High-throughput mAb purification with Fibro chromatography

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Introduction

Biomanufacturing is trending towards increased number of monoclonal antibody (mAb) projects and smaller batch sizes, with production of most mAbs expected to be below 150 kg/yr*. These trends are fueling demands to screen more clones faster and improve the efficiency of research and process development (PD). Current approaches to purify mAbs for high-throughput screening of lead candidates and optimize process conditions often use resin-based columns and liquid handling robots. This setup requires a large footprint and capital-intensive equipment.

Rapid cycling, fiber-based chromatography (Fibro) enables substantially reduced purification times in research and PD. Fibro chromatography supports relatively high capacity with residence times of seconds and allows full chromatographic runs in a few minutes per cycle. Here we describe how rapid cycling, using Fibro PrismA units together with an ÄKTA pure™ system and an autosampler, offers new opportunities in high-throughput purification for screening of lead candidates and process conditions.

* Data derived from BDO's BioProcess Technology Consultants bioTRAK™ database.

What is Fibro chromatography?

The Fibro adsorbent material has a cellulose fiber matrix with an open pore structure where mass transfer is governed by convective flow. This allows for high binding capacity (> 30 g/L) and residence times measured in seconds rather than minutes (Fig 1 and 2).

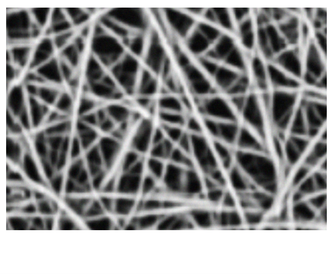

Fig 1. Conventional chromatography: diffusive flow. Mass transfer is restricted by the slow diffusion of molecules through the pores in the beads. Process flow is restricted by bead rigidity.

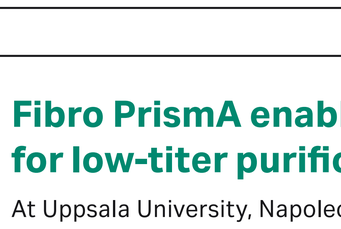

Fig 2. Fibro chromatography: convective flow. Mass transfer is fast due to convective flow. Process flow is restricted only by the size of the pores in the matrix material.

Fibro PrismA enables new opportunities for low-titer purification

At Uppsala University, Napoleone A. *et al.*† used Fibro PrismA to capture a bispecific mAb from low-titer cell culture supernatant. Eight liters of supernatant with a titer of only 7 µg/mL was loaded on a Fibro PrismA 3.75 mL unit. Figure 7 shows the wash, elution, and CIP phases. The purification cycle of 4.5 h is considered extremely long for Fibro. However, the corresponding purification on a protein A column would have taken more than a week, which is not feasible. Fibro PrismA allowed concentration and purification in one step, which resulted in high recovery and purity of the molecule.

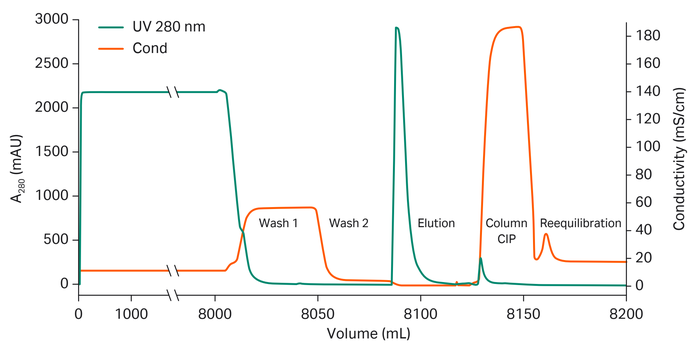

Fig 7. Purification of a bispecific antibody from a low-titer cell culture supernatant using a Fibro Prisma 3.75 mL unit.

† We thank Triinu Linkgreim, Antonino Napoleone, and Martin Lord, Group of Sara Mangsbo, Uppsala University, for permission to share their data. See their publication for details. https://doi.org/10.1016/j.nbt.2021.07.002

High binding capacity at short residence time and consistency over cycles

A scalable 4 mL Fibro unit immobilized with the protein A "PrismA" ligand can run a full chromatographic cycle in a few minutes with concentrated elution pool volumes of less than 3 matrix volumes (MV) (Fig 3). Consistent performance over cycles (recovery > 95%) was demonstrated by running 50 cycles with the 4 mL Fibro PrismA unit on ÄKTA pure™ 150 system (Fig 4). The elution peak has the same behavior over cycles and the pressure is stable.

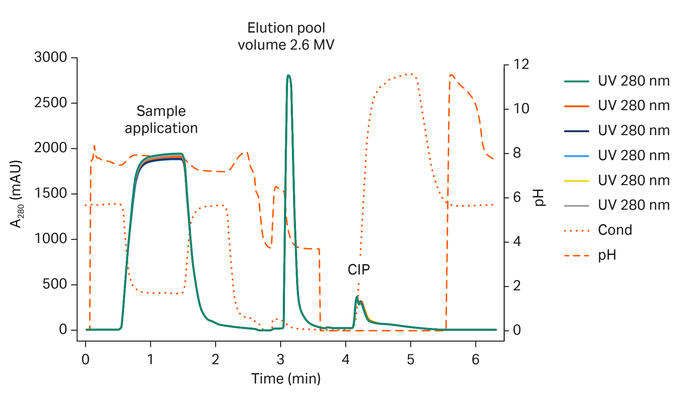

Fig 3. Protein A bind-elute profile on clarified cell culture mAb feed of 3.8 mg/mL purified on a ~ 4 mL Fibro PrismA unit: Overlay UV_{280} nm every 10th cycle, cycle time 6.3 min, flow rate 8 MV (CIP 4 MV).

