A Simple Solution to Chromatography for High-Purity Protein Preparations: The Modular Approach

Sean Taylor, Marie Nguyen, Farah Mavandadi, and Wei Liu,
Bio-Rad Laboratories, Inc., 6000 James Watson Drive, Hercules, CA 94547

Introduction

With the completion of the human genome project and the subsequent sequencing of the genomes of most model organisms, a wealth of transcriptional data has emerged to broaden our understanding of cellular mechanisms. Technologies including DNA microarrays, real-time PCR, ultra-high throughput DNA sequencing, and intergenic chip-on-chip arrays have allowed researchers to hone in on specific genes implicated in a number of diseases and cell signaling events. In the post-genomic phase, the focus is shifting toward proteomics studies and the characterization of the proteins encoded by the identified genes. Chromatographic purification is an important tool in proteomic studies and it is the first step of any functional characterization of proteins.

Traditional chromatography involves the separation of proteins by their inherent physicochemical properties, including pI, hydrophobicity, molecular weight, posttranslational modifications, and enzymatic activity. There is a range of instruments and reagents available to purify proteins based on these characteristics, with the goal being to achieve well over 90% purity for downstream biochemical and/or structural assays or for antibody production. However since many proteins have very similar properties, it is a challenge to achieve high levels of purification (Chapman 2005). This has led researchers to the use of affinity tag purification technologies whereby a sequence of amino acids (such as six or ten histidine residues) (Hochuli 1987) or an entire protein (such as glutathione-S-transferase) is expressed as part of a fusion sequence at either the N- or C-terminus of the target protein (Smith and Johnson 1988).

Typically, affinity tags will assure between 60% and 90% purity depending on the tag and the matrix of the sample in which the protein is expressed. However, maximizing protein purity becomes very important when the downstream application requires structural determination, enzymatic activity, interacting partners, or antibody production because all of these techniques can lead to artifactual results in the presence of contaminants. After initial affinity purification, a subsequent purification step such as ion exchange (to separate by pI), gel filtration (to separate by molecular weight), or hydrophobic interaction (to separate by hydrophobicity) can then be used. The major drawback to this tandem chromatography purification approach is that the experiments and instrumentation are complicated to set up, test, and run. As a result, some researchers accept undesirable purity with a single affinity purification while others perform separate steps of purifications that are time consuming, prone to error, and low in yield. A modular chromatography approach would simplify the purification process by automating the affinity purification and desalting step such that the eluted protein could be further purified with a gradient ion exchange chromatography step.

In this report, we compare protein purification using a single BioLogic DuoFlow Pathfinder™ instrument (for single-instrument tandem purification) againt the Profinia™ protein purification system plus the basic BioLogic DuoFlow™ instrument (for modular instrument purification). We show that the modular approach using the Profinia system for affinity purification and desalting and the basic BioLogic DuoFlow system for a subsequent ion exchange chromatography purification step is a simple, fast, efficient, and cost-effective means to achieve highly purified proteins compared to a tandem chromatography approach using a single instrument.

Materials and Methods

Reagent Preparation

A vial of Profinia *E. coli* control lysate (Bio-Rad Laboratories, Inc.) containing an overexpressed 51 kD polyhistidine fusion-tagged protein was dissolved in 12 ml of 1x IMAC lysis buffer (Bio-Rad). Control lysate (1.0 ml) was combined with 0.75 ml of an *E. coli* lysate overexpressing a polyhistidine-tagged 250 amino acid fragment of a putative glycosylase from *E. coli* (Q8XAA5) to give a solution containing the two polyhistidine fusion-tagged proteins of 51 and 24 kD.

Protein Purification

Single Instrument Approach — A BioLogic DuoFlow Pathfinder system was configured with 3 columns in tandem: a 1 ml IMAC column (for polyhistidine fusion-tagged protein purification), a 10 ml desalting column, and a 1 ml UNOsphere™ High Q ion exchange column (Figure 1).

