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PAT in Biopharmaceutical Processes

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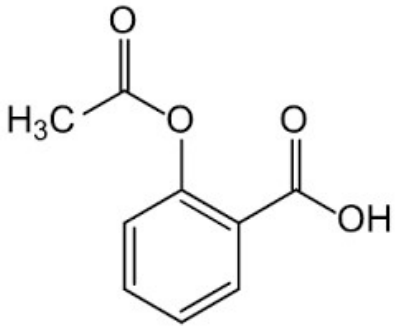
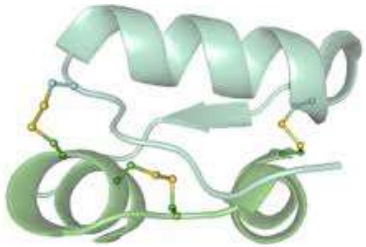
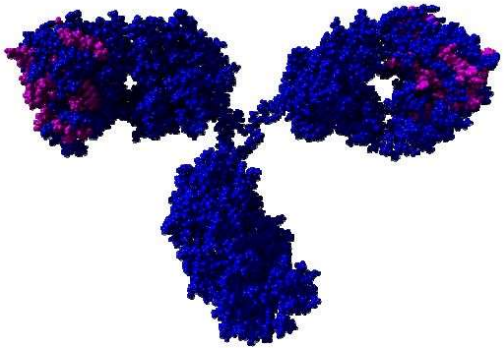
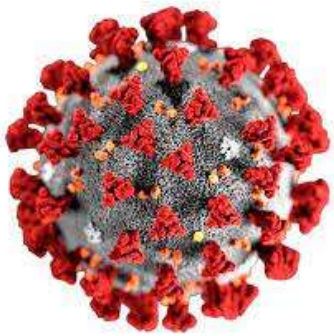
Pharmaceutical Products!









Insulin

Herceptin

COVID

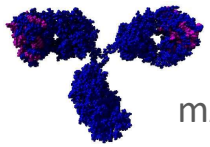
~ 0.2 kDa

~ 5 kDa

~ 150 kDa

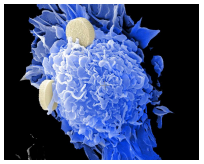
> 10^6 kDa

Biological Landscape

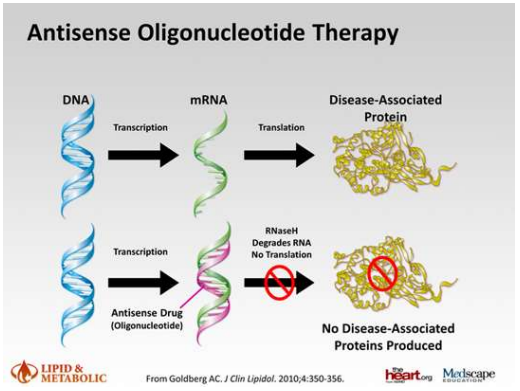


mAbs

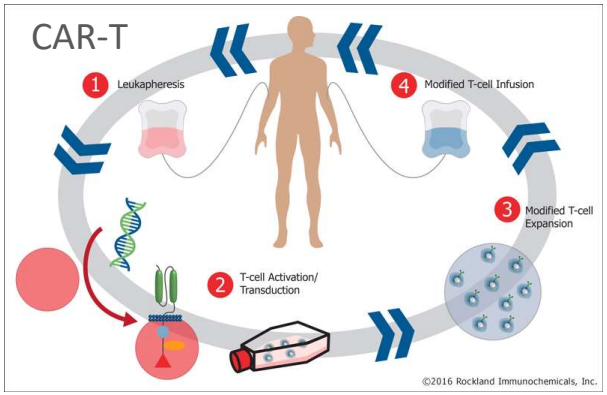
- **Proteins**
  - Recombinant proteins (e.g. insulin, EPO, Growth Hormone)
  - Monoclonal Antibodies (e.g. Avastin, Herceptin)
  - Antibody Drug Conjugates (e.g. Kadcyla, Adcetrix)
- **Vaccines**
  - Complete vaccines
  - Subunits
  - Proteins
  - mRNA



Vaccines



- **Oligonucleotides** (becoming very popular)
  - siRNA (antisense RNA)
  - Aptamers
  - mRNA (messenger RNA)
- **Gene/Cell Therapy** (increased popularity)
  - CAR-T Cell Therapy
  - Stem Cell Therapy
  - Etc.



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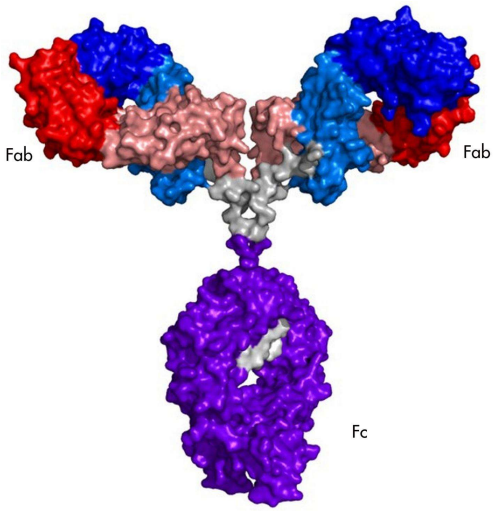
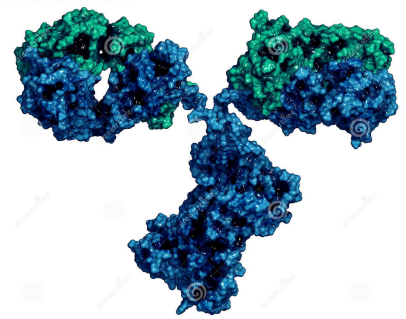
Monoclonal Antibodies

