SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **11/01/2016**
Revised Date:

[redacted]

Application Number: **2 R01 AI042783-16A1**

Principal Investigator

MUIR, TOM

Applicant Organization: PRINCETON UNIVERSITY

Review Group: **SBCB**
Synthetic and Biological Chemistry B Study Section

Meeting Date: **10/19/2016** ***RFA/PA:*** **PA16-160**
Council: **JAN 2017** ***PCC:*** **M38A**
Requested Start: **04/01/2017**

Project Title: **Peptide Autoinducers of Staphylococcal Pathogenicity**

SRG Action: **Impact Score:** [redacted]
Next Steps: **Visithttp://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
16	[redacted]	[redacted]
17		
18		
19		
20		
TOTAL	[redacted]	[redacted]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

2R01AI042783-16A1 Muir, Tom

BIOHAZARD COMMENT
COMMITTEE BUDGET RECOMMENDATIONS
Vertebrate Animals
ADMINISTRATIVE NOTE

RESUME AND SUMMARY OF DISCUSSION: This resubmission of a renewal proposes to provide fundamental insights into how a quorum sensing (QS) system such as the accessory gene regulator (agr) operates at the molecular level, and lay the foundation for the development of new strategies for treating *S. aureus* infections. The reviewers are extremely enthusiastic about all aspects of this application. Significance is exceptional, as what is discovered in this work will likely translate to other Gram+ pathogens. The response to the previous critiques is strong, with the previous animal studies removed (although the section describing vertebrate animals was apparently left in by mistake). That aim is replaced by innovative new experiments to identify pharmacological modulators. The investigative team and environment are unparalleled and this team has discovered much of what is known in this field. The rigor of the approach is extremely high, as both unbiased and targeted activities are proposed, and alternatives are provided for most experiments. Innovation permeates all of the methods and assays. There is a good mix of established techniques and new technologies. The panel found no weaknesses worth mentioning and had the highest enthusiasm for the potential impact of this application on understanding the *S. aureus* agr system and its potential application to various bacterial pathogens.

DESCRIPTION (provided by applicant): A research program will be undertaken to study agr signal transduction in the commensal pathogen, *Staphylococcus aureus*. The accessory gene regulator (*agr*) locus found in all staphylococci encodes a quorum sensing (QS) circuit that controls the expression of virulence and other accessory genes. It consists of two oppositely oriented transcriptional units, of which one encodes four proteins, AgrBDCA, involved in production and sensing of an autoinducer peptide (AIP), and the other encodes a regulatory RNA that is the effector of target gene regulation. The finding that staphylococcal virulence can be inhibited through antagonism of this QS pathway has fueled tremendous interest in understanding the detailed mechanisms at play throughout the circuit. Building on recent breakthroughs that have allowed us to reconstitute much as the quorum sensing circuit using purified components, we propose to integrate chemical, biochemical, biophysical and genetic tools for the purpose of obtaining a deeper understanding into the molecular processes underlying the production and sensing of the autoinducer peptide (AIP) pheromone that is central to *agr* regulation. The program will move forward in three directions: Aim 1, identifying the key missing players in AIP biosynthesis; Aim 2, understanding how agonism and antagonism of the QS system relates to newly discovered conformational changes in the AIP receptor, AgrC, and; Aim 3, identifying novel modulators of *agr* through sophisticated target-based screens. These studies will lay the groundwork for the development of therapeutic strategies targeting *agr*, but also contribute to a fundamental understanding of QS systems of this type, which are pervasive in the low-GC bacterial phylum, Firmicutes.

PUBLIC HEALTH RELEVANCE: Staphylococcus aureus (*S. aureus*) is an opportunistic pathogen capable of invading mucous membranes or soft tissue; once invasion occurs, the bacterium deploys a diverse arsenal of virulence factors to evade the host immune system and to facilitate spread of the infection in the host environment. A research program will be undertaken to study the central quorum sensing (QS) circuit, termed *agr*, which regulates the onset of virulence as a function of bacterial population size. Building on recent breakthroughs that have allowed us to reconstitute much of the circuit using purified components, we propose to integrate chemical, biochemical, biophysical and genetic tools to gain a deeper understanding into the molecular processes underlying *agr* regulation; these studies will provide fundamental insights into how a QS circuit such as *agr* operates at the

molecular level and will lay the foundation for the development of new strategies for treating *Staphylococcus aureus* infections.

