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Development of the PAT toolkit for continuous bioprocessing

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Development of the PAT toolkit for continuous bioprocessing

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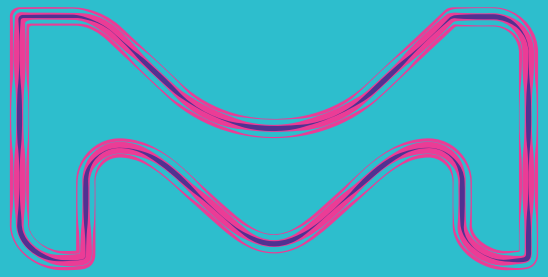


MERCK

Merck Vevey, Switzerland

- Manufacturing facility for **Bavencio®**, **Rebif®** and **Erbitux®** (drug substances)
- **Feed batch** processes
- Stainless steel large volume tanks
- Lack of space for expansion

- **Process development** & clinical manufacturing facility
- Implement properly **continuous manufacturing**
- Flexible production capacities
- Labs designed to be adaptable to new technologies evaluation

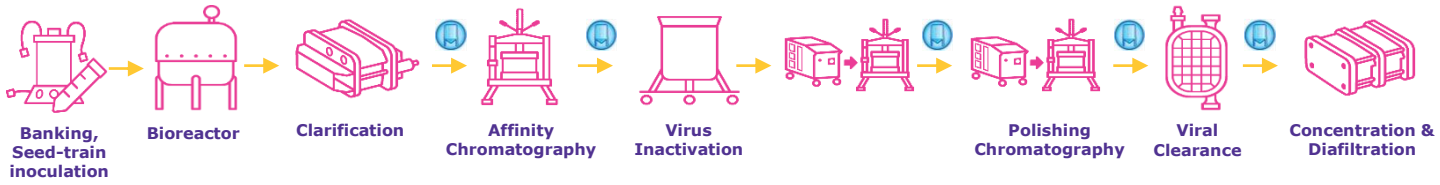
Develop processes in adequation to industry complexity & volatility

↗ Productivity

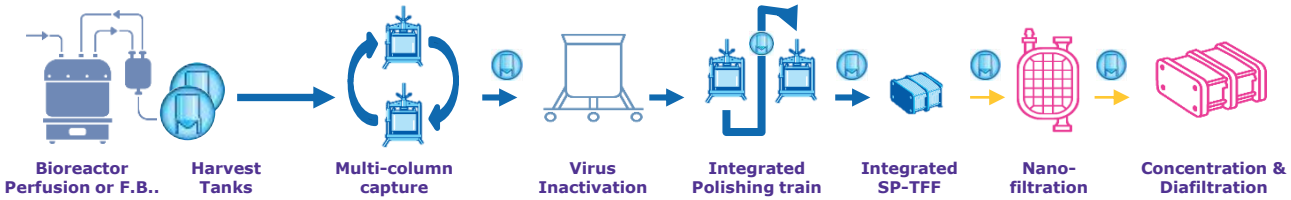
↘ Footprint & cost

Our journey to Continuous Manufacturing for clinical stages

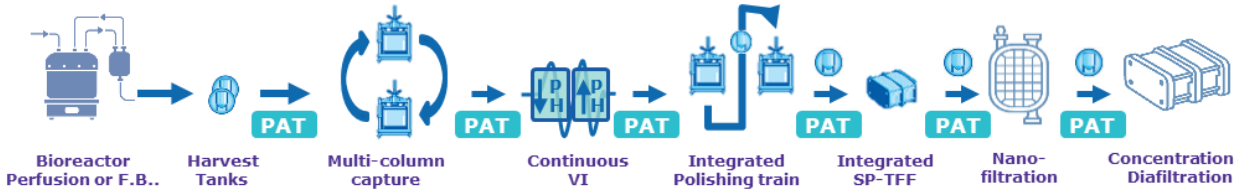
Batch



Today 'E2E'



Future 'E2E+'



Analytics

Mostly offline

Time consuming data analysis

Early PAT integration

Initiatives to unlock data science

Large PAT deployment

Data science incorporated in process

Observations

USP & DSP technologies for continuous manufacturing are mature

PAT to control / correct process needed acceleration to align with goals

Process Analytical Technology, strategy

Main measurement **categories** in Biopharma

- **Critical Process Parameters**
- **Performance Indicators**
- **Critical Quality Attributes**