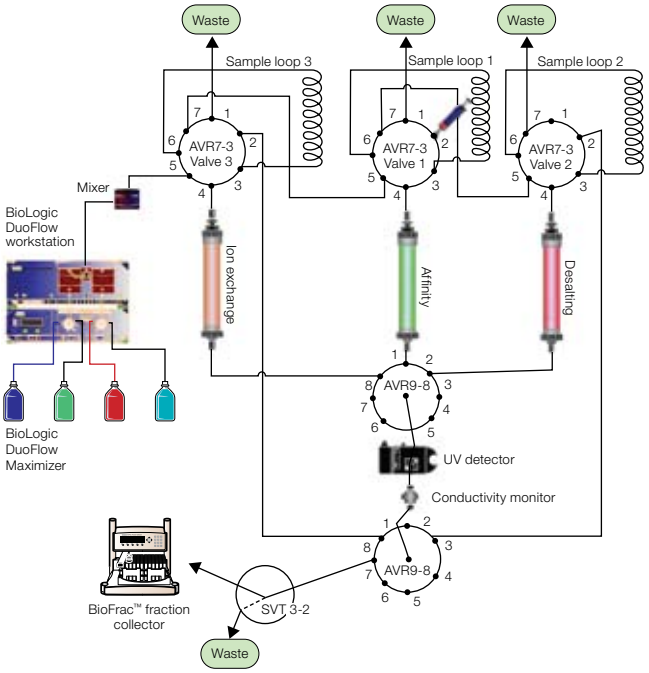

Fig. 1. DuoFlow Pathfinder configuration. Single instrument, triple column, tandem chromatography configuration. The three columns were connected to their respective injection valves (AVR7-3) such that the eluent from each could be diverted to the detector and conductivity monitored via an AVR9-8 valve. Detected peaks could then be diverted via a second AVR9-8 valve to either the fraction collector/waste valve (SV3-2) or the appropriate sample loop (AVR7-3).

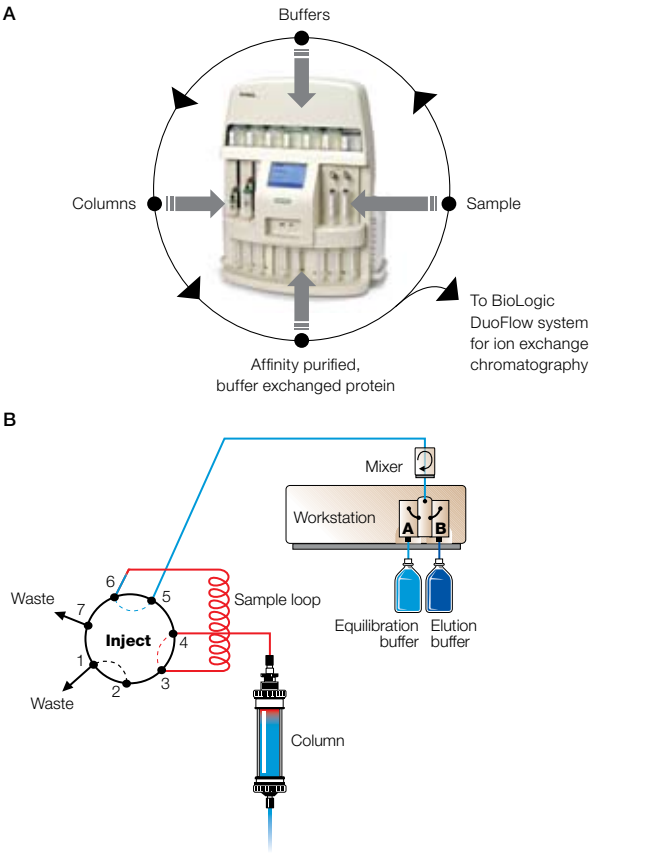

Fig. 2. Triple column modular chromatography configuration. A, The affinity and desalting columns were connected to the Profinia protein purification system. The preprogrammed IMAC plus Desalting method was selected for automated polyhistidine fusion-tagged protein purification and desalting. **B,** The eluent was then subjected to ion exchange chromatography using the basic BioLogic DuoFlow system, using the preprogrammed Ion Exchange method.

