

# Production of Recombinant Spider Silk

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## Perspective

In comparison to other fibre materials, spider silk threads exhibit remarkable mechanical qualities such as toughness, flexibility, and low density. Even compared to kevlar and steel, they outperform them. Long lengths and particular protein structures are the source of these exceptional properties. More than 20,000 amino acids are found in spider silk proteins. Polypeptide stretches make up more than 90% of the protein and can be repeated hundreds of times. The final qualities of the silk are determined by the function of each repetition unit. These characteristics make them appealing for medical and technical product development, as well as cosmetics.

Due to the cannibalistic behaviour of spiders, it is not viable to get huge volumes of silk through livestock rearing of spiders. Recombinant production is attempted in diverse expression systems such as plants, bacteria, yeasts, insects, silkworms, mammalian cells, and mammals in order to obtain spider silk proteins (spidroins) on a large scale. Cost-effective and efficient production systems are required for large-scale production to be viable. The several varieties of spider silk, their proteins, and architectures are described in this paper, as well as the generation of these difficult-to-express proteins in various host organisms, with a focus on plant systems.

Engineered *E. coli* has successfully created recombinant spidroins that are comparable in size and mechanical function to native silk proteins. Transgenic plants, rice, and alfalfa have all been used to express recombinant spidroins. In comparison to other host systems, these expression platforms may offer increased industrial-scale economic feasibility. Recombinant spidroin genes were incorporated into the genome of *B. mori* using CRISPR/Cas9-based genome editing. The chimeric fibres spun by the silkworms had a tensile strength that was comparable to native spider silk.

Somatic cell nuclear transfer was used to create transgenic sheep embryos carrying a recombinant spidroin gene. A hair-follicle specific promoter was used to express recombinant spidroins within wool fibres in the transgenic embryos. Transgenic progeny, on the other hand, have yet to be obtained. The mechanical properties of spider silk are well-known. It is one of the strongest biomaterials known, outperforming steel and Kevlar in terms of mechanical qualities. Farming spiders for silk, on the other hand, is impossible. As a result, producing recombinant spider silk proteins (spidroins) in more hospitable hosts is a fascinating subject of study. A

heterologous silk production system that is both highly efficient and cost effective is required for large-scale production to be sustainable. In addition to many other platforms, genes expressing recombinant spidroins have been expressed in bacterial, yeast, insect, and mammalian cells. The recent developments in leveraging an increasingly varied variety of host platforms in the heterologous creation of recombinant spidroins are discussed in this study.

## Spider silk's enchantment

Few materials have captivated the human imagination in the same way that spider silk has, from Greek tales to the realities of current synthetic biology. Spider silk has an amazing combination of mechanical qualities, including strength, hardness, and elasticity, despite its delicate look. Spider silk has been proven to be three times stronger than Kevlar and five times stronger than steel (on a weight-for-weight basis). Spider silk proteins are also soluble in water, biocompatible, and biodegradable. Spider silk, particularly dragline silk (see Glossary), has been used for a variety of applications, ranging from battery components to tissue scaffolds, because of its exceptional characteristics.

## Protein structure in spider silk

Spider silk proteins are commonly referred to as spidroins, a combination of spider and fibroin. Spidroins include three different domains: a glycine- and alanine-rich repeating core domain, bookended by nonrepetitive N- and C-terminal domains, and are typically between 250 and 350 kDa in size. The terminal domains each have about 100 amino acid residues and are highly conserved across species. The repeating domain, on the other hand, might contain hundreds of repeat modular units and shows sequence variety between the seven primary types of silk. The repeated region's modular units are typically made up of crystalline polyA or polyAG motifs, which provide strength, or less crystalline GGX or GPGXX motifs, which provide flexibility. To optimise the creation of recombinant spidroins, a range of synthetic biology approaches have been used, including bioinformatics, directed evolution, and the utilisation of diverse host systems. In addition to many other platforms, recombinant spidroins have been generated in bacterial, yeast, mammalian, and insect cells. The development of recombinant spidroins is still a hot topic in science. Mechanical qualities and innovative applications of silk fibres made with recombinant spidroins have gotten a lot of attention. As a result, little thought has been given to how the host system chosen may affect the production efficiency, mechanical characteristics, and commercialization of recombinant silk fibres and their constituent spidroins.

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