

SOFT CHAIN IN THE MOLECULAR STRUCTURE OF SPIDER SILK FIBERS IS A TARGET OF HIGH POTENTIAL FOR INTRODUCING NEW FEATURES

Aprelkova A.P. (ITMO), Mohamed A. (ITMO), Deeb N. (ITMO), Otinov G.D.(ITMO)

Supervisor – PhD in Biology, Assoc. Prof, Elena Koshel (ITMO)

Introduction: Developing new biomaterials with new features is crucial for tackling important problems in the field of biomedicine, such as combining durability, strength, and biocompatibility in one material. Spider silk, with its exceptional strength-to-weight ratio, biocompatibility, and elasticity, is a highly promising material for usage as a scaffolding material for medical fibers. While it was proven to be used successfully in biomedical applications, durability is still considered a limitation for extended periods of usage [1].

This research aims to enhance the properties of recombinant spider silk fibers through modification on the soft chain that plays an important role in maintaining the structural integrity of the silk fiber.

Main part: The poly-alanine β -sheets in spider silk contribute to the strength of the fiber, as a result of the stacking of β -sheets into structures of a higher order. Soft chains serve as a linker between the β -sheet structures and in some instances hold functional properties according to the silk type. Incorporating small motifs into the soft chain may improve the overall silk properties without compromising its unique features.

The mussel *Mytilus edulis* produces a Dihydroxyphenylalanine-rich (Dopa) adhesive Mussel foot protein Mfp. The general sequence of this protein family often contains repeated motifs where Dopa is present, typically in a context that might resemble: Poly(Gly–Ser–Gly–Tyr–Dopa) [2]. We hypothesize that the introduction of Dopa-containing motifs into the ADF-4 soft chain will enhance the fiber's strength and stability under stress conditions by promoting crosslinking and improved inter-fiber interactions.

This research investigates different positions and repetition strategies of the Mfp-3 motif within the ADF-4 protein to create a superior protein-based fiber with enhanced durability and stability under stress conditions. We are currently focused on constructing the genetic construct *in vitro* to incorporate it into a protein-producing strain of *E. coli*. The primary expected products are two recombinant proteins: an unmodified version of ADF-4 (control) and a modified version containing the Mfp-3 motif. These proteins will then be characterized and compared to evaluate the impact of the Dopa-containing motif on the mechanical, structural, and biocompatible properties of the resulting silk fibers [3].

Conclusion: The addition of new motifs to proteins via engineering genetic constructs is an effective method to introduce new features while conserving the structural integrity of proteins. The enhanced mechanical properties and biocompatibility of the modified silk could make it an attractive scaffold material for tissue engineering applications, supporting cell growth and tissue regeneration.

References:

1. Agnarsson, I., Boutry, C., & Blackledge, T. A. (2008). Spider silk aging: initial improvement in a high performance material followed by slow degradation. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 309(8), 494-504.
2. Liu, Y. S., Li, Y., Su, W. Y., Jian, T. H., Kao, C. T., Tsai, Y. Y., ... & Wei, Y. (2024). Uncovering mussel foot protein (Mfp-3)'s binding secrets: Enhanced biomimetic adhesives via mass spectrometry and residue labeling. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 702, 134953.

3. Bittencourt, D. M. D. C., Oliveira, P., Michalczechen-Lacerda, V. A., Rosinha, G. M. S., Jones, J. A., & Rech, E. L. (2022). Bioengineering of spider silks for the production of biomedical materials. *Frontiers in Bioengineering and Biotechnology*, 10, 958486.