How to Write A Competitive Grant Proposal

California Institute for
Quantitative Biosciences

Timeline for Proposal Preparation

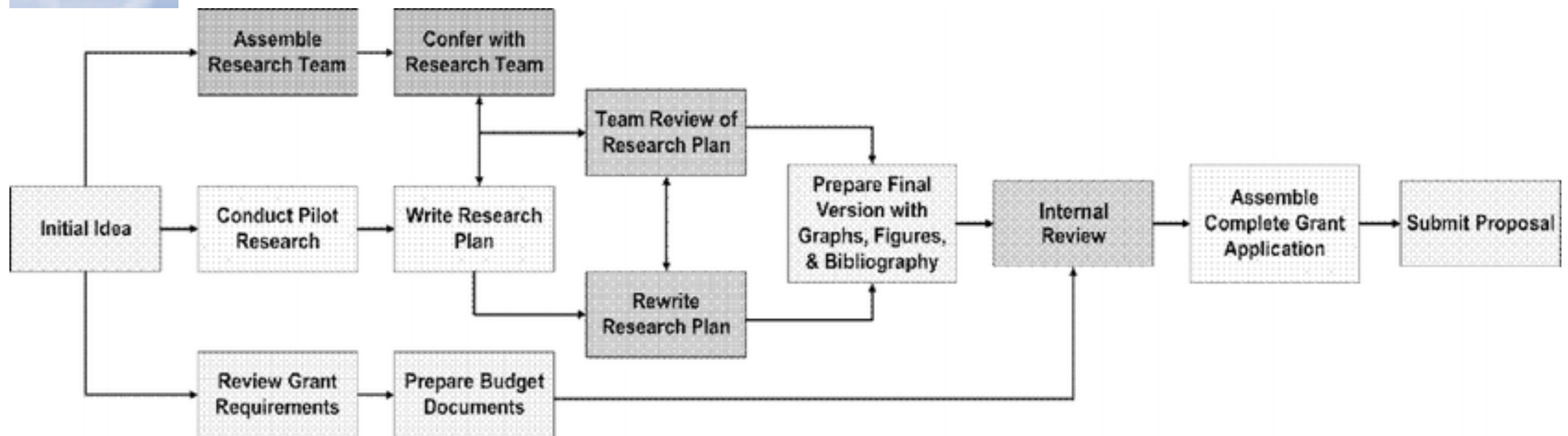

Figure 1 Steps of the NIH grant application process. Steps in preparing and submitting a grant proposal.

