E. COLI DERIVED SPIDER SILK MASP 1 AND MASP 2 PROTEINS AS CARBON FIBER PRECURSORS

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Utah State University

June 7, 2016

Project ID LM103

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Overview

Timeline

Start date: November 1, 2014
End date: October 30, 2016
Percent complete: 70%

Barriers

- 2.5.1. Lightweight Materials Technology (VTP MYPP 2011-2015)
  - Performance: Match carbon fiber using spider silk instead of PAN

Budget

- Total project funding
  - DOE: $1,490,744
  - Contractor share: $497,298
- Funding FY 2015: $997,758
- Funding FY 2016: $990,284

Partners

- U. of California, Riverside
- Oak Ridge Nat'l Laboratory
- Utah State University

Relevance

Overall Project Objective

Reduce the weight of vehicles thereby reducing green house gas emissions and the dependence on foreign oil through the use of carbon fibers produced from spider silk protein fibers

Project Goals

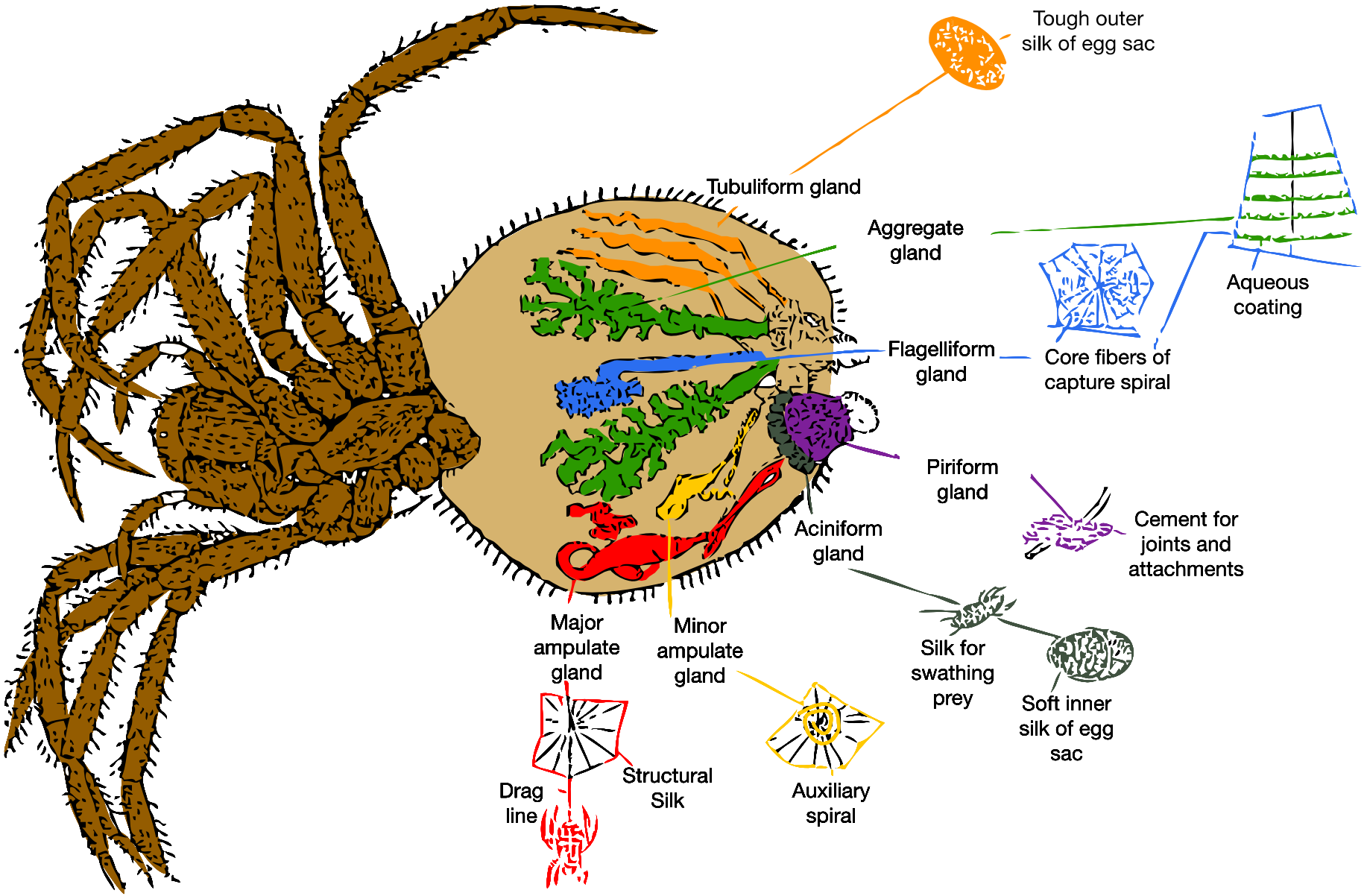
- Maximize protein production via *E. coli* while maintaining full-length protein
- Develop a Scalable Fiber Spinning process
- Improve spider silk fiber mechanical properties
- Generate transgenic silkworms producing silk with much higher strength
- Determine optimal stabilization conditions for spider silk protein fibers for conversion to carbon fibers
- Conduct techno-economic analyses to estimate costs