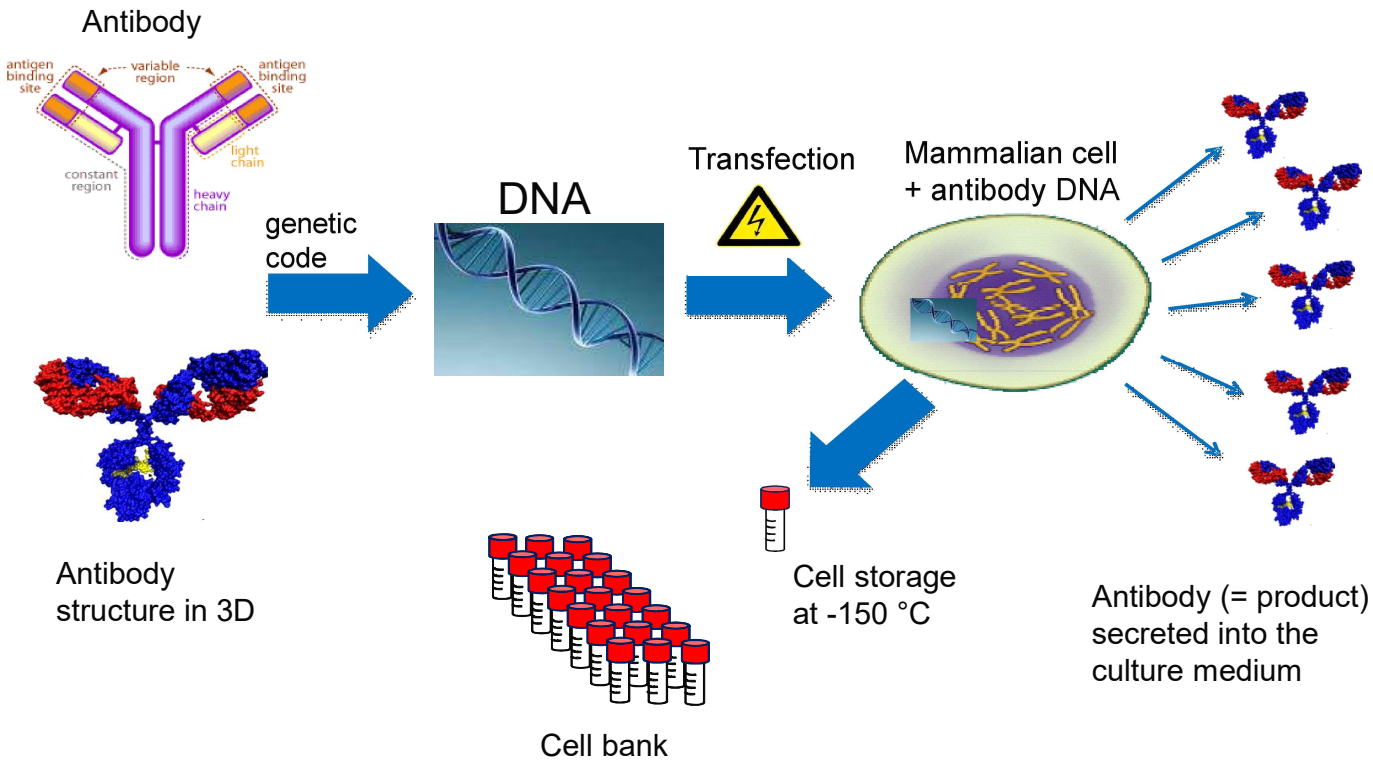


- Large biomolecules (150 kD)
- Contain both heavy (50 kD) and light (25 kD) chain
- Expression mainly in eucaryotic cell lines (NS0, PerC6, CHO, HEK or other cell lines)
- Mainly glycosylation at the C_H chains
- Activity determined by F_{ab} region

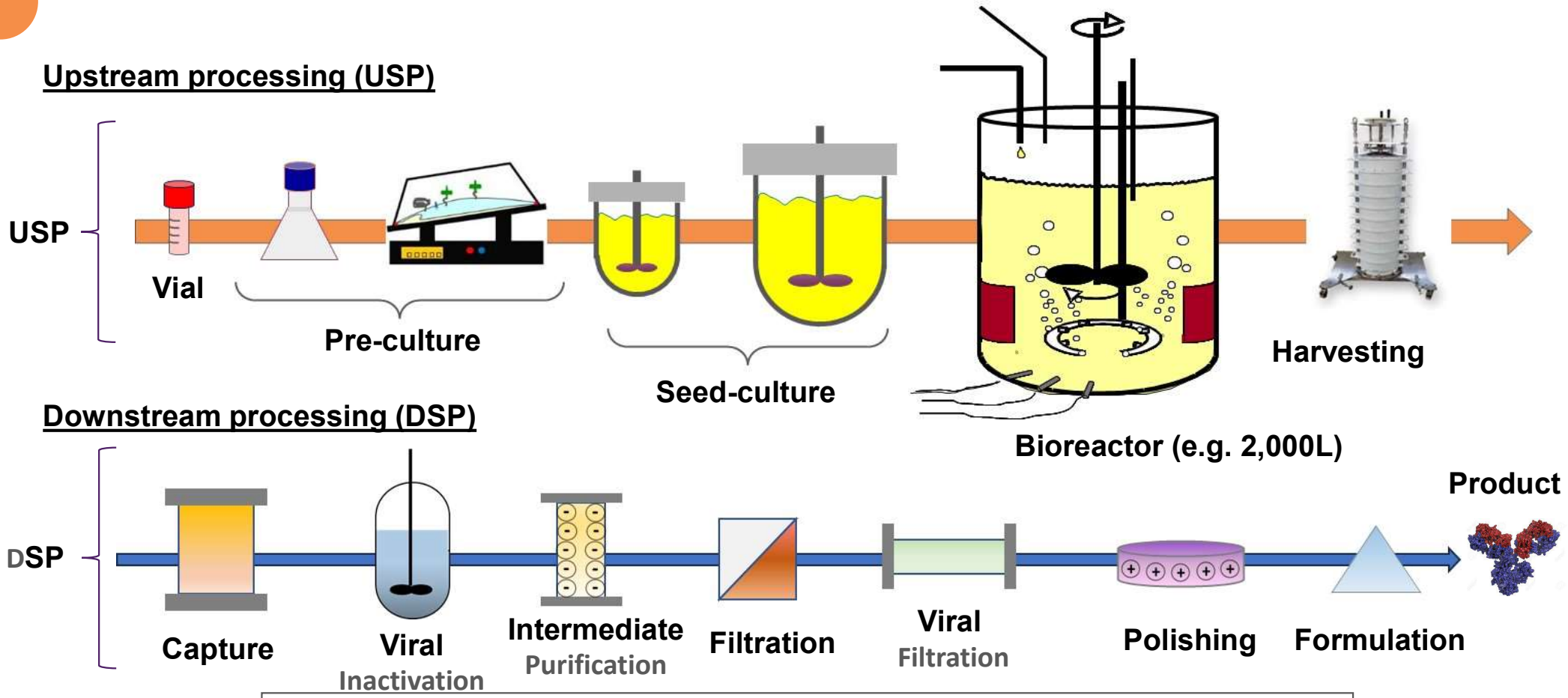


How are they produced?

