Basic Grantsmanship

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Outline of This Presentation

- The NIH grant submission process in brief
  - Types of grants
- Elements of a grant proposal
  - Writing the Description
  - Writing the Specific Aims
  - Writing the Background and Significance
  - Writing the Experimental Design
- Formatting tips
- The review process, submitting revisions, and success statistics

The NIH Grant Process in A Nutshell

- Submission
- Review by Study Section= IRG (3-4 months)
- Review by institute Council (+2 month)
- "Pink sheets" sent out
- Notice of grant award issued (+3-4 months)
- 9 months!

Receipt, Review, and Award Cycles

Types of Applications	Cycle I	Cycle II	Cycle III
Application Receipt Dates			
Institutional Ruth L. Kirschstein National Research Service (Kirschstein-NRSA) Awards * (**All** new, competing continuation, supplemental and revised applications) Training Grants	January 10	May 10	September 10
Academic Research Enhancement Award (AREA) (**All** new, competing continuation, supplemental and revised applications except those involving AIDS-related research)	January 25	May 25	September 25
New Research Grants and Research Career Awards	February 1	June 1	October 1
Program Project Grants and Center Grants (**All** new, competing continuation, supplemental and revised applications)	February 1	June 1	October 1
Competing Continuation, Supplemental and Revised: Research Grants and Research Career Awards	March 1	July 1	November 1
Small Business Innovation Research (SBIR), Small Business Technology Transfer (STTR) Grants (**All** new, supplemental, and revised applications except AIDS and AIDS-Related applications)	April 1	August 1	December 1
PRE+POSTDOC AWARDS **Individual Kirschstein-NRSA Awards (Standard)** **	April 5	August 5	December 5
Conference Grants and Conference Cooperative Agreements (**All** new, competing continuation, supplemental and revised applications)	April 15	August 15	December 15
AIDS and AIDS-Related Grants (**All** new, competing continuations, supplemental and revised applications; *includes* AIDS and AIDS-Related SBIR/STTR)	May 1	September 1	January 2
Review and Award Schedule			
Scientific Merit Review	June - July	October - November	February - March
Advisory Council Review	September - October	January - February	May - June
Earliest Project Start Date ***	December	April	July

* *Several NIH Institutes and Centers use only one or two of the receipt dates for Institutional NRSA awards. Please check the program announcement for "Institutional Research Training Grants (T32)" at http://grants.nih.gov/training/nrsa.htm.*

** *The National Research Service Award Individual Predoctoral Fellowships for **Minority Students** and **Students with Disabilities** have special receipt dates.*

*** *Awarding components may not always be able to honor the requested start date of an application. Therefore, applicants should make no commitments or obligations until confirmation of the start date by the awarding component.*

Kinds of NIH Grants, Research

- R01- investigator-initiated research
  - Average size is 225K per year direct costs
- PO1- program project grants
- R21- small pilot studies- 2 years
  - Only 100-150K per year
- There are many, many other kinds- R15, Pioneer Awards
- All compete for extramural funds within an Institute

Kinds of NIH Grants- Training

- F and T-series- training grants for postdocs
- K awards- for training and support of faculty awards
  - K05- Faculty development award
  - KO3- Mentored Research Scientist Award
  - KO8- Mentored Clinical Scientist Development Award
  - K23- Mentored Patient-Oriented Research Career Development Award
  - New -K99/R00- Pathway to Independence Award
    - 1-2 years of mentored support followed by 3 years of independent support
    - DP2- new innovators award (risky new PI projects)

NIH Resources- Do Your Homework First

- Find out success rates for different institutes and types of grants
- Find out what your competitors are doing (CRISP)- ie what type of research actually gets funded
- Identify the expertise of the various study sections and suggest a specific study section
- Find out if any institutes have RFAs into which your proposal would fit
- Tailor your proposal to an institute and to reviewers

The NIH Grant Process

- Submission
  - 3 times a year (dates vary by grant type)
  - Electronic submission dates
    - Your institution's deadlines precede the NIH's
  - RFAs (Request for Applications) can have special dates
  - Sent to CSR (Center for Scientific Review)
  - Keep submission date table (next slide) near your desk!

Planning and Writing

Planning a Grant: Timing

- Get preliminary data during the previous 6-12 months; prepare figures
- Sketch out possible specific aims at least 6 months ahead (can have extra)
- Beef up YOUR qualifications by publishing as much as possible in the best journals
- Allow 3 months minimum to write; ramp up effort to 100% in last few weeks (go to a quiet place!)

Experimental Design- Planning

- A focused project with a small number of related aims is more likely to be funded than a large, diffuse project or one containing unrelated aims
- Use three specific aims if possible (2- 4)
- Do not be overambitious! (common beginner failing is to include 10 years of work)
- Get advice from senior colleagues on potential aims during planning stages

Grant Mentoring

- Get as much help as possible at all stages of planning and writing
- You can't have too much help!
- Ask for examples of successful grants from more senior colleagues
- Ask for help from non-scientists to catch typos!

Novelty of Project

- Can be novel in methods or in hypothesis
- Novelty is a double-edged sword- it's good, but will reviewers believe in your paradigm shift?
  - Reviewers want to see other groups (peer review) agree with novel concepts first
  - Other papers (preferably from other groups) must have already been published in reputable journals!

Remember

- You should propose to do the precise experiments which move the entire field forward as directly and quickly as possible
  - Don't propose experiments just because they CAN be done, or are variants of things that were done before that just prove the same things

When to Submit A Grant
(ie stop doing experiments)

- You can show the need for the work in the field
  - Hypothesis-driven research *vs* cataloging (ie a specific, later developmental stage in a research project)
  - Important disparity in current research of others
  - RFA (Request for Applications) indicates interest by NIH
- You can show you can accomplish the work
  - Feasibility studies done; or you have publications with techniques
- Your preliminary results show promise (but need more data)
  - Do not do *everything* ahead of time!

Page Requirements (note that NIH may soon drop to 12-15 pages)

- Specific Aims- 1 page
- Background and Significance- 3 pages
- Preliminary Studies- 3-4 pages (no limits) OR Progress Report- usually 7-10 pages for a 5 year grant (no specific limits)
- Experimental Design – totals 25 pages including the above

The Cover Letter

- Used to direct your grant to a specific study section and/or institute- will almost always accomplish this task
  - CSR encourages this. Spend time researching IRGs
- Should be very brief: only states that you believe that the XXX study section has the requisite expertise to review your grant and/or that the work falls within the purview of the XX institute
- Having your grant reviewed by people who do the same kinds of studies (= like the topic; approve of the methods) is <u>critical</u>

Targeting

- If you have no cover letter, your title, abstracts, keywords, and aims are used to target your grants
- Diseases mentioned will target it both for funding by a specific Institute
- You may suggest the type of expertise required to review, but do not suggest specific people!

The Description (Abstract)

- This is the only thing most reviewers at the study section will read
- Introduce the subject, briefly explain what has been done, and what gaps remain
- Describe each of your aims succinctly, summarizing what you will learn
- Put the project into a clinical perspective
- Polish: remove extra words, and make it elegant!

Specific Aims

- A one page summary of the proposal (*vs* abstract which is a half-page summary- language can be duplicated)
- Specific aims test specific predictions: hypotheses which involve mechanisms are best
- Provide rationale and brief summary of work, and expected impact on field
- Refine and revise multiple times! Very important part of the proposal (second only to abstract/description)

A. SPECIFIC AIMS

The anthrax bacillus produces a three-component exotoxin of which an essential element is the protein known as protective antigen, or PA. PA binds to a cell surface receptor and is cleaved to generate a 63 kDa protein to which the one of the other anthrax toxins, LF and EF, can then bind. Proteolysis and binding of PA permits internalization of the PA-LF complex into the endosomes and then the cytosol, where LF is able to attack cellular machinery and cause cell death. Since proteolytic cleavage of the PA anthrax toxin is obligatory for the manifestation of toxic activity, this cleavage step represents a natural target for pharmacologic intervention. Previous research has shown that this cleavage is performed by a cellular surface enzyme known as furin, a member of the family of eukaryotic subtilisins. This proposal centers on the hypothesis that the cytotoxic action of anthrax toxin can be blocked through inhibition of the activating cleavage event, resulting in lessened toxicity and cellular protection.

In the last decade, several groups have shown that it is possible to block bacterial toxin cleavage using large molecule protein inhibitors of furin (such as mutated derivatives of alpha 1 antitrypsin, "PDX"; Jean et al., 1998). Our work has focused on identifying small molecule inhibitors of furin through combinatorial chemistry techniques, and we have recently identified a stable hexapeptide, D6R (D-hexa-arginine), which represents a potent inhibitor of furin (Cameron et al., 2000). Our preliminary data indicate that D6R can effectively inhibit furin-mediated cleavage of a bacterial toxin derived from *Pseudomonas*, thus blocking lethal effects, in both cell lines as well as live animals. Initial experiments indicate that anthrax toxin activation can also be blocked by D6R. We here propose to systematically apply our studies of furin inhibition to the blockade of anthrax toxin activation. Specifically, we will pursue the following specific aims:

Specific Aim I. Investigation of D6R as a potential therapeutic in the blockade of anthrax toxin cytotoxicity.

A. What is the time course and the ED50 for PA in the presence of the stable furin inhibitor D6R? How does preincubation affect D6R potency? How potent is this small molecule inhibitor compared to the engineered protein alpha-1- PDX?

B. Can direct inhibition of PA precursor cleavage by D6R be demonstrated using the iodinated PA precursor as a substrate for recombinant furin?

Specific Aim II. Examination of the structural requirements for inhibition of furin-mediated cytotoxicity by D6R-related molecules.