Batch

Off-line
Sample is tested outside of the production area

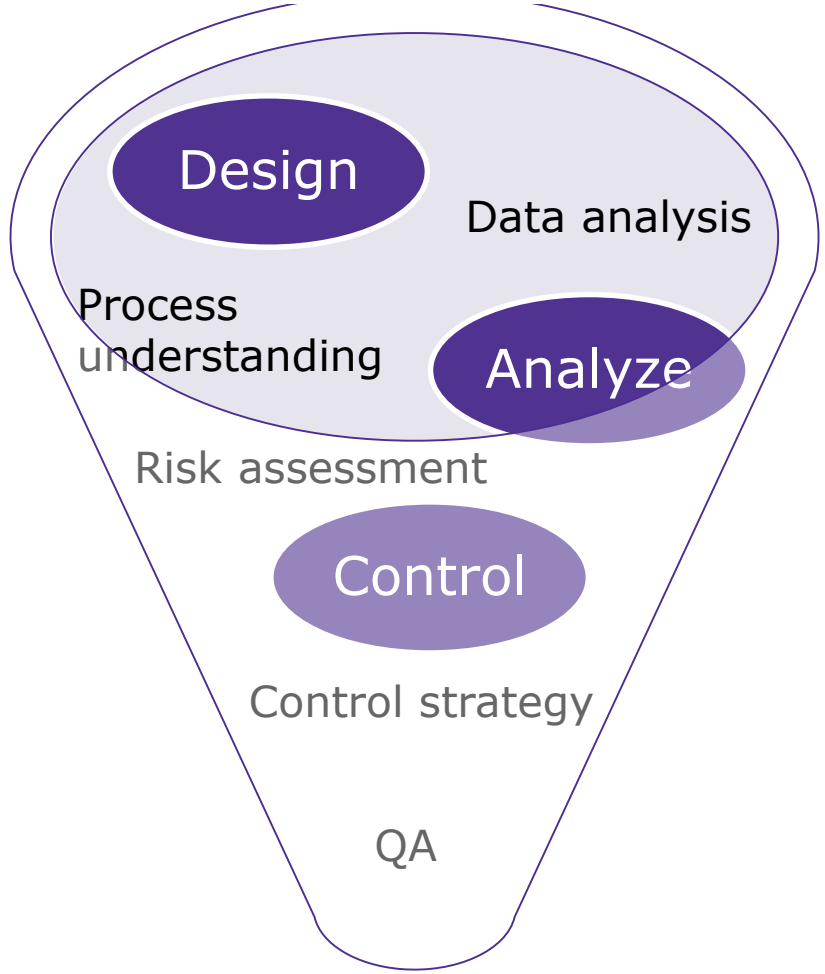
In-line
Sample is not removed from the process stream

Future 'E2E+'

In-line
Sample is not removed from the process stream

At-line
Sample is analyzed near the process stream

Off-line
Sample is tested outside of the production area



Reduce DS release time
Ensure product quality

Process Analytical Technology deployment

Off-line
Time response:
Days to weeks

SIMCA

Analyse

Acquire

In-line Testing:
Minute response

synTQ

Control through process automation systems

PAT-manager software

OSIsoft.

Data-historian

Archive

Understand
Monitor

Automated At-line Testing: Minutes to hour response

Acquire

Analyse

Off-line testing	Sampled and tested in **another lab**
At-line testing	Sampled and tested **on the floor**
In-line testing	**No sampling**, testing inline

MERCK

PAT manager: Harnessing the power of PAT

sample

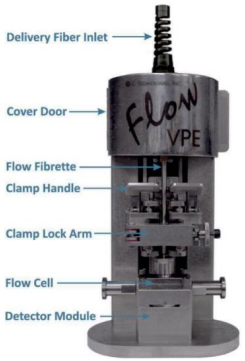
Master Controller

MAST Interface

sample

Nova FLEX2

RamanRXN2-Multichannel Analyzer
Kaiser Optical Systems



synTQ

PAT Manager

Models

Attribute	Quantity
Analyte 1	3 g/L
Analyte 2	5 g/L
Analyte 3	1 g/L
. . .	. . .

