NIH Proposal Overview

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Editor – College of Engineering
and Science
NIH – Proposal Components

- Title – Limited to 81 characters

- Narrative – Concerns how this grant is related to public health. Three to four lines

- Abstract – 30 line document detailing your research (Gap in understanding, long term goal, object of this application, hypothesis, rationale, relevance to NIH mission, specific aims, innovation and significance.

- Research Plan – Specific Aims, Research Strategy, Timetable, Future Directions

- CV – Four page document detail PI qualifications

- Facilities and Other Resources – Information relevant to why each item is important and sufficient to accomplish work undertaken

- Equipment - all items available for project, including that assigned to you and shared by other institutions on campus
Title

- 81 characters (spaces and punctuation marks)
- Use 10 most relevant words – create three titles – send to 5 people
- Choose the best of the three

Narrative

- Specifically concerns how this grant is related to public health
- No more than 2 or three sentences
- Be succinct and use plain language for an audience

"This proposed research is relevant to public health because the discovery of evolutionary conserved mechanisms is ultimately expected to enhance risk prediction of abnormalities. Thus, this research is relevant to NIH’s mission regarding developing knowledge to reduce burdens of human disability."
Abstract

• No more than 30 lines of text
• Summary of the activity suitable for dissemination to the public
• Briefly state the specific aims and research design
• Info on significance (i.e. gap the study is addressing and public health significance)
Abstract

Systematically Improving Healthcare Delivery: Comparing Educational Interventions

There is a gap as to how healthcare providers are trained to deliver health care and the actual outcome that results from such training. While changing processes is necessary, responsive change requires smart decisions and full staff involvement at multiple levels, which often does not occur. Additionally, while staff training is intended to facilitate positive changes in hospital delivery systems, the best method to conduct this training is notoriously difficult to determine. Therefore there is a critical need to compare the effectiveness of the traditional, formal lecture-driven training method to the “learn as you implement” project-based training method. As part of the researchers’ long term (career) goal to develop methods to better use information, knowledge development and process complexity in complex environments, the objective of this application is to compare two methods of implementing process improvement strategies within two discrete hospital units: MedAd and POS. The PIs will compare two education methods to determine which is best for training hospital staff to improve both the quantity/quality of the care they deliver. The central hypothesis, based upon our preliminary results, is that __________. The rationale for this work is that understanding ______________ will lead to ______________. To evaluate the effectiveness of these educational interventions, the researchers will investigate each in its ability to accomplish two specific aims: 1) Compare the two educational methods of achieving process improvement within both MedAd and POS at multiple facilities. We will provide two groups lecture-driven education in state-of-the-art process improvement methodologies used in engineering management and industrial engineering, and with two other groups, we will use project-driven education in process improvement methodologies through selected hospital-based projects; 2) Combine the strengths of both approaches to offer an innovative educational intervention that is tailored to and sustainable in the hospital environment. Based on our analysis, the most influential factors on education, learning, and team execution will be integrated, verified and proposed as a single intervention. The significance of these sub-aims will improve workflow processes and information coordination to increase patient safety and quality of care, while supporting AHRQ’s identified evidence gap in care coordination and NIH’s mission to save lives. The PIs will conduct this work at multiple facilities within the Greenville Hospital System. This innovative method involves combining engineering management interventions and comparing educational training provided to the study groups. Regarding specific outcomes, this research will yield best-practices that require understanding the context of the process, informed by increasing process-thinking. Such results are likely to have a positive impact in that understanding the need to align information flows with processes can reduce medical errors and improve patient care.
Research Plan

- Specific Aims (1 page)
- Research Strategy (Max 13 pages – 7 for R03s)
  - Significance
  - Innovation
  - Approach
    - Each Specific Aim (same format for each)
    - Intro Paragraph
    - Justification/Feasibility
      - Lit review/prelim studies
    - Research Design
    - Expected Outcomes
    - Potential Problems/Alternative Strategies
- Investigator
- Environment
EXAMPLE OF A HYPOTHESIS-DRIVEN SPECIFIC AIMS SECTION

Interferon gamma (IFN-γ) is central to the maintenance of homeostasis, as well as to host defense against a variety of pathogenic microorganisms and tumor cells. In addition, it can have an active role in the pathogenesis of a number of diseases. IFN-γ mediates all of these effects through a single binding protein (the γ subunit of the IFN-γ-receptor complex), which is present on the surfaces of all normal nucleated cell types (Granger & Coe, 1994). While it is known that the binding protein initiates signal transduction (Yu, 2000), and it is understood mechanistically how it does so (Griffin et al., 2004; Campbell, 2008), what is not clear is how this critically important protein is produced. Lack of such knowledge is an important problem, because, without it, acquiring the ability to modulate the number of receptors on cells pharmacologically is highly unlikely.