A. What are the potencies of modified furin inhibitors against recombinant furin in a fluorogenic assay?

B. What are the potencies of modified furin inhibitors in the RAW cell anthrax toxin cytotoxicity assay?

C. What is the specificity of the modified inhibitors for furin as opposed to the related convertases PC1, PC2, and PACE4?

Specific Aim III. To test the use of D6R and/or other stable furin inhibitors identified in Aim II in animal models of anthrax toxicity.

A. Does toxin-induced production of the cytokine TNF-alpha decrease upon administration of D6R?

B. Do D6R-related peptides protect against anthrax toxin in a rat model of intoxication?

C. Are D6R-related peptides toxic when administered acutely or chronically to animals?

B. BACKGROUND AND SIGNIFICANCE

Anthrax and the activation of anthrax toxins

The causative agent of anthrax is the bacterium known as *Bacillus anthracis*, a gram-positive, spore-forming, rod-shaped bacterium. Infection of mammals by anthrax spores results in germination within alveolar

Specific Aims can contain questions

Or not..

A. SPECIFIC AIMS

Within the past decade substantial progress has been made in deciphering the enzymatic mechanisms responsible for opioid peptide synthesis. Opioid peptide precursors are now known to be cleaved by prohormone convertases 1 and 2, subtilisin-like proteases restricted to neuroendocrine tissues, to produce small bioactive opioid peptides. Our work during the past two cycles of this grant has focused on the biochemical characterization of these enzymes. We have produced both enzymes in recombinant form and have defined their specificity on a model opioid peptide precursor, proenkephalin; we now know that PC2 is the enzyme responsible for the majority of small opioid peptide production from this precursor, while PC1 functions to generate large opioid-containing intermediates. Working with Richard Houghten, we have also identified potent inhibitors of both enzymes through the use of combinatorial peptide library screening. Most recently, in collaboration with Wolfram Bode, we have obtained the first crystal structure of this family of enzymes, the related enzyme furin; all of this work has been funded directly from this NIDA grant.

In this renewal application, we propose to apply the structural information which has been gained thus far to study mutants of PC1 and PC2, which will help us to understand the molecular basis of convertase specificity. We will continue to develop convertase inhibitors, and will pursue two practical applications of our ability to produce large quantities of recombinant convertases, crystallization and *in vitro* neuropeptide production. The proposed work consists of the following four specific aims:

Specific Aim 1. Structure-function analysis of PC1 and PC2: site-directed mutagenesis
We are now in possession of considerable structural information for the two convertases responsible for the synthesis of active opioid peptides. By exploiting this structural information through rational mutagenesis, we will further our knowledge of the enzymology of opioid peptide synthesis.

Specific Aim 2. Crystallography of PC1, PC2 and proPC2 (with W. Bode)
Our collaborative efforts with the Bode group have been highly successful with the recent crystallization of mouse furin. We now plan to extend these efforts to prepare milligram quantities of highly purified mouse PC1, PC2 and proPC2 for similar crystallographic studies.

Specific Aim 3. Characterization of small molecule inhibitors of PC2 (with R.A. Houghten)
Considerable evidence has accumulated to support the idea that PC2 is the enzyme responsible for opioid peptide production as well as for the production of other small bioactive peptide products. Successful inhibition of this enzyme with a small molecule inhibitor could be useful in several disease states. We plan to continue to screen combinatorial small molecule libraries obtained from this group, as well as to more fully characterize promising compounds already identified.

Specific Aim 4. *In vitro* synthesis of bioactive peptides from recombinant precursors (with M. Simon)
Our overexpression experiments have resulted in the production of chemical quantities of the opioid peptide precursors proenkephalin and prodynorphin; overexpression of proopiomelanocortin is now well underway. When combined with our purified recombinant processing enzymes, these precursors can be used as source materials to generate bioactive peptides that may be targets for orphan G-protein coupled receptors.

These aims build upon the increasing knowledge of the biochemistry and structure of proprotein convertases obtained during previous cycles of this grant, and should further our knowledge of the enzymatic mechanisms responsible for opioid peptide synthesis.

Introduction to Your Revised Grant -3 Pages

- Do not be argumentative. Accept responsibility for not making your arguments persuasive the first time!
- Yield on most if not all points by revising the proposal according to the wishes of the reviewers
- Re-state your previous score so the reviewers can improve it (both score and percentile)
- Outline precisely how you have responded and mark the grant with lines in margins
  - Not with italics or with different fonts!

Background and Significance

- Comprehensive and clear background for the scientific reader who is not in the field
- In
lit
- Co
discrepancies that the present grant will address
- Clinical relevance can go here as well as in significance section
- Persuasive rhetoric: at the end, the reader agree that the proposed studies are necessary and important

Minimize Acronyms and Jargon!

Rhetoric

- Definition: using language effectively to please or persuade
- Should be aware of this requirement continuously
- Will involve some repetition of key elements throughout grant

Preliminary Studies

- Should be closely linked to Specific Aims. So state directly! ("these data support our ability to perform the experiments outlined in Aim 2")
- Convince the reviewer that your ideas and methods are good
  - You *can* design logical and well-controlled experiments
  - You *can* present your results clearly
- Do <u>not</u> include small experimental details (5 ul)

Preliminary Studies

- Figures should be formatted nicely and located on the same page as the discussion. Use a conclusion for each title!
- Set them off by using a different typeface
- Number them for easy reference

Progress Report

- Format with respect to publications you had during the funding period
- Re-state all of the conclusions you came to as a result of each publication
- Include additional work you did which was not initially proposed, if it is relevant to the current grant
- Extremely important section -to show that you did not waste previously awarded monies
- Ends with a list of publications credited to the grant

Experimental Design

- Use tried and true format:
  - 1) Rationale
  - 2) Experimental design
  - 3) Anticipated results and interpretation
  - 4) Potential problems and alternative approaches
- The experimental design section ALWAYS follows the order given in the Specific Aims

Rationale

- Ties into the background section
- Provides brief explanation for the experiments which follow

The Rationale Begins the Design Section

initially succeed since many conditions will have to be tested. However our success with the related protein furin gives us confidence that we may have established initial conditions which may lead to success. In addition, the wide experience of our collaborator, who has a distinguished history of success with both serine proteinases as well as a variety of other proteases (see CV attached), bodes well for the success of these crystallographic efforts.

Summary and Significance, Aim 2. The proposed experiments to determine the crystal structure of the two most important neuroendocrine proteolytic processing enzymes will provide us with substantial new information on the enzymes which control neuropeptide processing. The structures will represent the only other mammalian family members which have been crystallized (although the distantly related Kex2 subtilase was crystallized this year; Holyoak et al., 2003) and so will provide us with the basic common elements of these enzymes as well as detail the molecular differences which confer specificity and other characteristics unique to each enzyme.

SPECIFIC AIM 3. CHARACTERIZATION OF SMALL MOLECULE INHIBITORS OF PC2

Rationale: Considerable evidence indicates that PC2 is the enzyme responsible for small opioid peptide production as well as the production of other small bioactive peptide products (Johanning et al., 1998; Miller et al., 2003a; Berman et al., 2000; Laurent et al., 2003). It is interesting that PC2 can be inhibited by two natural products sharing little sequence similarity: the CRES protein (Cornwall et al., 2003) and 7B2 (Martens et al., 1994). We have shown that combinatorial peptide and peptidomimetic libraries represent effective means to identify both natural and synthetic inhibitors of convertases (Apletalina et al., 1998; Cameron et al., 2000). We have already screened four peptide and peptidomimetic libraries for inhibition by PC2. The bicyclic guanidine library has yielded two potential targets, and we intend to follow up on these hits, as described below. At the same time, we will continue to screen other small molecule libraries supplied to us through our collaboration with Drs. Houghten and Appel of the Torrey Pines Institute for Molecular Studies (see attached letter and CV).

Experimental Design:

A. Small molecule library screens

A number of heterocyclic and small molecule positional scanning combinatorial libraries are available for screening against PC2 for the identification of inhibitory activity. The diversities for these mixture-

Experimental Design: What Constitutes a *Good* Experiment?

Unambiguously interpretable results!

- If result 1 is obtained, hypothesis is upheld
- If result 2 is obtained, a new (but still interesting) direction is indicated
- Stronger if two different approaches are used to confirm hypotheses

What Constitutes a *Good* Experiment? (II)

- Perhaps boring, but studies are necessary to be able to derive a mechanism
  - Pathways generally are great aims (unless impossibly complex)
- You have a corner on the market
  - No one else is using the approach/asking the questions that you are
  - You have a unique reagent/cell line/animal/technique

What Constitutes a *Bad* Experiment?

- PI makes claim for method that overextends method's reach, or is inappropriate, or outdated
  - I have a special calibrated string to measure the circumference of the earth
  - I have tested it locally and it works well
  - Therefore I can use it to measure the circumference of the earth
  - (note lack of detail as to how I will do this!)
- PI addresses a problem that reviewers think is trivial (this can sometimes be overcome by sending to a different study section!)

What Constitutes a *Bad* Experiment? (II)

- Riskiness
  - Reviewers do not believe that the experiment will come out in the manner predicted
    - (leads to risk of pyramid scheme)
  - Yeast two-hybrid (often yields no results)
  - "Proteomics"- NIH says it wants, but reviewers do not like (not hypothesis-driven!)
- Outside of current paradigm
  - There is a time when every experiment is novel yet begins to fit into current thinking; if not there yet is "premature"
    - ER degradation mechanism as example- before we knew about retrograde transport out of the ER, how could proteosomes be logically involved in secretory protein degradation?

