Guidelines for Preparing an NIH Proposal

Excerpts from:

The "Specific Aims" section is a short and self-contained summary of the research questions and the goals of the project. It should stand alone and function both to give an extremely brief introduction to your research project and to jog the memory of someone who has read your grant earlier and needs a brief reminder of the project.

The "Background and Significance" section should thoroughly but concisely make the case for why this research is important, provide a context for your work, and indicate how results from the project would affect the field. Because the reviewer may be an expert in a related but separate field, this section should serve as a review article for a sophisticated reader and bring him or her up to date in your field. NIH suggests that the style and tone of the magazine *Scientific American* is a good standard; this background should tell an engaging story, as described in the previous chapter.

The "Preliminary Results" section is a demonstration of your ability to perform the proposed work, illustrating technical tools and experimental prowess to approach your aims. For some grants (such as R03 and R21 applications) this section is optional because preliminary data are not required, but it is a central component of the standard R01 grant. For competitive renewals of R01 and other renewable grants, the "Progress Report" is also included in this section to demonstrate the progress toward the aims of the prior funded grant.

The "Research Design and Methods" section is a detailed outline of the proposed research, and should serve as a blueprint for the work to be performed if the grant is funded. It should provide enough detail to demonstrate a clear direction for the work, and the technical capability to perform it. All experiments should be organized under the "Specific Aims" section, demonstrating how each aim will be accomplished.

The "Literature Cited" section is an inclusive bibliography of published references pertinent to your work. While often ignored as being secondary documentation, it is actually important in the presentation and can affect the evaluation of your application.
Specific Aims

Introducing your project

The scientific portion opens with this section. Usually it is about one page, and contains no citations because the information is general in nature. In essence, this should be a highly condensed version of the next section, the "Background and Significance" section, ending with a listing of the actual "aims" of the application. As a rule, it is a synopsis of the information that explains to the reader why your proposed research will be important to pursue, and the context into which the work would fall. Once the reader has read the remainder of the grant application, he or she should be able to reread this section as an effective memory refresher for why the work is important and what questions it will answer.

Summarizing the rationale

The "Specific Aims" section begins with background information that presents the rationale for the application. This information should provide a broad context appropriate for a reviewer who may be a researcher in a related but separate field. The pertinent facts about what is known and unknown in the area of the research, the relevant questions to be addressed about this area, and the potential implications and ramifications of the work should be summarized in two or three short paragraphs. These paragraphs should be self-contained and should stand alone. Obviously, such a short synopsis will not explore all the fine nuances in the research area of interest, but it should provide a coherent explanation to a scientist
working in a related area. The following questions should be addressed:

1. What is the field of investigation?
2. Why is it important?
3. What is the state of knowledge in this area?
4. What important questions remain unanswered?
5. What would be the ramifications of answering these questions?
6. What limitations have posed barriers to research in this area?
7. What new or potential developments offer you a successful avenue to address your questions?

The end of the final paragraph should consist of a lead-in sentence such as "To approach these goals, we specifically propose:" followed by a listing of the actual "aims" for the project.

**Stating the aims of the project**

There should be two to four aims that are succinct statements precisely summarizing the research to be performed. Each aim should cover one basic research focus central to the topic of the grant. They should be thematically related and fit together to clarify the important issues raised in the introductory paragraphs. They may each explore a different idea, or different approach to the same idea, as long as they form a cohesive thematic unit, addressing an overall central idea or hypothesis. Each aim should be headed with a number ("Aim 1," etc.), to which later parts of the grant will refer.

**Types of aims**

The aims are one of two types. The first is the "hypothesis-driven" aim, based on a scientific hypothesis to be tested and substantiated or refuted by the data that will be generated in your proposal. The hypothesis should be reasonably justified by observations, either published or personal (given in the "Background and Significance" and/or "Preliminary Results" sections) that have interesting implications. This hypothesis-driven approach is favored, because it is immediately apparent what would be gained from the proposed work. The second type is the "exploratory" aim, which does not carry a specific hypothesis, but seeks to obtain useful data about a specific question. This type is descriptive and emphasizes gathering information or taking a "fishing" tact to allow future formation of hypotheses.

