

Large Scale Production of Spider Silk Protein in *E. coli*

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ABSTRACT

Spider silks have long been a focus of research due to their remarkable mechanical properties including strength, toughness and elasticity. Moreover, biodegradability and biocompatibility of spider silks make them beneficial to use in biomedical applications. Spiders cannot be farmed because of their territorial and cannibalistic nature. Hence, production of recombinant spider silks is the only feasible solution for large scale production

Large scale production is still challenging due to the small recombinant protein size, low yield and low water solubility of bio-synthetic spider silk. The current study reports our progress as well as evolution of effective protocol for large scale bio-synthetic production of spider silk protein in *E. coli*.

Our main spidersilk protein we seek to produce and purify in a large scale is MaSp2. Spider dragline silk is primarily composed of proteins called major ampullate spidroins (MaSps) that consist of a large repeat array flanked by nonrepetitive N- and C- terminal domains. All MaSp genes are co-expressed in the major ampullate gland of *Nephila clavipes*.

PROTEIN PRODUCTION USING BIOREACTORS

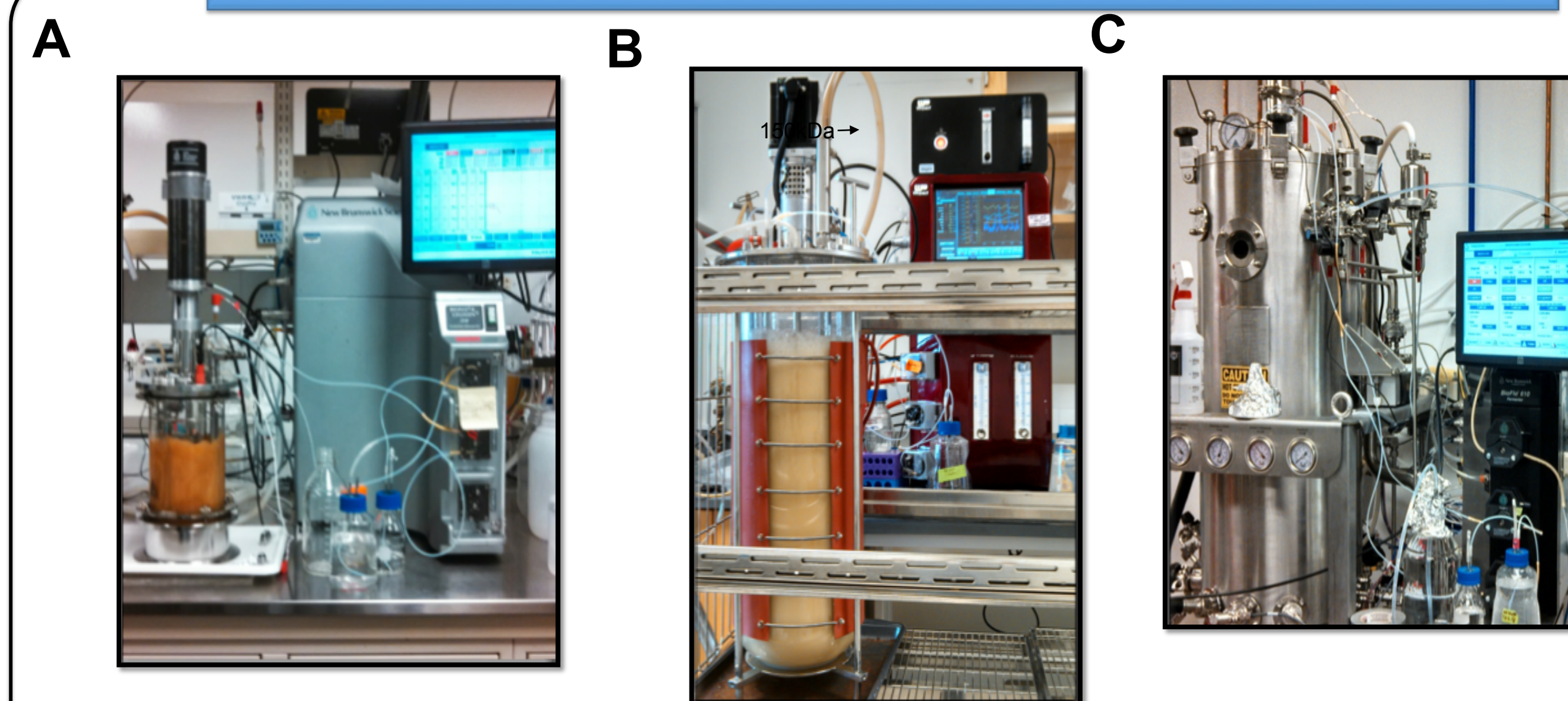
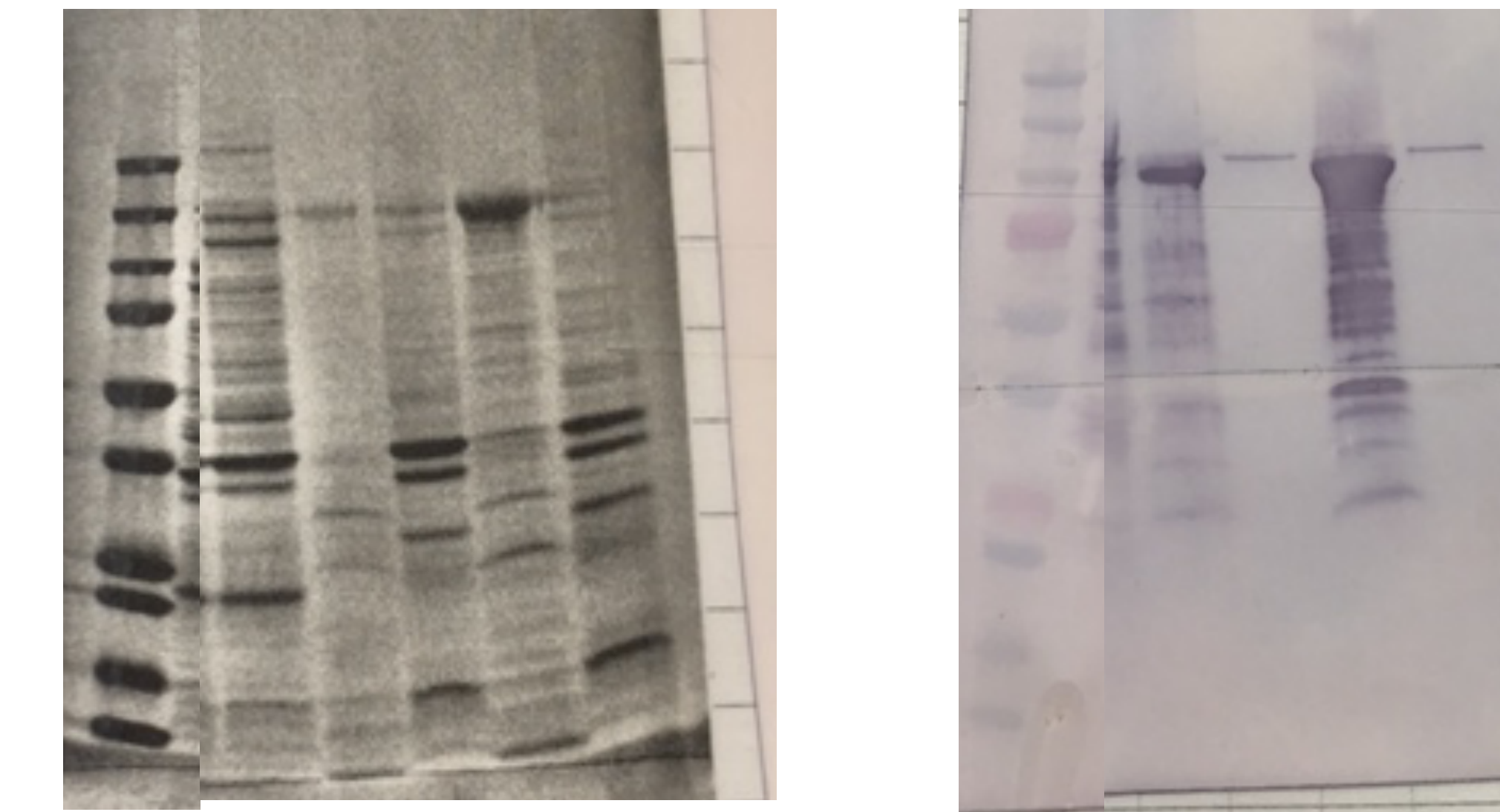
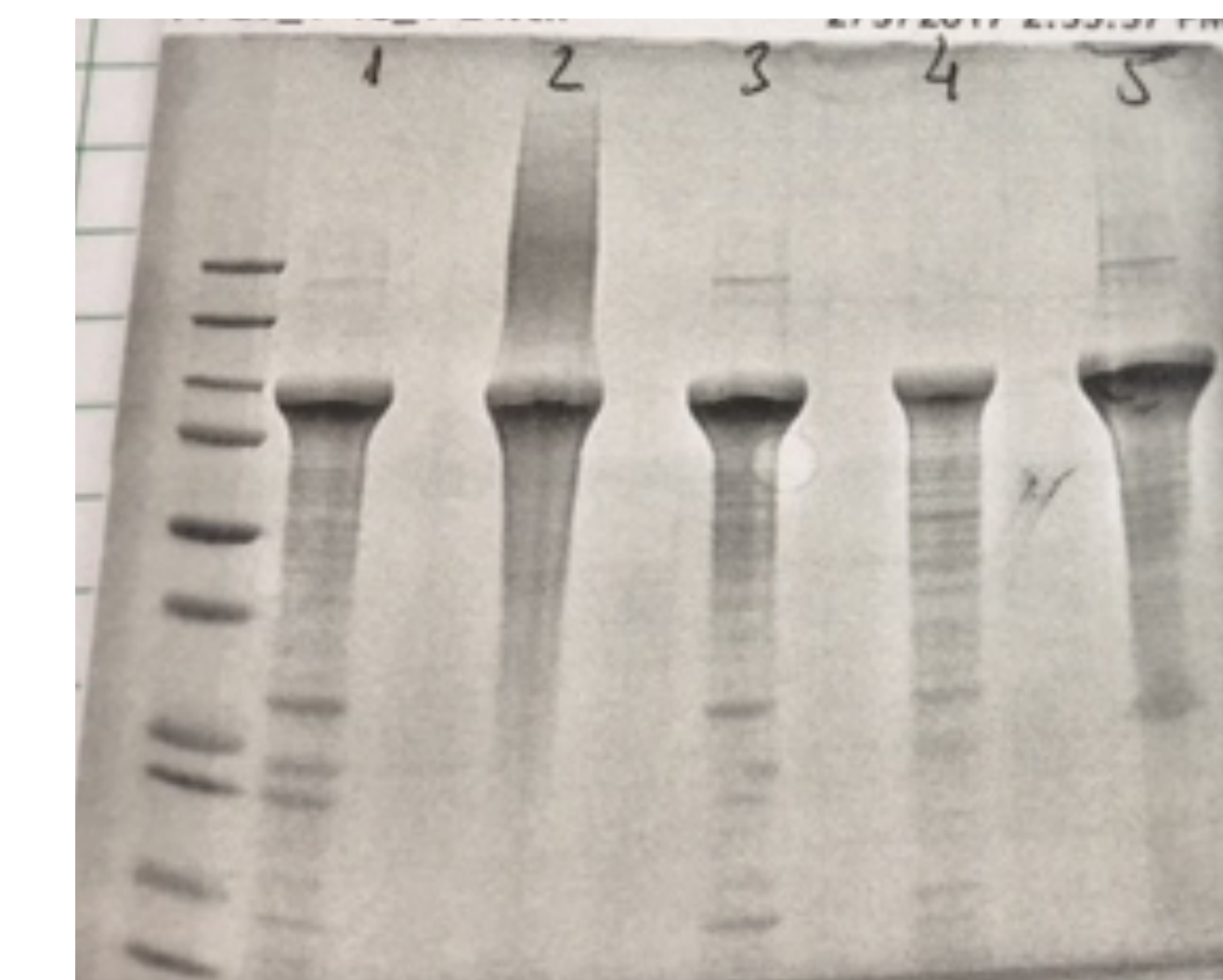


