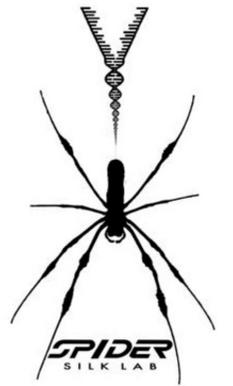




Elucidating the protein domains of recombinant hagfish alpha required for fiber formation.

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Introduction

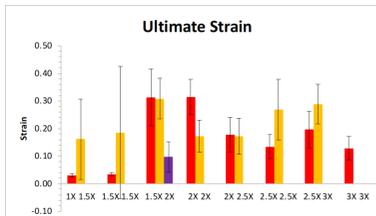
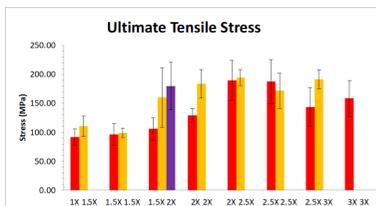
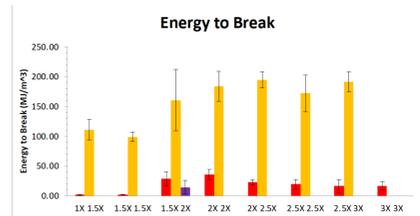
Hagfish intermediate filament proteins produced from a synthetic system show mechanical properties comparable to that of the native proteins. The success of small-scale expression of both the Alpha and Gamma subunits invites experimentation of the protein elements. In the natural system, the hagfish produces, transports, stores, and then excretes the proteins to produce their fibers. Whereas a synthetic system is not limited by these requirements and only needs to produce the protein in abundance. If removing one or both termini does not interfere with fiber formation, it may allow for a greater yield from synthetic systems, and open new applications of recombinant hagfish alpha proteins.

Hypothesis

Are the N and C termini of recombinant hagfish α required to form fibers with appreciable mechanical properties in a synthetic system?

Results: Alpha domain and Alpha with C-terminal

Recombinant Protein	Size	Purified Protein yield
HF Alpha	70 kDa	~ 8g/L
N-terminal and Alpha Domain	58 kDa	~ 14g/L
C-terminal and Alpha Domain	52 kDa	~6g/L
Alpha Domain	39 kDa	~12g/L
N-terminal and C-terminal	34 kDa	~1.7g/L



- Top left: The different recombinant variations of hagfish alpha, with size of the protein and yield from expressions
 - High lighted: fibers have been tested
- Top Right: Tensile stress experienced by the fibers before breaking
- Bottom right: amount of strain experienced by the fibers in percent elongated
- Bottom Left: Amount of energy required to break the fibers by stretching. (Stress * Strain)
 - Red: Alpha Domain with C-terminal
 - Yellow: Alpha Domain Alone
 - Purple: Full Hagfish Alpha Subunit

- Stretching the fibers before drying shows an increase in the amount of tensile stress the fibers can withstand before breaking
- Strain appears to increase with stretching, decreases and then increases again. Unique phenomenon to these fibers.
- The Alpha domain alone appears to show more tensile strength and requires more energy to break compared to the Alpha with C-terminal
- Alpha domain recombinant provided a higher yield than the hagfish alpha native recombinant protein

Conclusions to Date:

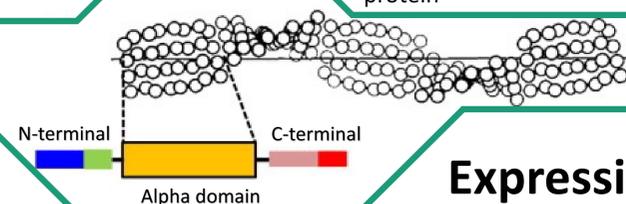
- Initial testing shows evidence that N and C terminal are not required for fibers to form.
 - However, further testing will elucidate the effect of the N and C termini on mechanical properties.
- The Alpha domain unit shows an increase in tensile strength compared to the Alpha C-terminal unit
- Smaller proteins result in greater quantities of purified protein (reducing cost of production).
- Results support the need for further testing of the Hagfish Alpha subunit

Past research

Bin Wang, Wen Yang, Joanna McKittrick, Marc André Meyers, Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration, Progress in Materials Science, Volume 76, 2016, Pages 229-318, ISSN 0079-6425

Brianne E. Bell, Isaac K. Burton, Jose Arreola-Patino, Thomas I. Harris, Paula Oliveira, Dong Chen, Randolph V. Lewis, Justin A. Jones, Scalable purification of recombinant structural proteins, hagfish intermediate filament α and γ , from inclusion bodies for fiber formation, Protein Expression and Purification, Volume 199, 2022, 106152, ISSN 1046-5928,

Paula E. Oliveira, Dong Chen, Brianne E. Bell, Thomas I. Harris, Caleb Walker, Haixia Zhang, Brittany Grob, Randolph V. Lewis, Justin A. Jones, The next generation of protein super-fibers: robust recombinant production and recovery of hagfish intermediate filament proteins with fiber spinning and mechanical-structural characterizations, Microbial Biotechnology, Microb. Biotechnol., Volume 14, Issue 5, 2021, Pages 1976–1989 ISSN 1751-7915



Expression and Fiber Spinning



- Protein variants were expressed in BL21DE3 in a 2 L Bio-flow reactor, expression was induced using IPTG
- After 4 hours of expression, the media and cells were centrifuged to recover the cells.
- Protein was purified through a series of wash buffers and centrifugal separations (inclusion body preparation).

- Purified protein was resuspended in formic acid in a 20% weight per volume solution (dope).
- The dope was extruded via syringe into a saltwater bath to produce the fibers

- Fibers were then wound through a stretching system (shown below), by varying the speeds, different stretch factors were produced
- Samples of the fibers were then measured and tested to determine their mechanical properties.

