REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
16-01-2018	Final Report	15-Jun-2014 - 14-Jun-2017

4. TITLE AND SUBTITLE
Final Report: Spider Silk Glue Proteins BAA 8.1

5a. CONTRACT NUMBER
W911NF-14-1-0267

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
63119-LS.1

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Randolph Lewis
UU	UU	UU	UU		19b. TELEPHONE NUMBER 435-797-9291

RPPR Final Report
as of 02-Feb-2018

Agency Code:

Proposal Number: 63119LS **Agreement Number: W911NF-14-1-0267**
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DUNS Number: 072983455 EIN: 876000528
Report Date: 14-Sep-2017 Date Received: 16-Jan-2018
Final Report for Period Beginning 15-Jun-2014 and Ending 14-Jun-2017
Title: Spider Silk Glue Proteins BAA 8.1
Begin Performance Period: 15-Jun-2014 **End Performance Period:** 14-Jun-2017
Report Term: 0-Other
Submitted By: Randolph Lewis Email: randy.lewis@usu.edu
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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 3 **STEM Participants:** 2

Major Goals: See uploaded documents.

Accomplishments: See uploaded documents.

Training Opportunities: See uploaded documents.

Results Dissemination: See uploaded documents.

Honors and Awards: See uploaded documents.

Protocol Activity Status:

Technology Transfer: See uploaded documents.

PARTICIPANTS:

Participant Type: PD/PI
Participant: Randolph V Lewis
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Kyle Berg
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N

Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Michaela Hugie
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Major Goals:

The overall goal of this proposal is to produce these proteins and measure their adhesive strength thus determining if this adhesive has potential for military applications as an emergency repair material or as a different adhesive product.

Specific aims

1. Generate full-length clones of ASG 1 and 2, especially the larger version of ASG 2.
2. Incorporate these genes into a baculovirus vector to transform Sf9 insect cells.
3. Produce sufficient amounts of the two proteins to allow adhesion testing.
4. Test the two proteins separately and in various ratio combinations for adhesive strength.

Accomplished:

Concise list of accomplishments:

1. Determined that ASG1 was not a part of the aggregate gland glue.
2. Obtained sufficient protein and DNA sequence for generation of a ASG2 gene with *E.coli* codon bias.
3. Produced ASG2 protein in *E.coli* and purified the protein to a sufficient level for adhesion testing.
4. Tested the adhesion properties of the ASG2 protein.
5. Generated all the necessary vectors for transformation of Sf9 cells.

Detailed accomplishments:

1. Despite substantial effort using PCR, cloning and 5'RACE we were unable to find an ASG1 clone of any larger size than originally reported. During this work Hayashi's group reported studies using large-scale exome analysis of the aggregate gland (Collin, M. A.; Clarke, T. H.; Ayoub, N. A.; Hayashi, C. Y. *Sci. Rep.* **2016**, *6*, 21589). Their data confirmed our results of ASG1 and showed that it was not aggregate gland or even silk gland specific and must have been a contaminant of the web when it was originally found.

Hayashi's group also reported a larger sequence for ASG2 which is what we predicted so we got permission from them to use their sequence to continue our studies. The figure below (Fig. 1) shows the figure from their paper with the sequences of the exomes they found and assembled. They found highly conserved repeat region (Fig. 1b), a linker region which appears to be similar to the repeats but now diverged substantially (Fig. 1c) and C-terminal non-repetitive region characteristic of spider silk protein genes.

