Micro scale-down technologies for high throughput development of chromatographic separations

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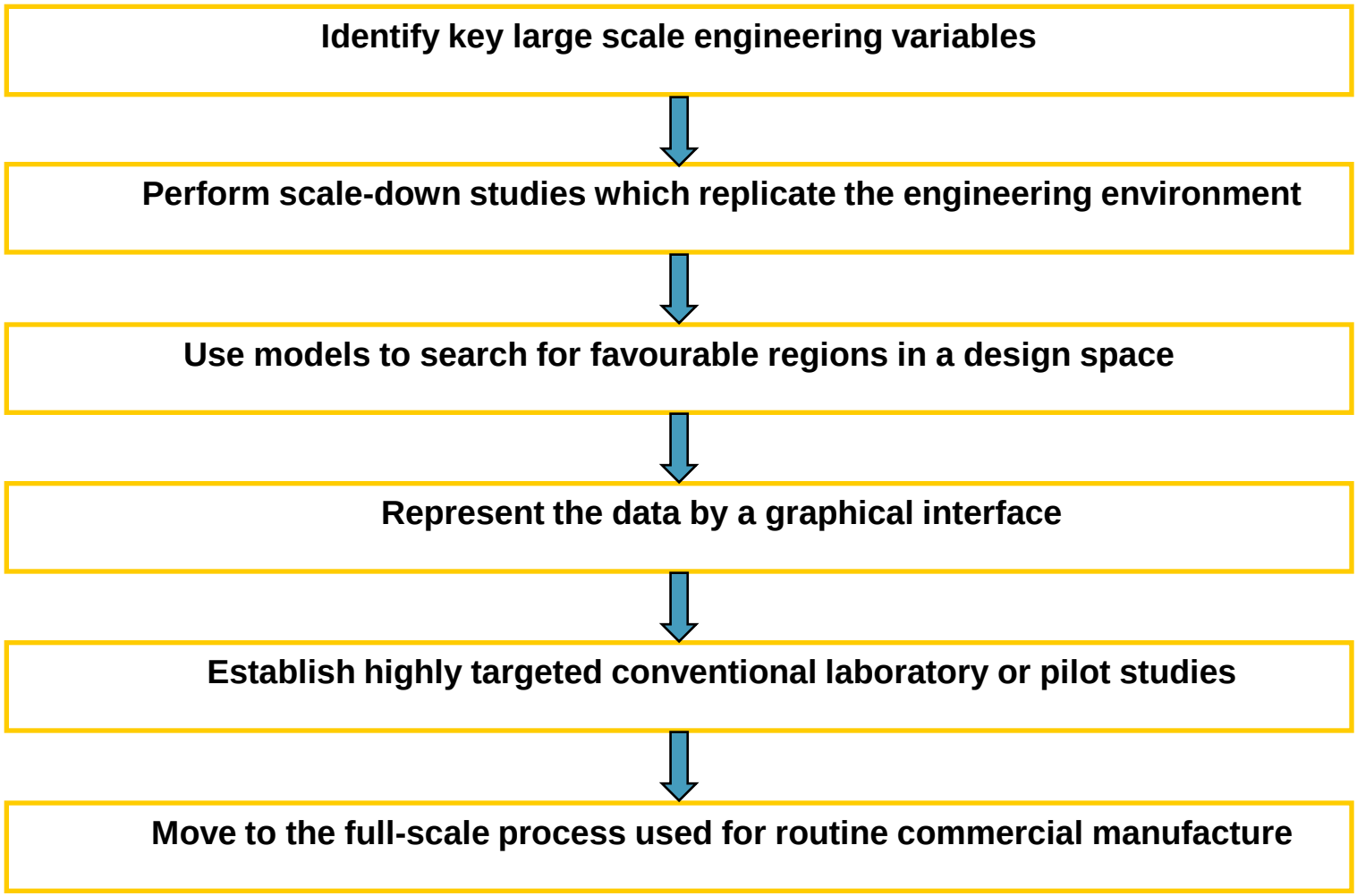
Contents

- Introduction
  - UCL strategy for micro biochemical engineering
- Overview of available technologies
  - Microlitre batch plate incubation
  - Miniature columns
  - Pipette tip chromatography
- Case studies
  - VLP purification
  - Antibody quantification
- Conclusions

What is micro biochemical engineering?

