5th B CELL WINTER SCHOOL

Hotel Franziskushöhe, Lohr am Main

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"SPECIFIC AIMS" PAGE

GUIDELINES

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GENERAL INSTRUCTIONS

- Please, add on the cover page at the beginning of the document the title of your project, your name, the name of your mentor and date, and describe in the box at the end of the document your overall goal of your thesis project. In a grant proposal, this part is called **Specific Aims.** We have included a brief list of guide lines taken from presentations by Dr. Whitney (Univ. of California), and Rita Balice-Gordon, Ph.D. Professor Dept. of Neuroscience, and the NIH website (the originals are included in the attached PDF named Examples_Whitney-Balice_NIH.pdf).
- Please contact us if you have any further questions.
- The page should be sent back to Dr. Agnes Giniewski as a WORD document by e-mail **no later than January 31, 2018.**

GENERAL GUIDELINES - "SPECIFIC AIMS" PAGE

TITLE

The title should create a good first impression, inform the reviewer of the proposed research topic, and engage the reviewer's interest.

SPECIFIC AIMS PAGE

1. The most important page of a grant proposal
   - ✓ It's the master plan for the rest of the proposal
   - ✓ You engage or lose the Reviewer on this page
2. Maximum - one page
3. Consists of three paragraphs

   Paragraph 1 - Introduction part

   - Introduce the big picture/relevance of your research
     (Educate the reviewer by summarizing the important knowledge.
   - Describe your long-range/term research or career goal(s).
   - State your overall project goal /what you hope to accomplish.
     - Ensure that the overall project goal addresses an identified gap in knowledge and represents a step toward achieving your long-range career goal.
   - Present your central hypothesis (or, alternatively, a statement of need).
     - Be sure that you present a true hypothesis – one that can be objectively tested to determine its validity – rather than a predetermined conclusion.
   - Explain your rationale for pursuing the project.
     - Indicate what it will be possible to accomplish when your research is complete.
   - Lead into Specific Aims.

   Paragraph 2 - List Specific Aims

   - Delineate your specific aims in a bulleted list.
   - Delineate a reasonable number of specific aims, presented in a logical order.
   - Ensure that specific aims correlate with your central hypothesis.
   - Whenever possible – test a hypothesis in the specific aim title
     - You want the Reviewer to know that your work is hypothesis driven
     - Don't make the Reviewer work to figure out what the hypothesis is
   - Ensure that all specific aims relate to and support your overall project goal.
   - Aims should test mechanistic hypotheses.
   - Provide conceptual rather than descriptive specific aims.
   - "Why" aims are generally stronger than "what" aims.
   - Specific Aims are not methods, and Aims should not be a list of experiments

- Define a clear purpose, working hypothesis or statement of need, and expected outcome for each specific aim.
- **Make sure no specific aim is dependent on the successful outcome of another aim. If possible Aims are not inter-dependent but rather supportive of each other.**
- Include brief description of approach after each Specific Aim.

Paragraph 3 - What can you do when all aims are reached?

- Identify the project's innovation, e.g., a unique approach or technology.
- Delineate the project's expected outcomes.
    - Should validate central hypothesis and resolve gap in knowledge.
- Summarize the project's significance
    - Provides segue to Background and Significance

SUMMARY - "SPECIFIC AIMS" PAGE for NIH Grants (1 page)

Paragraph 1

- **Opening Sentence** (*Should get the reader's attention*.)
- **Current Knowledge**
- **Gap** (*What is the unmet need?*)
- **Current Barrier to Progress** in the field/area
- **Long-term Goal**
    - Overall objective (*This is the next step to achieve the long-term goal.*)
    - Central hypothesis
- **Rationale** that underlies the proposed research. . .

Paragraph 2

- **Specific Aims** that link back to the central hypothesis
    - Specific Aim 1: *To determine. . .*

      Our working hypothesis is. . . To test this hypothesis, we will. . .