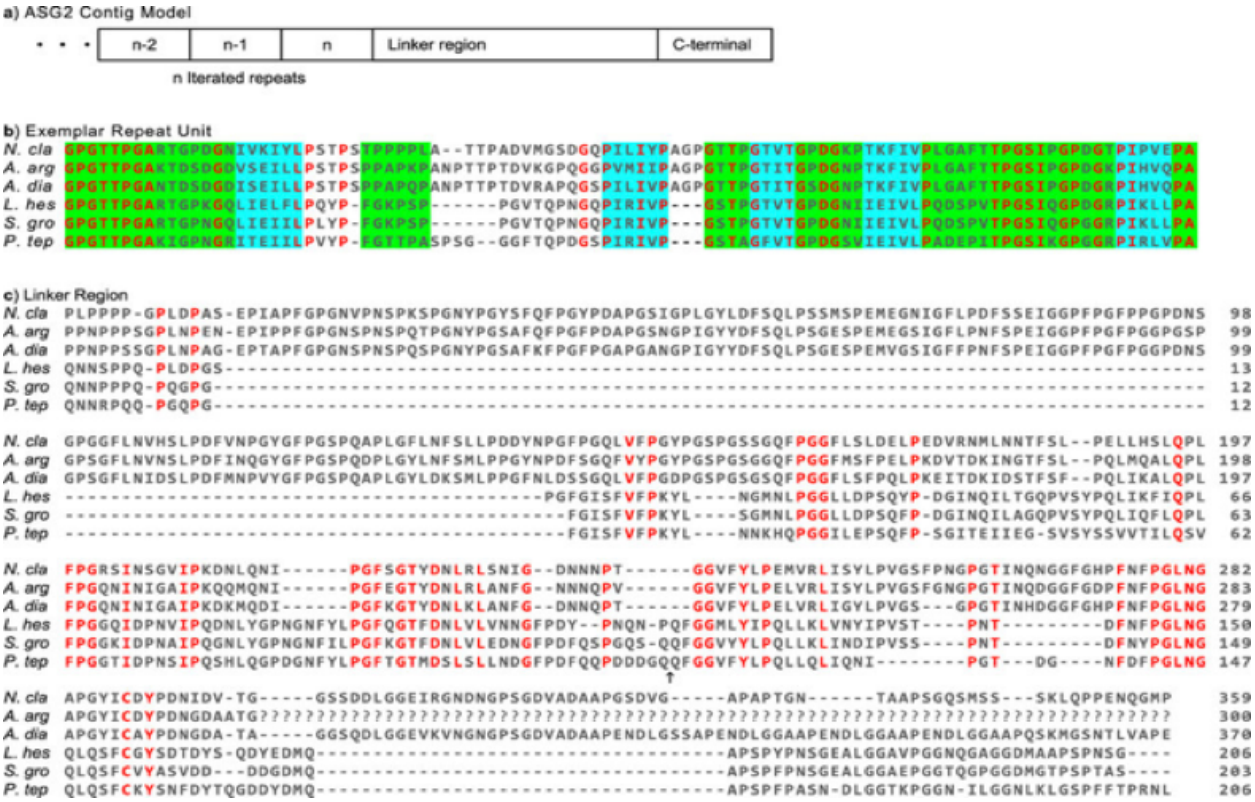

Figure 1. ASG2 sequences from several different species of spider.

2. Using the protein sequence from Hayashi's paper we created a gene with *E.coli* codon bias and had it synthesized. It was designed so that we could generate proteins with varying lengths of the repetitive region which will allow for determination of the role the length of this region plays in adhesion. As shown in Figure 2 below we made genes with 3, 6, 9 and 12 repeats. The successful genes are shown in the gels in that figure as well as the model for each. These genes were all sequenced at the ends to confirm the sequences were correct prior to using them for protein expression.

3. The genes were then used in flask fermentation expression studies to insure that protein of the correct size was being produced which it was. Then we moved to expression in a bioreactor to increase bacterial production and thus make enough protein to study. We chose one of the lengths (3X, roughly a 140kD protein) for initial studies. We were able to detect protein production in these fermentations but we saw very poor detection using our standard system with an antibody to the His tag we attach to all of our proteins to aid in purification. So a second antibody we have to the C-terminal non-repetitive region was used and showed good detection levels. So that antibody was used subsequently.

