iGEM at Utah State University

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iGEM: International Genetically Engineered Machine Competition

Regional Jamboree – Americas West: Stanford University
October 12-14, 2012

World Championship – MIT:
November 2-5, 2012

Introduction – iGEM

- International Genetically Engineered Machine (iGEM) Competition
  - Undergraduate synthetic biology competition
  - Founded in 2004 with 5 teams
  - 2011: 167 teams, 51 from US
  - 2012: 193 teams, 68 from US

- Design, build, and test simple biological systems made from standard, interchangeable biological parts-BioBricks

What is synthetic biological engineering?

An approach to engineering biology

Not what you make, but how you make it

Goal: to make biology easy to engineer

Synthetic Biological Engineering

- The design and construction of biological parts, devices, and systems

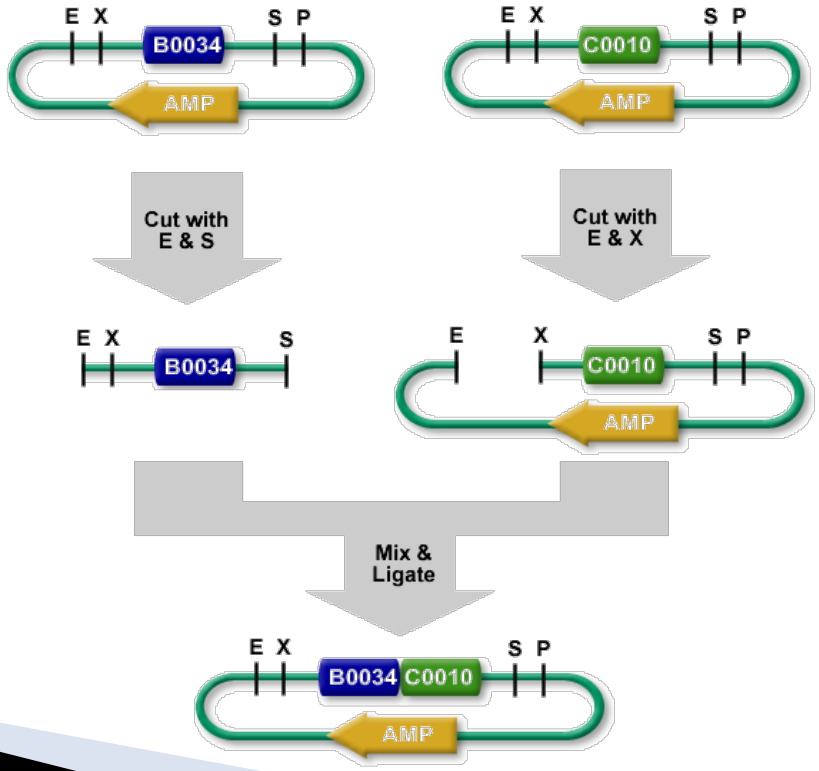
- The redesign of existing, natural biological systems for useful purposes

Fundamental principles of synthetic biological engineering

1. Recombinant DNA technologies
2. PCR
3. Automated sequencing
4. Automated DNA construction
5. Standards
6. Abstraction

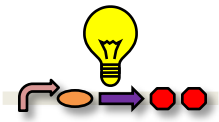
BioBricks

- Standardized and easily assembled parts of DNA for the design and construction of biological systems and devices.

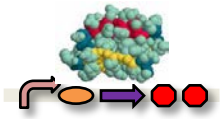


TECHNOLOGY ABSTRACTION

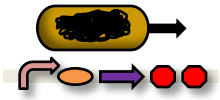
Devices



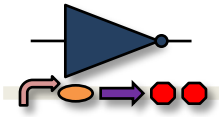
Reporters



Protein Generator

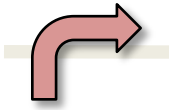


Secretion/Excretion

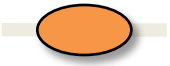


Regulatory

Parts



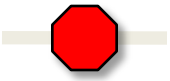
Promoters



RBS

Proteins

RNA



Terminators

DNA TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAG

BioBricks

- For the iGEM competition, teams are provided a library of standardized parts (*BioBricks*) and asked to design and build genetic machines

Over 1,000 DNA standardized parts are distributed to each team from the Registry of Standard Biological Parts at MIT

USU at iGEM

2008: Bronze Medal: Efficient Systems for Monitoring Polyhydroxybutyrate (PHB) Production in Microorganisms

2009: Gold Medal: BioBricks without Borders-Secretion Systems

2010: Gold Medal: CyanoBricks- Developing Cyanobacteria as a Biological Engineering Platform

2011: Gold Medal: CyanoBricks- Expression Testing and Bioproduct Development

Student Participation: 8 High school students
31 Undergraduate students
15 Graduate students

2012 iGEM Competition

- New in 2012:
  - Regional Competitions (total 5 regions)
    - Americas East (Pittsburg): 44 teams
    - Americas West (Stanford): 24 teams
    - Asia (Hong Kong): 56 teams
    - Europe (Amsterdam): 53 teams
    - Latin America (Colombia): 16 teams
  - World Championships
    - MIT

ArachniColi: production and purification of spider silk proteins in *E. coli*

Team Successes

-Gold medal
-Regional Finalist
-Won award Best Engineered BioBrick Device
-Selected to World Championships at MIT
-Won award for Best Manufacturing Project

Outline

Background

Team Goals

Design with BioBricks

The First Spider Silk BioBricks

Spider silk-GFP fusion protein

Protein Purification

Outreach, Conclusions, Future Work

Properties of spider silk

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	$8x10^4$

- As strong as Kevlar, 7 times more elastic
- 5 times larger energy to break than rubber
- Biocompatible
- Biodegradable

Potential spider silk applications

Motivation

Problem:

- Spiders are territorial and cannibalistic so producing spider silk in 'spider farms' is not an option
- Even if you could have farms, silk collection would be difficult
- The mechanical properties of natural silk are not ideal for all applications

Solution: Create spider silk BioBricks for expression in *E.coli*

Team goals

- Create the first spider silk BioBrick
- Create a method of purifying spider silk that is produced in *E.coli*
- Create spider silk-GFP fusion proteins for easy detection of products
- Manufacture spider silk protein in *E.coli*
- Manufacture spider silk fibers from protein
- Use tRNAs to optimize spider silk production