Data historian

DCS

GMP compliance

MERCK

Raman for USP – Multiple scales

Ambr 250

Bio 3.5 L

Perfusion 200-2000L

Strategy

- Raman presence in all process development scales
- High throughput process development with large design space

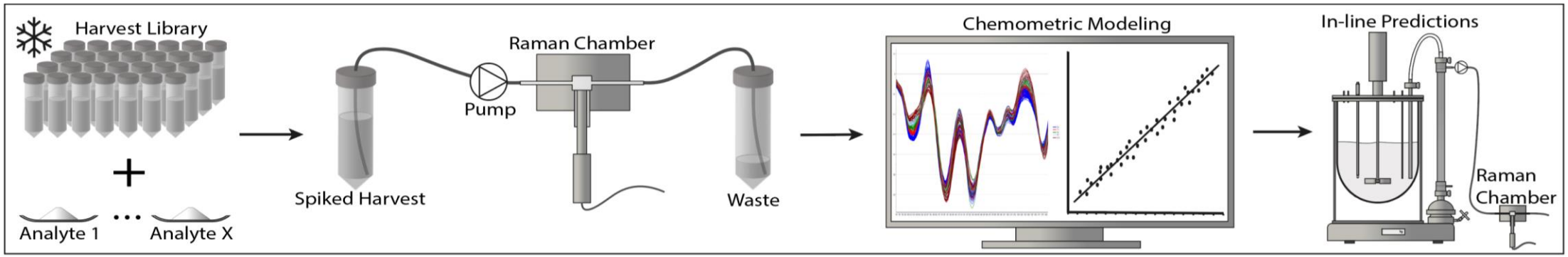
Target

- Time reduction in model building
- Media saving
- Time saving for other activities

Raman for USP – Multiple scales

Ambr 250

- Early process development
- Variability source
- Fed-batch & "perfusion"



Harvest Library

- Samples of multiple Bioreactor runs
- Samples from different working days
- Samples widely covering process variability

Spectral Acquisition:

- Harvest library mixed with analyte spikes
- DoE Spiking possible
- Flow cell for Raman Calibration

Modeling:

- Pre-processing
- PLS Regression

In-line Prediction:

- Flow cell in permeate
- Analyte monitoring
- Feedback loops

Romann, P., Kolar, J., Tobler, D., Herwig, C., Bielser, J.-M., & Villiger, T. K. (2022). Advancing Raman model calibration for perfusion bioprocesses using spiked harvest libraries. *Biotechnology Journal*, 00, e2200184. https://doi.org/10.1002/biot.202200184

