A Semi-Automated Chromatography Platform for Parallel Purification of High Quality Therapeutic Targets

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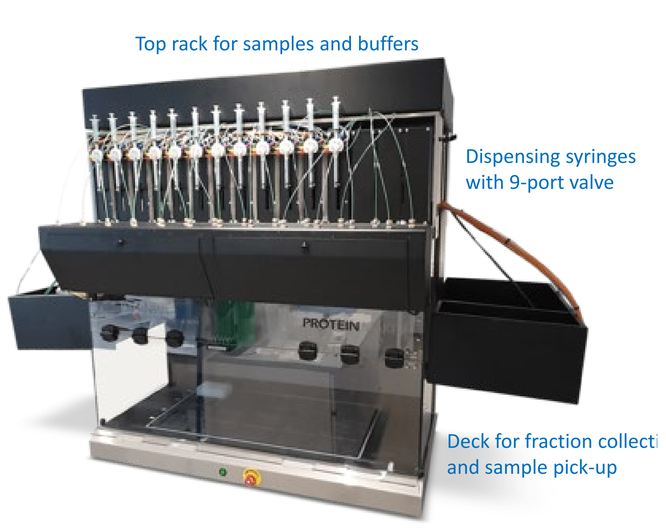
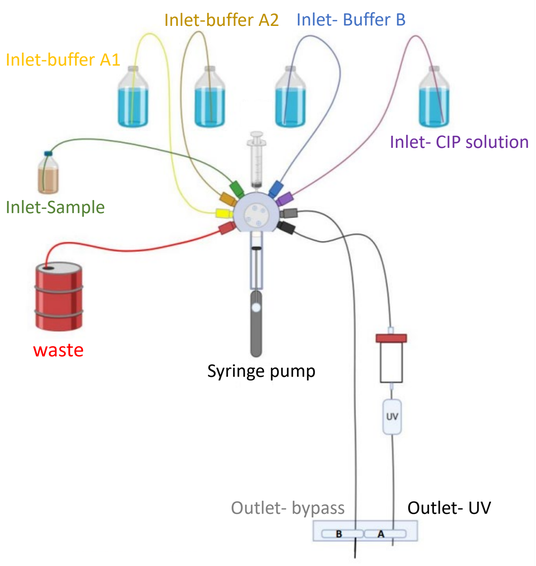
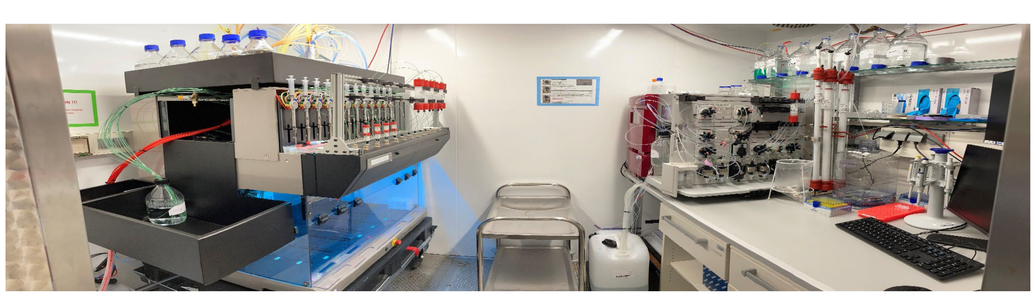
Abstract

High-throughput parallelized protein purification is essential in various applications during the several stages of the drug discovery process. Although multiple workflows are available for screening several constructs on a small scale, most of these workflows fail to yield high-quality products for use *in vitro* assays and structural studies. In order to achieve this goal, the Seattle Structural Genomics Center for Infectious Disease (SSGCID) has outlined a purification procedure that employs an automated chromatography system. This instrument enables the parallel processing of multiple lysates and buffers using 24 independent channels. We have enhanced the SSGCID methods by developing a workflow that runs capture, desalting, and reverse capture or ion-exchange chromatographic methods in a completely hands-free manner. The parallel chromatography platform is integrated with an ÄKTA chromatography system which utilizes an autosampler to process size exclusion runs in serial mode. To demonstrate the utility of this approach, two case studies are presented.

