**Specific Aims**

Blue Text = Guidance from [NIH SF424 R&R](https://grants.nih.gov/grants/how-to-apply-application-guide.html)

Orange Text and Outline = Adapted from [*The Grant Application Writer’s Workbook*](http://www.grantcentral.com/workbooks/national-institutes-of-health/).

State concisely the goals of the proposed research and summarize the expected outcome(s), including the impact that the results of the proposed research will have on the research field(s) involved. List succinctly the specific objectives of the research proposed (e.g., to test a stated hypothesis, create a novel design, solve a specific problem, challenge an existing paradigm or clinical practice, address a critical barrier to progress in the field, or develop new technology).

**First Paragraph: Why**

Opening sentence: *A sentence to immediately capture the reviewers’ attention and highlight an area relevant to the targeted program/funding agency.*

Current knowledge: *Information about what is known that will allow reviewers to understand the importance of the proposed research. Sets up the gap/unmet need.*

Knowledge gap or statement of need: *The subject of the proposal; must relate to the previous statements as a next step to advance the field. (Note: It is not essential to use the term “knowledge gap” in this sentence.)*

Consequence(s) of not addressing knowledge gap or need: *Explain why failing to address this gap/need will prevent vertical [as opposed to incremental] advancement of the field.*

**Second Paragraph: What**

Long-term goal: *The goal of your research over multiple funding periods. This must be broader than “overall objective.”*

* “Our *long-term goal* is to…”

Overall objective: *What will be accomplished through this project; must link back to the gap/need you are addressing.*

* “The *overall objective* of the proposed research is to…”

Central hypothesis: *What must be tested to attain the objective. This should be broad; details will be provided in specific aims.*

* “Our *central hypothesis* is that…”

Data to support hypothesis: *Your preliminary data (just the punchline), and work by others if relevant.*

Rationale: *What attaining your objective will allow you to do and how that will advance the field (vertically); must link back to knowledge gap/statement of need.* **[Include only if it is not repetitive of information in the "Why" paragraph.]** *For some mechanisms, it can be useful to instead briefly describe expertise or resources that make you/your team well suited to perform the proposed studies.*

**Third Section – List of Aims: How**

Specific Aims: *The aims paragraphs should each contain minimally a title and a working hypothesis. These should make it clear which component of the central hypothesis is tested in that aim—and why. Each title should be broad and open-ended; the working hypothesis can provide the focus of the aim. If you have no room to expand on how you will achieve your aim in an additional sentence or two, make sure that your working hypothesis gives a sense of approach and experimental readout.*

Aim 1: Title

* Working hypothesis:

Aim 2: Title

* Working hypothesis:

Aim 3: Title

* Working hypothesis:

**Fourth Paragraph: Payoff**

Expected outcomes & broader impact: *What your aims are likely to produce, how that would contribute to the overall objective, and what broader impact this would have on this area of research.*

* “The *expected outcomes* are …”

Broader impact

* “The *broader impact* is…”

Source: [NIAID Sample Applications](https://www.niaid.nih.gov/grants-contracts/sample-applications). PD/PI: Vernita Gordon, Ph.D., University of Texas at Austin. Application: “[Assessing the roles of biofilm structure and mechanics in pathogenic, persistent infections.”](https://www.niaid.nih.gov/sites/default/files/1-R01-AI121500-01A1_Gordon_Application.pdf)

**Specific Aims**

This proposal’s *objective* is to determine the impact of the spatial structure and mechanics of *Pseudomonas aeruginosa* biofilm infections, in chronic wounds, on virulence, antibiotic resistance, and immune evasion.

Most chronic bacterial infections are caused by biofilms, aggregated bacteria that are embedded in a matrix of polymer and protein. Unlike well-mixed, liquid cultures, biofilm infections have well-defined spatial structure. This spatial structure is given by the sizes of bacterial aggregates, the relative positions of aggregates, and matrix heterogeneity. Aggregates also have viscoelastic mechanical properties that are conferred by the matrix. Basic principles of material transport indicate that the spatial structure of biofilm infections must impact intercellular signaling, virulence, and antibiotic resistance; comparison of biofilm mechanics with known phagocytic forces indicate that resistance to deformation and breakup likely help biofilms resist immunological clearance. However, there is little to no in-depth, quantitative knowledge regarding the impact of spatial structure and mechanics on disease course. Completion of the work we propose here will open new possibilities for therapeutic strategies that specifically target biofilm structure and/or mechanics.

Our *long-term goal* is to find new strategies for remediating biofilm infections by addressing physical properties. Here, our *central hypothesis* is that spatial structure and mechanics are the major *physical* factors controlling the development of pathogenicity, antibiotic resistance, and immune evasion in biofilm infections. This hypothesis is based on a synthesis of our own and others’ published work. The *rationale* is that completion will identify key physical targets for preventing, disrupting, or ameliorating biofilm infections for an important biofilm-forming opportunistic human pathogen. The work we propose here will also develop experimental techniques and understanding of an important model system that together will constitute a widely-applicable platform for assessing the impact of biofilm structure and mechanics for other infecting organisms.

We will test our central hypothesis and attain our objective *via* the following *specific aims*:  
**1: Determine the spatial structure and mechanics of biofilm infections in wounds.** For this, we will use sophisticated imaging to determine, in three dimensions, the size, number, locations, and heterogeneous matrix content of bacterial aggregates in a mouse model of chronic wound infection. We will simultaneously measure the density and distribution of neutrophils around the biofilm aggregates. At present, no good technique for measuring the mechanics of biofilm infections exists. We will develop such a technique using AFM microindentation and abradement of *ex vivo* biofilms. *Working hypothesis:* The structure and mechanics of *in vivo* biofilm infections in chronic wounds will follow development trajectories arising from the matrix- producing capabilities of the bacteria and pressure from the host immune defense.

**2: Determine how spatial arrangements impact bacterial growth, biofilm microenvironments, antibiotic resistance, and virulence.** For this, we will use manipulative techniques that we recently developed to re- create biofilm structures found in *in vivo* and *in vitro* environments and measure the biological changes induced by specific structures. *Working hypothesis:* Virulence and antibiotic resistance will depend on key structural characteristics, such as the sizes of aggregates and the distances between aggregates, through the development of microenvironments that differentiate as a result of the material transport and consumption of growth substrate, bacterial products, and antibiotics.

**3. Determine the role of spatial structure and mechanics in biofilm-leucocyte interactions.** For this, we will add freshly-isolated human neutrophils to biofilms at different stages of formation and with different structures and mechanics and monitor the attack by neutrophils and the bacterial response. *Working hypothesis:* Biofilm tolerance and evasion of neutrophils and their action will depend both on the neutrophils’ ability and speed in breaking off and engulfing pieces of biofilm, and on the ability of biofilms to kill immune cells.

The *expected outcome* of this work is a comprehensive understanding of what structures and mechanics develop in biofilm infection of chronic wounds, and the degree to which these structures and mechanics give rise to pathogenicity, antibiotic resistance, and evasion of the immune system. The results will have an important *positive impact* because they lay the groundwork to develop a new class of targeted treatments.