Raman for USP – Multiple scales

Bio 3.5 L

OPC

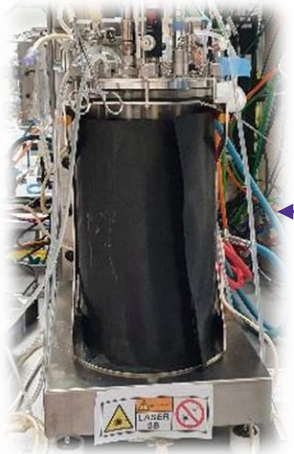
synTQ

SIMCA

Glucose predictor → Moving average → Glucose feed calculator

OPC

MFCS software

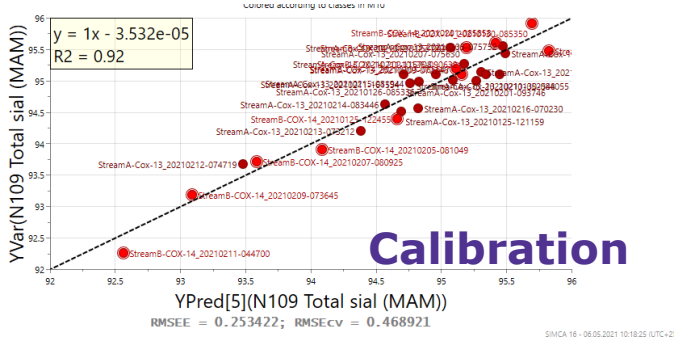


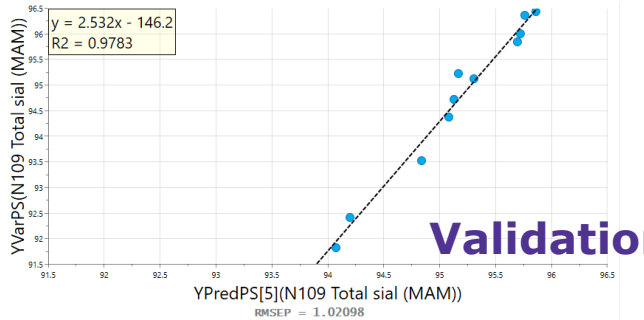
Bioreactor's glucose pump

MERCK

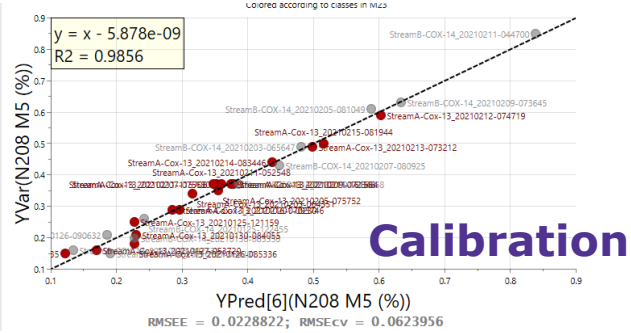
RAMAN - monitoring capabilities of glycosylation

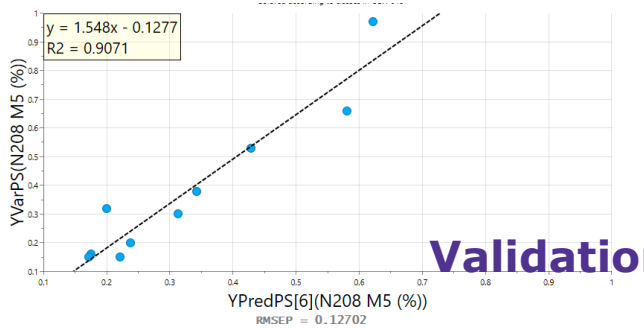
Total Sial





High Manose





CQA	RMSCV	R2-Calibration	RMSEP	R2-Validation	Calibration range	Validation range
Total N109 %	0.47	0.92	1.02	0.98	92-96	91-96.5
High Mannose %	0.06	0.99	0.13	0.91	0.1-0.9	0.1-1

Raman for USP – Multiple scales

Perfusion 200-2000L

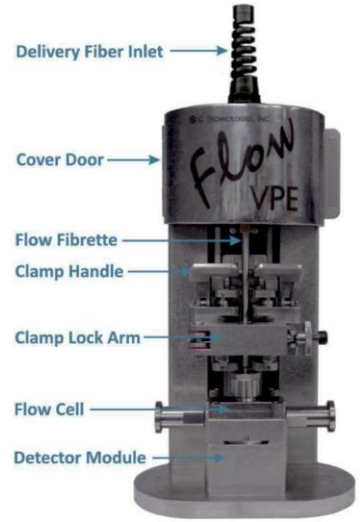
- Successful transfer and installation of Raman at clinical manufacturing site
- Models built on 3,5L scale, used in 200L scale

- Good alignment on glucose and lactate data
- Titer alignment show some discrepancies but overall trends are aligned

FlowVPE: In-line protein concentration

Current situation

Offline OD measurement with manual dilution to obtain protein concentration



In-line analysis

$$A = \varepsilon \, l \, C$$

The relation is not linear leading to false concentration measurement

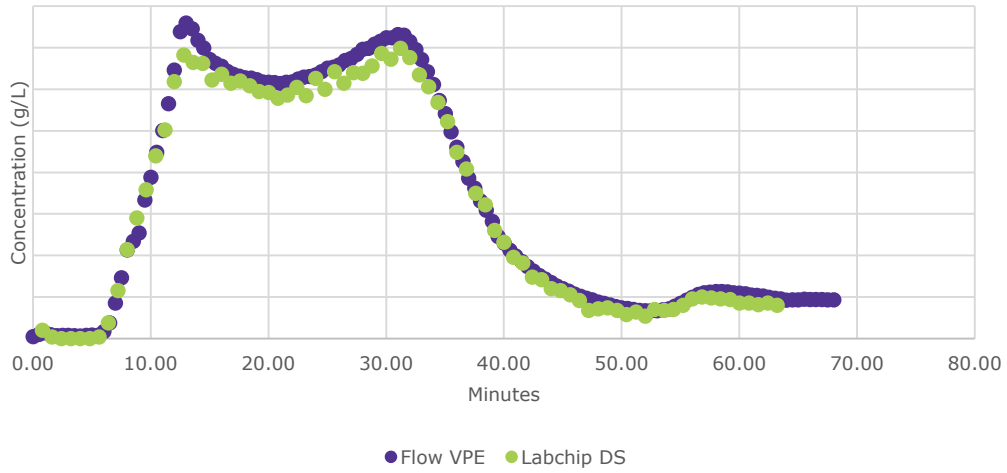
A: Absorbance of the solution for a given wavelength

