SPIDERMAN | SPIDrain Engineering with chromoprotein and natural dyes
I agree
Yes of course
Well...
Agreed
What would be the most optimal material to make a battle suit?
It's unfair...
WHY SPIDER SILK?

Strength, stiffness, toughness, and extensibility

Light and has great air permeability

Fully biodegradable
Comparison of the toughness of different materials

(Bourzac et al. 2015)
How to get spider silk for Spiderman suit?
Microorganisms support large scale fermentation and production.

Spiders are not applicable to large scale farming.

Spider silk DNA

1807 Simon Peers collection (Perkel et al. 2014)
HOW TO WEAVE A SPIDERMAN SUIT?

Spider silk spinning

Silk dyeing with pigments
SPLIDroin EngineeRing with cheloprotein And Natural dyes
How to produce spider silk?
pH and ion concentration cause NT and CT to trigger of silk formation

Rep region contains high levels of Alanine and Glycine, contributing to silk strength

(Andersson et al. 2017) (Ling et al. 2019)
Professor Anna Rising

- Provided reference papers
- Suggested us to use the combination of NT-2Rep-CT

(E. coli BL21(DE3))

(Andersson et al. 2017)
Purification by Ni-NTA method and yield

Average = 336 mg/L
= 46 mg/g
Spinning Dope

Silk fiber

coagulating bath
shear force

β-sheet conformation

(Otikovs et al. 2017)
Fiber spinning in coagulation bath

<table>
<thead>
<tr>
<th>pH</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatinous</td>
<td>Accumulation</td>
<td>Fiber-like</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

100% Isopropanol

Continuous fiber

(Heidebrecht *et al.* 2015)
Our hardware prototype

- Constant speed spinning wheel
- Stepper motor
- Beaker with spinning buffer
- Console
Visiting Professor Mengqing at Donghua University, Shanghai, China

1. Shear force needs to be applied
2. Longer time in coagulation bath
3. Optimum speed spinning wheel
Our Improved Hardware

- **Spinning wheel:** speed can be adjusted
- **Stepper motor**
- **Console**
- **Long tank for spinning fiber to form and be stabilized**
Coagulation bath

Flow rate: 0.7 mg/min

Spinning wheel
Our Fiber Spun by NT2RepCT spidroin

Diameter = 17.6 μm
More recombinant spidroins made!

(Zhou et. al. 2018)

Argiope trifasciata

Euprosthenops australis
Purification and comparison with NT-2Rep-CT
By NTWCT spidroin

Diameter = 7.9 μm

By NT3WCT spidroin

Diameter = 7.9 μm
How to color our silk?
Tour of factory to better understand dyeing process

Clothes dyeing factory in Quanzhou, China
Our finalized hardware

Clothes dyeing machine in Quanzhou, China

Dyeing tank added after spinning wheel
Environmental Damage

1. Water pollution
2. Heavy metal
3. Not degradable

Waste water treatment facility in Quanzhou, China
Use natural dye!
Our natural dye

Glucose → Tryptophan → Indole → Indoxyl → Indigo

tnaA, vioA, vioB, vioE, fmo, vioC

Protodeoxyviolacein acid → Deoxyviolacein

(Rodrigues et al. 2013)  (Hsu et al. 2018)
Our production
Deoxyviolacein Yield: 85.81±9.09 mg/L

Indigo Yield: 6.97±0.44 mg/L
Dyeing our silk with deoxyviolacein:

Original Silk

Silk with deoxyviolacein dye

Water bath
Chromoprotein Coloring
pET28a

- **sfGFP**
- **NT**
- **His-tag**
- **2Rep**
- **CT**

pET28a

- **NT**
- **2Rep**
- **CT**
- **sfGFP**

**MW kDa**

- 170
- 130
- 100
- 70
- 55
- 45
- 35
- 25

**Proteins**

- NT-2Rep-CT-sfGFP
- NT-2Rep-CT
- sfGFP-NT-2Rep-CT

**M**
A) sfGFP-fused spidroins

B) sfGFP-fused spider silk spinning at concentration 40 mg/ml

C) sfGFP-fused spider silk under UV light
sfGFP-NT2RepCT

Continuous sfGFP-fused spider silk

NT2RepCT-sfGFP

Discontinuous sfGFP-fused spider silk
However with eforRed...

Protein extraction failed
Indirect dying:
Rep-Chromoprotein + Spidroin

Rep-chromoprotein mixed silk formation mechanism
90% NT2RepCT
10% sfGFP

\[ \Phi = 25.49 \, \mu m \]

90% NT2RepCT
10% 2Rep-sfGFP

\[ \Phi = 9.57 \, \mu m \]

Recombinant sfGFP-fused spider silk under microscope
Recombinant eforRed-fused spider silk under microscope

95% NT2RepCT
5% eforRed

Φ = 31.65 µm

95% NT2RepCT
5% 2Rep-eforRed

Φ = 24.74 µm
Recombinant amilCP-fused spider silk spinning

90% NT2RepCT
10% 2Rep-amilCP

2 mm
Chromoprotein Mixture Dyeing

β-sheet

Colored Silk fiber
Entrepreneurship
Introducing our minimal viable product (MVP)

