Building Better Purifications: Automation-Enabled Optimization Across Chromatography Workflows

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INTRODUCTION

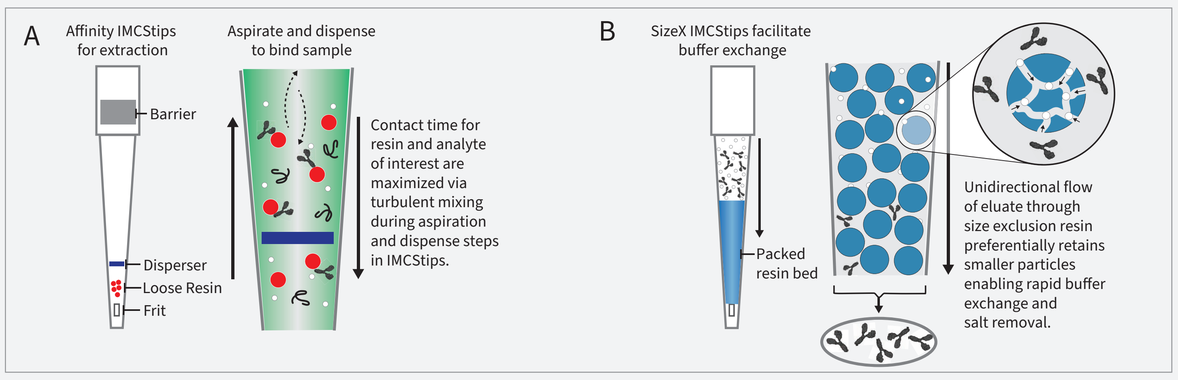
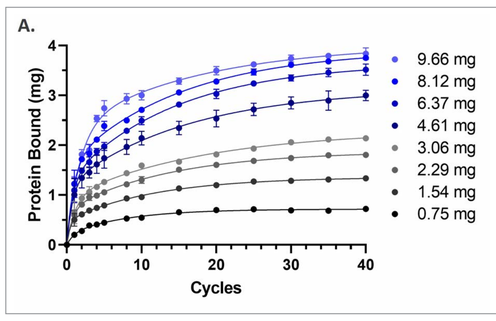


Figure 1. *Representation of the operation of the two types of IMCStips used in this study: Affinity or Ion Exchange IMCStips (A) and SizeX IMCStips (B).*

- Optimizing protein purification is essential for achieving high yield, purity, and stability. Conventional optimization relies on multiple instruments and manual steps, limiting throughput and reproducibility.
- This study evaluated IMCStips® as a high-throughput platform for automating purification optimization. Chromatography resins built into pipette tips enabled rapid screening of resin types, wash and elution conditions, and buffer exchange steps in a versatile, programmable format.
- Workflows were tested across multiple automated liquid-handling systems to assess reproducibility, recovery, and scalability.
- Together, these studies demonstrate a modular, automation-enabled framework for developing and integrating protein purification processes.

AFFINITY PURIFICATION - Optimization of Resin selection, Wash, and Elution Parameters for Antibodies

Automated Protein A purification using IMCStips® on the Hamilton STAR platform was used to evaluate resin performance and optimize purification conditions. Binding kinetics were assessed using human polyclonal IgG, while elution and wash parameters were optimized across four antibodies (mAb1–3, bsAb) to capture resin- and antibody-specific effects on yield and impurity clearance.

