it is unlikely to arise
  - alternative approaches you would try if it were to arise

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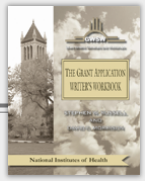
Suggested structure for Approach section



Potential Problems and Alternative Strategies paragraph:

- Example of language for this:

  Regardless of our hypothesis, the experiments within this aim will …, and whether … Given that the experiments within this aim use well established and routine genetic tools to determine …, it is unlikely that the experimental techniques will fail. However, if …, the interpretation of the results could be problematic. To overcome such a complication, we would … by …. For instance, our preliminary data indicates that…. Thus we would use … to identify which … .

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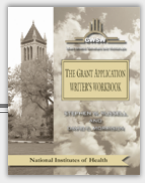
Suggested structure for Approach section



Timeline

- Comes after ***all*** of the Specific Aims
- *Purpose* — outline timeframe needed to complete each subaim (table or paragraph)
  - Grant applications are often rejected because overly ambitious.
  - Grant applications are often rejected because not ambitious enough.
  - Carefully thinking through and presenting a timeline (preferably including a schematic component) can circumvent these problems.

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Suggested structure for Approach section

Timeline example:

Timetable					
Aims/Tasks	**Year 01**	**Year 02**	**Year 03**	**Year 04**	**Year 05**
Aim 1: Characterize the function of…	←	—	→		
1.1 Identify which…	←	—	→		
1.2 Define when…		←	—	→	
1.3 Examine the effects of…		←	—	→	
Aim 2: Determine the cellular source of…		←	—	→	
1.1 Determine where		←	—	→	
1.2 Identify which…		←	—	→	
Aim 3: Identify the downstream…			←	—	→
3.1 Characterize the…			←	—	→
3.2 Identify the…			←	—	→
3.3 Examine the…				←	→

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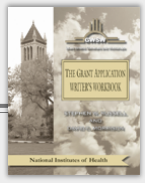
Suggested structure for Approach section



Future Directions

Purpose — to summarize:

- where you expect the science to be at end of the study
- how the results will complete next step in the continuum projected in *long-term goals* statement of Specific Aims page
- what the next steps in the continuum will be, and why they are important

Example of language for this:

…. The knowledge gained will provide the foundation for …. In the future we plan to probe the mechanisms of …. by …. We will also expand the studies outlined here to examine whether … Overall, such studies will help us attain our long-term goal of … by …

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Suggested structure for Approach section

EXAMPLE PROPOSAL 3

Risk Factors for Glaucoma: Uncovering their Genetic Basis Using Inbred Mouse Strains

complication, the selection of mice used in the genotyping arrays would become more tightly limited to mice representing only the extreme phenotypes – significantly high and low CEC density- which would increase the likelihood of identifying relevant loci. Another way to overcome this problem, would be the replacement of one strain with a different inbred mouse strain of like CEC phenotype. This would be possible because we initially phenotyped many different strains for CEC density.

Timeline

Aims/Tasks	Year 01	Year 02
Aim1: Uncover genes influencing CECs	←	→
1.1 Generate cohorts of intercrossed F2 progeny	←	
1.2 Phenotype cohorts for CEC density and CCT		← →
1.3 Map genetic loci influencing CECs		← →

Future Directions

The identification of genetic loci that influence CECs would greatly contribute to our understanding of the pathogenesis of glaucoma. An understanding for the genetic basis of CEC density coupled with the current ease of measuring CEC density in the clinical setting, our ability to assess risk for disease, treat disease, and prevent vision loss associated with glaucoma would be greatly enhanced. Furthermore, genetic manipulation of loci that influence CECs as well as pharmacological studies aimed at manipulating CEC density and function could also be pursued for new preventative measures and treatments for glaucoma and other CEC-related diseases. This early detection would enable us to identify high risk individuals, begin preventative treatments well before the onset of disease begins, and ultimately give us the opportunity to significantly reduce the incidence of vision loss associated with glaucoma.

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Suggested structure for Approach section

expression by sacrificing a subset of mice at post-operative day 5, 10 and 20, isolating the SAN and using confocal microscopy to stain for HCN4 (46), a SAN specific protein, and image eGFP expression.