Our long-term goal is to understand how the receptor for IFN-γ can be manipulated for preventive and therapeutic purposes. The objective here, which is our next step in pursuit of that goal, is to determine how production of the receptor’s γ subunit is regulated transcriptionally. Our central hypothesis is that both constitutive and stimulated regulation are required through different sets of cis-acting response elements in the gene’s promoter. Our hypothesis has been formulated on the basis of our own preliminary data produced using the promoter that we recently cloned (Galaway et al., 2008; see Justification and Feasibility sub-subsection under Research Strategy-Approach). In addition, the work of Adams & Seagram (2008) is supportive of the hypothesis. The rationale for the proposed research is that, once it is known how transcription of the γ chain’s gene is regulated, production of the subunit can likely be manipulated either up or down pharmacologically, resulting in new and innovative approaches to the prevention and treatment of a variety of diseases.

We plan to test our central hypothesis and, thereby, accomplish the objective of this application by pursuing the following two specific aims:

1. **Identify the DNA response elements that regulate constitutive transcription of the subunit’s gene.**

   Based on the preliminary data referred to above, our working hypothesis is that one or more Sp1 sites are critical to the regulation of constitutive transcription.

2. **Determine how stimulated transcription is up-regulated by different stimuli.**

   We postulate, again on the basis of our preliminary data, that cyclic AMP response elements (e.g., CRE and AP-2) regulate stimulated transcription of the subunit’s gene, regardless of how stimulated transcription is activated.

With respect to expected outcomes, the work proposed in aims 1 and 2 is expected to identify the full complement of response elements and the cognate transcription factors that are responsible for constitutive and stimulated transcription of the subunit’s gene. Such results are expected to have an important positive impact, because the identified components are highly likely to provide new targets for preventive and therapeutic interventions in addition to fundamentally advancing the fields of receptor biology and immunotherapy, as will now be detailed in the next section.
Research Plan – Specific Aims

• **State concisely the goals of the research and the expected outcomes, including the impact of these results on the fields involved**

• **List the specific objectives (e.g. test hypotheses, create novel designs, challenge an existing paradigm, address barriers preventing advancement, or creating new technologies)**

• **Most important section of the proposal**

• **Frame as hypothesis oriented infinitives (e.g. “To determine whether the X gene is overexpressed in disease Y” rather than “To perform PCR on patients with Y.”)**
Prostate cancer remains the leading cause of death in men, in spite of extensive efforts at periodic screening. Recent NCI data indicate that 200,000 new cases will be diagnosed in 2004 and that more than 80,000 men will die from complications.

Evidence indicates that prostate cancer cells are highly invasive and that complement receptor C1q-mediated signaling through NF-kB and AP-1 signaling pathways leads to secretion of matrix metalloproteinase-9 (mmp-9) that promotes prostate cancer. Of potential importance, Crocetin, a traditional Asian medication recognized for centuries for its potential in preventing or treating various human diseases, has been suggested as an effective therapeutic strategy to treat cancer.

Though this molecule from the Crocus plant shows significant anticancer effects in animal models, the manifestations of these mechanisms have not been determined.
Research Plan – Specific Aims

- **Sentence 5 – Critical Need – summarizes how to fill these gaps.**
  
  Given the polarization between skeptics disputing the efficacy of alternative medical strategies, and evidence supporting Crocetin, there is a critical need to provide scientific evidence to reconcile these perspectives.

- **Sentence 1 – Long Term Goal – career goal which is part of your application. Your long range goal and the funding agency’s mission are one.**
  
  Our **long term goal** is to develop novel therapeutic strategies for treating cancer based upon natural remedies.