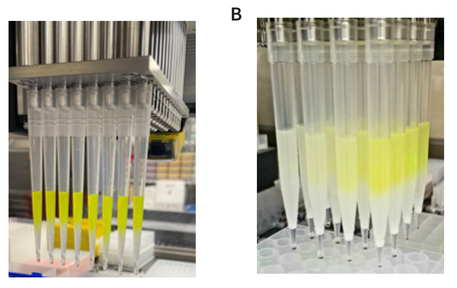


Resin	Manufacturer's DBC (mg/mL)	Tip-based q_{max} (mg/mL)	Tip-based Kd (mg/mL)
PrismA	80	80.3	0.23
HipH	60	52.4	0.40
MSSLX	60	72.4	0.51
DurA	80	71.8	0.58

Figure 2. *Binding kinetics and resin performance comparison for Protein A IMCStips®. Example kinetics for PrismA (**A**) illustrate protein binding across cycles at varying IgG loads to illustrate the approach used to model binding behavior.* ***Table 1:*** *Calculated theoretical capacity (q_{max}) and K_D values for multiple Protein A resins.*

AUTOMATED SIZE-EXCLUSION WORKFLOW – Across Sample Volumes and Biomolecule Sizes

Automated buffer exchange using SizeX IMCStips® on the Dynamic Devices Lynx enabled >80% recovery of proteins, plasmids, and viral particles across 100 µL to 1 mL volumes. The 10–20 minute workflow achieved high salt and dye removal without centrifugation or vacuum steps, offering a fast, reproducible solution for high-throughput desalting.



Sample Volume	Sample Protein	% Protein Recovery	Elution Volume	% Salt Removed
100 µL	GFP	84 ± 3	103 ± 3 µL	98
150 µL	GFP	83 ± 2	153 ± 2 µL	98
750 µL	GFP	90 ± 5	748 ± 41 µL	99
1,000 µL	GFP	85 ± 4	1034 ± 15 µL	99

Figure 6. *SizeX$_{100}$ and SizeX$_{150}$ IMCStips® are for sample volumes of 100–150 µL (**A**, using 1 mL tips) while SizeX$_{750}$ and SizeX$_{1000}$ IMCStips® are for sample volumes of 750–1000 µL (**B**, using 5 mL tips).* ***Table 4:*** *Consistent recovery (>80%) and >98% salt removal across varying sample volumes using SizeX IMCStips®.*

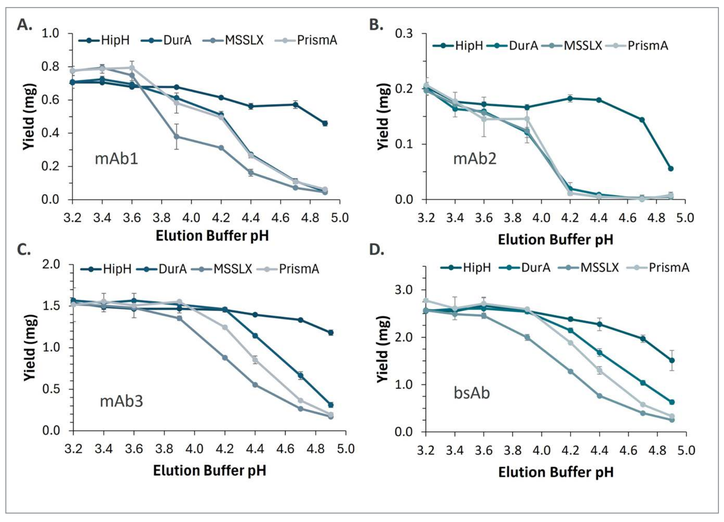


Figure 3. *Subsequent elution optimization was performed across four antibodies (mAb1–3, bsAb) using varying pH conditions, revealing resin-dependent recovery trends. HipH maintained higher yields under milder elution (pH ≥ 3.6), while others showed greater sensitivity to pH changes.*

