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Monitoring and control of reproducibility in quasi-continuous integrated production processes of Active Pharmaceutical Ingredients

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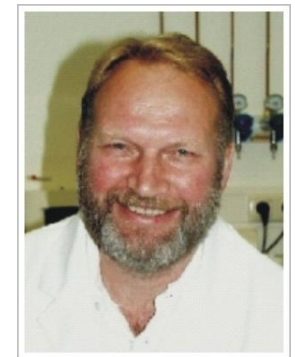
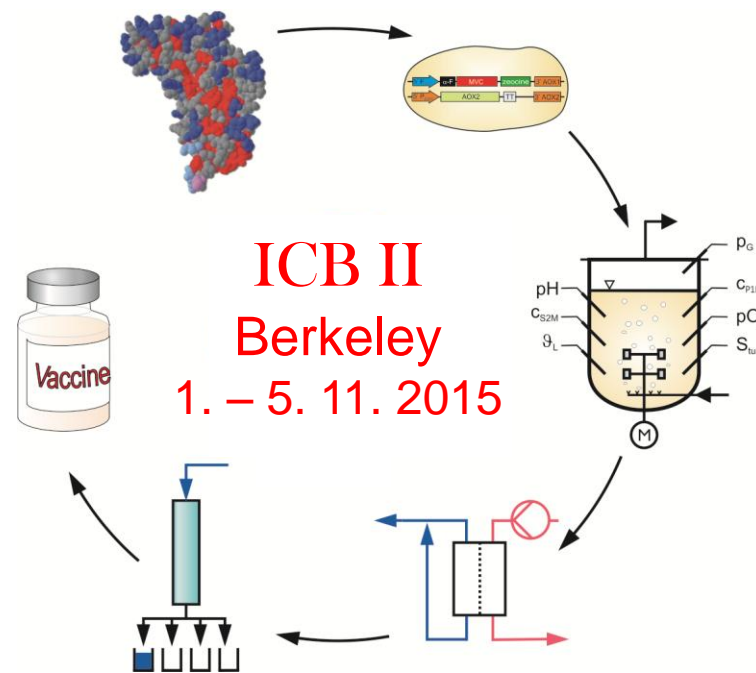
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Monitoring and control of reproducibility in quasi-continuous integrated production processes of Active Pharmaceutical Ingredients



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Outline

The three levels and nine tasks of Process Analytical Technology

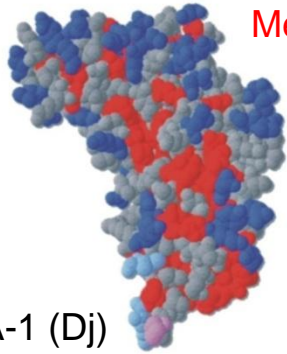
explained with a *process development for potential Malaria vaccine production*



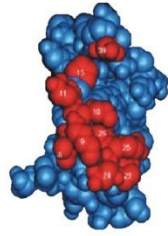
DiCo – Diversity Covering Malaria proteins – *host construction*

Malaria vaccine candidates

Apical Membrane Antigen 1



Merozoite Surface Protein 1



AMA-1 (Dj)