- **Sentence 2 – Objective of this Application – step to the long term goal, defines overall purpose of activities, defined endpoint, designed in such a way to match the critical need in the first paragraph**
  
  The **objective of this application** is to determine the molecular mechanisms of action and therapeutic efficacy of Croetin in inhibiting prostate cancer cell metastases.
Research Plan – Specific Aims

• **Sentence 3 – Central Hypothesis** - Strong linkage between statement of need and the objective. Provides direction for the application – objectively testable – no predetermined conclusion.

Our *central hypothesis* is that Crocetin inhibits C1q-dependent signaling pathways leading to cell migration. It does so by inhibiting activation of key transcription factors NF-Kb and AP-1 in-vitro, and that these activities will be reproduced in an in vivo animal model of prostate cancer metastases. We have formulated this hypothesis based upon our data suggesting an inhibitory effect of Crocetin extract on expression NF-Kb and AP-1, and observations that NF-Kb and AP-1 are required for enhanced secretion of MMP-9 from prostate cancer cells.

• **Sentence 4 – Rationale** - What will become possible that is not possible now

Our *rationale* for these studies is that developing scientifically-based evidence to support therapeutic benefits of Crocetin would provide a foundation for clinical trials to test potential benefits in cancer patients. We are well prepared to pursue these studies because of our published research with this compound and the commitment of our institution to develop novel strategies to prevent cancer. We propose two Specific Aims.
Research Plan – Specific Aims

*Provide a logical step-by-step development of the key activities (Aims/Goals/Objectives to address the “critical need.”)*

We plan to test our hypothesis by pursuing the *following specific aims*

1. **Specific Aim 1 – Identify the DNA Response Elements regulating constitutive transcription of the subunit gene.** Based on preliminary data, the working hypothesis is that one or more Sp1 sites are critical to regulating transcription.

2. **Specific Aim 2 – Determine how stimulated transcription is up-regulated by different stimuli.** We postulate from our data that AMP response elements regulate stimulated transcription of the subunit’s gene.

*Each Specific Aim set off as a bullet point, italics*

*Make sure they link back to your working hypothesis informed by data*

*Indent and Number each*
Research Plan – Specific Aims

Payoff Paragraph - Specific and credible, present tense; directly related to the area and funding agency identified in the opening sentence.

Sentence 1 – Expected Outcomes

Regarding expected outcomes the work in Aims 1 and 2 is expected to identify those response elements responsible for transcription of the subunits gene.

Sentence 2/3 – Positive Impact

Such results will have a positive impact because the identified components are highly likely to provide new targets for preventive interventions as detailed in the next section.

• Ensures easy transition into the Research Section which begins with Significance
Research Plan – Significance/Innovation

A) SIGNIFICANCE

A1) Importance: understanding fundamental aspects of virus infection processes controlled by receptor or antibody binding, and the controls of viral host ranges. The paroviruses include many human and animal pathogens, including the simian, B19 virus which causes the childhood fifth disease and more severe diseases of adults, as well as the recently identified human bocavirus and ParV4. The adeno-associated viruses (AAVs) are paroviruses that are not associated with disease, but are being developed as human gene therapy vectors and the same issues of receptor and antibody recognition are important for vector optimization. The viruses we are studying in this model are the canine parovirus (CPV) and its close relative canine panleukopenia virus (FPV), which bind to the host transferrin receptor type-1 (TfR) to infect cells [53]. The paroviruses have a 25 nm diameter T=1 capsid that is assembled from 60 copies of two or three versions of a single capsid protein, and the single stranded DNA of the virus is packaged into the pre-formed capsid by the action of the larger non-structural protein (NS1). Although those capsids are remarkably robust and survive in the environment, structural variation results in viruses with different properties, and those also show structural changes during the process of cell entry and nuclear trafficking. The simple and well defined structures of the parovirus capsids, the known properties of the TfR, and the well characterized antibodies available for these studies allow us to examine several processes of viral infection. Variant viruses with extended host ranges can cause new outbreaks or epidemics of infectious disease. The viruses that we are examining include the comparison of such a system, where one variant arose as a pandemic pathogen in a new host through the acquisition of mutations in the capsid protein that altered its structure to change host-specific receptor binding, and also to change its antigenic structure.