Berg, K.M, et. al. J Gen Int Med 2007;22:1587-95

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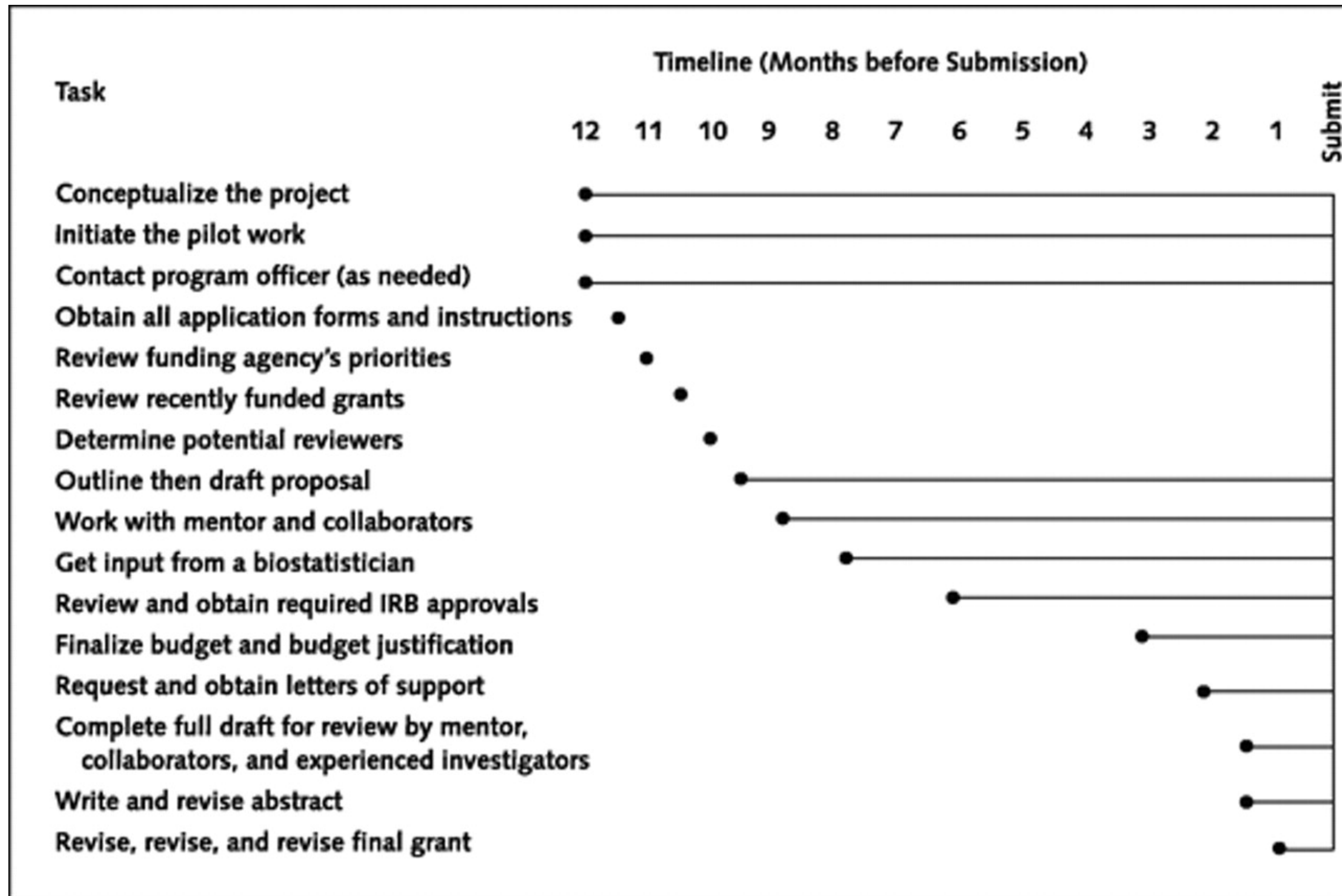
Timeline for Proposal Preparation



Inouye, S. K. et. al. Ann Intern Med 2005;142:274-282

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Most common reasons for not receiving funds*:

- Lack of new or original ideas
- Diffuse, superficial or unfocused research plan
- Lack of knowledge of published relevant work
- Lack of experience in the essential methodology
- Uncertainty concerning the future directions
- Questionable reasoning in experimental approach
- Absence of acceptable scientific rationale
- Unrealistically large amount of work
- Lack of sufficient experimental detail
- Uncritical approach

*Per Cheryl Anne Boyce, Ph.D. and Houston Baker, Ph.D., Grant Writing for Success, NIH Regional Grant Workshop

What are Your Goals?

- Specific
- Measurable
- Realistic

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Specific Aims

Your Specific Aims are the *cornerstone* of your entire proposal.

- The objectives of your research project.
- What you want to accomplish.
- Your project milestones.
- Testable concepts and ideas.
- Focused on an unresolved issue or on a roadblock to advancing the field.

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Specific Aims

Your specific aims are:

- The most important page of your proposal.
- Start with a brief problem statement to introduce your research question and state why it is important.
- Limited to 2-4 aims.
- Declarative (use short bullet points).
- Explain why this research matters.
- Not inter-dependent but supportive of each other.

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Specific Aims

Include, on 1 page:

Introduction:

-Big picture/relevance of your research.

