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Mechanical Properties of Natural *Osmia lignaria* Silk and Recombinant Expression of the Silk Proteins

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MECHANICAL PROPERTIES OF NATURAL OSMIA LIGNARIA SILK AND RECOMBINANT EXPRESSION OF THE SILK PROTEINS

by

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Capstone submitted in partial fulfillment of the requirements for graduation with

University Honors

with a major in
Biochemistry

in the Department of Chemistry and Biochemistry

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Abstract:

The use of petroleum-based plastic and the subsequent waste has led researchers to explore alternatives in the form of biopolymers. Spider silk and hagfish intermediate filaments show the potential to be advantageously utilized in textile and biomedical industries. Bee silk is a sparsely studied biopolymer that has shown potential to be added to these other impressive biopolymers. *Osmia lignaria,* is a solitary bee native to the western United States. Silk from these bees can be isolated while the larvae are spinning their silk. The silk from these bees has been mechanically tested for strength, stress, strain, and elasticity. Cocoon fragments were also analyzed for the amino acid composition. Comparing potential sequences to known bee silk protein sequences, the *O. lignaria* silk proteins were identified. DNA constructs were ordered, cloned, digested, and finally ligated into high expression vector pET-19k. After transformation into *Escherichia coli* BL21 (DE3) cells, expression was performed. Through a Coomassie Blue staining and western blot, no protein could be observed.

Acknowledgements:

I want to recognize and express my immense gratitude for my Honors mentor and research professor Dr. Justin Jones, as well as Oran Wasserman, Jayden Turner, Paula Oliveira for their assistance in my research. I thank the Utah State University Bee lab for providing *Osmia lignaria* larvae for the research. I want to recognize the office of research and Utah State University's Honors program for providing me with my URCO grant. Finally, I want to thank Aubrey Hampton for her help in editing.

Final Product:

Introduction:

Naturally and recombinantly occurring biopolymers, such as spider silk, silk from silkworms, and hagfish intermediate filaments, have been investigated for applications in industry to replace petroleum-based plastics (Wasserman et al., 2022)(Morley et al., 2022). In 2020, it was estimated that there was a total of 5 billion tons of petroleum-based plastics in landfills or in the environment as waste, and by 2050, an estimated 34 billion tons of plastics will be in the world (Mazhandu et al., 2020). The decomposition of petroleum-based plastics can take hundreds of years, leading to a waste management problem as well as many other potential issues like food contamination (Mazhandu et al., 2020). Biopolymers can be incorporated into the textile industry in bulletproof vests, clothing, and parachute chords (Lewis, 1996)(Maheshwari & Chopda, 2018). Biopolymers can also be incorporated into the biomedical industry in replacement ligament engineering, hypoallergenic sutures, and retinal membranes (Morley et al. 2022)(Harris et al., 2019)(Farinelli et al., 2019). The major challenge is finding a fibrous protein with good mechanical properties (that can be utilized in different applications) and can be produced at high yields (Oliveira et al., 2021). Recombinant production of naturally occurring biopolymers or naturally occurring fibrous proteins has been investigated due to their high production yield, making the use of recombinant biopolymers much more efficient (Olivera et al., 2021). There have been several studies on hagfish specifically, two proteins they produce that can be made into a synthetic fiber that has displayed similar strength to spider dragline silk (Olivera et al., 2021)(Wasserman et al., 2022). These hagfish proteins have been isolated by identifying the gene sequencing that encodes for the proteins, inserting them into a plasmid, and then expressing the gene in *Escherichia coli* (Oliveira et al., 2021). The resultant proteins from this process have proven to be more reasonable to reproduce efficiently due to the proteins being much smaller than spider silk (~65 kDa compared to >300 kDa) (Oliveira et al., 2021). The proteins also have less repetition of protein domains and less reliability on glycine and alanine (Oliveira et al., 2021).