- Addresses limitations of the current development paradigm
- Combines scale-down assessment and large scale verification
- Reduces feed requirements
  - Operation at the millilitre-scale or smaller
- Uses automation as far as possible
  - Enables parallel experimentation

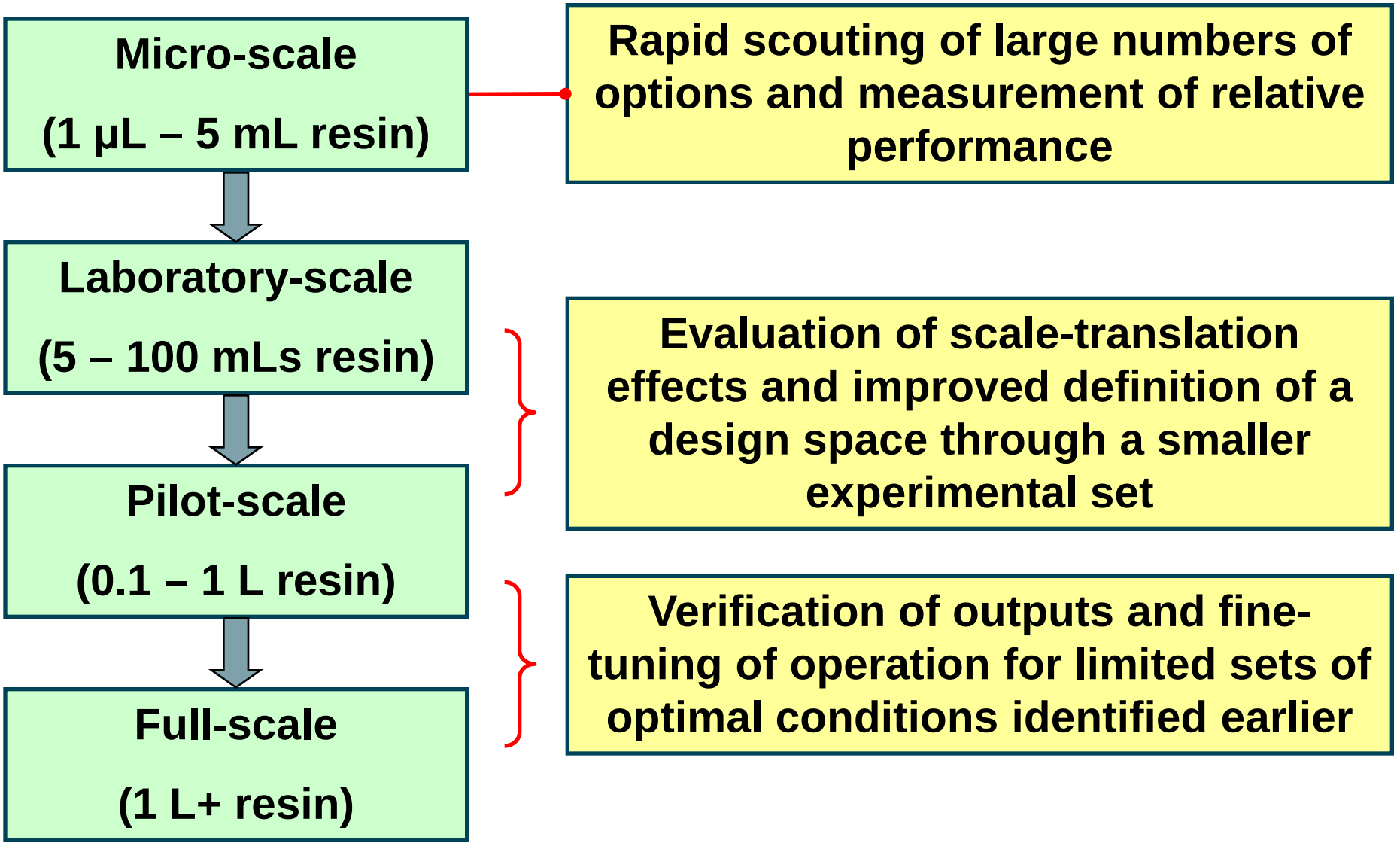
Micro biochemical engineering workflow



Challenges in chromatography development

- Many operating modes, chemistries and backbones
  - Affinity
  - Ion-exchange
  - Hydrophobic interaction
  - Multi-modal
  - Buffer selection
- Laboratory studies may require large feed volumes
- Many conflicting output metrics
  - Yield
  - Quality
  - Time
  - Economics

Micro-scale approach to chromatography



Automation of microscale chromatography

- Can run many separations simultaneously
- Unattended operation
- Simplifies preparation and clean-up
- Reduces manual workload to a manageable level
  - Removes some potentially time consuming and labour intensive tasks
- Can integrate with other equipment
  - Orbital shakers
  - Solid–liquid separation devices
  - Plate readers

Improved process understanding

- Screen many different resins and buffer conditions
  - Dynamic binding capacity, yield and purity
- Obtain kinetic, equilibrium and breakthrough data
  - Study how feed concentration and residence time affect separations
- Improve process characterisation
  - QbD, validation and support greater post-approval process flexibility