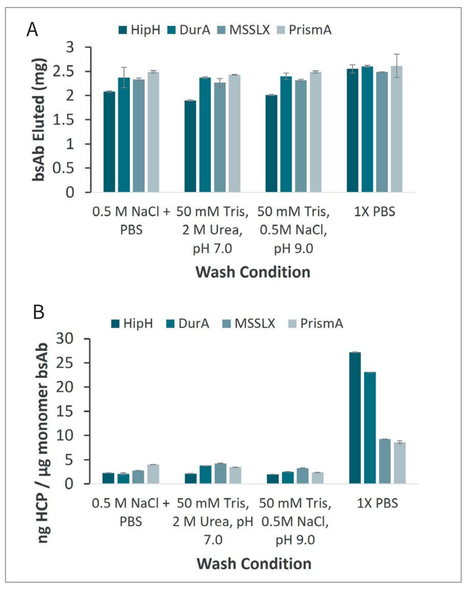


Figure 4. *Effect of wash conditions on recovery (**A**) host-cell protein (HCP) removal (**B**) during bispecific antibody purification, eluted at pH 3.4. Wash condition screening further demonstrated that buffer composition influenced host-cell protein (HCP) clearance. High-salt and chaotropic washes reduced HCP carryover.*

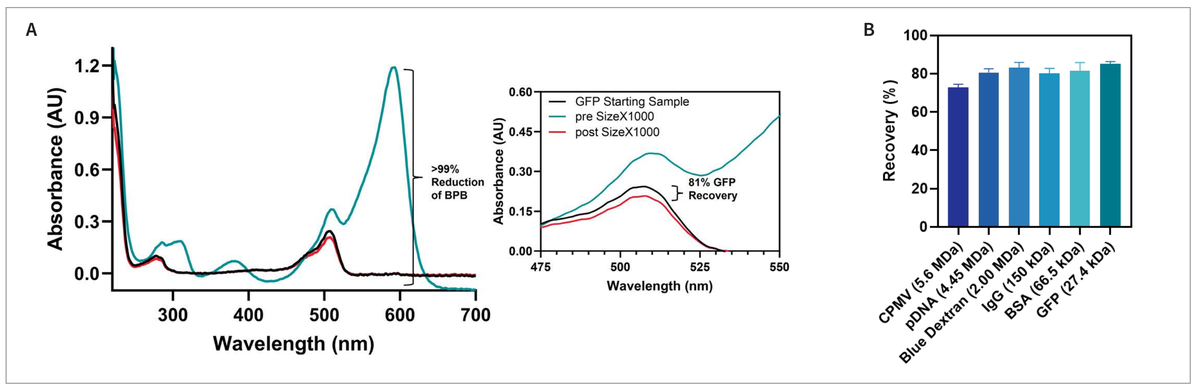


Figure 7. *(**A**) UV-Vis analysis of bromophenol blue (BPB at 505 nm absorbance) and green fluorescent protein (GFP, at 590 nm absorbance) before and after buffer exchange using SizeX$_{1000}$ IMCStips®. Greater than 99% of BPB was removed while 81% of initial GFP was retained. (**B**) SizeX$_{150}$ IMCStips were used to buffer exchange various samples with a range of molecular weights. The tips performed consistently across molecular weights from 27 kDa to 5.6 MDa, demonstrating broad applicability for biomolecules of varying sizes.*

CONCLUSION

- IMCStips® provide a modular, automation-ready framework for optimizing protein purification, with Affinity, Ion Exchange, and Buffer Exchange workflows delivering consistent recovery and reproducibility while eliminating manual transfer steps.
- Each run processed up to 96 samples in <60 minutes (Affinity and IEX), or <20 minutes (SizeX) demonstrating a high-throughput platform for rapid optimization.
- These workflows can also be combined, for example, using SizeX IMCStips® for post-Affinity, to create fully automated, end-to-end purification pipelines.
- Cross-platform compatibility demonstrates flexibility for diverse automation systems, supporting scalable and reproducible purification development.