In the process of searching for a biomaterial suited for synthetic production, a group based in Australia headed by Dr. Tara Sutherland has been studying silk produced by honeybees, specifically the species *Apis mellifera* (Maitip et al., 2015)(Wittmer et al., 2011). Their research has shown that the *A. mellifera* fibroin proteins can be used to produce high production yields of similar products as spider silk and hagfish fibers that can be used for textile and biomedical applications (Wittmer et al., 2011). The silk fibers produced by *A. mellifera* are composed of four proteins in coil-coiled formation (Maitip et al., 2015). These proteins have a lower molecular weight (30-40 kDa), less reliability on glycine and alanine, and a low repetitive sequence, allowing for a protein that is more balanced in amino acids (Morley et al., 2022)(Wittmer et al., 2011). The natural fibers from *A. mellifera* have shown an extensibility of 204% with a reduced tensile strength of 132 MPa when compared to spider silk and hagfish fibers (Maitip et al., 2015)(Wittmer et al., 2011). All four bee proteins can be reproduced recombinantly with ease, and have shown an increased tensile strength (strain and stress) compared to the natural fibers (Maitip et al., 2015).

Figure 1: Photo of *Osmia lignaria*, By Chelsey Ritner of Identification Technology Program

While extensive research has been done on social bee species such as A. *mellifera*, solitary bee species such as *Osmia lignaria* silk mechanical properties and suitability for recombinant expression remain unexplored. There may potentially be advantageous differences in the silk of a solitary

bee due to the different host environments of the cocoon. *O. lignaria*, a species native to the western United States, produces silk during development and has not been widely explored. The availability of *O. lignaria* and its silk-producing ability make it a prime subject of study. This research will work to bridge the knowledge gap of bee silk by exploring the physical and mechanical properties of the natural fibers, and the recombinant ability of *O. lignaria's* silk proteins and its ability to be spun into a fiber. This will result in another potential biopolymer that could help replace petroleum-based plastics in various uses.

Methods:

Natural Fibers:

Isolating fibroins proved to be quite difficult. Utah State University's Bee lab provided me with 24 pupating cocoons to isolate fibers from. With these cocoons I worked to isolate fibers under a microscope from the formed cocoon. The cocoons were first placed in a -80 ºC freezer overnight to euthanize the bees. Following this, the cocoons were cut open, and the bees were removed prior to fiber isolation. Using a light microscope to visualize the bees, the cocoons would be held in place by a pair of tweezers while another pair of tweezers would pull on individual strands to isolate them. Many fibers were collected through this process, but most of them proved too short or broke before they could be glued onto a plastic C-card. The fibers that were successfully isolated were then visualized under a digital light microscope to measure the diameter of the fibers under 400x magnification. The fibers were then mechanically tested in three groups: 0 hours after production, 24 hours after production, and 48 hours after production. These fibers were then mechanically tested for stress, strain, elasticity, and toughness. These measurements were collected using an MTS machine. The fibers were loaded into the machine and were clamped by both ends of the machine. The machine collected data as it pulled on the fiber until it eventually broke. Very few data points could be collected this way and so another method was created to isolate fibers.

The new method required the larvae to have not formed cocoons yet. The bee lab at Utah State University provided me with 48 larvae that were near their pupation stage. Similar to the previous method, the silk was collected using tweezers and a light microscope. However, the silk was collected as the larvae were forming the silk. After the cocoon had begun being formed, silk would be collected by putting the tweezers to the mouth of the larvae (near the secretion point of the larvae) so that the larvae would attach the silk to the tweezers. The silk was secreted as a hydrogel that quickly hardened when exposed to the air. Once the hydrogel-silk was attached to the tweezers, I gently pulled away from the larvae, extending the fiber until it eventually broke, or the larvae would stop spinning. This mimicked the extension that the larvae would normally perform when pulling the silk from one part of the cocoon. These fibers would be cut to fit onto C-cards and then mounted by taping them down and gluing them to the card. The fibers were then measured for diameter and were mechanically tested the same way as the first method.

Recombinantly Expressed Proteins:

The *O. lignaria* silk proteins were identified using the amino acid sequences that encode for the silk proteins for the honeybee *A. mellifera.* The *A. mellifera* sequences were plugged into the NCBI database to look for similar sequences in *O. lignaria.* Four sequences came up that I called OligF1, OligF2, OligF3, and OligF4, which is short for *Osmia lignaria* fiber protein 1-4. AlphaFold 2 was used to predict the tertiary structure of the four *O. lignaria* proteins. This rendering compared the predicted structure to that of other known silk proteins. The suspected sequences also were confirmed through amino acid analysis. Silk samples from natural *O. lignaria* cocoons were shipped to the University of California-Davis for analysis. Once the results returned, the compositions were compared to the composition of amino acids of the combined four OligF protein compositions. The four OligF proteins were combined because they would imitate the composition of the natural silk. The collected composition and the calculated

composition of amino acids were then compared to each other as well as to the protein composition of *A. mellifera.* This was done to confirm that the amino acid compositions were the same indicating the identified sequences encoded for the silk proteins.