The method development requires creating and testing a separate program for each column and finally running them together as a single method using the queuing function in the BioLogic DuoFlow 5.1 software. The sample was processed as follows (see diagram in Figure 1):

1. Injection of sample into loop 1.
2. Purification on the affinity column.
3. Diversion of fractions corresponding to elution peak to sample loop 2.
4. Desalting on column.
5. Diversion of protein elution peak to sample loop 3.
6. Purification on ion exchange column.

Modular Instrument Approach — The Profinia system was configured with 2 columns: a 1 ml IMAC column (for polyhistidine fusion-tagged protein purification) and a 10 ml desalting column in tandem (Figure 2). A basic BioLogic DuoFlow system was configured with a 1 ml UNOsphere High Q ion exchange column (Figure 2).

The Profinia system is a fully automated chromatography system designed to perform affinity purification then desalting, or affinity purification and desalting in tandem with no requirement for setup or programming. The basic BioLogic DuoFlow system was set up with a single ion exchange column and a single valve (Figure 2B) and the method was preprogrammed using the Ion Exchange method template in the BioLogic DuoFlow software. The sample was first purified over the affinity and desalting columns using the Profinia system and then run over the ion exchange column for further purification using the BioLogic DuoFlow basic sytem.

Experion™ Automated Electrophoresis System

The Experion automated electrophoresis system (Bio-Rad) was used to analyze all fractions from the purification process and gel images were generated from the electrophoresis profiles by the Experion software. The Pro260 chip kits were employed to detect and quantify the purity of target proteins according to the manufacturer's instructions.

The software automatically calculates the concentration and purity of each band in a lane and the purity data were analyzed to compare the consistency of purification between the two approaches. The % purity and standard deviation were calculated from three independent runs with data analysis from the Experion 3.0 software (Table 2).

Results and Discussion

Chromatography is the key step to prepare proteins with high purity for downstream functional and structural studies. Although many applications only require one-step affinity purification to achieve sufficient purity for downstream analysis (less than 80% purity), higher purity is often critical for more delicate or sensitive biochemical, structural, or interaction studies. Even minor contaminants of trace quantities of proteases, enzyme inhibitors, or contaminating proteins may alter the structure or function of the target protein and may significantly affect the downstream results.

A multistep protein purification can be a daunting task for researchers who are not familiar with chromatography techniques and instrumentation. The combined complexity of setting up the instrument and the potential risk of losing protein during the multistep purification process have led researchers to accept lower protein purity, thus compromising on technologies that require highly purified proteins.

Our study shows that both modular (two instrument) and tandem (single instrument) purifications generate highly purified proteins (Figures 3 and 4). With both approaches, the IMAC resin purifies the 51 and 24 kD proteins and the High Q ion exchange media results in elution of purified 24 kD at low salt concentrations and purified 51 kD protein at high salt concentrations. However, the amount of time and effort spent on the two parallel purifications of the same proteins are significantly different (Table 1). With the BioLogic DuoFlow Pathfinder system, a tandem column purification can take almost two full days to set up, create the method, and test (Table 1).

Table 1. Total hands-on time required for triple column purification using a single (BioLogic DuoFlow Pathfinder) and modular (Profinia with BioLogic DuoFlow) instrument approach.

	Labor Hours			
Activity	Single Instrument Setup	Single Instrument Post Setup	Modular Instrument Setup	Modular Instrument Post Setup
Valve setup	1	0	0	0
Tubing setup	3	0	0	0
Method development	4	0	0.2	0
Priming and flushing	0.5	0.5	0	0
Testing	4	2	0.3	0.3
Washing	0.5	0.5	0.1	0.1
Total Time	**13**	**3**	**0.6**	**0.4**

