Virus-like Particles - Projects Available

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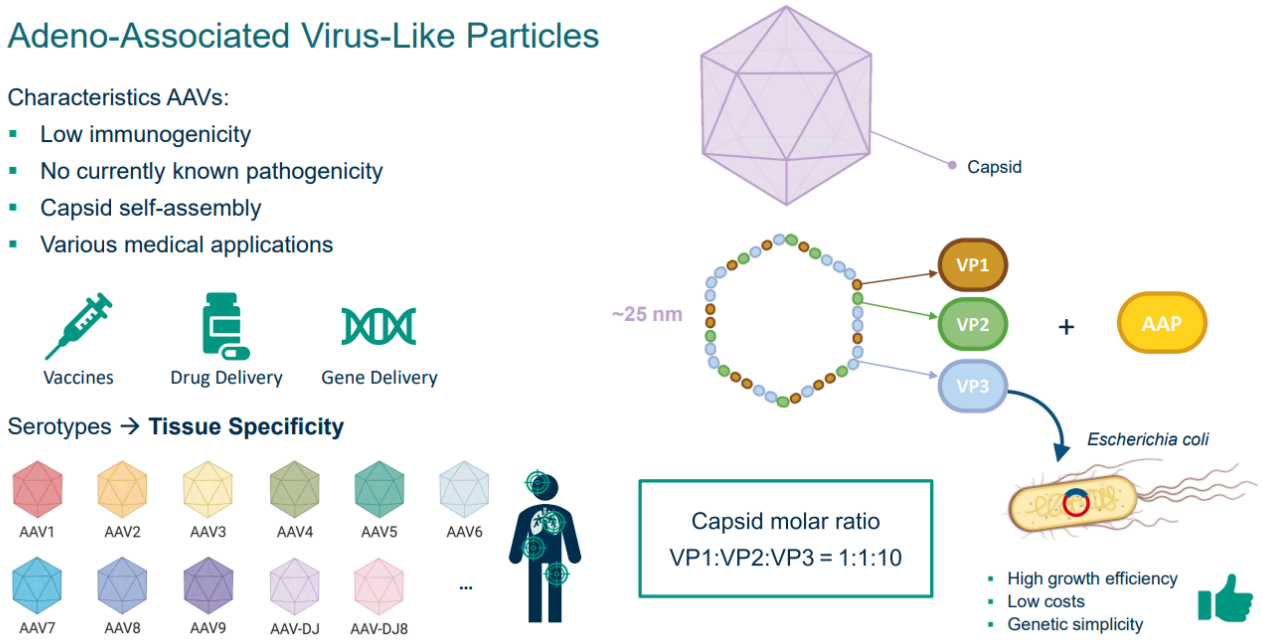
General Information

PhD project started 01.2025
Supervisors: Giulia Polazzo (giulia.polazzo@kit.edu) / Loulotte Waling (louise.waling@kit.edu)
Starting from: can be discussed
Language spoken: **English**

1. Introduction

Virus-like particles (VLPs) are nanostructures that mimic the organization and conformation of viruses, but they are non-infectious as they lack viral genetic material. Adeno-associated virus (AAV) vectors are among the most promising tools for gene therapy applications due to their safety and efficiency in gene delivery, but the production is challenging resulting in low yields and high costs (up to 2M$ per treatment). Previous research has demonstrated the potential of precipitation as capture step for VLPs.

AAV capsids are composed by three structural proteins, called "viral protein" (VP) with a ratio VP1:VP2:VP3 = 1:1:10. The VP3 protein is the main and shortest one, and can create VP3-only capsids. The structure self-assemble intracellularly with the help of the Assembly Activating Protein (AAP), expressed together with the three capsid proteins. Based on the conformation of the viral proteins, AAV can have 13+ serotypes with different tissues specificities.



