Automated Small Scale Protein Purification for Accelerated Development of Protein Therapeutics

Nico Verlinden[1], Lynn Jordan[1], Ross Beighley[1], Kevin McGuire[1], Brian Gerwe[1], Jeremy Lambert[1], Matteo Costioli[2], Clément Kaltenbach[2], Xavier LeSaout[2]

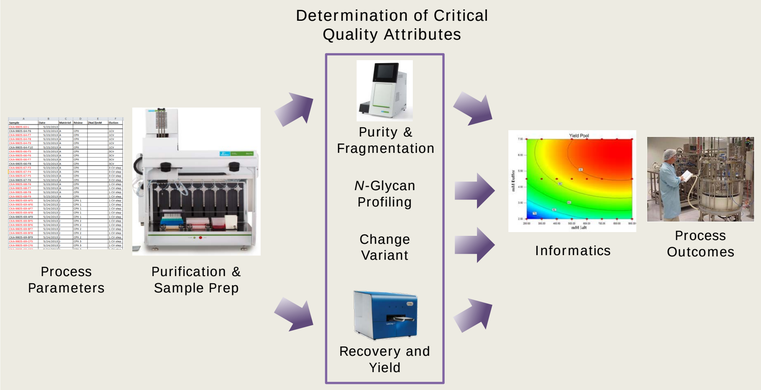
[1]PerkinElmer, Inc., 940 Winter Street, Waltham, MA USA
[2]Merck KGaA, Darmstadt, Germany

1 Overview

Optimization of expression conditions presents a key challenge towards the development of processes to efficiently produce biotherapeutic proteins. The ability to screen matrices of conditions critical to protein identity, titer, structure, and function requires systems for rapid purification and analysis.

The PerkinElmer JANUS® BioTx Pro Workstation was developed as an intuitive, flexible, automated device capable of performing parallel small-scale analytical protein purification. Here, we demonstrate the utility of the JANUS BioTx Pro Workstation through results obtained using the LabChip® DS and LabChip GXII Touch, for use in small scale purification of biotherapeutic proteins.

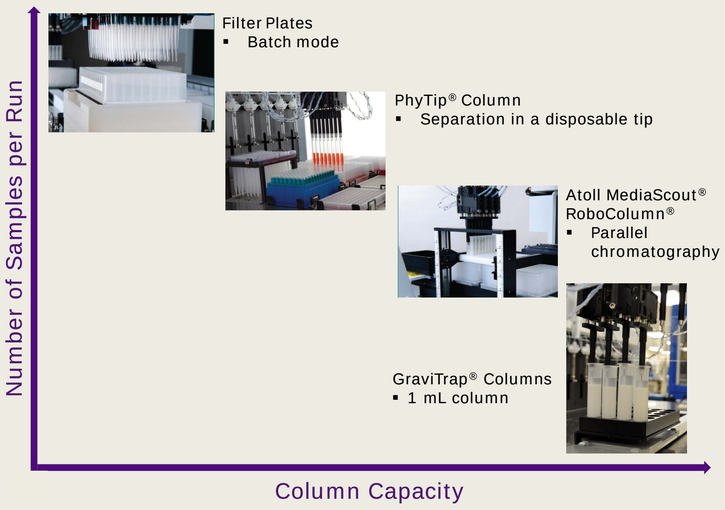
Small Scale High Throughput Process Development



2 Applications

The JANUS BioTx Pro, and Pro Plus platforms feature pre-programmed methods to automate a variety of commercially available small scale ion exchange, affinity and reverse phase chromatography solutions, including ATOLL MediaScout® RoboColumns®, PhyNexus® PhyTips®, and vacuum filtration plates including GE Predictor and Pall AcroPrep™ 96-well filter plates.

Automate Commercially Available Small Scale Solutions



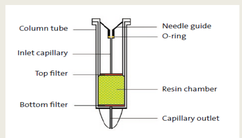
3 Instrumentation

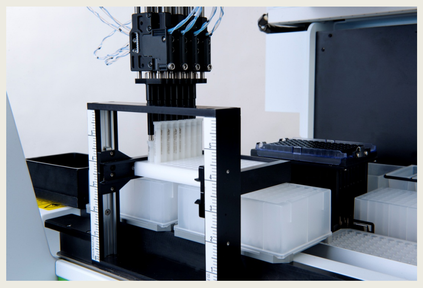
JANUS BioTx Pro:

- 12 deck positions
- 8-tip dispense arm with Varispan™
- Pipetting with both fixed tips and disposable tips
- Plate::shuttle for automated fraction collection