Once the gene sequences were confirmed to be the silk protein sequences, through the previous steps, constructs for the four genes were designed. The constructs were designed within a PMK plasmid and were designed to include the restriction site NdeI immediately upstream of the gene and the restriction site BamHI immediately downstream from the gene. The construct was also codon optimized for the organism *Escherichia coli* in preparation for cloning and expression.

Each of the four constructs were hydrated and then transformed into “One Shot Top10” *E. coli* cells for cloning. This was done via heat shock at 42 ºC for 30 seconds. SOC broth was added to the cells and incubated in a shaker for an hour at 37 ºC and 220 RPM and to help them recover from the heat shock. Then the cells were spread onto (1 µL/mL) kanamycin-LB agar plates and incubated at 37 ºC for 16-18 hours. A few cells were collected from each plate and added to 10 mL of (1 µL/mL) kanamycin-LB broth and incubated overnight for 16-18 hours at 37 ºC. The plasmids were then isolated from the cells using an IBI Scientific High-Speed Plasmid Mini Kit. Concentration of the DNA was then determined by UV-visualization spectrophotometry on a NanoDrop 2000 spectrophotometer.

Sample	OligF1	OligF3	pET-19k		OligF2	OligF4	pET-19k
DNA	3.1 µL	2.5 µL	5.9 µL		2.6 µL	3.1 µL	6.0 µL
10x rCutSmart*	5.0 µL	5.0 µL	5.0 µL		5.0 µL	5.0 µL	5.0 µL
NdeI*	1.0 µL	1.0 µL	1.0 µL		2.0 µL	2.0 µL	2.0 µL
BamHI-HF*	1.0 µL	1.0 µL	1.0 µL		2.0 µL	2.0 µL	2.0 µL
ddH_2O*	39.9 µL	40.5 µL	37.1 µL		38.4 µL	37.9 µL	35.0 µL

Table 1: The components making up the PMK + insert as well as the pET-19k digestion. All ingredients with an asterisk were combined in a master mix prior to DNA addition.

Each of the plasmids was digested (~1 µg) as well as pET-19k (~1 µg) using NdeI and BamHI-HF (high fidelity) in preparation to ligate the OligF insert into pET-19k. Samples were digested two at a time along with a pET-19k sample according to the makeup seen in Table 1. The plasmid pET-19k is a high expression plasmid containing a Histidine 10x tag, kanamycin resistance, ribosomal binding site, and a *lac* operon (Oliveira et al., 2021) (Morley et al., 2022). These components allow the plasmid to be a good expression vector for the OligF proteins. The solutions were then placed into a 37 ºC water bath for 1 hour. Electrophoresis was performed by adding the samples to a 10% agarose gel with Bulldog TAE dye added and letting it run. The electrophoresis allowed the samples to be separated and isolated. The bands were visualized under a UV light and the OligF and pET-19k bands were excised from the gel. All bands were combined for each unique sample. Every sample had a clean-up performed using the Promega Wizard SVGel and PCR Clean Up kit and protocol, using ddH_2O as the final eluent. This was done to isolate the DNA from the gel. UV-visualization was performed on each sample and calculations were performed to determine the necessary concentrations and volumes for a 1:3 mixture of pET-19k plasmid to OligF insert. For samples that were too low of a concentration, a condenser was used to concentrate the samples to a usable concentration.

Ligation was performed according to the recipe indicated in Table 2 with a 1:3 ratio of plasmid to insert. Quick ligase is added last to start the reaction. The reaction was allowed to run for 5 minutes at room temperature (25 ºC), and then 2 µL of ligation solution was transformed into BL21 (DE3) competent *E. coli* cells via heat shock. The transformation was performed using the NEB protocol "Transformation Protocol for BL21(DE3) Competent Cells (C2527)" (Biolabs). Then the 300 µL final volume was added evenly to 2 (1 µL/mL) kanamycin-LB agar plates. This was done for each sample totaling 8 plates intended for sequencing and expression. Plates were grown for 16-18 hours at 37 ºC in an incubator.