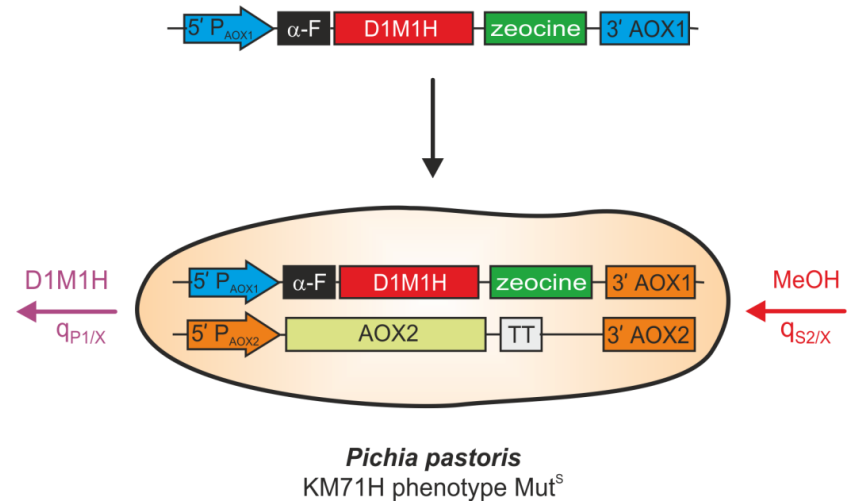
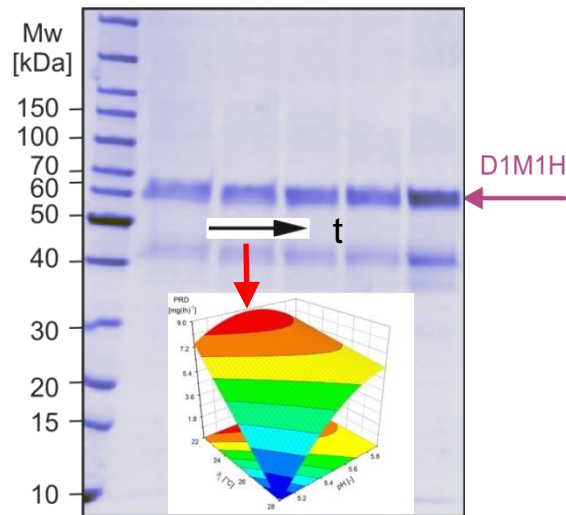
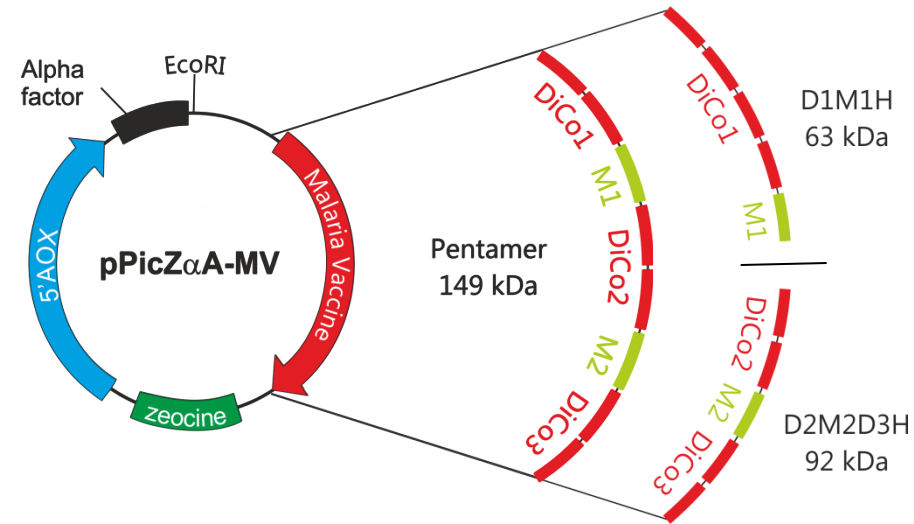
MSP-1 (Mi)



