From Optimization to Automation: Multidimensional (Multi-D) Histidine-Tag Protein Purification

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Abstract

The purification of proteins requires multiple iterations of individual column purification, fractionation, visualization, and pooling of fractions for the next column. Significant time is spent optimizing the first round of purification and using the information gained on subsequent batches of protein purification. A well-designed Multi-D method incorporates the optimizations of the individual column purifications steps into a single automated method, allowing hands-free reproducible high-fidelity purifications every time.

Here, a typical capture (immobilized metal affinity chromatography, IMAC), intermediate (anion exchange, AEX), and polish (size exclusion, SEC) purification workflow with an N-terminal small ubiquitin-like modifier tagged and C-terminal 6x histidine-tagged superfolder green fluorescent protein (SUMO-6xHis GFPsf referred to as just GFPsf going forward) on the NGC™ Chromatography System is used to show the development of an optimized automated 4-D Multi-D method highlighting the recovery and reproducibility of the final protein product.

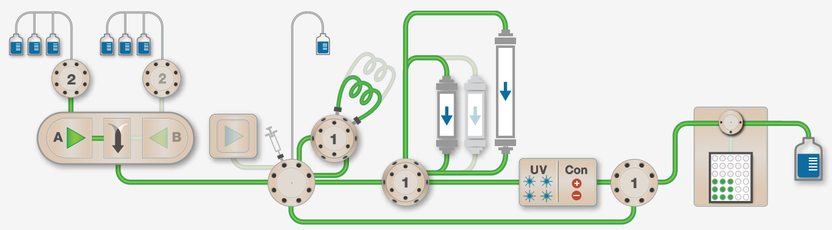
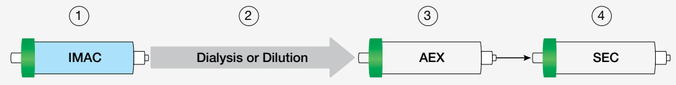


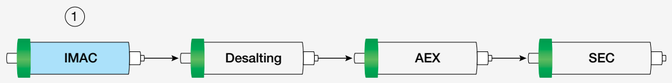
Fig. 1. Schematic of the 4-D Multi-D configuration of the NGC System. This schematic represents an NGC Quest™ 10 Plus System populated with a third tier, a sample pump, two buffer inlet valves, two column switching valves, and an outlet valve. One of the column switching valves is plumbed as a loop valve replacing the static loop on the inject valve. A fluidic line from outlet valve port 2 is plumbed into the injection port of the inject valve. All of the chromatography columns used are located on a single column switching valve. All of the static loops used in the Multi-D method are plumbed on the loop.

IMAC Purification Workflow

Traditional Sequential Workflow



Automated Multi-D Workflow



Optimization Parameters

Variables for Multi-D method creation:

IMAC Column
Sample volume, %B elution window, elution volume

Desalting Column
Sample volume, elution volume

IEX Column
pH, %B gradient elution profile, elution volume

SEC Column
Sample volume

IMAC Optimization

To determine IMAC conditions for an automated Multi-D method, optimal %B was determined by using the scouting feature of ChromLab™ Software to scout %B (Figure 2A) and by analyzing the effect of pre-elution wash %B on the subsequent AEX step (Figure 2B).