ε: Molar attenuation coefficient (specific for a given protein)

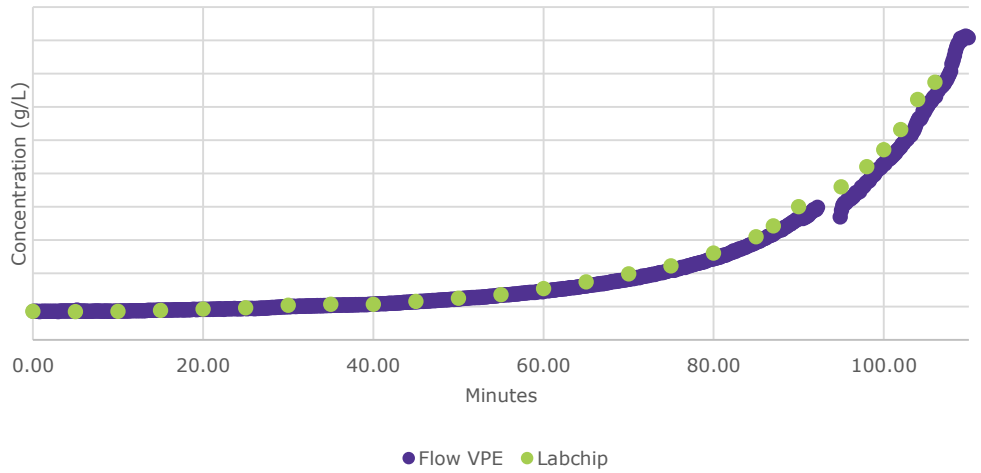
l: Optical pathlength → adjusted to avoid signal saturation