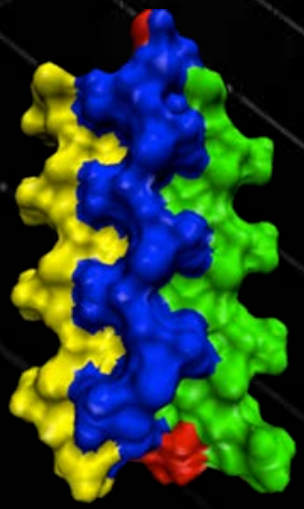
Spider silk proteins: a series of repeats

(GGYGPGAGQQGPGSQGPGSGGQQGPGGQ)GPYGPSAAAAAA **U**

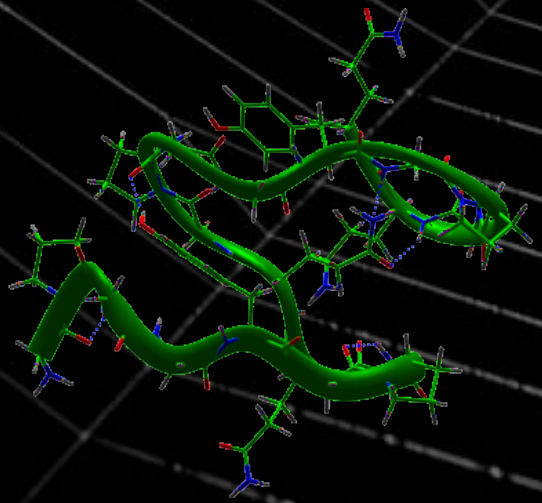
(GGYGPGAGQQGPGSQGPGSGGQQGPGGQ)(GGYGPGAGQQGPGSQGPGSGGQQGPGGQ)GPYGPSAAAAAA **W**

β-spirals and β-helices act like springs giving the silk high elasticity.

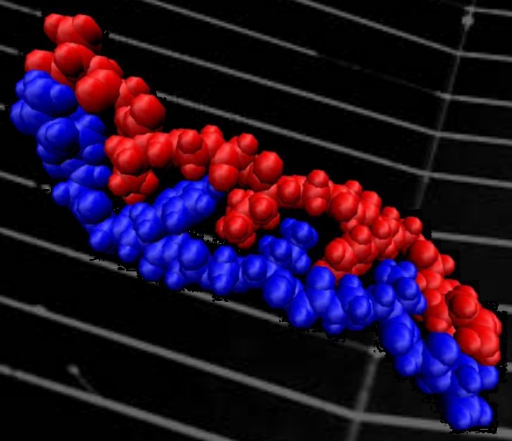
β–sheets give strength and stiffness properties to the fiber.



β - Sheets



β - Spiral



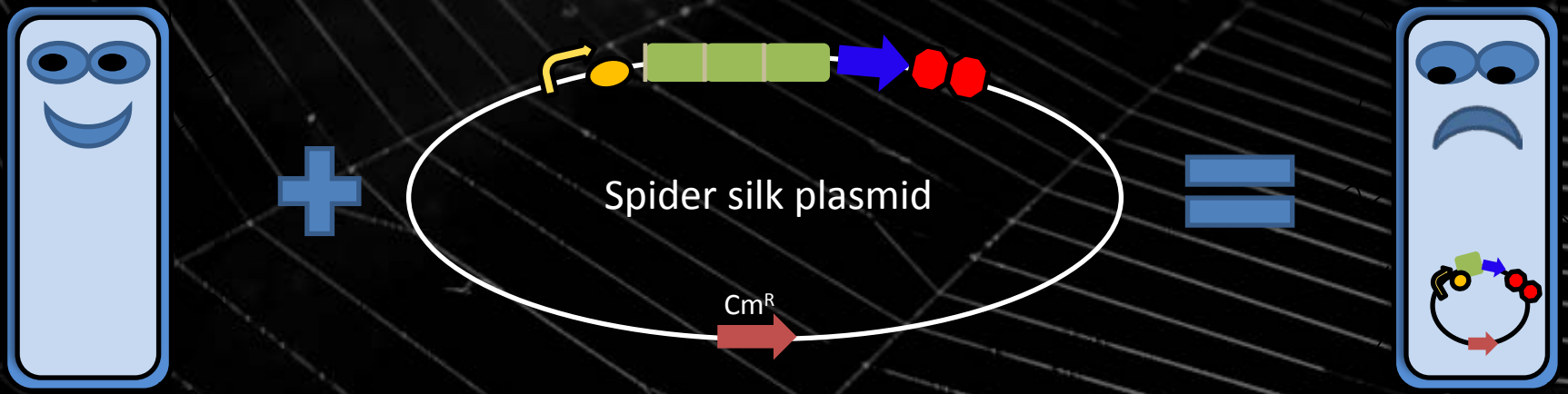
β - Helix

Spider silk amino acid composition

Amino Acid	Count	% Composition
Glycine (G)	30	44.1
Glutamine (Q)	12	17.6
Proline (P)	10	14.7
Alanine (A)	8	11.8
Serine (S)	5	7.4
Tyrosine (Y)	3	4.4

- The silk gene uses only six of the twenty different amino acids
- 44% of the amino acids are glycine, so having enough intracellular glycine is important for production

Designing a system to produce silk



- The bacteria works hard to produce the silk
- When the *E. coli* is overworked it no longer has sufficient tRNA's to make proteins it needs to survive, and product yield decreases

Amino Acid	% Composition	Codon	Unoptimized (W)	Fewest (F)	Balanced (B)
Ala (A):	11.80	GCT	25	0	0
		GCC	25	0	0
		GCA	25	100	100
		GCG	25	0	0
Gln (Q):	17.6	CAA	33	100	100
		CAG	67	0	0
Gly (G):	44.1	GGT	57	100	50
		GGC	40	0	0
		GGA	0	0	50
		GGG	3	0	0
Pro (P):	14.7	CCT	0	100	50
		CCC	0	0	0
		CCA	0	0	50
		CCG	100	0	0
Ser (S):	7.4	TCT	0	0	40
		TCC	0	0	0
		TCA	0	0	0
		TCG	0	0	0
		AGT	0	100	60
		AGC	100	0	0
Tyr (Y):	4.4	TAT	100	100	100
		TAC	0	0	0

