Vail Building, Room 507
Hanover, New Hampshire 03755-3842
Telephone 603-650-1247
Fax 603-650-1245
Email: robert.shanks@Dartmouth.edu

September 28, 2005

Dear Search Committee,

I am writing to apply for the position of Assistant Professor in the Department of Biology at Indiana University for the "Systems Biology / Microbiology" position advertisement listed at aaas.org.

I am a postdoctoral fellow in the laboratory of George O'Toole at Dartmouth Medical School in my third year of training. Here I have three research directions. First is the study *Staphylococcus aureus* biofilm formation with an emphasis on genetic approaches to identify factors that contribute to cell-cell adherence and biofilm development.

The second direction relates to the role of biofilms on human disease. I have had the opportunity to interact with physicians at Dartmouth Hitchcock Medical Center and we have developed fruitful projects that consider the impact of medical practice on biofilm formation. We have studied the impact of anticoagulants used in dialysis centers on biofilm formation, and the adherence of bacterial cells to ocular prostheses.

The third focus bridges my graduate training in eukaryotic genetics with my postdoctoral training in prokaryotic molecular biology. I have developed a powerful set of tools for manipulation of Gram-positive and Gram-negative genes that take advantage of *Saccharomyces cerevisiae* recombination. These have facilitated our research to a great extent.

I received a Ph.D. from the Tufts University Sackler School of Biomedical Sciences Department of Molecular Microbiology. There I worked in the laboratory of Dean Dawson and worked on budding yeast meiosis using genetics, cell biology, molecular biology and biochemistry. For my thesis I studied recombination, chromosome segregation, the cytoskeleton, motor proteins and meiotic checkpoints.

My goal is to establish an extramurally funded research program conducting biofilm research as well as engage in teaching. I hope to integrate hands-on laboratory work that reinforces concepts, and a focus on scientific reading and writing into my teaching of undergraduate students.

I have included my curriculum vitae, teaching philosophy and research overview and would be happy to send additional materials such as PDFs of manuscripts. Four letters of reference will be independently sent regarding my qualifications.

Lastly, I would like to inform you that I will also be applying to the Indiana University Department of Biology for their other current faculty search.

Thank you for your consideration,
Robert Shanks

Research Objectives Overview

Robert M. Q. Shanks
George O'Toole Lab
Dartmouth Medical School
Hanover, NH 03755
Phone: 603-650-1247
robert.shanks@dartmouth.edu

Training:

Graduate: yeast genetics, cell biology, molecular biology
Post Graduate: microbiology, bacterial genetics
Other: Cold Spring Harbor Genetics Courses

Research Directions: 1. Basic research of *Staphylococcus aureus* biofilm formation: determining factors that contribute to adherence to surfaces and post-attachment accumulation. 2. Effect of clinical practices on biofilm formation. 3. Building molecular tools to facilitate the study of *S. aureus* and other bacteria that enable researchers to tap into the power of yeast genetics.

Relevance: *S. aureus* is a major opportunistic pathogen and is one of the most common and serious nosocomial infectious agents. *S. aureus* is capable of both acute and chronic infections. *S. aureus* form biofilms that are highly resistant to antibiotic treatment and are a serious and expensive problem with implanted abiotic surfaces (catheters, artificial joints) as well as on biotic surfaces (e.g. heart valves, joints, and possibly lungs).

Goals: The aspiration of this research is to find genetic determinants of biofilm formation and to determine their mechanistic role in the process. These factors could ultimately be used as drug targets to help prevent biofilm formation and disrupt existing biofilms. A second goal is to examine the impact of clinical practices on biofilm formation on medical surfaces, e.g. catheters or contact lenses. The third is to create new molecular tools especially for the study of staphylococci. These vector-based tool kits are largely built and are detailed briefly below. They will allow researchers to study more genes, more thoroughly, in less time.

More information on these projects is listed below.

Research Program:

1. **Basic research into biofilm formation. Use of forward and reverse genetics to identify genes involved in biofilm formation and a combination of techniques to determine the role of these genes in biofilm formation and pathogenesis.** We have performed a mutant hunt for genes involved in *S. aureus* biofilm formation and isolated a number of novel genes with biofilm defective phenotypes, further characterization of these and other biofilm-related factors will be a research objective my laboratory. Currently, we are studying two adjacent open reading frames (ORFs) that we call StcA and StcB. These are cell-surface genes that have a role in cell-cell interactions, but not initial attachment to surfaces. Furthermore, overexpression of either of these genes confers a hyper-biofilm phenotype to *S. aureus* cells. We have a set of tools to characterize the role of these two proteins in biofilm formation, such as deletion strains,

complementing plasmids, epitope-tagged constructs, and antibodies. Our current model proposes a role for StcA and StcB in contributing to biofilm formation by making cells better able to adhere to one another, thereby promoting biofilm accumulation and tenacity. We are currently testing the hypothesis that these proteins act together to mediate cell-cell interactions. Additionally, we found other genes required for full levels of biofilm formation including unknown ORFs that are annotated as a putative transcription factor and a stress-response gene.