Sample	pET-19k	Insert	2x Buffer	Quick Ligase	ddH_2O
OligF1	3.7 µL	4.2 µL	10 µL	1 µL	1.1 µL
OligF2	3.7 µL	2.4 µL	10 µL	1 µL	2.9 µL
OligF3	3.7 µL	2.1 µL	10 µL	1 µL	3.2 µL
OligF4	3.7 µL	3.0 µL	10 µL	1 µL	2.3 µL

Table 2: The components making up the pET-19k + insert ligation.

A single colony from each plate was then added to 3 mL of (1 µL/mL) kanamycin-LB broth. The inoculated media was then placed in a shaker at 37 ºC and 220 RPM for 16-18 hours. This was done 8 times for each of the four plasmids. Following the overnight, a miniprep was performed on each of the samples to isolate the plasmids from the cells. Digestion was performed on the mini-prep samples using the ingredients in Table 3. This digestion was done to confirm successful ligation of the OligF insert and in preparation for Sanger sequencing. A master mix was used for all ingredients except for the DNA. Digested samples were mixed with 4 µL of loading dye, and then the whole sample was added to a 1% agarose gel with Bulldog TAE dye added. The gel was run at 100 V until the samples were about three-quarters of the way through the gel. The gels were imaged under a UV light. The samples were individually excised from the gel. A clean up procedure was performed on each sample using ddH_2O as the final eluent. Four positive samples for each OligF were sent for Sanger sequencing at the Yin lab at Utah State University (15 µL each). Each of the sequences was aligned and compared with the original construct sequence to determine which samples resulted in a positive clone with no mutations.

Ingredient	Volume
DNA	5 µL
NdeI*	0.4 µL
BamHI-HF*	0.2 µL
10x rCutSmart Buffer*	2 µL
ddH_2O*	12.4 µL

Table 3: The components making up the pET-19k + insert digestion. All ingredients with an asterisk were combined in a master mix prior to DNA addition.

A (1 µL/mL) kanamycin-LB spread plate was made for samples OligF1-2, OligF2-1, OligF3-4, and OligF4-2 incubating the plate at 37 ºC for 16-18 hours. Half of each spread plate was then scraped into 100 mL of a (1 µL/mL) kanamycin-LB broth. The flasks were then placed into a 37 ºC shaker rotating at 220 RPM. Cells were grown until the optical density reached 0.6 when the cells were induced for 4 hours. Induction was performed by adding 100 µL of IPTG to each flask. 5 mL samples were taken every hour, and at hour 4 the remaining sample was collected.

The cells were then lysed by pelleting 5 mL of each sample (hours 0-4) and discarding the supernatant, then the cells were resuspended into a lysis buffer (50 nM Tris and 200 mM NaCl). Using microtip sonication, the cells were lysed at 5 V for 15 seconds until they were the consistency of water. The lysate was then centrifuged at 1,000 G for 10 minutes; the supernatant was collected as the soluble fraction. The pellet was then resuspended in 250 µL of 8 M urea. The samples were centrifuged again at 1,600 G for 5 minutes; the supernatant was collected as the insoluble fraction. Each of the samples then had 50 µL of solution added to 50

µL of 2x sample buffer + β-mercaptoethanol (19:1). The samples were then incubated at 100 ºC for 5 minutes. Then samples were centrifuged for 30 seconds at 1,600 G. Then 20 µL of each sample was run on an SDS-PAGE (Tris-glycine) gel at 90 V for the 40% gel and 110 V for the 20% gel. The gel was then stained with Coomassie Blue stain to visualize the proteins. A western blot was also run utilizing the 10x His tag to visualize the OligF proteins using chemiluminescence antibodies.

Results:

Natural fibers:

There was a total of 67 fibers collected between the three groups of mechanical testing (0 hour, 24 hour, and 48 hour). The diameter, ultimate tensile strength, energy to break, elastic modulus, and ultimate strain are seen in Table 4. These data points can be compared to *A. mellifera* fibers which measure 9 µm in diameter, have a maximum strength of 132 MPa, energy to break of 400 MPa, and an ultimate strain of 0.4 MPa (Weisman et. al., 2010).