C: unknown in our case

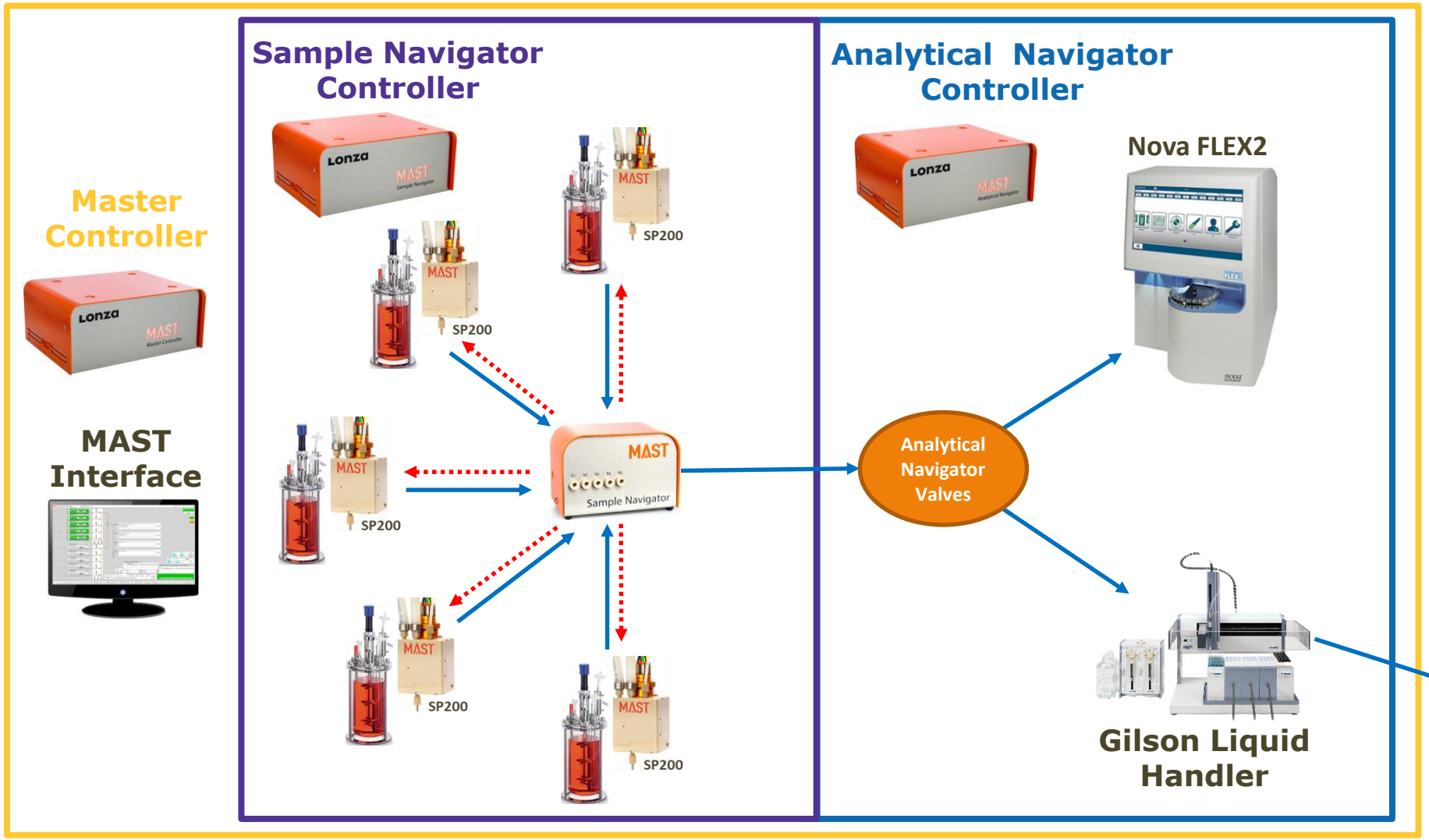
AEX Chromatography



Fusion protein Concentration

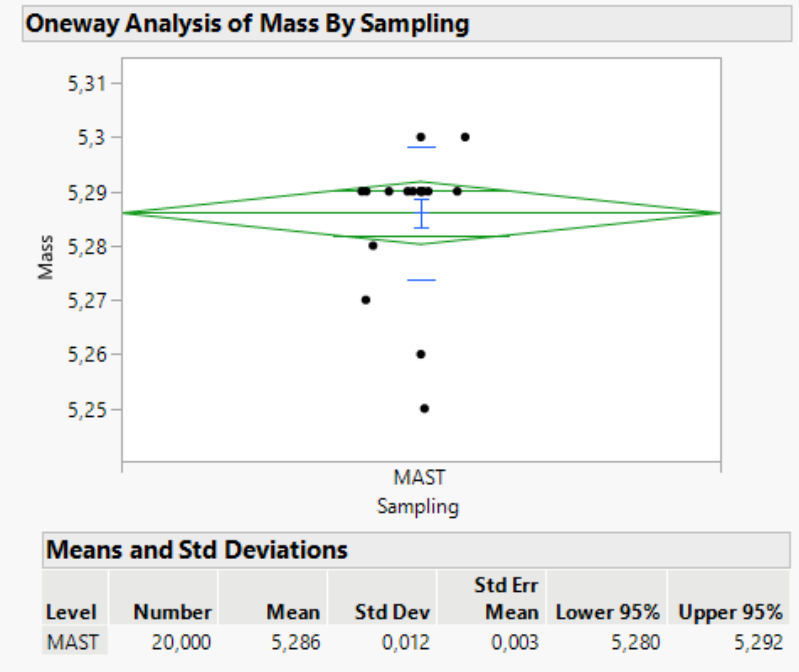


MAST autosampling device



- Aseptic sampling inside bioreactor, harvest tank or DSP intermediate tank
- Sample is collected, diverted & analysed based on process requirements
- Entry door for at-line analysis requiring sample preparation