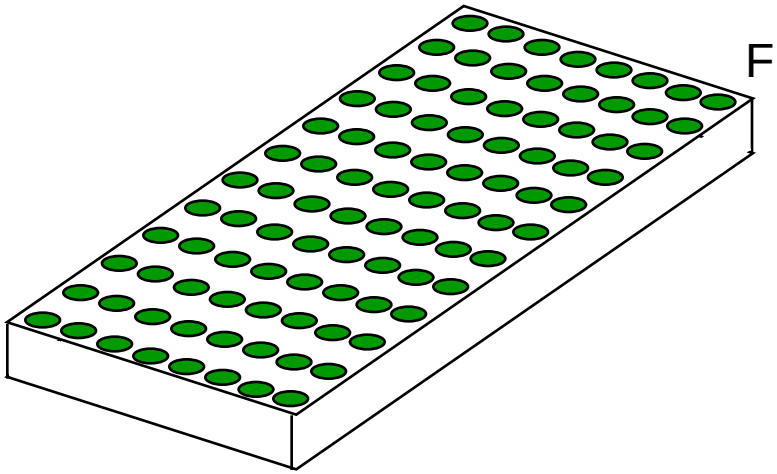
Microscale chromatography formats

Microlitre batch incubation

Miniature columns

Pipette tip chromatography

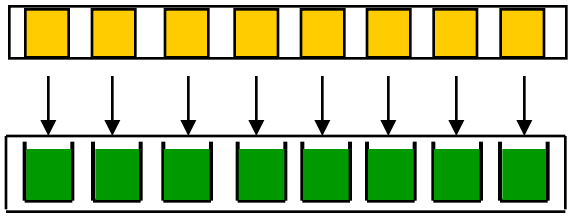
Microlitre batch incubation format



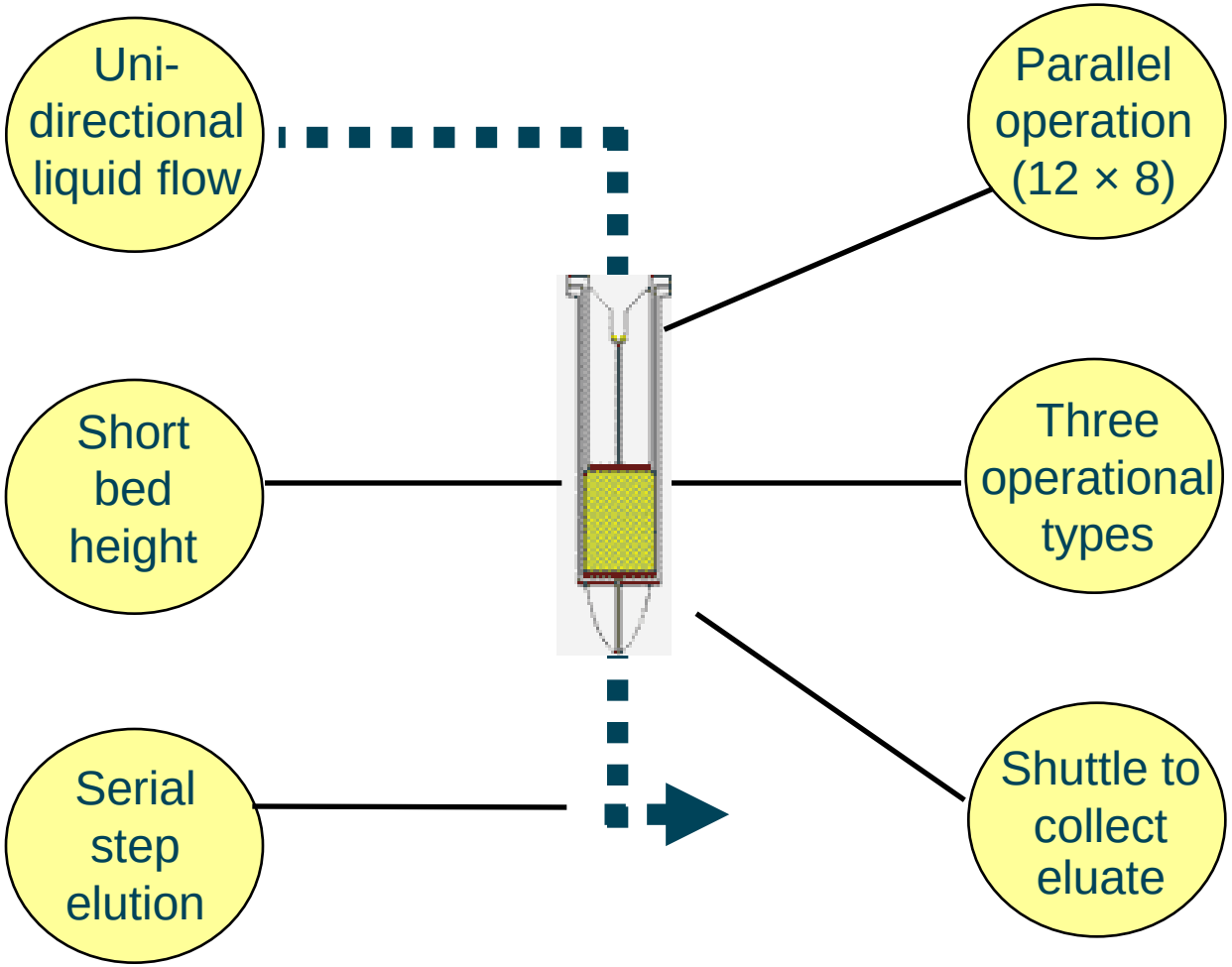
Filter plate containing up to a few hundred microlitres of matrix and incubated with feed material