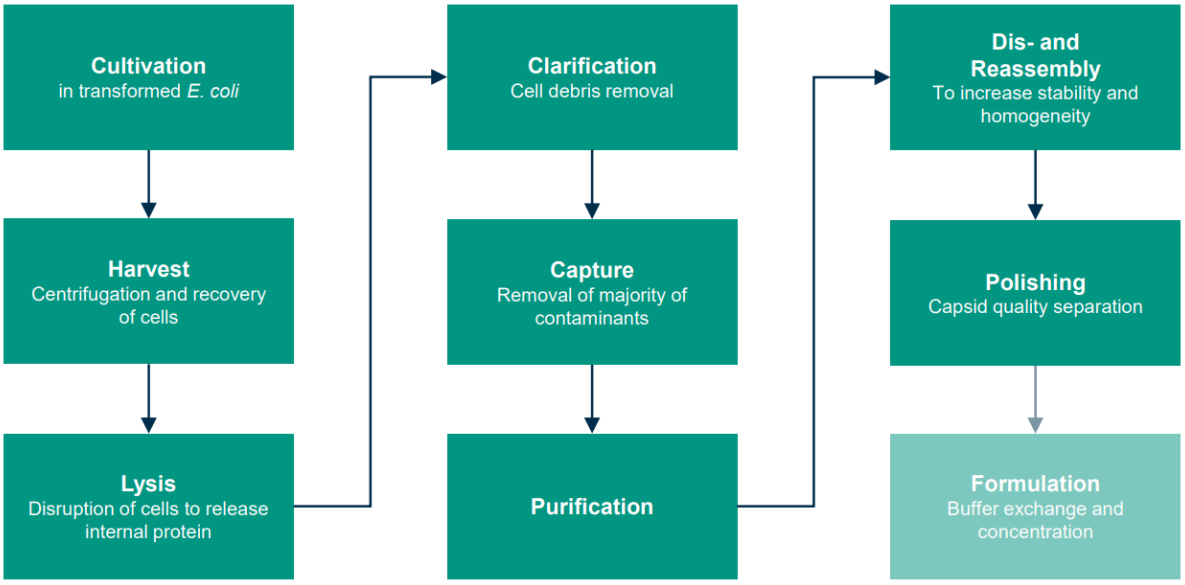
In industry, AAV-based vector are produced mainly in mammalian, insect or yeast cells, but high production costs, difficulties in assembling and variability in yield and quality make finding an alternative necessary. That's why we decided to develop a downstream processing using engineered *Escherichia coli*.

Downstream Process Development

Within a standard virus-like particle downstream process, the steps of clarification, capture, purification, dis-/reassembly and polishing are our main target:

- **Capture** - we need to find an optimal capture step with VLP recovery and cell debris removal as high as possible. We are currently investigating Aqueous Two-Phase Systems (ATPS) and precipitation.

- **Purification** - usually done by affinity chromatography. We need to find more efficient alternatives like multimodal Chromatography (mmSEC) or Steric Exclusion Chromatography.
- **Disassembly and Reassembly** - necessary to remove host cell DNA trapped inside the particles. We need to find the best and most efficient method for maximum VLP recovery.



Analytical Methods

Together with process development, the establishment of consistent analytical methods is essential. Currently, we are working with ELISA and SDS-PAGE, but they are expensive, time consuming, or only few samples can be investigated at once. Moreover, they don't allow to understand the *quality* of the capsids, for example due to aggregation. For this reason, we are currently investigating new methods and devices:

- **Gyros** - microfluidic device for automatic VLP titer - single capsid detection
- **Caliper** - microfluidic device for automatic CE-SDS - VP3 detection
- **SEC-HPLC** - analytical device that divides sample components based on dimension using MALS (multi-angle light scattering) or FLD (fluorescence) detector - single capsid vs aggregates

2. Projects Available

It is evident that a lot of projects can be created. Here some examples, but new ideas are welcome.

Experimental:

1. **Optimization of ATPS Parameters for AAV-VLPs Capture**, using high throughput liquid handling station *Freedom EVO® 200* (Tecan Group Ltd.)
2. **Optimization of Resolubilization and/or Disassembly Parameters for AAV-VLP**, using high throughput liquid handling station *Freedom EVO® 200*
3. **Development of a mmSEC Purification Step using CaptoCore**

Data Analysis:

1. **Analysis Method Development for Caliper** (CE-SDS)
2. **Analysis Method Development for SEC-HPLC**

Process Analytical Technology:

1. **Investigation of dis-/reassembly using FLD detector and PAT development**

More projects about Process Analytical Technology and Modelling will come soon.