Example: monoclonal antibodies



Overview of Complete Bioprocess

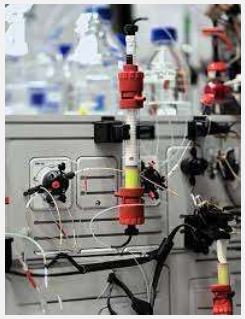


Complex process, many unit operations to develop medicines (~5-6 weeks)!

In Practice

Upstream

Fermentation → Harvesting



Downstream

Capture → Viral Inactivation

Intermediate Purification → Filtration → Virus Filtration → Polishing → Formulation → Product (e.g. Antibody)

Pharmaceutical Industry

How can PAT be implemented

Process Technologies Needed!



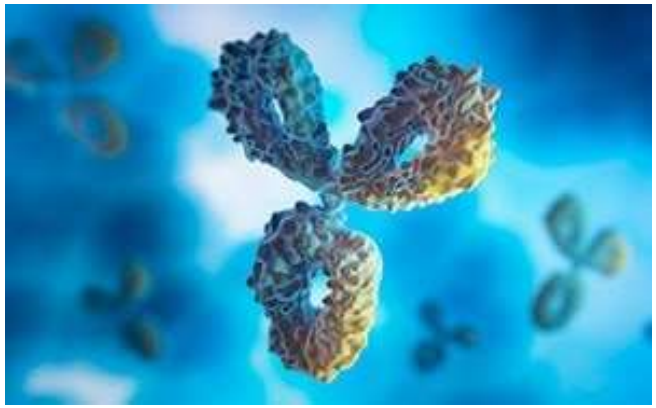

Biological Processes

Fermentation

Harvest

(cell free)

Purification



Monoclonal Antibody (MAb)

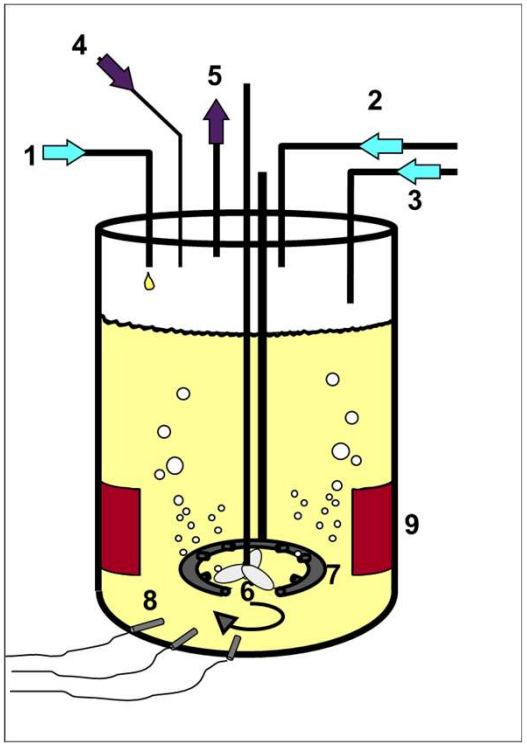
Lyophilized

Liquid Solution

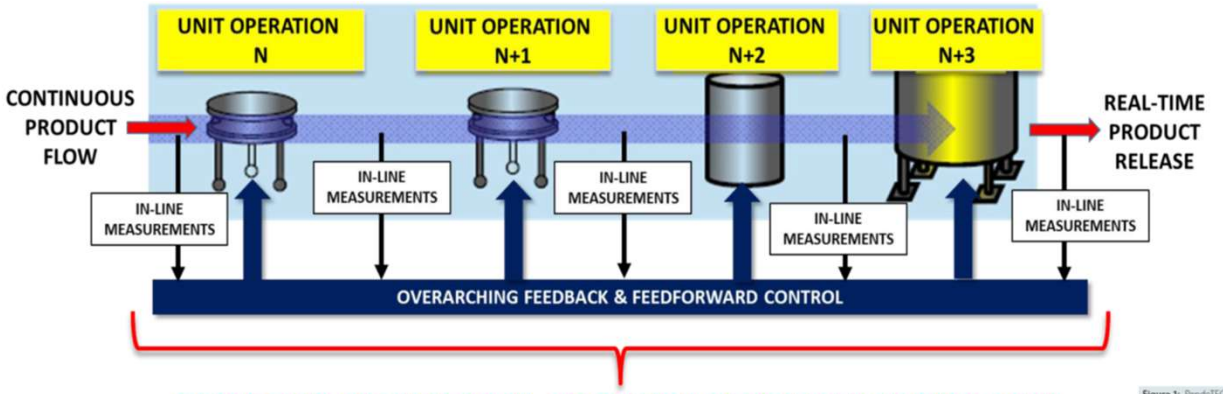
Process Analytical Technologies

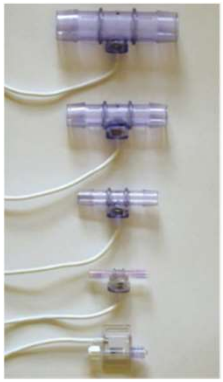
Process control by using sensors (in-line, at-line) from unit operation to unit operation is not developed yet.

- General parameters:
  - Temperature, pH, conductivity, pressure, gasses, etc.
- USP specific parameters:
  - Amino acids, glucose, lactate, etc.
  - Product content/modifications, glycosylation, etc.
  - Cell productivity/viability, etc.
- DSP specific parameters:
  - Product content/modifications, etc.
  - Product/process related impurities, etc.