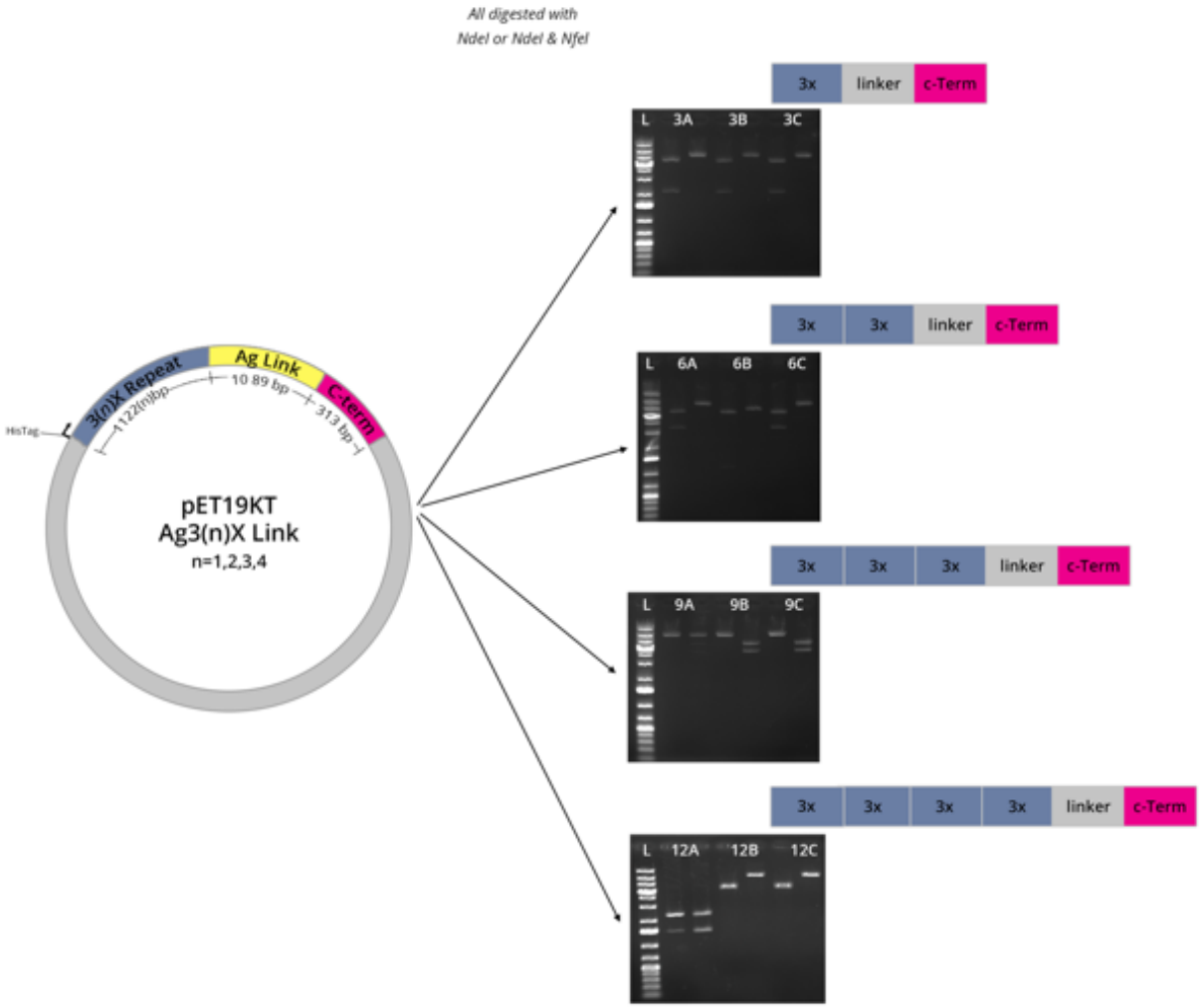

Figure 2. Gene constructs. On the left is the diagram of the vector and its parts. On the right at the gels showing the final complete vectors with the various size ASG2 protein genes in them.

Substantial effort was put into the purification procedures. In particular: 1) a variety of extraction buffers were used in order to extract the maximum amount of protein/g of cell pellet; 2) several precipitation conditions were also tested to achieve the highest ratio of spider silk protein to total protein left; 3) His tag affinity was tried but as noted above the His tag does not appear to be available on the protein surface so this was unsuccessful; and 4) finally an enthanol precipitation step was instituted to get the final protein product. The final product is shown in Figure 3 below.