Harvest mass at high cell-density sampled by MAST, N =20

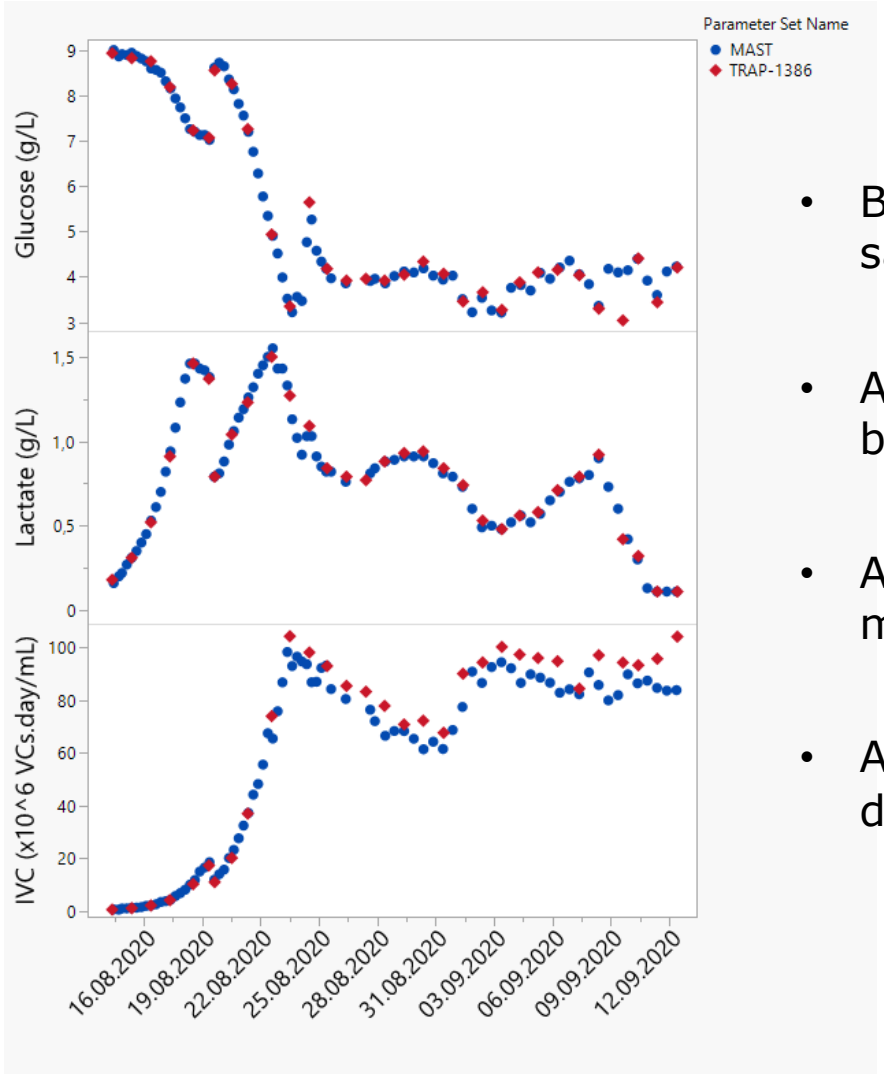


Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
MAST	20,000	5,286	0,012	0,003	5,280	5,292

- High confidence in setup sterility
- Robustness in sampled volume
- No issues at high cell densities

Bioreactor connected to Flex2 through MAST connection



- Both manual & automated sampling
- Automated sampling frequence between 6 & 12 hours
- Alignement of automatic & manual sampling results
- Ability to monitor drops/increase of analytes

MAST for at-line CQA testing

Sampling

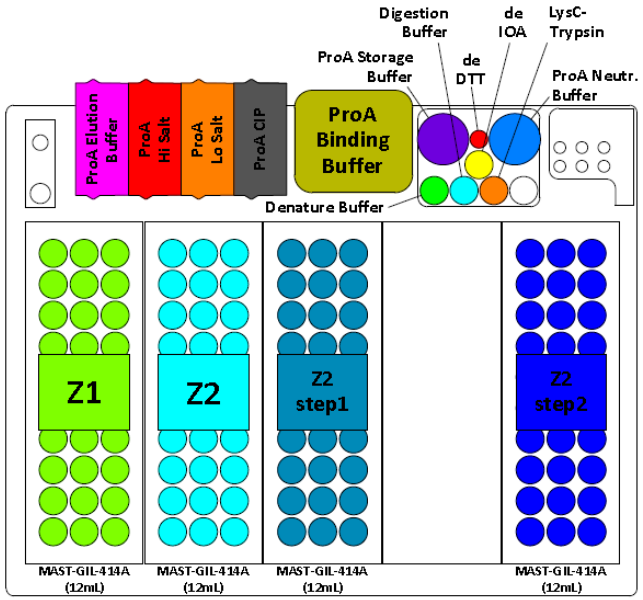
- Cell free

Sample preparation

- ProA capture
- Dilution with buffer/reagents

Analysis of CQA with LC / MS

- Titer
- HMW
- Oxidation
- Deamidation
- Glycans
- Misincorporation
- LMW



Gilson layout to allow at-line sample preparation

- ProA capture reagent
- Peptide mapping reagents
- Different sample storage zone for different temperatures

Priority to peptide mapping PoC due to multi-attribute capacity and analysis complexity