Overview of the semi-automated chromatography platform







Parallel chromatography platform

ÄKTA chromatography system integrated with an autosampler

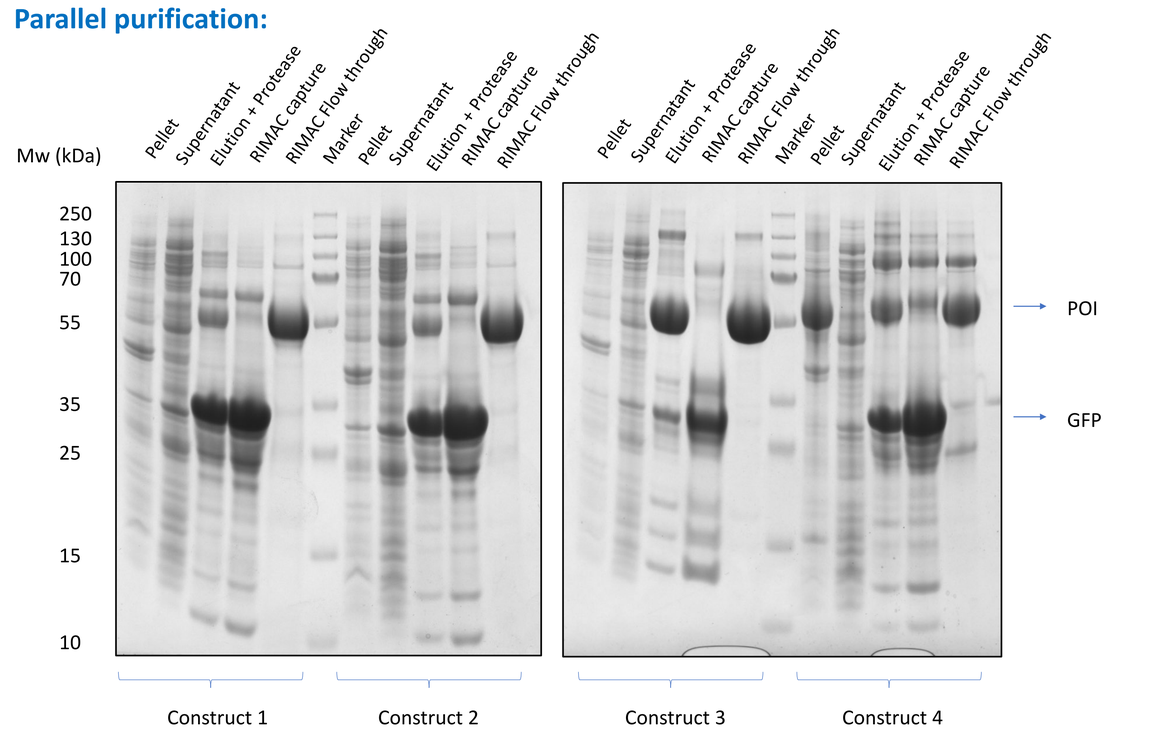
Case study 1: Parallel purification of GFP-tagged Acyl transferases

Construct: HIS – GFP – TEV – POI

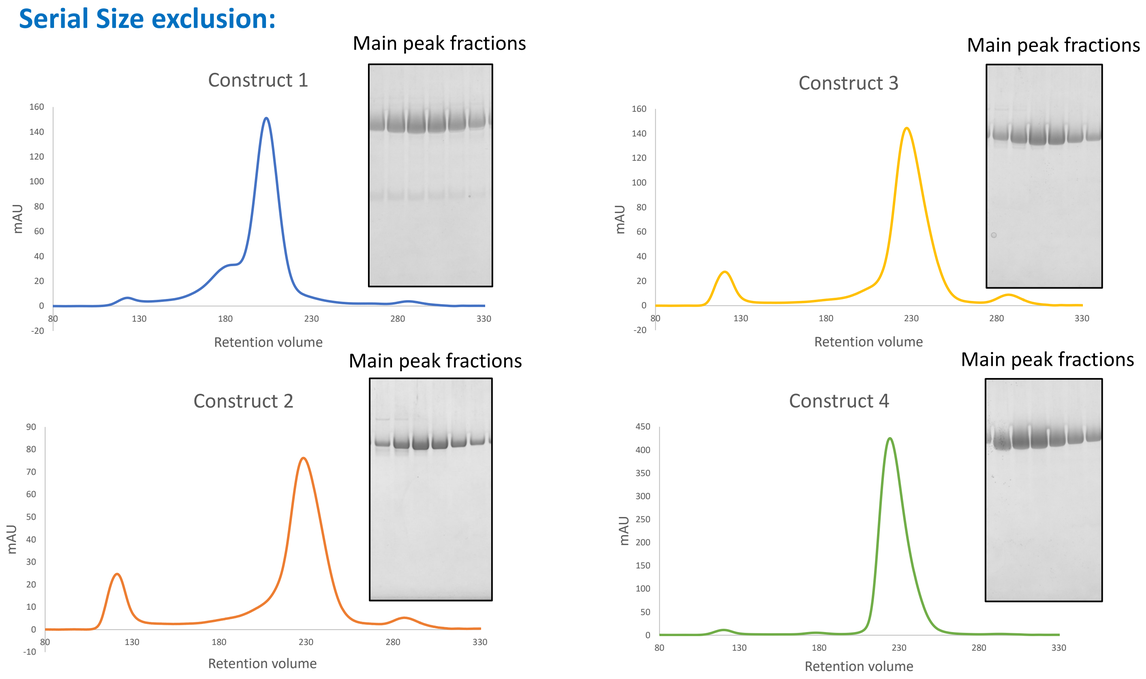
Expression: T7 driven *E. coli* expression in multiple 1L Thomson Ultra yield flasks

Expression Locus: Cytosolic

Parallel purification:

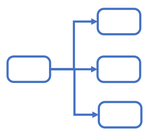


Serial Size exclusion:



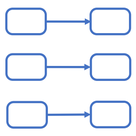
Applications

Multi-parameter



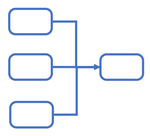
- Multiple conditions such as different lysis buffer conditions, Tag-cleavage conditions could be tested in parallel

Multi-Target



- Multiple constructs with various solubility tags, mutations or a set of related proteins could be purified in parallel

Multi-Scale Up



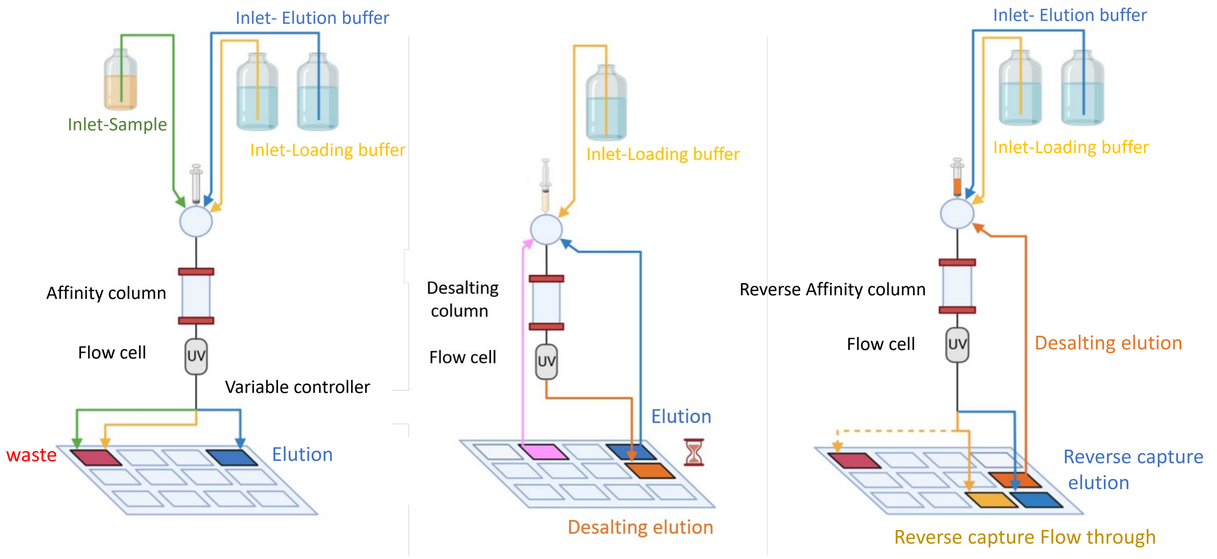
- Multiple scale ups can be purified in parallel and pooled to result in several 100 mg batch of purified sample

General Purification scheme

This method enables a single user to perform 12 purifications in 2 working days

Lysis → Capture → Desalting and Tag cleavage → Reverse Affinity → Size-exclusion

Hands-free



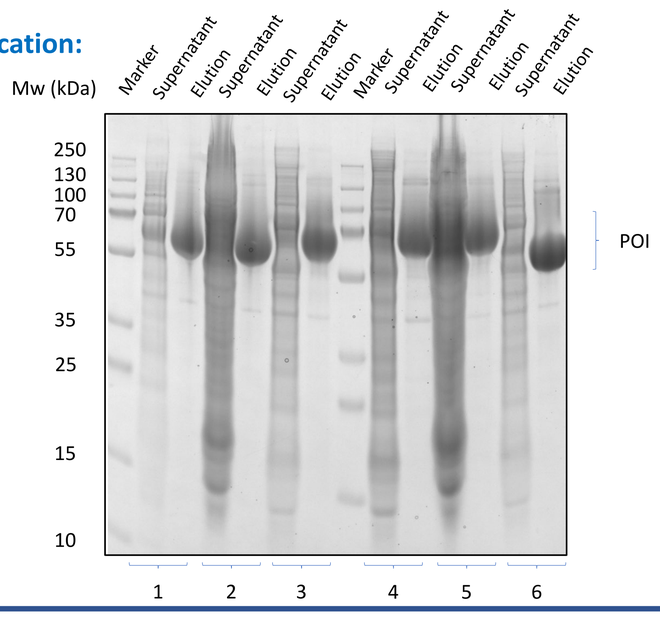
Case study 2: Parallel purification of Neuraminidase constructs

Construct: SP – POI – TEV – HIS

Expression: CMV driven HEK293 expression in multiple 1L Optimum growth flasks

Expression Locus: Secreted

Parallel purification:



Summary

- Existing work flow for parallel purification is improved up resulting in a completely automated workflow for capture, cleavage and tag removal.
- Automated workflow is integrated with a serial size exclusion platform resulting in a complete purification workflow for rapid purification of high-quality proteins expressed in E.coli, Insect and mammalian hosts
- Feasibility is demostrated by purifying multiple constructs in parallel expressed in various expression hosts.

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