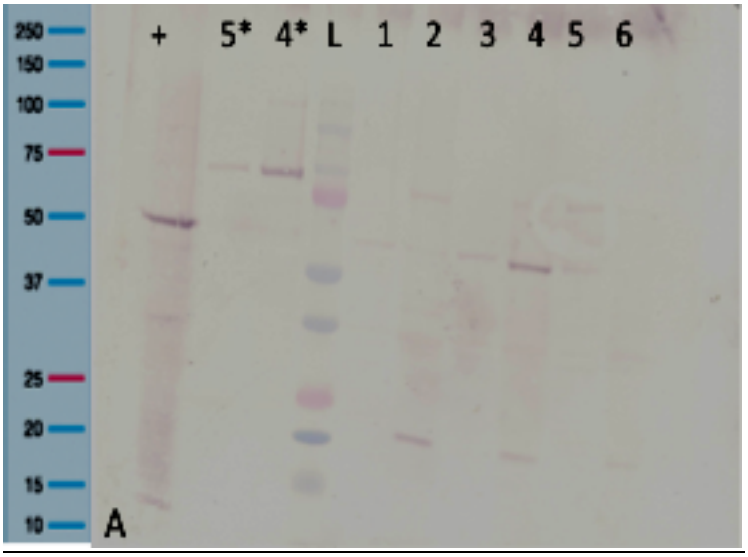

Figure 3. Western blot of the protein product of various purification protocols. Lane 4* is the final protocol used.

4. The purified protein was then used as an adhesive to glue polypropylene and polyurethane to each other (see Figure 4 below). The overlap testing method was used

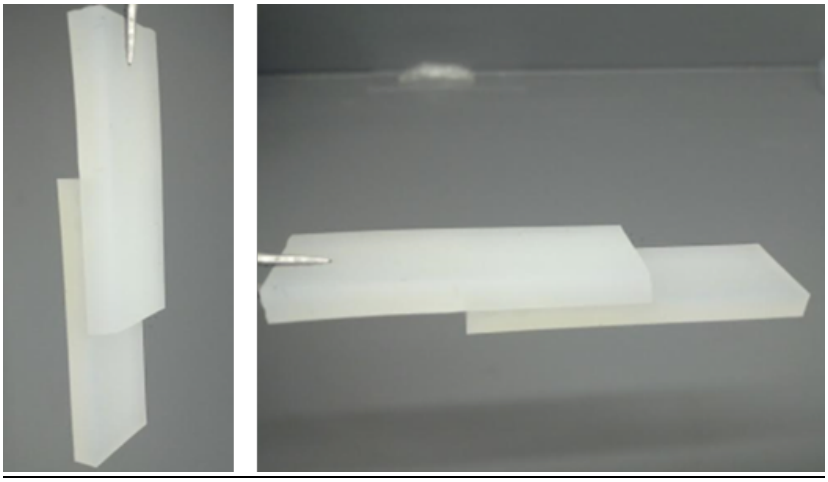

Figure 4. Examples of the overlap testing materials used.

as the best initial method. The results were not impressive, for polyurethane the breaking force was 4-7 kPa and for polypropylene even less around 1.3kPa. This was not unexpected as the protein is much shorter than the natural proteins and in addition has no glycosylation which is believed to be a key to adherence.

The next step, based on data from 2016 was to shift expression to a system that will glycosylate the protein we are producing. The insect cell line Sf9 seemed the most logical choice as it will produce glycosylated proteins which can be readily purified. To that end we designed the linker DNA segments needed to transfer these protein genes to a baculovirus vector for infection of the Sf9 cells. We have already been growing those cells in our laboratory for another project and thus have both a ready supply as well as experience in handling them.

The gene vectors were generated prior to the end of the grant. We will be transforiming the Sf9 cells in early 2018. It is likely that we will need to modify our current purification protocol for the proteins produced by the Sf9 cells due to the glycosylation on the protein as well as new impurity proteins. We are hopeful that expression in this system will produce a protein in which the His tag is exposed in which case we can utilize the His affinity tag system to more easily purify the proteins. If not we have substantial experience in spider silk protein purifications.

The proteins will be used for adhesion testing as well as biophysical studies.

Summary:

Although progress was not as rapid as expected we have laid an excellent foundation to move to the next steps to increase protein size and achieve glycosylated proteins to establish the ability of this protein in adherence applications.

Training:

One MS student Kyle Berg was on this project and he graduated this summer and will going to UCSD in their PhD neuroscience program. Another graduate student, Michaela Hugie has taken over the project. In addition 3 undergraduate students have worked on this project.

Dissemination:

One poster was presented by Kyle at the Materials Research Society meeting in Phoenix in March 2016.

Honors:

I was selected as the Undergraduate Research Mentor of the Year in 2016.

Tech Transfer:

A group based in our laboratory was awarded an NSF I-corps award in 2016-17. That effort was focused on adhesives and coatings with the adhesives area being very price driven in the commercial sector but less so in the medical/dental arena. We have been

working with two companies who are interested in novel adhesives but further work will be required the production of the proteins.