Milestones

Recipient Name: Randolph V. Lewis, Utah State University							
Project Title: Spider Silk MaSp1 and MaSp2 Proteins as Carbon Fiber Precursors							
Task #	Task Title	Milestone type	Milestone number	Milestone description	Milestone verification	Percent Completion	Expected Quarter
1	Fiber production	Milestone	1.1.1	1g/L protein	Purified protein recovered	100	Q2
1	Fiber spinning	Milestone	1.2.1	Tensile strength	Mechanical testing	100	Q3
1	Silkworm transgenesis	Milestone	1.3.1	Silk tensile strength	Mechanical testing	100	Q3
1	Spider silk production	Go/No		Tensile strength	Mechanical testing	100	Q4
2	Conversion	Milestone	2.1.1	Pre-treatment	Carbonization	50	Q5
2	Conversion	Milestone	2.2.1	Carbon fiber strength	Mechanical testing	25	Q6
2	Conversion	Go/No		Stabilized fiber	Thermal stability	25	Q7
2	Conversion	Milestone	2.3.1	Strength	Mechanical testing	0	Q8
2	Conversion	Milestone	2.4.1	Property relationships	Micro-structure	0	Q7
3	Technoecon	Milestone	3.1.1	Validation of sub models	Experimental Verification	100	Q1
3	Technoecon	Milestone	3.1.2	Engineering system model	Sensitivity Analysis	100	Q2
3	Technoecon	Milestone	3.2.1	TEA	Technology Comparison	100	Q3
3	Technoecon	Milestone	3.2.2	Process Optimization	Economic Viability	100	Q4
3	Technoecon	Milestone	3.3.1	LCA	Technology Comparison	75	Q6
3	Technoecon	Milestone	3.3.2	Vehicle Modeling	Impact Comparison	0	Q8

Approach/Strategy

- Create spider silk fibers with tensile strength of >750 MPa (Go/No Go with intermediate milestones) **Achieved Q4**
- Convert spider silk fibers to stabilized carbon fibers (Go/No GO, Q7 with intermediate milestones)
- Techno-economic analysis of estimated production costs (Final milestone Q8, with intermediate milestones)



