Ongoing genomics programs have driven both the development of protein expression methods and the generation of large numbers of recombinant proteins. A great deal of work is now directed toward characterizing the structure and biological function of these molecules. Such studies require that the proteins of interest be generated at desirable concentrations, be sufficiently pure, and in many cases, be biologically active. Protein purification can be a daunting exercise. One way to simplify the process is to attach a functional molecular affinity tag to the recombinant protein. Such tags bind to chromatographic affinity supports, so any tagged protein can easily be washed free of impurities, released from the support, and desalted.

Manual protein purification techniques are labor intensive and include a great number of time-consuming procedures. Most liquid chromatography systems are complex to learn and expensive. Either way, current approaches can be an inefficient use of research personnel. What is needed is a reliable means for automating and accelerating affinity-tagged protein purification without compromising protein quality or yield.

The Profinia protein purification system addresses this need. It offers a push-button alternative to existing lengthy methods of purification. The Profinia instrument is preprogrammed with methods that are optimized to work with prepackaged buffer and cartridge kits. The instrument and reagents together form a system that provides fast, dependable, and reproducible protein purification at a modest cost.
In two-step affinity-tagged protein purification, a protein is first purified by affinity chromatography, then desalted. In the Profinia system, the two steps are automated.

In the first step, a recombinant protein mixture is passed over a chromatography support containing a ligand that selectively binds proteins that contain an affinity-tag sequence (typically His or GST). Contaminants are washed away, and the bound protein is then eluted in pure form.

Affinity tags have different advantages. In IMAC, His binds with good selectivity to Ni\(^{2+}\) or other transition metals immobilized to the ligand; the tagged protein can be selectively eluted with imidazole. GST-tagged proteins bind to glutathione as the ligand, and are eluted with solutions of glutathione. Proteins with an enzymatically active GST fusion tag can only be purified under native conditions. In contrast, His-tagged proteins may be purified under native or denaturing conditions.

During the second step of desalting, affinity-purified samples can simultaneously undergo buffer exchange to remove salts in preparation for downstream applications.

A number of desalting techniques, including size exclusion chromatography, dialysis, and ultrafiltration, also allow buffer exchange. Desalting often includes the removal not only of salt, but also of other foreign substances, such as detergents, nucleotides, and lipids.

Affinity-Tag Purification Methods

The sequencing of genomic DNA over the past few decades has led researchers to investigate the structure and function of genetically encoded proteins. While recombinant DNA techniques allow the expression of proteins in quantities sufficient for investigation of protein structure and function, purification of these proteins remains a challenge. Many studies require proteins of interest to be homogeneous and contaminant free. Additionally, protein structural complexity can make purification a challenging task, since different proteins can behave very differently under the same conditions.

Advances in recombinant DNA techniques now enable the addition of affinity tags to a protein sequence* to facilitate purification. This advance has made possible the expression of huge numbers of proteins in a tagged format, which enables standard chromatography schemes to be used for purification (Gaberc-Porekar and Menart 2001, Hui and Usinger 2006, Nilsson et al. 1997, Terpe 2003, Waugh 2005). Tag-based purification methods are protein independent, which allows the application of a common methodology to all proteins a researcher might be interested in purifying. Affinity tag-based methods offer the possibility of easy single-step purification with minimal impact on protein structure and biological activity, applicability to a wide range of proteins, and yield of nearly homogeneous protein (90–99% purity) starting from the crude recombinant protein mixture (Terpe 2003).

The applications of affinity tagging are wide ranging. Affinity purification of protein complexes followed by downstream identification is a powerful tool for generating maps of protein-protein interactions and cellular locations of complexes. The method also enables the study and identification of posttranslational modification sites. If necessary, the affinity tag can be enzymatically cleaved from the purified protein using a protease cleavage site. For certain downstream assays, removal of the tag aids in determining the biological activity of the purified protein (for more on applications, see sidebar next page).

A number of affinity tag-based purification systems are available. The two most common approaches use immobilized metal affinity chromatography (IMAC) (Porath et al. 1975) for purification of polyhistidine (His)-tagged proteins, or glutathione S-transferase (GST) affinity tags (see sidebar above). Following affinity purification, proteins are typically desalted in preparation for downstream applications.