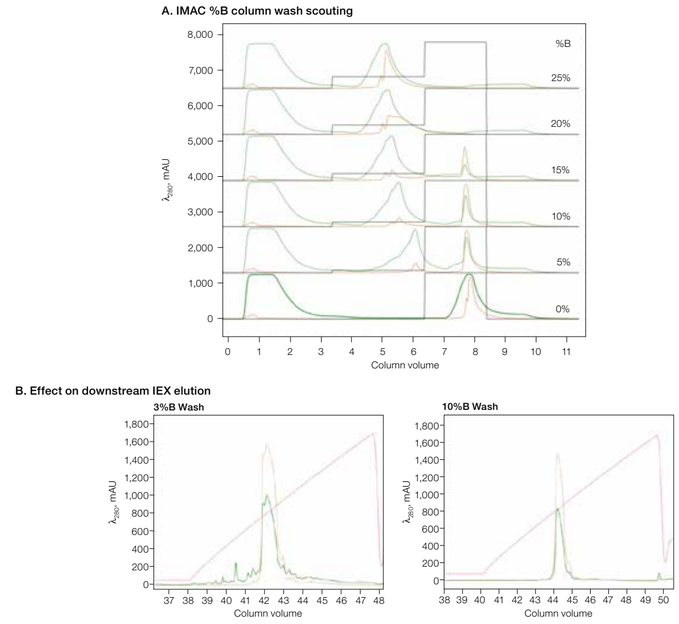


Fig. 2. IMAC %B wash scouting and its effects on downstream IEX elution. A, six scouting runs of column wash %B from 0–25%B in 5%B increments were run on a Bio-Scale™ Mini Nuvia™ IMAC Column using the ChromLab Software scouting feature. With increasing %B the leading shoulder of the 280 nM elution peak decreases without significant decreases in the GFPsf 495 nm trace up to 10%B. At 15%B wash we start to see significant GFPsf elution during the wash step and at 20%B all of the GFPsf has been eluted during the wash. **B**, ENrich™ Q Anion Exchange Column linear gradient elutions of IMAC purified GFPsf. The left represents the elution from a sample experiencing a default 3%B wash and the right from a sample that underwent a 10%B wash while bound to the IMAC column. The contaminant peaks seen with 3%B can cause complications when using threshold fraction collection. The lack of small impurity peaks on the leading shoulder of the 10%B sample allows for a low threshold value to be used for collection of the eluate to the loop resulting in maximum recovery and purity. Based on these data 10%B IMAC pre-elution wash was determined to be optimal and used to generate the Multi-D method. A_{280} (—); A_{495} (—); conductivity (—).

IEX Optimization

The ChromLab Software Anion Exchange template was used to load desalted GFPsf onto an Enrich Q Column. Optimal pH (Figure 3A) and %B (Figure 3B) were determined using the software's scouting feature.

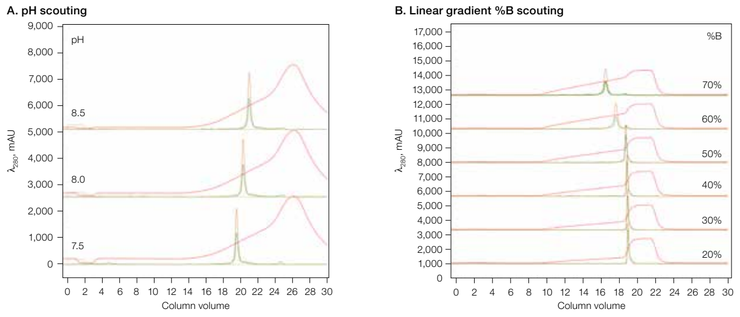


Fig. 3. ENrich Q AEX Column pH and %B scouting. A buffer blending module was inserted into the NGC Chromatography System and the Tris buffer system was used for the ENrich Q scouting. **A**, pH scouting runs using Tris pH 7.5, 8.0, and 8.5 generated by the buffer blending module for the duration of the entire method. Tighter GFPsf binding is demonstrated by increasing amounts of salt required to elute the protein as pH increases further from the theoretical protein pI of 6.8. **B**, using pH 8.0 from the previous scouting, the linear gradient %B endpoint was scouted in increments of 10%B. A GFPsf peak shift to the linear gradient begins at 50% and reaches completion by 70%B. 495 nm wavelength was used to account only for the GFPsf signal and not contaminants or buffer effects (for example, imidazole absorption at 280 nm). A_{280} (—); A_{495} (—); conductivity (—).

IMAC Multi-D Method Generation

An automated IMAC purification workflow Multi-D method was generated in ChromLab Software using the optimal conditions determined for each of the individual columns. Method phases (colored blocks in Figure 4) from existing tandem and Multi-D templates were used to assemble a custom IMAC purification method.

