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Affinity Purification of a 6X-His-tagged protein using a Fast Protein Liquid Chromatography System

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Abstract

Functional characterization of proteins requires them to be expressed and purified in substantial amounts with high purity to perform biochemical assays. The Fast Protein Liquid Chromatography (FPLC) system allows high-resolution separation of complex protein mixtures. By adjusting various parameters in FPLC, such as selecting the appropriate purification matrix, regulating the protein sample's temperature, and managing the sample's flow rate onto the matrix and the elution rate, it is possible to ensure the protein's stability and functionality. In this protocol, we will demonstrate the versatility of the FPLC system to purify 6X-His-tagged flap endonuclease 1 (FEN1) protein, produced in bacterial cultures. To improve protein purification efficiency, we will focus on multiple considerations, including proper column packing and preparation, sample injection using a sample loop, flow rate of sample application to the column, and sample elution parameters. Finally, the chromatogram will be analyzed to identify fractions containing high yields of protein and considerations for proper recombinant protein long-term storage. Optimizing protein purification methods is crucial for improving the precision and reliability of protein analysis.

Introduction

Numerous strategies are available for comprehending cellular biology. One approach involves a top-down strategy, wherein genetic mutations are introduced into a gene, followed by the evaluation of resulting phenotypic changes in a model organism. Conversely, a reductionist approach entails the initial elucidation of molecular mechanisms and enzymatic functions of a particular protein, accompanied by the characterization of its interactions with other cellular components. Subsequently, the impact of this protein on a biological pathway is assessed. Although each research approach possesses its inherent advantages and limitations, achieving a comprehensive understanding of a biological pathway necessitates interdisciplinary investigations.

With DNA being the genetic blueprint of life, understanding the mechanisms of DNA duplication and genome maintenance has been an area of active interest for over seven decades. Studies in the field of DNA replication have yielded copious data concerning the individual structures and

functions of numerous replication proteins. These inquiries, which encompass mechanistic aspects and biochemical activity assays, have been made feasible through the purification of these proteins, enabling their meticulous examination in an *in vitro* milieu. Consequently, protein purification emerges as an indispensable and ubiquitous technique in the majority of research endeavors geared toward unraveling mechanistic insights into DNA replication.

This article presents a methodology for isolating a DNA replication protein tagged with 6X-histidine, which has been overexpressed in bacterial cells. The protein of interest is human flap endonuclease 1 (FEN1), a structure-specific nuclease that plays a pivotal role in lagging strand replication and is also a critical participant in DNA repair pathways like base excision repair (BER)[1,2,3]. FEN1's primary function is to cleave at the base of a 5'-displaced flap structure, an intermediate that arises during DNA replication or BER. Initially, biochemical investigations assessing the enzymatic activity of FEN1 suggested a "tracking" mechanism, wherein the nuclease would recognize the free 5'-phosphate end of a flap structure and then follow along the flap to its base before cleaving it[4]. Subsequent research revealed that FEN1 operates via a "threading" mechanism, wherein it first binds to the base of the flap and then threads the free 5' end through its active site prior to cleavage (**Figure 1**)[5]. The ability to overexpress and isolate recombinant FEN1 has facilitated these breakthroughs, enabling researchers to employ it in biochemical and structural investigations.

Affinity chromatography is a commonly used separation method to purify DNA. This technique uses the reversible binding affinity of target proteins towards ligands immobilized on a resin to specifically trap the protein of interest. One of the most widely used bio-affinities is the robust interaction between the amino acid, histidine, and metal ions such as nickel and cobalt and hence, can be captured onto a resin charged with Ni^{2+} or Co^{2+}.

The DNA sequence encoding a string of 6-9 histidine residues (His) is frequently incorporated into the plasmid construct which encodes the protein of interest (at either the N-terminus or C-terminus), tagging the protein with a 6X-His-tag or a poly-His tag. The His-tagged protein can then be easily purified by immobilized metal affinity chromatography (IMAC), a subtype of affinity chromatography whereby metal ions on the resin capture proteins with an affinity tag, which can later be eluted using appropriate elution agents. Transition metal ions such as Ni^{2+} and Co^{2+} can be immobilized onto agarose or silica gel matrices derived from *N,N,N'*-tris-(carboxymethyl)-ethylenediamine or nitrilotriacetic acid (NTA) groups[6].

Metal ligands are known to be robust against degradation by physical, chemical, and biological factors and hold this advantage over other types of ligands[6,7]. Additionally, the His-tag is a relatively small tag and does not significantly impact protein structure or function[7]. However, in a bacterial expression system, many chromosomally expressed proteins have an affinity towards metal ions and may co-purify with the target protein. Nickel and cobalt are the typical metal ions used in IMAC matrices. The Ni-NTA resin and the TALON cobalt-based resin are commonly used for the purification of His-tagged proteins.

Ni-NTA versus TALON

The respective metal ions of both Ni-NTA and TALON are immobilized on the resin through NTA ligands. Ni-NTA is thought to have a higher binding capacity, binding up to 100 mg/mL of protein. This can result in a higher protein yield, with the caveat that contaminant proteins may be co-purified. In contrast, the resin has a higher binding specificity towards

His-tagged proteins and may be able to produce fractions of higher purity. In this study, we aim to compare the purification efficiency of both resins using the referenced automated fast protein liquid chromatography system, the NGC system (see the **Table of Materials**).