Paragraph 3

- **Payoff** (*These aims will yield the following expected outcomes. . .*)
- **Impact** (*These outcomes are expected to have an important positive impact because…)*

DOS and DON'TS FOR "SPECIFIC AIMS" PAGE

- **Dos**
  - Discuss them with colleagues
  - Try to limit this section to one page – it's a roadmap to the rest of the proposal and it must include the logic behind your aims.
  - Don't assume your Reviewer is an expert in your particular area – so write Aims for a non-expert compared to the rest of the proposal
- **Don'ts**
  - Don't state a hypothesis that you cannot actually test with the experiments you are proposing
  - Avoid using phrases like: To correlate… To describe… To develop; these help get your grant pegged as "too descriptive"
  - Avoid wishy-washy, passive tense, or flowery language – instead write your aims in active form with strong meaningful verbs
  - Don't write aims that can be viewed as "a fishing expedition"

EXAMPLE TEMPLATE - "SPECIFIC AIMS" PAGE

(in blue: notes, in black: actual text)

Start with preamble that describes the unmet medical need and/or gaps in our biomedical knowledge and why this is an important topic of study. For example:

The generation of immunological memory after vaccination is essential for long-lasting protection against future infections. Our long-term goal is to understand ____. The specific objective of this proposal is to ____. The central hypothesis is that ____. We formulated this hypothesis, in part, based upon our strong preliminary data, which shows that _____. The rationale for the proposed research is that once it is known how ____. We will pursue these studies in three Specific Aims:

- *Aim 1 INSERT TEXT.*
  Our *working hypothesis* for this Aim is that ____.
- *Aim 2 INSERT TEXT.*
  We will test the *hypothesis* ____.
- *Aim 3 INSERT TEXT.*

In these studies, we will examine the *prediction* that ____. The proposed work is innovative because it capitalizes on ____. At the completion of this project, we expect that the combined work proposed in Aims 1 and 2 will ____. We also expect that Aim 3 will establish ____.

PRACTICE EXAMPLES - "SPECIFIC AIMS" PAGE

Taken from: Rita Balice-Gordon, Ph.D. Professor Dept. of Neuroscience

Okay:

Specific Aim 1: To test the hypothesis that neurons in the GluR1 knockout mouse will have delayed dendritic maturation.

Better:

Specific Aim 1: To test the hypothesis that GluR1 signaling is necessary for dendritic maturation.
(or is sufficient)

Taken from: Erica Whitney - How to Write a Competitive Grant Proposal: Specific Aims - Research and Career Development Manager, QB3

Paul Hagerman, University of California, Davis, 5UL1DE019583-04

Specific Aims – what is wrong?

Aim X. To use targeted gene replacement to create a BRCA1-/- knockout mouse.

Aim X. To analyze gene expression profiles using microarray analysis in normal vs. cancerous prostate cells.

Aim X. To overexpress several components of the telomere enzyme in *S. cerevisiae* and measure DNA repair efficiency at telomere ends.

Specific Aims – what is wrong?

Aim 1. Show that p53 is upregulated in a chronic inflammation mouse model.

Aim 2. Demonstrate that downregulation of p53 using siRNA decreases inflammation.

Aim 3. Characterize additional p53 binding targets due to upregulation in response to chronic inflammation.

GOOD EXAMPLES FROM FUNDED NIH GRANT PROPOSALS

A. SPECIFIC AIMS

1. **Investigations of the mechanism of a1-b2 regulation of *IME4*.**
   a) Determine if the **a**1-b2 site controls expression of the *IME4* antisense transcript;
   b) Determine if expression of the *IME4* antisense transcript blocks *IME4* expression at a transcriptional or post-transcriptional step.

2. **Investigations of the role of Mcm1 phosphorylation.**
   a) Investigate the role of phosphorylation of specific residues in Mcm1;
   b) Identify proteins involved in phosphorylation of Mcm1.

3. **Investigations of the role of Mcm1 in regulation of osmotic stress and cell wall genes.**
   a) Identify genes that require the Mcm1 NT arm for proper regulation;
   b) Identify cofactors that require Mcm1 regulation of the cell wall and osmotic stress.

Taken from: Erica Whitney - How to Write a Competitive Grant Proposal: Specific Aims Research and Career Development Manager, QB3

Paul Hagerman, University of California, Davis, 5UL1DE019583-04

Aim 1: **To develop oligonucleotide-based approaches to reduce FMR1 RNA in vivo.**

Experiments will test predictions of our hypothesis that reductions in FMR1 RNA levels in neurons and/or astrocytes will ameliorate the neuropathology of FXTAS. This aim also tests the location (nuclear vs. cytoplasmic) of the cellular pathology caused by expression of the exCGG.

Aim 2: **To define the timing and reversibility of pathogenic responses to expression exCGG RNA.**

Tet-inducible neural cell models will be used to elucidate the time course of pathogenic responses to expression of the exCGG RNA and the extent to which they are reversible, thereby guiding Projects 2 and 3 in the development of new therapeutic interventions.