CRITIQUE 1:

Significance: 1
Investigator(s): 1
Innovation: 2
Approach: 3
Environment: 1

Overall Impact: This is an extraordinary proposal from an experienced world leader in peptide chemistry. Along with the co-I, the PI has shaped our current molecular-level understanding of how the S. aureus Agr quorum sensing (QS) pathway functions. The proposal not only takes an innovative and thoughtful approach to addressing the issue of drug-resistant bacterial infections, but also outlines a rigorous approach to revealing certain aspects of the Agr system that have been elusive. A multitude of biophysical, biochemical, and synthetic approaches address these current gaps in our comprehension of the Agr pathway. The methods are well described, well supported with preliminary data, and significant evidence is presented that the investigators will be successful. There are very few weaknesses in this resubmitted proposal; the previous reviewer concerns have been adequately addressed, and this reviewer is most enthusiastic.

1. Significance:

Strengths

- Antibiotic resistant bacterial infections are becoming rampant and increasingly untreatable, and although there are many drugs to treat Gram-positive infections, S. aureus kills more humans today than any other bacterium besides M. tuberculosis (MAJOR).
- It has been noted that the virulence of S. aureus can be altered by disturbing the Agr QS pathway; however, there are no medicinal interventions yet available; this proposal would lay significant foundational work (MAJOR).
- There remain critical unanswered questions regarding the Agr system, such as some missing players and the consequences of recently discovered receptor conformational changes; this serves as the premise of the proposal (MAJOR).
- Lessons learned on the S. aureus Agr system will most likely transfer to other notorious Firmicutes pathogens, such as those from the Streptococcus, Bacillus, Listeria, and Enterococcus genera (MAJOR).

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- The PI is a recognized leader in chemical biology (MAJOR).
- The PI has considerable experience in the synthesis of proteins and has developed new methods to do so (MAJOR).
- The PI has been working on the Agr QS pathway for a while and is intimately familiar with it (MODERATE).

- Co-I Novick is also a recognized leader in molecular pathogenesis, especially S. aureus, which is pertinent to this proposal given that he provides complementary expertise to the PI (MAJOR).
- Co-I Novick actually discovered the Agr QS pathway (MAJOR).
- Since 1999, the PI and Co-I have published no fewer than 14 papers together on the S. aureus Agr QS pathway. The majority of these papers appear in highly respected, multidisciplinary journals (MAJOR).

Weaknesses

- None noted.

3. Innovation:

Strengths

- Although Agr QS inhibitors have been known for a while (and some even described by the PI and Co-I, e.g., Lyon et al., PNAS, 97,13330, 2000), no global, small-molecule activators of Agr QS have been developed (which would be expected to disrupt biofilm formation)(MAJOR).
- A unique blend of chemical biology approaches is used to identify the proteases responsible for AgrD processing (MODERATE).
- The assay described in figure 6c is quite clever and will be informative if successful(MAJOR).
- The peptide synthetic methods described in Aim 3.2 are also perceived as a new innovation (MODERATE).

Weaknesses

- Contrary to what is claimed in the innovation statement, there already have been small molecule inhibitors of S. aureus virulence. These compounds block biosynthesis of staphyloxanthin, an antioxidant pigment that shields S. aureus from ROS produced by the immune system (see PMCID: PMC2747771) (MINOR).

4. Approach:

Strengths

- The experimental approach in all aims is well articulated, thoughtfully designed, and rigorous (MAJOR).
- The investigators have amassed a large amount of preliminary data that supports the majority of the proposed work. This lowers the overall risk of the project (MAJOR).
- The approach overall is rigorous, with proper controls described, alternative explanations given, and contingency plans nicely outlined (MAJOR).