Buffers and compatibility

Buffers are required during protein purification for cell lysis, sample preparation, resin equilibration, and elution of the captured protein from the resin. Tris, MOPS, and HEPES buffers up to a concentration of 100 mM are the known compatible buffers for the Ni-NTA resin. Buffers often include reducing agents to prevent the oxidation of protein and protein aggregation. However, above a threshold limit, reducing agents could strip the resin of metal ions. The recommended concentration of reducing agents such as beta-mercaptoethanol (BME) or dithiothreitol (DTT) is below 1 mM for the above-mentioned nickel- and cobalt-based resins.

The buffers for the purification of hFEN1 are Tris buffers containing NaCl, BME, phenylmethylsulfonyl fluoride (PMSF), EDTA, and glycerol. NaCl maintains the protein in soluble form and disrupts molecular interactions such as DNA binding. BME reduces oxidized proteins and thereby prevents protein aggregation. PMSF) is a protease inhibitor that prevents protease-mediated degradation of target protein. EDTA eliminates divalent cations from the sample, preventing their access to nucleases and proteases. Glycerol enhances the stability of the protein in aqueous form. Additionally, the lysis buffer contains complete protease inhibitor tablets to ensure maximum protection of target protein from degrading proteases during cell lysis. The equilibration and elution buffers contain imidazole, with the elution buffer containing higher quantities for the imidazole to displace the bound protein from the resin during elution.

Next Generation Chromatography (NGC) system

This automated, medium-pressure chromatography system designed for fast-flow protein liquid chromatography (FPLC) uses two pumps to simultaneously pump two different buffers and is capable of injecting a wide range of sample volumes from 250 µL to 100 mL. The sample loop (known as the Dynaloop in this system), makes it possible to inject larger sample volumes. The system can be operated using the Chromlab software, which facilitates customized method creation, manipulation of purification runs, and analysis of UV peaks and protein fractions.

Protocol

1. Sample preparation

1. To purify recombinant FEN1, express the construct (pET-FCH-FEN1) in BL21(DE3) cells as previously described by Ononye et al.[8].
    1. Inoculate LB media (**Table 1**) with 1% overnight culture and grow the cells at 37 °C until the OD reaches 0.6.
    2. Induce with 0.4 M isopropyl-beta-D-thiogalactoside (IPTG) and grow the culture for an additional 3 h.
    3. Harvest the cells by centrifuging the culture at 5,000 × *g* for 15 min at 4 °C. Store the pellet in a -80°C freezer.
    4. On the day of the purification, remove the cell pellet from the -80 °C freezer, allow it to thaw on ice, and resuspend the pellet in 100 mL of lysis buffer (**Table 1**).

        NOTE: The cell lysate should always be placed on ice during handling to prevent proteolysis. Once thawed, the entire purification process must be

completed on the same day. The cell lysate should not be stored in the refrigerator overnight.

2. Transfer the suspension to a 250 mL beaker and sonicate on ice with 30 s ON and 30 s OFF at 35% amplitude for 15 min.
   NOTE: Consult the manufacturer's settings to identify the amplitude.

3. Transfer the sonicated sample to a centrifuge tube and spin at 27,000 × *g* for 30 min at 4 °C to obtain a clear lysate. Place the cell lysate on ice in the 4 °C refrigerator until sample injection.

2. Preparing affinity columns

1. Fit the columns (one for the Ni-NTA resin and one for the cobalt-based resin) to a clamp stand such that the columns are held as straight as possible, avoiding any slanting of the columns. Ensure that the bottom of the columns are plugged in with a stopper and add 1-2 mL of starting buffer to the bottom of each column to prevent air bubbles during column packing.

2. Shake the resin bottles gently until the resin is fully resuspended. Open the top of the columns and pour in 10 mL of each slurry in a continuous manner to avoid the inclusion of air bubbles. Pour the resin against a metal spatula or glass rod held at the rim of the column to avoid air bubbles in the column.

3. Column packing using a flow adaptor

1. Unplug the stopper from the column and connect the bottom to one end of the tubing. Connect the other end of the tubing to the UV port of the FPLC system.

2. Ensure that the bed support housing has been pulled into the flow adaptor body and slide the flow adaptor body onto the top of the column (**Figure 2A**). With the cam latch in position 1, lower the flow adaptor taking care not to insert it into the buffer (**Figure 2B**). Switch the cam latch to position 2 (**Figure 2B**). Connect the tubing to the system and start the flow of the starting buffer for 1-2 min to rid the tubing of air and prevent air bubbles during packing.

3. Switch the latch back to position 1 and lower the flow adaptor into the buffer in one smooth motion, avoiding the inclusion of air bubbles, and switch the latch back to position 2. This allows for some buffer to enter the flow adaptor and removes any remaining bubbles.

4. Switch the latch to position 3 and tighten the cam base and locking ring (**Figure 2B**).

5. Start the flow of buffer at 10 mL/min to pack the column. Continue this step for 3 min or until the resin has been well packed.

4. Priming the sample loop of the FPLC system

1. Connect the tubing from the top of the sample loop to Loop E on the FPLC system (**Figure 3**).

2. Connect the tubing from the bottom of the sample loop to Waste 2 on the FPLC system.

3. Insert one end of the tubing into the column port of the FPLC system and place the other end in the waste bottle.

4. Insert the tubing from pump A in filtered double distilled water (ddH_2O).

5. Double-click on the **pump settings** using the software (**Figure 4A**, labeled 2) and select **system pump inject loop** (**Figure 4C**).