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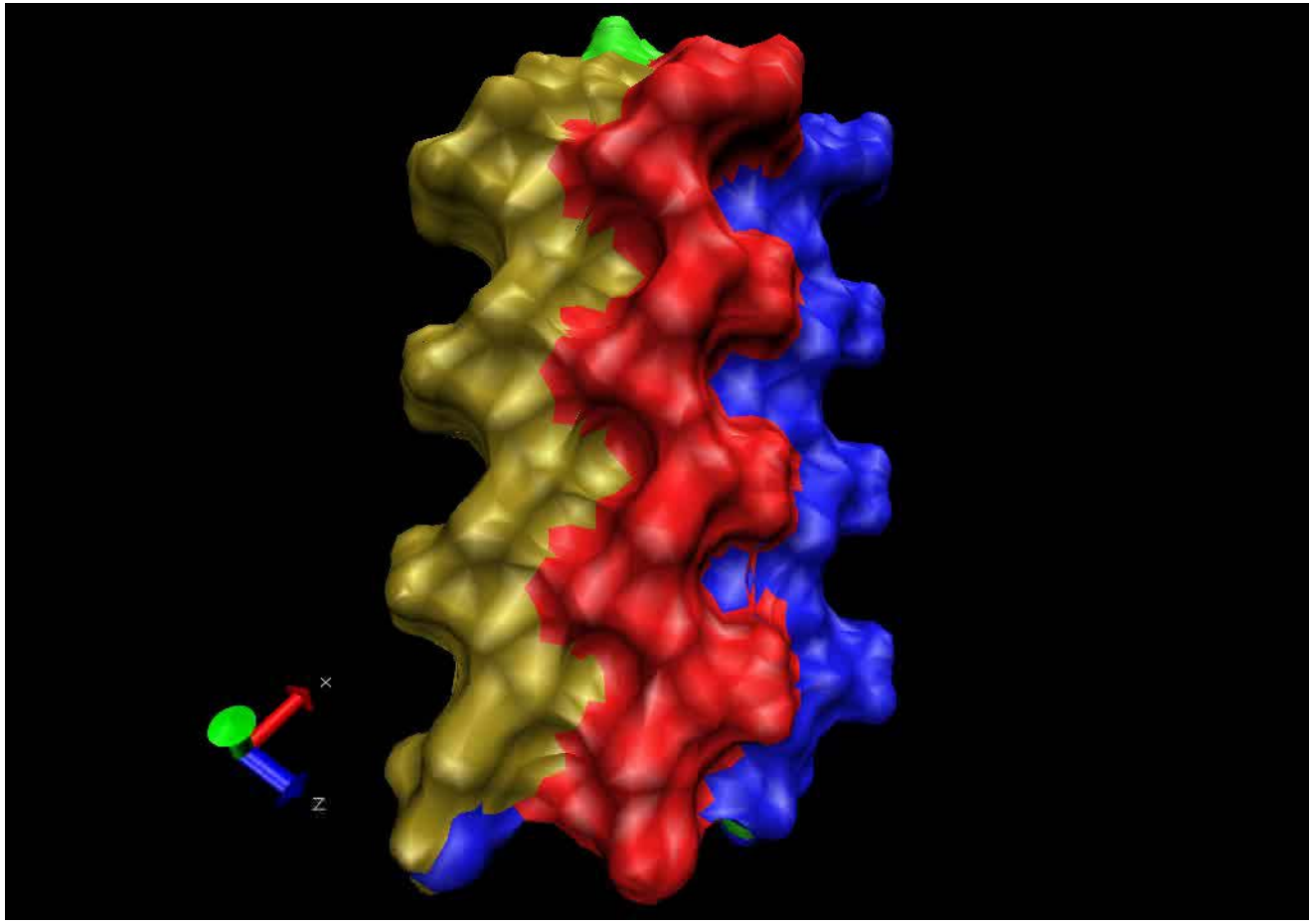