Weaknesses

- Part of Aim 2.1 proposes to solve the X-ray structure of an integral membrane protein, ArgC. The PI states several homologs have been purified and are active. Without a crystal or diffraction data, there are serious doubts whether this line of experimentation (solving the transmembrane regions) will be successful (MODERATE).

5. Environment:

Strengths

- The environment at Princeton is ideal to carry out the proposed work (MAJOR).

Weaknesses

- The PI recently moved from NYC to Princeton. The Co-I is still in NYC. The loss of proximity has the potential to slow down this highly fruitful collaboration (MINOR).

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

- Animal studies are not proposed in this revised A1 application, although some vertebrate documentation is included. This may have been accidentally included since the A0 application did propose animal work (Unacceptable).

Biohazards:

Unacceptable

- This proposal involves the use of pathogenic bacteria; biosafety procedures are not explicitly described. That said, the PI and co-I clearly have experience working with these hazards.

Resubmission:

- Aims I and II were received favorably, with concerns mostly pertaining to Aim III. Here, the animal studies were deemed premature. The replacement of those experiments with attempts to identify a global Agr QS activator are innovative and interesting.
- The Introduction to Application adequately addresses all other concerns previously raised by reviewers.

Renewal:

- The PI was fairly productive during the previous grant period, as evidenced by five publications found using NIH Reporter (Plasmid 2012, Mol Cell 2014, Chembiochem 2015, PNAS 2015, and Cell Chem Biol 2016). Several of these papers detail significant advances for the field.

Authentication of Key Biological and/or Chemical Resources:

Unacceptable

- None are given, nor a justification.

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

- Money is requested for animal work but animal work is not proposed.

CRITIQUE 2:

Significance: 1
Investigator(s): 1
Innovation: 2
Approach: 1
Environment: 1

Overall Impact: This proposal outlines studies to explore how the virulence-associated quorum sensing cascade, agr, is regulated at the molecular level through a series of interesting and well-reasoned experiments. Of particular significance is the role of this circuit in *Staphylococcus aureus* virulence, which can be attenuated by inhibition of the pathway. The PIs have longstanding expertise in the study of this pathway and as such, have acquired substantial preliminary data to provide strong premise for the proposed studies. The productivity during the previous grant period is good. Score-driving factors include the strong significance of the proposed work and compelling evidence for each of the stated aims (strong premise). The PI adequately addressed the previous reviews and removed animal studies, which were thought to be premature.

1. Significance:

Strengths

- *Staphylococcus aureus* is an opportunistic pathogen that causes a large number of infections each year, with increasing concern caused by drug-resistant strains. (moderate)
- Detailed examination of secretion and proteolytic processing of the AIP will yield critical information about the mechanisms of these processes, confirm the roles of each actor and potentially indicate new targets for therapeutic intervention with a focus on antivirulence strategies. (major)
- Characterization of the sensory domains of histidine kinases is notoriously difficult and the devised studies are likely to yield critical information about this feature of ArgC. This will yield important information about AIP binding and also provide one of only a limited number of examples of the structural characterization of stimuli-HK interactions. (major)
- The preliminary data provide strong premise for the proposed studies. (major)

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- The PIs are accomplished investigators with a longtime collaboration to study *S. aureus* quorum sensing and the agr system. They have complementary expertise, enabling in-depth studies such as those in this application.
- Productivity in the past grant period was good.

Weaknesses

- None noted.

3. Innovation:

Strengths

- Development of a clever strategy for the biosynthesis of AgrD-thiolactone independent of AgrB enabled the postulate that an alternative transporter is required for thiolactone secretion. (moderate)
- Use of new technology, such as nanodiscs, to reconstitute a two competent circuit is innovative and exciting. (major)
- Their hypothesis that opposing mechanical motions regulate the kinase activity of AgrC and the proposed methods of study are intriguing. (moderate)
- Targeting a virulence pathway for potential therapeutic development has the potential to be highly efficacious. (minor)

Weaknesses

- None noted.