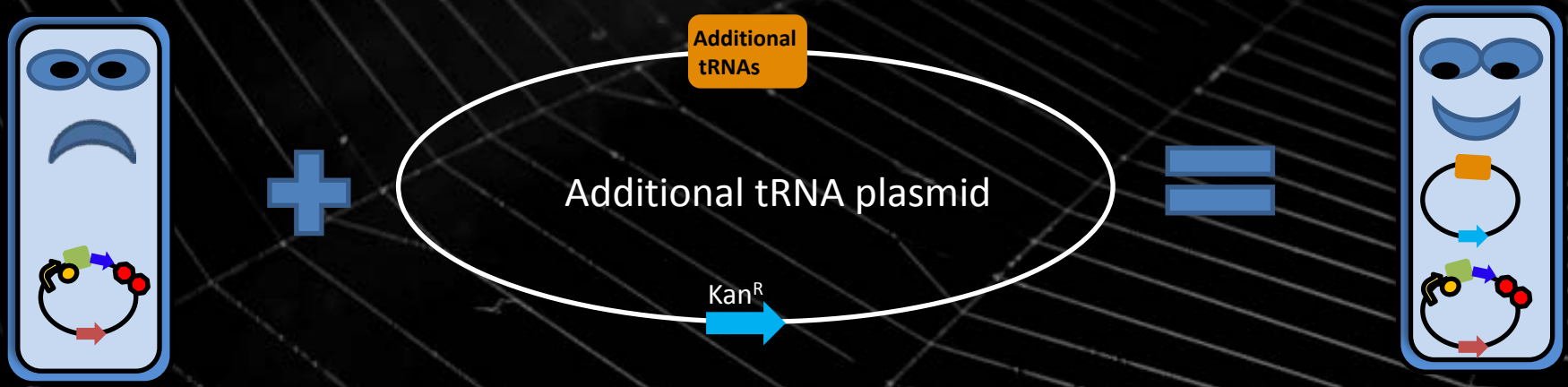
tRNAs

DNA construct containing the six tRNAs for enhanced spider silk expression in ‘F’ construct

G-Glycine (GGT codon)
Q-Glutamine (CAA codon)
P-Proline (CCT codon)
A-Alanine (GCA codon)
S- Serine (AGT codon)
Y-Tyrosine (TAT codon)

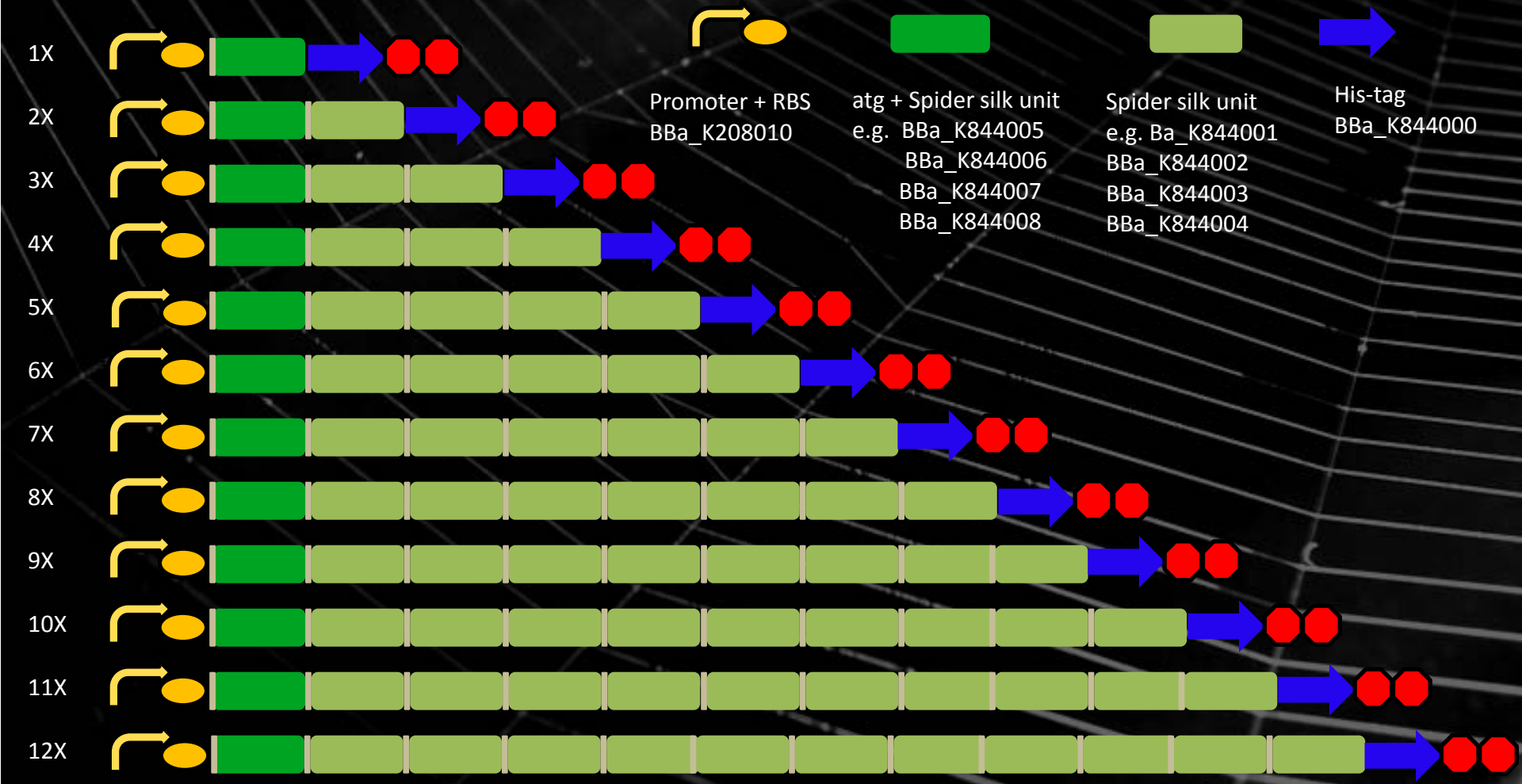
Unoptimized (W)	Fewest (F)
25	0
25	0
25	100
25	0
33	100
67	0
57	100
40	0
0	0
3	0
0	100
0	0
0	0
100	0
0	0
0	0
0	0
0	0
0	100
100	0
100	100
0	0

The benefit of tRNAs



- With additional tRNAs the bacteria should be able to handle our spider silk BioBricks
- The tRNA plasmid is pSB3K3