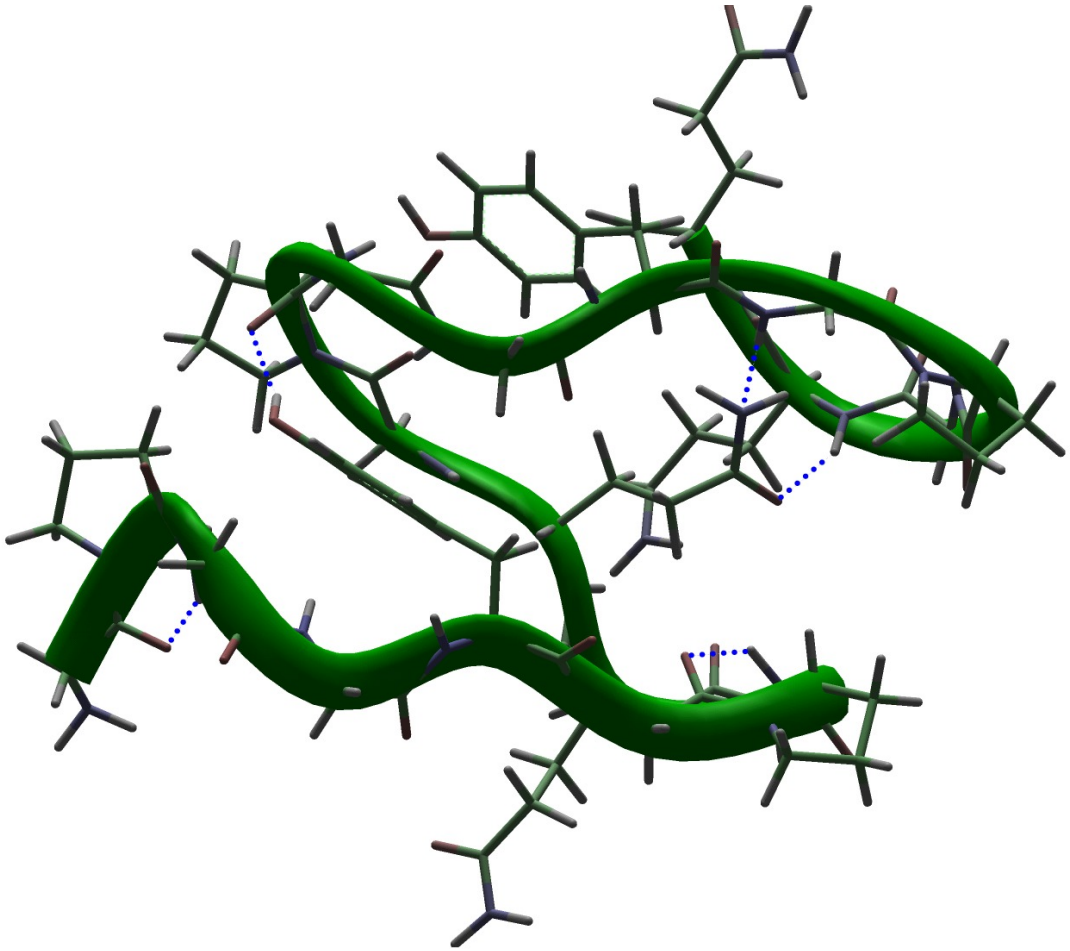
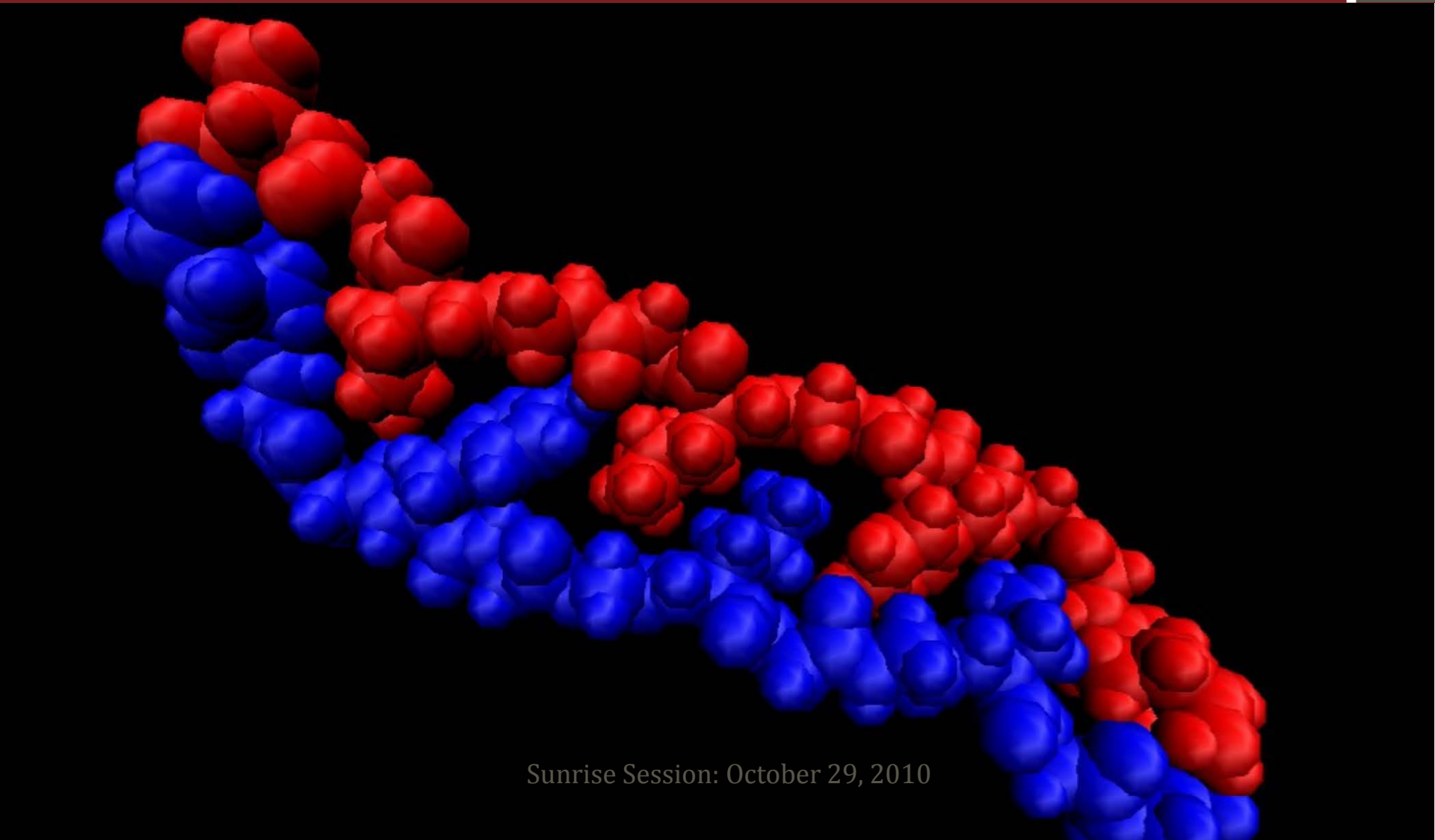
Lego