Agitate at a defined rate and for a defined time period

Centrifugation or vacuum filtration

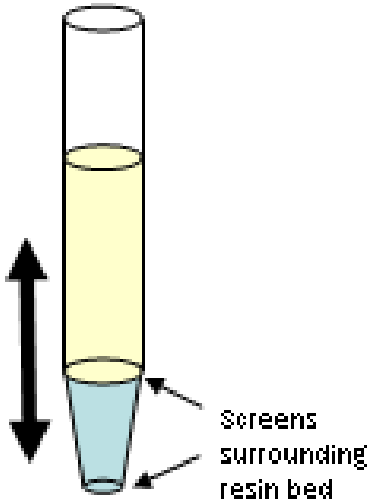


Miniature column format



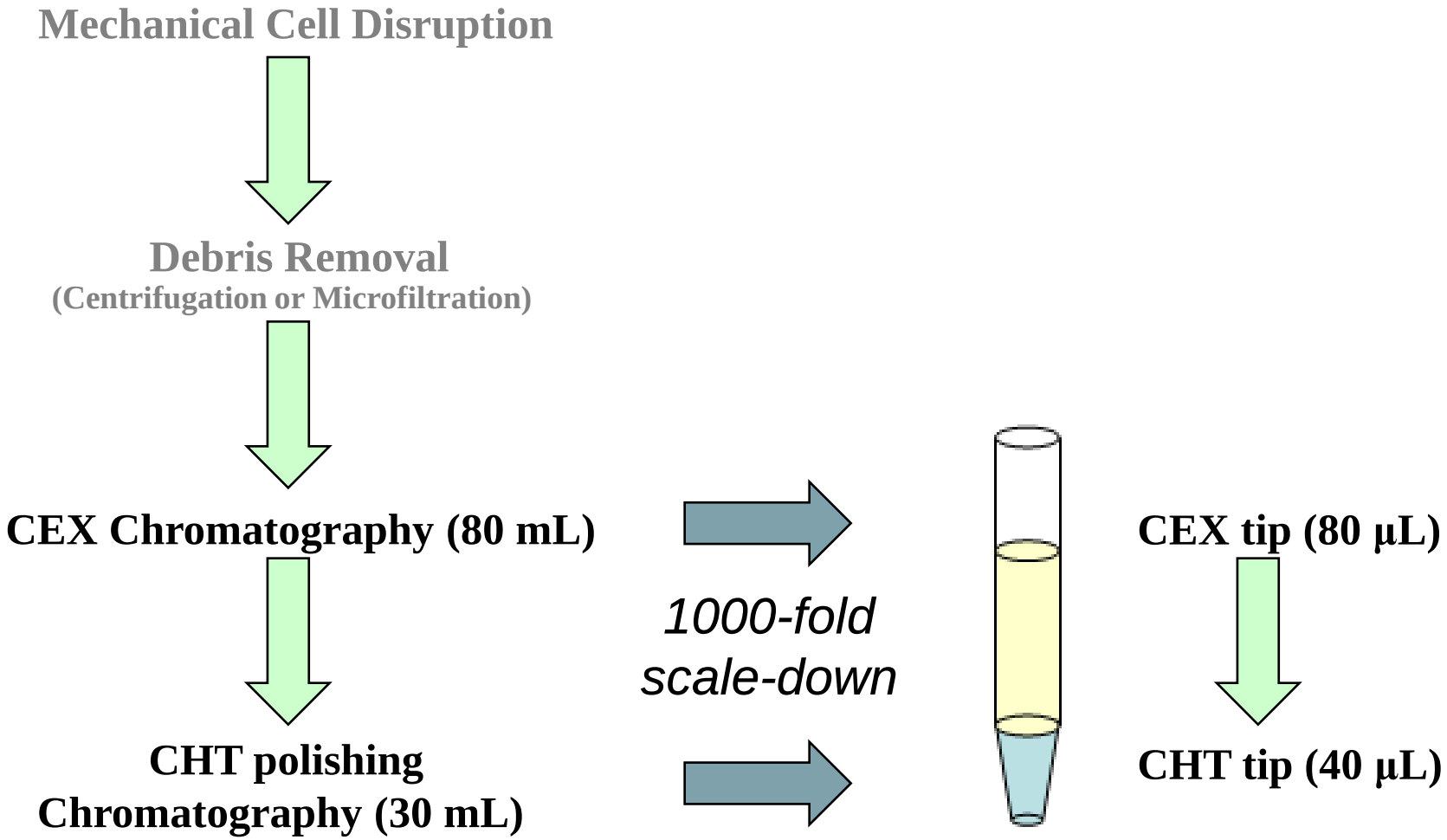
Wiendahl et al. (2008), Chemical Engineering and Technology, 31, 893–903