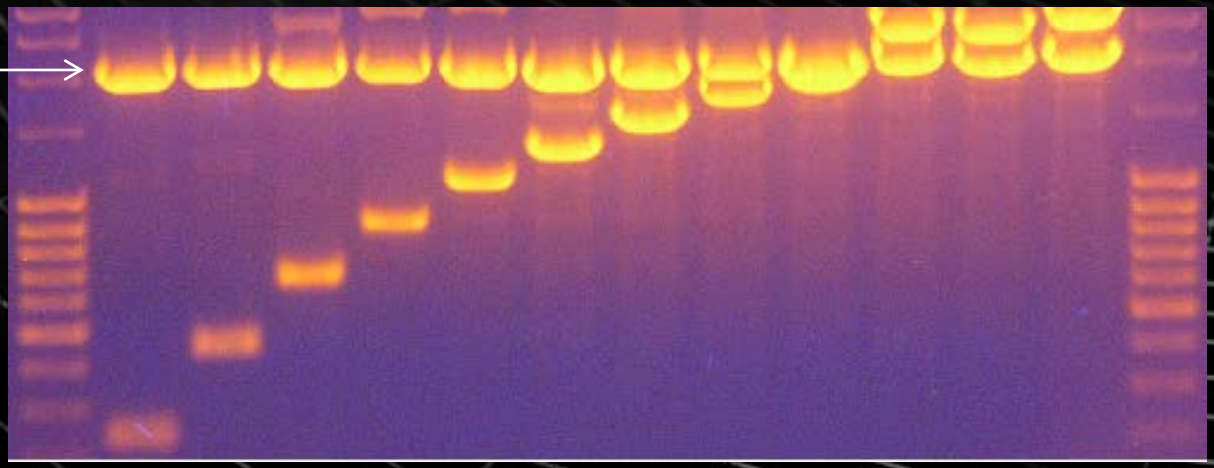
Addition of repeating units



Spider steps

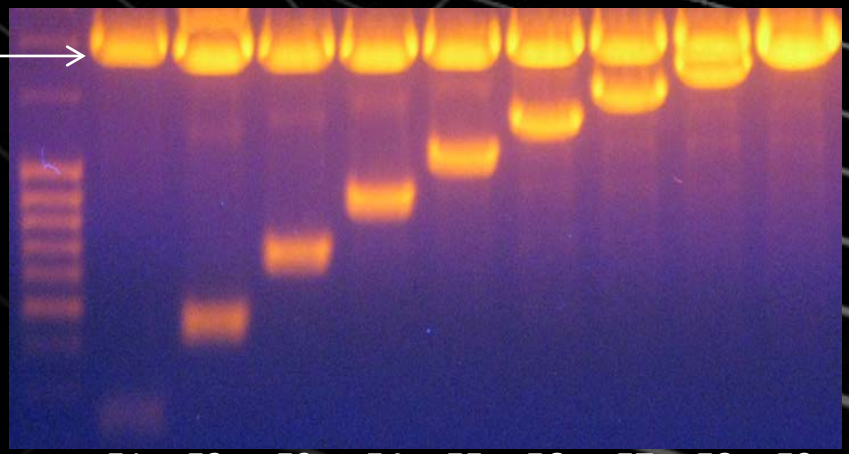
MassRuler DNA ladder

Linearized pSB1C3 vectors with spider silk gene insert cut out



B1 B2 B3 B4 B5 B6 B7 B8 B9 B12 B13 B14

Linearized pSB1C3 vectors with spider silk gene insert cut out

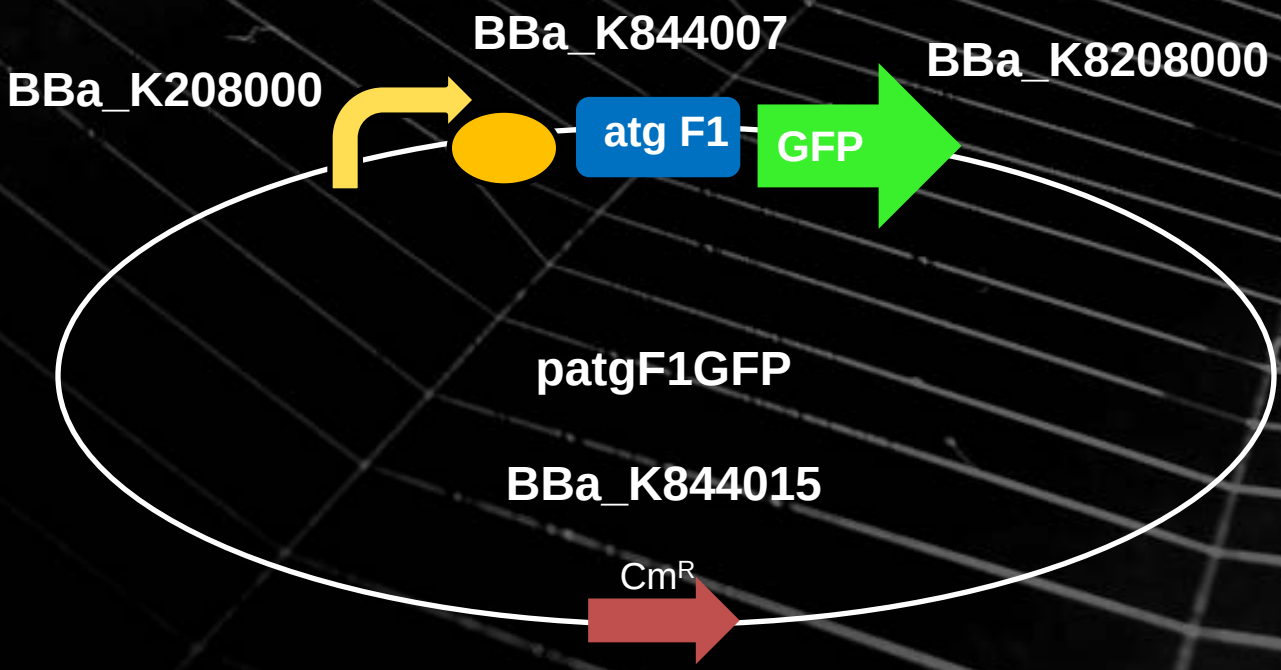


F1 F2 F3 F4 F5 F6 F7 F8 F9

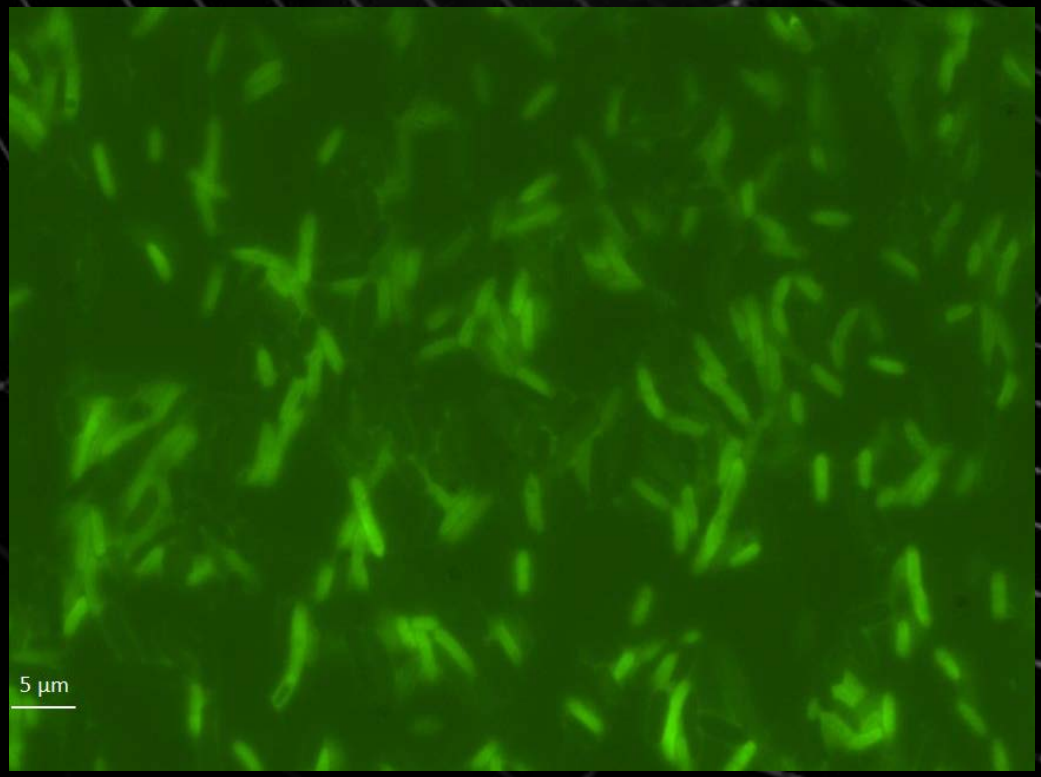