A2) Critical barriers: to antiviral therapy and vaccination success. Animal viruses are complex biological machines that engage host cell receptors and undergo a series of varying structural and functional changes to allow cell penetration and release of the genome for replication. Those infection processes are key to the success of any virus, and are targets of various anti-viral drugs. A better understanding of the details of the general processes involved will likely allow the development of more effective and broadly acting antiviral drugs. Although antibodies are critical components of immune responses of all vertebrates, in many cases they are poorly effective so that viruses maintain persistent infections or vaccines do not work well. Understanding the underlying rules that determine how antibodies bind to viruses and block the processes of cell infection will reveal how effective antibody responses might be elicited against different viruses.

A3) Improvement of scientific knowledge: understanding fundamental viral mechanisms and clarifying textbook knowledge of virus structure and functions. This project addresses several mechanisms important for all viruses of humans and other animals. In general terms those include understanding viral recognition of cell receptors, how changes in receptor binding sites lead to alterations of binding and host range, capsid and receptor structures and control of entry and trafficking within cells. During each of these steps the viral proteins must assume the correct conformation, bind receptors with the correct contacts, and in the process undergo a variety of structural transitions to release internal peptides, protein domains, and the viral DNA. Here we will investigate the roles of flexible and variable structures in the parovirus capsid and show how receptor and antibody binding control cell infection.

B) INNOVATION

This is an unusually complete model for understanding virus structures and functions involved in cell infection and host range control, as there are few other viruses where the ancestors and descendents of a host-switching virus that caused a pandemic of disease in its new host are available for analysis. The laboratories presenting this proposal are the only ones working with this model in any detail, but we have been able to explain many aspects of the process, from the evolutionary processes allowing emergence, to the identification of specific receptor binding as a key step that lead to the extended host range of the virus [reviewed (52)]. There are several fundamental issues being investigated in these studies, including being able to obtain a detailed understanding of the variation and dynamic properties of viral proteins, how those structural changes control receptor attachment and cell infection, and that analysis is complemented by studies of antibody binding and its effects on receptor binding and infection. By analysis of naturally variant or mutant viruses, receptors, and antibodies we will gain a better understanding of the functional properties of the capsid-ligand structures and alterations in binding properties. We use a variety of advanced methods and structural, biophysical, biochemical and functional assays in these studies. While many of the methods we propose are being used in viral studies (including by our laboratory), the proposed studies include combinations of those methods that are being used in novel ways to gain a complete understanding of the mechanisms involved.
Research Plan – Significance – Part 1

- **Why is this study important?**

- **How will your findings change science/medicine?**

- **Examples: Lives will be saved or quality of life improved (state how), new rationales for treatments tested (state why they’re needed)**

- **Emphasis positive impact as directly related to the mission of NIH.**
Research Plan – Significance – Part 1

Provide a critical analysis of the literature to expand on what was briefly stated in Specific Aims, and validate the importance of the problem to be resolve. Detail the existence of the gap/need.

The α subunit of the IFN-γ receptor complex binds this important cytokine to cells (Yang, 1977) and is the initiator of transmembrane signaling (Telifer and Gomez, 1988; Homer et al., 1995). Through it, IFN-γ exerts a wide range of immunologic effects. For example, serious infectious disease problems are experienced by those who become deficient in the cells that produce this cytokine, either because they are lost due to disease (e.g., AIDS; Galbreath, 2002; Toliver, 2003), during chemotherapy (Aikens and Osada, 2000), or as a natural consequence of aging (James and Kary, 1997; Zeleny et al., 2004). In addition to its homeostatic and defensive roles, IFN-γ appears to have a pathogenetic role, either because there is an overabundance of the cytokine (Alcott and Cochrane, 1998; Jones et al., 2000) or because cells become hyper-reactive to normal, physiologic concentrations (Sandoval, 1999). Responsiveness to IFN-γ is related to the number of its receptors that are on a cell’s surface (Carlson et al., 2003). This suggests that the effects of IFN-γ could be modulated by altering the number of receptors that are available to bind it. How to effect such modulation is not known. Our contribution here is expected to be detailed understanding of how production of the α subunit – the one that binds IFN-γ and initiates transmembrane signaling – is regulated transcriptionally.
Research Plan – Significance – Part 2