Pipette tip chromatography format

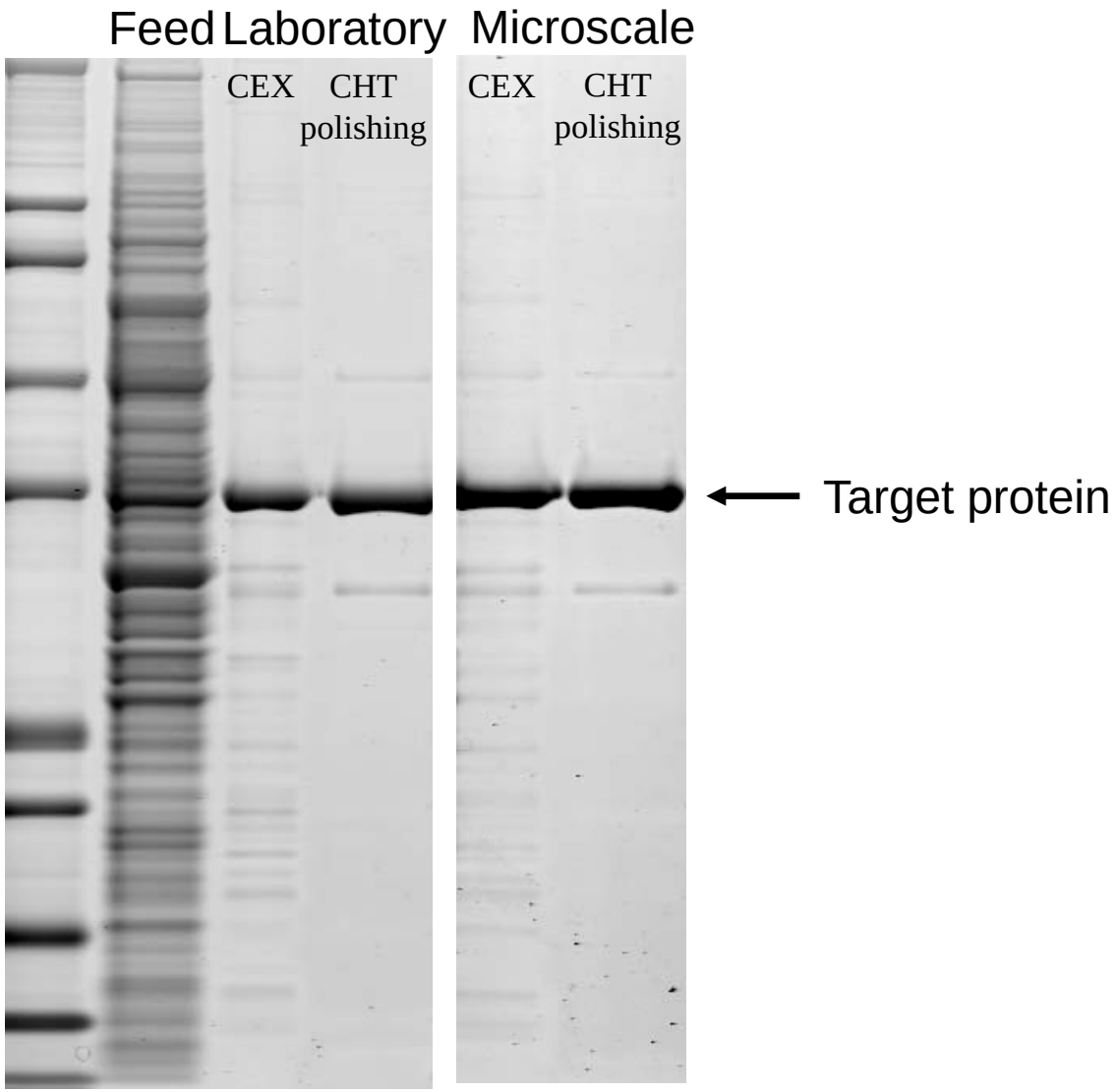


- Supplied pre-packed for any custom or off-the-shelf resin by PhyNexus
- Resin is packed into the base of the tip and held between two screens
- Feed or buffers are held in a 96-well plate
- These are aspirated and dispensed repeatedly in a bidirectional fashion for the required residence time
- Optimise robotic parameters
  - Flowrate (v)
  - Number of bidirectional cycles (N)

