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TITLE: The Effect of Novel Biofilm Inhibitors on Antibiotic Resistance in *E. coli*

PRINCIPAL INVESTIGATOR: Valerie Daggett

CONTRACTING ORGANIZATION: University of Washington

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Fort Detrick, Maryland 21702-5012

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Valerie Daggett

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14. ABSTRACT

E. coli is a prevalent multi-drug resistant (MDR) pathogen associated with trauma-related injuries of military personnel. Biofilm formation is associated with many of these MDR strains of E. coli. Curli, the first identified functional amyloid, are the major proteinaceous component of E. coli biofilms and they are implicated in cell attachment, virulence, and providing structural stability to biofilms. The amyloid biogenesis system in E. coli is remarkably adept in its ability to restrict amyloid formation to the cell surface. Through rapid polymerization and dedicated secretion machinery, these bacteria have evolved to generate large quantities of biofilm amyloid fibril scaffolding while minimizing the risks of self-toxicity through accumulation of intracellular aggregates. The protein CsgA is the dominant protein in these amyloid fibrils. We hypothesize that populations of transient α-sheet oligomers, composed of CsgA, exist within Gram negative biofilms, and that these structures can serve as targets for designed α-sheet peptides to suppress fibril formation in the EM. In turn, suppression of biofilm formation should improve antibiotic penetrance and reduce resistance.

15. SUBJECT TERMS
Approved for Public Release; Distribution Unlimited

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E. coli is a prevalent multi-drug resistant (MDR) pathogen associated with trauma-related injuries of military personnel. Biofilm formation is associated with many of these MDR strains of *E. coli.* Curli, the first identified functional amyloid, are the major proteinaceous component of *E. coli* biofilms and they are implicated in cell attachment, virulence, and providing structural stability to biofilms. The amyloid biogenesis system in *E. coli* is remarkably adept in its ability to restrict amyloid formation to the cell surface. Through rapid polymerization and dedicated secretion machinery, these bacteria have evolved to generate large quantities of biofilm amyloid fibril scaffolding while minimizing the risks of self-toxicity through accumulation of intracellular aggregates. The protein CsgA is the dominant protein in these amyloid fibrils. We hypothesize that populations of transient α-sheet oligomers, composed of CsgA, exist within Gram negative biofilms, and that these structures can serve as targets for designed α-sheet peptides to suppress fibril formation in the EM. In turn, suppression of biofilm formation should improve antibiotic penetrance and reduce resistance.

We are initially focusing on the UTI89 strain of *E. coli*, which was isolated from the urogenital tract of an infected patient. We have four main reasons for this choice of model system: (1) this strain is prevalent in antibiotic-resistance catheter-associated urinary tract infections; (2) this strain forms abundant amyloid fibrils and robust biofilms; (3) the growth and behavior of this strain in response to changes in environment have been well characterized; and (4) an important control CsgA knock out strain has been developed, ΔCsgA. Previous work has established the importance of growth conditions on the development of amyloid fibrils in UTI89 biofilms, providing an important benchmark for the proposed studies.

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

E. coli is a prevalent multi-drug resistant (MDR) pathogen associated with trauma-related injuries of military personnel. Biofilm formation is associated with many of these MDR strains of *E. coli.* Curli, the first identified functional amyloid, are the major proteinaceous component of *E. coli* biofilms and they are implicated in cell attachment, virulence, and providing structural stability to biofilms. The amyloid biogenesis system in *E. coli* is remarkably adept in its ability to restrict amyloid formation to the cell surface. Through rapid polymerization and dedicated secretion machinery, these bacteria have evolved to generate large quantities of biofilm amyloid fibril scaffolding while minimizing the risks of self-toxicity through accumulation of intracellular aggregates. The protein CsgA is the dominant protein in these amyloid fibrils. We hypothesize that populations of transient α-sheet oligomers, composed of CsgA, exist within Gram negative biofilms, and that these structures can serve as targets for designed α-sheet peptides to suppress fibril formation in the EM. In turn, suppression of biofilm formation should improve antibiotic penetrance and reduce resistance.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Biofilm, alpha-sheet, multidrug resistant, amyloid

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The major goal of this study is to test the ability of α-sheet amyloid peptide inhibitors to suppress biofilm formation in a clinically relevant *E. coli* strain and to determine whether combined administration with antibiotics increases bacterial mortality in comparison to purely antibiotic treatment.