• Direct statement of the significance of your project (concrete benefit relevant to NIH’s mission). Middle of the section – MOST IMPORTANT SENTENCE OF THE APPLICATION

transmembrane signaling – is regulated transcriptionally. This contribution is significant because it is the first step in a continuum of research that is expected to lead to development of pharmacologic strategies that will allow the number of surface receptors to be regulated, either positively or negatively. Once such strategies become available, there is the promise that in diseases that are associated with hyper-responsive ness to IFN-γ, cellular responsiveness could be down-regulated by reducing the number of receptors that are available to bind the cytokine. Decreasing availability of the α subunit, alone, is likely to be sufficient for this purpose. Conversely, when greater responsiveness is needed – for example, to ward off pathogenic microorganisms or, in the case of immunocompromised patients, opportunistic invaders – responsiveness of host-defensive cells to IFN-γ, could be increased. Thus, important advances in the therapy of diseases and complications that are associated with cellular-immune dysfunction could be expected. It is also expected that what is learned will be equally applicable to the prevention/treatment of diseases of agriculturally relevant animals. In addition, the research will be of significance because what is learned will contribute to broader understanding of how other receptor complexes can be modulated as an approach to therapy. Furthermore, better fundamental understanding of how receptor proteins are transcribed can be anticipated.

• Emphasis (unlike innovation) is on the concrete benefits from the advancement, not the advancement itself
Research Plan – Significance – Part 3

• Positive aspects – stems from benefits relevant to NIH’s mission

Details how your contribution enables future work

Benefits expected to accrue from the contribution you will make

Once such strategies become available, there is the promise that in diseases that are associated with hyper-responsiveness to IFN-γ, cellular responsiveness could be down-regulated by reducing the number of receptors that are available to bind the cytokine. Decreasing availability of the α subunit, alone, is likely to be sufficient for this purpose. Conversely, when greater responsiveness is needed – for example, to ward off pathogenic microorganisms or, in the case of immunocompromised patients, opportunistic invaders – responsiveness of host-defensive cells to IFN-γ, could be increased. Thus, important advances in the therapy of diseases and complications that are associated with cellular-immune dysfunction could be expected. It is also expected that what is learned will be equally applicable to the prevention/treatment of diseases of agriculturally relevant animals. In addition, the research will be of significance because what is learned will contribute to broader understanding of how other receptor complexes can be modulated as an approach to therapy. Furthermore, better fundamental understanding of how receptor proteins are transcribed can be anticipated.
Research Plan – Innovation

• A new and very different method of considering an important public health related problem that departs from the status quo - positive impact stems from the unlikeliness of advancement without this departure

EXAMPLE OF AN INNOVATION SUBSECTION

Although the potential importance of the IFN-γ receptor as a therapeutic target has been appreciated since 1995 (Homer et al.), no approach to date has been effective in moving beyond the status quo, which is little or no therapeutic effect. For example, Golum (1998) experimented with inhibitors of its transmembrane signaling activities. However, those approaches were only marginally effective because of alternative pathways that bypassed the inhibitors (King and Pomplun, 1999). Furthermore, many unacceptable side effects were experienced (Poulos, 2000). Blockage of the receptor with altered ligand that was incapable of initiating transmembrane signaling was also ineffective (Barnet and Cox, 2000). General inhibitors of protein synthesis were indiscriminate (Bobo and Thom, 2001) and antisense drugs (DNA – Good, 2002; RNA – Benchley and Cox, 2002; and chemical analogues – Kennedy et al., 2003) failed to inhibit receptor synthesis sufficiently to have measurable effects. The research proposed in this application is innovative, in our opinion, because it represents a new and substantive departure from the status quo, namely the approach of transcriptional regulation. Our preliminary studies (see Approach subsection) strongly suggest that this approach will be effective in down-regulating the receptor’s α subunit, which is expected to overcome problems that have been associated with other means of modulating the number of IFN-γ receptors on cell surfaces. As a consequence, an efficacious therapeutic approach based on the IFN-γ receptor is expected to result.
Research Plan – Innovation

