**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 (drageine) silk, which is composed of β-spirals, which impart elasticity, and α-sheets, which improve the fiber’s strength.

Architectural properties of drageine silk, compared to other materials:

<table>
<thead>
<tr>
<th>Material</th>
<th>Strength [kN/cm]</th>
<th>Elasticity [%]</th>
<th>Toughness [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drageine silk</td>
<td>4500</td>
<td>55</td>
<td>20</td>
</tr>
<tr>
<td>Rubber</td>
<td>1000</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Spider silk</td>
<td>200</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

[β-spirals and α-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’.

**Amino Acid Sequences**

- gGGAG
- pGGAG
- sGGAG
- pGGAG
- sGGAG
- pGGAG
- sGGAG

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**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 RNA 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 show silks on the cell by eliminating the cloned plasmid pools for the six amino acids used by the silk subunits. Addition of the tRNA supplementation plasmid releases fxx stress.

**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.

**High School Outreach**

Outreach to high school students from both our area and those in Utah and Idaho. The students synthesized and engineered biology were involved in spider and demonstrated how spider silk grows in the presence of a fluorescent bacteria.

**Human Practices**

- Spin and test fluorescent spider silk
- Build a platform for spider silk manufacturing
- Developed improved HiTag for better protein purification
- Developed a system of tRNA addition on silkworm

**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 Hi-Tag for better protein purification
- Developed a system of tRNA addition on silkworm

**Future Goals**

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

**Spider Silk Generator**

BBa_K844003: Lox/PTG integrative promotor and ribosome

BBa_K844004: Spider silk LAS Subunit U with ATG

BBa_K844005: Spider silk LAS Subunit W

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).

**Photo showing the first spider silk thread produced from BioBricks.**

**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.**

**SOS PAGE gel showing purified spider silk proteins.**

**SOS PAGE gel showing spider silk proteins.**

**Table showing the effect of protein size on mechanical properties.**

**Figure showing the effect of protein size on mechanical properties.**