For larger grant applications by independent investigators, such as R01s, the proposal should contain at least one major hypothesis-driven aim, and preferably
be mostly or all hypothesis-driven. Such grants are intended to fund a focused and targeted attack in a research area, and thus should contain distinct testable hypotheses. However, it may be entirely appropriate for a portion of such a project to be exploratory as well, as long as the grant is not primarily exploratory. For smaller type grants such as R03 and R21 applications, a predominantly exploratory focus may be appropriate, because these projects are intended to be preliminary and higher risk. Still, it is useful to incorporate hypothesis-driven work when possible even in these projects, as this gives the proposed work direction and focus. Even if an aim is exploratory and not hypothesis-driven, it is crucial to justify the rationale for how the information acquired would open an important field of investigation and lead to new hypothesis-driven work.

**Giving further details on the aims (or not)**

As a matter of preference, some authors give additional brief descriptive information under each aim. In this case, a brief paragraph is provided under each listed aim, to summarize the hypothesis and approach to be taken. If this is done, the information should be sufficient to stand alone, and should not be cluttered with details (particularly methodologic) that distract the reviewer from the central point of the aim. Because the “Specific Aims” section should be very concise, it may be better to restate the aims with these additional details at the end of the “Background and Significance” section (see Chapter 8).

**An example**

For example, the “Specific Aims” section for a hypothetical grant addressing “Idiopathic acute pan-cerebral necrosis” (not a real disease) could begin with a paragraph explaining the incidence, prevalence, and impact of this disease, continue with a paragraph concerning the epidemiologic data, and finish with a paragraph on the specific scientific facts that are pertinent to the aims, such as description of the available models and current thinking on pathogenesis of this disease, concluding with:

... In light of our recently enhanced understanding of the neuron explosion cascade (NEC) and its likely role in Idiopathic Acute Pan-Cerebral Necrosis (IAPCN), identifying abnormally expressed neuronal factors is likely to shed light on the pathogenesis of IAPCN. The central focus of this application will be to apply newly developed ultrasensitive techniques to identify abnormal genetic and protein expression of neuronal factors and investigate the role of these factors in the development of IAPCN. Specifically, we propose:

1. To quantify and localize expression of NEC factors in an established murine model of IAPCN;
2. To evaluate the in vivo effect of small molecule inhibitors and enhancers of NEC components in the murine model of IAPCN; and
3. To assess the metabolic activity in relevant brain areas in humans at risk for IAPCN.

As the opener to the science portion, this section will set the first impression for the reader. The reviewers will read it carefully, because it serves as an orientation for the rest of the grant application. This impression thus will be especially important to point the reader in the right direction and set the tone for the rest of your application.
Background and Significance

Laying the foundation for your project

This section serves as a foundation for your scientific ideas and research plans. It is usually two to four pages, and contains important facts backed by literature citations. These facts should lead the reader down the logical path to concluding that the specific aims are worthwhile to pursue. For grant applications that are written in response to an RFA or PA, it is particularly important that this section address the relevance of your project to the stated goals of the RFA or PA. The “Background and Significance” section, when well written, should serve as a stand-alone review article that gives the expert reader all the necessary information to explain:

Why the area of research is important. The rationale for your research should be clearly delineated. The scientific issues you address should be tied to some important fundamental problem, for example, pathogenesis or prevention of a threatening disease, a poorly understood basic molecular process used by many bacteria or viruses, a potential explanation for a phenomenon of unknown mechanism. The reviewer should be convinced that the aims, if achieved, would contribute to a fundamentally important topic with implications that unravel a key unanswered scientific question, or potentially provide a new clinical avenue. To continue the fictional example in the previous chapter, delineating the pathogenesis of IAPCN may be important because this rare disease shares important pathologic principles with Alzheimer’s or Parkinson’s Diseases and light will be shed on these more common syndromes. Perhaps IAPCN is a dysregulation of normal neuronal death during brain development, and understanding the processes will shed light on poorly understood central questions about brain ontogeny. Perhaps IAPCN is a localized form of
Spontaneous Human Combustion Syndrome, which has been a central mystery to combustionologists all over the world. Perhaps lives can be saved because, although rare in the general population, the incidence of IAPCN is high among new assistant professors, and the research will lead to useful preventive measures or therapies that will have far-reaching effects on society by saving this important group of people. Although scientific knowledge is important and interesting for its own sake, your research will carry more weight if you can tie it into some larger scientific or clinical picture (even if the work does not have immediate application).