• *Explain how the current application challenges seeks to shift current research*

• *Describe any novel theoretical concepts, approaches or methodologies, instrumentation or interventions to be used or developed that are an improvement over the existing state of the art*

• *Explain refinements, improvements, or new applications of theoretical concepts, approaches, methods, instruments, interventions*

• *Novelty of your study? Not all need be innovative, if the timing is right*

• *Half a page sufficient*
Research Plan – Innovation – Part 1

• **Document literature of what the norm is to this point.**

Although the potential importance of the IFN-γ receptor as a therapeutic target has been appreciated since 1995 (Homer *et al.*), no approach to date has been effective in moving beyond the status quo, which is little or no therapeutic effect. For example, Golum (1998) experimented with inhibitors of its transmembrane signaling activities. However, those approaches were only marginally effective because of alternative pathways that bypassed the inhibitors (King and Pomplun, 1999). Furthermore, many unacceptable side effects were experienced (Poulos, 2000). Blockage of the receptor with altered ligand that was incapable of initiating transmembrane signaling was also ineffective (Barnet and Cox, 2000). General inhibitors of protein synthesis were indiscriminate (Bobo and Thom, 2001) and antisense drugs (DNA – Good, 2002; RNA – Benchley and Cox, 2002; and chemical analogues – Kennedy *et al.*, 2003) failed to inhibit receptor synthesis sufficiently to have measurable effects.

• **If innovation stems from new approach, support by discussing previous approaches that were unsatisfactory**

• **If innovation from alternative interpretation, resulting in different conclusions, discuss citations mentioning shortcomings of earlier interpretations and how they prevent advancement**
Italicized statement on innovation

This proposed research is innovative, in our opinion, because it uses combined concepts of Six Sigma and HFACS to uniquely marry the concept of human factors to continuous quality improvement for use in a hospital setting, to standardize care in a way that was hitherto impossible. Our preliminary studies (see Approach section) strongly suggest that this novel approach can be used to elucidate all levels of the patient-treatment system, from the unsafe act itself to the organizational influences that constrain the human-machine system.

Include “In our opinion” because you do not want to be perceived as projecting your own subjective opinion onto the reviewers.

Complete the italicized sentence by stating what objectively sets it apart.

Must result in a positive impact as next explained
Positive Impact - stems from advancement that would have been unlikely without substantive departure from status quo

Emphasis unlike significance) is on the substantive departure that enabled the advancement, not the benefits themselves
Research Plan - Approach - Strategies

• Describe the overall strategy, methods, etc. used to accomplish specific aims. Include how data are collected, analyzed, and any resource sharing plans

• Discuss potential problems and alternative strategies and benchmarks for success

• If product in early stage of development, describe strategies to establish feasibility and address management of high risk aspects of the proposed work

• Point out procedures, situations, materials that may be hazardous, and precautions to be used
Research Plan – Approach - Strategies

• Describe your studies briefly
  ➢ Only enough background and methods to make the study understandable (1-2 sentences)
  ➢ Only enough to convince
  ➢ Emphasize conclusions

• Write the descriptions backwards
  ➢ What were the conclusions that support your study?
  ➢ What data are essential to support those conclusions?
  ➢ What background information has not been presented already?

• Arrange the sentences in background – data – conclusions order
Approach - Research Plan - One possible approach

• Each Specific Aim Overview/Intro – Investigator readiness/environments
  • Preliminary Studies:
    ➢ Review of Relevant Literature
    ➢ Justification and Feasibility
  • Research Design
  • Potential Problems/Alternative Strategies
  • Expected Outcomes
  • TimeLine

AND REPEAT FOR EACH AIM.
Aim 1 - Introduction paragraph

The proposed project is founded on several multi-year existing collaborations between groups studying infection and metabolism using C. elegans (Ausubel and Ruvkun), and computational groups focused on developing algorithms for biomedical research (Wahlby, Carpenter, and Golland), making us uniquely situated to accomplish the proposed aims. As shown in Figure C.1, our interdisciplinary team is highly interactive and our approach to image assay development is a highly iterative process to ensure robust real-world performance. Each proposed aim is independent, but in several instances, improvements made for one aim will benefit the others. Later sections detail our proposed algorithm development for each aim, which will occur in the rich, collaborative, interdisciplinary environment of algorithm and software development at the Broad Institute and MIT. Here we outline the team and the approach.
Aim 1 - Preliminary Studies – Lit review/justification