Test Group	Diameter (µm)		Ultimate Tensile Strength (MPa)		Energy to Break (MJ/m^3)		Elastic Modulus (GPa)		Ultimate Strain (mm/mm)	
	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
0 hr	7.07	2.24	67.45	35.11	7.67	8.71	6.59	5.32	0.18	0.12
24 hr	4.54	0.91	95.03	37.51	15.30	10.37	8.17	5.72	0.29	0.11
48 hr	5.22	1.68	94.06	31.34	12.34	8.84	8.74	5.29	0.22	0.16

Table 4. The measurements and mechanical test result means and standard deviations for the natural *O. lignaria* fibers measured at 0 hours, 24 hours, and 48 hours after spinning.

Recombinant proteins:

The amino acid composition was compared to the amino acid composition of the honeybee *A. mellifera.* The sequences that were hypothesized to be fiber protein encoding sequences were also compared to the amino acid composition of the native silk, and both matched up well. Compared to the *A. mellifera* composition, the Ala and Gly compositions ranged outside of the error bars. However, Ala and Gly compositions were added together and were found to be extremely similar to that of the *O. lignaria* natural silk composition, as seen in Figure 2. The AlphaFold2 rendering of the hypothesized sequences can be seen in Figure 3. The sequences hypothesized can be seen in Appendix 1.

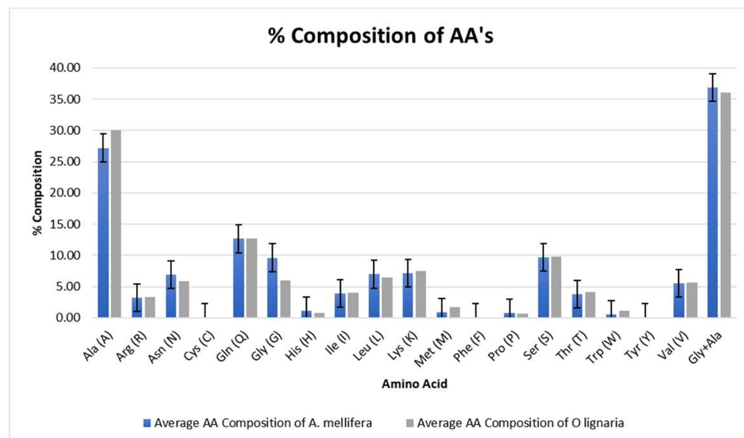

Figure 2: Average amino acid composition comparison between *O. lignaria* and *A. mellifera* showing similar amino acid composition of silk.

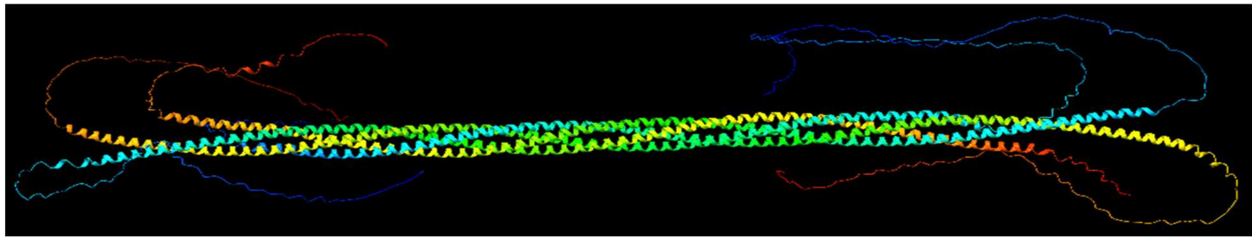

Figure 3: An AlphaFold2 rendering of the 4 *O. lignaria* silk proteins.

Following the digestion of OligF1-4 and the PMK plasmid, the electrophoresis showed bands in every lane. Figure 4 shows the electrophoresis gel for the digestion of the pET-19k + OligF1 ligation samples. The figure shows two bands, one near 5,000 bp and one near 1,000 bp. OligF2, OligF3, and OligF4 all show bands near 5,000 bp and 1,000 bp. All except OligF1-1 showed two bands in each of the lanes.