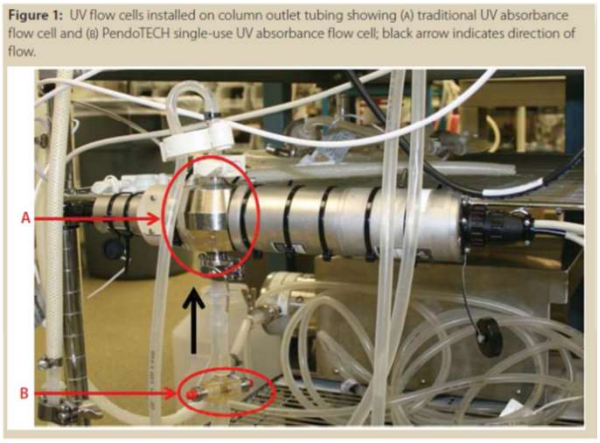


Process Analytical Technologies

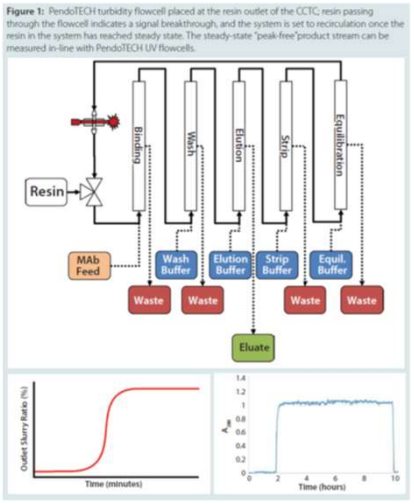




Pressure sensors



UV sensors

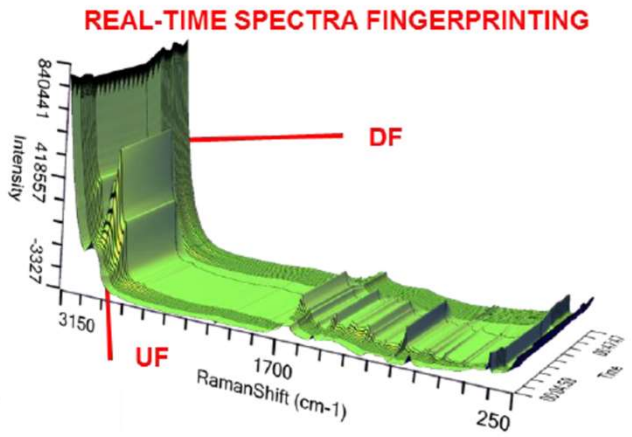


Turbidity sensors

Process Analytical Technologies

In-line quality and buffer composition measurement

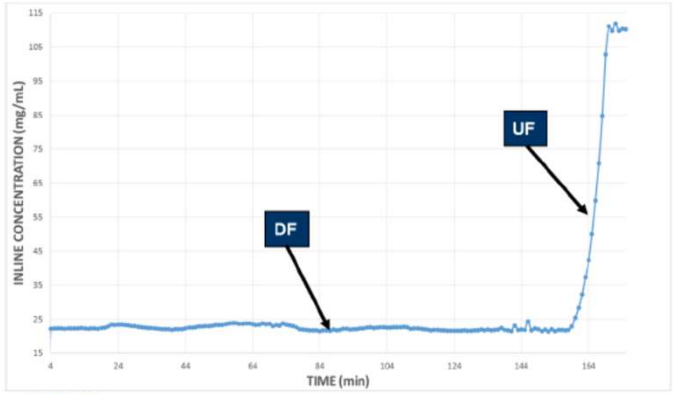
- Raman spectroscopy: fingerprinting method

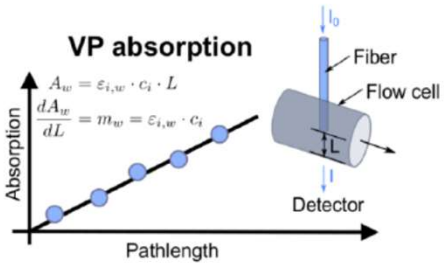


What about Product?