What Constitutes a *Bad* Experiment? (III)

- Cataloging data ("Descriptive")
  - Data must already fit into a hypothesis
- No quantitation proposed
  - How will different models/hypotheses be statistically distinguished?
  - How will experimental bias be avoided?
- All controls are not included
  - How can results be arrived at artifactually?
- Be your own worst critic!

Experimental Design

- *Why* did you choose the approach that you did?
  - Convince reviewers that it is the best approach of all that are currently available. Cite the success of other investigators -with specific references.
  - Remember Rhetoric!

After Experimental Design: "Anticipated Results and Interpretation"

- Use "anticipated results section" to convince reviewer that you will move science forward -no matter how experiments come out
- Most common failing of grants is to omit the interpretation section
  - Make it obvious what you will learn from each set of experiments; and how this moves the field forward: rhetoric!

Results and Interpretation Section

experiments, see NIH CV.

Results and Interpretation: These experiments will provide information on the molecular determinants of PC2 specificity as well as on many other biochemical characteristics of this important opioid synthesizing enzyme. We will learn whether PC2-specific residues in the binding pocket also contribute to the lowered pH optimum and the lowered calcium requirement of this enzyme. We will also learn whether the binding of 21 kDa 7B2 is affected by the mutations, which will further localize the site of interaction of these two proteins. We will confirm or refute the location of the binding of the 7B2 CT peptide as the P4 region of the pocket; and, by analyzing the rate of autoactivation in the various mutants, will be able to observe whether activation- an autocatalytic process which exhibits many important enzymatic differences to substrate cleavage- is affected by a given mutation.

Potential Problems and Alternative Approaches: It is possible that our cross-enzyme modeling efforts are erroneous because they are not based upon the actual structures of PC1 and PC2 but on the structure of furin. We are currently engaged in a more highly refined modeling effort together with the Bode laboratory; other potential mutations may become apparent when these efforts are more refined, although the models generated to date do support the proposed mutations. Finally, when the crystal

Use words like : will provide, will learn, confirm/refute, understand etc… ie you will move the field forward!

Potential Problems (or pitfalls) and Alternative Approaches

- Use "pitfalls section" to anticipate possible problems- then try to persuade that they are not serious because you have alternative approaches (or because others have data showing this)

Potential Problems and Alternative Approaches

Identify the problems before your reviewers do- then say why you don't believe they will be obstacles, but if they are, what you will do

will be identified that will require testing *in vivo*; as discussed below, the ability to enter cells will play a significant role in inhibitory capacity, and this may not be strictly correlated with inhibitory potency *in vitro*.

Potential problems and alternative solutions: The ability of the various compounds to enter cells and to inhibit proenkephalin cleavage intracellularly may represent a potential problem in these studies. It should be noted that many of these compounds are arginine derivatives; polyarginines, along with other basic peptides such as TAT, are known to efficiently cross cell membranes (Mitchell et al., 2000) and are indeed used as "protein transduction" reagents (Matsui et al., 2003). We have been able to use added D-polyarginines to block furin-mediated steps within cells; the cytotoxic cleavage of *Pseudomonas* exotoxin A in HEK293 cells (Sarac et al., 2002) is thought to occur within the endosomal pathway (reviewed in Molloy et al., 1999). Finally, if uptake presents a serious problem, we will consider derivatization of the molecules with a lipophilic group (such as decanoyl; this was previously found to be effective for the chloromethylketone furin inhibitor [Hallenberger et al., 1992]).

Summary and Significance, Aim 3: Our goal in these studies is to identify new small molecule inhibitors of PC2 which can be used to target various endocrine pathologies, including diseases of excess hormone production. These include ectopic peptide production in small cell carcinoma, insulinoma, and pituitary Cushing's. Blocking the production of glucagon- largely a PC2-mediated process- could also benefit

Summary: What Is an Overall Good Grant? (NIH criteria)

- Significance
  - Addresses an important problem
  - Advances our knowledge
  - Will impact field
- Approach
  - Appropriate to question and is state-of-the-art; controls and statistics are always considered
  - Problem areas considered and alternatives given
- Innovation
  - Novel concepts or technologies are a plus IF they are experimentally secure
- Investigator- has a good track record and has right expertise
- Environment- is supportive, provides needed equipment

Common Sense Items

- Step back and look at your reasoning. Would you buy it from someone else?
- Accept criticism from your colleagues even if you think it is wrong: it means you did not get your point across
- Don't perfect the beginning at the expense of the end- work on the last aim alone some days!
- Polish, polish, and polish again. Remove excess words; construct clearer sentences; improve formatting
- Give yourself enough time!

Timetable

- This section is only a few lines and describes the order in which you intend to carry out the experiments
- Most clear with a graphic format, although with simple grants a few sentences will suffice
- Not strictly necessary

References

- You must include the titles of all references
- Check to make sure that your references are accurate!
- Any format ok

Vertebrate Animals

- There are 5 specific points you must address
- You must provide justification for numbers you plan to use and also species
- Animal Care certification is required (can get after submission, but must be in place prior to award)

Budget

- Modular applications
  - $25,000 modules up to $250,000
  - No budget justifications
- Non-modular
  - everything above $250,000
  - Budget justifications included

The Budget

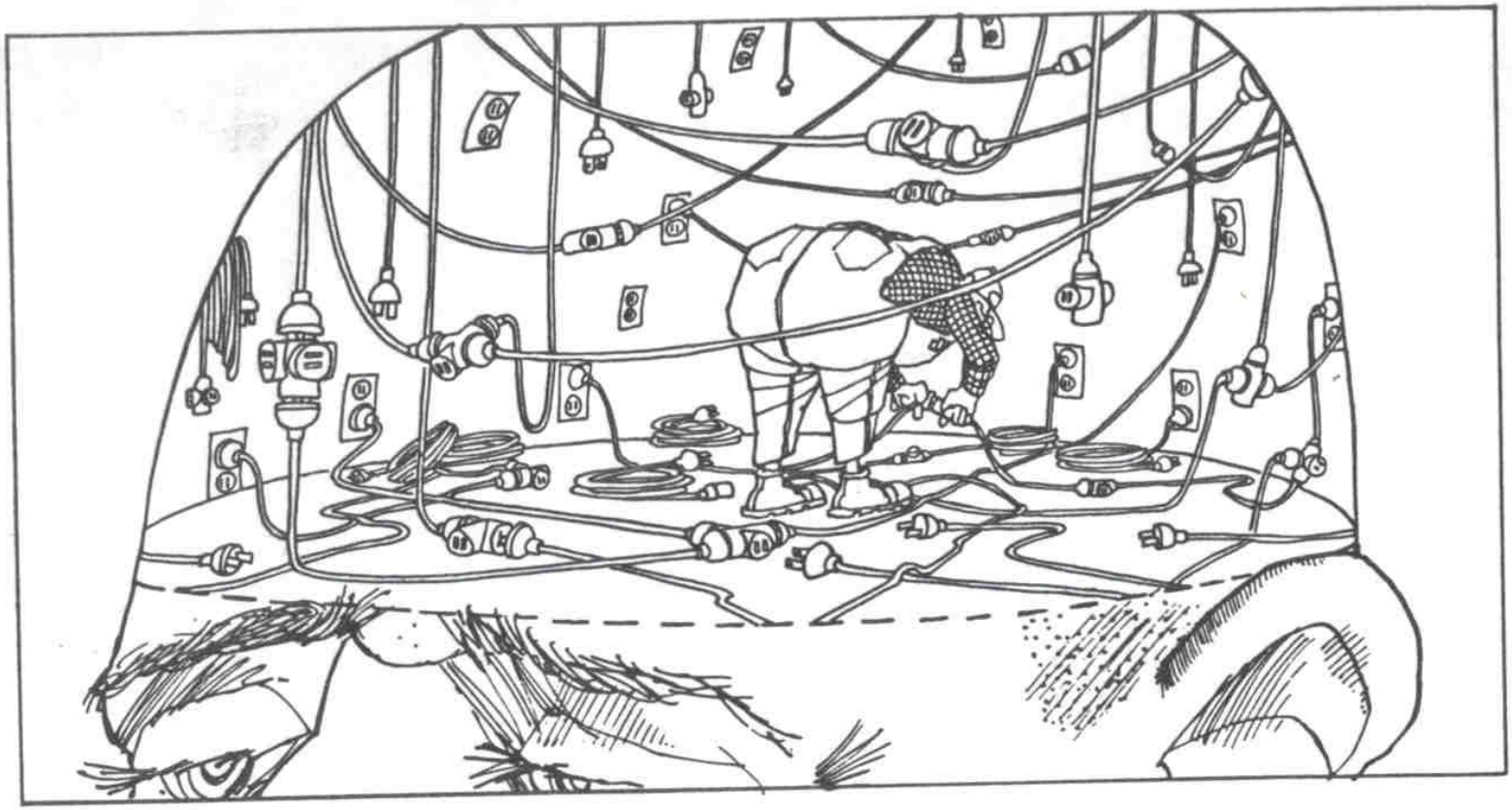
- For equipment, document convincingly why the piece is essential and why the specified model is required.
- For personnel:
  - Document the unique and essential role in the grant that each will play, and state how their qualifications match with their roles.
- Do not be afraid to include personnel and equipment justifications even though the guidelines say you don't need to have them- the reviewers will appreciate the clarification they provide

Budget

- Ask for realistic numbers of people and support
  - $$ and people should bear a reasonable relationship to the work proposed
- Assign each person (FTE) certain tasks (can split effort between aims or grants)
- Supplies- usually 12-15K per FTE is ok
- Equipment- request one large piece in your first grant
- Travel- only 1K per year x 2 FTEs allowable
- Secretarial support <u>not</u> allowable in most cases

Appendix

- Can include up to 3 PDFs of your relevant papers.
- THAT's ALL! (new)



How the brain works.