Task 1: Establish and optimize bacterial system: (a) Establish conditions for *E coli* growth and biofilm formation for WT UTI89 and ΔCsgA strain; (b) Ensure conditions are robust and reproducible over time; and (c) Quantify amyloid formation in WT and deletion strain.

Projected completion date: May 1, 2019, Done on time.

This task was completed on schedule. We engineered robust bacterial growth conditions when grown in LB broth, and optimal conditions for biofilm formation were established and are reproducible when using YESCA media supplemented with 4% DMSO. Amyloid formation in the wild-type and deletion strains have been quantified using a thioflavin T assay developed in the lab.

Task 2: Test biofilm inhibition by peptide inhibitors: (a) Screen peptide designs to determine their ability to decrease amyloid fibril formation; and (b) Determine dose-response for 5 best inhibitors

Projected completion date: September 1, 2019. Completed on time.

This was done, but in so doing we found that a specific design feature on top of our alpha-sheet template outperformed the others and we pursued improvements on that template. In addition, we discovered that some of our designs were being clipped by proteases. We determined where this was occurring and we redesigned to remove the protease site. We technically completed this task as written but the work led us to improved inhibitors and we have designed and synthesized many related variants and the labs were shut down because of COVID-19 and the work stopped. They remain to be tested and characterized under different situations.

Task 3: Test effect of antibiotics on bacteria: (a) Screen antibiotics to determine their ability to kill bacteria; and (b) Determine antibiotic minimum inhibitory concentration (MIC) values.

Projected completion date: January 1, 2020, ~50% complete

Gentamicin is the most potent antibiotic when used in conjunction with alpha-sheet peptides. MIC values have been determined for gentamicin and ciprofloxacin. We found that erythromycin and amoxicillin have little to no effect on UTI89 without alpha-sheet peptide, even at very high concentrations. This is when we also discovered that some of the alpha-sheet designs were being hydrolyzed by the bacteria. We began testing with new peptides in which the clipping site was redesigned, and we were seeing much improved effects, but then the lab was shut. We have restarted this work but so far with only one person able to work part time because of lab distancing and safety regulations.

Task 4: Test effect of biofilm inhibitors and antibiotics in tandem: (a) Test combined effect of biofilm inhibitors and antibiotics at optimal inhibitor concentration while varying antibiotic concentration; and (b) Quantify cell death with respect to each condition to see if the MIC is improved with co-administration of α-sheet designs.

Projected completion date: July 1, 2020. 15% complete. We established the protocols and we have synthesized the new peptide designs, but the actual combined testing began just before the pandemic began in Seattle.

Task 5: Analyze results: (a) Determine whether co-administration of biofilm inhibitors and antibiotics improve efficacy; and (b) Determine best combination of inhibitor and antibiotic for this system.

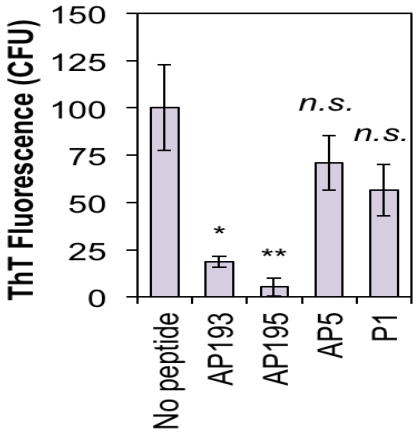
Projected completion date: July 31, 2020. 5% complete. We have been analyzing results throughout but this task is directed at the final analysis of the various combinations.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