Figure 2. Production of spider silks at (A) 5L (B) 20L (C) 100L volumes. Note: 1L bottle to scale

RESULTS



Expression of MaSp2 24 pET19KT 28 AF_T_R before TFF
Lane 1 Ladder, Lane 3 supernatant before homogenization, Lane 4 Pellet before homogenization, Lane 5 Supernatant after homogenization, Lane 6 Pellet after homogenization
3/22/2017



Purification of MaSp2 32T Spider Silk Protein after use of Nickel Column.
Protein was washed with a specific concentration of imidazole to wash out proteins not containing His tag, Our protein (containing his tag) remained bound. Washed with higher concentration of imidazole (.5 M) collected fragments, prepared samples and ran western for analysis.

Gel Prepared 2/3/2017
1. 70L run 20161129 28AFT TFF
2. 20161129 28 AFT Parr Vessel and TFF
3. 60L run 20170118 28 AFTT TFF
4. 64L run 20170125 28 AFT TFF
5. 20170125 Parr Vessel
The Parr vessel was used to concentrate and desalt our protein. Our protein was already fairly pure after ammonium sulfate precipitation. Parr vessel could only handle 200mL at a time. Too time consuming to wash out all the salts and have small runs using the pellet.

STRUCTURAL MOTIF AND PROTEIN PURIFICATION



A) TFF Column B) 500L Bioreactor C) Homogenizer

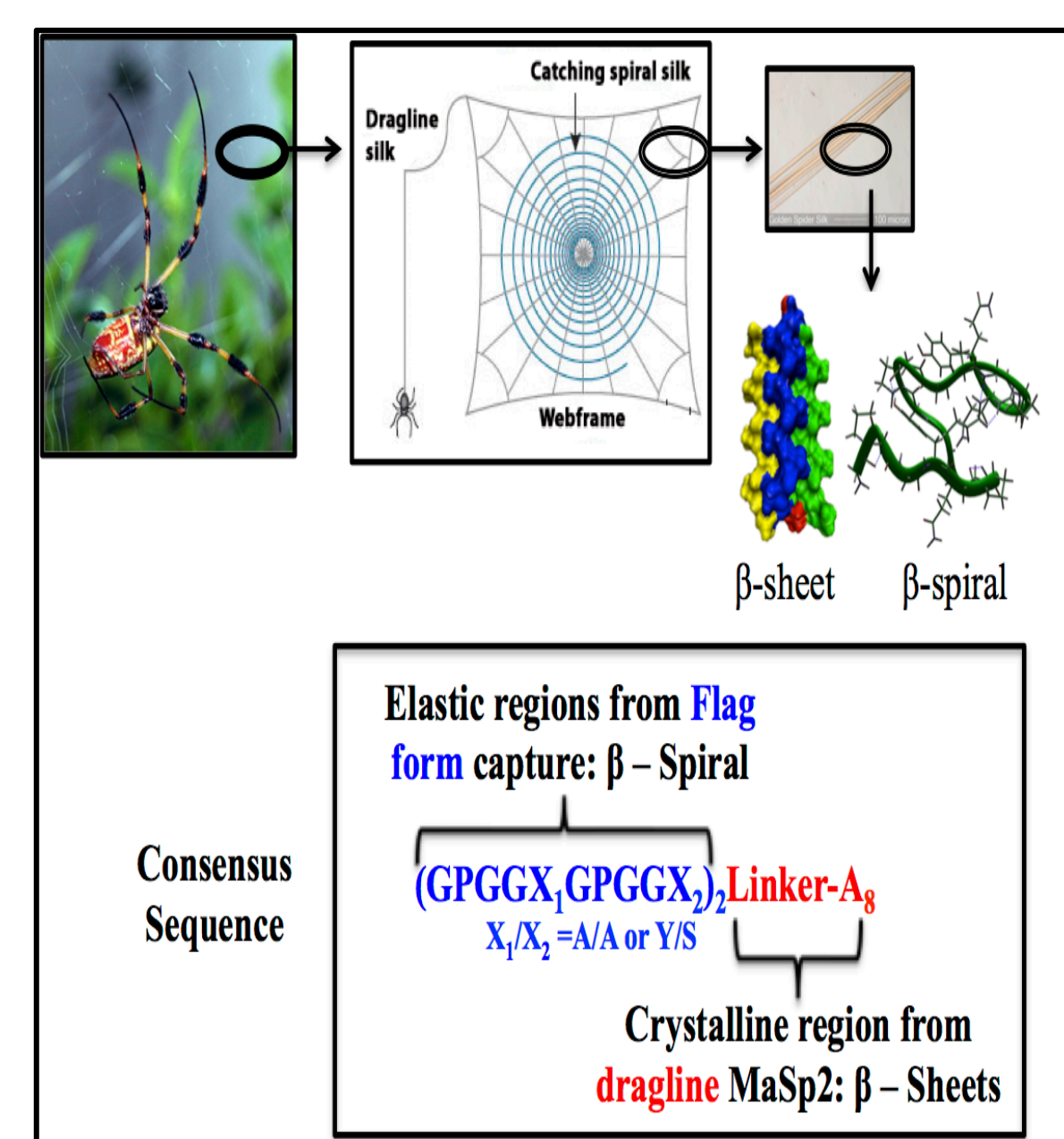


Figure 1. The golden orb weaving spider *Nephila clavipes* produces the major ampullate 1 (MaSp1) and major ampullate 2 protein (MaSp2) that confer strength



D) Parr Vessel

MATERIALS AND METHODS

Discussion

Initially transgenic goats were utilized to produce spidersilk protein MaSp1 and MaSp2. Our goal of large scale protein production and purification could not be met ideally by this method. It is very time consuming and inconvenient. For example in *E. Coli*, it is relatively easy to make multiple constructs but for a goat it would take a long time. Goats also cost more money to maintain (food, land, resources). Use of Spidersilk protein commercially would be more difficult using a transgenic animal such as goats due to regulations and negative stigma toward GMOs, as opposed to mass producing via *E. Coli*. Using bacteria such as *E. Coli* is beneficial due to its much lower cost margin, short generation time, and well understood and researched methods of inserting genes and producing desired proteins.

Methods of Protein purification

