USING SYNTHETIC BIOLOGY TO PRODUCE SPIDER SILK

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Why we study spider silk

Understand protein structure/function

Create a new biomaterial for:

tendon and ligament repair/replacement

drug implantation

airbags and tire cords

athletic gear

military materials, e.g. parachute cords

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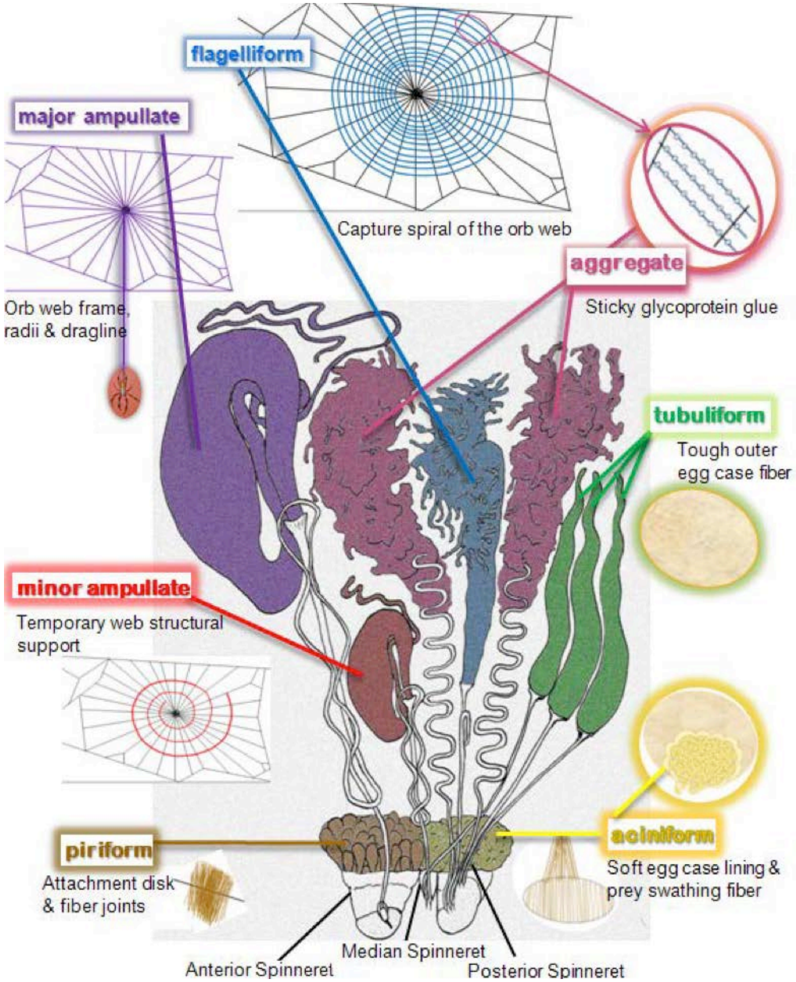
Why Spider Silks?



Spiders can make six types of silk with very different mechanical properties.

Material	Strength (N/m^2)	Elasticity (%)	Energy to Break (J/kg)
Major Ampullate Silk	4×10^9	35	1×10^5
Minor Ampullate Silk	1×10^9	-	3×10^4
Flagelliform Silk	1×10^9	200	1×10^5
Kevlar	4×10^9	5	3×10^4
Rubber	1×10^6	600	8×10^4
Tendon	1×10^9	5	5×10^3

Note the differing properties of the silks and their superiority to manmade fibers.

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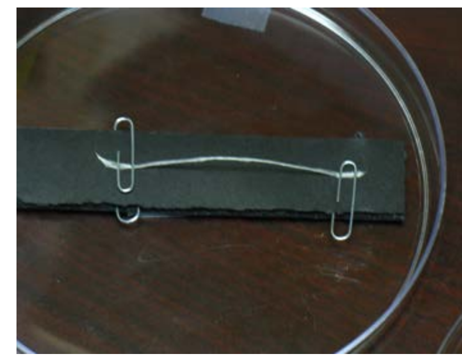
How to produce large amounts of spider silk

System	**Protein Concentration**	**Total Protein Yield**	**Production Time**
Bacteria	100mg/L; 30,000L fermentor	3kg/run	2-4 months
Goats	15g/L; 8L/day; lactation of 150 days	18kg/goat/year	1-2 years
Alfalfa	1% of soluble protein, 10T/acre	218kg/acre/year	4-5 years
Silkworm	5% < ? < 85%	Unlimited	2 years

Transgenic goats

Normal (L) and transgenic (R) cocoons

100 strands of synthetic spider silk



50 µm thick film of spider silk protein

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“Bulletproof”