Slinky



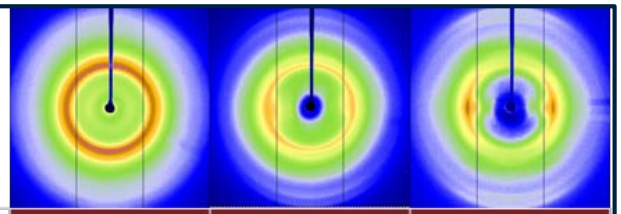
Zipper



Sunrise Session: October 29, 2010

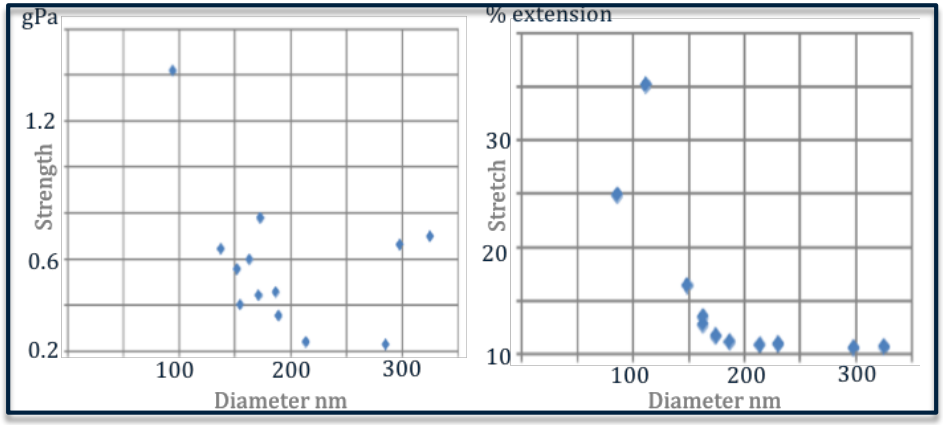
Technical Accomplishments and Progress

- Create spider silk fibers with tensile strength of >750 Mpa



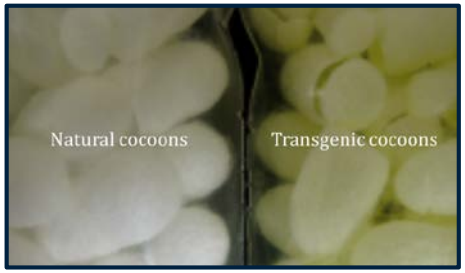
	As Spun	1.5x MeOH / 1.5x H_2O	2x MeOH / 2x H_2O
Max Stress (MPa)	14.3	164.1	257.7
Max Strain (%)	1.5	66	40
Toughness (MJ/m³)	0.092	92.3	84.4
Orientation Factor	NA	0.502	0.635

Post spin stretch of spider silk protein fibers with corresponding X-ray diffraction patterns showing increases crystallinity and orientation.

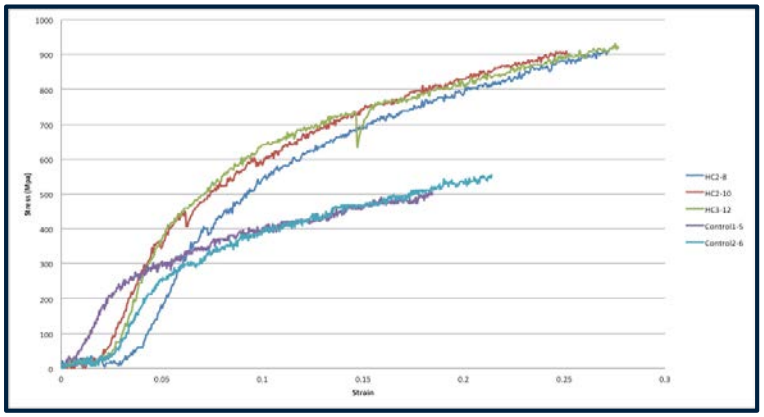


Electrospun spider silk protein fibers ranging from 100-350nm with corresponding tensile strengths and elongations. Note the non-linear behavior of both properties.