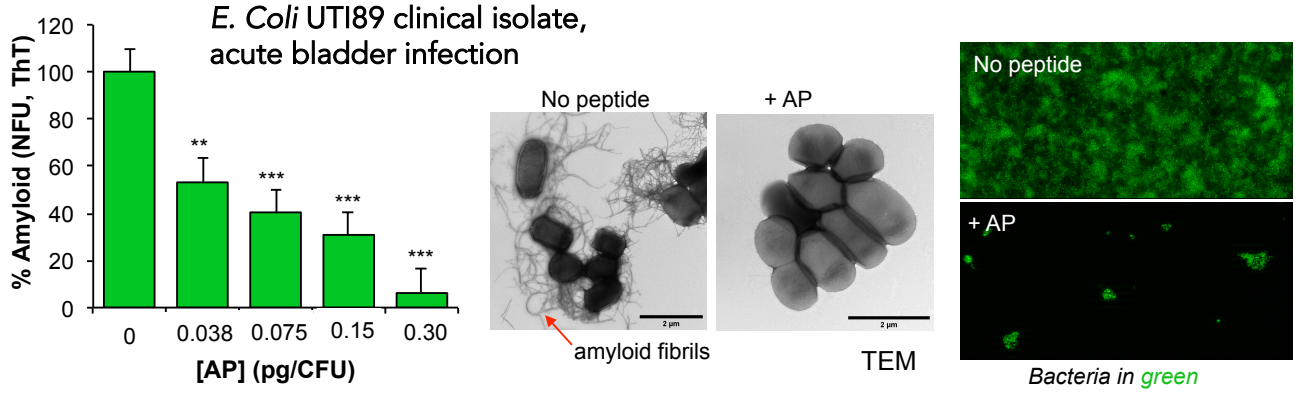
Major Activities and Significant Results

We have established the conditions for robust biofilm production by UTI89 bacteria for the proposed experiments. The amyloid content is quantified using a method developed to the in vitro and then carried re-engineered for use in live bacteria. This allows us to screen our designs and rank them based on how effect they are at inhibiting amyloid formation.

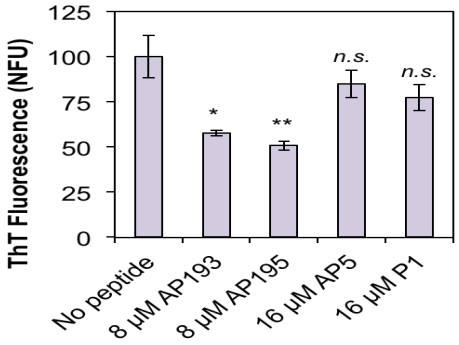


Some of our results are provided in the figure to the left to illustrate how they perform. The amyloid assay is based on thioflavin T binding to beta-sheet fibrils, which leads to an increase in fluorescence. In the absence of alpha-sheet peptide the bacteria make abundant fibrils and the addition of AP193 and AP195 lead to a dramatic drop in the fibril content of the biofilms. AP5 was less effective and P1, a random coil control also indistinguishable from *E. coli* without our designs. Note that baseline correction to account for nonspecific fluorescence in the cells is done by subtracting the signal of the ΔCsgA strain, which does not form fibrils. AP195 is a redesigned variant of AP5, and it is much more potent. AP5 is one of our early designs, and it is quite potent against a number of mammalian amyloid species *in vitro* and *in vivo* in animal models.

More results for AP195 are shown below. The panel at far left shows amyloid formed as a function of alpha-sheet peptide applied. Inhibition of amyloid fibril formation increases with increasing amounts of AP195 with essentially 100% inhibition at 0.30 pg/CFU (baseline correction using ΔCsgA reading). Thus, a dose-response relationship is obtained with nearly complete inhibition of amyloid at 0.3 pg per CFU bacteria. TEM images are provided next to the curves. The TEM images reveal extensive amyloid formation in peptide-free biofilms and biofilms grown in the presence of P1, but not those grown in the presence of 0.3 pg /CFU AP195 (Scale bars = 2 μm). In addition, green fluorescent (UTI89 SLC-719) biofilms exhibit far less adhesion to glass slides when grown in the presence or absence of our designs (scale bars = 50 μm). The images on the far right show the near absence of biofilm after administration of our peptide.