"Spider Step" Inserts

The first BioBrick Silk-GFP fusion protein

- To demonstrate the possibility of fluorescently tagging spider silk, the F1 spider silk protein construct was fused to GFP



Spider Silk GFP fusion protein



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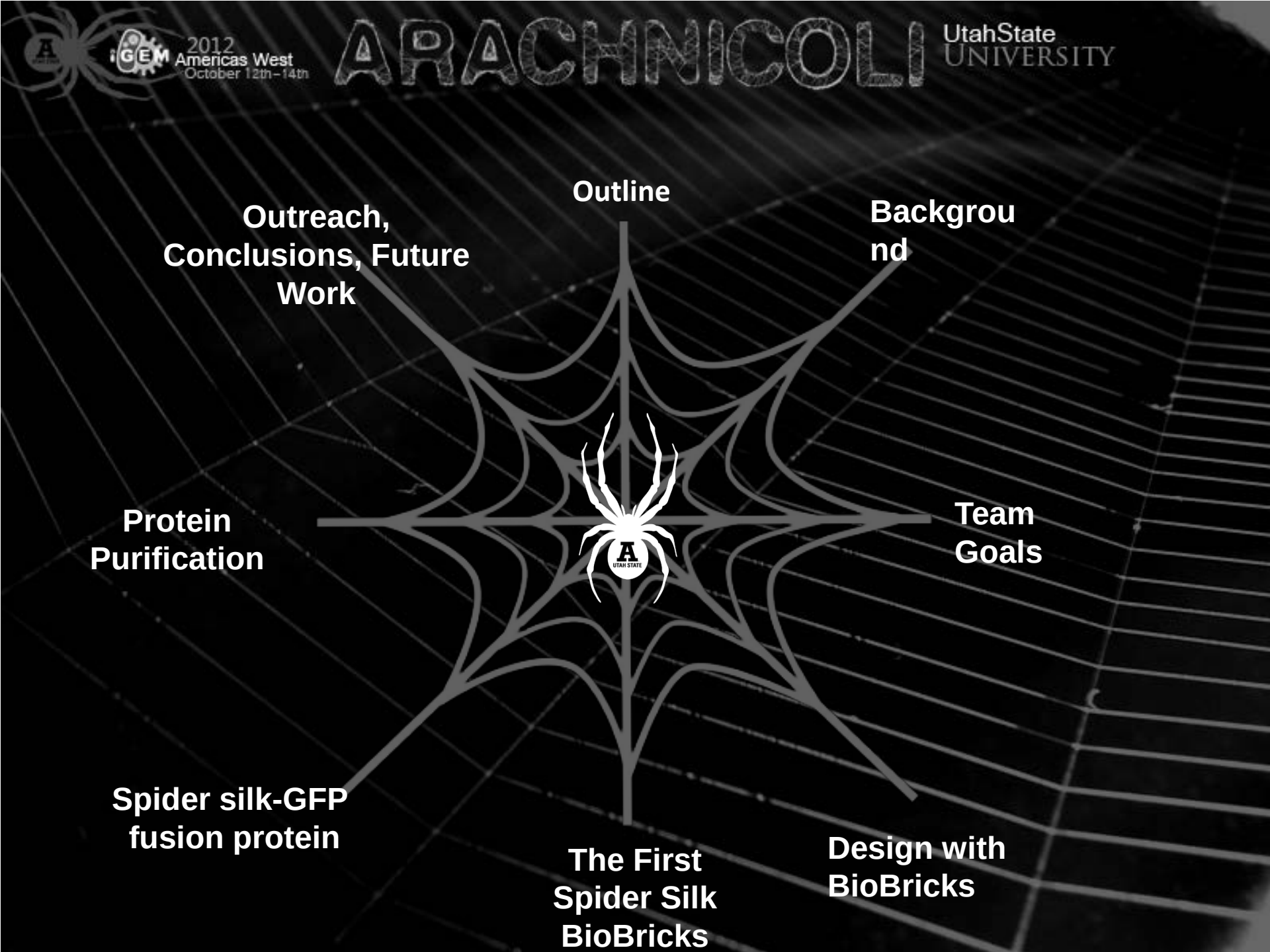
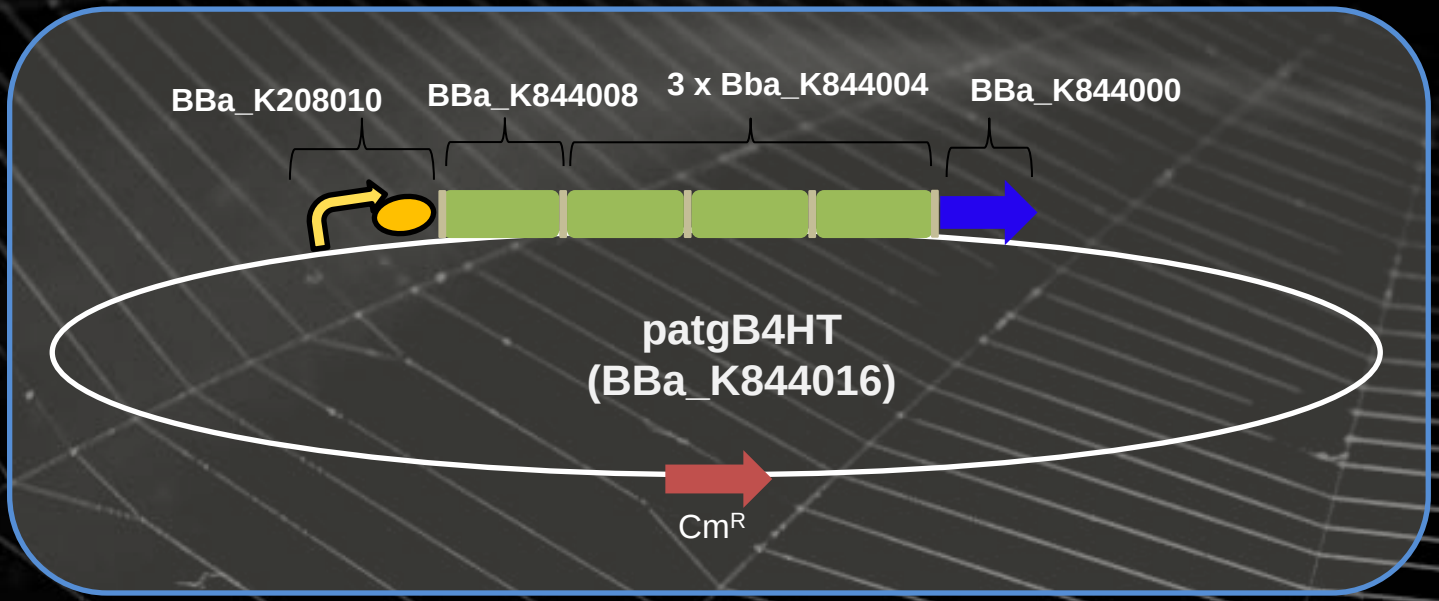
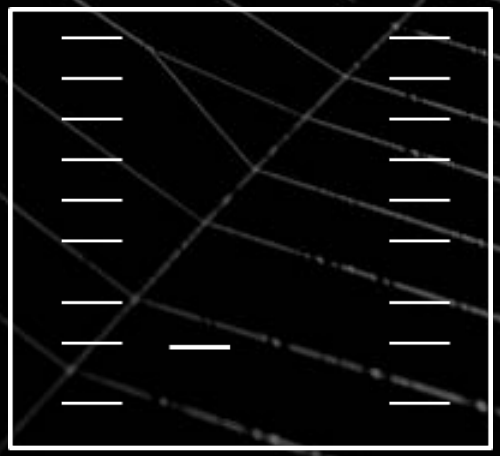




