Versatile modules enable automated multi-column purifications on the ÄKTA pure chromatography system

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Introduction

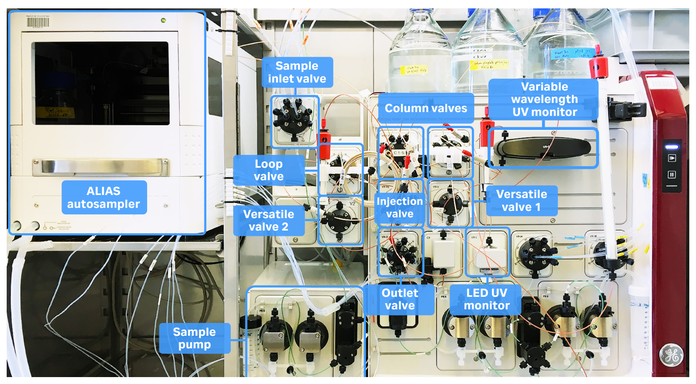
Protein purification processes in basic research (e.g., cytosolic and membrane proteins from small-scale expression cultures) typically include repetitive, single sample injections and simple, one-column purifications. Complex processes, involving multistep purification protocols and other manual steps, often become time consuming.

To streamline and accelerate downstream protein production, an autosampler and modular sample in-line dilution was used to automate two of the most commonly performed purification strategies: 1) ion exchange chromatography (IEX)/IEX/size exclusion chromatography (SEC), and 2) immobilized metal ion affinity chromatography (IMAC)/IEX/SEC. In addition, novel system configurations using the possibility to operate system components at user defined commands (e.g., operating two column valves independently) allowed for the design of purification loops that increased the purity of the target protein.

Incorporation of the ALIAS autosampler into the purification workflow

The ALIAS™ autosampler (ALIAS-AS) BIO Prep was connected to the loop valve of the ÄKTA™ and utilizes the ÄKTA system pump to inject the sample. Crucial modules are framed in blue in the system image (Figure 1).

The sample transfer from the vial to the internal loop was initialized by the SparkLink control software and took ~ 4 min for one vial with a volume of 7 mL. To prepare ALIAS-AS for injection of samples into the ÄKTA, the UNICORN™ method sequence was adapted to perform the necessary advanced operation instructions. To achieve volume injections > 7 mL, multiple sets of two phases can be connected in series in the method sequence.



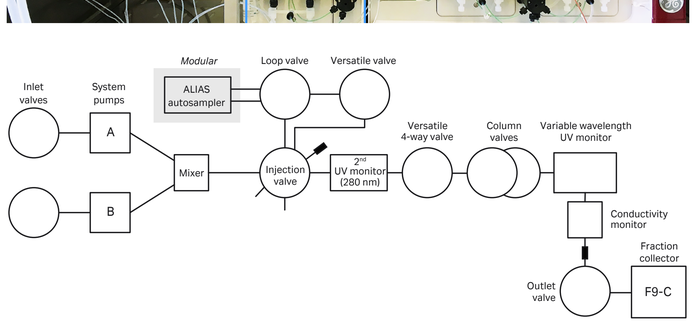


Fig. 1. Automated, multistep purification flow scheme using ALIAS autosampler and ÄKTA pure.

Application: screening of expression cultures

Automated, multi-column purification schemes using ÄKTA and ALIAS-AS enabled the purification of an array of small-scale expression batches produced in insect cells necessary to develop and optimize isotope-labeling strategies (^{2}H, ^{13}C, ^{15}N) for nuclear magnetic resonance spectroscopy applications.

Figure 2 shows the processed and deconvoluted mass spectra of purified, unlabeled, ^{2}H- and ^{15}N-labeled green fluorescent protein (GFP) samples showing the m/z values of unlabeled and labeled GFP. The peaks marked with an asterisk contain an additional N-terminal methionine (+131.2 Da).