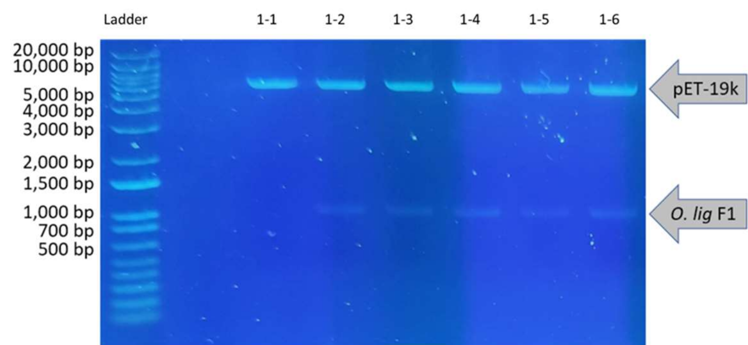

Figure 4: Electrophoresis gel following digestion of the OligF1 plasmid. 1-2 – 1-6 show both pET-19k (5,776 bp) and the insert (1,014 bp)

Only 4 samples of each OligF were sent for sequencing. OligF1 insert sequence ranged from base pair 133-1227. OligF1-2, 1-3, 1-4, and 1-5 showed full sequences. OligF2 insert sequence ran from base pair 133-1233. OligF2-1 and 2-2 showed full sequences. OligF2-3 and 2-6 did not show full sequences. OligF3 insert sequence ranged from base pair 133-1176. OligF3-4, 3-5, 3-6 showed full sequences. OligF3-7 did not show the full sequence. OligF4 insert sequence ranged from base pair 133-1284. There were a few issues with the samples so only sample OligF4-2 showed a full sequence. Samples that showed full sequences were used moving forward for expression.

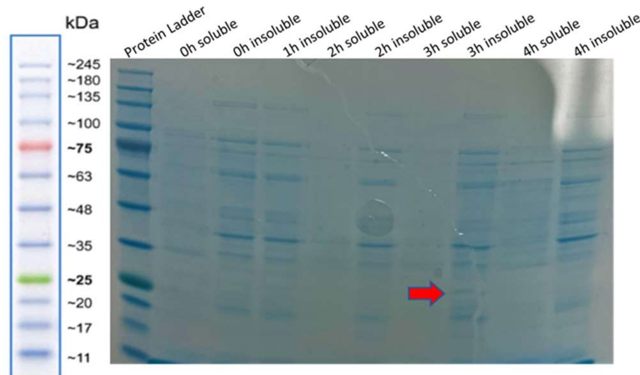

Figure 5: SDS-PAGE gel stained with Coomassie blue showing a protein at ~31 kDa, near same size as the predicted OligF1 protein.

To this point in the experiment, only expression for OligF1 and OligF2 have been run. During expression the flask containing OligF2 got cracked between hour 1 and hour 2. This led to a cessation of OligF2 expression until a future point. On the other hand, OligF1 ran a full expression. The SDS-PAGE gel can be seen in Figure 5. A single band can be seen near 31 kDa in the 3-hour insoluble fraction. However, this band cannot be seen in any other fraction. The subsequent western blot showed no coloration on any fraction

Discussion:

The measurements and mechanical tests of the *O. lignaria* fibers were taken to measure a baseline to compare the recombinant fibers to once fibers can be spun. Comparing the data to that of *A. mellifera,* the fibers showed no significant difference in fiber diameter. However, there was a lower ultimate strength and energy to break and a higher ultimate strain. The reasoning

for these differences is currently unknown. Due to the higher Ala and lower Gly composition, it would be reasonable to conclude that there would be a higher alpha helix content in the *O. lignaria* fibers. However, the lower tensile strength and higher elasticity do not support this theory.

By performing the amino acid analysis and comparing the composition of the natural fiber to the composition of the hypothesized sequence, I could see that there was a near-identical match of the amino acid composition. This was further confirmed by the comparison of the *O. lignaria* composition to that of *A. mellifera* as well as the AlphaFold2 rendering. The *A. mellifera* comparison shows that the *O. lignaria* sequences hypothesized contain very similar amino acid composition to that of a known bee silk. The Alphafold2 rendering (Figure 3) depicts all four OligF proteins, with high alpha helix secondary structure, intertwined to form a coil-coiled fibroin. It is also interesting to note that each protein is staggered, giving an opportunity to build onto the fibroin, extending the fibroin into a longer fiber. With this information, I felt confident that the identified sequences did encode for the *O. lignaria* silk proteins.