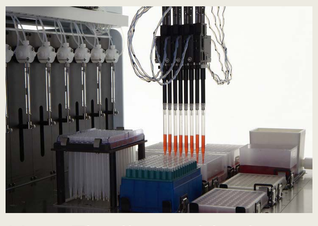
JANUS BioTx Pro Plus:

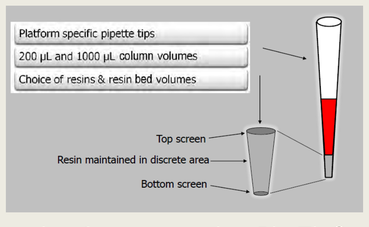
- 24 deck locations
- 8-tip dispense arms with Varispan™
- Plate::shuttle for automated fraction collection
- MDT 96 tip head for parallel pipetting with disposable tips
- Integrated plate gripper
- Vacuum manifold





VariSpan tips and plate::shuttle for automating MediaScout® RoboColumns with bed size of 50-600 µL.



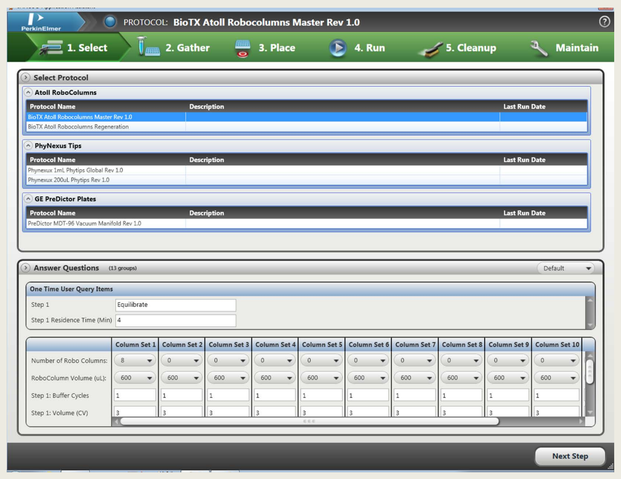


VersaTip disposable tip processing for automating PhyTip® columns with bed size of 5-160 µL.

Integrated plate gripper on 96-channel disposable tip head for automating filter plate processing with vacuum manifold on deck (JANUS BioTx Pro Plus) or offline (JANUS BioTx Pro).

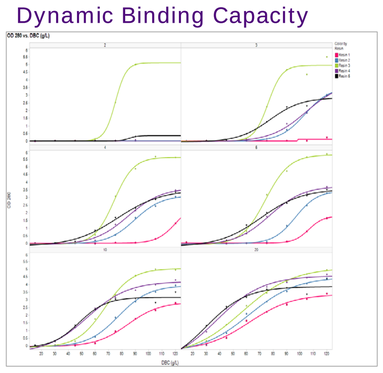
4 Software Interface

An intuitive graphical user interface was developed to enable rapid iterations of variables for optimization of purification techniques. A simple, form-based questionnaire guides the user through a set of questions, dynamically programming pipetting functions to control conditions critical to model purification predictable of large scale purification performance.



5 DBC and Screening Studies

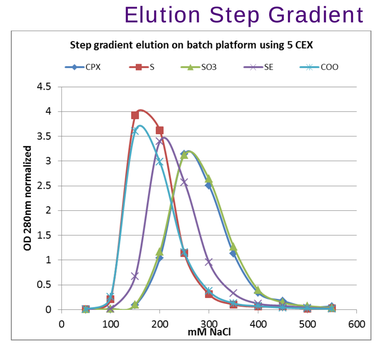
Dynamic Binding Capacity



A study was performed on the JANUS BioTx Pro Plus using MediaScout® Robocolumns® to understand the dynamic binding capacity of 5 CEX resins. Fractions of the load flow through were collected in deep well plates using the Plate::Shuttle and OD 280 was measured for each fraction collected. Using the graphical user interface on the BioTx Pro Plus, flow rates were adjusted to control residence times for each of the 5 resins evaluated.

Elution Step Gradient

In order to understand the impact of NaCl concentration on the ability to elute fractions from various resin types. A stepwise gradient of NaCl concentrations was performed using prepared buffers of increasing concentration. Eluted fractions were collected and measured for protein concentration using OD measurement.



6 Summary

The JANUS BioTx Pro and BioTx Pro Plus workstations enable automated small scale (sub-microgram to tens of milligrams) protein purification in a volume range of 100 µL – 48 mL using a selection of industry-standard purification formats designed to support process development for protein therapeutics.