1. *E.coli* producing spider silk

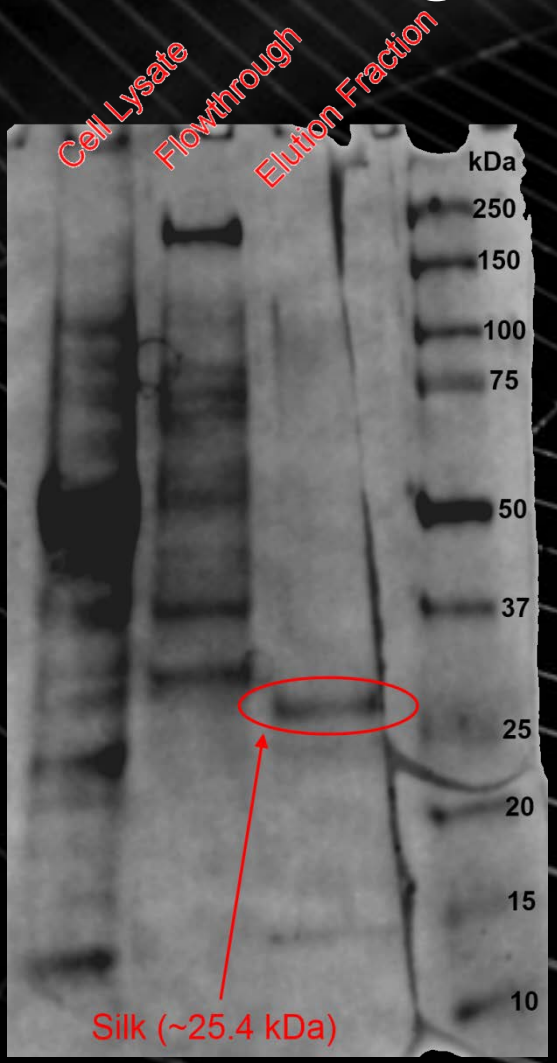
2. Purify spider silk using Nickel column