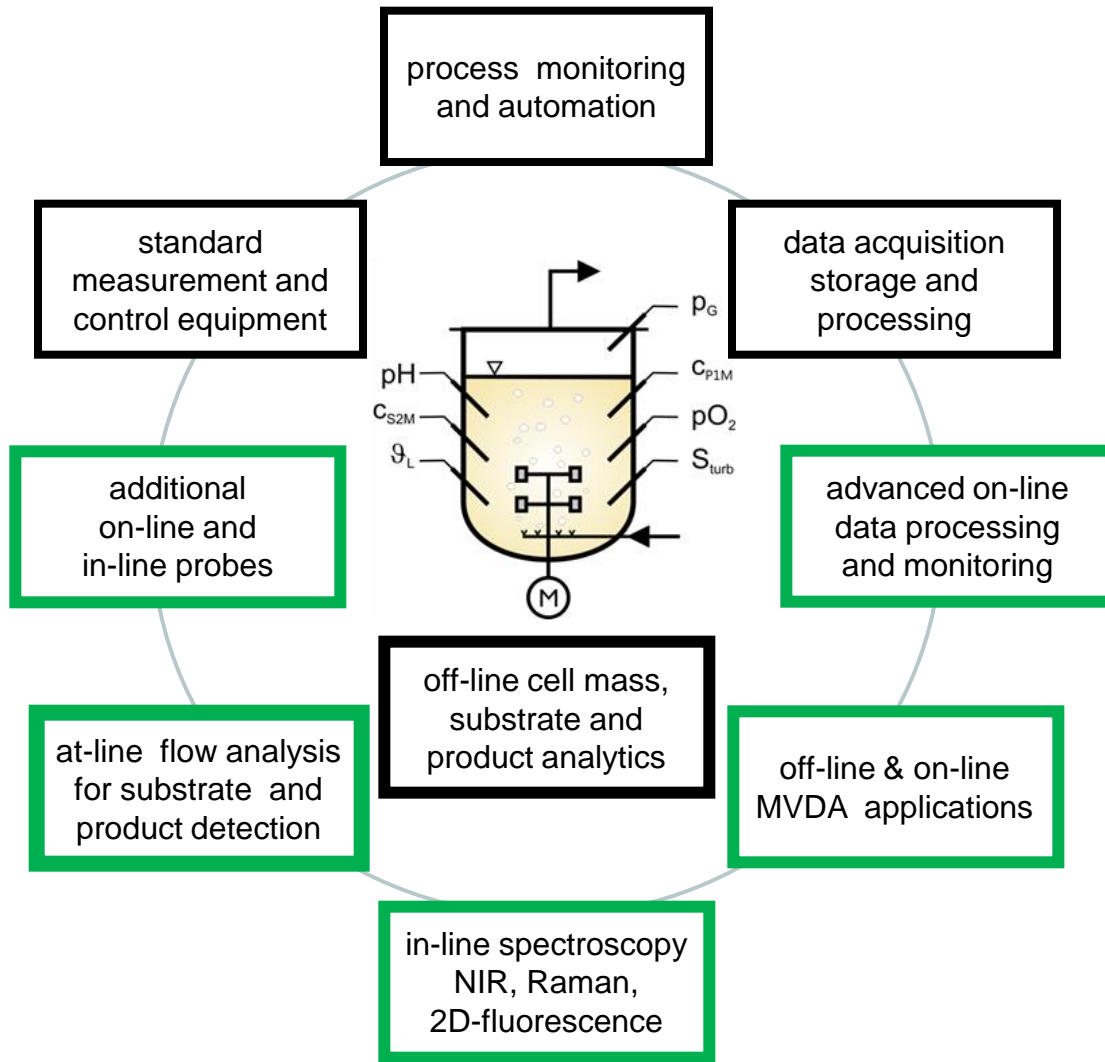
B. W. Faber

diversity covering *PfAMA1*

modified form of MSP1-19



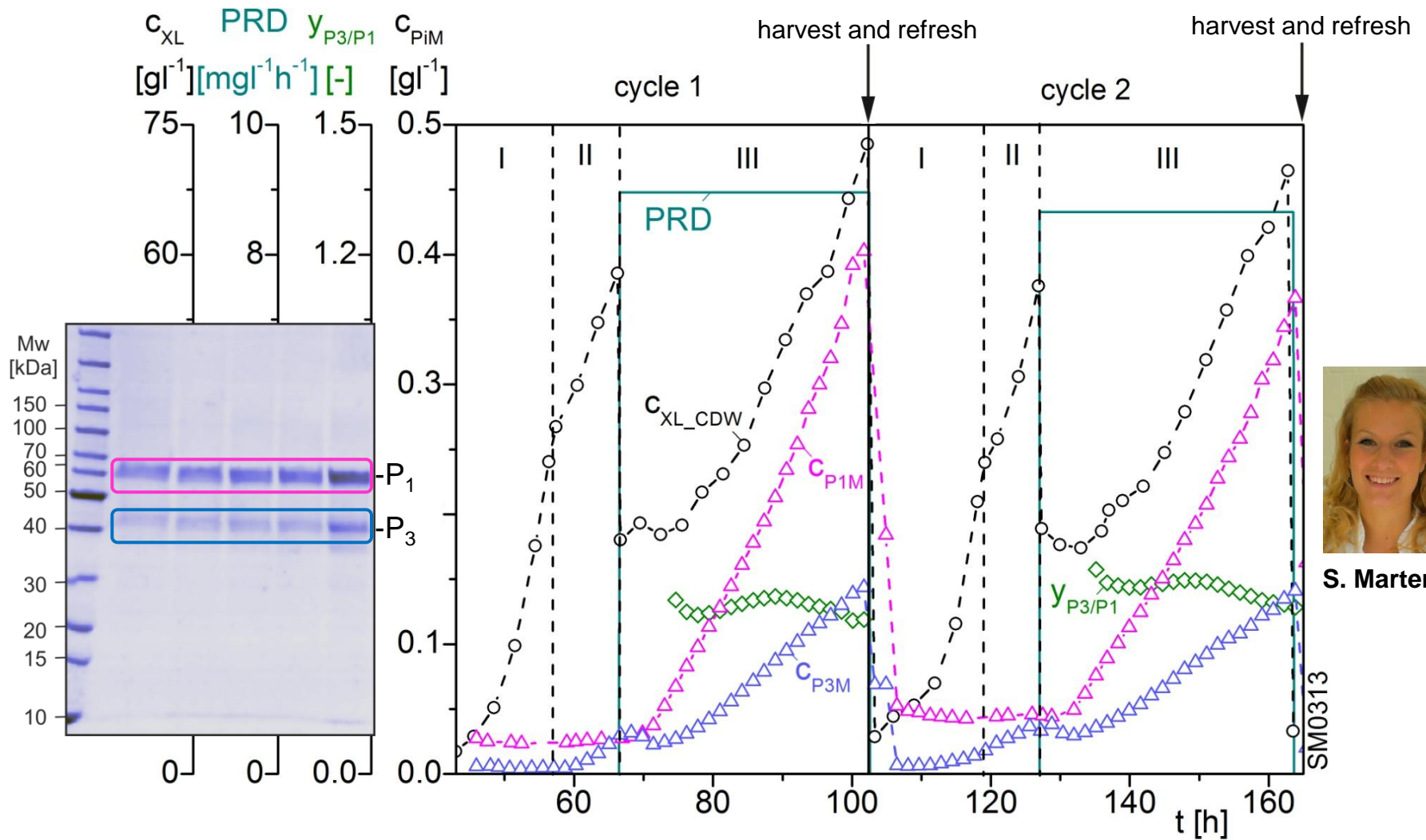
Enhanced process development – *The instrumentation level of PAT*