- Hermosa pink eforRed+sfGFP
- Pure White No chromoprotein
- Fluorescent green sfGFP
- Pink eforRed
- Orange sfGFP+eforRed
- Yellow-green sfGFP
- Small scale (automatic) fiber spinning machine
## Competitors and SWOT analysis

<table>
<thead>
<tr>
<th><strong>Strength:</strong></th>
<th><strong>Weakness:</strong></th>
</tr>
</thead>
</table>
| • Chromoprotein dying  
• Flexible DNA model for design | • High threshold for entering the market  
• Difficult to increase scale of production |

<table>
<thead>
<tr>
<th><strong>Opportunities:</strong></th>
<th><strong>Threat:</strong></th>
</tr>
</thead>
</table>
| • Growing need for innovative ideas for textile | • Hard to lower the price  
• Low trust for GMO |
Core Technologies

- We create coloring assembly for chromoprotein dying, many future extension
How to publicize our products?

• What kind of biosynthetic products will the public find the most approachable?

• What kind of language might be more acceptable to the public?
Public surveys

How well can you accept GMO soybean oil? (sample size 280)

- Don’t care: 26.6%
- Cannot Accept: 29.2%
- Accept: 44.2%

How well can you accept GMO textile such as spider silk? (sample size 280)

- Don’t care: 20.0%
- Cannot Accept: 6.1%
- Accept: 73.9%

- Public acceptance for GMO textile > Public acceptance for GMO food
Public Concerns

Natural dye artist in Alemany farm, San Francisco

“Humans haven’t developed sustainable and controllable way to manage the technologies for GMO”

Xiaolei, natural indigo dye artist, Shenzhen, China

“The culture tradition have to be carried on by someone, or it will be lost.”

“Unnatural dye is what I cannot accept, even though it may be the trend for future”
Interaction with Xiaolei

We presented Xiaolei with some of our synthesized pigment, and she demonstrated traditional indigo tie-dye with few members in our team.
Interaction with Xiaolei

The Xiaolei came back she used our deoxyviolacein to dye a dress

“The color of the dye is better I have expected, the quality of color fixation is good too.”

- All the dyed materials STRICTLY FOLLOWED the do not release policy
Interaction with Xiaolei
Other Public Engagement

Public speech in 4 schools

Workshops in our Lab
HOW TO WEAVE A SPIDERMAN SUIT?
Achievements

Successful spider silk protein purification

Well-designed hardware for automatic silk spinning

Elaborate-planned entrepreneurship

Synthesis of natural dyes indigo and deoxyviolacein

Novel chromoprotein method for coloring our spider silks.

Meaningful integrated human practices and public engagement
## Our Functional Spidroin Part Collection

<table>
<thead>
<tr>
<th>Name</th>
<th>Part Number</th>
<th>Part description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT2RepCT</td>
<td>BBa_K3264000</td>
<td>Recombinant spidroin</td>
</tr>
<tr>
<td>NT</td>
<td>BBa_K3264001</td>
<td>Protein domain of spidroin</td>
</tr>
<tr>
<td>CT</td>
<td>BBa_K3264002</td>
<td>Protein domain of spidroin</td>
</tr>
<tr>
<td>Rep</td>
<td>BBa_K3264003</td>
<td>Protein domain of spidroin</td>
</tr>
<tr>
<td>W</td>
<td>BBa_K3264004</td>
<td>Protein domain of spidroin</td>
</tr>
<tr>
<td>NT4RepCT</td>
<td>BBa_K3264005</td>
<td>Developed recombinant spidroin with more extensive repetitive added.</td>
</tr>
<tr>
<td>NTWCT</td>
<td>BBa_K3264007</td>
<td>Recombinant spidroin</td>
</tr>
<tr>
<td>NT3WCT</td>
<td>BBa_K3264009</td>
<td>Developed recombinant spidroin with more extensive repetitive added.</td>
</tr>
<tr>
<td>sfGFP-NT-2Rep-CT</td>
<td>BBa_K3264018</td>
<td>Developed recombinant spidroin with more chromoprotein added.</td>
</tr>
<tr>
<td>NT-2Rep-CT-sfGFP</td>
<td>BBa_K3264019</td>
<td>Developed recombinant spidroin with more chromoprotein added.</td>
</tr>
<tr>
<td>2Rep-amilCP</td>
<td>BBa_K3264012</td>
<td>Recombinant chromoprotein for coloring the silks formed by spidroin.</td>
</tr>
<tr>
<td>2Rep-sfGFP</td>
<td>BBa_K3264013</td>
<td>Recombinant chromoprotein for coloring the silks formed by spidroin.</td>
</tr>
<tr>
<td>2Rep-eforRed</td>
<td>BBa_K3264014</td>
<td>Recombinant chromoprotein for coloring the silks formed by spidroin.</td>
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</table>
Acknowledgements

Professor Anna Rising  
Professor Qing Meng  
Colorist Xiaolei
Thank You!
References:

Any questions?