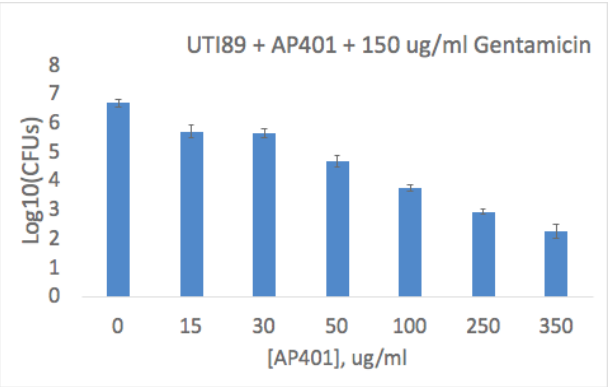
As can be seen above, the different peptides have different degrees of inhibition of amyloid. Very similar behavior is seen in another clinical isolate from the Seattle Children's Hospital. This GERB319 strain is resistant to gentamycin and ciprofloxacin. As with UTI89 above, the P1 and AP5 peptides had little effect, but P193 and AP195 caused a significant decrease in ThT fluorescence. As mentioned above, AP195 is a re-designed version of AP5 and it provides 100% inhibition at half the dose in this resistant strain, rendering it susceptible to both gentamycin and ciprofloxacin. These GERB319 results highlight the broad antimicrobial utility of synthetic α-sheet peptides obtainable irrespective of bacterial resistance profile.

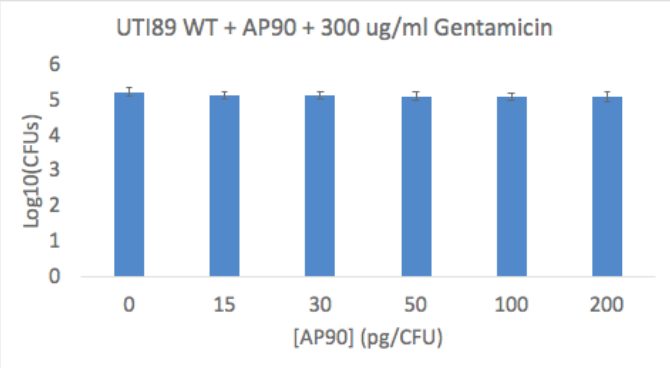


Synthetic α-sheet peptides inhibit fibril formation in GERB319 biofilms, a clinical UTI isolate with resistance to gentamicin and ciprofloxacin. Error bars represent the standard deviation from the mean of at least three replicates. Note that the background fluorescence is at ~50% in the ΔCsgA strain that does not form amyloid fibrils and nonspecific fluorescence has not been subtracted off. AP195 completely inhibits fibril formation. 8 μM AP195 = 0.3 pg/CFU.