Aim 3: **To evaluate the roles of astrocyte and neuronal dysfunction in the pathogenesis of FXTAS.**

Biochemical and pharmacological experiments will test our hypothesis, which predicts that loss of normal astrocyte function, possibly impairing glutamate uptake and/or alterations in neuronal glutamate signaling pathways, contribute subtle to severe changes in neuronal morphology and cell loss. These studies will provide mechanistic understanding of clinical interventions with memantine and lithium (Project 3).

Taken from Erica Whitney - How to Write a Competitive Grant Proposal: Specific Aims Research and Career Development Manager, QB3

Specific Aims: Boris Striepen, University of Georgia "Biology of the apicomplexan plastid"

Specific Aim 1: **Dissect the mechanism of apicoplast protein import.**

The bulk of the ~500 apicoplast proteins is nuclear encoded and post-translationally imported across four membranes. We (and others) have described three mechanistically distinct candidate protein translocons that reside in the three inner membranes of complex plastids. In the current funding period we will focus on a newly discovered mechanism that was derived from the ERassociated degradation system (ERAD) of the algal endosymbiont. We will use conditional gene disruptions and complementation assays to establish the importance of individual components and to define the energy source of the translocation process.

Specific Aim 2: **Understand the function of the apicoplast ubiquitination pathway.**

The ER-localized ERAD pathway goes hand in hand with the ubiquitination and subsequent proteasomal degradation of translocated proteins. Our preliminary data indicates that aspects of this protein modification pathway are still present in the apicoplast. What is the enzymatic machinery involved in this process? What are its substrates? And most importantly, what is the molecular function of apicoplast ubiquitination? A combination of genetic and biochemical approaches will be used to answer these important questions.

Specific Aim 3: **Discover a comprehensive set of apicoplast proteins and characterize their function.**

Mining comparative and functional genomic information we have assembled an extensive list of proteins for which we hypothesize a role in apicoplast biology. We will establish the localization of their protein products for a comprehensive set of these candidate genes by epitope tagging. In the previous funding period we have found conditional null mutants to be highly informative to study apicoplast protein function and we have developed phenotypic assays to detect defects in apicoplast protein import, apicoplast division, and apicoplast metabolism. We will apply this genetic approach to a prioritized list of validated candidates. To increase the throughput of our analyses we will develop and test a new mutagenesis approachbased on promoter replacement.

EXAMPLE OF FUNDED NIH GRANT PROPOSAL WITH NOTES

http://www.niaid.nih.gov/ncn/grant/app/default.htm

This sample was created using the PHS 398. It does not conform to all the requirements for electronic application, including page limits and other items. We will be posting samples of electronic applications soon.

SPECIFIC AIMS

Staphylococcus aureus is a well-armed opportunistic pathogen that produces a diverse array of virulence factors and causes a correspondingly diverse array of infections. The pathogenesis of *S. aureus* infections depends on the coordinately-regulated expression of two groups of virulence factors, one of which (surface proteins) allows the bacterium to evade phagocytes and colonize host tissues while the other (extracellular toxins and enzymes) promotes survival and multiplication at a localized site of infection. Our long-term goal is to elucidate the regulatory mechanisms controlling expression of these virulence factors as a prerequisite to the development of therapeutic protocols that can be used to attenuate the disease process. **The specific hypothesis behind the proposed research is that the staphylococcal accessory regulator *(sar)* is a major regulatory switch controlling expression of *S. aureus* virulence factors.** That hypothesis is based on the following observations. **First**, *sar* encodes a DNA-binding protein (SarA) required for expression of the *agr*-encoded RNAIII regulatory molecule (27). The SarA-dependency of RNAIII expression is important because RNAIII modulates expression of many *S. aureus* virulence factors (29). **Second**, phenotypic comparison of *sar* and *agr* mutants indicates that *sar* also regulates expression of certain *S. aureus* genes in an *agr*-independent manner (11, 21). An example of particular relevance to this proposal is the *S. aureus* collagen adhesin gene (*cna*). **Third**, mutation of *sar* results in reduced virulence in animal models of staphylococcal disease (8, 10, 28). Moreover, as anticipated based on the preceding discussion, *sar/agr* double mutants have reduced virulence even by comparison to *agr* mutants (8, 24). **Based on these observations, the experimental focus of this proposal is on the *sar* regulatory locus.** The specific aims are designed to provide a comprehensive assessment of the *agr*-independent regulatory functions of *sar*:

1. Correlate the production of each sar transcript with the production of functional SarA. The only recognized protein product of the *sar* locus is the SarA DNA-binding protein. However,

Uses short sentences, is neat, is clean with no typographical errors. Uses bullets and numbered lists for effective organization. No header or footer, since those are added automatically later. Stays within page limit **(in original version, prior to annotation.)**

Specific aims start with the background for the informed non-expert, writing at about the level of *Scientific American*. Gives summary for non-primary reviewers. Puts less technical information first.