Here, we describe the independent and collaborative research completed within and among the Wahlby, Carpenter, Golland, Ausubel, and Ruvkun groups that provides the foundation for this proposal. High-throughput C. elegans microscopy screen for regulators of Enterococcus faecalis infection. We recently published the first whole-animal C. elegans microscopy screen analyzed by automated image analysis. Building on a smaller, manually-scored screen65, we tested 37,214 chemicals for their ability to rescue C. elegans worms from an otherwise lethal E. faecalis infection. We acquired fluorescence images of the dead worms stained with SYTOX dye, plus brightfield images showing the entire worm population. Although the image-analysis approach was relatively simple, the screen uncovered six structural classes of compounds that are “anti-infectives” and appear to cure C. elegans animals without directly affecting the growth of E. faecalis. Three of these are novel structural classes of compounds that were not found in in vitro screens for antimicrobial compounds. This validates a major premise of our proposal, that image-based screens in the whole organism C. elegans will reveal compounds acting through novel mechanisms of action, in this case, mechanisms that are only manifest when the complex host/pathogen relationship is intact.
Approach - Research Plan

Aim 1 – Research Design - Intro

To score chemical perturbants for their ability to rescue C. elegans from an otherwise lethal infection by the pathogen Microsporidia, we will develop algorithms to count live and dead worms in each sample. These algorithms will delineate individual worms from clusters of worms and extract shape features that can distinguish curvy, live worms from straight, dead worms. The successful C. elegans viability screen described in Preliminary studies relied on measuring a fluorescent viability stain (SYTOX) across the population without needing to identify individual worms. However, for this Microsporidia assay, and other future live/dead screens, it is preferable to instead classify each animal as live or dead based on its shape in brightfield images; SYTOX staining adds reagent costs and sample preparation time and it is a less reliable indicator of viability from a biological perspective. In addition, SYTOX stains some pathogens we plan to screen as well as some types of debris, thus obscuring the signal from the worms.
Aim 1 – Research Design – Experimental Approach

While non-touching worms can usually be delineated in brightfield images based on the differences in intensities between foreground and background, image intensity alone is not sufficient for touching and overlapping worms. The high-throughput screening assays addressed here require algorithms that separate touching and overlapping worms in static images, where motion cues are unavailable. Moreover, edges and intensity variations within the worms often mislead conventional segmentation algorithms. On the other hand, while the varying postures of the worms introduce significant extrinsic geometrical differences, the worms have similar intrinsic geometrical properties (such as length and width profile). We propose a probabilistic shape model that captures this type of knowledge in an automated segmentation method. The key ideas are the construction of a low-dimensional shape-descriptor space and the definition of a probability measure on it. Closely related approaches for shape representation include the active shape model (ASM) and its variants, and medial axis transform methods for capturing shape variability in anatomical structures and other objects and others. We learn the possible shape variations from N training worms obtained by automated segmentation of a subset of worms that do not touch or overlap.
Aim 1 – Research Design – Experimental Approach

1. **Construct a low-dimensional worm shape descriptor** from the skeleton of the shape and its distances to the boundaries, given by the medial-axis transform

2. **Reduce dimensionality by Principal Component Analysis (PCA):** Align descriptors by similarity transformation (i.e., rotation and translation, no scale or skew) by minimizing the sum of the Euclidean distances of corresponding points along the skeletons.

