ARACHNICOLI

Production and Purification of Spider Silk Proteins in *Escherichia coli*

UtahStateUniversity iGEM 2012

Introduction

Spider silk is the strongest known biomaterial due to its combination of high tensile strength and elasticity. It has a large variety of potential applications including: biomedical sutures, athletic gear, parachute cords, air bags, and other yet undiscovered applications.

Spiders, however, cannot be farmed because they are territorial and cannibalistic. Thus, an alternative to manufacturing spider silk must be found. We have used BioBricks to engineer *E. coli* to produce this highly valuable product.

Spider silk manufacturing in *E. coli* has been limited primarily due to the highly repetitive nature of the amino acids in the spider silk protein. To overcome this obstacle, we have used various synthetic biology techniques to boost spider silk protein production and increase cellular fitness.

Properties of Spider Silk

Spiders can produce six different types of silk, each with unique mechanical properties. These properties come from proteins that have a repetitive and highly complex molecular structure. The silk used in this study is major ampullate (dragline) silk, which is composed of ß-spirals, which impart elasticity, and ß-sheets, which improve the fiber's strength.

Mechanical properties of dragline silk, compared to other materials.

Material	Strength (N m^{-2})	Elongation (%)	Energy to break (J kg^{-1})
Dragline silk	4000x10^6	35	40x10^4
Kevlar	4000x10^6	5	3x10^4
Rubber	1x10^6	600	8x10^4

Adapted from Foo CWP and Kaplan DL. 2002. Adv Drug Deliv Rev 54:1131-43.

Amino Acid Sequences

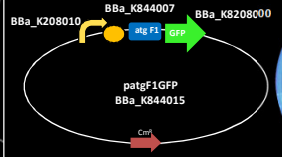
(GGYGPGAGQQGPGSQGPGSGGQQGPGGQ)GPYGPSAAAAAA **U**

(GGYGPGAGQQGPGSQGPGSGGQQGPGGQ)$_2$GPYGPSAAAAAA **W**

β-spirals and β -helices act like springs giving the silk high elasticity. β -sheets give strength and stiffness properties to the fiber. 'U' has 1 elastic unit (1E) and 'W' has 2 elastic units (2E). 'F' and 'B' are codon optimized based on 'W'.

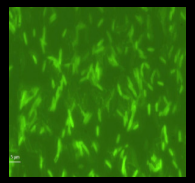
Spider Silk Translational Coupling

To demonstrate the possibility of fluorescently tagging spider silk, the F1 spider silk protein was fused to GFP. This allows us to indirectly measure silk production rates *in vivo*. The GFP is fused downstream of the silk protein so that we only detect fully translated silk constructs.



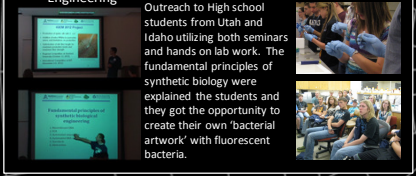
Plasmid diagram of translationally coupled spider silk and GFP proteins.

Petri dishes with *E. coli* cells expressing translationally coupled spider silk and GFP proteins. This demonstrates silk-GFP fusion functionality.



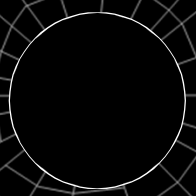
Fluorescent micrograph image of *E. coli* cells producing translationally coupled spider silk and GFP proteins.

High School Outreach

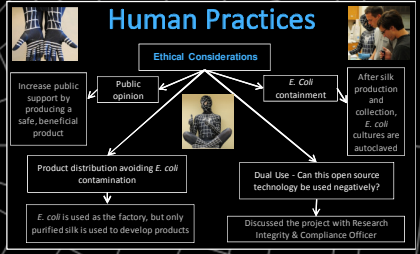


Outreach to High school students from Utah and Idaho utilizing both seminars and hands on lab work. The fundamental principles of synthetic biology were explained the students and they got the opportunity to create their own 'bacterial artwork' with fluorescent bacteria.