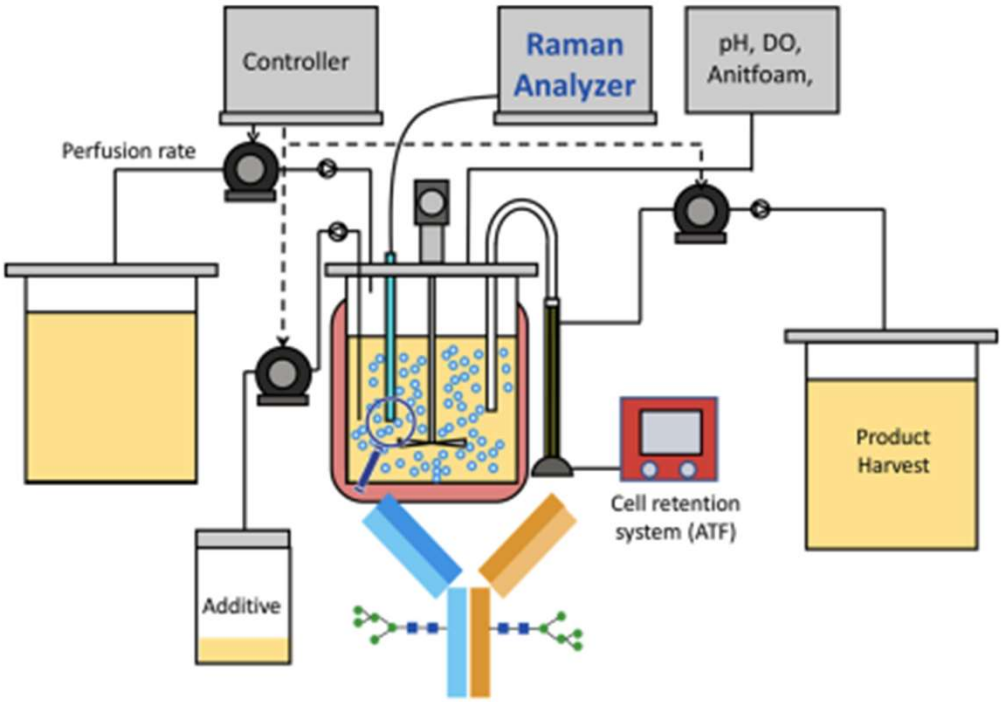
Real time concentration measurement

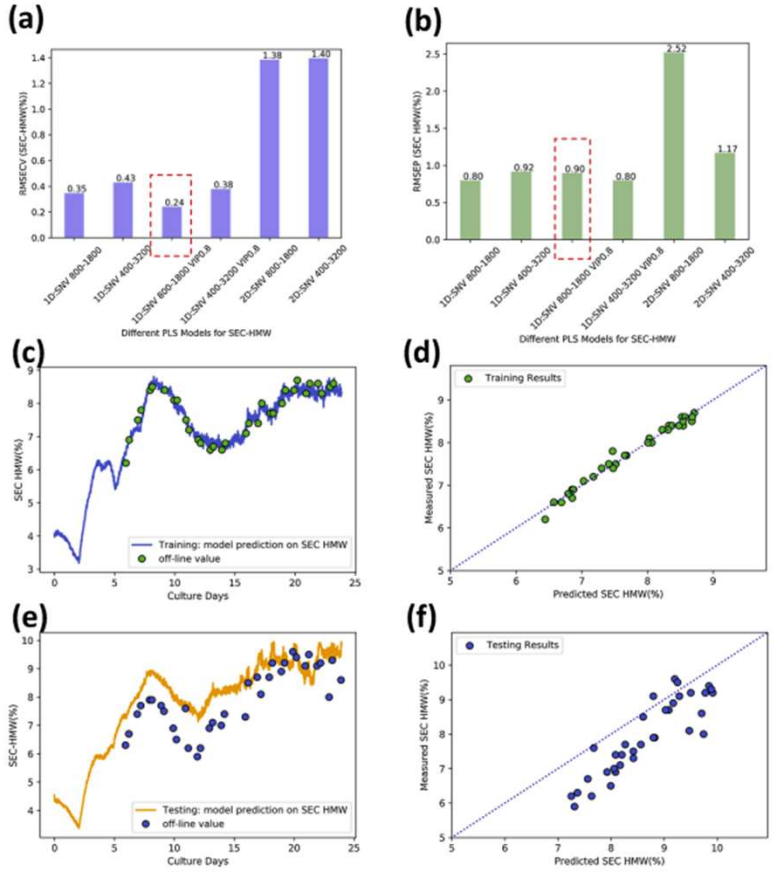
- UV/Vis slope-spectroscopy (FlowVPE)
- Large linear range: no dilution





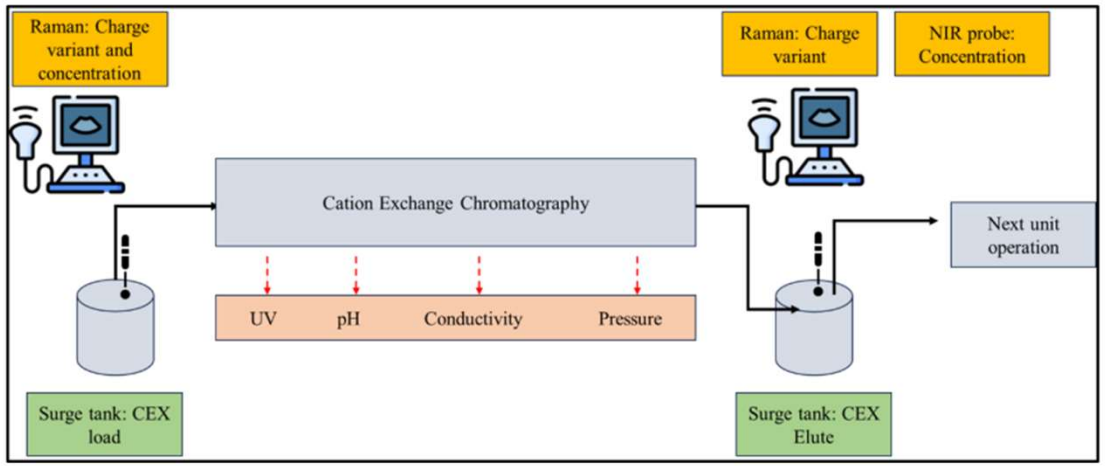
Raman Spectroscopy in Cell Culture Fermentation

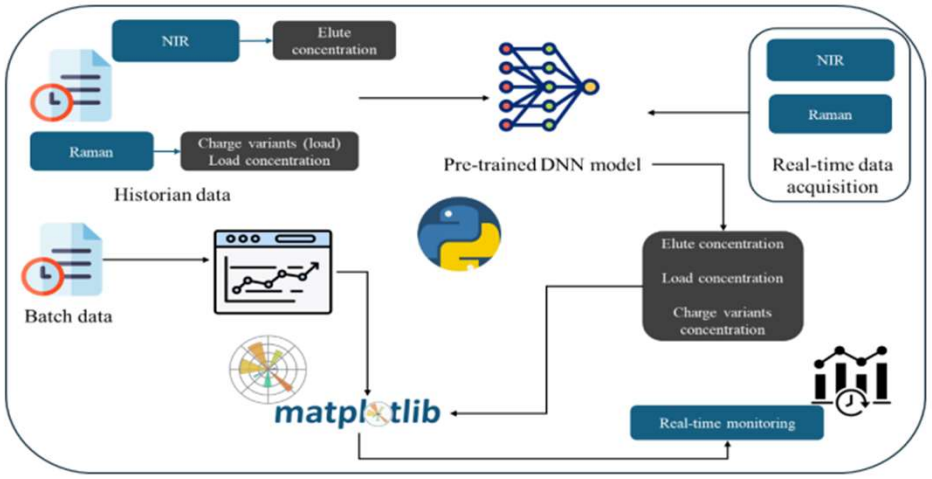




Liu et al., Biochem. Eng. J., 2021

NIR and Raman Spectroscopy in Downstream Processing





Nitika et al., Pharmaceutical Research, 2024
Nitika et al., Int. J. Biol. Macromol., 2021

What is the strategy in Manufacturing?