Pipette tip chromatography example 1: VLP purification (UCL and Merck)

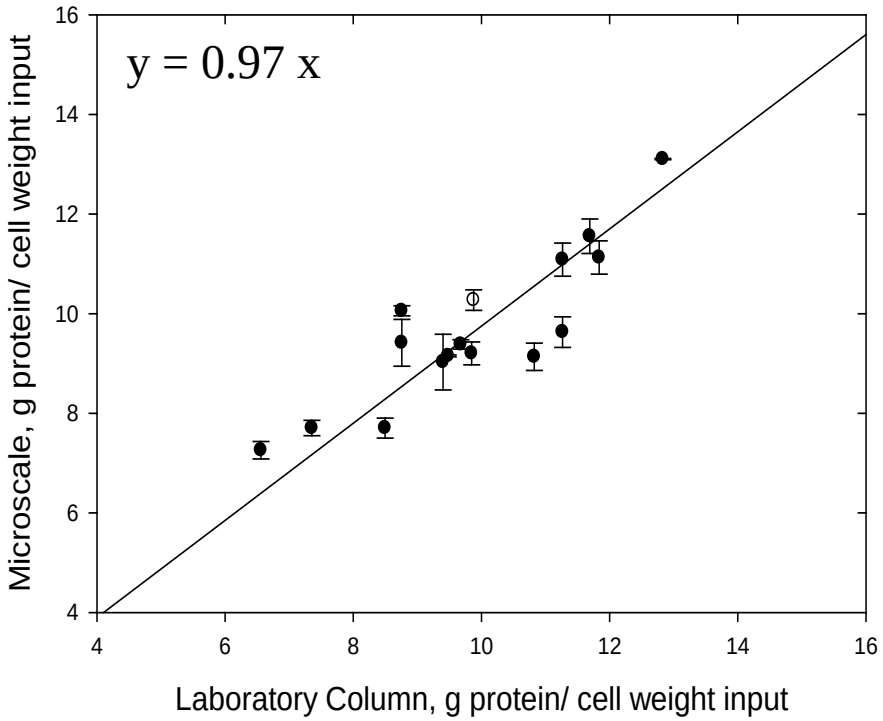


Comparability of microscale chromatography



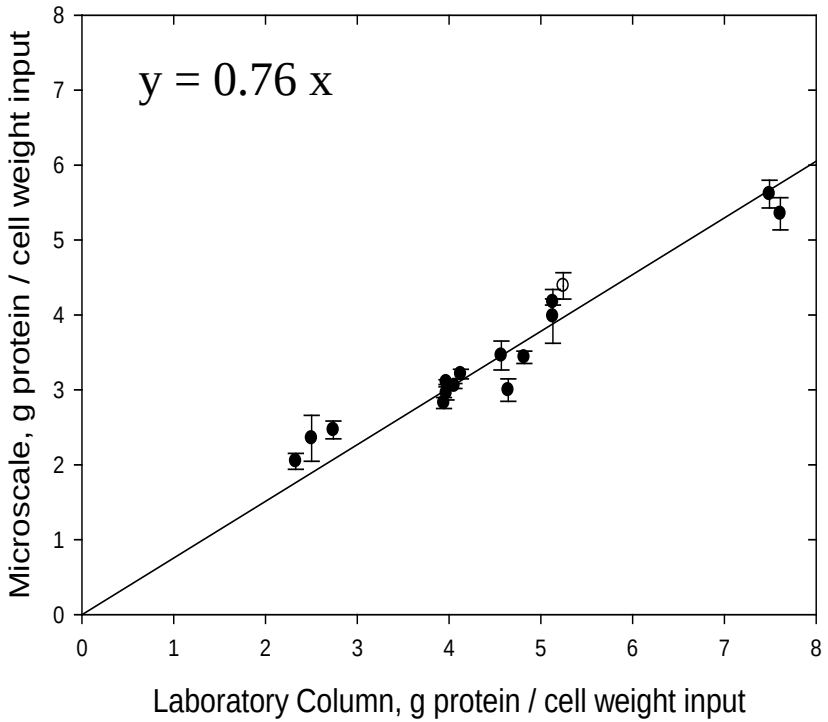
Correlation of microscale yield to lab scale