6. Click on the **flow rate settings** (**Figure 4A**, labeled 1) and change the **flow rate** to **10 mL/min**. Enter **0%** in the **%B** box (**Figure 4B**).
7. Start the flow to flush the sample loop with water, which will push the sliding seal downwards.
8. Once the sliding seal reaches the bottom of the sample loop, stop the flow. Switch pump A from ddH_2O to the starting buffer. Connect the tubing from the top of the sample loop to Waste 2 and that from the bottom to Loop E. Leave the tubing from the column port in the waste.
9. Start the flow at 10 mL/min. Once the sliding seal pushed upwards by the incoming starting buffer reaches the top of the sample loop, stop the flow. The sample loop is now ready for sample injection.

5. Sample injection

1. Double-click on the **pump settings** and select **Manual load loop** (**Figure 4C**).
2. Unscrew the tubing from Loop E and place it in the waste container.
3. Connect the tubing from the column port to the tubing on top of the flow adaptor.
4. Draw up the sample using a 30 mL syringe; then, attach the syringe to the injection port adaptor.
5. Screw the syringe into the injection port and inject the sample, ensuring that it is visibly injected into the sample loop. Store 100 µL of the sample (label **input lysate**) in a microcentrifuge tube at -20 °C for SDS-PAGE analysis
   NOTE: Depending on the total sample volume, 2-3 injections may be required.
6. Plug the injection port with the column cap.
7. Connect the tubing from the bottom of the sample loop back into the Loop E port.
8. Place the tubing from pump B in the elution buffer.

6. Method creation and analysis using the FPLC system-linked software

1. Navigate to the Home tab and select **New method**.
2. Select the **Method settings** tab on the left column, navigate to the **Column type** dropdown menu, and select **Custom** (**Figure 5**).
3. Enter the **column volume** (**5 mL**).
4. Enter the required **flow rate** for the run (**1 mL/min**).
5. Navigate to **Unit selection** and select **CV (column volumes)** as the **method base unit**.
6. Ensure that **Inlet A** is set to **Buffer A** and **Inlet B** is set to **Buffer B** (the default setting).
7. Select the **Method Outline** tab on the left side menu. Add the steps of the purification in the following order: equilibration, sample application, column wash, and elution.
   1. Under **equilibration**, set the **equilibration volume** to **10 CV**. Ensure that the **buffer setting** is kept at **0% B**.
   2. Under the **sample application** tab, set the sample volume by observing the sample loop. The position of the sliding seal indicates the sample volume.
   3. Under **column wash**, set the **wash volume** to **5 CV**. Disable fraction collection for this step; collect the wash as a whole in a beaker during the run, rather than collecting as fractions.
   4. For **elution**, set the **gradient volume** to **14 CV** starting from **0% Buffer B** to **100% Buffer B**.

8. Save the method and start the run.
9. Ensure that the fraction collector starts at the position of the first fraction.
10. Enter the **run name** and click **Start**.
11. During the equilibration phase, monitor the real-time UV curve. Ensure that all connections are secure with no leakage; confirm a complete circuit by looking for the buffer flowing into the waste container. If the UV curve has not stabilized during the last minutes of equilibration, extend the equilibration step until stabilization is achieved (**Figure 6**) .
12. At the time of sample application, switch the tubes going into the waste container to a clean beaker. During this step, collect the unbound proteins flowing out of the waste tubes as the flowthrough (label as **FT**).
13. Look for a sharp increase in the UV curve that likely reaches the maximum possible reading of 3,000 maU (**Figure 6**).
    NOTE: A typical curve plateaus at 3,000 maU through most of the sample application step.
14. During column wash, as residual unbound proteins are being washed off with only bound proteins remaining on the resin, look for a sharp drop in the UV curve (**Figure 6**). If the UV curve has not dropped by the end of the wash step, extend the step by clicking **hold step** until the UV has dropped sufficiently. At the beginning of this step, transfer the waste tubes from the **FT beaker** to another clean beaker labeled **Wash**.
15. When the elution step starts, ensure that the fraction collector moves into position and begin collecting fractions. Visualize the elution of protein by looking for peaks in the UV curve (**Figure 6**).
16. After run completion, navigate to the **Home** tab and select **Open Run**. Open the run to view the chromatogram and select **peak integration** on the top menu to mark the peaks obtained during the run.
17. Select the **Fractions** tab to visualize the fractions containing eluted protein.

7. SDS PAGE analysis

1. Mix 15 µL of each fraction containing protein with 5 µL of SDS sample loading buffer (**Table 1**). Heat the samples at 95 °C for 5 min.
2. Load samples into precast gels and run at 160 V for 45 min along with prestained protein marker.
3. Stain the gels in Coomassie brilliant blue staining solution for 30 min (**Table 1**).
4. Wash the gel in ddH_2O (repeat 3x).
5. Destain the gel in destaining solution for 1 h (**Table 1**).
6. Observe protein bands; detect FEN1 at the 42 kDa mark.
7. Pool fractions containing FEN1 and concentrate the pool using a 10 kDa centrifugal filter. Aliquot and store in the -80˚ °C freezer.