Hypothesis is easy to locate in bold type and includes health importance of the project. Provides three reasons for hypothesis, with references.

Scope of research is limited to three specific aims listed in bold and followed by a brief description of how each aim will be accomplished. These aims are the steps designed to prove the hypothesis.

Lists give guideposts to reviewers; indents and bold add readability.

Northern blot analysis reveals three *sar* transcripts (sarA, sarB and sarC), all of which include the entire *sarA* gene. Expression of each transcript is growth-phase dependent. The functional significance of this differential regulation will be assessed by correlating the production of each transcript with the production and activity of SarA.

A. The temporal production of SarA will be assessed by Western blot of *S. aureus* whole cell extracts with an affinity-purified anti-SarA antibody.
B. The DNA-binding activity of SarA will be assessed by electrophoretic mobility shift assays (EMSA) using whole cell extracts and DNA fragments known to include SarA-binding sites (e.g. *cis* elements upstream of the *agr* P_2 and P_3 promoters).
C. The function of SarA as a transcriptional activator will be assessed *in vivo* using transcriptional fusions between each of the *agr* promoters and a promoter-less *xylE* reporter gene.

2. Characterize the mechanism of sar-mediated regulation of the S. aureus collagen adhesin gene (cna). We have established that *sar* is the primary regulatory element controlling *cna* transcription and that this effect involves a direct interaction between SarA and *cis* elements upstream of *cna*. However, unlike SarA binding to the *agr* promoter region, SarA binding represses *cna* transcription. We will correlate the production of each *sar* transcript with the production of SarA and with the regulation of *cna* transcription. We will also define the *cis* elements upstream of *cna* that constitute the SarA DNA-binding target.

A. Complementation of the cna transcriptional defect will be done by introducing plasmids encoding the sarA, sarB or sarC transcripts into a cna-positive sar mutant. Once the SarA-binding site upstream of cna has been defined (see below), the complementation studies will be correlated with SarA binding to cis elements upstream of cna.
B. The SarA DNA-binding site(s) upstream of cna will be localized by EMSA using purified SarA. The specific binding site(s) will be identified by DNA footprinting and characterized by EMSA using cna sequence variants and purified SarA.
C. The in vivo significance of SarA binding will be assessed using transcriptional fusions between sequence variants of the cis elements upstream of cna and a promoter-less xylE reporter gene.

3. Identifify S. aureus virulence factor genes under the direct control of SarA. The scope of SarA as a regulatory protein is not well-defined because the identification of SarA targets has been restricted by the availability of gene probes and/or appropriate phenotypic assays. Our successful purification of SarA in a form capable of binding appropriate DNA targets (e.g. *cis* elements upstream of *agr* and *cna*) will allow us to define the DNA determinants required for SarA binding using a functional selection. We will then identify SarA binding sites within the *S. aureus* genome and evaluate SarA regulation of the genes *cis* to these binding sites.

A. PCR-assisted binding site selection will be used to functionally select DNAs with SarA binding sites from a random pool of synthetic DNA fragments. The consensus binding site will be determined by computer-assisted alignment of functionally selected DNAs.
B. The consensus sequence for a SarA-binding site will be used in homology searches of existing S. aureus genomic databases. The search will be extended to include the entire S. aureus genome as it becomes available.
C. SarA regulatory control of the genes cis to putative SarA-binding sites will be tested by Northern blot analysis of wild-type strains and their corresponding sar mutants.

S. aureus is among the most persistent of all human pathogens. The continued emergence of antibiotic-resistant strains emphasizes the need to identify new therapeutic targets for the treatment of *S. aureus* infections. We believe the *sar* regulatory locus may be an appropriate target in that disruption of *sar*-mediated regulation has the potential to attenuate the bacterium to the point that it is more susceptible to clearance either by the normal host defense systems or existing antimicrobial agents. Accomplishing the specific aims outlined in this proposal will provide the foundation required to assess that possibility by establishing the correlation between *sar* transcription and SarA production and activity (Specific Aim #1), elucidating the mechanism by which *sar* controls expression of a specific target gene (*cna*) (Specific Aim #2) and identifying additional SarA targets within the *S. aureus* genome (Specific Aim #3).