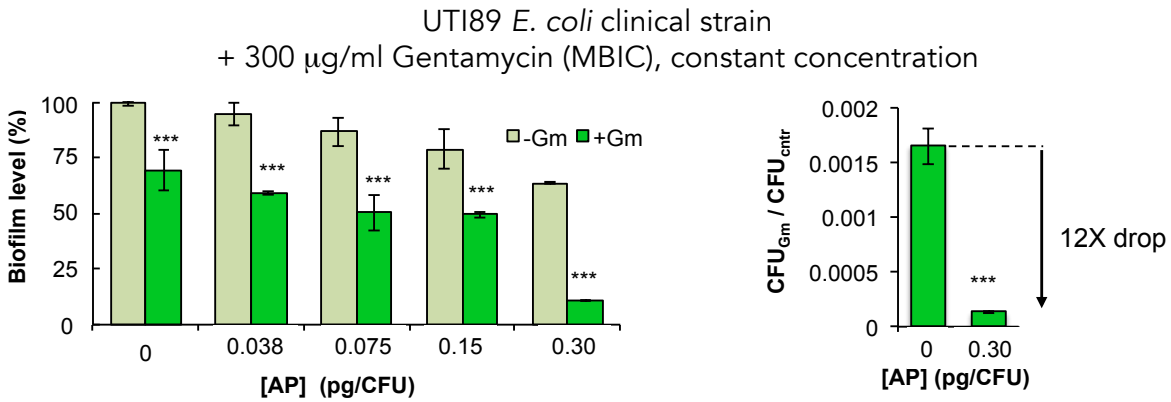
We have screened a number of different peptides and they fall into essentially two groups: those like AP5 and newer ones like AP193 and AP195. We also recently found out that E. coli contains a protease that protease that clips some of our peptides, which affects the abiity of AP5 to inhibit amyloid formation. As an example of this is AP90, which is very similar to AP5. We determined where the peptide was hydrolyzed and redesigned the turn in of the alpha-sheet hairpin. That peptide is called AP401, and it is much more active, as shown below. AP401 shows a dose-response in the drop of bacterial count of UTI89 with a constant gentamycin concentration of 150 μg/ml. AP401 is the same sequence but with flipped main-chain chirality in the turn as AP90. AP90 shows no activity at double the gentamycin concentration. The AP193 and AP195 peptides are dimers and the turns

appear to be protected from proteolytic attack. Nonetheless, we have combined the two design approaches, resulting in inhibitors that are both more stable and more potent.

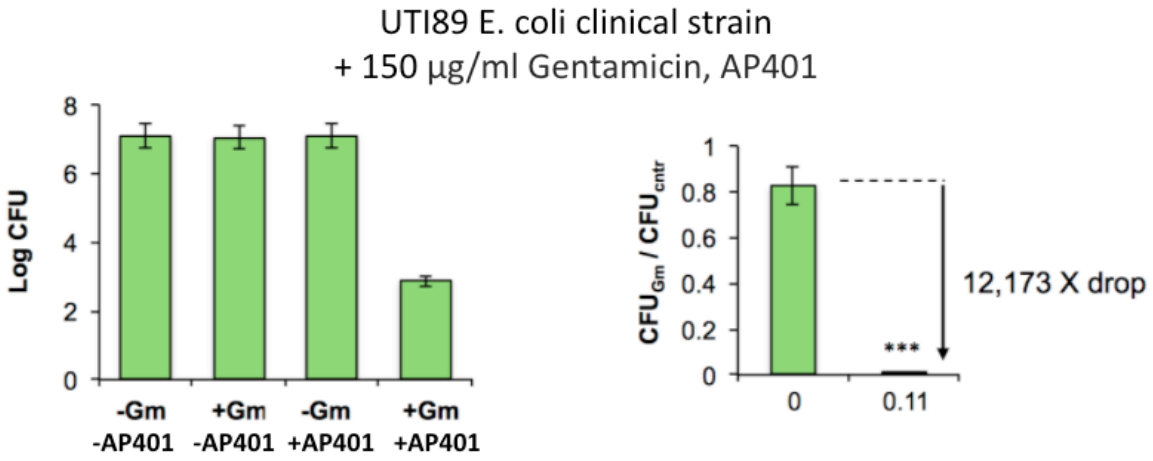




As an example of the results we are obtaining when combining antibiotics and alpha-sheet designs, below are results for AP195. It shows a dose-response curve for the drop in biofilm (in this case biofilm mass, amyloid content with and without 300 mg/ml gentamycin, with the most potent effect seen with AP195 0.30 pg/CFU. This corresponds to a 12X improvement in the drop in colony forming units with AP195 versus gentamycin alone.



