Section Mass Spectrometry and Proteomics

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Digital Membrane Chromatography — A New Way to Rapid Antibody Purification for Top-Down Mass Spectrometry

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Introduction

Monoclonal antibodies are essential therapeutic biomolecules that require efficient purification for production and analytical workflows.[1,2] Protein A affinity chromatography uses acidic elution, which risks antibody damage and is incompatible with mass spectrometry (MS).[3,4] Conventional desalting adds time, dilution, and sample loss.[4] To overcome these issues, a Protein A membrane system that uses a voltage application for elution, was used (DMC). This approach avoids harsh buffers and yields eluates directly suitable for top-down MS (TD-MS).

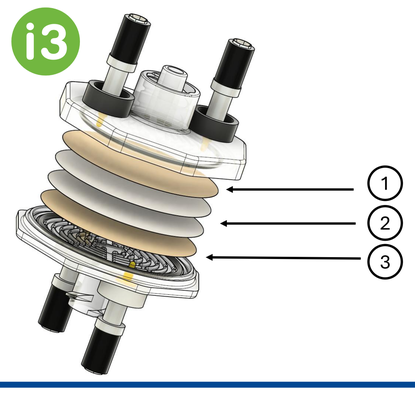

Fig. 1: **Schematic illustration of DMC^{PrA} Membrane Adsorber in exploded view.** (1) Gold-coated membrane electrode (upper side); (2) Regenerated cellulose membrane functionalized with Protein A; (3) Gold-coated membrane electrode (lower side).

Operating Principle

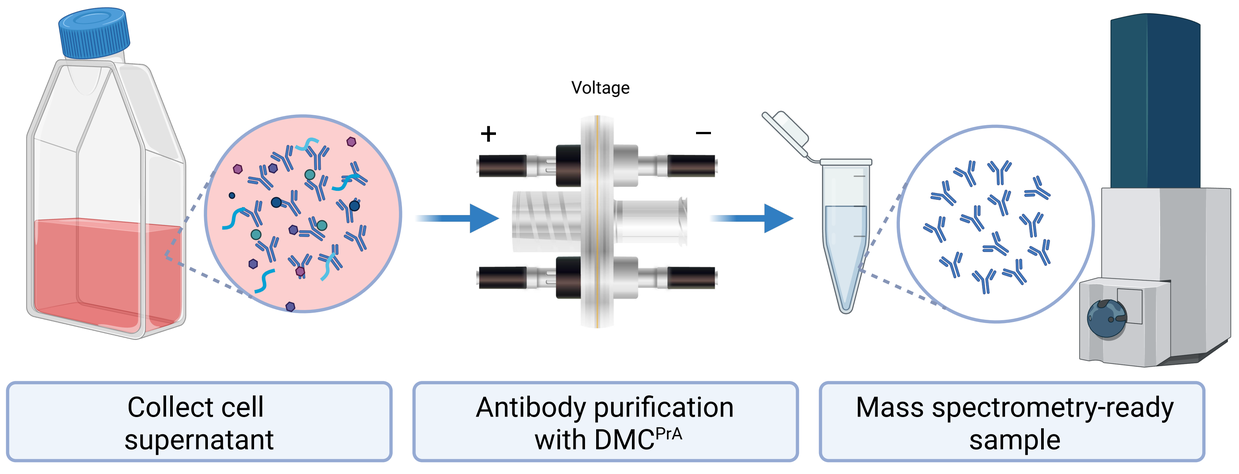

Fig. 2: **Schematic workflow of antibody purification for TD-MS analysis.** Starting from cell supernatant from antibody expression system, purification is performed using a DMC^{PrA} Membrane Adsorber for binding and washing. Antibodies are then eluted by voltage-triggered elution, and the purified sample can be directly analyzed by MS. Created with BioRender.com.

Results

- ❑ Top-down ESI-Q-TOF MS of DMC eluate shows clear intact antibody signals (Figure 3A) and deconvolution yielded a mass of ~148 kDa consistent with intact Trastuzumab (Figure 3B)
- ❑ DMC purified antibodies show equivalent binding performance compared to Protein G purified antibodies, demonstrating preserved functionality and binding capacity (Figure 4)
- ❑ DMC purification produced intact antibody spectra in minutes, while conventional columns require lengthy equilibration, buffer exchanges, and desalting steps (Figure 5)