3. Run protein gel to verify purity

4. Spin spider silk to make fibers

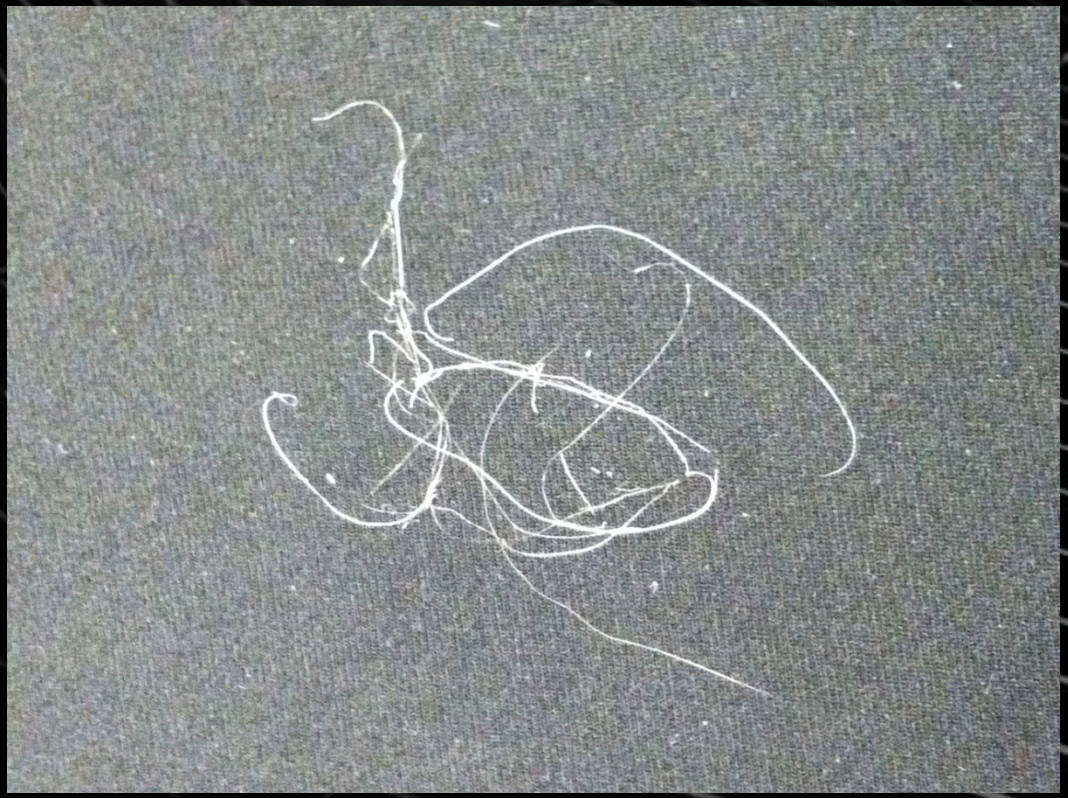
Protein gel



Coomassie blue stained SDS PAGE gel

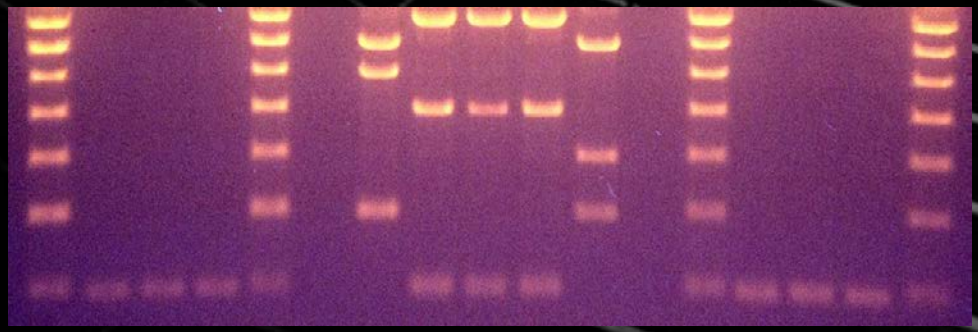

Got
Silk?

First spun spider silk from BioBricks



Team Successes

- First ever spider silk BioBrick parts
- First spun spider silk fiber from composite BioBrick part
- Improved His-tag for better protein purification
- First spider silk GFP fusion protein from BioBrick parts



BioBrick parts

			Name	Type	Description	Designer	Length
❤		W	BBa_K844000	Tag	10x-Histidine (10x-His) Tag with double stop codon (TAATAA)	Kathleen Miller	36
❤		W	BBa_K844015	Generator	lac/IPTG inducible Spider Silk 1x "F" Subunit fused to GFP	Brian Smith	1158
❤		W	BBa_K844016	Generator	Spider Silk Generator - 4x "B" Silk Construct with His Tag	Brian Smith	1116
			BBa_K844001	Coding	Spider Silk 1x 1E Subunit "U" (native sequence)	Ryan Putman	120
			BBa_K844002	Coding	Spider Silk 1x Subunit "W" (native sequence)	Federico Carlos Rodriguez	204
			BBa_K844003	Coding	Spider Silk 1x Subunit "F" (Fewest tRNA codon optimized)	Charles Barentine	204
			BBa_K844004	Coding	Spider Silk 1x Subunit "B" (Balanced tRNA codon optimized)	Andrea Halling	204
			BBa_K844005	Coding	Spider Silk 1x 1E Subunit "U" with Met (ATG) start codon	Ryan Putman	123
			BBa_K844006	Coding	Spider Silk 1x Subunit "W" with Met (ATG) start codon	Federico Carlos Rodriguez	207
			BBa_K844007	Coding	Spider Silk 1x Subunit "F" (Fewest tRNA codon optimized) with Met (ATG) added	Charles Barentine	207
			BBa_K844008	Coding	Spider Silk 1x Subunit "B" (Balanced tRNA codon optimized) with Met (ATG) added	Andrea Halling	207
			BBa_K844010	Regulatory	Enhanced tRNA Promoter for "E. coli"	Kathleen Miller	40
			BBa_K844011	Terminator	tRNA Terminator for "E. coli"	Kathleen Miller	15
			BBa_K844012	Generator	tRNA expression cassette for spider silk "F" proteins	Andrea Halling	812
			BBa_K844013	Generator	tRNA expression cassette for spider silk "B" proteins	Ryan Putman	668