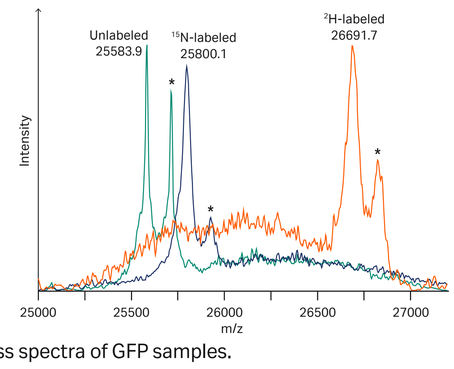


Fig. 2. Mass spectra of GFP samples.

Design of three-column purification

During in-line dilution the IMAC eluate flowed back via the outlet valve towards the mixing tee, where it was mixed with cation exchange chromatography (CIEX) buffer from the sample pump to reduce conductivity, before being re-captured on the CIEX column (Figure 3). During CIEX elution, a watch function by peak detection switched the column valve from bypass to SEC column position to allow the injection of turkey β1-adrenergic receptors (tβ1-AR) onto the SEC column. One purification cycle including cleaning in place (CIP) ran for ~ 4 h 40 min.

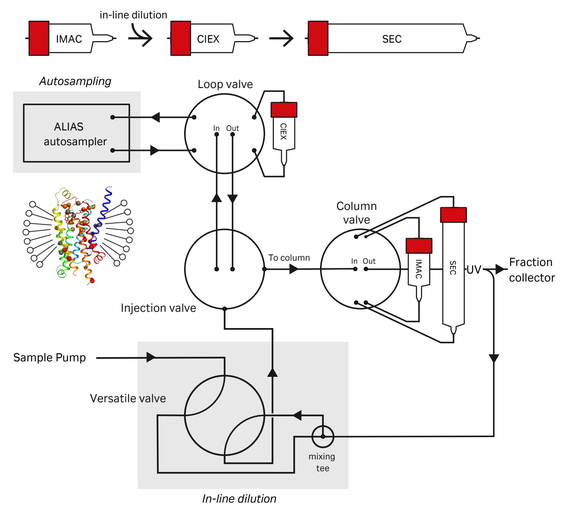


Fig. 3. Fluidics of the automated IMAC-CIEX-SEC scheme used to purify tβ1-AR.

ALIAS-AS was connected to the loop valve of the ÄKTA pure and utilizes the system pump to inject the sample (Figure 4). During anion exchange chromatography (AIEX) elution, a watch function by peak detection triggered the column valve to switch from bypass to SEC position to allow the injection of GFP onto the SEC column. To minimize the risk of column damage by excessive delta-column pressure and overall system pressure, flow rate needs to be reduced during SEC loading. One purification cycle including CIP ran for ~ 2 h. Figure 5 shows a representative run result.

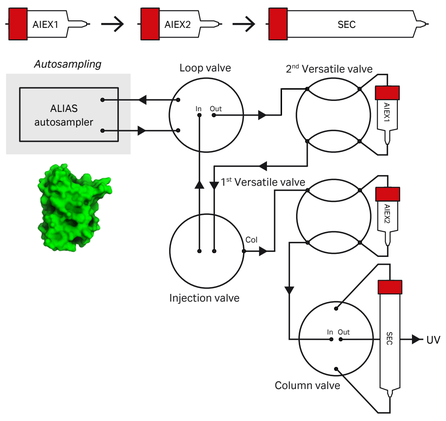


Fig. 4. Fluidics of the AIEX1-AIEX2-SEC protocol used to purify GFP.