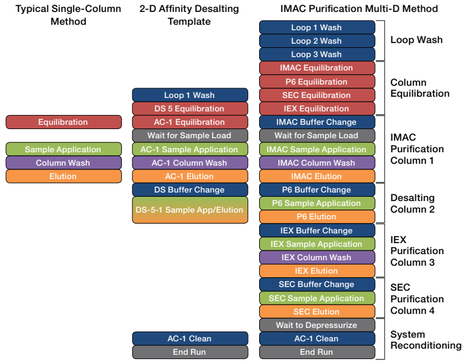
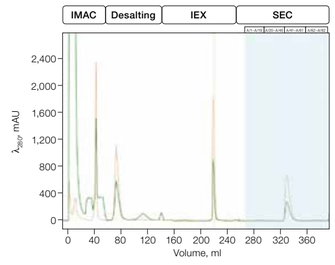


Fig. 4. Graphical representation of the conversion of a single-column method into a ChromLab 2-D template and IMAC purification workflow Multi-D method. The backbone of a single column method is the equilibration, sample application, column wash, and elution phases. These phases are modified and recycled to make the second column in the ChromLab 2-D templates. Additional system wash phases are generated to equilibrate the loops at the beginning of the method, clean the IMAC column, and flush the system at the end of the method. The template is further expanded to generate the IMAC purification workflow Multi-D method. The expansion occurs by recycling the same phases from the 2-D template to account for the additional columns in the final Multi-D method. 96% of the Multi-D method phases shown here (24 of 25) are recycled directly from the 2-D template with IEX elution being the only phase generated by hand.

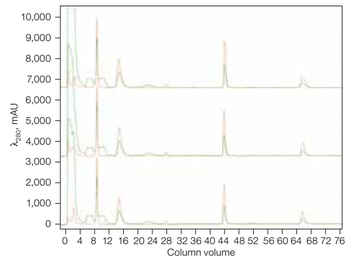
Final Automated IMAC Purification

The IMAC Multi-D method created in ChromLab Software (Figure 4) was used in a validation run (Figure 5A) as well as in three additional runs to assess method reproducibility (Figure 5B). ChromLab Software peak integration was used to determine percentage recovery of GFPsf at the IMAC, IEX, and SEC steps (Figure 5C and D).

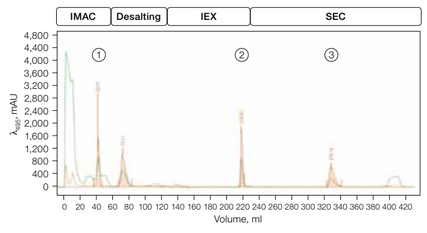
A. Multi-D IMAC purification workflow



B. Multi-D method reproducibility



C. Multi-D method recovery



D. Percentage recovery

Column	Peak Area (495 nm)	% Recovery*
IMAC	5,753.51	100.0
IEX	5,523.5	96.0
SEC	5,169.46	89.8

* Defined by the area of the peak of interest divided by the area of the IMAC peak, multiplied by 100.

Fig. 5. The IMAC purification Multi-D method. A, 2 ml of clarified GFPsf lysate was initially loaded onto a 5 ml Nuvia IMAC Column using the sample pump. The protein was washed with 10%B, step eluted from the column, and stored in a 10 ml static loop on the loop valve position 1. The protein was loaded from the loop onto the 50 ml Bio-Scale™ Mini Bio-Gel® P-6 desalting column and eluted isocratically with the protein collected into a 20 ml static loop at position 2 of the loop valve. The 20 ml protein sample was loaded onto a 1 ml ENrich Q Ion Exchange Column and eluted with a linear gradient. Using threshold collection the protein was shuttled into a 5 ml static loop on position 3 of the loop valve. The protein was then loaded onto a 120 ml Sephacryl S300-HR column in a single injection and eluted isocratically collecting 1.5 ml fractions. **B**, offset chromatograms of three separate 11.5 hr IMAC purification Multi-D method runs show high purification reproducibility. **C** and **D**, percentage recovery from a single run calculated using ChromLab Software generated peak area values. 495 nm wavelength was used to account only for the GFPsf signal and not contaminants or buffer effects (for example, imidazole absorption at 280 nm). A_{280} (—); A_{495} (—).

Conclusion

- Automated Multi-D methods enable consistent and reproducible hands-free protein purification
- Optimized methods for each step are vital to the construction of Multi-D methods to maximize reproducibility and recovery
- Multi-D methods can be constructed using the Tandem/2-D templates in ChromLab Software following the standard equilibration, sample application, column wash, and elution process of a single-column method

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