Following the successful cloning and ligation as indicated by the electrophoresis gels, Sanger sequencing showed that there were no mutations in at least one of the samples for each of the OligF protein. The expression however, does not show as successful of results so far. The OligF1 protein should have been expressed in the insoluble fraction, similar to hagfish proteins expressed recombinantly (Olivera et al., 2021). The SDS-PAGE gel shows only one band near the expected 33.7 kDa molecular weight of OligF1. This band is only found in the 3-hour insoluble fraction. Because the band disappears in the 4-hour fraction, I do not have strong confidence that this is the OligF1 protein. I would expect expression to go up over time, not to appear at 3 hours and then disappear. The band seen in the 3-hour insoluble fraction also appears to be lower, closer to 30 kDa. This was confirmed in the western blot with no protein visualized by the 10x His antibody stain. Moving forward, I plan to express OligF2 again and OligF3 and OligF4 using the same protocol. Upon successful expression, I will do an expression in a continuous culture fermentation tank, develop a purification protocol for the protein, and then spin the purified protein into fibers that can be measured and mechanically tested. If expression continues to yield no protein, I will attempt expression in a different cell line, try a different expression time, and attempt expression at a different temperature to see the optimal expression conditions for the *O. lignaria* silk proteins.

Reflection:

I began my college career in the Honors program with the goal to graduate, go on to medical school and become a doctor. Those goals haven't really changed, but I have. My experience through classes, in the honors program, involvement in research, studying, and interacting with other students and professionals, I have changed. I feel that my experiences throughout college have helped me become someone who is passionate about the world. I have developed a passion for science, medicine, the last lecture, and ultimately, for learning. Throughout my journey I may not have changed my goals, but my purpose of those goals, the reason why I do things, and my perspective have changed. I now view the journey as more important than the end goal. I think less about how I can quickly accomplish my goals and more about the legacy I build along the way. I think about the lives around me, the difference I can make, the knowledge I can learn, and the relationships can make.

Towards the end of my freshman year I got involved in research with Dr. Justin Jones in the biology department. I had always enjoyed science and completing experiments, so in preparation for medical school I wanted to get involved in research. Out of all the different areas of research I was fascinated by the subject of artificially made spider silk. I was fortunate enough to be offered an undergraduate research position in the lab working with two graduate students on their projects. To this day I am so thankful for Dr. Jones and his willingness to accept me onto his team. I also am so thankful for his supportive nature because after a year of

working in the lab, one of my graduate lab partners and I developed the idea that is now my Honors capstone project. Dr. Jones did the opposite of clipping our wings; he encouraged us to do the project, giving us ideas and helping us along the way. For this I am so grateful for him and his impact on my life. I feel interactions like this are largely what Honors is all about. I not only have learned about mentor-mentee relationships and professional development, but I have learned, through Dr. Jones' example, about being a leader and lifting others to reach their full potential. I learned from experience how helpful, and meaningful, it is to have a mentor/leader that has an open door policy and is willing to answer any question that you come with. These same traits can easily be seen in my graduate lab partners as well. They not only trained me and helped me to learn how a lab works, but they did so with such patience and made me feel like an equal. These are traits I aim to emulate myself because of the examples provided by these amazing people.

After extensive research and hours of reading research papers (including on vacation which led to harassment from my brothers), I had developed my project. I then applied for and received Utah State's Undergraduate Research and Creative Opportunity (URCO) grant. This allowed me to begin my project on the recombinant expression of *Osmia lignaria* silk proteins. I learned a lot about the scientific process and the more technical points, like grant writing and background research. Very quickly we realized that we would need to include research on the native fibers as well. Through the USU bee lab, we got cocoons that we could isolate fibers from. After 3 months of tedious isolation, two data points were obtained. It was painful to see months of work disappear in less than an hour. This part of the experiment gave us insight as to a more effective way to isolate *O. lignaria* fibers. It led to the hypothesis that over time these fibers would get brittle and break very easy. It led to understanding how the fibers are made by the bee first as a hydrogel like product that hardens into a fiber. The most important thing I learned was how exciting it felt to have success after such a discouraging defeat. I had learned growing up that hard work is rewarding. However, it is harder to have the opportunity to work for months just to seemingly fail, and then later succeed through perseverance. This experience allowed me to begin thinking critically and to problem solve myself. New plans to isolate the natural fibers were made and the following spring I was able to isolate four times as many fibers in a matter of two days. I cannot begin to describe the excitement I felt. I remember jumping up and down explaining it to my friends.

