

SPLIT INTEIN SYNTHETIC BIOLOGY PLATFORM FOR SCALABLE MICROBIAL PRODUCTION OF HIGH- PERFORMANCE BIOMIMETIC TEXTILES, ADHESIVES AND OTHER MATERIALS

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Brief Description

A team of engineers at Washington University in St. Louis have developed a synthetic biology platform for low-cost, sustainable production of high-performance composite materials. This technology uses a split intein assembly system to synthesize high molecular weight polymers (e.g., spider silk and mussel foot adhesive) that cannot be made with traditional microbial techniques.

Problem

Most synthetic polymers on the market today are derived from petroleum. Biosynthesis of these materials from microorganisms using low-cost, abundant feedstocks could offer a scalable, environmentally-friendly alternative production method. In addition, nature has evolved a wide array of protein-based materials with delicate nano-scale structures that could confer high performance properties to composite materials such as silk, elastin, collagen and adhesives. The remarkable properties (e.g., strength, elasticity and adhesiveness) of these polymers arise from the multi-scale assembly of a narrow set of basic, repetitive peptide sequences. In other words, the more repeats that are assembled, the higher the molecular weight and the higher the molecular weight, the better the performance.

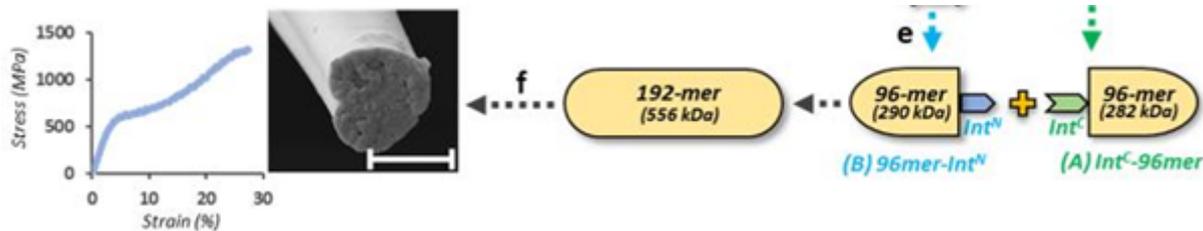
In many cases, if bacterial cell factories could be engineered to stably and efficiently produce larger proteins, those proteins can outperform current biosynthetic materials with lower cost and more sustainable production than petroleum-based polymers. However, it has not been possible to engineer recombinant microbes to produce high molecular weight biosynthetic polymers using traditional genetic techniques.

Solution

This technology utilizes split intein-mediated ligation to assemble high molecular weight proteins from smaller subunits. Because the ligation occurs post-translation, large polymers can be stably produced by *E. coli*. This system enables reliable production of large proteins with high-performance properties beyond their natural counterparts or with novel functions. The inventors have demonstrated this approach in two use cases: microbially produced spider silk fibers that are as strong and tough as natural spider silk; and mussel foot proteins (Mfps) with higher underwater adhesivity than natural Mfps. The general split intein platform offers the opportunity to provide unlimited supplies of natural materials from inexpensive, renewable feedstocks, presenting a sustainable and low-cost alternative to

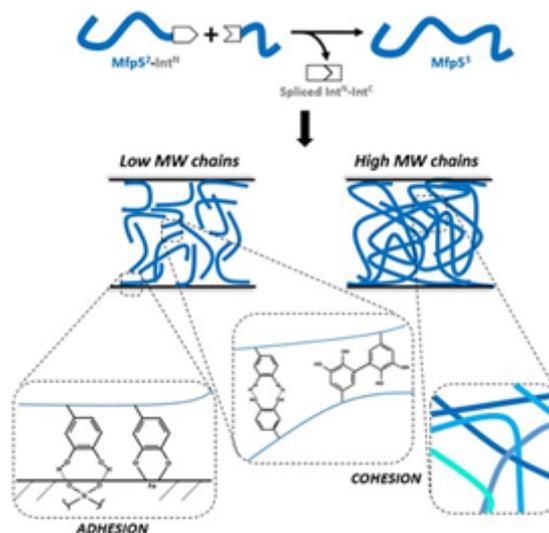
petroleum polymers for numerous mechanically demanding applications.

Spider silk proteins with unprecedented molecular weight and mechanical properties.