What is known thus far about the area of proposed research. Work pertaining to the area of interest should be summarized and clearly cited in a comprehensive manner. This includes scientific discoveries leading up to the questions being asked, previous attempts to address similar questions, current theories, previous limitations that have prevented resolution of the area, and recent advances that may assist in pursuing the aims. There should be sufficient information here to educate the reader about how your work would fit into the field and advance it. The information should be comprehensive and clearly organized, with citations crediting anyone who has contributed to the field (see also Chapter 11). This review should be as objective as possible, and avoid subjective statements that might alienate a reviewer with an opposing view. Instead, it is better to give the clearly established facts, acknowledge controversy, state the prevailing hypotheses in the field, and add your interpretation of the facts with reference to supporting data. Focusing on the positive (why your viewpoint could be correct) is always better than the negative (why others are incorrect). The goal is to convince the reviewer that you are thinking clearly and objectively about the issues, and have a firm grasp of the field.

Implications of the proposed work in the field. A brief explanation should be given as to how accomplishment of the aims would broaden or change the field. This should be related to what is already known, and how your data would enhance or change the current state of knowledge. If applicable, mentioning potentially useful applications of your findings is also helpful, although great care should be taken to avoid making claims that are tenuous or unsupportable.

Organization and Structure

As discussed earlier, an effective writing style is to break this section into multiple paragraphs that follow an outline form, with the topic sentences underlined. This is particularly important for the “Background and Significance” section, where the reader needs to assimilate a large amount of information quickly. Each topic sentence should convey the entire point of its paragraph, with the other sentences elaborating on the data or rationale for that statement, making the key points easy to absorb at a glance. This provides the reviewers with visual cues for quick recall of the information (that they may have read weeks earlier) when skimming the application during the review meeting.
An example

Some authors conclude this section by listing the aims again, in the context of what has just been described. The restatement and expansion of the aims here can strengthen this section by directly tying your proposed research to the information presented. After each aim, a few sentences now can be given to summarize the experimental approach and how it relates to the rest of the background information. To continue the fictional example from the last chapter, we could conclude the “Background and Significance” section with:

... Specifically, we propose:

1. To quantify and localize expression of NEC factors in an established murine model of IAPCN.
   While the importance of the NEC in IAPCN has been clear, standard immunohistochemical methods have lacked the sensitivity and quantitative precision to define NEC dysregulation in IAPCN. Recently, our group has developed ultrasensitive methods to perform quantitative in situ localization of NEC factor transcripts and proteins. We will apply these methods to a well-established murine model of IAPCN to evaluate the regulation of NEC factors in different anatomic locations during various stages of disease development.

2. To evaluate the in vivo effect of small molecule inhibitors and enhancers of NEC components in the murine model of IAPCN.
   Novel small molecule inhibitors and enhancers of factors in the NEC are available in our laboratory, and we have utilized them successfully as tools to study murine Parkinson’s Disease. To evaluate whether downregulation or upregulation of NEC components have a causal role in IAPCN, we will treat control and IAPCN mice with these small molecules. The results will be correlated to those of the first aim.

3. To assess the metabolic activity in relevant brain areas in humans at risk for IAPCN.
   While evaluation in the murine model will allow precise localization of NEC dysregulation, such studies are not feasible in humans. We have established a collaboration with a neuroradiology laboratory that specializes in positron emission tomography (PET) scanning to localize metabolic activity in neuropathogenesis. Our cohort of IAPCN subjects will be evaluated with PET to explore metabolic abnormalities in this disease, and the findings will be correlated to those of the first and second aims.
Research Design and Methods

Describing your experimental plans

This is obviously the heart of the application and will receive the most scrutiny during review. The emphasis of the reviewers will vary somewhat depending on the type of grant. For training grants (F and K series), this section will be judged primarily on whether these experiments will be a useful exercise in learning skills through performing research; for independent investigator grants (R series), the emphasis will be on the appropriateness of the plans to achieve the stated specific aims. In both cases, this section should be highly organized and clear.

Importance of logical organization

The “Background and Significance” section describes the research plans for the entire project in a format that clearly conveys to the reviewers your plan of action to tackle the specific aims you have proposed. Experiments to address your hypotheses should be organized in a logical manner that displays a methodical approach to the scientific issues, with sufficient detail to convince readers of feasibility. This section should be a roadmap for research during the years of the proposed project, and clearly show the reviewers how you intend to proceed, with alternative routes if you hit roadblocks.