Representative Results

BL21 (DE3) cell lysates expressing hFEN1 were passed through equilibrated Ni-NTA and TALON resins. The Ni-NTA resin is charged with Ni^{2+} ions and has a high binding capacity. The results show that the Ni-NTA resin yields a higher quantity of FEN1 compared to the TALON resin (**Figure 7**). The Ni-NTA resin is also known to non-specifically bind to other chromosomally expressed proteins. Cell lysate passed through the cobalt-based resin, was purified with high purity but a lower yield, compared to the Ni-NTA. Like

many other DNA-binding proteins, FEN1 was further passed through size exclusion resin to remove the impurities. hFEN1 can be identified at the 43 kDa mark.

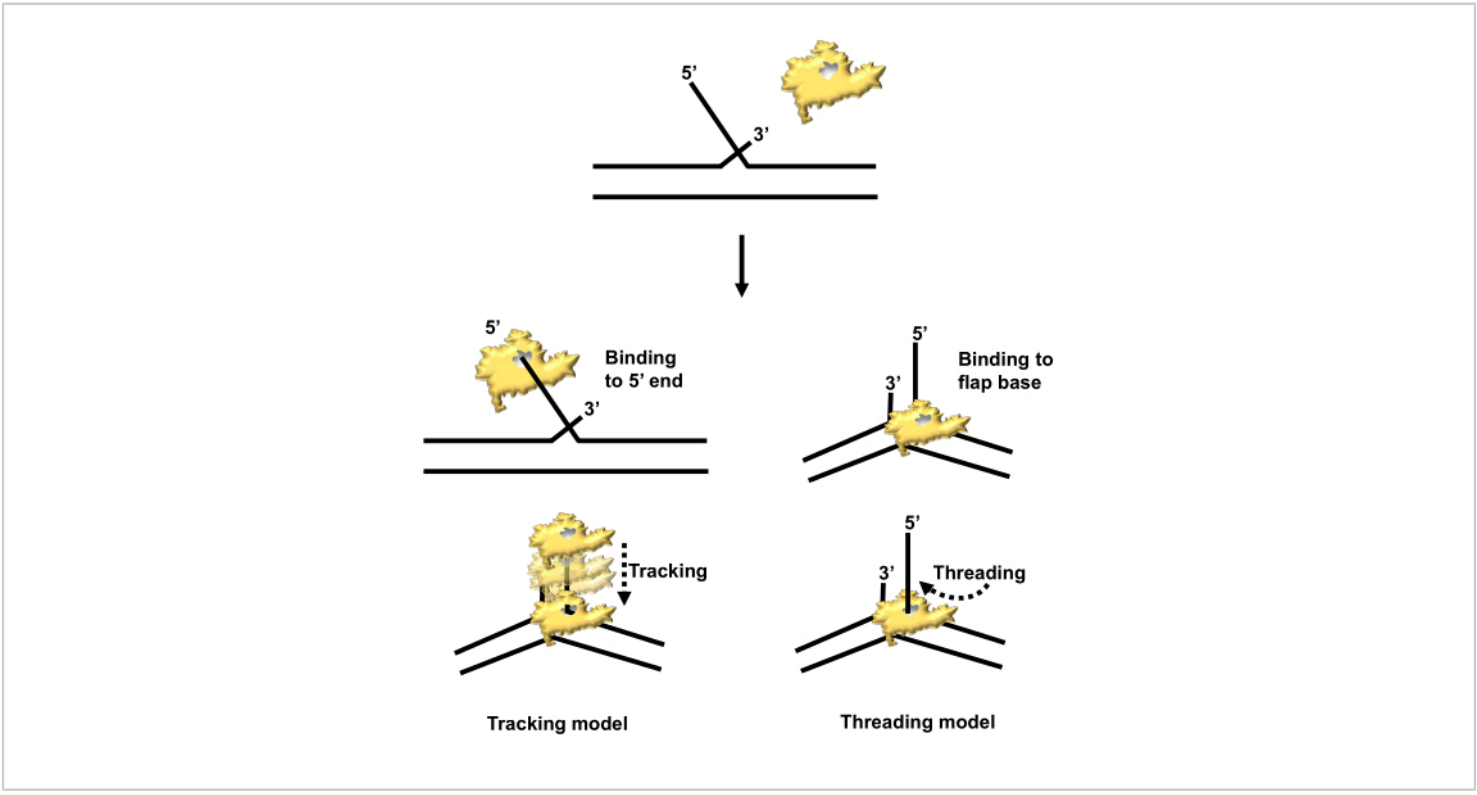

Figure 1: Flap cleavage mechanism by hFEN1: tracking versus threading. FEN1 (depicted in yellow) was initially thought to bind to the 5' end of the flap structure and track through it to the flap base (left). Later studies revealed that FEN1 first binds to the flap base and threads the 5' flap through its active site (right). Abbreviation: hFEN1 = human flap endonuclease 1. Please click here to view a larger version of this figure.

