Automated, multistep column chromatography on an ÄKTA pure system using in-line sample dilution

Leonildo Delgado[1], Bastian Franke[1], Tuomo Frigård[2], and Shin Isogai[1]

[1] *Focal Area Structural Biology and Biophysics, Biozentrum, University of Basel, Switzerland*

[2] *Cytiva, Björkgatan 30, 751 84 Uppsala, Sweden*

Introduction

Structural analysis of proteins often involves repetitive purification of the same protein with minor changes, for example, point mutations and other modifications as a result of protein engineering. When working with repetitive, multi-step protein purification protocols, significant manual labor is needed when interacting with the instrument and preparing the sample for the next step. In this study, automated purification methods, including up to four chromatography and two in-line buffer dilution steps, were developed.

Customized ÄKTA pure fluidics

We designed an ÄKTA™ pure system fluidic scheme for performing automated, multistep in-line dilution and purification (Figure 1). Pressure regulators are labeled with rated pressures. The outlet port is connected to a 4-way versatile valve through a mixing tee for re-injection. The flow directions are indicated with arrows.

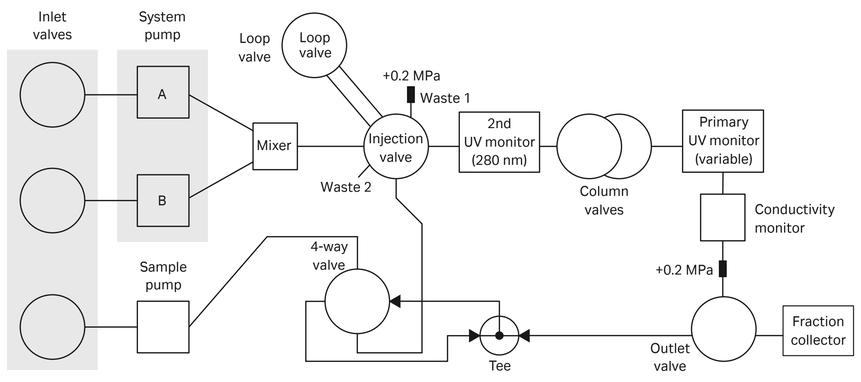

Fig 1. Automated, multistep in-line dilution and purification flow scheme.

Automated, multistep purification using in-line dilution

Methods

The flowpaths (Figure 2) used in the individual steps are color-coded according to the colors shown in the table. The columns used during the method are shown in the upper right along with port positions for each column. Flow positions of critical ÄKTA system valves in each step are shown in the table. Arrows indicate direction of the sample transfer between columns (except for the first step where the sample is loaded from the sample pump). The lower figure is a representative chromatogram from one automated run with column loading, elution, and column wash represented by horizontal bars above the diagram. Results from four separate methods were combined to yield a single chromatogram. A secondary UV trace is shown for the CIEX1 to ALAC (alprenolol ligand affinity column) transfer. Conductivity changes are shown as dashed lines. Elution peaks of the target protein are highlighted by bars above the peaks. Absorption peaks from air bubbles are marked with an asterisk. (For further interpretation, the reader is referred to the published version of these results).

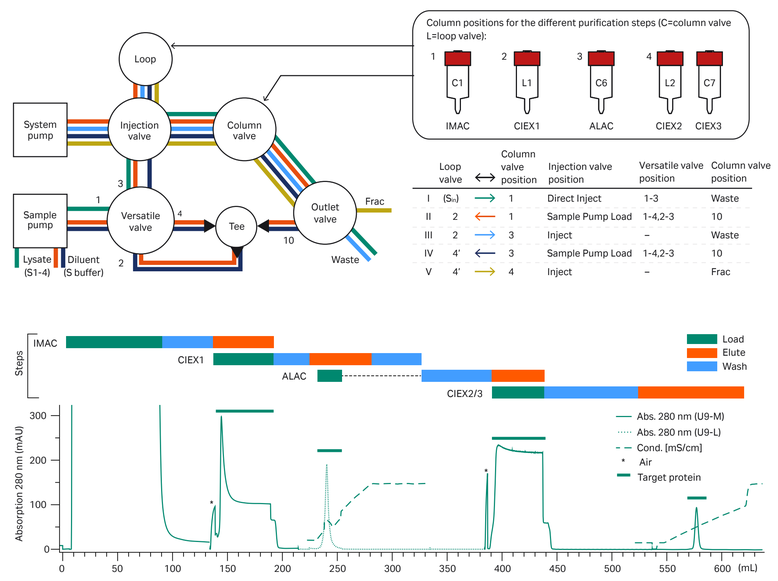

Fig. 2. Schematic showing flowpath diagram of each chromatography step.

Automation advantages

Figure 3 (A) shows the timeline of a sequential four-sample purification run. Four input lysates are captured on separate IMACs, which took ~ 4 × 1 h. The refinement stage consists of multiple blocks (CIEX1-ALAC-CIEX2/3), each with a run time of ~ 19 h. All columns used in the purification were either cleaned (CIP) in between the refinement steps or at the end of the method.

In Figure 3 (B) the chromatograms are slightly offset in the y-axis direction to help visualize all chromatograms. The values on the x-axis are not modified.

Figure 3 (C) depicts the yield comparison between four consecutive runs. The yield is obtained by calculating the area under curve of the eluting peak in the CIEX2/3 chromatogram. For testing purposes, identical lysates are fed through different sample inlet ports (s1-4). The error bars indicate standard deviation from three experiments, except for sample s3, where only two experiments were recorded. (For further interpretation, the reader is referred to the published version of these results).