Old/Current Facilities

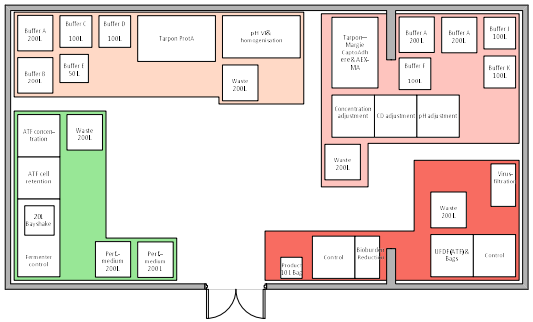
- Batch
- Stainless steel
- Cleanrooms C & D class
- e.g. 20,000L bioreactors

Single Use Facilities

GE Healthcare's KUBio (Graphic: Business Wire)

- Batch
- Single use (e.g. plastics)
- Cleanrooms C & D class
- e.g. 2,000L bioreactors

Intensified Facilities

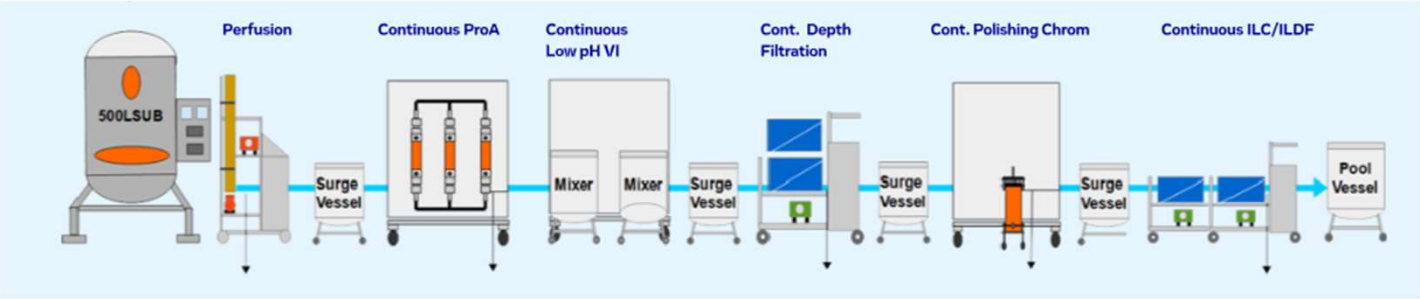


- Intensified (semi-continuous)
- Single Use (e.g. plastics)
- Cleanrooms B & C & D class
- e.g. 100 – 500L bioreactors
- Smaller Footprint

Focus on Intensified Processing

1. Further reduction in Manufacturing Footprint and CAPEX
2. Process Robustness (higher degree of automation)
3. Reduction in Inventory (days at hand)
4. No scale up during drug development required

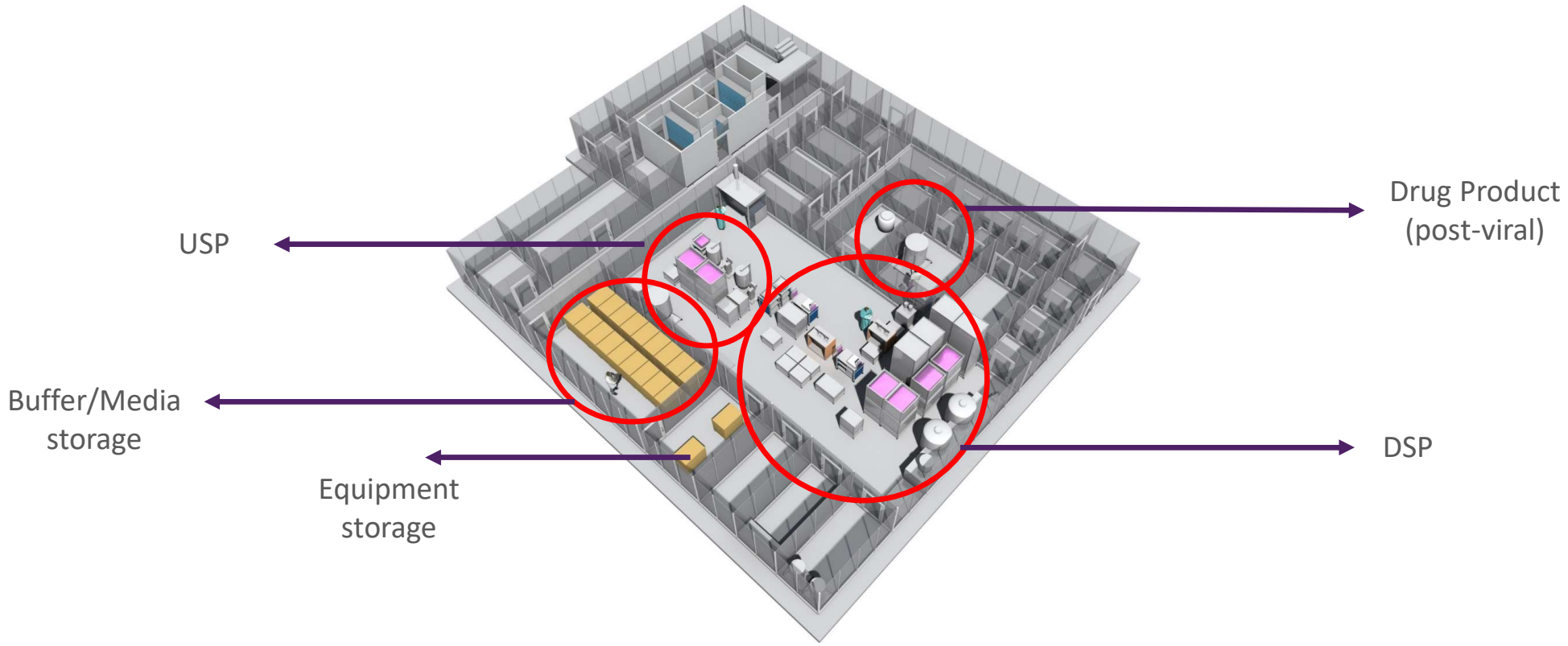
(e.g. Just-Evotec)