3 PAT levels: *instrumentation*



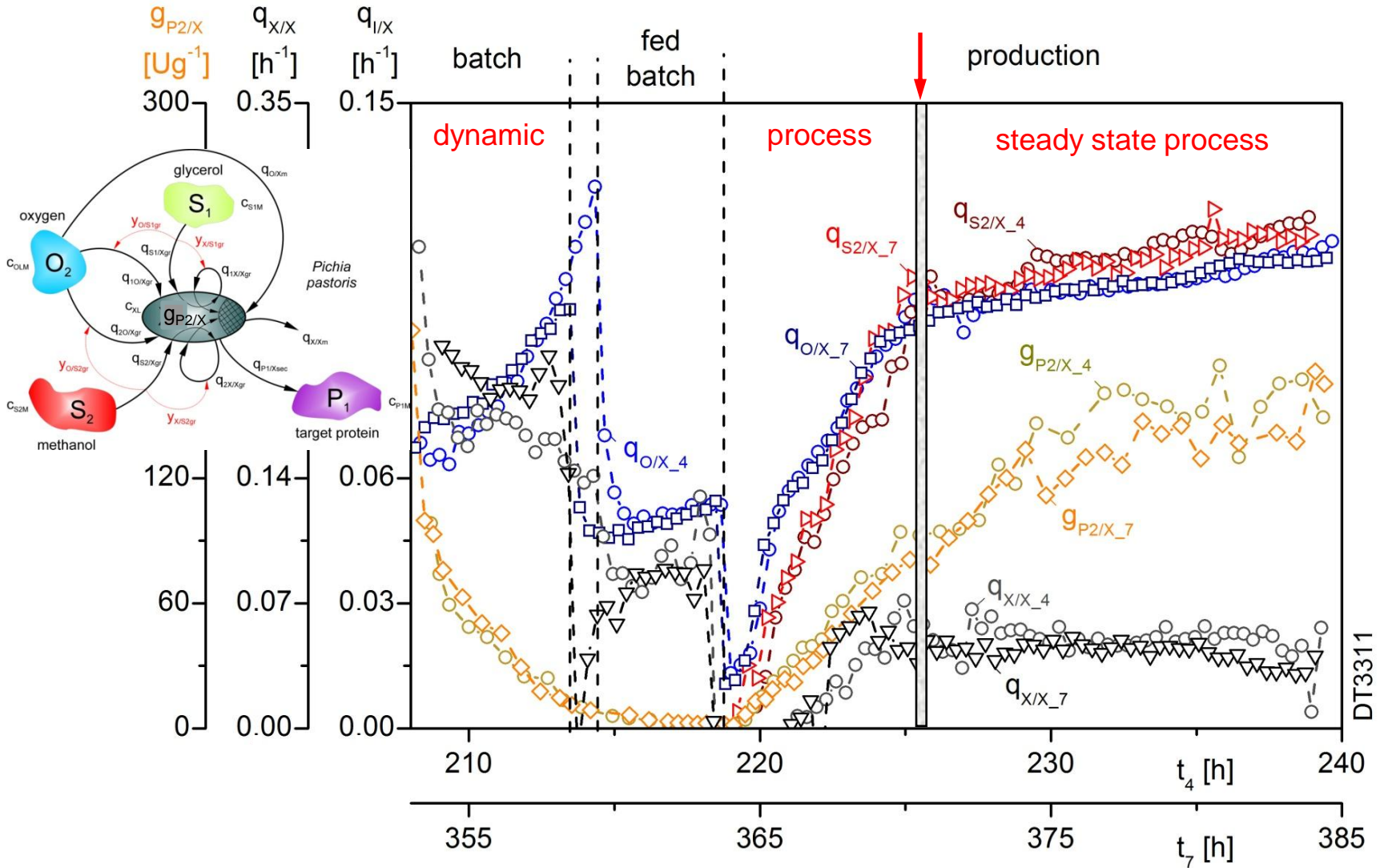
Circular processing features with consistent production quality