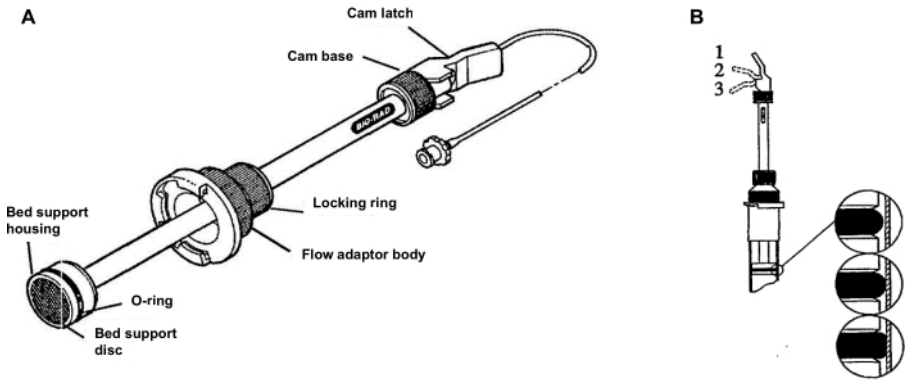

Figure 2: Flow adaptor. (**A**) Components of the flow adaptor used for column packing and purification process; (**B**) the three positions of the cam latch used to tighten/loosen the O-ring and fix the adaptor in place. The zoomed-in image shows the state of the O-ring (loose/tight) at positions 1,2, and 3 from top to bottom. Images were taken with permission from [9]. Please click here to view a larger version of this figure.

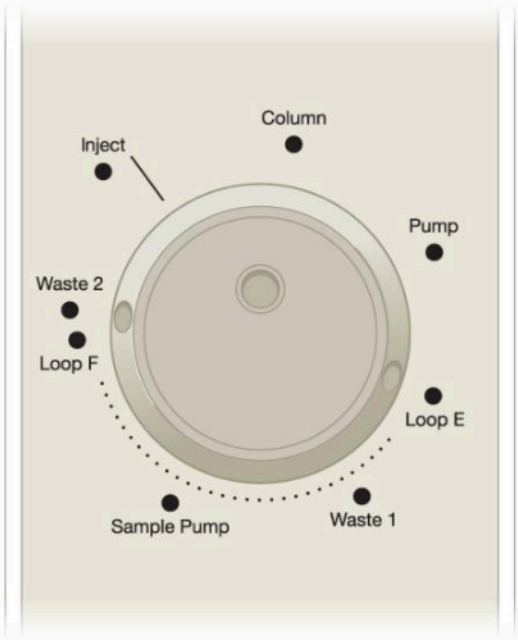

Figure 3: Injection module of the Fast protein liquid chromatography system. The various ports of the injection module connect the fast protein liquid chromatography system to the column, sample loop, and waste. The image was taken with permission from [10]. Please click here to view a larger version of this figure.