2. Clinically related biofilm studies.

a. Biofilm formation on renal dialysis catheters.

b. Biofilm formation on intraocular lenses.

Interaction with physicians at Dartmouth Hitchcock Medical Center has led to some fruitful experimentation regarding the formation of *S. aureus* biofilms on medically relevant surfaces and in the presence of anticoagulants that could be or are used as catheter lock solutions. One objective of research in my laboratory will focus on prevention of biofilm formation on medically important surfaces, such as indwelling medical devices (figure 1).

We have found that a common catheter lock solution, sodium heparin, stimulates biofilm formation on abiotic surfaces in vitro. This has led us to examine the impact of other potential catheter lock solutions and anticoagulants on biofilm formation in vitro. We have tested a number of these and found some that prevent bacterial growth, such as sodium citrate and sodium EDTA at high concentrations, but stimulate biofilm formation at low concentrations, some that have no effect upon biofilm formation, and one that both inhibits biofilm formation and dissociates preexisting biofilms (figure 2 and 3).

This work has also had a positive impact on our basic research. We examined the stimulation of biofilms by heparin and found that it enhances cell-cell interactions in a protein-synthesis dependent manner, suggesting that it directly or indirectly stimulates the expression of some adhesive factor(s). We used genetic and biochemical approaches to determine whether known biofilm factors are required for heparin-dependent biofilm stimulation and found that they were not, suggesting that heparin stimulates biofilms through a novel mechanism. A mutant hunt to isolate candidates not stimulated in biofilm formation is underway with the help of an undergraduate student.

Characterization of the sodium citrate effect on biofilms is also underway. A candidate genetic approach was used to identify regulators necessary for stimulation of biofilm formation by low levels of sodium citrate. We have found that the SarA regulator of virulence is necessary for this response, while many other known regulators and adhesion factors are not.

We plan to augment the genetic analysis with a proteomic approach. Surface protein preparations of cultures exposed to heparin, stimulatory levels of citrate and a control will be analyzed.

Biofilm formation on intraocular lenses is a serious concern to ophthalmologists. We have acquired a number of keratitis-associated clinical isolates of *S. aureus* and *S. epidermidis*, and are testing their ability to form biofilms in vitro, first under conditions we know promote biofilm formation to determine the capacity for these strains to form biofilms, and then under conditions that staphylococci might encounter in the clinic, such as formation on intraocular lenses.

3. **Molecular tools for pathogenic bacteria: vector-based tool kits for Gram-Positive and Gram-negative bacteria**. For all the work that has been done on the study of *S. aureus*, there is a surprising lack of molecular tools. I have made a unified collection of tools for *S. aureus* research. These vectors are novel in that they take advantage of the efficient homologous recombination system of the budding yeast *Saccharomyces cerevisiae*. They are useful for a range of genetic and biochemical research applications such as homologous recombination based cloning, site-directed mutagenesis, neutral site chromosomal-integration, inducible expression constructs, two-step gene replacement and one-step gene disruption. These plasmids are made in such a way that a single set of primers can direct recombination into almost all of the vectors, such that one amplicon can generate tools for many applications. Our Gram-positive plasmids are based on pC194, pE194, pT181, and pWV01 temperature sensitive and wild-type replicons. They have four different selectable markers. They will greatly increase our competitiveness; we will be able to ask more questions in less time. We have already used these to mutate several in *S. aureus*, including *stcA*, *stcB*, *spa*, *fnbA*, *fnbB*, and *sdrB*. I will always be on the look out for new techniques to facilitate research in my laboratory.

   I also have made an extensive tool kit useful for a wide range of Gram-negative and have included origins of replication including ColE1, p15a (pACYC), RK2, oriR6K, pSC101, and pWV01. These have a wide range of copy number and incorporate a number of different selectable markers. We have used these to mutate many genes in *Pseudomonas aeruginosa* including, but not limited to *rhlB*, *clpP*, *clpX*, *crc*, PA1131, and *fadL*. These genes all have roles in bacterial biofilm formation, motility, and/or pathogenesis. Additionally expression vectors have been made and utilized.

   As an example of the potential of this system we have recombined 5 pieces of DNA with greater than 85% efficiency. To the original vector in a single step we added an inducible promoter/repressor system, a GFP gene into which we added two site directed mutations resulting in F64L and S65T residue changes and a C-terminal 3HA epitope tag. The process takes little time and is very easy.