2b. Determine whether I_{MCU} inhibition protects against SND.

I will test whether DN-MCU Tg mice are protected from SND in our Ang II infusion model (17). I hypothesize that by limiting Ca^{2+} influx into mitochondria Tg mice will have less cell death and SND vs WT mice. To test this hypothesis, I will implant ECG telemeters in Tg vs WT mice and a miniosmotic pumps infusing Ang II (3 mg/kg/day) or saline (17). Pumps will be in place for up to three weeks. During this time I will measure resting and activity related HR and arrhythmias. Mice will be unrestrained and I will have continuous 24-hour ECG recordings. Mice will be evaluated for episodes of severe bradycardia, a hallmark of SND, which will be defined as a HR less than 200 beats/min during an activity level of one or greater, as we described (17). After three weeks, mice will be sacrificed and whole hearts will be isolated. Isolated hearts will be Langendorff-perfused, overdrive-paced, infused with ISO and evaluated for sinus node recovery time (SNRT) (17), which is a measure of SAN health. For immunohistochemical staining, whole hearts will be excised. The right atrium and superior vena cava will be removed, flash frozen, sectioned, and stained for apoptosis (TUNEL) and reactive oxygen species (DHE) (45). HCN4 (46), an SAN enriched protein, staining will be used to mark the SAN. I will measure atrial fibrosis by performing a Masson's trichrome stain on sectioned right atrial tissue (49). I will detect apoptotic activation by performing caspase-3 and caspase-9 activity assays in tissue samples.

Anticipated Outcomes and Possible Complications

I expect that DN-MCU Tg animals and DN-MCU painted mice will have similar HRs to WT and eGFP painted mice at baseline, but lower HRs during periods of exercise or after ISO injection. I would be surprised if the opposite were true, but it could be interpreted that blocking mitochondrial Ca^{2+} entry makes Ca^{2+} more available in the cytoplasm, activating CaMKII, and increasing release of Ca^{2+} from the sarcoplasmic reticulum. This is a scenario that my lab is uniquely suited to study. The alternate hypothesis would remain an exciting and important conclusion because there is no data available about the effect of inhibiting I_{MCU} in an animal model. Any data generated from this study will add to our understanding of HR determination.

I expect that DN-MCU mice will be protected from SND and SAN cell death, showing maintenance of HR at baseline levels, lower SNRT, and fewer apoptotic cells. If the opposite is found to be true, it would remain an exciting finding because of the novelty of this study. If I_{MCU} inhibition promotes SND, which I do not expect, perhaps there is a lower threshold for mitochondrial Ca^{2+} that must be maintained in order to have cell viability. Completing this sub-aim will provide important information about the role of cardiospecific MCU during pathophysiologic conditions.

Future Studies

My proposed studies will provide many answers about the involvement of MCU in SAN physiology and dysfunction. It is undeniable that many more questions will arise after completion of this exciting body of work. I have already made mutant MCU constructs that are likely resistant to CaMKII phosphorylation. While outside of the scope my proposal, these constructs will help me study the relationship between CaMKII and cardiac MCU in the future. I plan to learn the patch-clamp technique before finishing my project because this may be an important technique for my future studies and Dr. Anderson's laboratory has many experts in the technique of patch clamping. I am excited and motivated to complete the work outlined in my proposal. Completing this body of work will provide important information about cardiac mitochondria and will allow me to learn basic scientific principles on which I can build an independent scientific career.

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Suggested structure for Approach section

Future Studies
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- *My studies will provide answers to…*
- *…many additional questions will arise.*
- *While outside of the scope of my proposal, these xxx will…*
- *In future…*
- *Completion of this work will provide important information about…*
- *…and will allow me to learn xxx on which I can build an independent scientific career.*

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neutralization using standard methods (46). The affinities of binding can be directly measured for the antibody expressed on the yeast surface using flow cytometry to estimate on- and off-rates (9, 67).