Spider silk is a natural material that is as strong as some steel alloys with toughness greater than Kevlar. The inventors reduced the highly repetitive core of a natural dragline silk protein to a single common unit repeated 96 times (“96-mer”). Using the split intein technology, two 96-mers can be produced separately in *E. coli* and then ligated together to yield a 192-mer, 556 kDa spidroin product (about twice as large as any previous biosynthetic spider silk). Fibers spun from this 192-mer protein replicated the unparalleled combination of high strength and toughness exhibited by natural spider silks.

Strong underwater adhesives using engineered microbes.



Mfp5 has the highest adhesion strength of mussel foot proteins (Mfps), a family of repetitive proteins that help mussels strongly adhere to a variety of surfaces while underwater. High molecular weight Mfps are expected to entangle into a more robust network of interactions than low molecular weight proteins. However, large Mfps are difficult to produce synthetically in *E. coli* due to limitations in the number of covalently bonded Mfp units. To make an Mfp5 molecule with multiple units, the inventors produced a 1-unit Mfp5 and 2-unit Mfp separately in microbes. Then, using split inteins, these two smaller components were covalently joined to form a large 3-unit Mfp5 protein (i.e., Mfp5³). The adhesion forces of the resulting Mfp5³ were comparable or greater than previously reported Mfp-mimetic adhesives.

Key Advantages

- **Low-cost, sustainable production** – microbial production from readily available, inexpensive feedstock (e.g., cellulosic biomass and simple sugars)
- **Scalable process**
- **High-performance materials** – producing a high molecular weight product enhances performance in a variety of systems, for example:

- biosynthetic spider silk with superior mechanical properties (high tensile strength, toughness, elastic modulus and extensibility)
- biosynthetic mussel foot protein with superior underwater adhesion
- **Tunable properties** - biosynthetic production:
 - allows for editing the protein sequences and engineering the material assembly to fine-tune the properties of the material
 - enables novel applications not possible with conventional production

Applications

- **Synthetic biology** – microbial production of biosynthetic replacements for petroleum-based fibers, adhesives and other composite materials
- **End user applications for high-performance biosynthetic spider silk:**
 - Defense - impact resistant textiles such as body armor/bullet-proof clothing
 - Consumer textiles – high performance clothing
 - Medical – surgical sutures for wound closure
 - Aerospace - lightweight cables and ropes
 - Automotive – airbags and seatbelts
- **End user applications for high-performance biosynthetic mussel foot protein:**
 - Adhesives, particularly for underwater repair
 - Medical – surgical glue
 - Coatings

Stage of Research

The inventors have demonstrated microbial production with split intein assembly for two different high-performance biomimetic materials, high strength spider silk and high adhesive mussel foot protein.

- **Biosynthetic spider silk** – The inventors produced large recombinant spidroins (556 kDa with 192 repeat motifs) in *E. coli* and spun it into silk fibers that replicated the mechanical properties of their natural counterparts: tensile strength (1.03 ± 0.11 GPa), modulus (13.7 ± 3.0 GPa), extensibility ($18 \pm 6\%$), and toughness (114 ± 51 MJ/m³). They continue their research to improve the process, improving scalability, lowering costs and increasing efficiency.
- **Synthetic mussel foot adhesive** – The inventors used *E. coli* to produce high molecular weight synthetic Mfp proteins (3 consecutive Mfp5 sequences) with high underwater adhesive properties: DOPA-modified Mfp5³ displayed a high adhesion force (201 ± 36 nN μm^{-1}) and a high adhesion work (68 ± 21 fJ μm^{-1}) for 200 s cure times, which are higher than those of previously reported Mfp-mimetic adhesives. In addition, they observed a positive correlation between Mfp5's molecular weight and its measured adhesion force, offering a path to engineering even stronger underwater adhesives.

Publications

- Bowen, C. H., Dai, B., Sargent, C. J., Bai, W., Ladiwala, P., Feng, H., ... & Zhang, F. (2018). [Recombinant spidroins fully replicate primary mechanical properties of natural spider silk.](#) *Biomacromolecules*, 19(9), 3853-3860.
- Kim, E., Dai, B., Qiao, J. B., Li, W., Fortner, J. D., & Zhang, F. (2018). [Microbially Synthesized Repeats of Mussel Foot Protein Display Enhanced Underwater Adhesion.](#) *ACS applied materials & interfaces*, 10(49), 43003-43012.

Patent Application

- [Split intein mediated polymerization and production of mussel foot adhesive protein materials](#)
(U.S. Patent Application Publication No. US20200071368A1)

Website

- [Zhang Lab](#)