3. **Find posture probabilities and resolve clusters by graph search algorithm:** The weights $w$ of the training worms define a probability measure on the feature space of the worm deformations: $p(x) \sim \exp(w^T L w)$, where $L = \text{diag}(1 \ldots L)$ as in162.
Aim 1 – Research Design – Experimental Approach

Validation, evaluation, and benchmarks To validate and evaluate the proposed algorithm we will use a set of 6000 expert-annotated brightfield images from a previous screen with images from the Microsporidia screen. Our goal is to achieve “screenability” in terms of both accuracy and computational speed. **Accuracy:** We will use metrics accepted in the screening field to assess accuracy based on the ability to distinguish control wells with worm populations of known phenotype—hundreds of these controls are included in each experiment. If the assay readout is Gaussian, we will aim for a Z’-factor above 0.5 (>0.2 would still be acceptable); if not, we will use classification sensitivity and specificity, overall aiming to avoid visual examination for 90–95% of the samples. During the iterative process of algorithm and assay development, we will also validate individual steps of the image analysis pipeline (foreground/background segmentation, worm cluster resolution, live/dead scoring) as appropriate, comparing algorithm results to “ground truth” provided by our worm experts. **Speed:** Image processing should keep pace with image acquisition; given current image acquisition rates and cluster computing costs, our goal is 6 CPU-minutes or less per image on a typical CPU. The methods proposed are likely to meet this goal, but there are many ways to reduce computational costs if needed.
Aim 1 – Potential Problems and Alternative Strategies

Initial foreground/background segmentation is a prerequisite for the proposed cluster separation. If local adaptive thresholding is not sufficient, we will rely on more advanced methods, such as level-sets for foreground/background separation (Aim 2).

Cluster skeletonization may not coincide with the centers of the worms, skewing the cluster separation. A distance transform of the binary image can guide the merging step, forcing it to preserve ridges located at a worm's thickness from the cluster edge.

Scoring viability from clusters: If individual worms cannot be segmented, we will measure the proportion of cluster area occupied by straight worm segments by a simple algorithm that fits long line segments inside the cluster.
Approach - Research Plan

Aim 1 - Expected Outcomes - highlight the return on investment – more extensive than in Specific Aims. Single short paragraph - ½ page.

Expected outcomes from this aim include:
• An innovative and standardized methodology to identify near misses and sentinel events.
• A methodology for investigating these near misses and sentinel events through RCA techniques.
• A methodology for documenting these near misses and sentinel events in a way that can be tracked, analyzed and monitored.

Aim 1 – Timeline – a few lines here. No gantt charts – not enough room

Timeline: Work on Aim 1 will take place during the first two years. Work on Aim 2 will commence six months after funding and will be finished by the end of the third year. Work on Aim 3 will begin halfway through the second year and will be finished by the end of year 5.
Biosketches – Personal Statement

- Specific to each proposal—you can no longer use the same biosketch for every submission

PERSONAL STATEMENT:

Dr. Carlucci, an Early Stage Investigator, will be the Principal Investigator of this project. He was Chief Resident during his final year of residency. His infectious-diseases fellowship included one full year of research with Dr. James Bonoffri, a leading NIH-funded investigator in the area of staphylococcal bactere mia. As a result of Dr. Carlucci’s research, nosocomial staph infections in neonatal intensive-care units began to be recognized as a burgeoning problem. The current proposal is part of a continuum of independent research that traces its roots to those earlier investigations. Of particular importance to the feasibility of this project is his mastery of quantifying staphylococcal bactere mia in antibiotic-treated neonates. His administrative experience as the PI of an NIH-funded grant (R01AI12345), as well as his tenure as Assistant Head of Children’s Hospital’s Infectious Diseases Laboratory, prepare him to meet the administrative needs of the proposed project. His membership in the campus-wide Infectious Diseases Cooperative, and the fact that neonatal bacteremia has been declared a priority focus by Children’s Hospital, assure that the assembled research team will have access to a wide range of expanding resources (see Facilities & Other Resources and Equipment sections). Dr. Carlucci has worked and published with other members of the proposed research team, especially Dr. Jasper Gussmann, who will be a Co-Investigator.
Research Plan – Approach - Preliminary Studies

• *Discuss the PI’s preliminary studies, data and experience relevant to this application.*

• *Preliminary data can be an essential part of a research grant application to establish the likelihood of success. Exceptions are:*
  
  - R21/23 – Exploratory/Development Grants
  - R03 – Small Research Grants
  - R15 – Academic Research Enhancement Awards
  - R41/43 – Small Business Research Grants

• *Early Stage Investigators include preliminary data – for R01s, reviewers instructed to place less emphasis on prelim data for ESI than on prelim for more established investigators*