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 purification 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 NCG™ 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.

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).

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.

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).

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 0-D templates in ChromLab Software following the standard equilibration, sample application, column wash, and elution phases of a single-column method.