S. Martens



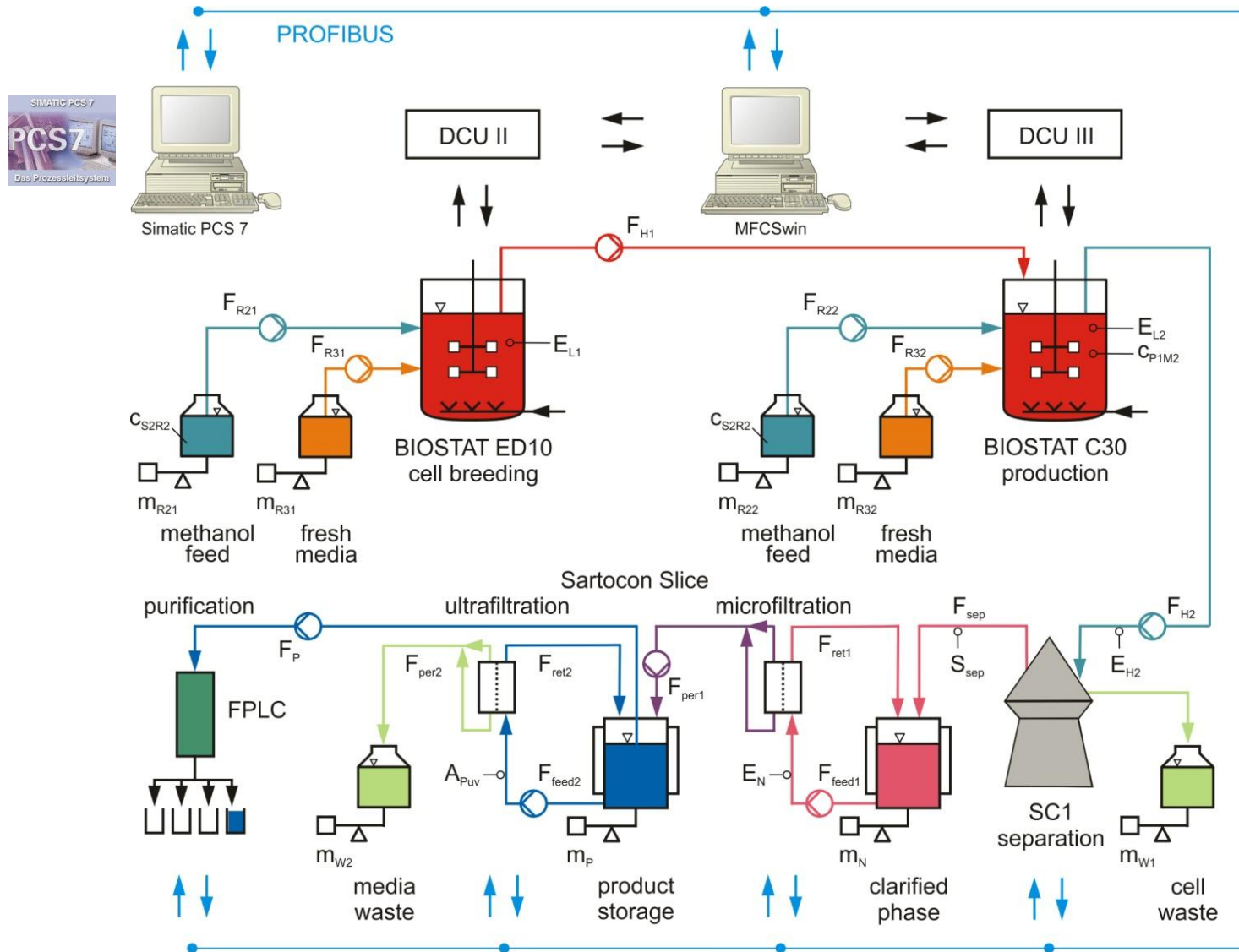
Cell specific reaction rates in reproducible experiments



