Automated affinity purification and subsequent buffer exchange of recombinant proteins in a single system

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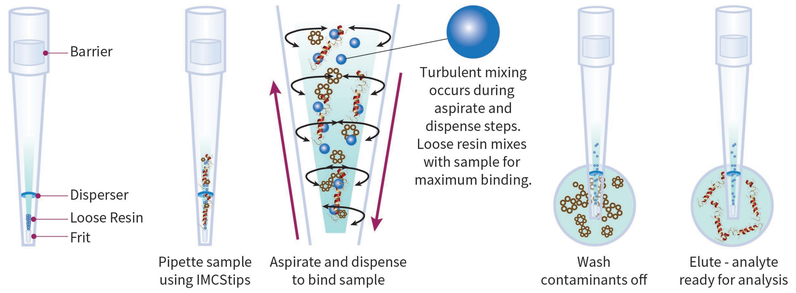
Introduction



- An effective purification of recombinant proteins is through the affinity ligand-tag system.
- The elution step often involves the use of small molecules at higher concentration and/or with higher affinity to the resin ligand.
- The small molecule needs to be removed before downstream analysis
- Here, we demonstrate a fully automated, high-throughput affinity purification followed by buffer exchange on a single robotic platform.

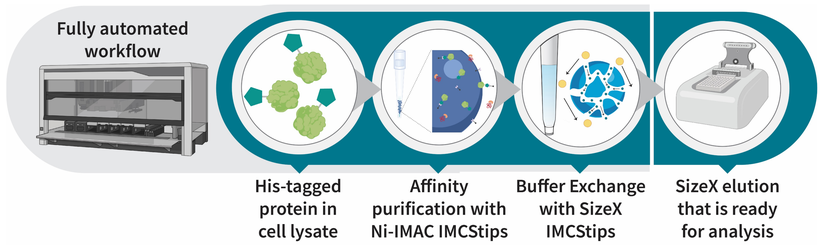
Instrumentation

Three histidine-tagged proteins were purified using affinity IMCStips filled with 15 µL resin bed and SizeX$_{150}$ IMCStips operated on Hamilton Microlab STAR. Below shows deck layout.

- A Tip Carrier
- A1 Affinity IMCStips
- A2 Hamilton 1000 µL Tips
- A3 Hamilton 300 µL Tips
- B Tip Carrier
- B1 SizeX150 IMCStips
- B2 Hamilton 300 µL Slim Tip
- C Plate Carrier
- C1 SizeX Equilibration Buffer Trough
- C2 Affinity Equilibration Buffer Trough
- C3 Affinity Wash Buffer Trough
- C4 Affinity High Salt Wash Buffer Trough
- C5 Affinity Elution Buffer Trough
- D Plate Carrier
- D1 Waste Trough
- D2 Equilibration Plate
- D3 Sample Plate
- D4 Wash 1 Plate
- E Plate Carrier
- E1 Wash 2 Plate
- E2 Wash 3 Plate
- E3 Affinity Elution Plate
- E4 SizeX Elution Plate

Methodology

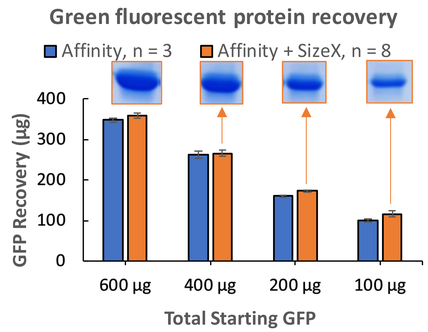
Total processing time: < 1 hour and 30 minutes

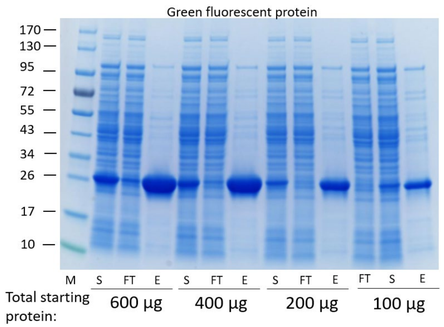


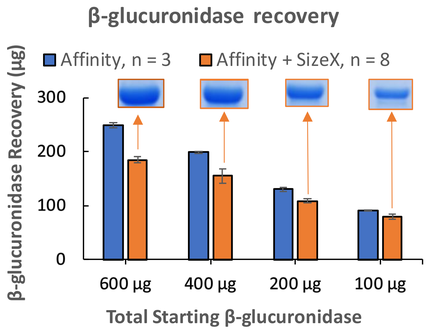
Results

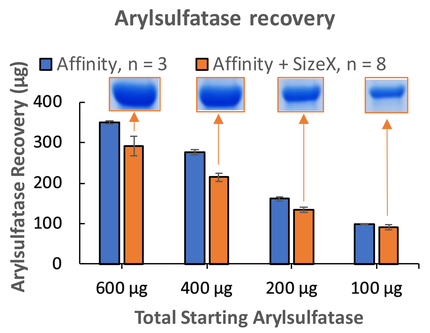
Protein recovery was measured using NanoDrop at 280 nm. SDS-PAGE gel shows high purity of recovered proteins. Protein band intensities correlate to protein recovery measurement.

* Affinity vs Affinity+SizeX$_{150}$ results were not from the same runs









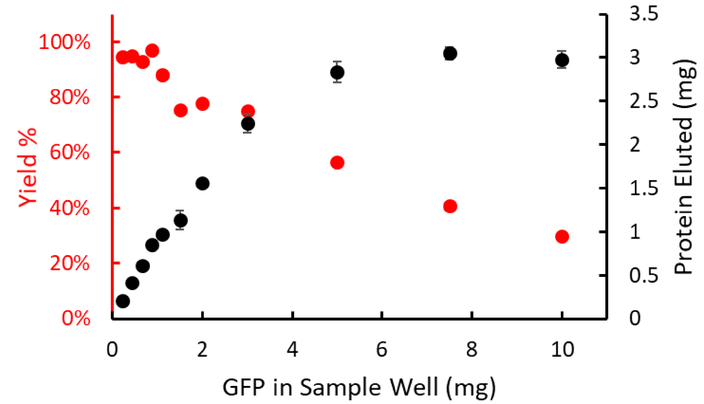
M = Marker, S = Sample, FT = Flowthrough, E = Elution

Results

Less than 1 mM imidazole in protein solution after buffer exchange. Started with 300 mM imidazole from Ni-IMAC elution. Measured using NanoDrop (Abs @ 230 nm)

Protein Recovery Profile

The profile generated for 41 µL resin bed in IMCStips



Profile of recovered GFP in total protein eluted (**black**) and percent yield as a function of initial sample load (**red**). D*ata from Ref 1.*

Conclusion

- Fully automated protein affinity purification and buffer exchange in a single liquid handling system
- Up to 96 crude lysate can be processed and desalted, purified proteins in less than 1 hour and 30 minutes.

  > 90% recovery of 100 µg recombinant protein using IMCStips filled with 15 µL Ni-IMAC resin, followed by SizeX$_{150}$ IMCStips
- SizeX$_{150}$ IMCStips automate buffer exchange of proteins removing > 99% of imidazole

Reference

1. P. A. Kates, J. J. Tomashek, D. A. Miles, L. A. Lee, *BioTechniques* **2020**, *68*, 148-154.