Need for New Technology

While affinity-tag chromatography has provided a basis for effective protein purification, current techniques, especially traditional chromatographic techniques, can require significant time, specialized expertise, and large expense. Researchers are presented with a range of purification technologies, from manual methods to sophisticated systems based on high-end

* The tag sequence is incorporated into an expression vector alongside the DNA sequence encoding the protein of interest. Induction of the vector results in expression of a fusion protein — the protein of interest fused to the affinity tag, which can then be purified from the cell lysate.
Why Use Affinity Chromatography?

Understanding cellular processes requires knowledge of the structure, function, posttranslational modifications, and interactions of proteins. Until recently, progress in understanding proteins has been slowed by the difficulty in purifying proteins. The advent of easy-to-use and high-throughput methods for protein purification should accelerate proteomics research.

Useful applications of affinity-tag purification schemes include:

- **Developing and producing proteins for therapeutic applications.** The human augmenter of liver regeneration (hALR), a hepatotrophic protein that can stimulate hepatic cells to grow regardless of genus, has been expressed and purified from *Escherichia coli* (Sheng et al. 2007). The expression of hALR enables further study of its biological function, and also suggests that recombinant hALR could be developed for repair of hepatic damage.

- **Developing vaccines.** Purification of a His-tagged spike glycoprotein, one of the major structural proteins of SARS-associated coronavirus (CoV), allowed production of a large amount of the protein, which maintained antigenicity and immunogenicity and induced strong IgG responses in mice (Zhao et al. 2005).

- **Generating sufficient protein for crystalization and structural analysis.** Studies of *Mycoplasma pneumoniae* aim to find novel protein structures that may have similar functions across species (Chen et al. 2004; see also *BioRadiations* 117).

- **Identifying protein binding partners to elucidate functional pathways.** Hardwidge et al. (2006) created GST-fusions with virulence proteins from enteropathogenic *E. coli* (EPEC), an enteric human pathogen responsible for much worldwide morbidity and mortality. These fusion proteins were expressed in *Saccharomyces cerevisiae*, and the yeast proteins that interact with the proteins were isolated by affinity purification against the GST tag. These complexes were subjected to isolectro-coded affinity tagging combined with electrospray ionization-tandem mass spectrometry, and the peptide sequences were searched against a database, which provided a list of proteins that bound specifically to each EPEC virulence protein.

- **Determining cellular localization, identifying posttranslational modifications, and biochemically characterizing proteins.** Tristetraprolin (TTP), an anti-inflammatory protein that destabilizes mRNA, has not been adequately characterized, due to the difficulties in protein purification.

- **Purification of a fusion protein expressed in *E. coli* (Cao 2004) allowed generation of antibodies that could be used for cellular localization of the protein. Additional studies measured the binding affinity of TTP under different conditions, which suggested that phosphorylation and other posttranslational modifications reduce TTP’s mRNA binding affinity by half.

- **Characterizing protein properties via site-directed mutagenesis.** Site-directed mutagenesis was used to target the putative NADP binding site of maize photosynthetic NADP-malic enzyme (Detarsio et al. 2003), which catalyzes the oxidative decarboxylation of L-malate to yield pyruvate, CO₂, and NAD(P)H. In maize and other C4 plants, this enzyme is involved in a CO₂-concentrating mechanism that increases photosynthetic yield.

- **Following expression in *E. coli* and purification of the recombinant protein, the participation of mutated residues in substrate binding and the catalytic reaction was inferred by kinetics and by circular dichroism and fluorescence spectra.**

**References**


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Yan Y et al., Characterization of a novel vaccine candidate and serine proteinase inhibitor from *Schistosoma japonicum* (Sj serpin), *Vet Parasitol* 131, 53–60 (2005)


instrumentation. Each approach has advantages, but each is also characterized by an intrinsic set of limitations that makes it a less than satisfactory solution.

Current manual purification methods use spin and gravity-flow columns. These methods require hands-on time, can lack reproducibility, and do not facilitate collection of data during purification. They also require a second manual desalting procedure to deliver purified proteins. Low-pressure liquid chromatography systems provide more automation and can achieve faster throughput than the corresponding manual methods. Unfortunately, these systems can require a significant amount of chromatography expertise and user involvement, especially in programming and maintenance. While low-pressure systems can automate the desalting run, manual intervention is still necessary to program a separate desalting method. In addition, the affinity-purified eluate must be transferred onto the desalting column manually.

The considerable amount of time and attention demanded by these purification methods limits the effort that can be spent on the downstream activities that generate the proteomic data — the ultimate objective of the investigation.