It is advisable to begin with an “Overview” subsection of a paragraph or two. This should serve as orientation to the research as a whole, and present the big
picture. This overview should integrate the questions posed by all the specific aims, after summarizing the “Background and Significance” section in a few sentences to serve as a reminder and transition for the reader. The overall approach for each aim should then be painted in broad strokes. Reading this subsection should provide a “forest” context for the “trees” to follow.

Each specific aim is then covered in an individual section, beginning with a verbatim restatement of the aim. The information provided in this section should elucidate the idea being explored, the specific experimental approaches and protocols, and the plans for interpreting the results. Different authors follow various organizational schemes, but including the following subheadings under each aim ensures coverage of all the key elements.

Subsections under each aim

Hypothesis. This is a succinct (one or two sentence) statement of the hypothesis being explored in the aim. This statement should be a clear expression of a theory, and not a question or a vague description of an area to be explored. You should articulate the hypothesis alone without supporting statements here; the evidence should be clear from the “Background and Significance” and “Preliminary Results,” sections as well as being briefly summarized in the following “Rationale.” Lengthiness defeats the purpose of providing an easily remembered central idea here.

Rationale. This is a concise (one or two paragraph) explanation supporting the hypothesis. High points among the evidence for the hypothesis (already covered in the “Background and Significance” section and Preliminary Data) should be summarized, so that reviewers can appreciate the thought process without being bogged down in details (that are available in the other sections). The information should be succinct and efficiently convey a reasonable case for the hypothesis.

Experimental Approach. This section describes the experiments to be performed to address the aim and its hypothesis, and is the meat of each described aim. It should be further broken into subsections that contain separate experiments or procedures. Important reagents and/or their derivation may warrant their own subsections, if they pose a potentially significant hurdle to the research. The degree of detail to include in each subsection should be as brief and clear as possible to avoid distracting the reader with trivialities, but maintain enough information to convince the reader that the approach can work and that you have the requisite technical ability and reagents. Standard assays in common usage may be abbreviated, especially if they are demonstrated successfully in the “Preliminary Results” section or an appendix, but any experimental procedures that are non-standard should be described in detail. Methods that are not already successful in the laboratory should be supported with enough information to convince the
reader that they can be achieved readily; either demonstration of closely related techniques in the “Preliminary Results” section or documentation of technical support from a collaborator are usually needed. The sources of all key reagents should be made clear, for example, by listing catalogue numbers/company names, or by referring to appended letters of support from collaborators. The listed experiments should fall into ordered categories and have a sensible flow, leading the reader through an organized train of thought to approach the issues raised in the aim. For complicated experiments that involve multiple arms or repetitions under different conditions, it is often clearer to show a table that demonstrates the various permutations, rather than trying to explain them through a running commentary.

Interpretation of Results. This is a crucial section that is often either entirely overlooked or underdeveloped by many grant writers. Although it may be obvious to you what the experimental results would indicate, it is often not so clear to the reviewers. Whereas you (as a researcher in this area) have these issues in mind constantly, it is important to remember that the reviewers may be thinking about this topic for the first time, and the implications of the experiments you propose may not be intuitively clear to them. Thus, even the most elegant experimental plans can have little impact unless their interpretation is clearly delineated. In fact, especially novel approaches may be paradoxically perceived as irrelevant if their link to the hypotheses is not made obvious.

Depending on the results expected, this subsection may be very brief or require detailed explanation. Generally the more tightly focused and obvious the aim, the less explanation will be required. For example, if you have already explained in the “Hypothesis” and “Rationale” subsections why it is important to identify binding partners for a particular protein, and your aim is to identify these partners through several techniques, a brief comment about anticipated results may suffice. However, if your aim is to perform a multivariate analysis of a patient population, it may require many paragraphs to explain the meaning of the many possible findings. The typical “Interpretation of Results” subsection falls between these extremes, and runs one to three paragraphs. Finally, a crucial yet often neglected consideration for this subsection is the possibility that a hypothesis presented for this aim is incorrect. Provision should be made for this possibility, and it should be made clear that the interpretation of the data will be able to distinguish this circumstance and allow revision of the hypothesis.