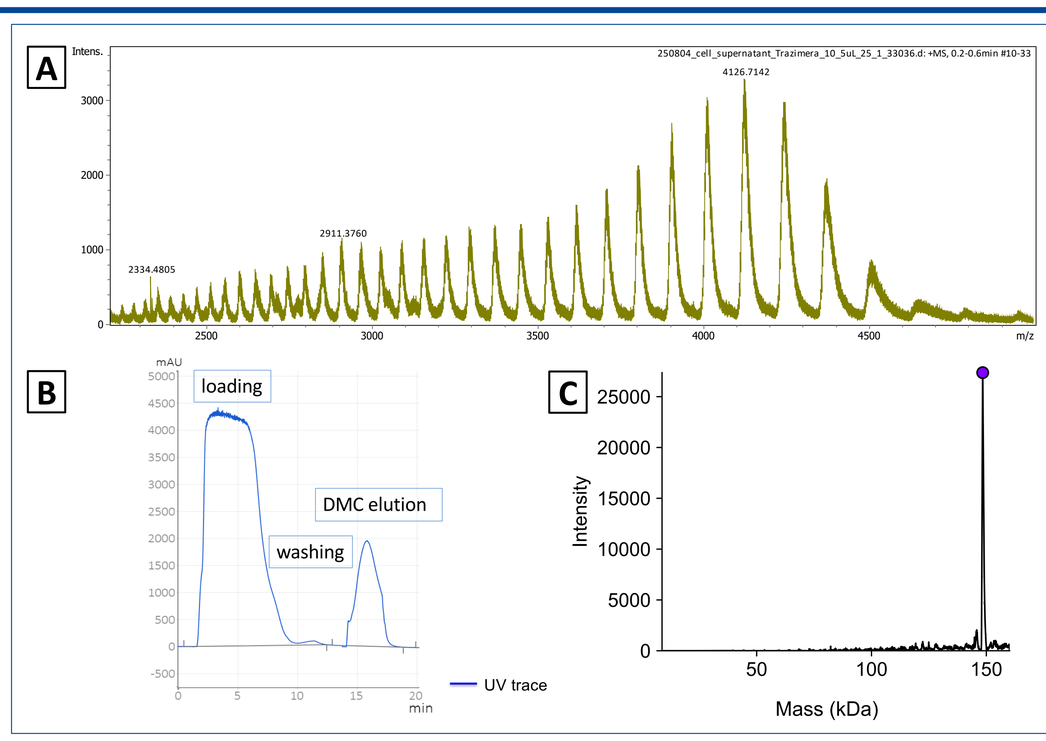

Fig. 3: **ESI-Q-TOF analysis and deconvolution of DMC-cleaned Trazimera®.** (A) Direct injection into ESI-Q-TOF of cleaned Trazimera® sample from cell culture supernatant. (B) Cell culture supernatant spiked with Trazimera®; elution of unbound protein from DMC^{PrA}, washing, and DMC elution. (C) UniDec deconvolution of ESI-Q-TOF spectrum of cleaned Trazimera® sample out of cell supernatant, showing main molecular weight at 148.42 kDa.

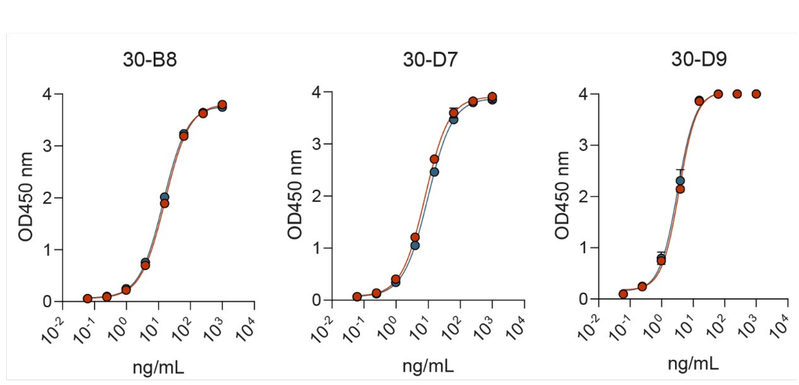

Fig. 4: **Functional analysis of mAbs isolated from IgG-expressing cell supernatants using DMC^{PrA}.** 30-B8, 30-D7, and 30-D9 IgGs were expressed in HEK293F cells and isolated from supernatant using either DMC^{PrA} membranes (red) or Protein G resin (blue). Binding to the PcrV protein was assessed by ELISA. Antibodies were serially diluted (four-fold) starting at 1 µg/mL.

Fig. 5: **Commercial column versus DMC^{PrA}.** Comparison of total processing times for antibody purification using a commercial column versus the DMC^{PrA} membrane adsorber. Created with BioRender.com.

Conclusion

DMC enables gentle and rapid antibody purification directly from commercial formulations and complex matrices such as cell culture supernatants.

The method produces eluates that are ready for mass spectrometric analysis without requiring additional desalting steps.

DMC eluates retain binding capacity equivalent to commercially established Protein G purification.

Overall, DMC provides a promising new strategy for antibody enrichment and clean-up in structural proteomics, bioprocessing monitoring, and therapeutic quality control.

References

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