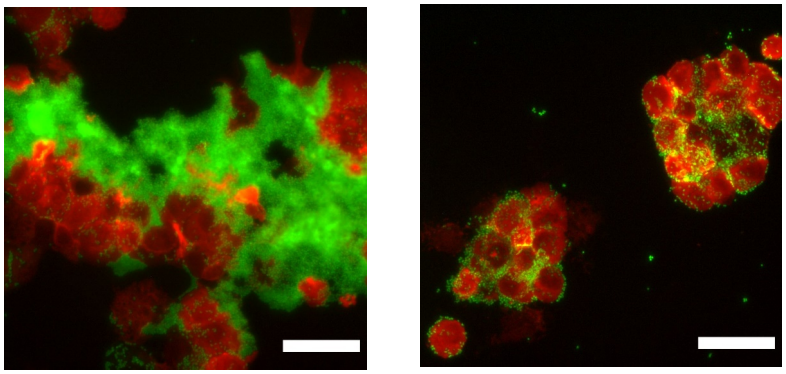
The effect improves 3 orders of magnitude when the turn is redesigned to prevent proteolysis, as described above. AP401 provides a 12,173X effect relative to gentamycin alone at half the gentamycin concentration and one-third the original peptide concentration.



We are finding that our alpha-sheet peptides render different strains of E. coli more susceptible to antibiotics, even strains deemed to be resistant. We then tested whether the bacteria also become more susceptible to the host immune response due to the loss of the stabilizing structural scaffold of the curli amyloid fibrils. For this we posited that these structural changes would increase the availability of bacteria for phagocytic clearance by immune cells. To investigate this idea, biofilms were cultivated in microtiter plates with the synthetic α-sheet peptide AP193 added to the growth medium. A fluorescent UTI89 derivative strain (SLC-719, carrying chromosomal vsfGFP) was substituted for these experiments to facilitate visualization. After 48 h, mature biofilms were washed and macrophages (RAW 264.7, stained for red fluorescence) were applied to the biofilms for 1 h at a multiplicity of infection (MOI) of ~1:100 (macrophage:bacteria) prior to resuspension of the entire sample. Macrophages were separately co-incubated with planktonic bacteria as a positive control.

E coli *E coli* + AP

Biofilm protects bacteria (green) from macrophage (red)



Human macrophage ingesting bacteria

Analysis by flow cytometry and fluorescence microscopy revealed that macrophages were able to phagocytose far more *E. coli* when biofilms were cultivated in the presence of AP193. The images above illustrate the dramatic effect our compound has on the ability of macrophages to gain access and engulf bacteria.

Overall, we have experimented with a range of antibiotic concentrations, but our comparisons with our best twice redesigned alpha-sheet inhibitors found that combinations with gentamicin are the most effective. We see a 4X, 10X, 25X and 12,000X improvement in antibiotic susceptibility with our alpha-sheet designs for amoxicillin, erythromycin, ciprofloxacin and gentamycin, respectively. However, note that none of these studies have been done yet with the newest designs that are dimeric and have the redesigned turn---the first 3 are dimers without the new turn and the 4th, and best, is a monomer with the redesigned turn. We are anticipating further improvements with our newest designs. Nonetheless, while some of these effects are relatively minor, we found that UTI89 is resistant to amoxicillin, erythromycin, and ciprofloxacin up to 1 mg/ml in the absence of our designs. Although we have more screening to do before drawing firmer conclusions, we are seeing an increase in both susceptibility to antibiotics and the host immune system with administration of our compounds.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The research began with a graduate student, Alissa Bleem, who generated the preliminary data for the proposal application. She graduated with her PhD 5 months after the grant began. Alissa trained a new graduate student, Tatum Prosswimmer, to take over the work. Alissa did an excellent job training Tatum, which in turn added to her professional development. Tatum was the recipient and her expertise has grown over time. Before the shutdown we took on a new graduate student for this project (shared with Dr. James Bryers in my department) and Tatum began training her. In this way we were able to bring in another person and the experience also helped with Tatum's professional development. Unfortunately the new student has not been allowed to work in the lab since our shutdown in March, as new students do not qualify as essential critical personnel under the current COVID-19 policies.