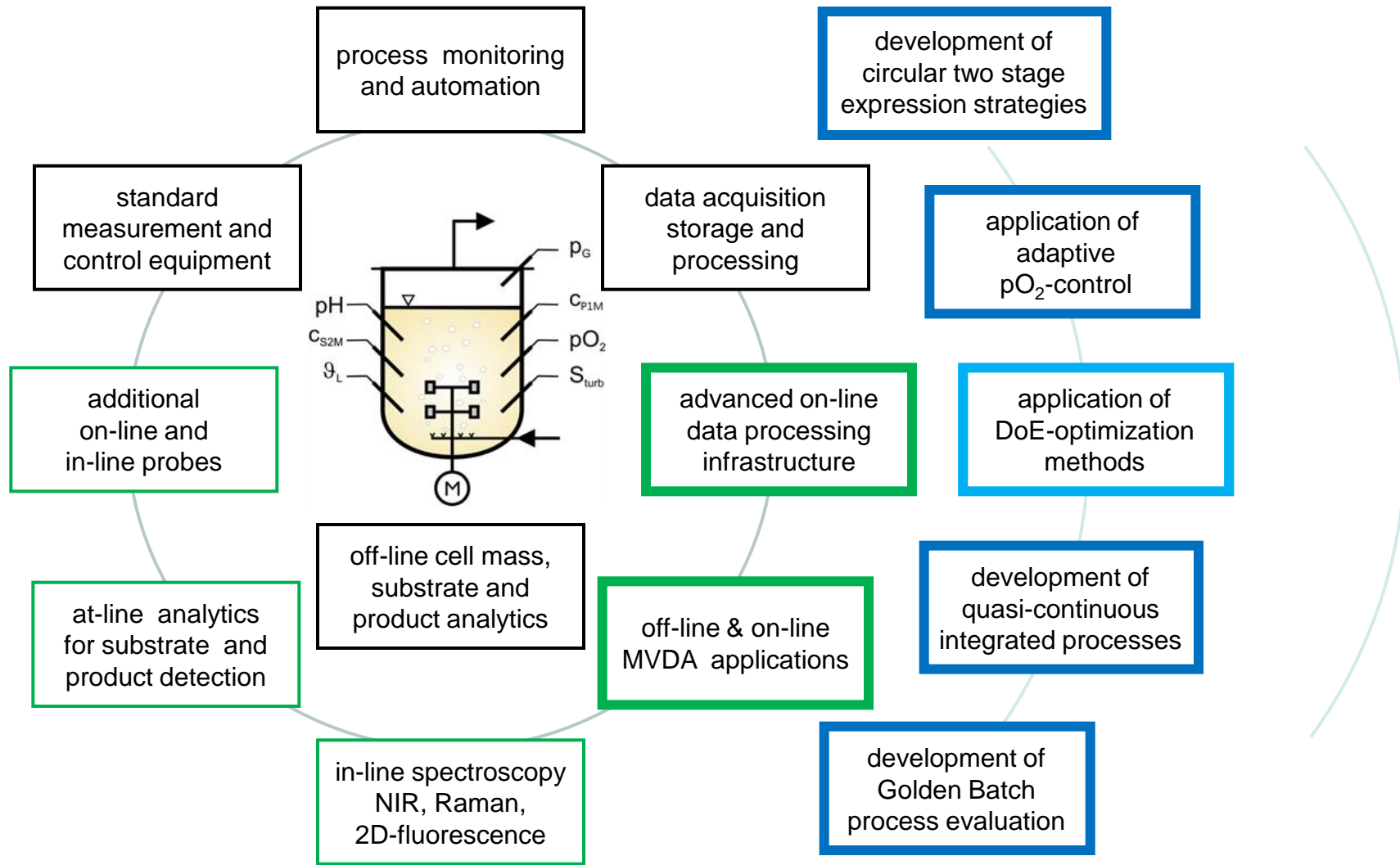
Industrial compatible *Integrated Scale-down Production Plant*



Unit operations of production of secreted pharmaceutical proteins



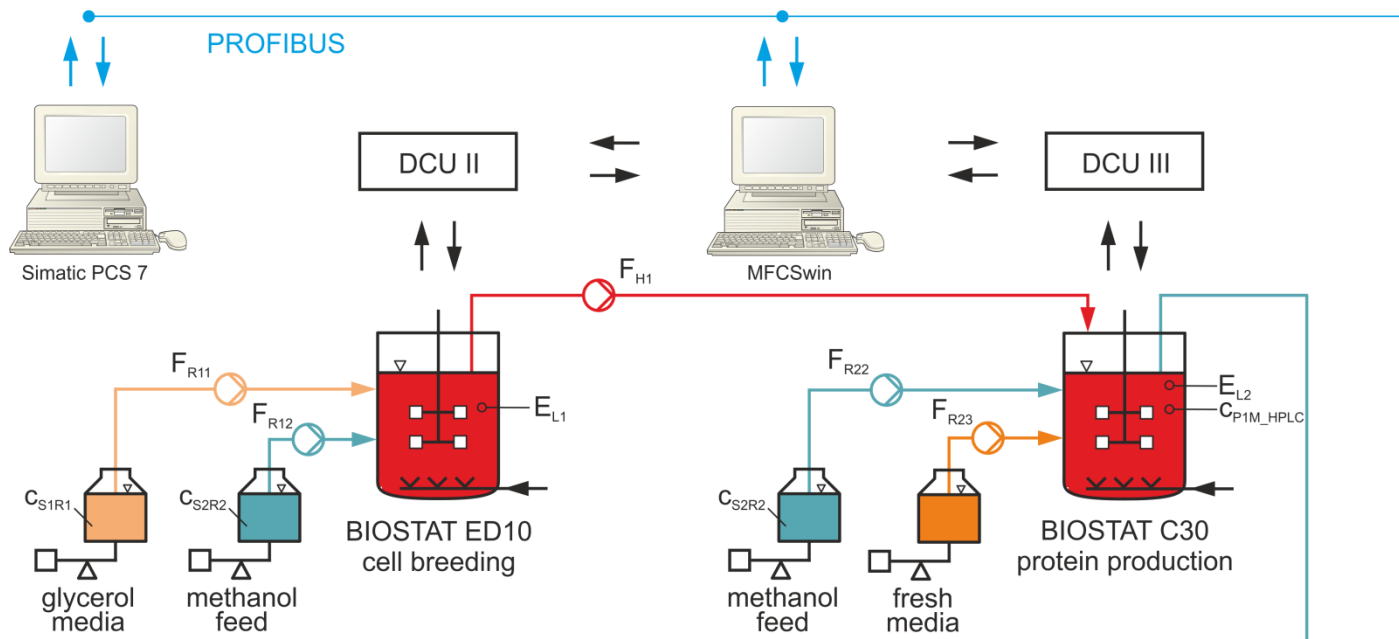
Enhanced process development – *The process development level of PAT*