CEX Chromatography



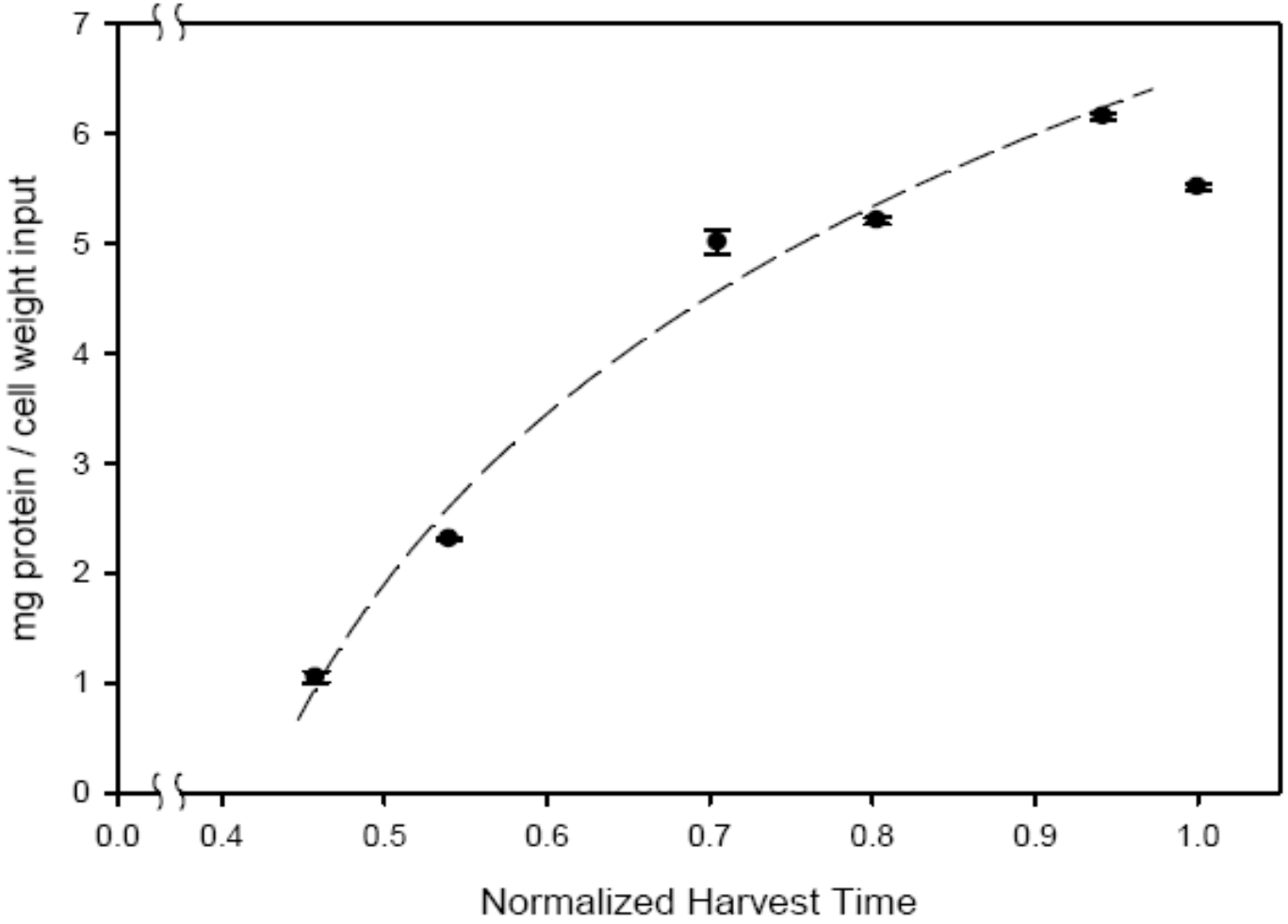
Strong one-to-one correlation between microscale and laboratory scale

Polishing Chromatography



Consistent offset following polishing chromatography allows use of a correction factor

Determination of Harvest Time



Wenger et al. (2007), An automated microscale chromatographic purification of virus-like particles as a strategy for process development, *Biotechnology and Applied Biochemistry*, 47, 131–139

