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Structure-function studies of Giant proteins from *Apilactobacillus kunkeei*

Apilactobacillus kunkeei is the dominating bacterial species in the honey gut of bees. Genome sequencing of different strains of *A. kunkeei* identified that they all had four to five genes encoding giant extracellular proteins of 3000-9000 amino acids¹. We hypothesize that these proteins function in attachment of the bacterium to the honey gut. In addition, the bacteria have biosynthetic gene clusters that allow production of antibiotics, which are effective against bee pathogens². Thus, the Giants may be key to a probiotic role of *A. kunkeei* in the honey bee.

In an on-going collaboration between the groups of Maria Selmer <https://www.icm.uu.se/structural-biology/selmer-lab/> and Siv Andersson <https://www.icm.uu.se/molecular-evolution/andersson-siv/> we characterize the structure and function of these proteins. Ultimately, we hope to understand why these bacteria invest a large part of their genome in encoding proteins that are among the largest bacterial proteins.

Structure prediction using AlphaFold2 has produced tentative models of the architecture of the Giant proteins, built by a combination of globular domains and imperfect repeat structures that lack similarity to other known protein structures (Fig. 1). We can express and purify parts of the Giant proteins in *E. coli* and preliminary data shows that some of the domains are stabilized by sugar ligands.

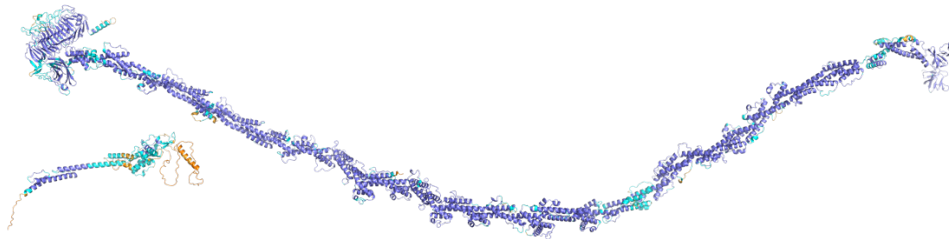


Fig. 1. AlphaFold2 model of one of the Giant proteins from *A. kunkeei*

The aim of this project is to characterize interesting parts of the Giant proteins to gain insight in the architecture of the proteins, and the function of their different domains. The student will get experience of working with a wide range of protein methods. The project can be adapted to the interest of the student and may involve:

- Ligation-independent cloning
- Construction of GFP fusions of the two ends of the Giant proteins for binding and experiments with bacteria and bee cells
- Protein expression and purification
- Stability studies using circular dichroism and differential scanning fluorimetry
- Screens for stabilizing ligands that give clues to the function of different domains
- Small-angle X-ray scattering
- Protein crystallization & crystallography
- Cryo-EM
- Structural bioinformatics

References

1. Tamarit, D. et al. Functionally structured genomes in *Lactobacillus kunkeei* colonizing the honey crop and food products of honeybees and stingless bees. *Genome Biol Evol* 7, 1455–1473 (2015).
2. Dyrhage, K. et al. Genome Evolution of a Symbiont Population for Pathogen Defense in Honeybees. *Genome Biol Evol* 14, 1–15 (2022).