-The problem you are addressing.

- Focus of project/what you hope to accomplish.

- Overall hypothesis.

- Lead into Specific Aims.

Specific Aims:

-Propose to test mechanistic hypotheses.

-State what you propose to determine or test.

- Specific Aims are not methods; include brief description of approach *after* the Specific Aim.

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2. SPECIFIC AIMS

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

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 ____.

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Introduction

Dr. James R. Alfano, University of Nebraska at Lincoln

Application 1-R01-AI069146-01A2: Suppression of innate immunity by an ADP-ribosyltransferase type III effector

Eukaryotic innate immune systems act as effective barriers to infection by microorganisms. Understanding the mechanisms that bacterial pathogens employ to circumvent innate immune systems will improve our ability to control disease.

Plants and animals use specific pattern recognition receptors (PRRs) to recognize conserved molecules of microorganisms (known as PAMPs). Plants have numerous PRRs that can recognize specific virulence proteins specifically present in pathogens (known as Avr proteins). Many Gram-negative bacteria use type III protein secretion systems to inject effector proteins into host eukaryotic cells. We have shown that a primary role for many *Pseudomonas syringae* type III effectors is to suppress innate immunity.

However, the enzymatic activities and the mechanisms that type III effectors use to suppress innate immunity are not well understood. Identifying the enzymatic activities of type III effectors and their substrates is essential to identify important components of innate immunity and to improve strategies to control bacterial diseases.

Our *long-term goal* is to elucidate the molecular basis for suppression of innate immunity by type III effectors. The objective of this application is to identify targets of the *P. syringae* type III effector HopU1, a mono-ADP-ribosyltransferases (ADP-RTs), and to determine its roles in bacterial pathogenesis. *The central hypothesis of the proposed experiments is that the targets of the HopU1 ADP-RT type III effector will be components of innate immunity.* We formulated this hypothesis based on the literature and on our research on other type III effectors as well as our preliminary data showing that HopU1 suppresses outputs of innate immunity. Recently, we have shown that HopU1 can use several *Arabidopsis* RNA-binding proteins as high affinity substrates in *in vitro* ADP-RT assays. Based on our preliminary data, one of these proteins, *At*GRP7, plays a role in innate immunity. A major goal of this application is to elucidate the function of this protein as it relates to innate immunity. We are prepared to undertake the proposed research because we have extensive experience in manipulating type III systems, and we were among the first to report that certain type III effectors suppress innate immunity. In addition, our preliminary identification of HopU1's substrates has positioned us well to perform the experiments described in this application. Our research team includes experts in the following areas: type III secretion systems, proteomics and mass spectrometry, Affymetrix microarrays, plant glycine-rich RNA-binding proteins, and animal pathogen ADP-RTs. This qualified group of investigators will insure that our discoveries are linked to basic concepts of pathogenesis and immunity in both plants and animals.

Introduction

Mark S. Smeltzer, University of Arkansas

Big picture/ Rele-vance

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.

Goal

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.

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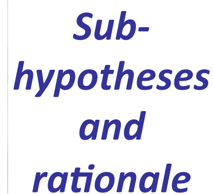
Overall hypothesis



Sub-hypotheses and rationale

Short-term goal

Lead-in to Specific Aims

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:*

Specific Aims

Specific Aim 1. We will test the prediction that X…..

1A. We will manipulate X and measure Y …
Our hypothesis predicts …

1B. We will do X assay to determine ….
We expect to find …

Specific Aim 2. To characterize the mechanism of …

2A. We will test whether X happens by Y….
We predict that …

2B. We will test the hypothesis that X happens by carrying out Y methods …

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Specific Aims

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.

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Specific Aims

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.

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Specific Aims

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).

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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 ER-associated 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 Aims

Boris Striepen, University of Georgia
"Biology of the apicomplexan plastid"

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 Aims

Boris Striepen, University of Georgia
"Biology of the apicomplexan plastid"

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 approach based on promoter replacement.