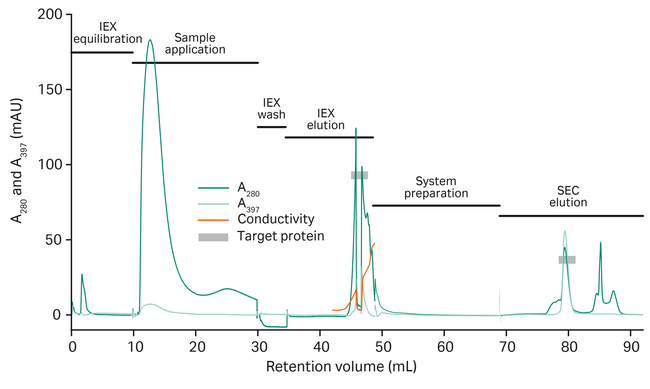
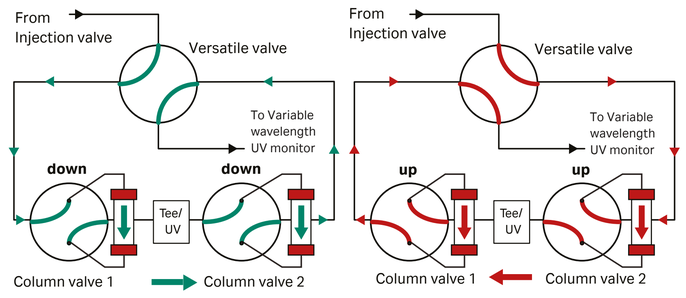


Fig. 5. Representative chromatogram from one automated GFP run.

Novel system configuration allows for independent operation of two column valves

A customized system configuration file enabled independent operation of two column valves and allowed design of purification scheme loops (Figure 6). A 4-way versatile valve was placed upstream of column valves to control flow directions. To allow column downflow operation the two column valves were either switched to up- or down flow depending the position of the versatile valve. This way, sample can be transferred back and forth between two columns. In addition, a mixing tee or UV-monitor can be placed between the column valves for in-line dilution or for monitoring the sample during transfer.



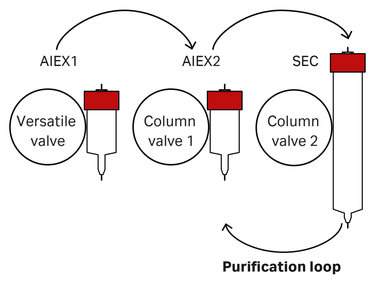


Fig. 6. Column setup used in the purification scheme.

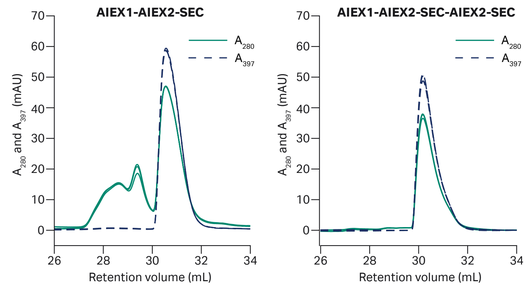


Fig. 7. Chromatogram overlay of of A_{280} (solid green line) and A_{397} (dashed blue line) SEC traces performed in triplicates.

GFP was recycled up to two times and representative chromatograms are shown in Figure 7. A_{397} SEC traces revealed minimal sample loss during transfer (Figure 8). One-cycle chromatograms were slightly shifted in the x-direction for overlay.

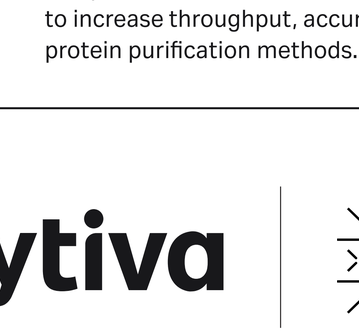


Fig. 8. One-cycle (orange) and two-cycle (dark blue) A_{397} SEC traces.

Summary

- The ÄKTA pure platform provides a solid scaffold for the development of complex protein purification strategies.
- The applied in-line dilution IEX chromatography method allowed for the design of multi-step purification schemes, thereby reducing purification cycle time and human intervention.
- Manual, single injections were replaced by an ALIAS-AS to automate the purification of large arrays of protein lysate samples. ALIAS-AS offers a reliable and robust module to increase throughput, accuracy, and reproducibility of protein purification methods.

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