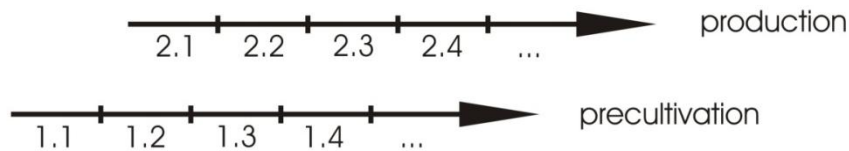
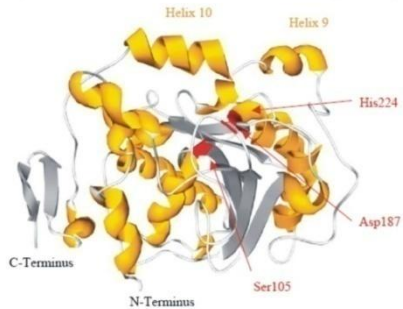
3 PAT levels: *instrumentation* *process development*



Up-scale into a two reactor strategy with sequential/parallel cultivation



Lipase B from *Candida antarctica* (CALB)



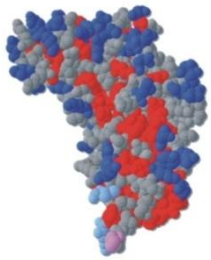
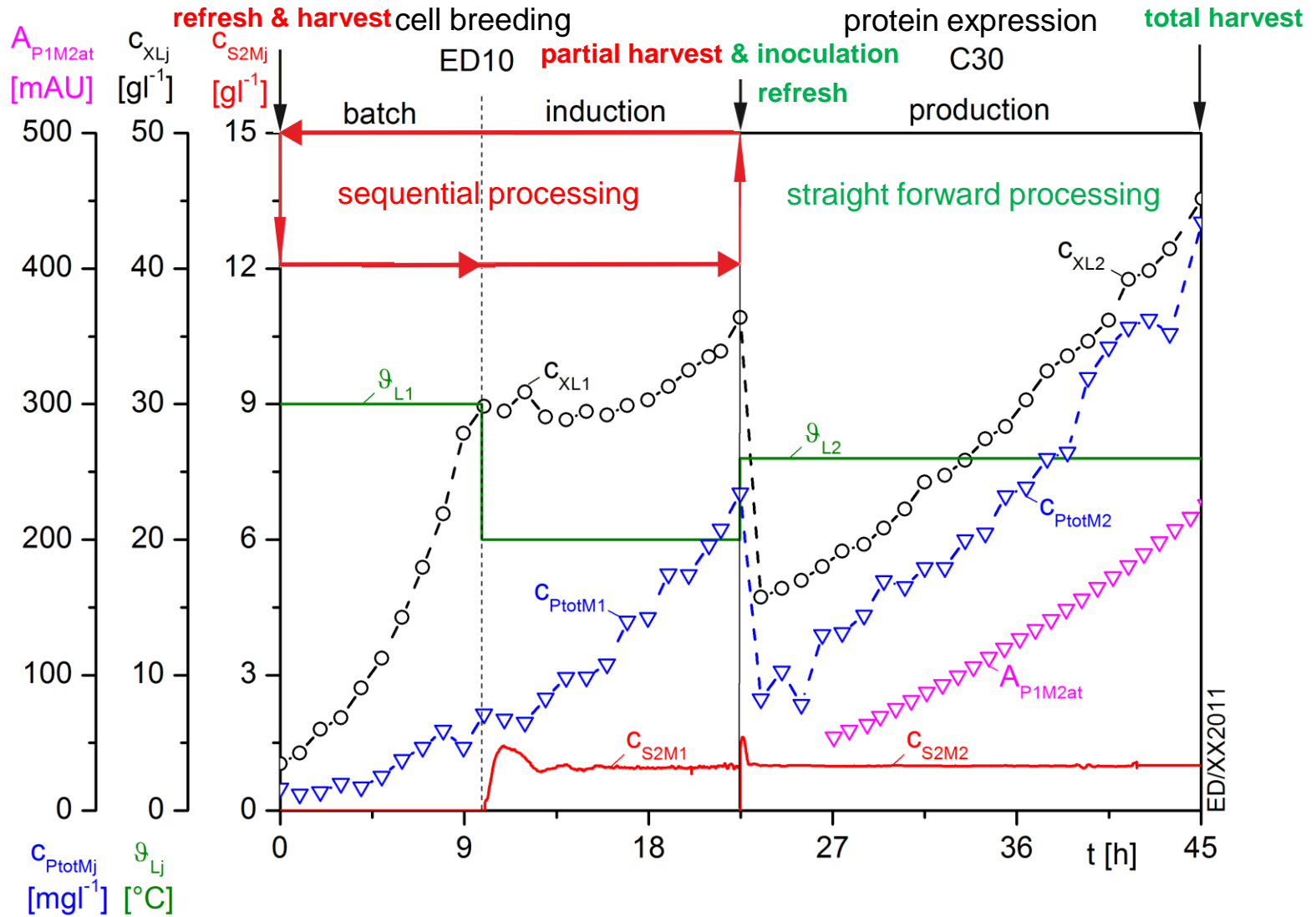
K. Lögering