Specific Aims: Adding Sub-Aims

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, 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* P2 and P3 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.

Specific Aims: Adding Sub-Aims

Specific Aim 1A. Does the fMRI response at the trailing edge of a moving object reveal a deblurring mechanism? Based on pilot results, we hypothesize that parametrically correlating psychophysical data to fMRI responses will reveal the neural mechanism of deblurring in V1 and/or MT+ (Experiments D. 1B-D.1E, & D.1G). TMS will be used to stringently test whether the psychophysically measured modulations in perceived position are causally linked to the neural responses revealed in the fMRI studies (Experiment D.1H).

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Specific Aims: Adding Sub-Aims

Specific Aim 1B. The precision of position coding in MT+: Some models of localization suggest that position and motion information are integrated in MT+. On the other hand, MT+ is thought to be only coarsely retinotopic. To reconcile these differing views, we will (1) test whether MT+ plays a key role in coding object position (not just motion) and (2) establish the spatial resolution at which MT+ is able to code position (Experiment D.1C).

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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.

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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.

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Diagram Specific Aims

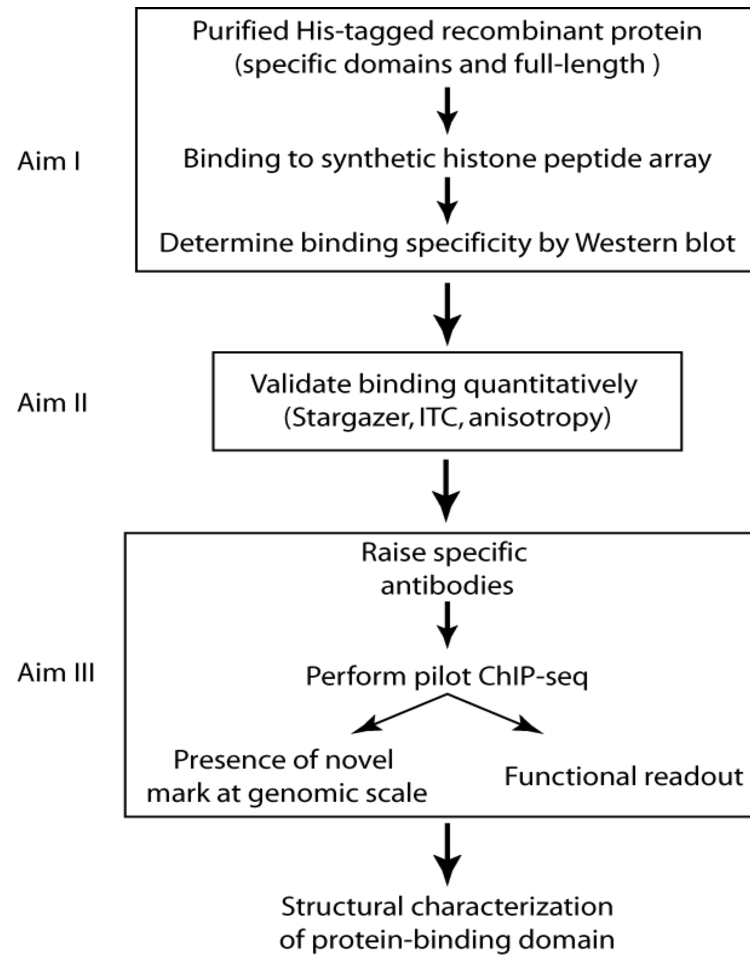

Fig. 1. Schematic flowchart for the current proposal. The main steps along each specific aim are indicated. See text for details.