4. Approach:

Strengths

- The investigators presented strategies to ensure an unbiased approach to the proposed studies, often including both a method to perform targeted studies, as well as untargeted experiments to assure that the success of the proposed work is not unnecessarily affected by existing hypotheses (scientific rigor).
- Studies to identify the protease responsible for degradation of AgrD and secretion of the thiolactone are well thought-out and thorough. (major)
- The proposed studies to examine the mechanism by which membrane receptor ligation activates or inhibits intracellular His kinase activity are both exciting and well conceived. Enthusiasm is strengthened by strong preliminary data. (major)
- The proposed strategy to identify global activators of agr, including the chain inversion method, are clever and have a high likelihood of providing such compounds. (major)

Weaknesses

- None noted.

5. Environment:

Strengths

- Excellent for conducting this multidisciplinary research, which is evident from the prior accomplishments of this collaborative team. (major)

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

- Statement is included but no animal work is proposed in the Research Strategy section.

Biohazards:

Unacceptable

- Biosafety considerations are not discussed although use of pathogens is proposed.

Resubmission:

- The PI addressed the previous comments and provided additional preliminary data to support this resubmission and address previous concerns about feasibility.
- Animal studies were removed as recommended and replaced with experiments to further detail the interactions on a molecular level.

Renewal:

- Productivity is good as judged from publications (5 were noted), several of which were highly impactful.

Authentication of Key Biological and/or Chemical Resources:

Unacceptable

- not included

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

- Funds for animal's studies should be removed given that these experiments are no longer proposed.

CRITIQUE 3:

Significance: 1
Investigator(s): 1
Innovation: 1
Approach: 1
Environment: 1

Overall Impact: This renewal is a tour de force by an exceptional research team (Muir/Novick) that focuses on elucidating the molecular mechanisms underlying *agr* signal transduction in *Staphylococcus aureus*, a human pathogen that can infect any organ and is responsible for thousands of community- and hospital-acquired infections in the United States alone. The questions being addressed in this proposal are highly significant not only because this system is important for *S. aureus* virulence and the work may provide new approaches to prevent and treat infection, but also because of the fascinating chemical and biological questions that are being tackled. Innovation is present throughout the proposal as evidenced by the questions being addressed and methods being utilized, and both hypothesis/candidate-driven approaches and unbiased approaches are presented for many

experiments. The scientific rigor is exceptional. The likelihood for success is apparent from the prior track record of accomplishments in this area by the research team, productivity in the prior period of support, preliminary data supporting the proposed work, and the multifaceted approach. This application is a resubmission and the criticisms from the prior review are adequately addressed. Overall, enthusiasm for this ambitious and creative proposal is extremely high.

1. Significance:

Strengths

- The scientific premise of the proposal is excellent. There is exceptional justification for the proposed studies, and provided (likely) success, important knowledge gaps will be filled.
- Understanding the *agr* system at the molecular level is important because it regulates virulence in a human pathogen of particular concern, and insights may provide new approaches for preventing and treating infection. (major strength)
- Biochemical and biophysical characterization is likely to provide new paradigms that may have broad impact; for instance, a particularly noteworthy idea put forth in this proposal is a connection between cellular proteases and quorum sensing. (major strength)
- Pharmacological modulators of the agr system will inform whether this system is a viable target for new antibiotic strategies, and both inhibition and activation of this system are considered in the context of this proposal. The latter strategy may have utility in preventing biofilm formation, which is a complication on medical devices and surfaces. (moderate strength)
- Identification of small molecule inhibitors as well as activators of the *agr* quorum sensing system will overcome issues with thiolactone-based peptides, and provide new tools to the research community and potential leads for new therapeutics for non-traditional targets**.** (moderate strength)

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- Exceptional research team – Muir and Novick have been collaborating for >15 years and have co-published many papers on the S. aureus Agr QS system, indicating that they are enormously productive and creative. (major strength)
- Novick discovered the Agr QS system. (major strength)
- Muir has all of the needed expertise in chemical biology and method development required for the proposed work. (major strength)
- Collaboration with Prof. Haw Yang (Princeton) for FRET/TIRF experiments will enable experiments in Aim 2.1. (moderate strength)

Weaknesses

- None noted.