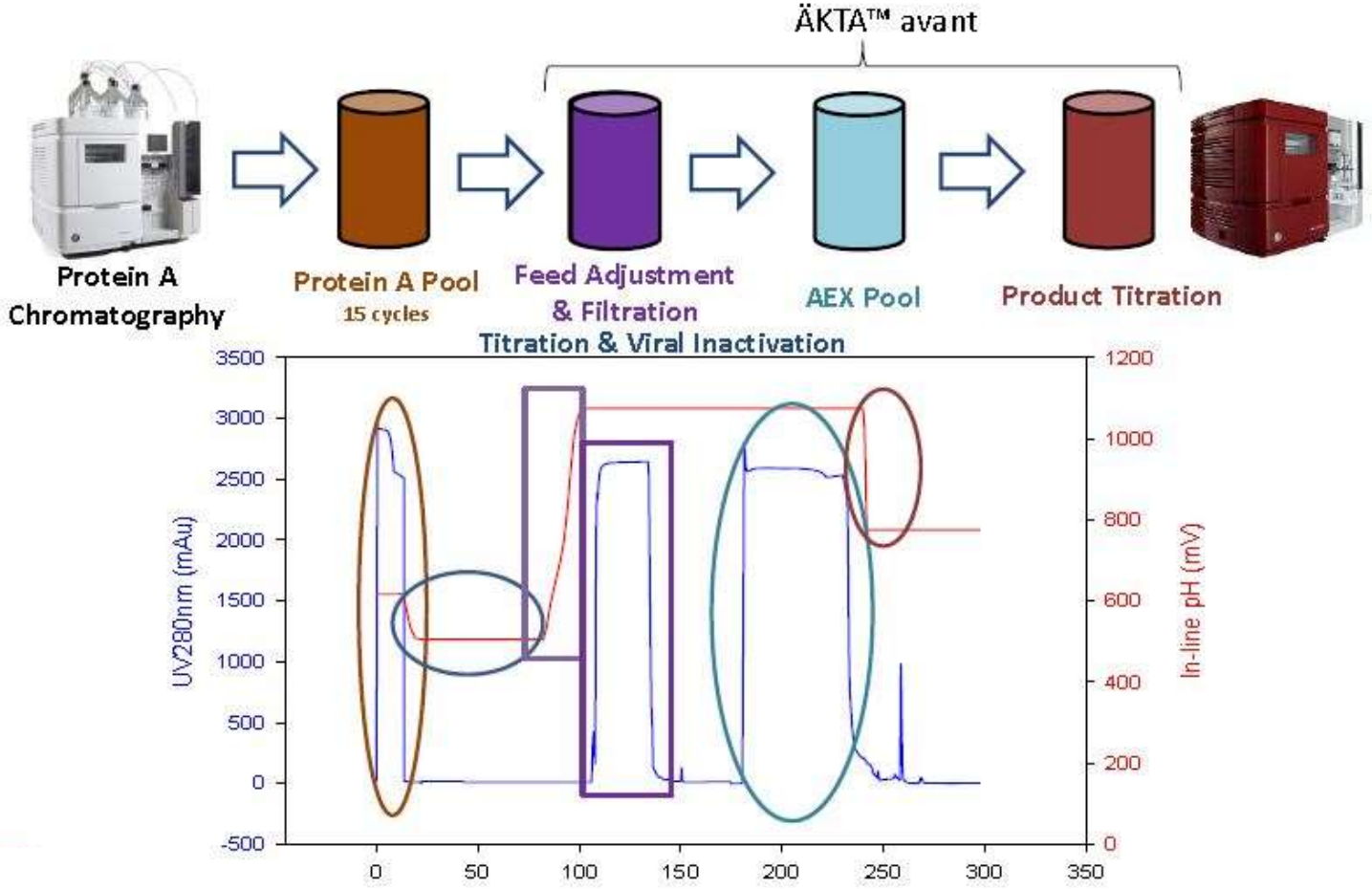
(semi-continuous)

(e.g. simAbs)

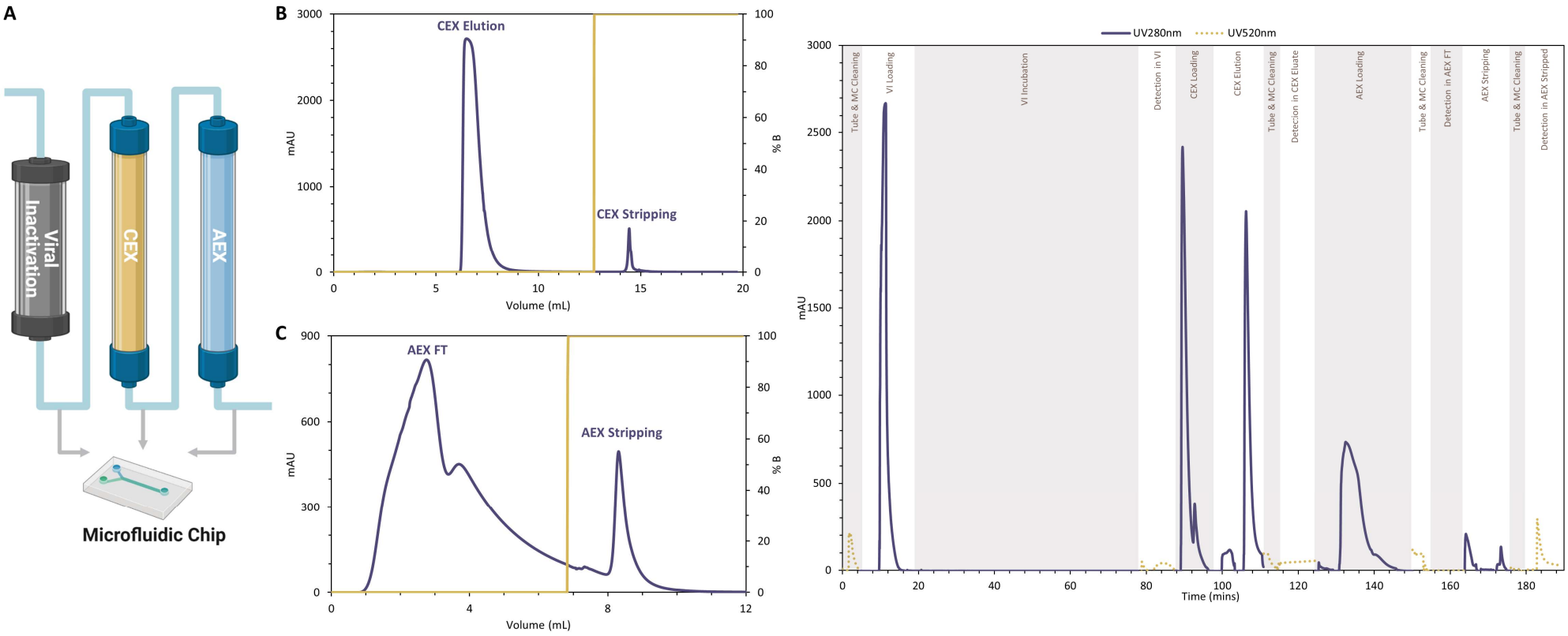
Plant Layout of Intensified Manufacturing