I was scheduled to speak about this work at an international conference in April 2020, but the conference was cancelled/postponed due to COVID-19. Meeting details: UK Microbiology Society Annual Conference 2020, Session on Identifying novel eukaryotic drug targets and mechanisms of action. Edinburgh International Convention Centre, Edinburgh, Scotland, March 30 – April 3, 2020. *Postponed*

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

COVID-19 has had a big impact on our ability to do research. The work was progressing nicely. In our screening of our designs we found a particularly good template for these studies and we found that some peptides were being clipped. These two things led us to redesign peptide sequences and create a new small library of designs that are much more effective against the biofilms in *E. coli*. This may have slowed us down a bit, but it has led to better inhibitors. Then the work was cut short. We plan on finishing what we proposed. The protocols and methods have been established and the primary student on the project has recently been allowed back in the lab under strict safety regulations. We are on track but the restrictions on personnel in the lab will continue to slow us down but I am confident that we can complete these studies with a no-cost extension.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report at this time

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report at this time

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report at this time, although we are eager to pursue translation if warranted when the work is completed to a start-up company, to partner with a company, or pursue government-supported clinical trials.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report at this time

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Our approach and objectives have not changed; it is our ability to do the studies that changed and this is due to a lab move and, mostly, COVID-19.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

COVID-19 and a lab move significantly affected our ability to complete this project on time. We were making good progress prior to these events.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The lab closure has had a significant impact on our work and ability to hire. There is a hiring freeze and we have severe limitations on who is allowed in the lab to do research, which also means we aren't spending on supplies and services, etc. Also, with the lab down the main student working on this project was paid to be a TA one quarter instead of from this grant.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable aside from not using them because of being shut down.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  We have a manuscript in preparation.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  No

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  No

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Not applicable, too early at this time.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Not applicable at this time

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

In preparation, we may include some of the results in an application involving our alpha-sheet designs

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

None at this time

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	Alissa Bleem
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5
Contribution to Project:	Ms. (Dr.) Bleem did the initial work on this project, establishing the assays and growth conditions for the bacteria.
Funding Support:	NSF Fellowship

Name:	Tatum Prosswimmer
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	11
Contribution to Project:	Ms. Prosswimmer has performed the studies described here, first with Alissa Bleem and then she took over the research.
Funding Support:	As our lab was not operational after a move in December of 2019, Tatum was supported Winter Quarter as a TA but she was able to do some work in a collaborator's lab and she trained Sara Nick. She went back on this grant Spring Quarter and then we were shutdown by COVID-19. She worked from home but could not resume experimental work in the lab until recently.

Name:	Sara Nick
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3

Contribution to Project:	Ms. Nick was being trained by Ms. Prosswimmer when the lab was shut down and it is anticipated that she will continue with the project when we open back up for nonessential personnel.
Funding Support:	Department of Bioengineering

Name:	Delaney Wilde
Project Role:	Undergraduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Ms. Wilde was a student helper of Ms. Prosswimmer. She will continue with the project when we open back up for nonessential personnel.
Funding Support:	Department of Bioengineering

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Active Funding:
1. PRMRP Award PR182247 (V Daggett, PI) CDMRP, 2/1/19 – 7/31/20, NCE requested, The Effect of Novel Biofilm Inhibitors on Antibiotic Resistance in E. coli, Goal: Suppression of biofilm formation with alpha-sheet compounds
2. Gift, Washington Research Foundation (V Daggett, PI), 3/1/19-9/30/20, SOBA: An early diagnostic test for Alzheimer's Disease, Goal: Testing human samples with SOBA
3. UW Center for Translational Muscle Research, NIH/NIAMS P30 AR074900-01, M. Regnier PD, 4/19 – 3/24, The goal of this center is to provide a unifying resource and state of the art approaches to enhance skeletal muscle research and facilitate novel insights to muscle pathologies.
4. R01-AG067476 (V Daggett, PI) NIH/NIA, 4/14/2020-3/31/2025, Conformational heterogeneity and alpha-sheet: Determinants of toxicity in Abeta variants, Goal: Characterize Abeta toxic oligomers in vitro and human samples, effect of alpha-sheet designs

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

None

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*