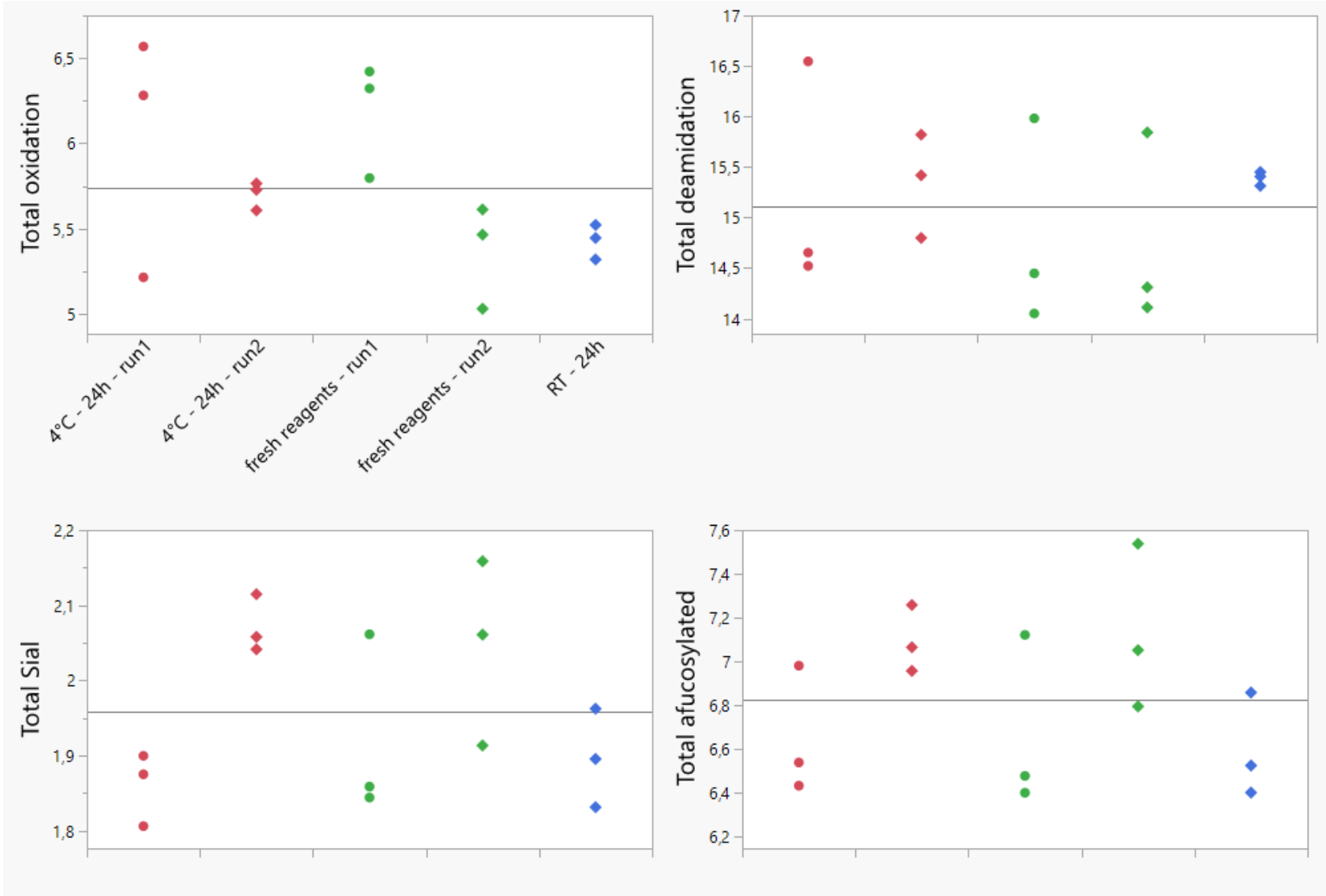
Peptide mapping PoC results

Major challenges

- Reagents stability
- Results alignment with offline routine testing

- Early peptide mapping results on QA show reagents stability

- Variability observed on some results mainly due to raw data processing



*PAT implementation is a transformation **journey** not only from a technology standpoint but also from facility, quality, skills and regulatory*

Reliable inter-systems communication

Collaborate with providers for PAT deployment and fill gaps

Move from PoC to PD support and to manufacturing

Cross-scale method validation

Acquire process knowledge while exploring design spaces

Model building with adequate variability

Technologies evaluation / PoC

Thank you

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