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Diagram Specific Aims

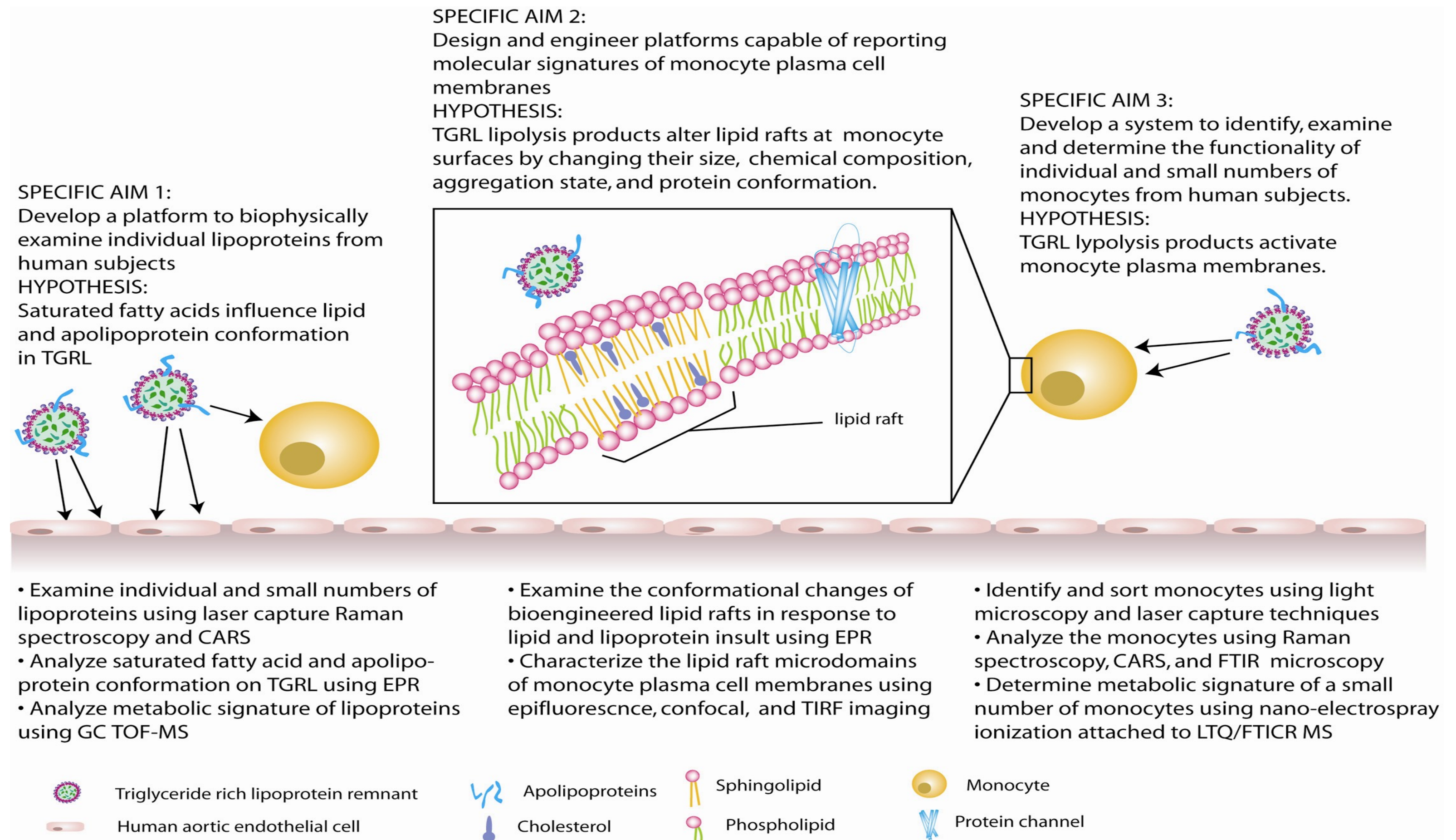

Figure 1. Research overview for the Bioengineering Research Partnership on lipid-membrane interactions.