Any Questions?

Formatting

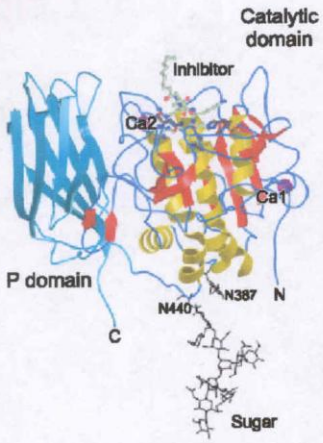
The Package Is As Important as the Content

- Reviewers cannot extract a great experiment from a hard-to-read page
- Do not use "busy" fonts or column layout
  - Use Sans Serif such as Arial for Figures (10 point) and a Serif font (Times Roman or Palatino at 11 point) for all the rest of the text
- Do not combine bold, underline, italics and many different font/font sizes on one page (and never underline! it is very difficult to read)
- Separate all paragraphs with empty space- make it look like a book (ie, easy to read)

Make it Easy!

- Reviewers will read your grant over several days or even weeks
- Construct discrete sections which can be understood alone
- They will not remember a rationale you presented only in the Background and Significance section when they are reading the Experimental Design

Figure 2. Crystal structure of mouse furin (from Henrich et al, 2003)



The furin crystal structure **(Figure 2)** reveals many interesting aspects, not the least of which is an extremely acidic substrate-binding groove. This extended region of negative charge is sufficient to explain the highly inhibitory properties of polyarginines previously discovered in our combinatorial peptide library screening efforts. Another interesting finding was the novel structure of the P domain, a beta barrel which abuts the catalytic domain and affects catalytic parameters, and was found to be formed by a jellyroll-type beta barrel (shown in blue in Figure 2 above). The furin structure was published this year in Nature Structural Biology (Henrich et al 2003, #7).

2. CRES collaboration (with G. Cornwall). The protein CRES is a testes-specific cystatin-related protein which is however inactive against cysteine proteinases due to its lack of certain critical residues (Cornwall et al 2002, #6). We have collaborated with Dr. Gail Cornwall of Texas Tech to examine the inhibition of this protein against PC2; Dr. Cornwall initially contacted us because PC2 is colocalized with CRES in testicular tissue. Our experiments surprisingly showed that CRES represents a potent inhibitor of PC2, with inhibitory activity in the nanomolar range. Other serine proteases and other proprotein convertases were not affected. We are continuing to collaborate with Dr. Cornwall on structure-function analysis of CRES inhibition (Cornwall et al, 2000; #6).

3. Development of the mini-RIA. In order to facilitate radioimmunoassay of a large number of fractions derived from size fractionation of precursor cleavage products, we developed a "mini-RIA" procedure in which tubes are handled in 96 well racks during the majority of the RIA. This technique, which saves considerable operator time, has now been published in Analytical Biochemistry (Laurent and Lindberg, 2002; #5). This technique will be useful in analyzing the effect of mutations on specificity in Aim 1.

4. Overexpression of CPE, PAM and POMC. We have recently initiated the overexpression of rat CPE (vector obtained from Dr. Lloyd Fricker of AECOM) using the CHO cell system, and have received PAM-3-overexpressing CHO cells from Dr. Betty Eipper of the University of Connecticut, which we are in the process of amplifying further. Expression has been confirmed using enzyme activity assay as well as Western blotting. The current estimated expression levels of CPE and PAM-3 are about 0.1 mg/liter (based on the known specific activity of purified recombinant CPE and PAM-3 previously obtained from the Fricker and Eipper laboratories; we have also obtained recombinant PAM-3 from Unigene); however, we have only begun methotrexate amplification, and expect to ultimately achieve 10-20 times these levels. These studies support our ability to perform the experiments described in Specific Aim 4.

PROJECT GENERATED RESOURCES: During the course of this work we have generated milligram quantities of convertases (mouse PC1, mouse PC2, and human and mouse furins) and developed inhibitors useful both *in vivo* and *in vitro*. We make limited quantities of both purified proteins and peptides (where financially feasible) available to the scientific community. In addition, we have generated recombinant prodynorphin, proenkephalin, proenkephalin antisera, and proenkephalin

Lots of white space between small paragraphs

The Package Is As Important as the Content

- Be extremely clear- few abbreviations, a simple layout, **repeat/rephrase** your necessary justifying statements throughout
- No jargon!- it is not likely that the reviewer is exactly in your field
  - hold down the number of acronyms please!
- Perfect spelling and grammar show that you can pay attention to detail

Consider putting experimental detail in a separate section at the end so that the flow of experiments is not interrupted

Methods Section (an NIH-acceptable 10 point font)

to synthesize and test for biological activity.

SPECIFIC METHODS

Protein overexpression We typically employ the dihydrofolate reductase-coupled method of protein overexpression in CHO cells (Lindberg and Zhou, 1995), a method which results in the transfected protein reaching levels of expression which are between 1-3 mg/ liter (secreted into the medium). We are also now testing the more rapid glutamine synthetase-coupled method of expression in which the inhibitor methoximine is used to drive expression levels to even higher levels (10-50 mg/liter) (Cockett et al., 1990); preliminary data indicate that this is indeed the case.

PC2 assay PC2 is assayed in a 50 ul total volume in round-bottomed polypropylene microtiter plates in 0.1 M sodium acetate, pH 5.0, containing 2 mM calcium chloride, and 0.1% Brij, a nonionic detergent. Five to ten ng of PC2 are typically employed per assay. The substrate used is 0.2 mM pyr-RTKR-aminomethyl coumarin, and the fluorescence of the highly fluorescent proteolytic product MCA (methylamino coumarin) is measured in a Fluoroskan kinetic plate reader thermostatted at 37C at 380 nm excitation and 460 emission. The fluorescence is calibrated against a curve of free MCA.

Co-immunoprecipitation of PC2 and 7B2 Stable transfection of wild type PC2 and PC2 mutants will be performed in AtT-20 cells as previously described (Apletalina et al., 2000). The cells will be labeled with [S35]methionine for 20 min and chased for 30 min. The cells will then be extracted with coimmunoprecipitation buffer, immunoprecipitated with our PC2 antiserum, and subjected to gradient SDS PAGE and autoradiography to gauge the extent of interaction of the molecules with each other.

PC1 assay PC1 is assayed identically to PC2 except that the pH of the acetate buffer is 5.5, the optimum pH for this enzyme. Approximately 100 x more PC1 (500-1000 ng) than PC2 is used in order to obtain a reasonable fluorescent signal within a 2 h time frame.

PC1 truncation rate The rate of carboxyl terminal cleavage of wild-type and mutant PC1s to the 66 kDa form will be assessed by incubation at 37C in PC1 reaction buffer, followed by Western blotting with antiserum 2B6 which detects all forms of PC1 (Vindrola and Lindberg, 1992).

PAM assay The PAM assays previously worked out by others are used (Kolhekar et al., 1997, Miller et al., 1992). Briefly, these involve amidation of [125I]-YVG in the presence of 5 uM cold YVG, then separation of product by extraction into ethyl acetate and quantitation. Only linear rates are used to calculate specific activities (by comparison with purified recombinant PAM obtained from B. Eipper or A. Consalvo of Unigene).

CPE assay The CPE assays involve the use of the substrate dansyl-FAR in sodium acetate buffer at pH 5 (Fricker, 1995); following incubation, acidified chloroform is added to extract dansylated product lacking basic residues,

Summaries

- Use summaries throughout the grant to help the reviewer see what the grand goals of each aim are
- Use a summary at the end of the grant to rephrase again how this proposal will move science forward ("tell them what you told them")
- Again, writing a grant is an act of rhetoric: you must persuade

Use of Summaries

be effective for the chloromethylketone furin inhibitor [Hallenberger et al., 1992]).

Summary and Significance, Aim 3: Our goal in these studies is to identify new small molecule inhibitors of PC2 which can be used to target various endocrine pathologies, including diseases of excess hormone production. These include ectopic peptide production in small cell carcinoma, insulinoma, and pituitary Cushing's. Blocking the production of glucagon- largely a PC2-mediated process- could also benefit diabetics, as glucagon acts in opposition to insulin, and insulin is predominantly synthesized by PC1. We have already demonstrated that stable polyarginine inhibitors of furin can be useful in inhibiting convertase-mediated pathological processes both in cell lines as well as in live animals (Sarac et al., 2002). These data suggest that therapeutic inhibition of convertases may indeed represent a viable pharmacological approach. Lastly, the identification of potent PC2 inhibitors that are both specific and active *in vivo* will represent a major step forward for the future study of this convertase in that we will be able to identify physiological pathways that depend upon the generation of PC2-specific peptides.

This reinforces your message as to the point of the aim!

Always Get Multiple Outside Opinions

- You should have other people look at your grant at several stages
  - Specific Aims can be discussed with colleagues even prior to beginning to write
- Give your first draft to as many colleagues, both expert as well as non-expert, senior and non-senior, who will agree to read it (give them 2 weeks!)
- Give the final draft to someone who is very good at finding typos and sentence errors (1-2 days)

Self-Check

- Did you provide persuasive language in every section?
  - Do not use highly self-aggrandizing language
- Did you make sure the last Aim is as well-written as the first?
- Did you polish sufficiently?