Spools of bacterially produced spider silk protein, 350m of 8-fiber thread.



Comparison of natural and transgenic silkworm cocoons under UV light so the fluorescent probe attached to the spider silk protein can be used to identify the transgenic silkworms.



Stress-strain curves for control and transgenic silkworm silk. The samples are the same as described above in the table above. Note both the similar shapes and values for the different transgenic silkworm lines which is very similar to the variation in the controls.

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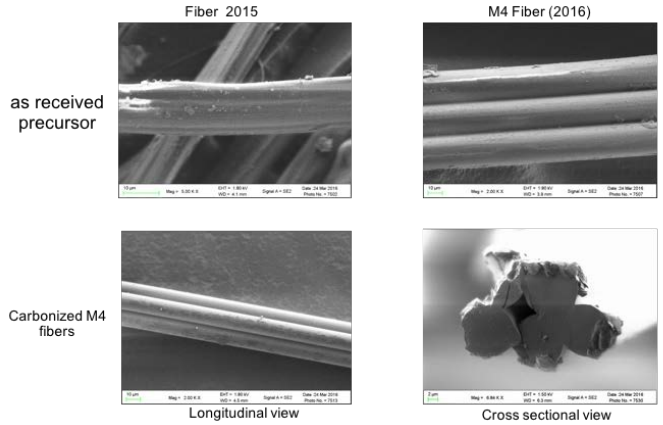
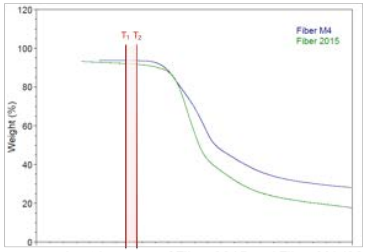
Technical Accomplishments and Progress

- Convert spider silk fibers to stabilized carbon fibers (Go/No GO, Q7 with intermediate milestones)



Stretch Study of Fiber 2015 and Fiber M4 (Jan 2016)



Thermogravimetric curves of Fiber 2015 and Fiber M4 (2016) in N_2:

Produced carbon fibers from M4 precursor can be easily wrapped around a core with a diameter of 1"

- M4 showed higher yield than previous fiber
- Stretching window has been identified between T_1 and T_2 for both materials at the beginning of the process
- New precursor Fiber M4 has shown better stretch characteristics during process. (this potentially leads to higher mechanical performance)
  - Fiber 2015 : Max 4.6%
  - Fiber M4 (2016) : Max 25.6%

→ M4 Fibers can be stretched typically up to 20% during process (carbonized batched obtained on Apr 5th, 2016)

Conversion and Mechanical properties:

Successful carbonization for both types of silk fibers

Feb. 2016 → first successful carbonization of "M4 fiber" (several carbon fibers have been produced since then)
March 2016 → first carbonization of "2015 fiber"

Sample ID	Data type	Diamter (µm)	Peak Stress (ksi)	Modulus (msi)	Strain (%)
M4 Prec	Mono filament	28.42 (1.18)	24.6 (1.7)	1.1 (0.0)	17.73 (6.17)
Fib. 2015	Bundle: raw data	50.55 (38.44)	14.8 (14.6)	4.1 (3.6)	0.34 (0.08)
M4 0006	Bundle: raw data	52.5 (8.4)	23.8 (9.6)	0.7 (0.3)	3.40 (1.14)
	*Bundle: CS reassessed**	*25.46 (0.00)*	*98.9 (36.0)*	*2.7 (0.2)*	*3.40 (1.14)*
M4 0009	Bundle: raw data	48.9 (14.9)	27.5 (15.7)	0.8 (0.4)	3.23 (0.77)
	*Bundle: CS reassessed**	*25.46 (0.00)*	*82.8 (21.6)*	*2.6 (0.7)*	*3.23 (0.77)*
M4 0010	Bundle: raw data	54.8 (11.1)	25.9 (12.1)	0.6 (0.2)	4.49 (1.66)
	*Bundle: CS reassessed**	*25.46 (0.00)*	*120.3 (54.6)*	*2.5 (0.5)*	*4.49 (1.66)*
M4 0011	Bundle: raw data	47.8 (4.6)	33.1 (7.0)	0.6 (0.1)	5.27 (1.19)
	*Bundle: CS reassessed**	*25.46 (0.00)*	*111.6 (38.8)*	*2.0 (0.5)*	*5.27 (1.19)*