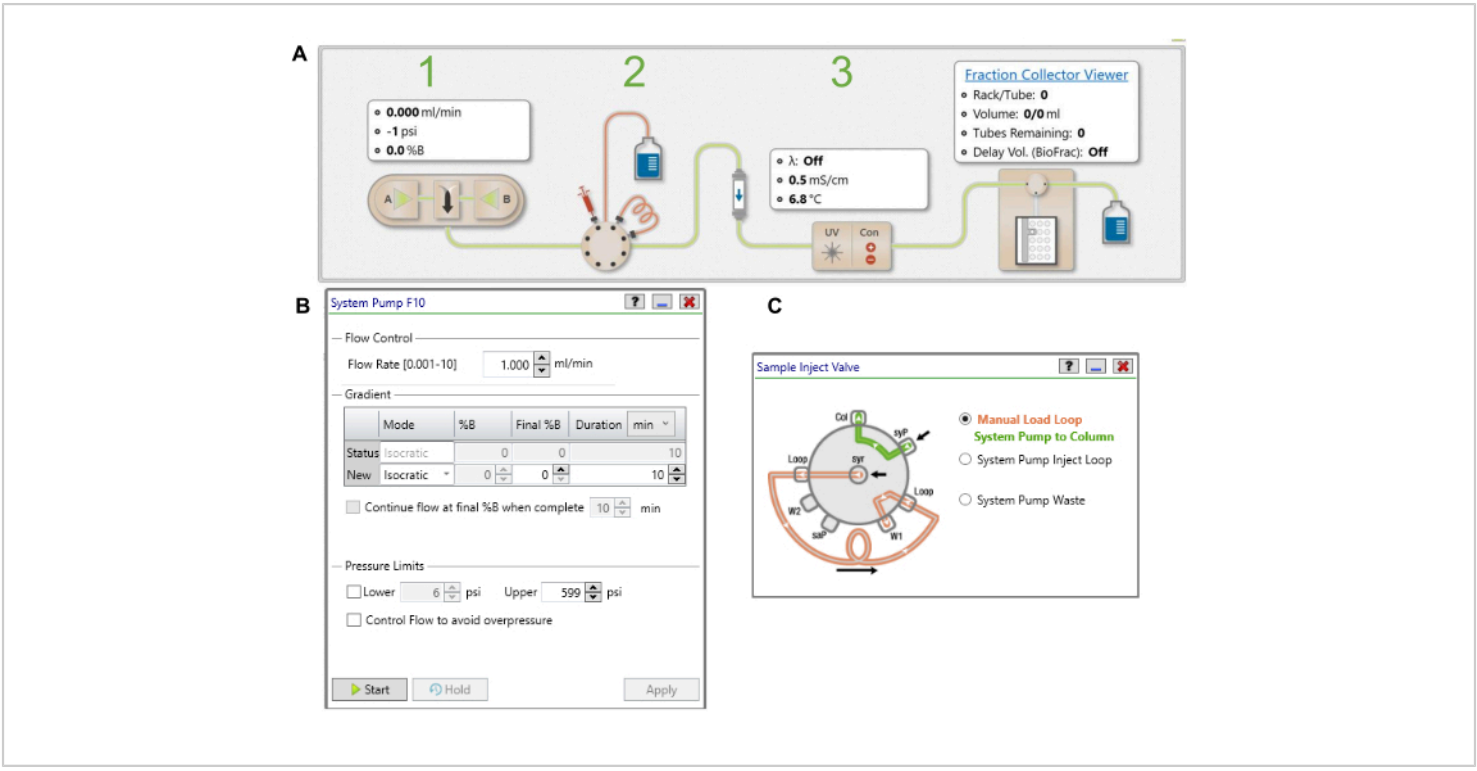

Figure 4: System control. (**A**) System control tab in which flow settings can be manipulated and flow path can be seen; (**B**) flow rate settings window; (**C**) pump settings window. Please click here to view a larger version of this figure.

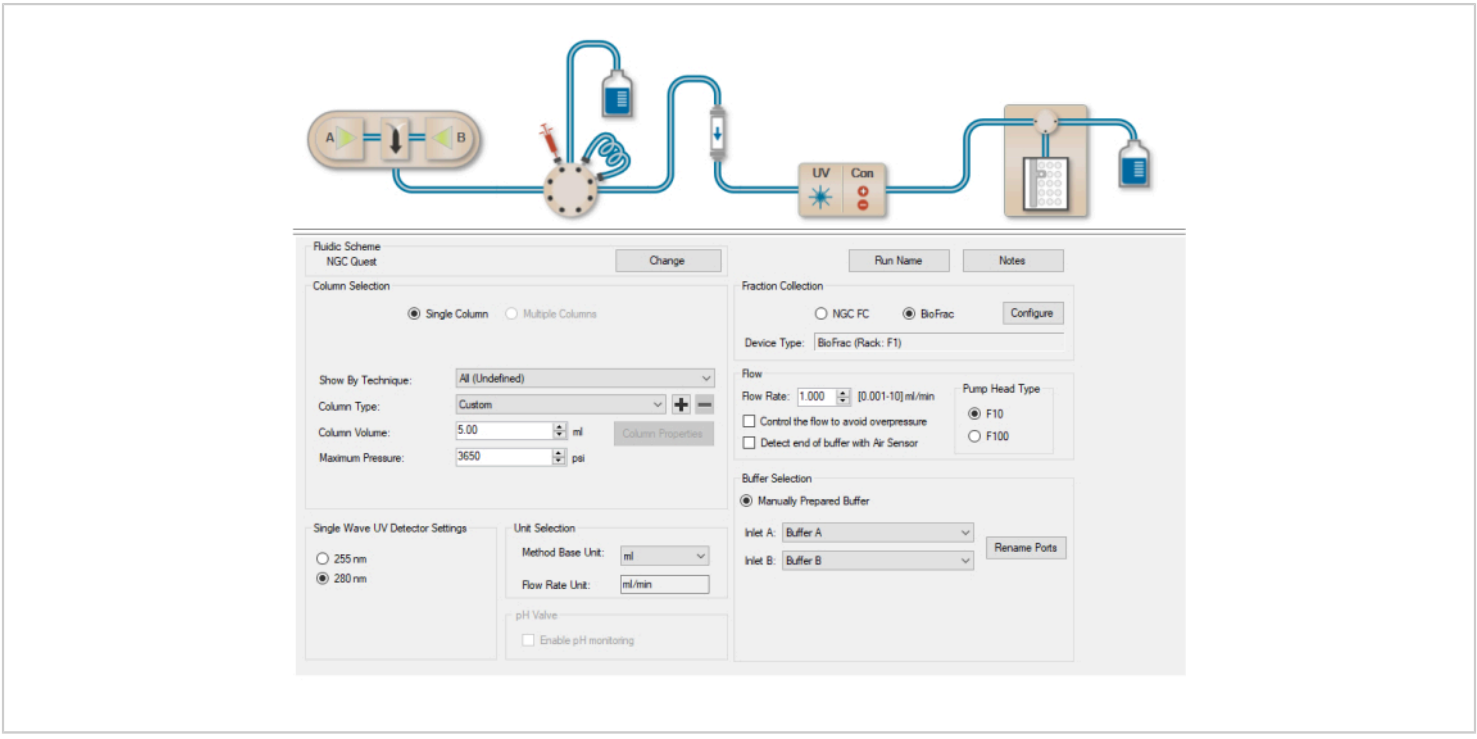

Figure 5: Method settings window. A window to enter purification details such as column type, column volume, method base unit, and flow rate for the run. Please click here to view a larger version of this figure.