Our proteins MaSp1 and MaSp2 have a polyhistidine tag, which is a string of histidine residues typically at either N terminus or the C terminus of a recombinant protein. Originally to purify our protein we used a Nickel Column. Nickel columns are used for immobilized metal affinity chromatography for the purification of recombinant proteins with a polyhistidine tag at either terminus. Our protein would bind to the nickel column and then collected via elution with a high concentration of Imidazole. This process however was too expensive for large scale protein production and purification.

For a while, we used Ammonium Sulfate precipitation. Following fermentation, cell pellets were collected via centrifugation and suspended in a lysis buffer (.5M NaCl 200mM Tris). The cell suspension was sonicated for an hour and the disrupted cells were incubated at 70-80°C for 30 minutes to precipitate *E. coli* proteins. the protein was precipitated by adding ammonium sulfate. The purified protein pellet was washed till the conductivity of the wash reaches 10 microsiemens and lyophilized. Ammonium sulfate precipitation was also too inconvenient and time consuming. We would also expose our protein to a lot of salt that would take a lot of time to wash.

Currently, the cheapest and most efficient method of purifying our protein, that complements large scale production is by the use of TFF columns. Tangential flow filtration (TFF) is a rapid and efficient method for separation and purification of biomolecules. Our protein is around 90 kDa. The pore size in the TFF columns we use are 750 kDa and 50 kDa. The 750 kDa column washes away larger molecules, while we further remove smaller impurities in the 50 kDa column as well as concentrating our protein. We "back flush" the 50 kDa column to collect our protein. TFF columns can be used over and over again with low maintenance and can filter samples from 10mL to thousands of liters.

CONCLUSIONS

- Goal is produce 250 grams of purified spidersilk protein
- Currently we reach around one gram of protein per liter fermented in our bioreactor.
- We are optimizing the TFF protocol to recover more spidersilk protein. By working with different trans-membrane pressures (TMP) increase shear stress (stress induced on the liquid to degrade the protein) by trying a stronger pump, and flow rates.

ACKNOWLEDGEMENTS

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