Properties of precursor and carbon fiber

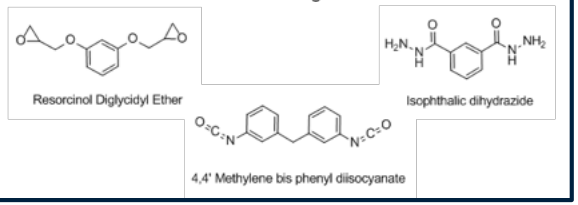
- Average fiber diameter is **around 9 µm** (this value is not firm)*;
  → Carbon fibers from M4 **potential of 100 ksi tensile strength**
- **Elastic Modulus of 4.1 Msi** has been achieved on initial trials.
- Fused filaments have been observed. Common issue in fiber process, **Solution: silicon based finishing application on precursor**

* Cross Section (CS) of single fiber are measured from SEM images ~9 µm. In calculations of bundle surface area, single fiber diameter assumed to be 9 µm and bundles consist of 8 fused filaments.

Crosslinking agents and suggested synthetic pathways for obtaining crosslinking of the MaSp fibers are selected based on the type and concentration of reactive groups. (acidic, basic and hydroxyl groups) present in MaSp1&2 molecular chains.

Reactivity class	Target functional group	Potential reagent
Amino -reactive	$-NH_2$	N-hydroxysusccinimide (NHS) ester, Resorcinol diglycidyl ether (Di-epoxides)
Carboxyl-to-amine reactive	-COOH	Carbodiimide
Hydroxyl reactive	-OH	4,4' methylene bis phenyl diisocyanate (MDI)

Potential Crosslinking chemicals:



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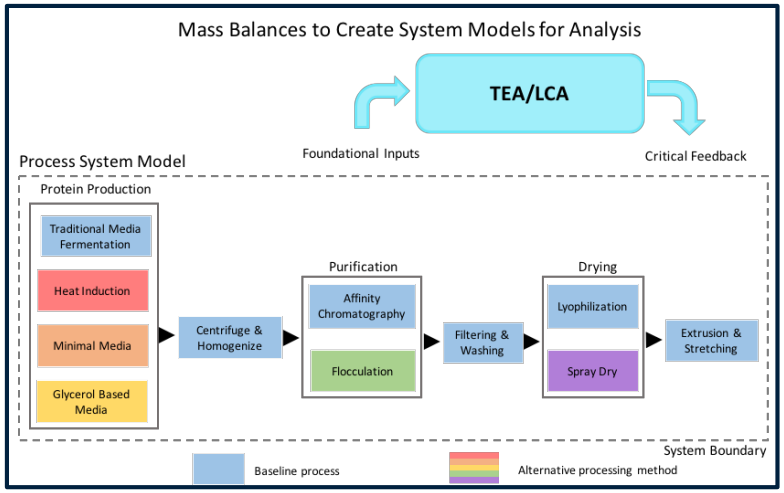
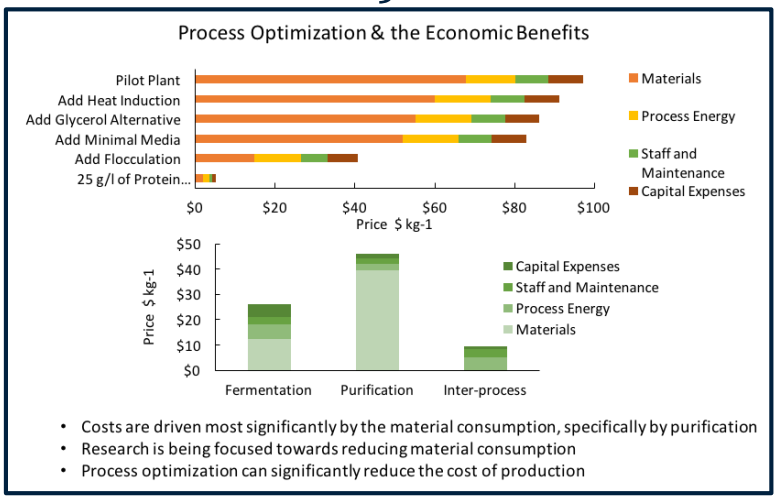
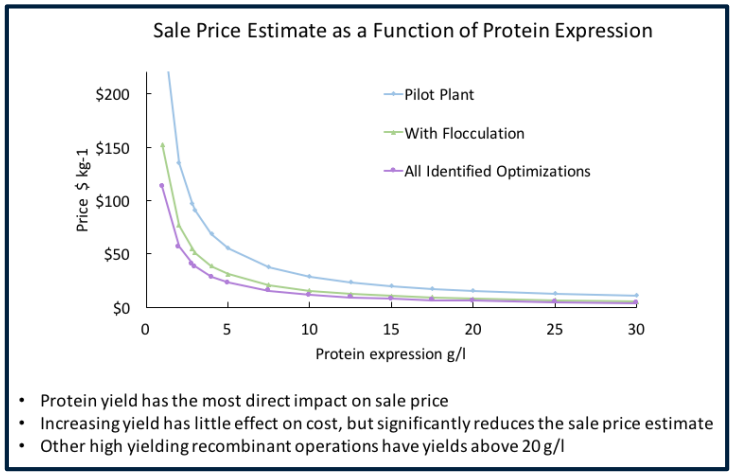
DOE June 7, 2016