The Profinia Protein Purification System

A Total Solution for Affinity-Tagged Protein Purification and Desalting

The Profinia protein purification system is an easy-to-use, automated liquid chromatography system for the purification and desalting of affinity-tagged proteins (Figure 1). The Profinia system replaces manual and more complex automated affinity purification methods with preprogrammed, reproducible, and time-saving protocols that enable completely unattended operation (Figure 2).

A variety of purification kits are available for the Profinia instrument; they allow use of widely used purification methods, such as IMAC, GST, and desalting. Reagents are specially formulated to work with the instrument’s preprogrammed (Bio-Rad) methods, and are designed to successfully and automatically carry out equilibration, binding, wash, and elution steps. Routine maintenance procedures, such as cartridge cleaning and storage, occur automatically at the end of each run. Three main reagent kit configurations are available: the Profinia purification, starter, and buffer kits. All kit components, including buffer bottles and cartridges, are designed for simple plug-in installation.
Fig. 1. Simple, automated, and reproducible affinity-tagged protein purification. The Profinia system works with prepackaged buffer and cartridge kits. The instrument features a small benchtop footprint (58 x 33 cm) and a space-saving compartmental configuration that houses all reagents, sample and collection tubes, and cartridges on the instrument. A central compartment provides convenient storage for a touch-screen stylus and access to a USB drive for run data capture and export.

Automation That Lets You Focus on Furthering Discovery
On the Profinia system, up to two samples can be run in sequence, with the choice of using a single cartridge for both samples or individual cartridges for each. The two-step purification works by automatically detecting, selecting, and diverting the main affinity peak from the affinity cartridge to the desalting cartridge for buffer exchange (Figure 3). Flow-through, wash 1 and 2, and elution fractions are collected in designated individual fraction tubes, making it easy to locate the purified protein without the need to pool fractions. Once elution is complete, an estimate of protein yield and concentration is displayed on the instrument touch screen. This information can be transferred to a USB portable memory device for import into a PC with optional Profinia software installed. For real-time data acquisition, the instrument can be connected to a PC running Profinia software.

The optimized Bio-Rad methods for the most common affinity applications (programmed into the instrument) are accessed through a touch-screen interface that guides selection and setup. These automated templates can be edited in the system’s program-method mode. The default values of system parameters, such as flow rates and wash times, can be adjusted to optimize the yield of a specific protein of interest. Once a program method has been customized (Figure 4), it can be stored and recalled as a saved method.

Fig. 2. Time and labor savings with the Profinia system over manual methods of affinity-tagged protein purification. Typical ranges in time required for purification of target proteins are shown. The Profinia system’s self-contained hardware and preprogrammed methods accelerate His- and GST-tagged protein purification. The system combines affinity purification with automated desalting, compared to separate processes for affinity and desalting in the manual methods.  

Fig. 3. Automated two-stage affinity and desalting protein purification. The chromatogram illustrates the Profinia instrument’s ability to automatically detect, select, and transfer the main affinity fraction eluting from the affinity cartridge (peak 1) to the desalting cartridge. When it elutes as a purified and desalted protein (peak 2), it is collected in a single fraction tube.

Fig. 4. Optimization of Profinia IMAC purification. Overlay of UV absorbance profiles of three IMAC purifications performed on the Profinia system. A 30 kDa His-tagged protein was purified three times in sequence, with the flow rate for sample application adjusted to optimize yield. As the flow rate decreases, yield increases, as seen in the elution peak profile and the results table (inset). Run 1, —; run 2, —; run 3, —. The Profinia system allows adjustments in the methods, like flow rate, to accommodate desired optimization.

<table>
<thead>
<tr>
<th>Run</th>
<th>Method</th>
<th>Flow Rate</th>
<th>Yield (Increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bio-Rad</td>
<td>2.0 ml/min</td>
<td>6.0 mg</td>
</tr>
<tr>
<td>2</td>
<td>Program</td>
<td>1.0 ml/min</td>
<td>7.5 mg (25%)</td>
</tr>
<tr>
<td>3</td>
<td>Program</td>
<td>0.5 ml/min</td>
<td>8.4 mg (40%)</td>
</tr>
</tbody>
</table>
Yield and purity for both IMAC and GST purifications using the Profinia system were equivalent to results obtained using both manual and automated methods. An SDS-PAGE comparison (Figure 5) showed that the 51 kD GST-tagged protein was purified to apparent homogeneity using either the Profinia system or a manual method. Table 1 summarizes the results of both affinity methods used and demonstrates the utility of the Profinia system compared to low-cost manual solutions, as well as slightly higher-priced instrumentation that requires chromatographic expertise.