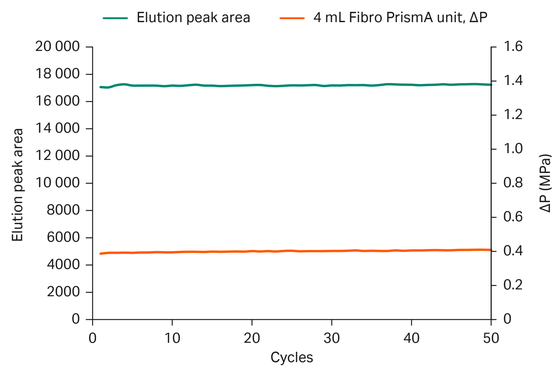

Fig 4. Trend curves of max delta column pressure (ΔP) and area of elution peak performance over cycles. The UNICORN™ software trending tool uses data from a large number of chromatography cycles to generate trend curves for area of UV peaks and pressure in a selected phase.

High throughput, automated one- and two-step purification of mAb samples with laboratory-scale HiTrap Fibro™ PrismA unit

When screening multiple clones or optimizing a process, it is critical to have automated, efficient purification solutions with minimized cross-contamination risk.

Purification of 10 different mAb samples was set up on an ÄKTA pure™ system (Fig 5) with a Teledyne™ autosampler. The 0.4 mL HiTrap Fibro™ PrismA unit was compared with a 1 mL HiTrap™ MabSelect PrismA™ column. To check for carryover between runs of different samples, an SDS-PAGE analysis was performed on blank runs after the protein A step (Fig 6). No protein bands were visible, indicating lack of carryover. With the HiTrap Fibro™ PrismA unit, the capture step time was reduced from ~ 60 min to ~ 10 min (including ~ 5 min to clean and prepare the autosampler between samples), with similar recovery and purity to the HiTrap™ column packed with MabSelect PrismA™ resin.

An automated 2-step tandem purification of mAb feed samples was set up with the captured peak from the HiTrap Fibro™ PrismA unit directly transferred to 2 × 5 mL HiTrap™ Desalting columns. The setup included a versatile valve and a second UV to track sample from the second step. High recovery between the capture and the desalting steps was obtained, and cycle time was reduced more than 3-fold compared with HiTrap™ MabSelect PrismA™ (Table 1).

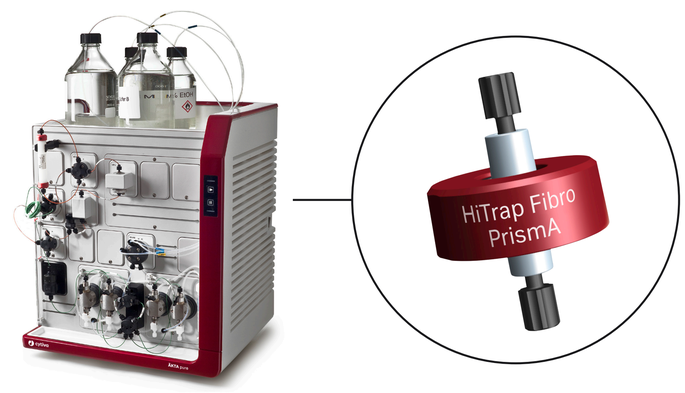

Fig 5. This picture illustrates an ÄKTA pure™ system and the HiTrap Fibro™ PrismA unit.

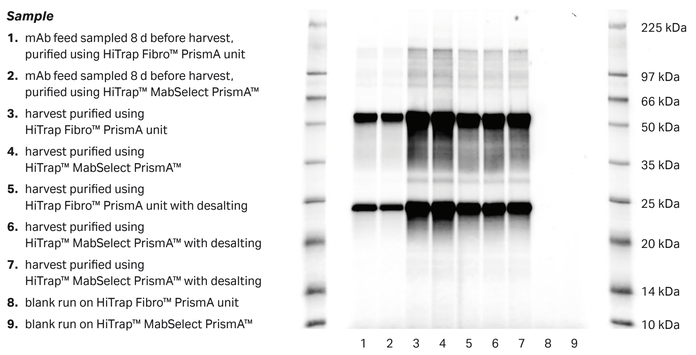

Fig 6. SDS-PAGE analysis after protein A step. Blank runs are samples 8 and 9.

Table 1. Recovery and run time of 2-step tandem purification

Purification format	Recovery – UV measuring elution area of affinity step (mL × mAU)	Recovery – UV measuring elution area of desalting step (mL × mAU)	Total run time, including autosampler and CIP (min)
HiTrap™ MabSelect PrismA™ column, 1 mL	1036	1027	84
HiTrap Fibro™ PrismA unit, 0.4 mL	1175	1109	22

Conclusions

Here we describe a technology for high-throughput, automated purification with the potential to reduce time-to-market:

- The open porous structure in the fiber adsorbent material enables purification cycle times of a few minutes.
- Serial set-up with autosampler on ÄKTA™ systems provides full chromatograms in high-throughput mode, which automates purification of large numbers of samples.
- Fibro PrismA enabled high-speed sample preparation for research purposes in 4.5 h. Purification would not be feasible with protein A resin chromatography, as it would take > 1 wk.

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