Potential Pitfalls and Alternative Approaches. This subsection is often omitted altogether in many grant applications, despite being perhaps one of the most important in the eyes of reviewers. It is almost never appropriate to leave this out. Here you discuss possible roadblocks to your experimental plans, and how you would adjust your approach to get around them. Being realistic about your limitations is crucial here, and you should show awareness of the potential problems you face in your plans, with an appropriate balance of confidence and caution. Being able to anticipate the criticisms of a reviewer and to trump them with
foresight and discussion of alternatives is an opportunity to demonstrate how clearly you have thought through your project. Failure to see obvious weak points in your experimental plans can give the impression that your approach is careless and therefore less likely to be successful.

Even if you have considered all the pitfalls during the design of your experimental plan, this will not be apparent to the reviewers unless you explicitly discuss them. Regardless of whether the plans for the aim are obviously achievable or fraught with risk, this subsection should be included. Even if the experimental approach for the aim is entirely standard for your laboratory and all the reagents are already in your hands, it is appropriate to make a short statement to this effect to indicate the importance of these issues in your mind. If the experimental plan is unavoidably high risk, discussing the possible points of failure and why this is still the best approach will show the reviewers that you are not blindly pursuing this path, and that it is a worthwhile approach to a difficult question despite the risk. This will also prevent the reviewers from assuming that you are taking this tactic because you cannot see the problems it entails. The key is to reveal your thought process, and ability to troubleshoot.

One major pitfall that should be addressed, if appropriate, is the possibility that a hypothesis being explored is incorrect. As in the “Interpretation of Results” subsection, this possibility should be given clear consideration. Here it is pivotal to explain how the experimental plan would be modified or the research direction changed if this is the case; otherwise the entire aim loses its validity if the hypothesis is wrong.

**Overall Summary and Significance.** After the final aim, many authors end this section with a subsection entitled “Overall Summary and Significance.” This is a concluding statement that revisits the big picture and discusses how the results from all the aims pertain to the general questions posed in the grant application. Depending on the complexity of the topic, this discussion may be one or several paragraphs. It should not be unnecessarily verbose, but should concisely provide a clear context for assessing the importance of the work. The importance of the results and the impact on the field should be discussed, concluding the section by stressing the relevance of the work.
Use of Literature Citations

Importance of documentation

Documentation is an important aspect of the application. As mentioned earlier, the “Background and Significance” and the “Preliminary Results” sections are intended to bring the reviewer up to date in your research area and to demonstrate your technical ability to perform the proposed work. Published findings in support of both are essential, just as for a review article. The use of literature citations is therefore central to supporting these sections and putting your work into context within your field.

Format

There are no strict guidelines for the format of the citations and bibliography, as there are for specific journals. The choice of format is therefore a matter of personal style. In line with the concept that ease for the reader and clarity are paramount, many writers choose to use the “author-year” format, where in-text citations are referenced by the last name of the first author and year of the publication. For a reviewer who is somewhat familiar with the literature in your research area, this is often the easiest format to read, because this information is enough to bring the relevant work, if not the specific paper, to mind. Because the “Literature Cited” section does not apply to the page limit of the scientific section, the references (authors, title, year, journal, volume, pages) should be completely provided in the bibliography.
Demonstrating familiarity with the literature

Beyond the purpose of literature citations in the documentation of what you write, a sometimes-neglected aspect is the role of these citations in demonstrating your familiarity with your research area. The work you cite is a reflection of your command of the information in your topic, and it is therefore important that you be thorough in acknowledging all relevant publications. While it may not be crucial to cite widely known facts (e.g., HIV/AIDS has killed millions of persons worldwide), citing primary data that support your hypotheses or experimental approach is very important. This shows that you are well versed in all the relevant information and cognizant of all pertinent aspects. While your reasoning seems crystal clear to yourself, it may not be so clear to the reader unless you lead him/her through all the information that led you to your conclusions.

Acknowledging the work of others

Another less direct impact of your literature citations is giving credit to others in the field. Although one citation for a key fact may be enough to provide documentation, failing to provide other relevant citations may leave a negative impression. Omission of those citations can suggest to the reader that you are not thorough in your knowledge of others’ work in your field. This also may give the impression that you do not properly consider all the available data. Keep in mind that your reviewers in fact may be among those others working in this area.