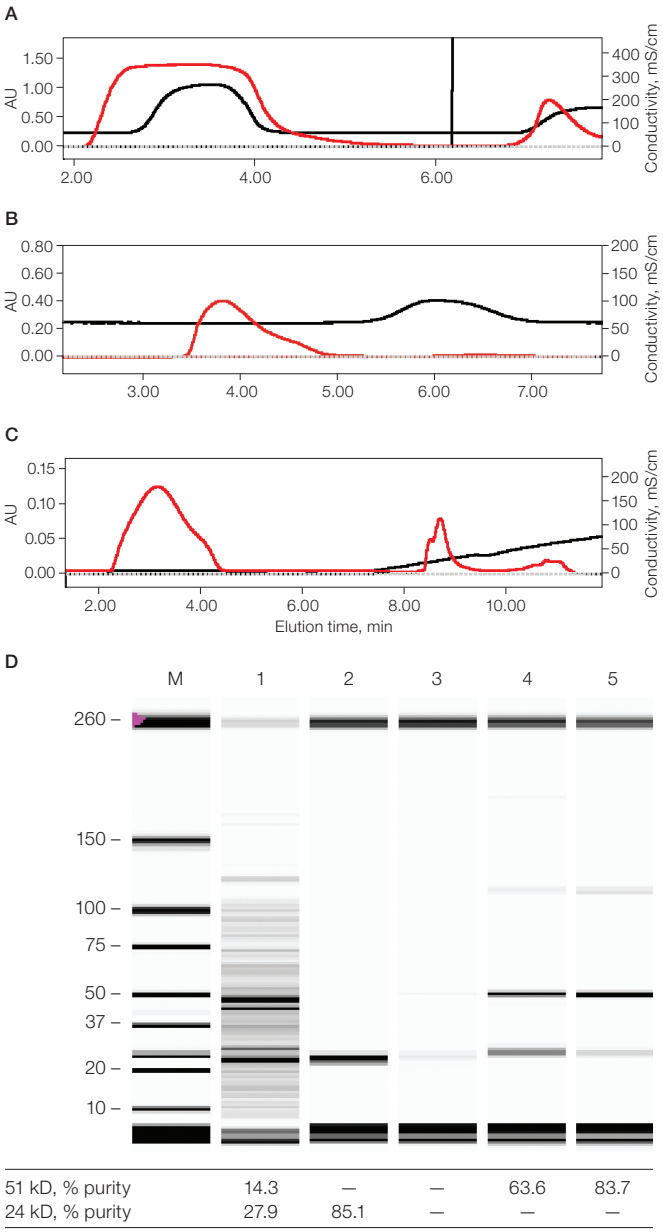

Fig. 3. Triple Column Single Instrument Chromatography. A, Affinity chromatography of the protein lysate; **B**, Desalting chromatography of the affinity purified protein; **C**, Ion exchange chromatography of purified protein; (—) A_{280}, (—) conductivity; **D**, Gel electrophoresis analysis of protein fractions. The gel image was generated by the Experion system. M, molecular weight marker; lane 1, protein lysate; lanes 2–5, ion exchange purification with the flowthrough in lane 2 and salt gradient in lanes 3–5. Table below gel image indicates the purity (in %) of each protein in the corresponding fractions.

Table 2. Summary of protein purity from single (BioLogic DuoFlow Pathfinder) and modular (Profinia with BioLogic DuoFlow) instrument approaches with triple column purifications from three independent experiments.

Protein	Approach	Purity, %	Std. Dev.,%
24 kD	Single	91.8	6.7
51 kD	Single	84.9	8.1
24 kD	Modular	98.7	1.1
51 kD	Modular	91.2	6.7