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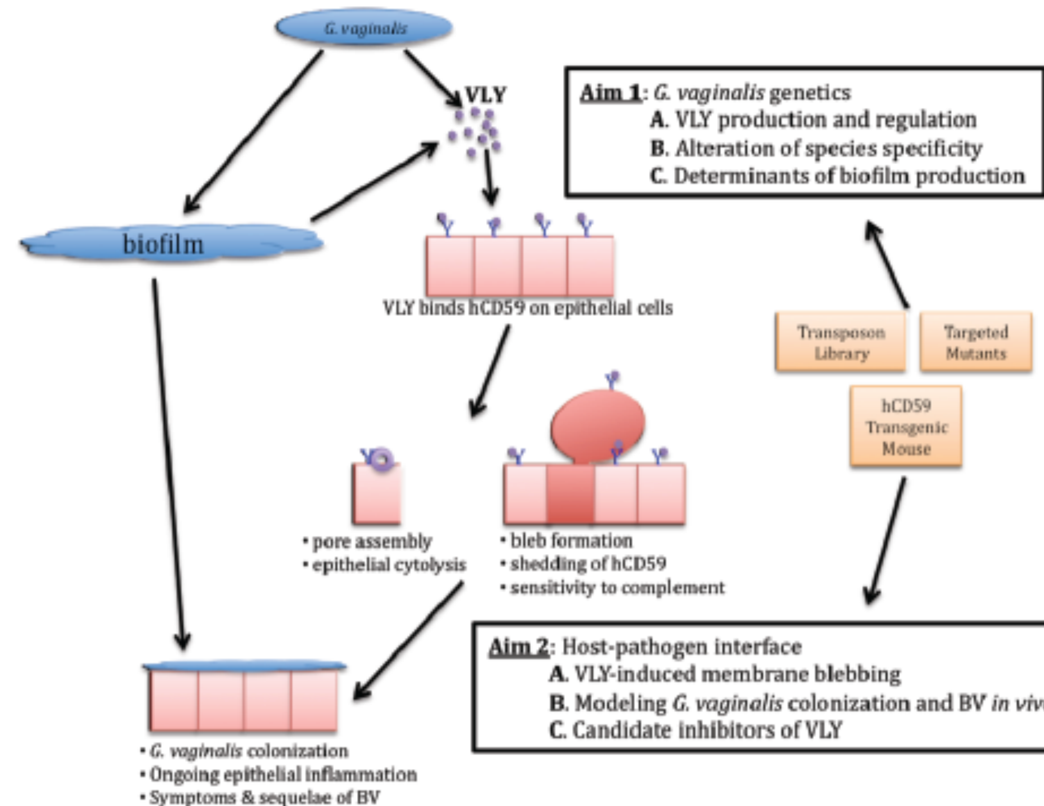
Diagram Specific Aims



Ratner, AJ. R01 HD061371-01, *"Gardnerella vagina/is:* toxin production and pathogenesis"

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Look at Funded Specific Aims

PI and Grantee Institution	Application and Summary Statement
Dr. James R. Alfano University of Nebraska at Lincoln	Resubmission of an unsolicited application: Application 1-R01-AI069146-01A2: Suppression of innate immunity by an ADP-ribosyltransferase type III effector
Dr. George Louis Drusano Ordway Research Institute, Inc.	Application responding to a request for applications: Application 1-R01-AI079729-01: Resistance Suppression for Influenza Virus With Combination Chemotherapy
Dr. Christopher D. Huston (new investigator) University of Vermont	Resubmission of an unsolicited application: Application 1-R01-AI072021-01A2: Molecular Mechanism of *Entamoeba histolytica* Phagocytosis
Dr. Michael G. Rossmann Purdue University at West Lafayette	Resubmission of an unsolicited application: Application 1-R01-AI076331-01A1: Structure and Function of Flaviviruses

Look at http://funding.niaid.nih.gov/researchfunding/grant/pages/appsamples.aspx

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