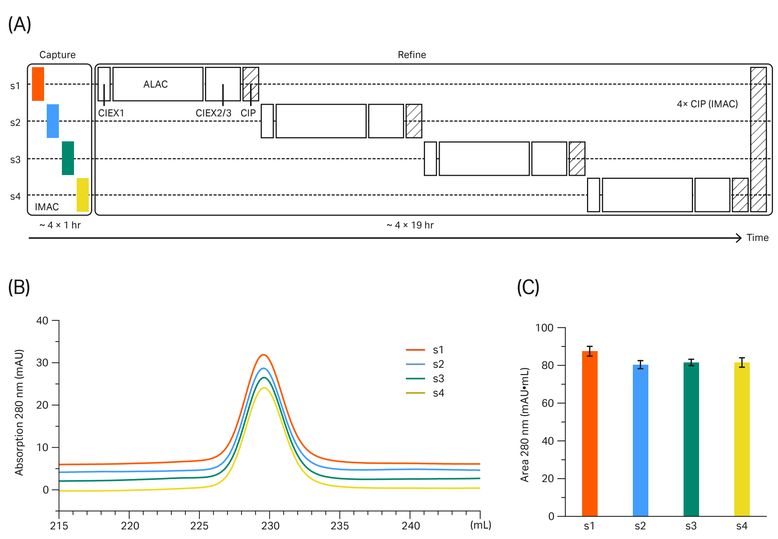

Fig. 3. Timeline of the purification run (A), chromatogram overlays (B), and bar chart showing yield comparisons between four consecutive runs (C).

Summary

- An automated, modular, four-column purification protocol was developed with in-line capabilities for protein purification.
- The automated purification method is up to six times more efficient than manual procedures.
- The method is flexible and can be run without modifying fluid paths or adding external components.

Performance tests of batch and in-line dilution methods

The batch dilution occurs in a reservoir (Figure 4A) through four consecutive steps (sample fractionation, diluent dispense, recirculation, and sample loading). The in-line dilution (Figure 4B) uses a simple static mixing tee and combines two flows from the system pump (sample eluate) and the sample pump (diluent).

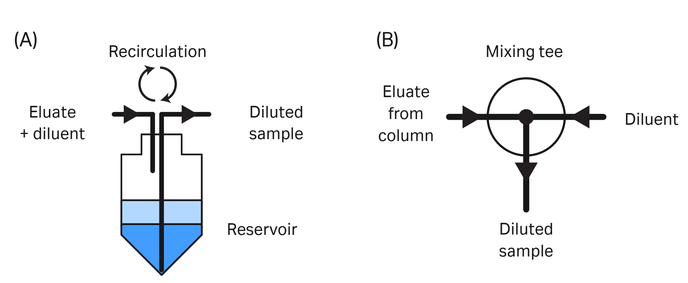

Fig. 4. Batch (A) and in-line dilution (B) methods.

Batch solution method: 60 mL of a 1 M NaCl solution was placed in the reservoir and diluted with 40 mL of distilled water. At the dispense phase, the conductivity monitor records the conductivity of the diluent solution. At the recirculation phase, the conductivity initially rises due to the high conductivity of the sample fraction before dilution and quickly decreases by mixing (Figure 5). The conductivity stabilizes at the target conductivity after ~ 2 min.

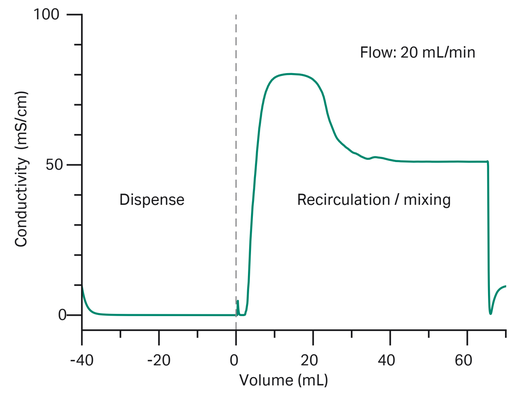

Fig. 5. Conductivity during the batch dilution method.

In-line dilution method: The high salt solution (B buffer) containing 1 M NaCl was run from the system pump and distilled water was run from the sample pump at the flow speed ratio indicated on the x-axis as a ratio of buffer B in a combined flow. Different flow rates were tested and plotted as indicated in Figure 6. The y-axis shows the measured conductivities. The fluctuation of the conductivity during the tests was much smaller than the markers in the plot, and were therefore omitted from the figure.

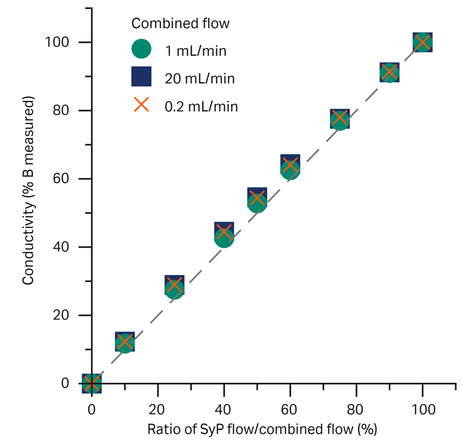

Fig. 6. Conductivity under different flow rates.

Table 1. Comparision between batch and in-line dilution methods

	Batch	In-line
Fluidics	Outlet > reservoir > inlet	Dedicated path with mixing tee
Sample transfer	Via reservoir	Direct sample transfer between columns
Phases	4 (Fractionation, dispense, mix, reload)	1 (Elution, dilution, loading combined)
Repeatability	Single use	Repeatable (washable)
Conductivity control	Can be adjusted to desired value during the experiment	Pre-calibrated
Dilution speed	Slow (approx. 10 min)	Instant
Method programming	Easy	Complex
Required modules	Sample pump, outlet selector, inlet selector	Versatile valve (4-way valve), sample pump, outlet selector, inlet selector