Allow Time for Institutional Processing

- Varies from 2 days to 2 weeks depending on institution
- In-house grants people will make sure that your numbers add up and that your indirect cost figures are correct
- They must now send in every grant you submit electronically

Submitting Additional Material Prior to Review

- Do not submit this just a few days before meeting, because reviews are already written
- Send it 2-3 weeks before the study section meets
- A 1-2 page update is sufficient (2 is max)
  - Papers newly accepted for publication
  - New experimental findings that support feasibility or importance of the work

Review

Receipt by NIH

- Number assigned:
  - 1 R01 DA 123456 -13A1 type (new=1, competing=2 etc), mechanism, institute, identifier #, year, and revision
- Direction toward a specific IRG for merit review
  - Each of the 20 Initial Review Groups has 5-10 SRGs or Scientific Review Groups (120 total)
  - Each is headed by an SRA or Scientific Review Administrator – get to know yours!
- Direction toward most related Institute for funding
  - Program Officers divide up grants and try to attend IRG meetings
- 77,000 grant applications per year (up from 45,000 after doubling of budget)

IRG = study section =Scientific Review

- About 12-20 scientists chosen to represent a cross-section of various fields of expertise
  - Make sure your grant can be understood by someone whose work only distantly relates to yours
- Get six weeks to read 8-12 grants; 75-100 grants are a typical load for a study section
- Half are "streamlined"= "triaged"=UN=not scored. These are not discussed at the meeting; do receive full review)

The Study Section

- Scores of the 2-3 assigned reviewers are given at the very beginning and again after reviews are presented (primary, secondary and [optional] reader) – websites now facilitate agreement
- 15-20 min discussion per grant
- Reconciliation of differing scores among 3 reviewers typically occurs prior to the general vote
- Study section members then "vote their conscience"
- Average of all members' score is used to calculate (outliers may be removed at the SRA's discretion)
- Budget is then discussed

Grant Review

- Lower half are triaged- not subjected to discussion- but do receive full reviews. Not scored (just say "bottom half" or UN= unscored)
- Scored applications:
  - 1-1.5 "outstanding" (very rare)
  - 1.5-2.0 "excellent" (most common: fundable grants are often closely clustered in this range)
  - 2.0-3.0 "very good" to "good"
  - 3 - 5 below average
- Only 1.0- 1.5 will now be funded

After Review

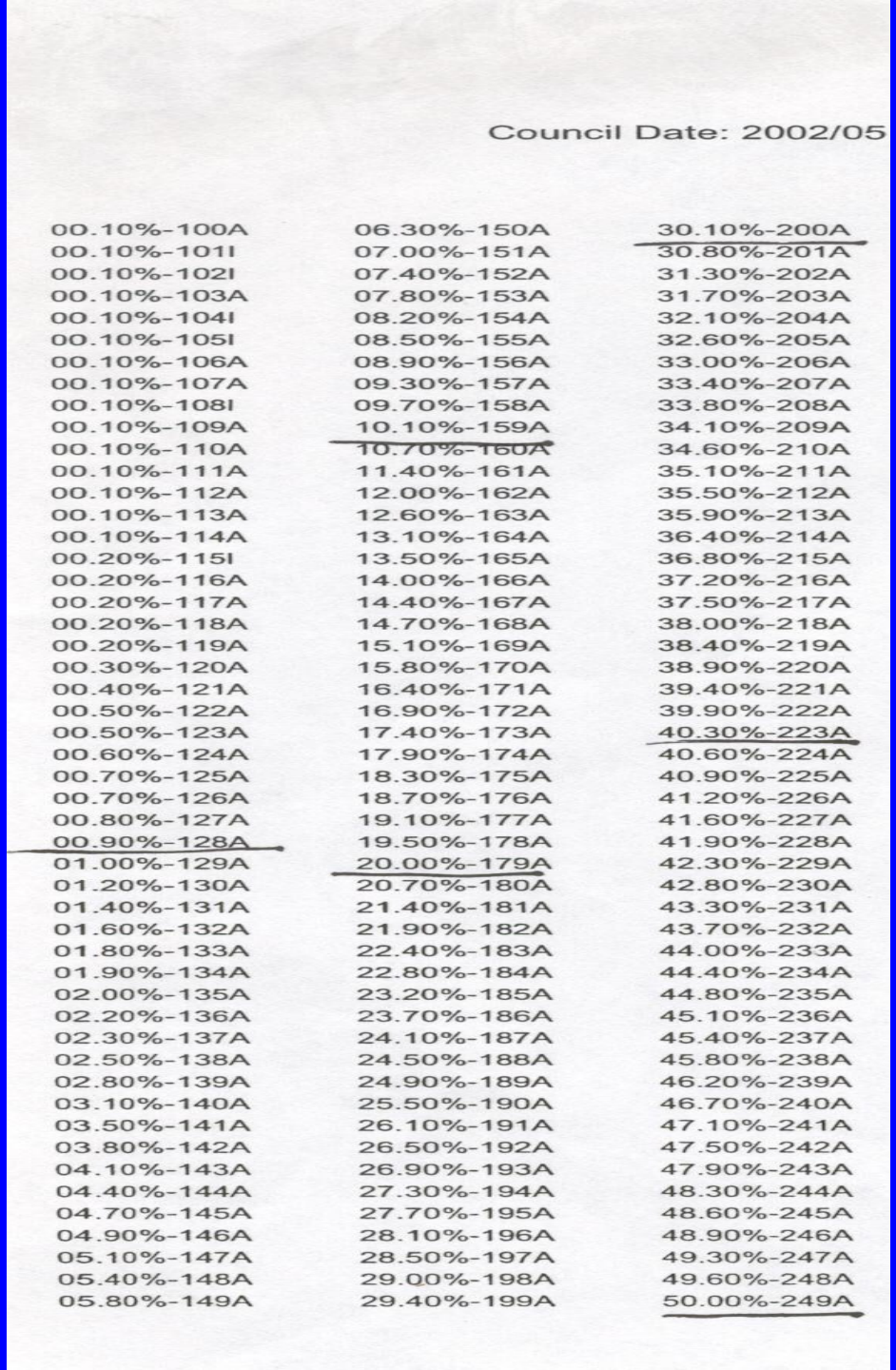
- SRA will average all priority scores, then calculate percentiles (bubble sheets)
  - This results in a comparison of these grants with those in the past two cycles
- SRA prepares "pink sheet" which summarizes the various reviews and includes text from all of them
  - You receive this four to eight weeks after review
- After review of your grant, the SRA is no longer your contact; contact your Program Officer (PO) to find out your score and what it means with regard to funding

Scientific Council

- Four to five months have elapsed since you submitted your grant
- Two months after review Council meets, generally supports the IRG's decisions
  - May recommend funding out of turn if work is of especial interest to Institute

Funding

- Notice of Grant Award (NGA) is the official notification of funding (electronic)
  - Often received AFTER official start date
- It takes 9-10 months to get a grant funded
  - With one revision, almost 2 years…so start now!



Council Date: 2002/05

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49.30%-247A
49.60%-248A
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Most funded grants now receive scores between 1.2 and 1.6!

(little discrimination since only 0.4 units of a 5 unit range is really used)

The pink sheet (no longer pink!)

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SUMMARY STATEMENT
(Privileged Communication)

Application Number: 2 R01 DA05084-12

Review Group: ZRG1 MDCN-5 (1)
CENTER FOR SCIENTIFIC REVIEW SEP

Meeting Dates: IRG: OCT/NOV 1998 COUNCIL: JAN/FEB 1999 RR/TNL
Requested Start Date: 04/01/1999

LINDBERG, IRIS, PHD
LOUISIANA STATE UNIV MED CTR
DEPT/BIOCHEM/MOLECULAR BIOLOGY
1901 PERDIDO STREET
NEW ORLEANS, LA 70112

Project Title: OPIOID PEPTIDE SYNTHESIZING ENZYMES

IRG Action: Priority Score: 151 Percentile: 7.8
Human Subjects: 10-NO HUMAN SUBJECTS INVOLVED
Animal Subjects: 10-NO LIVE VERTEBRATE ANIMALS INVOLVED

GENDER, MINORITY, & CLINICAL TRIAL CODES NOT ASSIGNED

PROJECT YEAR	DIRECT COSTS REQUESTED	DIRECT COSTS RECOMMENDED	ESTIMATED TOTAL COST
12	175,051	154,980	216,620
13	154,793	154,793	216,358
14	160,764	160,764	224,704
15	163,975	163,975	229,192
16	244,780	244,780	342,136
TOTAL	899,363	879,292	1,229,010

NOTE TO APPLICANT FOLLOWS SUMMARY STATEMENT.

RESUME AND SUMMARY OF DISCUSSION:

The Committee scored this competing continuation in full time and slightly reduced amount to study the cell biology and biochemistry of opioid peptide processing enzymes PC1 and PC2. This proposal has several strengths. The PI has shown excellent productivity in the last funding period. It is a well controlled study and a logical extension of the previous work. There is good likelihood of valuable information coming out of these studies. The PI has made several interesting observations in the last funding period which are worth pursuing. The proposed studies are sound and the questions being asked are relevant. However, the Committee had some doubts about the applicability of the to be identified inhibitors of PC1 and PC2. Similarly, the mechanistic aspects of PC1 and PC2 are over generalized to all prohormones. Nevertheless, these concerns are minor and the expertise of the PI ensures a successful completion of this project. The Committee recommended reduction in budget due to deletion of graduate student and related tuition fee costs.

DESCRIPTION (from applicant's abstract):

Opioid peptides such as enkephalins and endorphins are formed through the

Do Not Take Reviews Personally!

- Sometimes you fail to hit the right study section
  - There can be widely different perceptions of the merit of a given proposal among study sections
  - Sometimes there is an element of arbitrariness/luck with a given reviewer's perceptions
- Sometimes your timing is off
  - Get more preliminary data and go back in!
- Often you just need to jump through a few hoops to satisfy the reviewers

To RECAP: the best proposals..