First Spider Silk Thread Produced with BioBricks



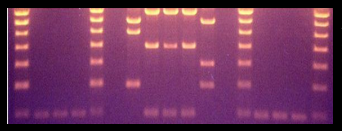
Human Practices



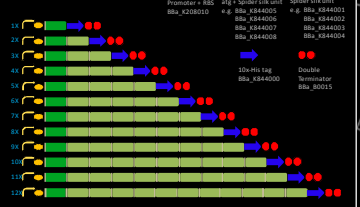
Genetic Design and Metabolic Manipulation

Spider silk protein subunits contain only six different amino acids. Larger numbers of repeating subunits impart greater elasticity and strength to the fiber, but could potentially drain the cell's tRNA pool. We have codon optimized the spider silk proteins for increased production, but instead of optimizing to *E. coli*'s ratios we reduced the number of codons used for each amino acid, and created a construct to supplement tRNAs that recognize those codons. This tRNA pool manipulation should allow for increased silk production.

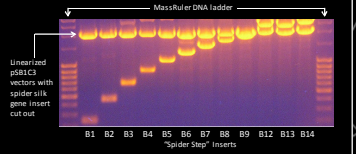
Diagram of cells co-transformed with silk producing plasmid and tRNA supplementation plasmid. Addition of only the silk plasmid can put stress on the cell by exhausting the charged tRNA pools for the six amino acids used by the silk subunits. Addition of the tRNA supplementation plasmid relieves this stress.



Agarose gel we ran to make the letter U, S, and U which stands for Utah State University (USU). The first 'U' consists entirely of 'F' BioBrick units, the second 'U' consists of only 'B' BioBrick units and the 'S' is a mix of both 'F' and 'B'.



Design diagram for spider silk proteins with various numbers of subunits. All constructs are preceded by a lac promoter, and followed by a 10x-His tag.



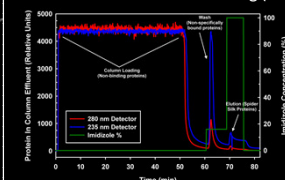
Agarose gel showing spider silk genes made up of an increasing number of silk subunits. 'B' denotes the type of tRNA optimization performed and the numbers indicate the number of subunit repeats.

Spider Silk Generator

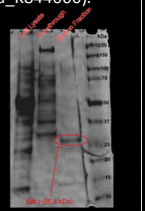
BBa_K208010: Lac/IPTG inducible promoter and rbs
BBa_K844008: Spider Silk 1x Subunit "B" with ATG
BBa_K844004: Spider Silk 3x Subunit "B"
BBa_K844000: 10x His-tag

His-tag and Spider Silk Purification and Spinning

After producing spider silk protein, we lysed the cells and used a nickel column to purify our protein with the C-terminal 10x His-tag (BBa_K844000).



Nickel Resin Column Elution Graph. Red and blue lines indicate amount of protein in column effluent. Green line indicates the concentration of the elution buffer.



SDS PAGE gel showing purified spider silk protein.

The 10x His-tag binds more tightly to the affinity column, which allows the use of higher concentration rinses to remove contaminating proteins.

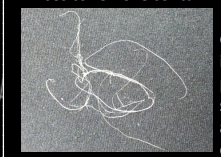


Photo showing the first spider silk thread produced from BioBricks.

The purified silk is dissolved and the solution is loaded into a modified plastic extruder. The solution is forced through a small bore needle into a coagulation bath. This process organizes the molecular structure of the silk, creating a fiber.

Team Successes

- ✓ Built first ever spider silk BioBrick parts and spun first spider silk fiber from composite BioBricks
- ✓ Built a platform for spider silk manufacturing
- ✓ Developed improved His-tag for better protein purification
- ✓ Expressed first silk-GFP fusion protein from BioBrick parts

Future Goals

- Increase number of silk subunits
- Analyze the effect of protein size on mechanical properties
- Characterize effects of tRNA addition on silk yield
- Spin and test fluorescent spider silk

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