CSI: NY

SCIENCE NATION

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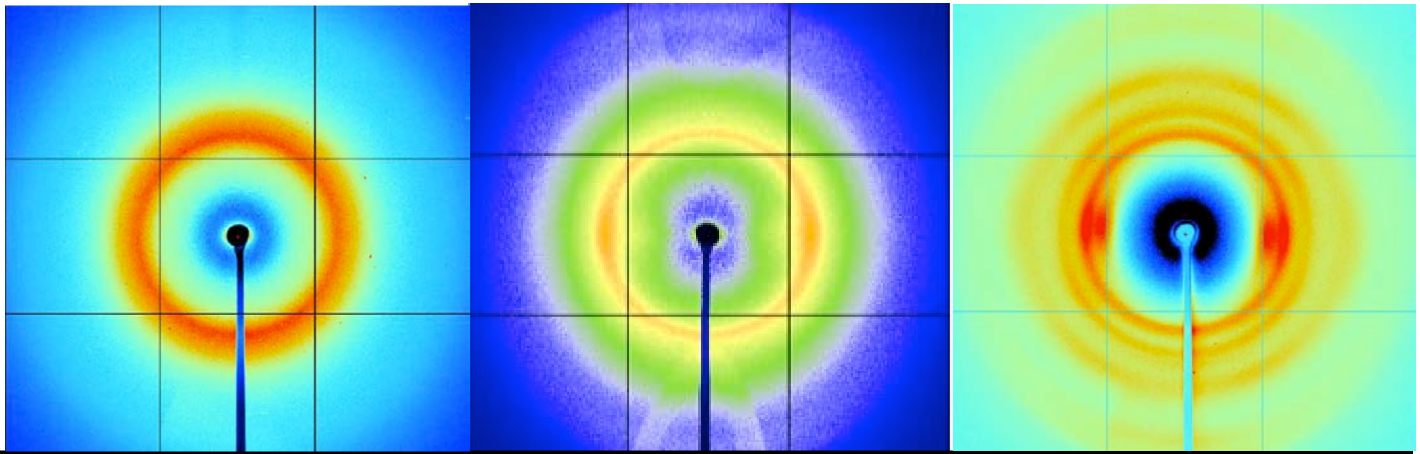
Properties of synthetic fibers

Sunrise Session: January 20, 2012



	Step 1	Step 2	Step 3
Diameter (microns)	68	34	28
Tensile Strength (Megapascals)	28	102	128
Elasticity (Percent)	2	19	52
Toughness (joules/meter^3)	.3	19	55