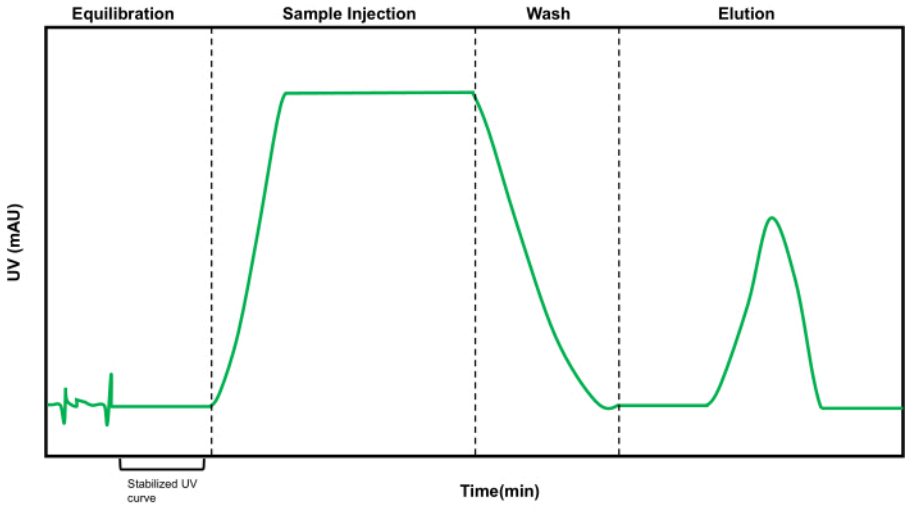

Figure 6: Schematic of the UV curve during FPLC: The UV curve rises and falls according to the purification phase. At the beginning of equilibration, the UV curve can get distorted due to the presence of air bubbles or contaminants in the tubing. As equilibration continues, the curve should stabilize as a flat line; During sample injection, as proteins unbound to the resin flow out, the UV curve rises until a maximum threshold is reached. The wash step, which flushes out residual unbound proteins, shows a gradual decrease in the UV measurements. Finally, during the elution phase, when the target protein is eluted, a peak is seen in the UV curve. Please click here to view a larger version of this figure.

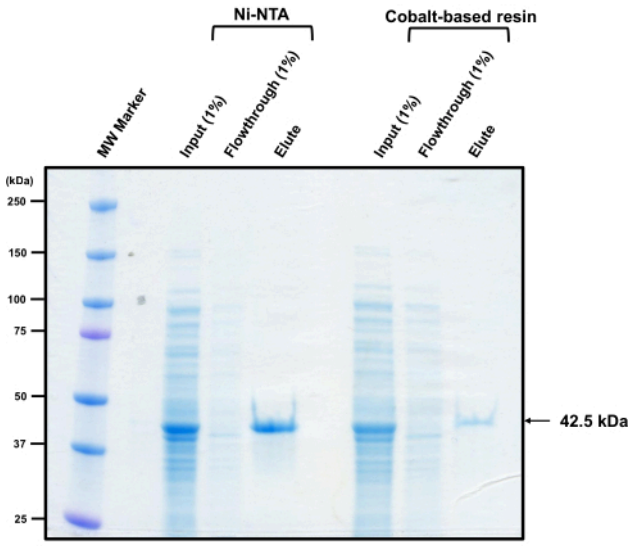

Figure 7: SDS PAGE gel image. Lane 1-Molecular weight marker; Lane 2-1% Ni-NTA Input (cell lysate); Lane 3-1% flowthrough from the Ni-NTA resin; Lane 4-hFEN1 purified from Ni-NTA resin; Lane 5-1% Input (cell lysate); Lane 6-1% flowthrough from the cobalt-based resin; Lane 7-hFEN1 purified from cobalt-based resin; black arrow represents FEN1 at 42.5 kDa. Abbreviations: SDS-PAGE = sodium dodecyl sulfate; MW = molecular weight; hFEN1 = human flap endonuclease 1; NTA = nitrilotriacetic acid. Please click here to view a larger version of this figure.

Buffers and solutions	Components
Lysis buffer	50 mM Tris–HCl [pH 8.0]
	500 mM NaCl
	1 mM beta mercaptoethanol (BME)
	1 mM phenyl-methylsulfonyl fluoride (PMSF)
	1 mM EDTA
	1 tablet complete protease inhibitor
	10 mM imidazole
	10% glycerol
Equilibration/Wash buffer	50 mM Tris–HCl [pH 8.0]
	500 mM NaCl
	1 mM BME
	1 mM PMSF
	10 mM imidazole
	10% glycerol
Elution buffer	50 mM Tris–HCl [pH 8.0]
	500 mM NaCl
	1 mM BME
	1 mM PMSF
	1 M imidazole
	10% glycerol
SDS sample loading buffer	900 µL 4x Laemmli sample buffer
	100 µL BME
Coomassie brilliant blue staining solution	50% ddH_2O
	40% Methanol
	10% Acetic acid
	0.1% Coomassie brilliant blue R-250

Destaining solution	50% ddH_2O
	40% Methanol
	10% Acetic acid

Table 1: Buffer composition.

Discussion

Affinity chromatography is a widely used technique to purify DNA-binding proteins. Immobilized metal affinity chromatography (IMAC) is a specific type of affinity chromatography that uses metal ions to capture the histidine residues of a peptide sequence. This is why the "6X-His tag" or "poly-His tag" is attached to the N-terminus or the C-terminus of proteins to be purified. Nickel and cobalt are the most commonly used metal ions and vary in their compatibility with reagents such as BME and DTT normally used in purification. Although purifications have been carried out at concentrations of 1 mM BME or DTT, the use of these reagents can still be unreliable. Incompatibility can be observed during equilibration. If the buffer that flows through the resin appears brown in color, it indicates oxidation of the metal ions and may strip the metal ions from the resin. This could drastically reduce the binding capacity of the resin.

To ensure high purity and yield of protein, affinity interactions between metal ions and the His-tag should be favored over other possible charge-based interactions. This will ensure that all of the protein is eluted at once instead of eluting over different phases of the elution. To achieve this, the protein should carry a very low charge during the purification. If the pH values of the buffers are closer to the isoelectric point of the target protein, the charge of the protein can be kept low. As FEN1's isoelectric point is approximately 8.8, our buffers are maintained at pH 8.