Fully Automated Downstream Process (ProA-VI-Filtration-AEX)



Aggregate detection with inline microfluidic chip



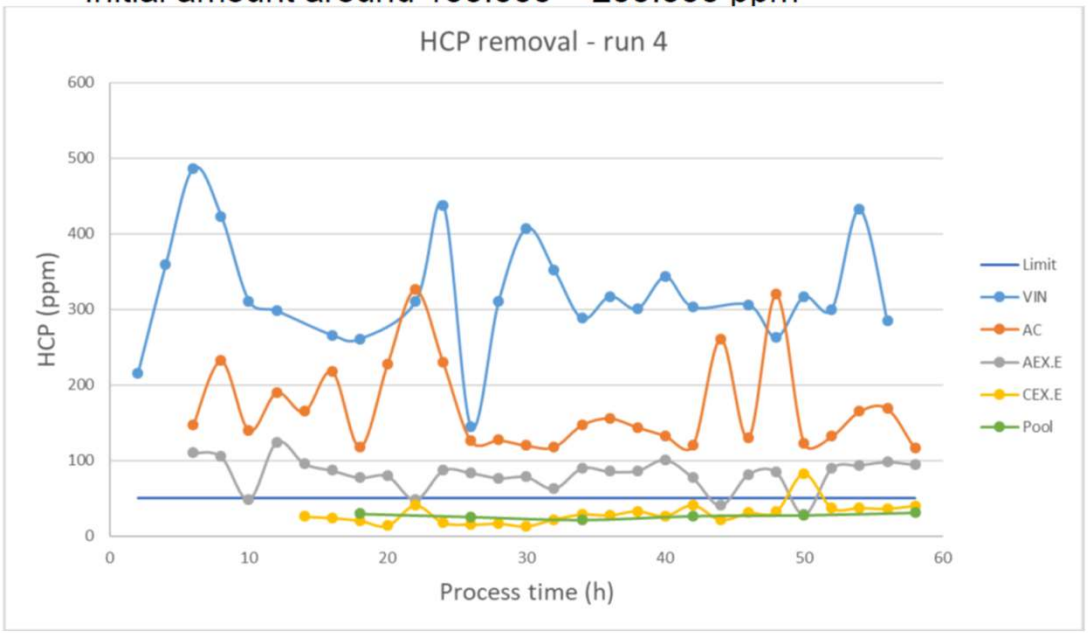
Schematic representation of integrated downstream processing (left) and chromatographic profile with aggregate detection (right)

Sao Pedro et al., J. Chem. Technol. Biotechnol., 2021

Fully automated control of Host Cell Impurities (HCP) removal from the process

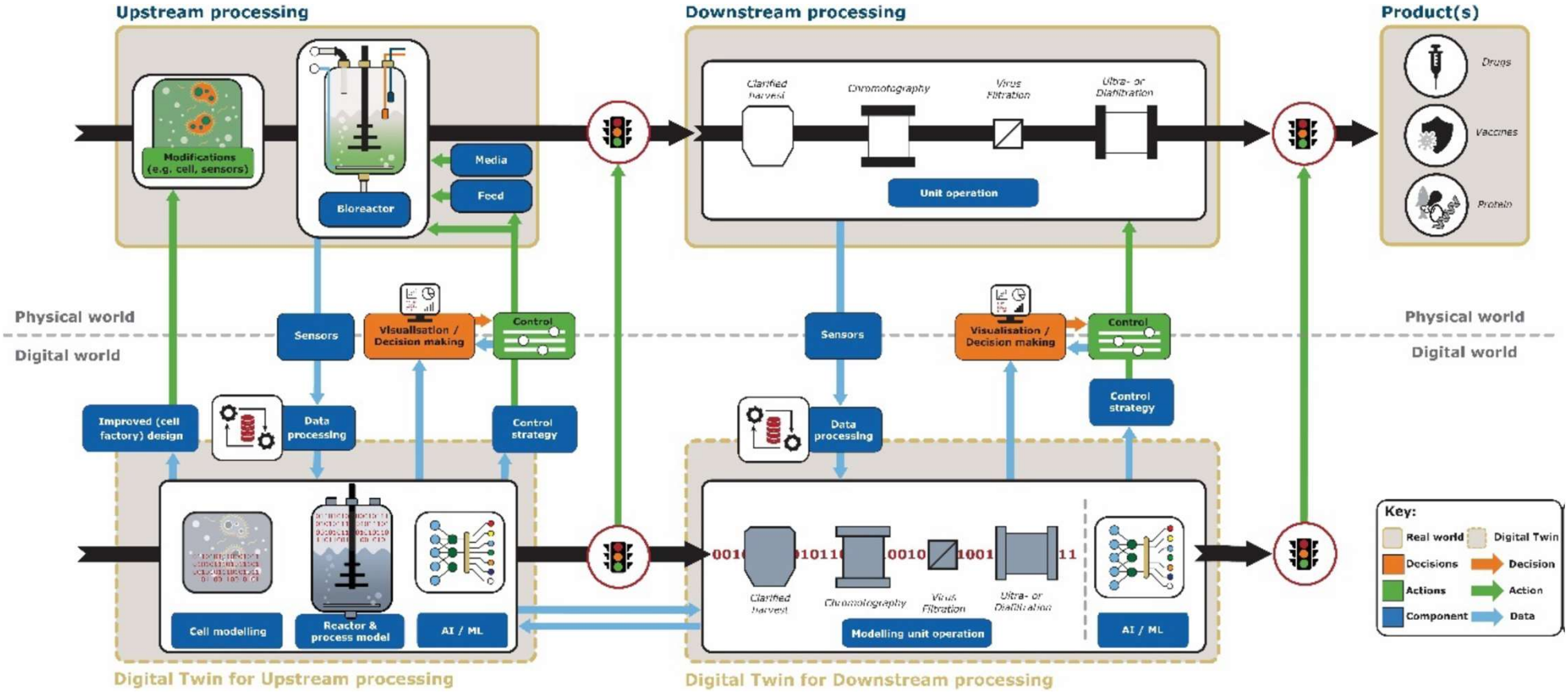
Impurity removal - HCP

- Efficient removal of HCP in different steps of DSP
  - Initial amount around 150.000 – 200.000 ppm



What can we do with these PAT data?

Digital Twin



Questions?