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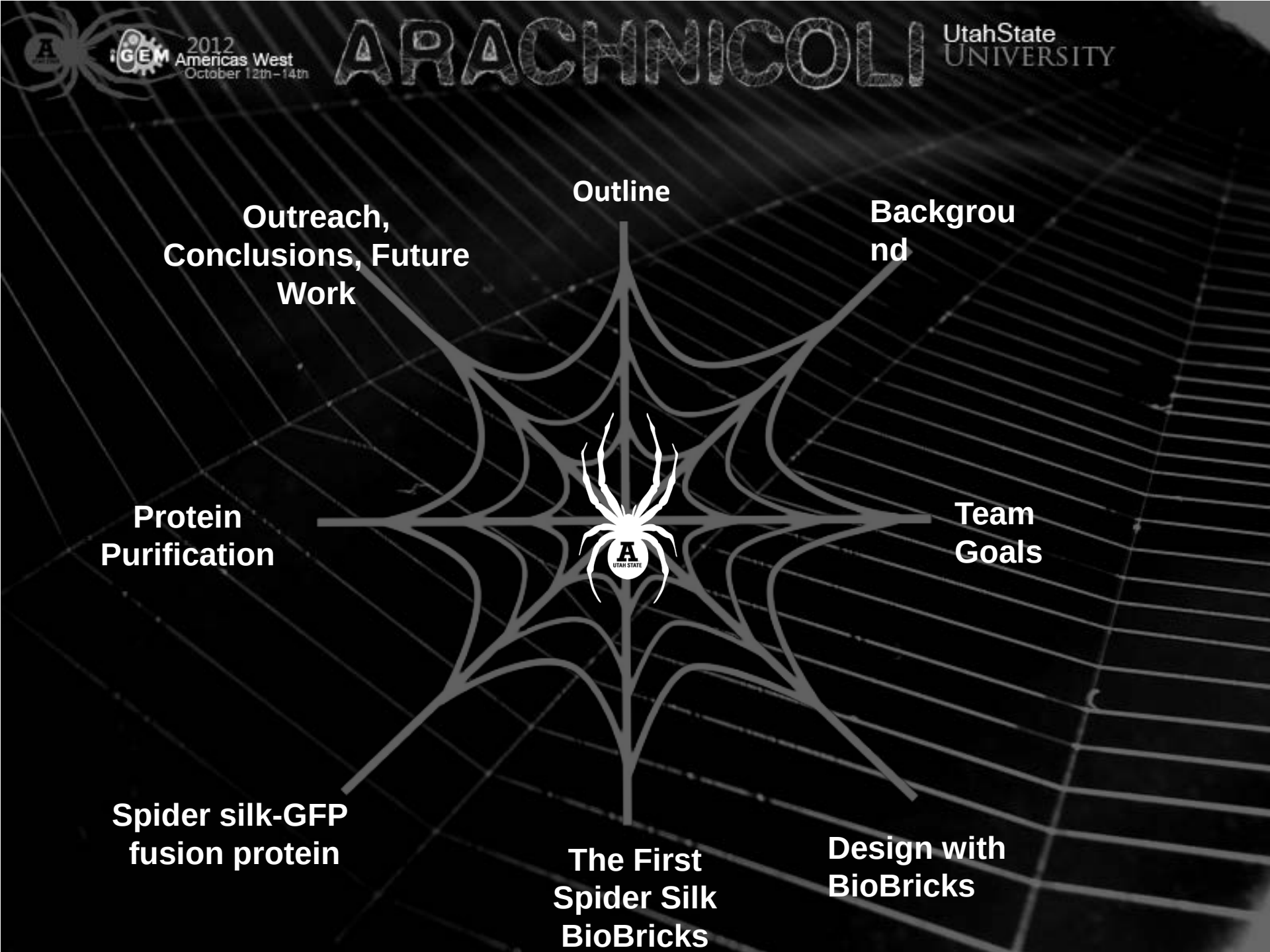
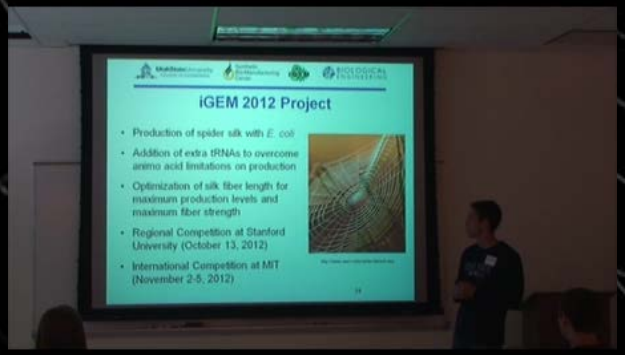
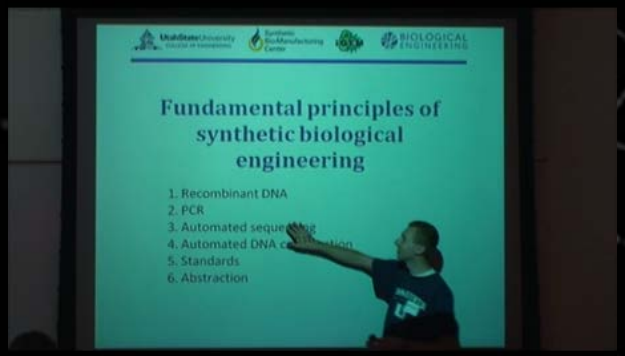


High School Outreach

- Outreach to High Schools from Utah and Idaho

Discover Biological Engineering





Engineering State

Human Practices

Ethical Considerations

Public opinion

E. Coli Containment

Increase public support by producing a safe, beneficial product

After silk production and collection, *E. coli* cultures are autoclaved

"With great power, comes great responsibility..."

-Uncle Ben

Product distribution avoiding *E. coli* contamination

Dual Use - Can this open source technology be used negatively?

E.coli is used as the factory, but only purified silk is used to develop products

Discussed the project with Research Integrity & Compliance Officer

Acknowledgements

First year we had team members from four different institutions:

Cooper Union, NY

University of Utah, UT

Logan High School, UT

Utah State University

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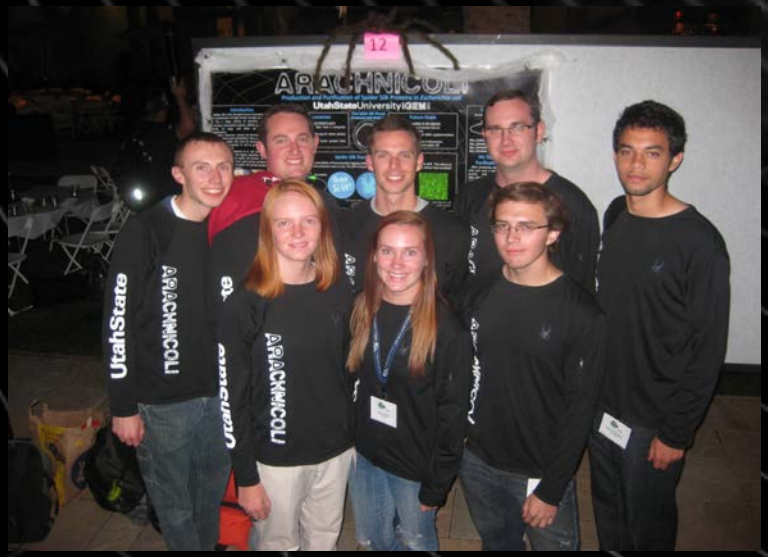

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Sponsors

SPYDER

GenScript
Transforming Biology Research

PBI Pressure BioSciences Inc.

IDT
INTEGRATED DNA TECHNOLOGIES

Synthetic Bio-Manufacturing Center

SWBEC
Sustainable Waste-to-Bioproducts Engineering Center

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Questions?