The Ni-NTA resin, charged with Ni ions, is thought to have a greater protein-binding capacity and is expected to yield a higher quantity of protein compared to the cobalt-based resin TALON. This also means that there is a greater potential for the binding of non-specific proteins and therefore, lower purity. TALON's lower binding capacity is complemented by higher specific binding and the yield of high-purity protein. This is consistent with our results showing a higher yield of protein purified from Ni-NTA compared to the TALON.

Regardless of resin, purity, and yield are significantly impacted by the column packing. Efficient packing can be achieved using the flow adaptor connected to the NGC automated chromatography system[9]. At high enough flow rates, the flow adaptor can effectively pack the resin and prevent the accumulation of buffer in the column. The flow adaptor along with the NGC allows for the accurate comparison of both resins due to the regulation of flow rate, pressure, and elution gradient, as well as the efficient collection of protein fractions.

Through the Chromlab software, run conditions can be monitored in real time[10]. The flow rate can be changed at any time throughout the run. During sample application, system pressure may exceed the threshold (600 psi). In this case, lowering the flow rate during the sample application step can reduce the pressure. The software also features a **Hold step** function through which any step can be extended as required. This function is particularly helpful during column wash and

elution steps. During the column wash, the UV graph is expected to dip down and almost reach 0-this signifies that most of the unbound proteins have been washed out of the resin. In case the UV line continues to rise or fails to ramp down, the wash step can be extended until the expected graph is seen. Similarly, during elution, the elution of the target protein can be visualized through peaks in the UV graph. If unexpectedly, no peaks are visible, the elution step can be extended until anticipated peaks are observed.

Tips and tricks

During the equilibration step, the buffer must be injected into the column and the waste buffer should be continuously released into the waste bottle; this ensures that all connections are tight and leakproof. In case of any leakage from any site of the NGC, all the ports surrounding the area must be checked, since the origin of the leak may be different from where the leak is noticed. If the flow has been started but no buffer is entering the column, it is likely that air is trapped within the tubing. Purge the system before continuing further.

During sample injection, if the back pressure from the system is resisting the injection, the tubing must be unscrewed from Loop E and placed in the waste bottle for the duration of the injection. It is important to screw it back into Loop E before starting the program. In case the equilibration buffer or sample is accumulated in the column during the run, the run must be paused and the flow adapter removed. The tubing must be unscrewed from the bottom of the column and the buffer or sample allowed to flow through. If the sample flows through, make sure to collect it as the flowthrough.

If fractions run on SDS PAGE gel show high levels of contaminant proteins, fractions could be run on either side of the peaks seen in the chromatogram. Often, for low-expression proteins, pure fractions do not show significant peaks in the UV.

Understanding the differences between the properties of the nickel-based resin and the cobalt-based resin allows for the selection of the resin best suited for the future applications of the purified protein. For example, proteins used in *in-vitro* biochemical assays are expected to be of the highest possible purity, a situation in which the cobalt-based resin is suitable. However, if the protein is used for the production of antibodies, a significant yield of purified protein is required for the process. The Ni-NTA resin could serve as a better option in this case. This article aimed to bring out the differences in these resins through the purification of FEN1.

Disclosures

The authors have no competing financial interests or other conflicts of interest.

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References

1. Balakrishnan, L., Bambara, R. A. Flap endonuclease 1. *Annu Rev Biochem.* **82**, 119-138 (2013).
2. Asagoshi, K. et al. FEN1 functions in long patch base excision repair under conditions of oxidative stress in vertebrate cells. *Mol Cancer Res.* **8** (2), 204-215 (2010).
3. Lyamichev, V., Brow, M. A., Dahlberg, J. E. Structure-specific endonucleolytic cleavage of nucleic acids by

eubacterial DNA polymerases. *Science.* **260** (5109), 778-783 (1993).

4. Bornarth, C. J., Ranalli, T. A., Henricksen, L. A., Wahl, A. F., Bambara, R. A. Effect of flap modifications on human FEN1 cleavage. *Biochemistry.* **38** (40), 13347-13354 (1999).
5. Xu, Y., Potapova, O., Leschziner, A. E., Grindley, N. D., Joyce, C. M. Contacts between the 5' nuclease of DNA polymerase I and its DNA substrate. *J Biol Chem.* **276** (32), 30167-30177 (2001).
6. Wen-Hui, K., Kuo, Chase, H. A. Exploiting the interactions between poly-histidine fusion tagsand immobilized metal ions. *Biotechnol Lett.* **33** (6), 1075-1084 (2011).
7. Charlton, A., Zachariou, M. Immobilized metal ion affinity chromatography of native proteins. *Methods Mol Biol.* **421**, 25-35 (2008).
8. Ononye, O. E., Njeri, C. W., Balakrishnan, L. Analysis of DNA processing enzyme FEN1 and its regulation by protein lysine acetylation. *Methods Mol Biol.* **1983**, 207-224 (2019).
9. Bio-Rad. *Econo-column flow adaptor instruction manual.* https://www.bio-rad.com/webroot/web/pdf/lsr/literature/M7380014.pdf.
10. Bio-Rad. *NGC Chromatography systems and ChromLab software instrument guide version 3.3.* https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10026252.pdf (2015).