3. Innovation:

Strengths

- Innovation is found in all aspects of this proposal as highlighted by the creative methods, the hypotheses, and the approaches being taken to understand the agr system. (major strength)

- The idea presented in Aim 3 of identifying a global autoinducer peptide (AIP) activator, proposed as a new means to block biofilm by *S. aureus*, is novel. (moderate strength)
- Use of the reconstituted two-component circuit in mechanistic studies is innovative. (moderate strength)
- The concept of a link between quorum sensing and protein turnover, including the hypothesis that a Clp protease or another AAA+ protease degrades $AgrD^C$ to provide the thermodynamic driving force for AIP production, is novel and intriguing. (moderate strength)
- Small molecule screens using ArgC reconstituted in nanodiscs is a creative use of nanodisc technology that should have broad application to other types of problems/screens. (moderate strength)
- The combination of chemical biology, structural methods, genetic approaches, and screening presented in this proposal is unusual and spectacular. (major strength)
- The balance of hypothesis/candidate-driven and unbiased approaches is arguably an innovative aspect of this proposal and keeps the door open for the option that a hypothesis is proven incorrect or that a line of reasoning takes experiments in the "wrong" direction. (minor strength)

Weaknesses

- None noted.

4. Approach:

Strengths

- The proposed experiments are backed by extensive assay development (including creative assays) and preliminary data from the research team; the likelihood of success and new findings is extremely high. (major strength)
- Balance of hypothesis/candidate-driven and unbiased approaches is a strength of the Approach. (moderate strength)
- The combination of methods enables the proposed work and is expected to advance the field. (moderate strength)
- All of the above strengths contribute to the highly rigorous nature of the proposed work, which ensures a robust and unbiased approach. (major strength)

Weaknesses

- None noted.

5. Environment:

Strengths

- The environment at Princeton University and NYU for conducting this research at the chemistry/biology interface is excellent, as exemplified by all of the prior accomplishments of the research team. (major strength)

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

- A vertebrate animal section is included (and addresses the four points), but no animal studies are proposed in the grant. They are suggested as a line of future work. Of note, animal studies were in the original A0 application and in response to the reviewer concerns, these studies were removed from the A1 application.

Biohazards:

Unacceptable

- It is noted that BSL2 facilities/approval for Staphylococcus aureus are not explicitly noted in the facilities/resources section. Nevertheless, the co-PIs have many years of experience handling these organisms.

Resubmission:

- The concerns expressed in the critiques of the original submission were thoughtfully and convincingly addressed in this revision, and additional preliminary data was included.
- The greatest reviewer concern seemed to be the animal studies originally proposed in Aim 3. The applicant has removed these studies and included new experiments to identify a global Agr regulator.
- The concerns about innovation in the proposal were adequately addressed and the application provided a perhaps needed reminder that innovation is found throughout the proposal in terms of hypotheses and experimental design.

Renewal:

- A publication list was not included in the application, but a literature search indicates the research time has been productive and published 5 papers, including work in top journals like PNAS and Cell Chem Biol.

Authentication of Key Biological and/or Chemical Resources:

Unacceptable

- Not included.

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

- Amend budget because animal studies are budgeted for, but not part of the proposed work.

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

BIOHAZARD COMMENT: Biosafety considerations are not discussed although use of pathogens is proposed.

V**ertebrate Animals:** While this application is coded for vertebrate animals and a vertebrate animal section is included there are no plans described for using vertebrate animals

COMMITTEE BUDGET RECOMMENDATIONS:
The budget should be reduced by the amount budgeted for animal studies, since they not part of the proposed work.

ADMINISTRATIVE NOTE:
Applications submitted for due dates on or after January 25, 2016 are required to include a new PDF attachment describing plans for Authentication of Key Biological and/or Chemical Resources that will be used in that research study (see NOT-OD-16-011). Reviewers were asked to consider information provided in this attachment as part of their evaluation of your application. This attachment was missing from your application and could not be assessed.

Footnotes for 2 R01 AI042783-16A1; PI Name: Muir, Tom

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.