Monoclonal antibodies (mAbs) are used in the clinic to treat a variety of diseases, including various cancers, multiple sclerosis, and rheumatoid arthritis. To develop a single therapeutic mAb hundreds of candidates are purified and screened. The downstream functional assays used to characterize candidate mAbs require milligram quantities of highly pure protein in appropriate buffers. Low pH, for example, can lead to mAb degradation, and cell-based functional assays require specific salt concentrations and pH. These stringent requirements have made mAb purification a bottleneck in the discovery process.

To increase the throughput of our existing mAb purification protocol we coupled an NGC Discover™ Pro Chromatography System to a Teledyne CETAC ASX-560 Autosampler. The NGC™ Discover Pro System’s multidimensional (Multi-D) chromatography function allows automated multicolumn purification workflows while the ASX-560 Autosampler accommodates up to eighty-four 50 ml conical tubes for automatic sample loading. The NGC Signal Import Module (SIM) was used to facilitate communication between the two instruments.

We show that, using this medium-throughput automated process, 84 samples can be purified in two days, yielding pure protein in a highly reproducible manner.

Materials and Methods

NGC System Configuration

We used an NGC Discover Pro System. The modules required for this tandem purification setup were the system pumps with two buffer inlets, a mixer valve, an inject valve, a sample pump with one sample inlet valve, two column switching valves, a multi-wavelength detector, an outlet valve, and a pH valve. The buffer inlets were used for buffers and cleaning solutions. The outlet valve was used to collect flowthrough. A BioFrac™ Fraction Collector collected the purified mAbs.

Figure 1 summarizes the connections made between the autosampler and the NGC System via the SIM.
Increasing Throughput of a Tandem mAb Purification by Adding a High Volume Autosampler to the NGC™ Chromatography System

Chromatography
A 1 ml HiTrap MabSelect SuRe Column was used for the initial capture step with HBS wash buffer (50 mM HEPES, pH 7.5, 150 mM NaCl) and 0.1 M acetate elution buffer (50 mM acetate pH 3.5, 150 mM NaCl). For the tandem method, the protein was eluted off the affinity column directly onto two 5 ml HiTrap Desalting Columns plumbed in tandem for pH neutralization and buffer exchange. All columns were cleaned in place between samples using three column volumes of 0.5 M NaOH and re-equilibrated using HBS.

SDS-PAGE Electrophoresis
Fractions containing purified mAbs were run on TGX Stain-Free™ Gels and imaged using the Gel Doc™ EZ Imaging System.

Liquid Chromatography-Mass Spectrophotometry (LC-MS)
Purified antibodies were analyzed using an Agilent 1200 HPLC System followed by electrospray ionization mass spectrometry using an Agilent Q-TOF 6520 Instrument. A Poroshell 300SB-C8 Column was used for desalting and separation.

Results
Increased Throughput and Associated Recovery Rates
mAbs were captured using a Protein A resin followed by buffer exchange on a desalting column. Using the NGC Discover Pro System’s Multi-D chromatography function, these columns could be run in tandem and automatically equilibrated and cleaned. At maximum loading flow rate, the full capacity of the ASX-560 Autosampler allowed purification of 84 samples in less than two days in a fully automated fashion with approximately 30% sample loss (Figure 3). Sample loss could be reduced to 6% by lowering the flow rate to 2 ml/min, allowing 70 samples to be purified over a two day period (Figure 3). Both methods yielded milligram quantities of protein, sufficient for downstream cell-based assays.

CETAC AScript Software
The ASX-560 Autosampler is controlled by AScript Software, a step-based software with line-by-line commands (Figure 2). The following is the AScript script that allows sample loading onto the NGC System:

Step 1: Define Label
Step 2: Wait (Time)
Step 3: Wait for Port Input
Step 4: Move to Next Tube
Step 5: Lower Sample Probe
Step 6: Wait (Time)
Step 7: Wait for Port Input
Step 8: Move to Rinse
Step 9: Wait (Time)
Step 10: Pump Off
Step 11: If Position... Go to Label

The first Wait for Port Input (Step 3) is the opening signal from the NGC System during equilibration telling the autosampler to go to the sample and lower the sample probe. The second Wait for Port Input (Step 7) is the closing signal from the NGC System during sample application telling the autosampler that the sample has finished loading and to wash the probe. The last step, If Position... Go to Label (Step 11), is what makes a loop out of the script so that multiple samples can be loaded automatically. This script can be used for as many samples as the ASX-560 Autosampler can hold without cross-contamination from sample to sample.

Fig. 2. AScript Software script for coupling the ASX-560 Autosampler to the NGC Chromatography System.

Fig. 3. Throughput and associated recovery rates. At maximum flow rate 84 samples could be purified with approximately 30% sample loss in less than two days. Lowering the flow rate to 2 ml/min reduced sample loss to 6%. Recovered protein (●); samples/48 hours (■); ASX-560 Autosampler maximum sample capacity (∙∙∙).
High Purity and No Sample Cross Contamination
Our method yielded 2–6 mg of protein at >95% purity, as assessed by SDS-PAGE (Figure 4A). To ensure that this fully automated tandem chromatography platform did not result in sample cross contamination, three purified mAb samples were analyzed by LC-MS. No cross contamination could be detected (Figure 4B), demonstrating that this method is suitable for the drug discovery process.

Highly Reproducible pH and Buffer Composition
The downstream cell-based functional assays used to characterize candidate mAbs require strict and uniform pH and salt conditions. A comparison of eight mAbs purified using our automated high-throughput platform showed uniform final buffer composition and elution profiles, validating the reproducibility of this method and demonstrating that the purified mAbs are suitable for functional studies (Figure 5).

Simple Automation and NGC System/ASX-560 Autosampler Integration
The NGC System can easily be configured for Multi-D purifications (Bio-Rad bulletin 6674) and tandem methods can be adapted using the existing tandem method templates of the NGC System’s ChromLab™ Software. Once such a method has been developed and the NGC System and autosampler are communicating with each other through the NGC SIM, purification of multiple therapeutic mAb samples is a fully hands-off process that requires three simple steps to begin an automated run: (1) priming the sample probe line; (2) entering the number of mAb samples in ChromLab Software; and (3) entering the number of runs in the Ascript Software’s method. In the ten months since installation of this automated system, we have purified approximately 500 antibodies using the NGC System with the ASX-560 Autosampler.
Conclusions

By coupling the NGC Discover Pro System to the ASX-560 Autosampler, we have created a robust, fully automated purification workflow. Each run takes 30–45 minutes, allowing purification of 84 antibodies over a single weekend. This workflow is currently being used at NGM Biopharmaceutical and has greatly accelerated our therapeutic mAb discovery process. The method is simple to implement and can easily be extended to applications that require more complex purification schemes, such as 3-D and 4-D purifications, by taking advantage of the NGC System’s Multi-D feature.

Visit bio-rad.com/web/mAbNGC for more information about the NGC System and chromatography automation.

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