- Are well-written
  - Easy to read (can put down and pick up easily without losing train of thought)
  - Focused on only a few goals
  - Persuasive
- Are scientifically intriguing: provide an important piece of a biologically relevant puzzle
- Have strong personnel
  - PI has (many) good publications over a long time period
  - Personnel have good training and publication track record
  - PI has excellent collaborators who have included strong letters of support (often you will have to tell them what you need in the letter)

Most Common Reasons Scores Are Bad

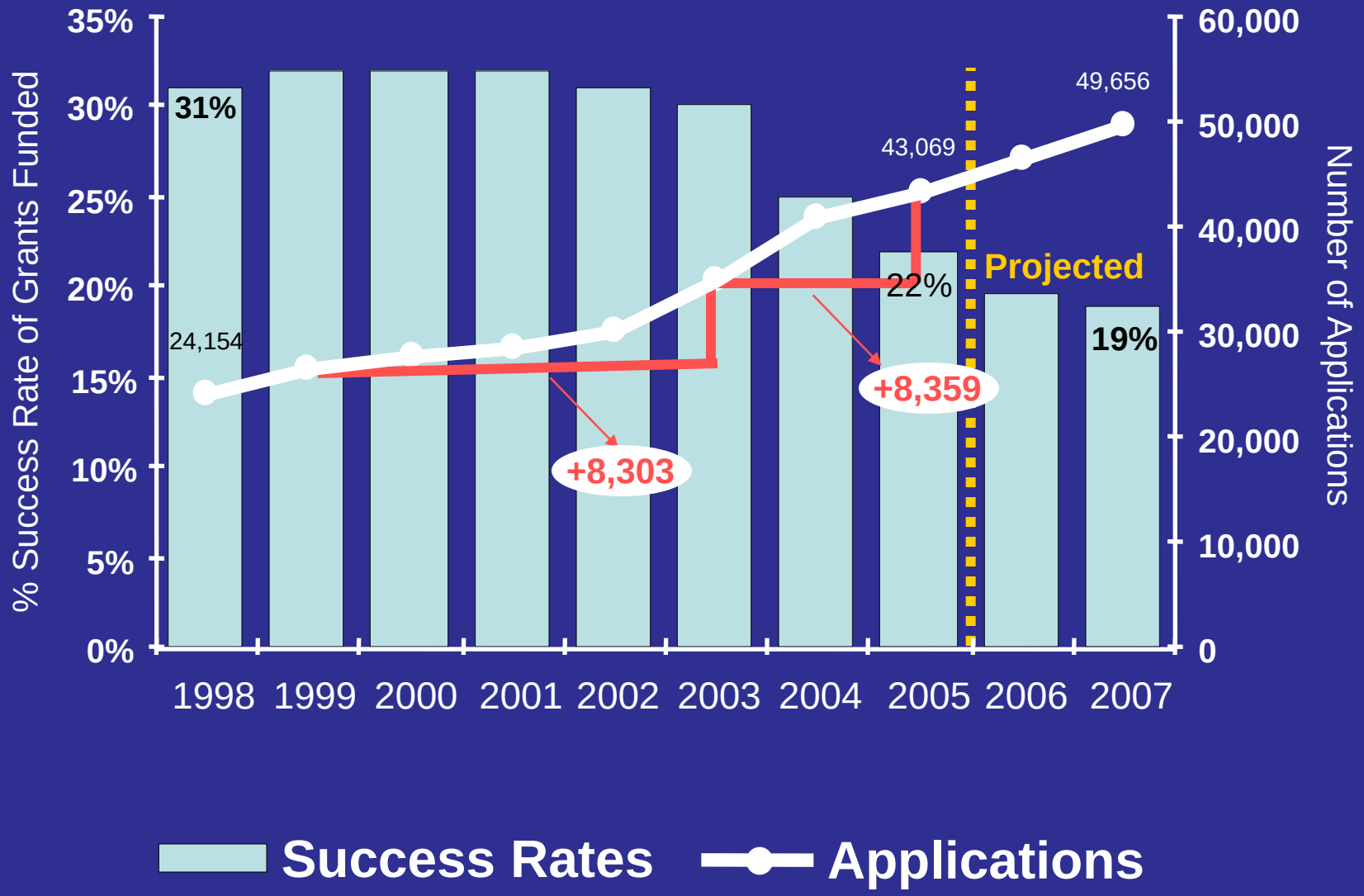
- New investigators are overambitious (less is definitely more!) NUMBER ONE REASON
- No interpretation of results- SECOND REASON
- Unfocused- experiments do not relate to each other, or to any defined hypothesis
- Fishing expedition/data collection (no actual hypothesis)
- Too risky- a pyramid scheme
- Too novel- hypothesis does not fit into the currently accepted paradigm
- PI has not published much or in good journals

Statistics

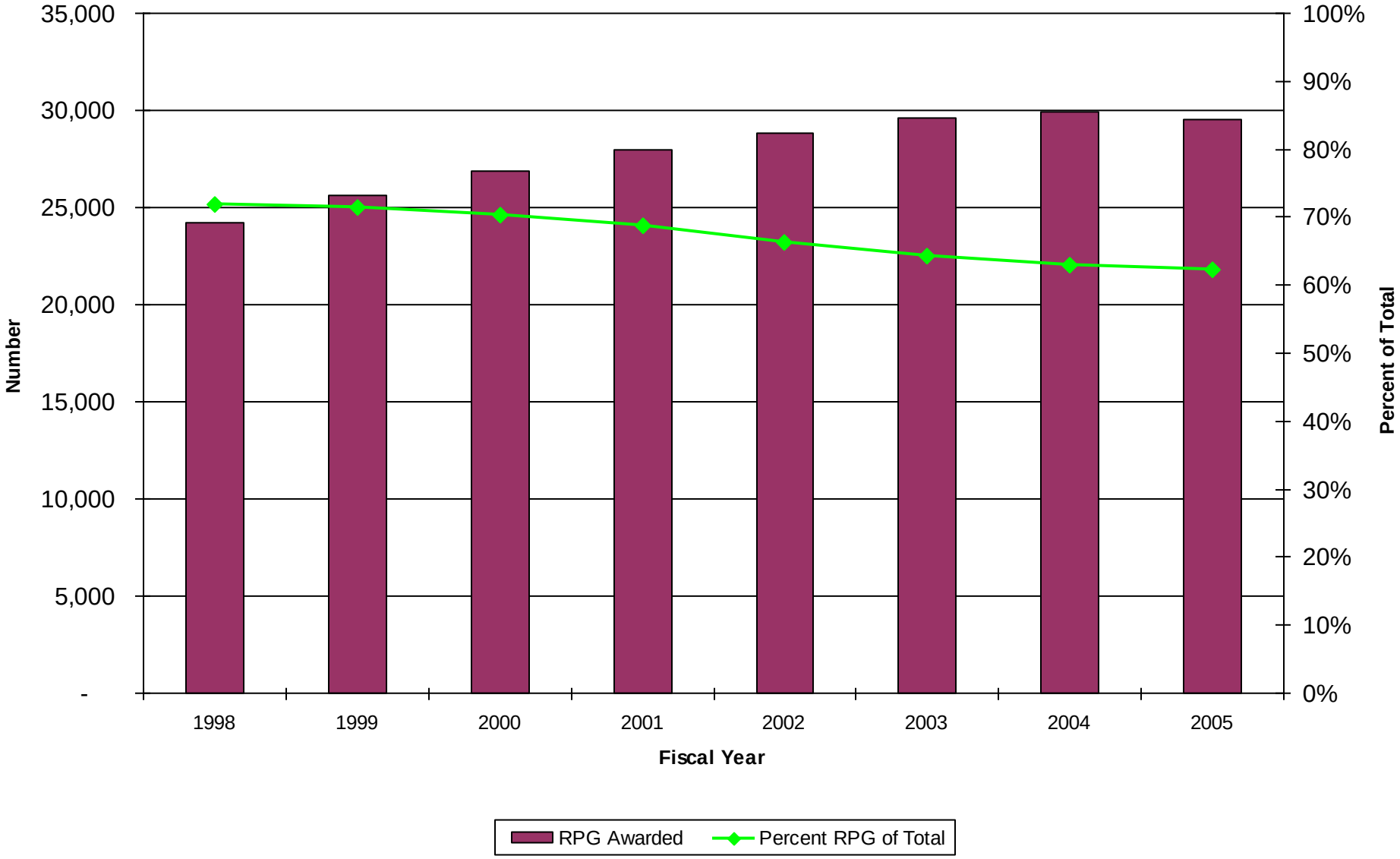
- The following slides are from the NIH website
- There are many more interesting slides there

