ATPS Master Thesis Proposal

Starting from **May/June 2025**
PhD project started 01.2025
Language spoken: **English**

Title:

Enrichment and separation of Adeno-Associated Virus-Like Particles (AAV-VLPs) using Aqueous Two-Phase Systems (ATPS)

1. Introduction

Adeno-associated virus (AAV) vectors are among the most promising gene delivery vehicles for therapeutic applications. Virus-like particles (VLPs) represent a valuable tool in AAV research, providing a non-infectious platform for studying vector assembly, stability, and purification. Previous research has demonstrated the potential of aqueous two-phase systems (ATPS) for enriching and separating recombinant AAV particles produced in HEK293 cells. However, further exploration is required to assess the efficiency of ATPS in purifying AAV-VLPs produced in *Escherichia coli*.

2. Research Objectives

This thesis aims to replicate and expand upon the screening performed in the referenced study by:

- Evaluating the effectiveness of ATPS in enriching and purifying AAV5-VLP and AAV8-VLP.
- Optimizing phase composition for maximum yield and purity of AAV5-VLP and AAV8-VLP.
- Comparing the separation efficiency of different ATPS formulations.
- Assessing the impact of ATPS parameters (e.g., pH, polymer concentration, salt type) on VLP integrity and recovery.

3. Methodology

- **Expression and Production of AAV5-VLP and AAV8-VLP:** VLPs will be cultivated in *E. coli* using recombinant expression systems, followed by cell lysis and crude extract preparation.
- **Aqueous Two-Phase System Screening:** Various polymer-salt ATPS compositions will be tested for their ability to partition AAV5-VLP and AAV8-VLP between phases, automating the procedure with the liquid handling station *Freedom EVO® 200* (Tecan Group Ltd.).
- **Optimization and Characterization:** The most promising conditions will be optimized, and purified VLPs will be analysed using techniques such as ELISA kit, SDS-PAGE and dynamic light scattering (DLS).
- **Comparison and Data Analysis:** Separation efficiency, yield, and purity will be quantified and compared between AAV serotypes and ATPS conditions.

4. Analytics and Tools

- Robotic liquid handling station (*Freedom EVO® 200*, Tecan Group Ltd.)
- Recombinant protein expression in *E. coli*
- SDS-PAGE electrophoresis system
- ELISA kit
- UV/Vis spectroscopy
- Dynamic Light Scattering

5. Timeline

1. **Month 1** – Literature Review and Experimental Setup
   - Review relevant ATPS and AAV purification literature.
   - Design experimental plan, order reagents, and prepare *E. coli* expression system.
2. **Month 2-3** – Production and Initial ATPS Screening
   - Express and purify AAV5-VLP and AAV8-VLP in *E. coli*.
   - Perform initial ATPS screening to identify promising conditions.
3. **Month 4** – Optimization and Characterization
   - Fine-tune ATPS parameters for improved yield and purity.
   - Conduct analytical techniques (ELISA, SDS-PAGE, DLS) to assess particle integrity.
4. **Month 5** – Data Analysis and Conclusion
   - Compare results, finalize dataset, and interpret findings.
   - Discuss scalability and feasibility of the method.
5. **Month 6** – Thesis Writing
   - Compile results, write and finalize the thesis.