The dramatic difference in purification time is attributable to the fully automated Profinia system operations that require no user handling or intervention between the application of sample and collection of the purified and desalted protein (Figure 2). With manual methods, protein purification and desalting are two separate operations requiring manual transfer between the two columns. In addition, Profinia prepackaged reagents and other kit components are ready to use and do not require additional preparation time. Neither of these time-saving advantages applies to the low-pressure chromatography system or to manual methods. Furthermore, with the other methods, buffers and other kit reagents must be prepared by the researcher, so the hands-on requirements of these procedures add processing time.

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**Fig. 5. SDS-PAGE analysis of purification fractions.** M, markers (Precision Plus Protein™ dual color standards); L, lysate (unfractionated); FT, flowthrough; W, wash 1; P, eluted protein. Note comparable results for the two methods.

**Table 1. Yield and purity data for a 51 kD protein.** Protein was purified and desalted with the Profinia consumables (IMAC or GST starter kit) and instrument, a low-pressure chromatography system with the manufacturer’s buffers, and manually using an affinity gravity-flow and desalting spin column kit with appropriate buffers. For more information, refer to Berkelman and Urban (2006), Hui and Usinger (2006), and Petersen and Usinger (2007).

<table>
<thead>
<tr>
<th>Purification Method</th>
<th>Affinity</th>
<th>Desalting</th>
<th>Average Yield</th>
<th>Average Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IMAC and Desalting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Profinia system — IMAC starter kit and Profinia instrument</td>
<td>34 min (combined)</td>
<td>7.0 mg</td>
<td>96.6%</td>
<td></td>
</tr>
<tr>
<td>Low-pressure system — IMAC cartridges and His buffer kits</td>
<td>55 min</td>
<td>20 min</td>
<td>7.3 mg</td>
<td>96.7%</td>
</tr>
<tr>
<td><strong>GST and Desalting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Profinia system — GST starter kit and Profinia instrument</td>
<td>50 min (combined)</td>
<td>4.76 mg</td>
<td>96.2%</td>
<td></td>
</tr>
<tr>
<td>Manual — gravity-flow and spin column kits</td>
<td>90 min (combined)</td>
<td>4.34 mg</td>
<td>96.1%</td>
<td></td>
</tr>
</tbody>
</table>

**Gauging Profinia System Performance**

Ideally, delivery of purified and desalted proteins for downstream applications should meet three criteria equally: quality (purity, homogeneity, activity), yield, and throughput. Since quality is absolutely essential, one or more of the other performance criteria are often compromised to achieve high-quality results.

As demonstrated empirically using the Profinia system, there is no longer any need for a trade-off: A 51 kD dual His- and GST-tagged protein was affinity purified and desalted using the Profinia IMAC or GST starter kit or with a low-pressure chromatography system and manual kits (gravity flow for affinity and centrifugation for desalting). Identical volumes of rehydrated *E. coli* lysate containing a 51 kD protein were used for all separations. Five consecutive purification runs involving IMAC or GST-based purification followed by desalting were performed using columns and buffers from the respective kit or system’s manufacturer. The results obtained with the Profinia system demonstrate good reproducibility (similar results for different runs on the same purification cartridge) for GST-tagged proteins. The elution time for both the affinity and the desalted peak varied by no more than 10 sec (Berkelman and Urban 2006).

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Conclusions
Affinity tagging of recombinant proteins has made it possible to purify a large number of diverse proteins using a single purification technique. Until now, the technologies available for purifying affinity-tagged proteins have required a significant investment in hands-on time and expert technique to deliver purified recombinant proteins suitable for downstream applications. The Profinia system is the solution for fast, reproducible, and cost-effective purification of affinity-tagged proteins. As an automated system that is easy to learn and easy to use, the Profinia system enables the redirection of research time from purification activities to downstream applications and high-level tasks. This is accomplished by speeding the purification process and dramatically reducing the amount of hands-on time required, without sacrificing either the yield or the quality of the purified protein.

For more information, visit us on the Web at www.bio-rad.com/affinitypurification/

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Purification and preparation of fusion proteins and affinity peptides containing at least two adjacent histidine residues may require a license under U.S. patents 5,284,933 and 5,310,663, including foreign patents (assignee: Hoffmann-La Roche).

Expression and purification of GST fusion proteins may require a license under U.S. patent 5,654,176 (assignee: Chemicon International).