

# Recombinantly Expressed Protein Fibers from Hagfish and Honeybees

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## Introduction

The US Navy has contracted Dr. Justin Jones and his lab to work with hagfish slime proteins. They desire to use hagfish slime to stop incoming ships through nonviolent methods and keep both servicemen and US equipment safe. Hagfish are scavenging slime eels that live near the bottom of the ocean. When threatened they can eject a combination of slime and protein threads from their pores that expand in size roughly 1000x clogging the gills of its attacker and forcing them to release the hagfish<sup>1</sup>.

Hagfish produce two types of protein threads: the alpha and gamma proteins<sup>2</sup>. Through testing performed in the Spider Silk Lab at USU under the direction of Dr. Jones, we have found the gamma protein threads to be significantly “weaker” than the alpha recombinant silks (figure 1).

The goal of this research is to increase the gamma recombinant protein thread’s strength and production. This is being done by recombinantly expressing different sections of the protein (the N-terminal + C-terminal, N-terminal + gamma protein, gamma protein + C-terminal, and the gamma protein alone). The mechanical testing of these different combinations is underway, and data is not currently available. When combination with the greatest mechanical improvement is found, the next step will be researching how best to improve production rates.

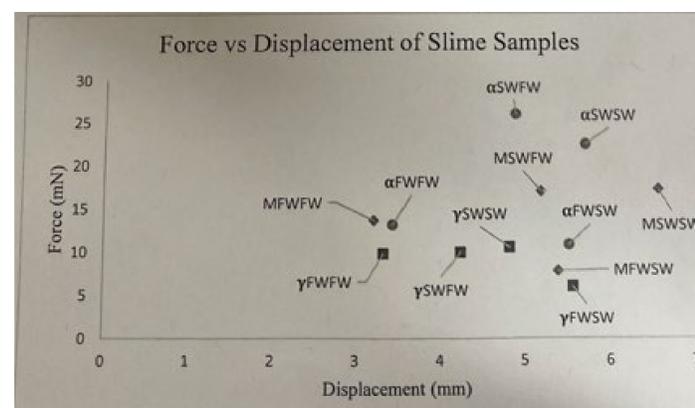
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## Methods

DNA of the proteins of interest was acquired. The DNA was put through a PCR reaction to increase the quantity of the DNA present. After that the solution was digested, cleaned up and quantified. Gel SDS was performed to ensure the correct DNA size was present and then the DNA was analyzed and compared to the correct sequence. Once evaluated and quantified, the DNA was transformed into replication cells of *E. coli*. The *E. coli* was cultured, and the cells were lysed to extract the DNA. The DNA was extracted, and returned to the previous procedure of analyzing, quantifying and purifying the extracted DNA to make sure it was transformed into *E. coli* correctly. Once the transformation was successful the DNA was then transferred into expression cells that produce significantly more proteins.

**Figure 1** – This graph depicts the force over displacement that different threads were exposed to. They were extruded and tested in combinations of freshwater (FW) and saltwater (SW) to better understand their properties in a water environment. Gamma proteins and mixes of gamma and alpha proteins (marked M) were not as readily able to handle the force that alpha proteins did. This can be seen by the difference in force levels along the Y-axis that gamma protein threads achieved.



## Upcoming Research Project

There are many other types of biological silks. One of the newer silks that the lab is beginning to research is honeybee silks. Honeybee larvae produce a shell around them made of a small tetrameric coil-coiled protein. Honeybee silk from *Apis mellifera* has previously been investigated via recombinant production and showed impressive production yields and great mechanical properties when spun into fibers<sup>3</sup>. Honeybee silk is roughly 30 kDa which makes protein expression in convenient vectors (like *E. coli*) much more feasible than other silks (such as the orb weaver major ampullate silk). However, since this silk has not been researched sufficiently by the scientific community, there is still a lot of work to be done before we understand this silk. Oran Wasserman has been working to start research under Dr. Jones that will analyze the silk of the *Apis Dorsata* honeybee in depth. Much of the effort put into research over the last two months by Oran and myself has been to get this project ready to research by summer of 2022. We anticipate that the silks will have similar compositions to hagfish and other biological silks and will attempt to replicate the production process in a similar manner to determine if they are a reliable source of biological recombinant protein silks.

## References:

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- [3] [8] Maitip, J., Trueman, H. E., Kaehler, B. D., Huttley, G. A., Chantawannakul, P., & Sutherland, T. D. (2015). Folding behavior of four silks of giant honey bee reflects the evolutionary conservation of aculeate silk proteins. *Insect biochemistry and molecular biology*, 59, 72-79.