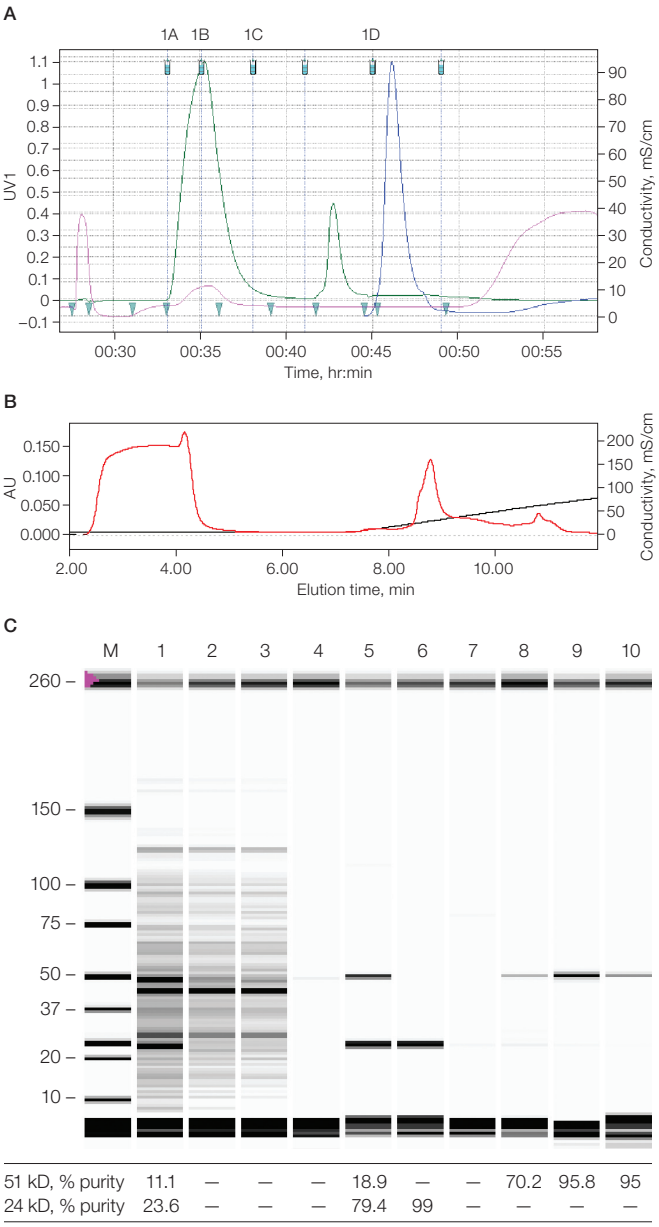

Fig. 4. Triple Column Modular Instrument Chromatography. A, Affinity purification of the polyhistidine fusion-tagged protein (green trace) with tandem desalting (blue trace) on the Profinia system; **B**, Ion exchange purification of the protein on the single column BioLogic DuoFlow system (red trace); **C**, Gel electrophoresis analysis of protein fractions. The gel image was generated by the Experion system. M, molecular weight marker; lane 1, protein lysate; lane 2, flowthrough from affinity column; lanes 3 and 4, washes from affinity column; lane 5, affinity purified and desalted protein; lanes 6–10, ion exchange purification fractions with the flowthrough in lane 6 and salt gradient in lanes 7–10. Table below gel image indicates the purity (in %) of each protein in the corresponding fractions.

Once the instrument has been set up, there is still a significant amount of labor time required for subsequent protein purifications, which can be complicated and can require up to three hours especially when purifying different proteins (Table 1). In addition, purification of the target protein in separate steps on different columns can increase risk of losing protein. On the other hand, the modular setup with the Profinia purification and basic BioLogic DuoFlow systems automates the affinity and desalting steps with subsequent ion exchange on the basic BioLogic DuoFlow instrument requiring minimal setup time. Furthermore, the post setup labor time with the modular approach is very simple and takes typically less than 20 min. Finally, the purity level from both the single and modular instrument approach is not compromised (Table 2).

In conclusion, the modular chromatography approach using the Profinia protein purification system and basic BioLogic DuoFlow system is a better alternative for protein purification for researchers who value less hands-on operation time and less complexity. The two instruments achieve comparable quality of highly purified proteins with the high-end BioLogic DuoFlow Pathfinder system at a lower cost (Table 2). In addition, the two systems operate independently and can be used separately for different purification purposes.

References

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