An additional variant on this protein engineering approach would involve determining the virus-specific contacts and specificity determinants of the antibodies. We have already solved the X-ray crystal structure of the Mab 14, which specifically binds CPV but not FPV (21). Other specific antibodies do not recognize certain natural or antibody-selected antigenic variants (escape mutants) of the capsid (63). We would therefore use the same yeast expression library and mutagenesis to select antibody mutants that recognize those variant capsids, and thereby determine the antibody structural determinants of site-specific recognition. This would allow better models of the antibody-capsid contacts to be obtained.

C3c1) Functional testing of the mutated antibodies. To determine the effects of the different changes in the capsids and antibodies on the process of infection and the relationship to neutralization, we would examine the effects of the purified antibodies or antibody domains (as scFv or Fab) on the viral functions. We would purify the wild type and mutant forms of the capsids and antibodies. Antibodies would be tested for their ability to compete with TfR in binding assays, and for their ability to neutralize standard virus preparations. Wildtype or mutant capsids would also be used, and treated with proteases to cleave varying proportions of the VP2 in some studies. Those would then be examined for their ability to bind the TfR in solid phase or on cells, for uptake into the normal pathways of cell entry, or to be neutralized, using our well established methods (24, 46).

C3d) Expected outcomes, potential problems and their solutions or alternative approaches.

These studies will use existing well-characterized antibodies and engineered variable domains to answer important questions about the mechanisms of antibody attachment, recognition of specific capsid structures, and their interactions with receptor binding leading to neutralization. We already have the antibody-capsid complex structures of a representative set of 8 antibodies (21), and have several of the antibodies expressed in either bacteria or yeast and have confirmed that those are expressed and bind the viral capsids (e.g. Fig. 8). We are therefore well positioned to carry out the studies proposed.

While the lack of binding by some of the antibodies to sites with cleaved loops has not been formally shown, that is highly likely given the properties of the known escape mutations and binding sites. The preparation of the gold-labeled Fabs should be quite straightforward, while the TfR labeling may be more challenging. However, the TfR ectodomain dimer, as expressed, displays two 6-His tags on the underside of the receptor, and that should be readily labeled by Ni^{2+}-gold conjugates. The selection for antibody domains with altered affinities should be straightforward given that we already know that the most interesting antibody pairs are expressed in a functional form on the surface of yeast, and our close collaborator, Dr. Moonsoo Jin, has used all of the methods proposed for preparing and screening mutant libraries for ligands with altered affinities (see letter of collaboration).

C4) Overall Summary and Conclusions.

These studies will integrate our understanding of the structures of viral capsids with a more detailed knowledge of the binding properties and functions of host cell receptors and antibodies. The results would be correlated with biochemical and structural analyses of the capsid flexibility, variation, and/or asymmetry. These are central questions that apply to any non-enveloped animal virus, and have parallels to the structural changes and interactions seen for many enveloped virus glycoproteins, and so the results will clarify some of the underlying rules about how viruses interact with their host ligands and infect cells.

The work builds on a solid intellectual and methodological foundation resulting from our previous studies, and we have most of the materials and background information required. For each of the projects we combine well established methods with new approaches, and have alternative approaches for each of the experiments where the technology is novel or untested. Studies already underway would be continued in the first phase of the funding period, while studies requiring the development of reagents or information from previous studies will be done later in the project.

TIMELINE

This project would take 5 years to complete. The sequence of studies will initiate in years 1 and 2 with the preparation of the capsid mutants and their testing, along with development of the new methods for sample preparation for cryoEM, and collection of the cryoEM data for analysis. Analysis of the role of cleaved and stabilized capsids would initiate with the currently available mutants, and continue through years 3 and 4. Mutant forms of the TfR and antibodies would be prepared in the first years, and tested in later years up to year 5. The preparation of capsids with altered receptor binding sites (peptides or domains), and selected on mutant receptors, would occur during years 3 to 5.

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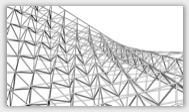
Topics

NIH instructions

Fellowship Research Strategy

Rigor and Reproducibility



How to Structure the Research Strategy

Examples of Wording

Rigor & Reproducibility

Other Aspects

Questions?

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