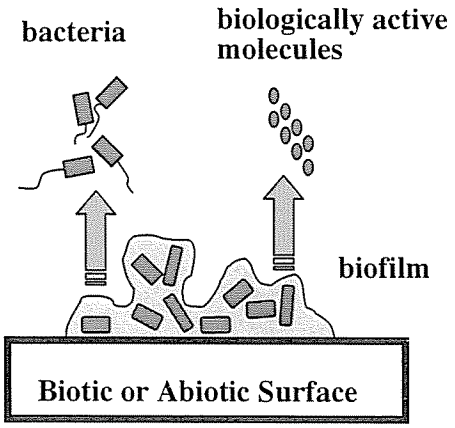

Figure 1. Biofilms form on biotic or abiotic surfaces. Biofilms afford protection to bacteria (green) and can shed bacteria and biologically active molecules (red) leading to persistent or acute diseases.

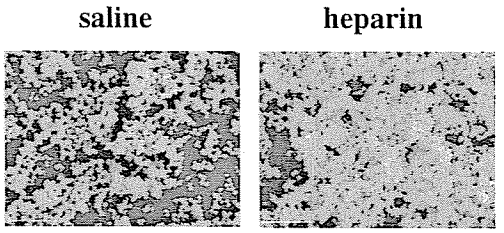

Figure 2. Scanning electron micrographs of *S. aureus* biofilms on PVC plastic. Biofilms formed in the presence of sodium heparin (1000U/ml) exhibit more surface coverage and 3-dimensional architecture. Heparin is commonly used as an anticoagulant in dialysis catheters. We have recently submitted a manuscript in which we survey alternative catheter lock solutions.

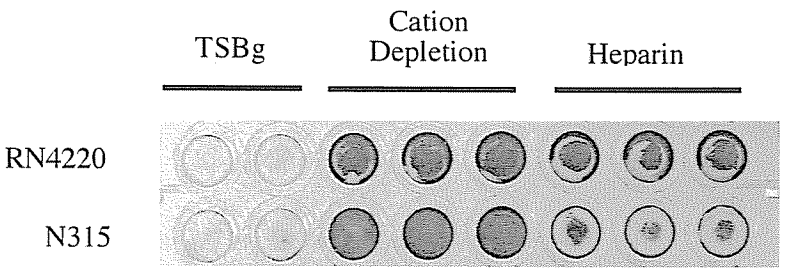

Figure 3. Find Biofilm Factors in Different Conditions. Depicted is a photograph of crystal violet stained *S. aureus* biofilms (strains RN4220 and N315, an MRSA) formed under different environmental conditions in a microtiter dish. We are taking genetic and biochemical approaches to identify factors important in biofilm formation under these and other conditions.

Teaching statement **Robert Shanks**

I look forward teaching and mentoring undergraduate and graduate students. I enjoy teaching and have endeavored to incorporate teaching in every step of my scientific career. I believe that as a researcher it is my responsibility to communicate my knowledge, experience and enthusiasm for science to inspire the next generation. Furthermore, I think that by interacting with students, one is constantly challenged and forced to think of biological problems with a different perspective.

As a graduate student, I was a laboratory assistant for dental, veterinary and medical students for three years. In this microbiology course, I was responsible for a short lecture at the start of each class covering the highlights of the previous classroom lecture, as well as directing the students in techniques of microbiology. The students isolated bacteria from their own bodies and identified them, thereby learning a number of basic microbiology and microscopy skills. I was also a teaching assistant for a medical school molecular biology class for three years. In this position I was responsible for leading weekly question and answer sessions as well as grading tests. I tutored several students for the medical school molecular biology class. I was also a tutor for a graduate level genetics course for two years in which I worked with individual students and the entire class discussing scientific papers and working out genetic problems.

As a post-doctoral researcher and graduate student I have had the pleasure of mentoring eight first-year rotation students, several undergraduate summer students, and a women in science program (WISP) student for several years. In this capacity I have in several cases found a project for the student as well as directing the day-to-day course of laboratory research of these students. I find this one-on-one mentoring particularly enjoyable. It is delightful to see the progress of an undergraduate student over the course of several years.

My research experience in several different fields of biology (microbial genetics and physiology, molecular biology, eukaryotic genetics, cell biology, and biochemistry) as well as teaching and mentoring students will help me to be a successful educator that has the ability to communicate knowledge and enthusiasm for science to students.

I think that the teaching of science benefits from visual teaching styles and rigorous hands-on laboratory opportunities. My experiences at two Cold Spring Harbor courses, once as a teaching assistant and the other as a student have strongly influenced this view. At those courses the students were able to enjoy the excitement of participating in real and fruitful research projects. This excitement in answering novel questions in a laboratory fosters the learning (the intercalation) of concepts much more so than lectures alone.

Based on my scientific experiences, I feel that I am most qualified to teach microbiology, molecular biology, genetics and biotechnology courses. However, I would be comfortable teaching other courses. In these courses I would emphasize the reading, writing and discussion of scientific material.