Transgenic silk worm data

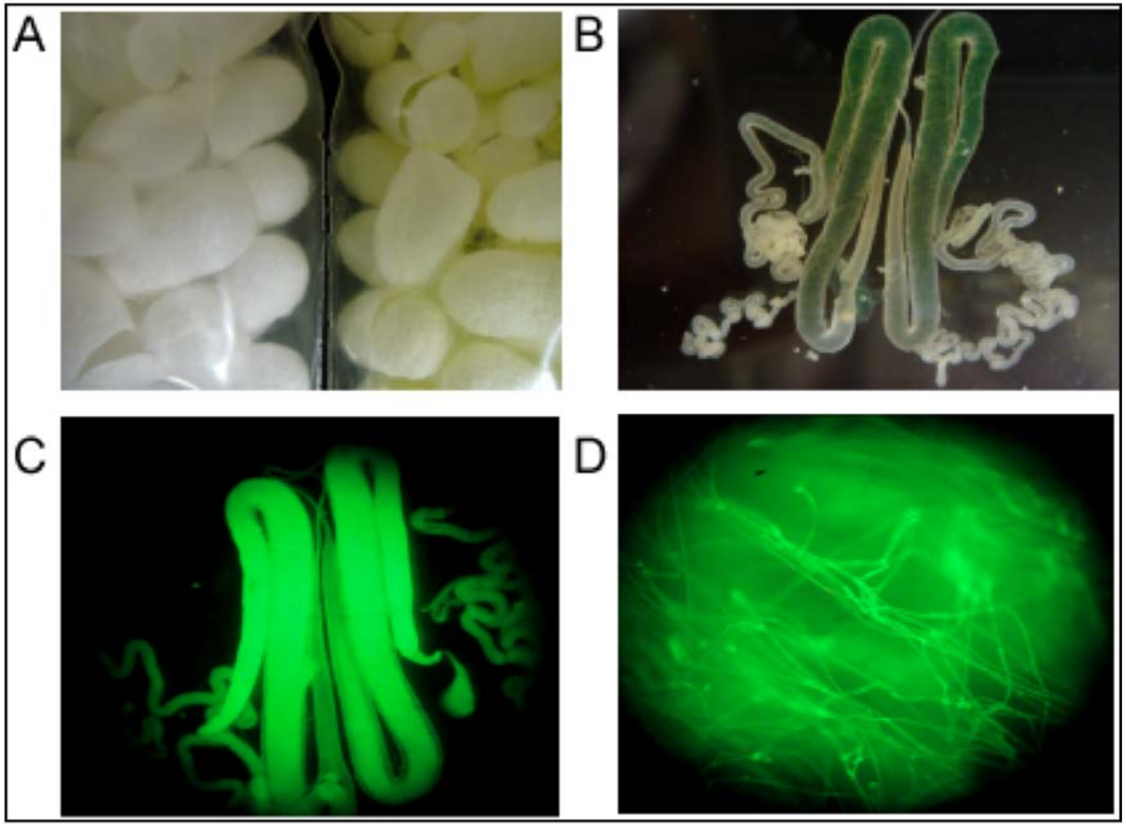


Figure 3. GFP expression in (A) eggs, (B, C) silk glands, and (D) silk fibers of transgenic silkworms.

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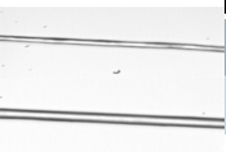
MECHANICAL PROPERTIES	SILKWORM SILK (DEGUMMED)						DRAGLINE SILK *Nephila clavipes*
	NON TRANSGENIC	TRANSGENIC BLEND					
		Chimeric spider silk – GFP		Chimeric spider silk + GFP			
	pnd-w1	P6- Line 7		P6+ Line 4		Ratio to spider silk	
Max Stress *MPa*	198.0	315.3	*159%*	338.4	*171%*	*45%*	744.5
Max Strain *%*	22.0	31.8	*145%*	31.1	*141%*	*102%*	30.6
Toughness *MJ/m³*	32.0	71.7	*224%*	77.2	*241%*	*56%*	138.7

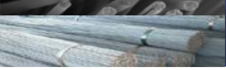
Processed spider silk



Material	Extensibility	Strength	Toughness
$A1S8_{20}$	70%	.15	94
$Y1S8_{20}$	80%	.14	62

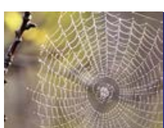
Man-made materials





Material	Extensibility	Strength	Toughness
Kevlar	3%	3.6	50
Carbon fiber	1%	4	25
High-tensile steel	1%	1.5	6

Natural spider silk



Material	Extensibility	Strength	Toughness
Dragline silk	35%	4	150
Flagelliform silk	270%	.5	150

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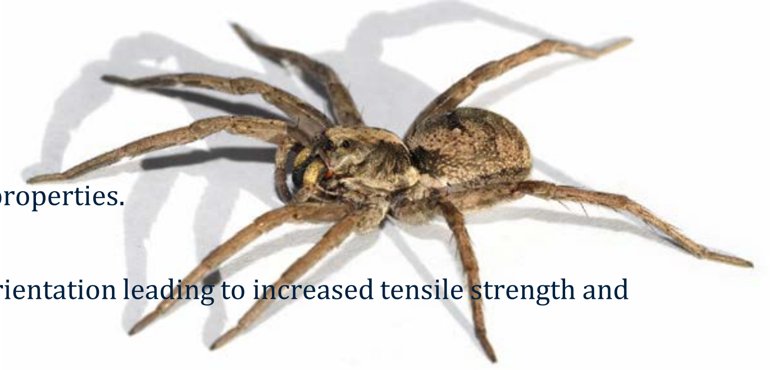
Conclusions

- • The longer the GPGXX sequence the larger the elongation and the higher the poly-Ala percentage the higher the tensile strength.



- Longer proteins give both better tensile strength and elongation.
- Proper spinning conditions eliminate variation in fiber mechanical properties.
- Post-spin draw increases ß-sheet content and w/ water increases orientation leading to increased tensile strength and elongation regardless of sequence.
- Electro-spun nanofibers show increased tensile strength with decreasing fiber diameter reaching 1 Gpa (not seen in any biological fiber reconstituted in any fashion) and, in contrast to most materials, elongation does not decrease leading to possible "super fibers".
- 3-5% spider silk co-spun with the naturally spun silkworm silk has significant effects on both tensile strength (80% increase) and elongation (50%).
- Spider silk has the highest thermal conductivity of any organic material tested.
- At low extension rates (0.5-5 mm/min), MA and MI silks have similar failure energies to aramid and UHMWPE but at high rates (5000 m/sec), both MI and MA silks significantly outperform those high performance fibers.
- Simultaneous multi-fiber spinning achieved.