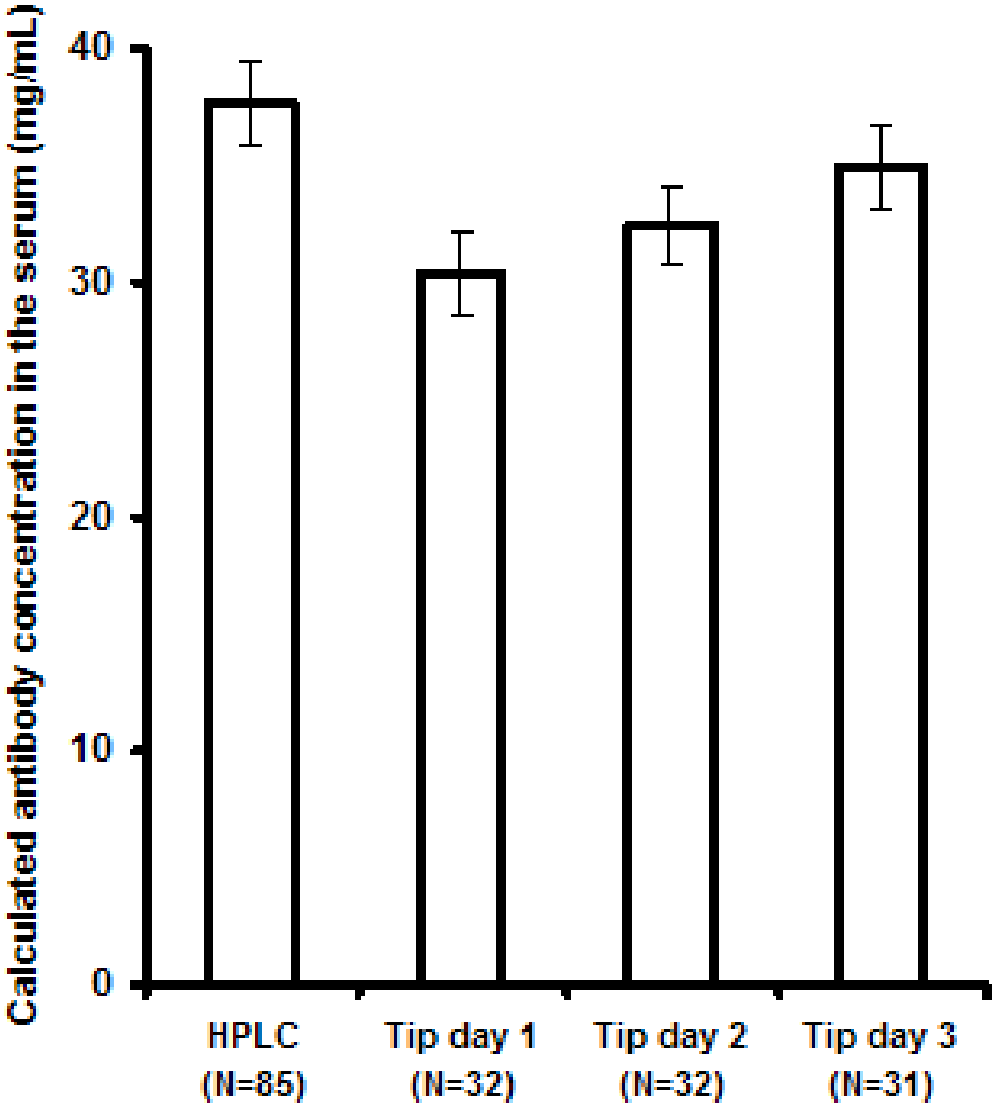
Pipette tip example 2: antibody analysis

- Robotic tip screening for ovine polyclonal antibody capture
  - Maximising dynamic binding capacities
- Protein G HPLC for antibody quantification
  - Manually-intensive
  - Time-consuming
  - Required litre scale- buffer volumes
  - Conducted on separate system to the robot
- Required a faster, more integrated alternative

Robotic tip analysis

- Protein G tip method operated on Tecan robot
  - 8-channel pipetting system
  - 40 µL packed resin bed
- Protocol set-up to integrate with main experiment
- Evaluated properties of method with ovine pAb
  - Linearity
  - Range
  - Specificity
  - Accuracy (versus reference Protein G HPLC)
  - Precision

Sample data – accuracy and precision



Tip versus HPLC comparison

Characteristic	Protein G tips	HPLC
Samples processed simultaneously	8	1
Analysis run time / sample (minutes)	5	13
Buffer volumes	mL-scale	L-scale

Tip versus HPLC comparison

Robotic pipetting capacity **[Number of liquid handling channels]**	**% tip – HPLC time saving**
8	62
12	69
96	94

Chhatre et al. (2010), An automated packed Protein G micro-pipette tip assay for rapid quantification of polyclonal antibodies in ovine serum, *Journal of Chromatography B*, 878, 3067–3075

Continuing challenges at microscale

- Potential for data overload
  - Require efficient ways to store and manage data
  - Need to select test points carefully to minimise analysis
    - Prevent analytical bottlenecks
- Need to minimise sample volume
  - Microscale unit operation linkage must account for volume consumed in hold-up or analysis

Potential solutions

- Mathematical tools for efficient design space search
  - Simplex algorithm for early development or where analysis is time-consuming
- Smart deployment of current range of analytical methods
  - Use fast assays (e.g. total protein) at first for coarse screening
  - Leave time-consuming assays (e.g. ELISA) until a good region has been identified
- Microfluidic assays developments
  - Reduce sample volume requirements
  - Lab-on-a-chip (e.g. Agilent Bioanalyser for electrophoresis)

Conclusions

- Microscale chromatography
  - Rapid data generation
- Automation
  - Increased throughput
  - Reduced manual intervention
- Requires rapid integrated assay techniques
  - Avoid shifting bottleneck over to analysis