New Grant Applications and Success Rates

During and After Doubling Period



Number of R01 Equivalents and Percent of Total Research Grants

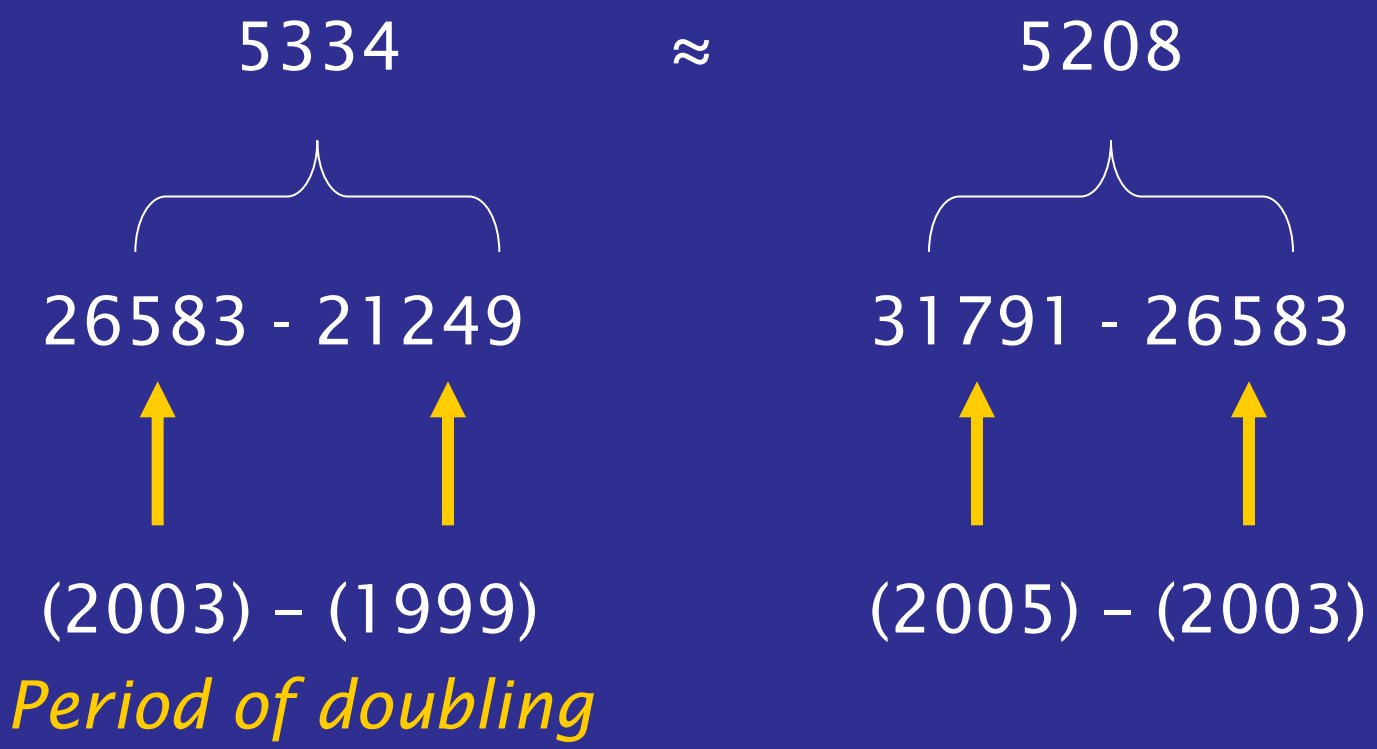


R01 Equivalent* Includes R01, R23, R29 and R37

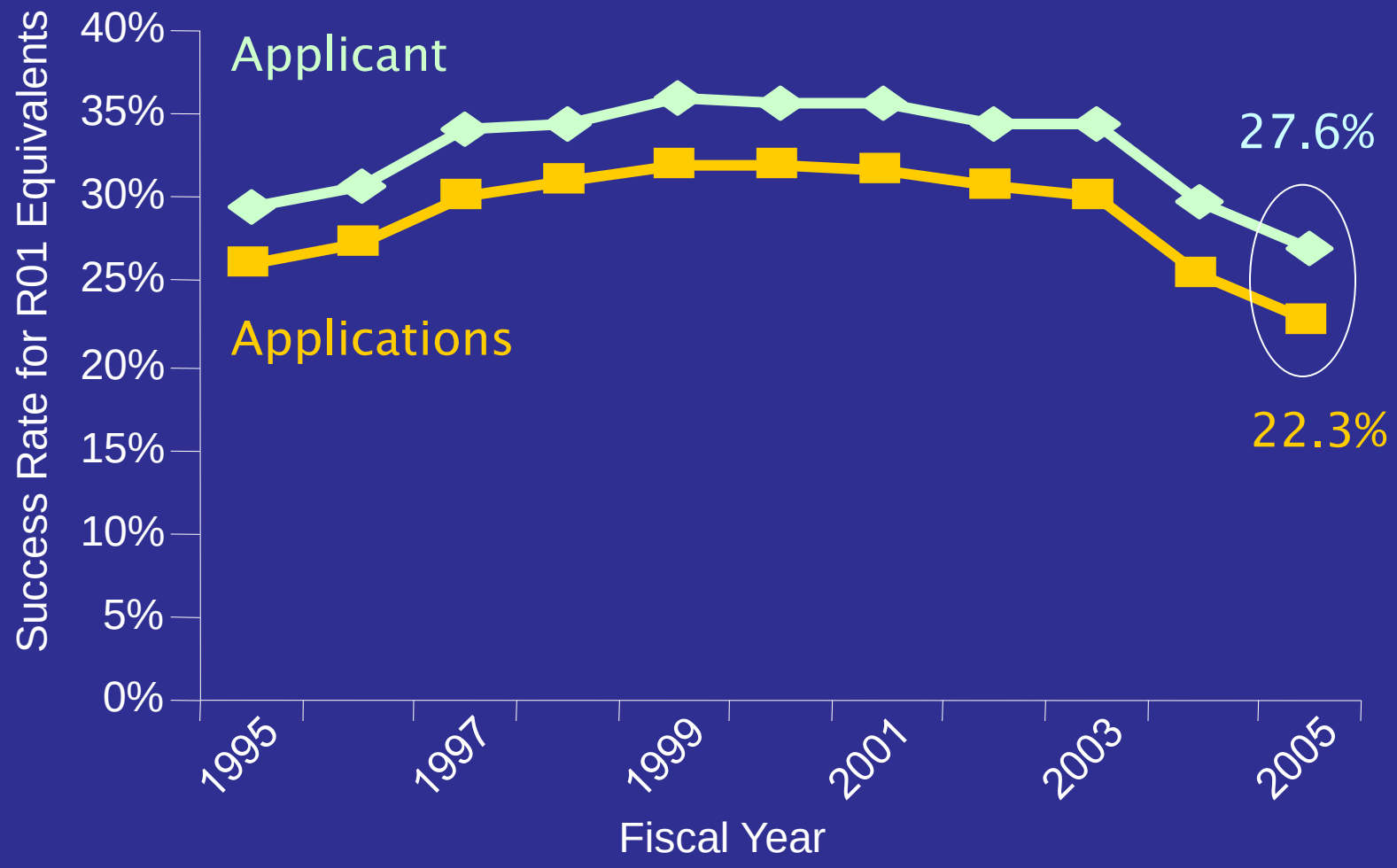
What Is Really Happening? 3 Fundamental Drivers

- Large capacity building throughout U.S. research institutions and increase in number of tenure-track faculty
- Appropriations below inflation after 2003
  - Increases of +3 % in '04, 2.2% in '05 and 0% in 06
  - Biomedical Inflation in 2004 was ~ 5%
- Budget cycling phenomenon

As Many *Applicants* in Past 2 Years as During Previous 5 Years!

