# ESR 1

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| **Project title and research strand:** | Fibroin from tobacco cells for medical use.<br>Strand 2: Fibers for medical application. |  |
| **Name:** | Lara Bitar | |
| **Supervisors, affiliation:** | Luisa Bortesi; Maastricht University (NL)<br>Alexander Kopp; Fibrothelium (DE) | |

## Abstract

Fibroin, the main component of silkworms silk, is a natural biocompatible and biodegradable material with exceptional mechanical properties what makes it a valuable material in the biomedical industry. However, the process of recovering fibroin from Bombyx mori silk has quality, purity, sustainability and ethical drawbacks. Therefore, the necessity to develop a more sustainable production platform for recombinant vegan fibroin of improved quality. The fact that fibroin is a large and repetitive protein –at both the nucleic acid and amino acid levels- poses serious challenges to its recombinant production. We propose an optimized plant-based expression system for the recombinant production of fibroin. By combining two strategies: a DNA mediated assembly, [generating a versatile library of vectors creating a series of modular golden gate cloning steps to gradually assemble the fibroin gene] and an intein mediated protein assembly for the assembly of larger fibroin polymers, [exploiting an inteins mediated assembly to ligate polypeptide fragments into larger fibroin proteins] we successfully produced, purified and quantified vegan recombinant fibroin polymers (60 kDa, 115 kDa) from the tobacco plant *N. Benthamiana*. These recombinant proteins are now anticipated for industrial and medical testing.

## Visual Summary – Poster

Maastricht University

Faculty of Science and Engineering

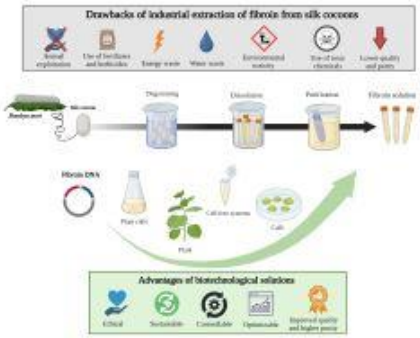
# Silk fibroin production from tobacco for medical use

Lara Bitar [A], Alexander Kopp [B], Luisa Bortesi [A]

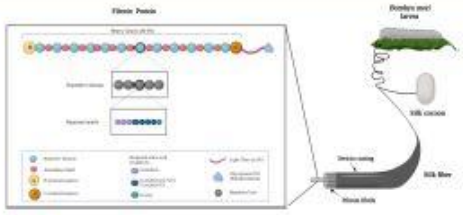
[A] Maastricht University, Aachen-Maastricht Institute for Biobased Materials, Molecular and Applied Biotechnology
[B] Fibrothelium GmbH, Aachen Germany

### Introduction

Silk fibroin is a natural biocompatible and biodegradable material with exceptional mechanical properties. Hence its industrial exploitation as base polymer biomaterial for the production of biomedical products. However, the animal based process of recovering fibroin from *Bombyx mori* silk is not sustainable and alters the proteins intrinsic physical and mechanical properties resulting with an impure final product of lower quality. Therefore the necessity of an alternative plant based production platform for improved pure recombinant vegan fibroin proteins of higher quality.




Due to its challenging large size (+15Kbp, +391kDa) and complex repetitive structure, no previous research has ever produced the full length recombinant fibroin. Our solution is creating an optimized plant based expression system, as plants are flexible and versatile platforms for recombinant protein production and suited for the expression of highly repetitive constructs due to their genetic stability.



### Aims

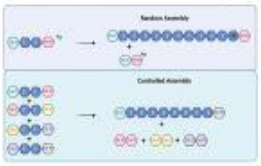
We aim in this study to produce recombinant fibroin in tobacco plants using molecular engineering techniques to later implement these raw materials into the established manufacturing routines of biomedical industries.

### Methods

For the construction of the repetitive protein a combination of two implementing strategies is planned; first a Genetic assembly method of the fibroin gene the golden gate cloning procedure followed by an Inteins mediated assembly that will ligate the polypeptide fragments forming the full fibroin protein using the inteins abilities in protein splicing. The expression of Fibroin was tested in different compartments in the *Nicotiana benthamiana* after its transformation by *Agrobacterium tumefaciens* and in cell free expression systems (ALICE), after detection by western blot, proteins were purified by heat precipitation, quantified using a BCA assay and characterized for biomedical usage.


Genetic assembly-Vector Library

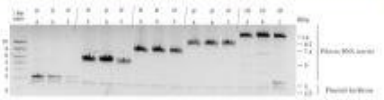
Inteins mediated assembly



Expected outcomes

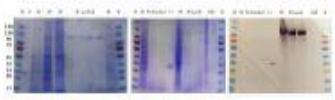
### Results

The library of DNA vectors was prepared and used for the genetic assembly the long and repetitive fibroin DNA sequences (3; 5; 7,4; 9,7; 14 Kbp) that were tested by restriction digest on agarose gel followed by sequencing .



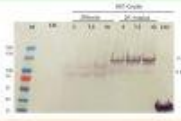
The successful production of 2F (90 kDa) and 4F (180 kDa) fibroin polymers from *Nicotiana benthamiana* was detected via western blot.

After purification, the samples were visualized on comassie gels followed by western blots. The final yields of pure recombinant 2F is estimated to be 2,5mg /g of fresh plant leaves.



The Intein mediated random assembly showed the successful production, in one pot, 2F (90 kDa) and 4F (180 kDa) polymers in the vacuole.

The expression of 2F (90 kDa) and 4F (180 kDa) fibroin polymers in the cell free system from the genetic assembly and intein mediated assembly was detected via western blot.



References

Correspondence to:
Lara Bitar
l.bitar@maastrichtuniversity.nl
Maastricht University

Aachen Maastricht Institute for Biobased Materials (AMIBM)
www.amibm.org
+3143 38 82 568

Brightlands Chemelot Campus
Urmonderbaan 22
6167 RD Geleen, The Netherlands