I had no idea how many doors this capstone would open for me. Through poster presentations I met people from all around campus that would be interested in what I was doing and I would be able to learn things about their research and discipline as well. I learned how to become a good presenter. I learned how to share my ideas with others who have different levels of understanding about a subject. Because of my research and this project, I was given the opportunity to present to the incoming freshmen at a "Science United" conference and share my passion for research with them. I also was able to learn things in the research lab that helped me in my undergraduate classes such as biochemistry and microbiology. I felt so much more prepared in class when I not only knew about a topic, such as recombinant expression, but had performed it. I was able to help my classmates and lab partners understand what we were doing better on our assignments.

As I prepare to go to medical school I feel so much more prepared than I would have without these experiences. I feel that through the Honors program as well as my capstone project I am more prepared to persevere through challenges, to think outside the box and solve problems on my own, to dare to learn things, and to pay attention along the way. My capstone project was something that I planned to do whether it was for some sort of credit or not. Because of this I feel it truly does encapsulate the whole of my Honors and college experience. It touches every part of my journey because it became something that developed from blooming passions. It is something that dared me to push my boundaries and explore new things. I feel it is the perfect crowning jewel to place atop my college career. (Word Count: 1,095)

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Appendix:

Appendix 1.

OligF1
1 mkipallatc lfvwgsvsas dghhggkgip llemakggas itlsaavsak aglragqvae
61 asqkeaaiqs gaaageaaka rgladqtakl seqsamlqsq aaakskaaee aataeqnaql
121 earaiaaqat aaakeaasga rsaaeaasta aleaaiaaka vqvqaalslk skekameaan
181 kaaaasnaak aaaqanraav milakataak aearaamaal asakvaardg ansaaeaear
241 nevaqliaai dqksreidag lsmkaqaiak ssarnneaav iganidsakr ivkvpqkvii
301 pkhesswqss keekievdvk qvdvknvhse ssgswsh

OligF2

1 mkipailvts lltwglvgas idddhsmlsq kdghslekml lskvmtsamr hengapmlgl
61 gkkvhislgr tkasaaaeek aaglvkasam niadavvktt arsaalsaka aaavkqalml
121 qekaealaqa alevqteqla lssraeaags vaqaalqrtq gaaqvataaq nlasnyqerv
181 naaaaaeaaa teraleiadv sralgqiass lsaaaagaqa kttqsseata aeagraasla
241 sdadsaqqaa tneaqvaari egraareasa nsasqdtdaa qleasaaakt taaaaigdga
301 ivglgedgga gaqeivqaka lakasawlgk ggsskkgwe

OligF3

1 mkipallvts llvwglaegr vvgvenshgl ekssvssssi kvgtarvrgd asdagaisvq
61 dalnvvrage svglnvdlga aartaakaaa sqaadtenae agakaailma iskreeaikl
121 seiarqrltt aakaaealvs aarraaelta aakaatqasa ttaeaaaqaq vkanadsiia
181 kkaqaaeaka aaeaevkakm aanaaaqlla karlaakeea latklaaiaq vaiararnav
241 ekalsaqsga ttqssnavki egqaanaegt avgrlqtlla iiqtvaaaea daaskasswa
301 kqisssksdv qvkgwksgsv

OligF4

1 mkipallaas llvwglanad gsigqesnhg tvkkkqvqvq vrekkewnsg wdskssnvwd
61 sksstgwesq skgkavaieg aavgtgmaet aaasgeaian nlgtgeaaan sqasaatqsa
121 iaaqsagtas alsmdaanla anaagaqgka aaqsekamkl slatrnaamq aedivdkaka
181 aagraevlar naaaanaraa lqseranela qaedaaaaea qaktaaaava tkvalqlaqi
241 avkaeaaaas aaaaaakatr iadaaaaraa svnaiaqaev easaqaenta gvsqaaasas
301 aetmavaasa sataeaaaeg gaekgelslp ksskveiqsk kiittekvdk assgwd

Biography:
Jackson Morley has attended Utah State University as a first-generation student for the past four years, majoring in Biochemistry and minoring in Biology. His passion for academics led him to receive the Irving Condie Frost Award in Organic Chemistry, the 2023 George H and Billie Bush Emert Scholarship for Biochemistry as well as the College of Science Undergraduate Ruth L. Novack Scholarship. As an active researcher at Utah State University, he has participated in numerous scientific presentations and looks forward to continuing his research regarding bee silk after graduation. After completing his undergraduate degree Jackson intends on attending Medical school and pursuing a career in cardiothoracic surgery.