ION EXCHANGE CHROMATOGRAPHY – Automated Screening of Anion-Exchange Resins

Automated ion-exchange purification was performed on a Hamilton STAR using IMCStips® containing four commercial anion-exchange resins with different chemistries and pore structures. Bovine serum albumin (BSA) was used as a model protein to evaluate binding kinetics and resin performance. Fractogel TMAE HiCap (M) achieved the highest capacity (~155 µg protein/µL resin) with the lowest dissociation rate, while POROS 50 HQ and Sepharose Q Fast Flow exhibited intermediate binding behavior. Comparison with long-term equilibrium data confirmed that automated binding achieved nearly the same equilibrium capacities as 72-hour batch incubations, demonstrating rapid equilibration and reproducible, resin-specific performance within the IMCStips® format.

Resin Type	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_a (M^{-1}) (k_{on}/k_{off})
POROS 50 HQ	8.35E+04	3.62E-01	2.31E+05
Fractogel TMAE HiCap (M)	1.81E+04	2.28E-02	7.92E+05
Fractogel TMAE (M)	6.59E+04	4.49E-01	1.47E+05
SMT-DEAE (BDEAE)	5.17E+04	4.27E-01	1.21E+05

Table 2. *Binding kinetics (bimolecular rates) for resins.*

Resin Type	K_{D30} (µg/mL)	q_{max30} (µg/µL)	K_{D72h} (µg/mL)	q_{max72h} (µg/µL)
POROS 50 HQ	177	65.4	47.3	101.8
Fractogel TMAE HiCap (M)	255.9	155.3	46.7	185.1
Fractogel TMAE (M)	450.2	89.7	58.7	97.1
SMT-DEAE (BDEAE)	622.7	113.8	374.1	76.3

Table 3. *Binding isotherm comparison between 30 binding cycles using IMCStips (K_{D30}, q_{max30}) and 72-hour equilibrium binding in the tube (K_{D72h}, q_{max72h}). Units are q_{max}: µg/µL; K_D: µg/mL.*

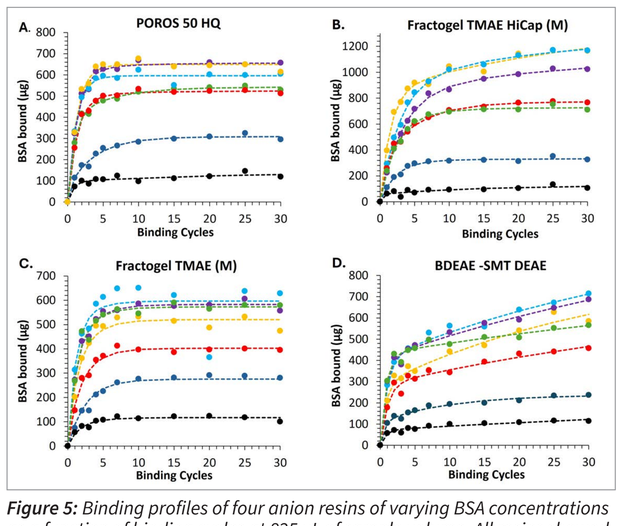


Figure 5: *Binding profiles of four anion resins of varying BSA concentrations as a function of binding cycles at 825 µL of sample volume. All resins showed rapid binding, reaching equilibrium within ~10 aspiration–dispense cycles. Initial protein loads: ●1619.48 µg ●1593.10 µg ●1268.85 µg ●880.28 µg ●851.40 µg ●444.68 µg ●210.38 µg*

	HAMILTON	dynamicdevices	TECAN	INTEGRA	opentrons	analytikjena
Affinity	•	•	•	•	•	•
$SizeX_{50}$			•			
$SizeX_{100}$, $SizeX_{150}$	•	•	•		•	•
$SizeX_{750}$, $SizeX_{1000}$		•				
Nucleic Acids	•	•	•		•	•
Ion Exchange	•	•	•	•	•	•
Reverse Phase	•	•	•	•	•	•
Phosphopeptide	•	•	•	•	•	•
Custom Solutions	•	•	•	•	•	•

Table 5. *IMCStips® availability across automated liquid-handling systems for Affinity, Ion Exchange, Buffer Exchange, and other chromatography applications.*

Contact: inquiries@imcstips.com

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