Technical Accomplishments and Progress

- Techno-economic analysis of estimated production costs (Final milestone Q8, with intermediate milestones)







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Response to Previous Year Reviewer's Comments

This project was not presented at the 2015 Annual Merit Review.

Partners and Collaborators

- Dr. Cheryl Hayashi, U. of California, Riverside, co-PI.
  Gene sequences and comparisons for spider silk protein gene choices to produce.

- Drs. Soydan Ozcan and Felix L.Paulauskas, ORNL co-PIs.
  Spider silk fiber conversion to carbon fiber and analyses of those fibers.

- Dr. Jeff Yarger, Arizona State University, collaborator.
  NMR, Raman and X-ray diffraction.

- Argonne National Laboratory, facilities.
  X-ray diffraction facility

Remaining Challenges and Barriers

- Convert spider silk fibers to stabilized carbon fibers (Go/No GO, Q7)
- Further improve the strength of the spider silk fibers
- Increase spider silk protein production to drive costs down

Proposed Future Work

Based on the three remaining challenges the following is the future work.

- Convert spider silk fibers to stabilized carbon fibers (Go/No GO, Q7)
  - Optimize the oxidation process with regard to the temperature ramping, final temperature and time of heating.
  - Test crosslinking agents to better stabilize the proteins.
  - Use different spider silk proteins with higher carbon content.

- Further improve the strength of the spider silk fibers
  - Introduce the multi-fiber spinning head (24 fibers).
  - Determine the effects of photo-crosslinking of the proteins during spinning.
  - Improve spinning conditions via additives as well altering spinning physical conditions
  - Breed top silkworms and induce partial knockout of silkworm silk gene

- Increase spider silk protein production to drive costs down
  - Generate higher cell densities by optimizing carbon feed rate
  - Use higher induction levels to increase protein production/ unit of bacteria
  - Add additional antibiotic at induction to prevent loss of resistance

Summary

- Maximize protein production via *E. coli* while maintaining full-length protein
  - Protein production has gone from 0.5g/L to as high as 4.0 g/L
  - Purification process developed with 17-fold lower costs
- Develop a Scalable Fiber Spinning process
  - Up to 1000m of 8 fiber thread has been spun
  - Moving to a 24 fiber thread spinning head
- Improve spider silk fiber mechanical properties
  - Improved from 200 MPa to over 400 MPa
- Generate transgenic silkworms producing silk with much higher strength
  - Improved from 600 MPa to over 900 MPa with stable transmission
- Determine optimal stabilization conditions for spider silk protein fibers for conversion to carbon fibers
  - In process
- Conduct techno-economic analyses to estimate costs
  - Nearly complete for the fiber production prior to conversion to carbon fibers

Technical Back-Up Slides

Special Mechanical Properties of Spider Silks

Material	Strength (MPa)	Strain (%)	Toughness (KJ/kg)
Dragline silk	4000	35	400
Minor Ampullate silk	1000	5	30
Flagelliform	1000	>200	400
Tubiliform silk	1000	20	100
Bombyx mori silk	600	20	60

[a]Data from Gosline,Lewis, Altman

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Production Methods

System	Protein Yield per Year	Production Time
Bacteria	12 kg per run	2-4 months
Goats	18 kg per goat	1-2 years
Alfalfa	218 kg per acre	4-5 years
Silkworm	??	2 years