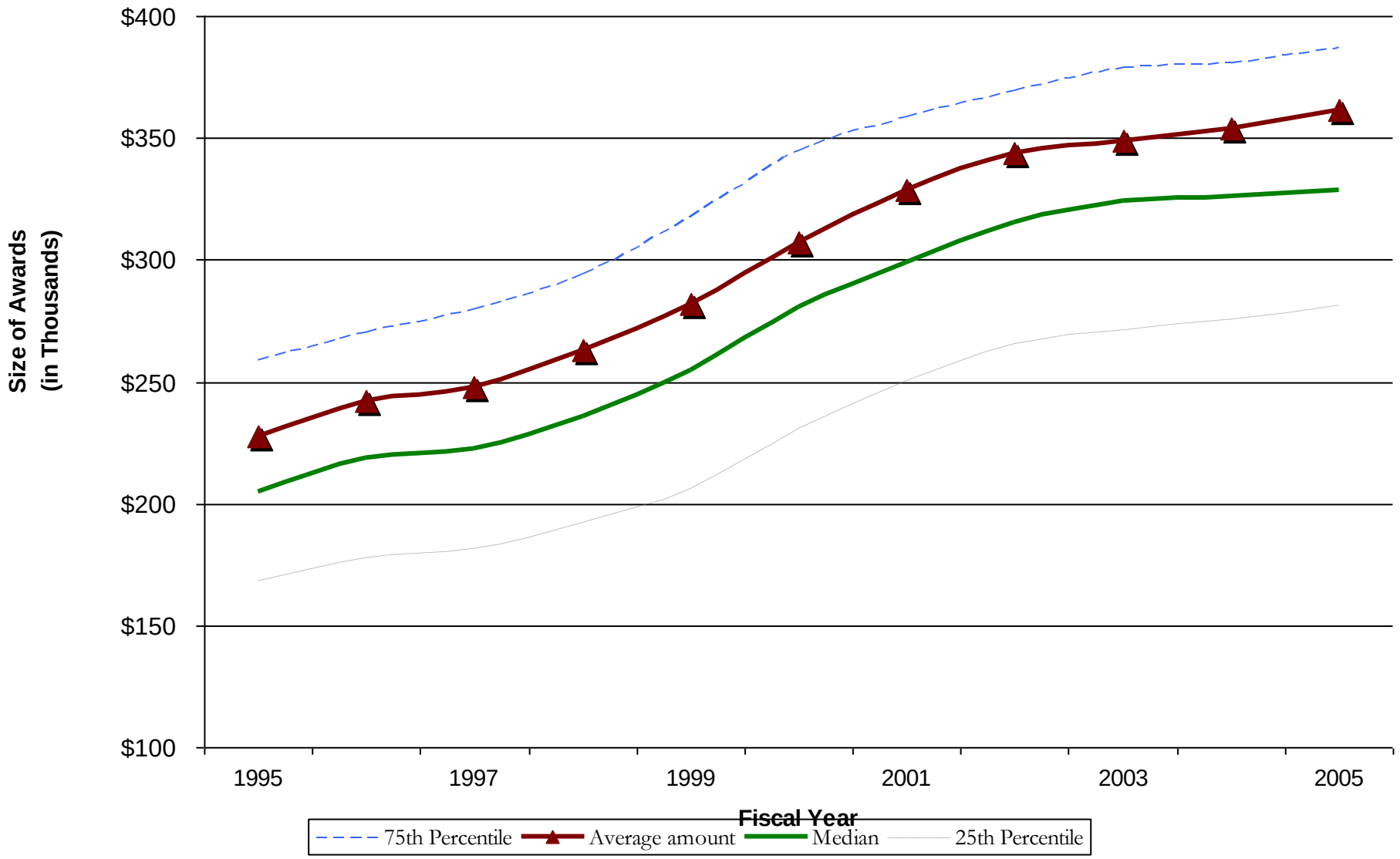


Success Rate per Application Understates Funding Rate per Applicant

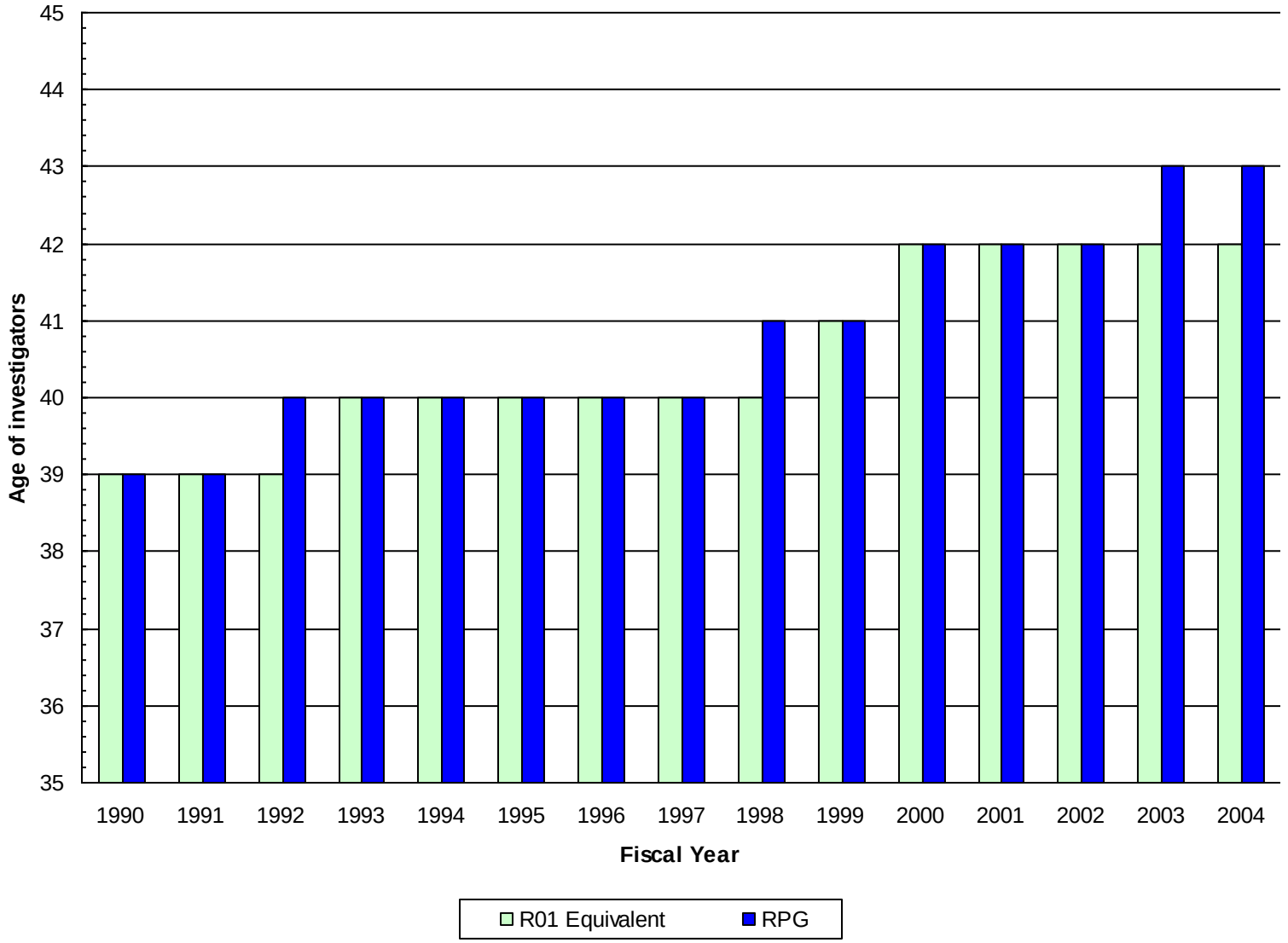


Success Rate files as of May 3, 2006. Program srf_indiv_060103_rfm
Individuals are determined using the pi_profile_person_id in IMPAC-II

Size of NIH Competing RPG Awards - R01s



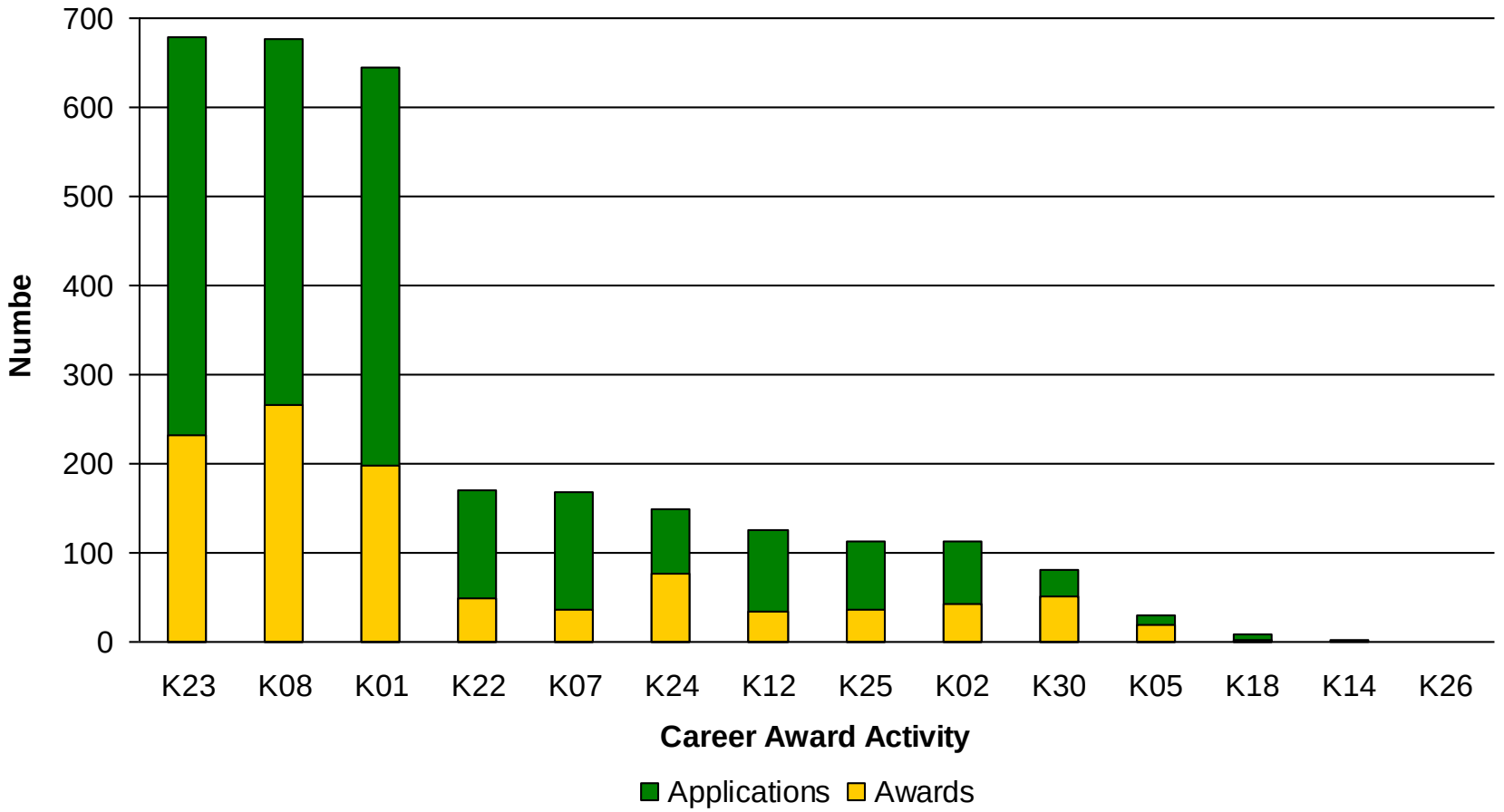
Average Age of First Time R01 Equivalent and RPG Investigators



RPG = R01, R03, R15, R21, R22, R23, R29, R33, R34, R35, R36, R37, R55, R56, RC1, P01, P42, PN1, U01, U19, UC1 and NIGMS P41.

R01 Equivalent* Includes R01, R23, R29 and R37

Number of NIH Competing Career Awards and Applications by Activity, Fiscal Year 2005



Additional Resources

- See each Institute websites
- University of Pittsburgh website

Good Luck!