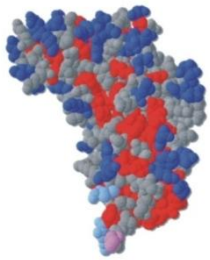
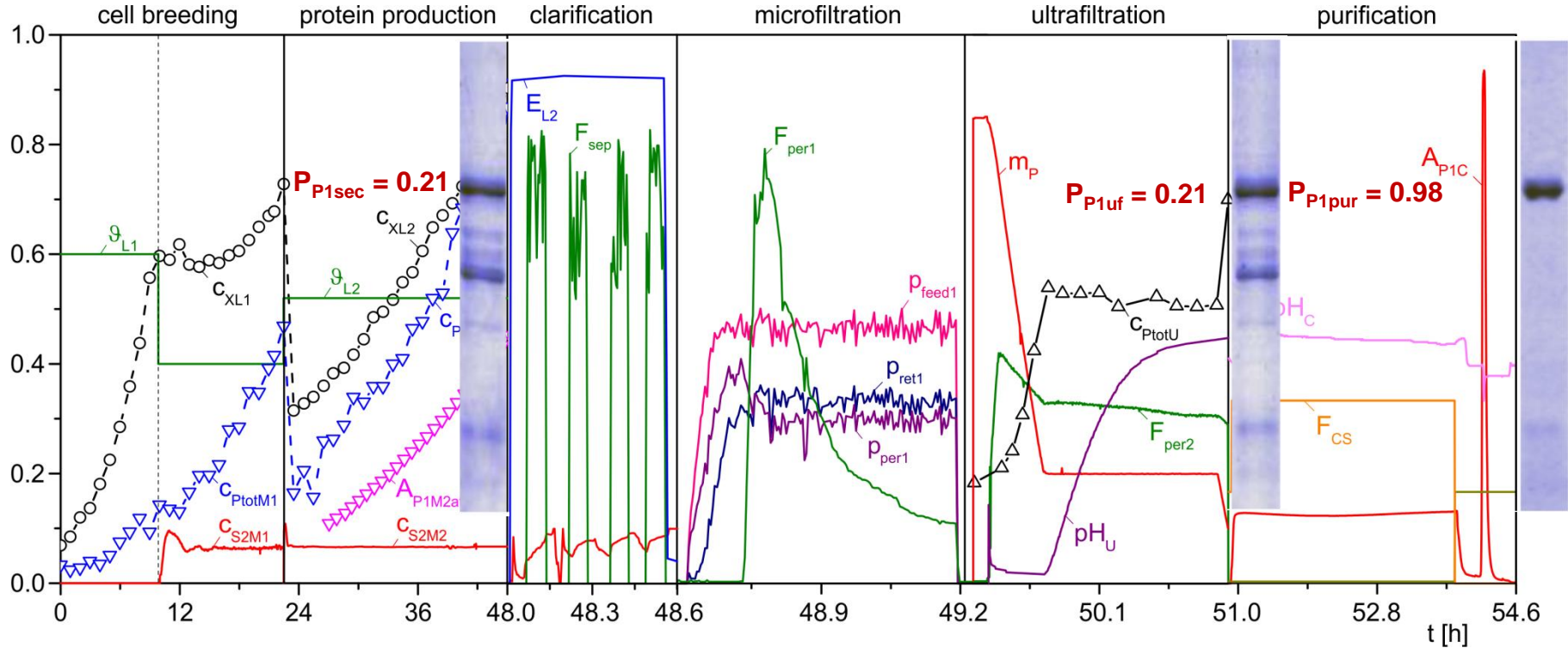
C. Müller



Two reactor *sequential/parallel D1M1H production upstream strategy*

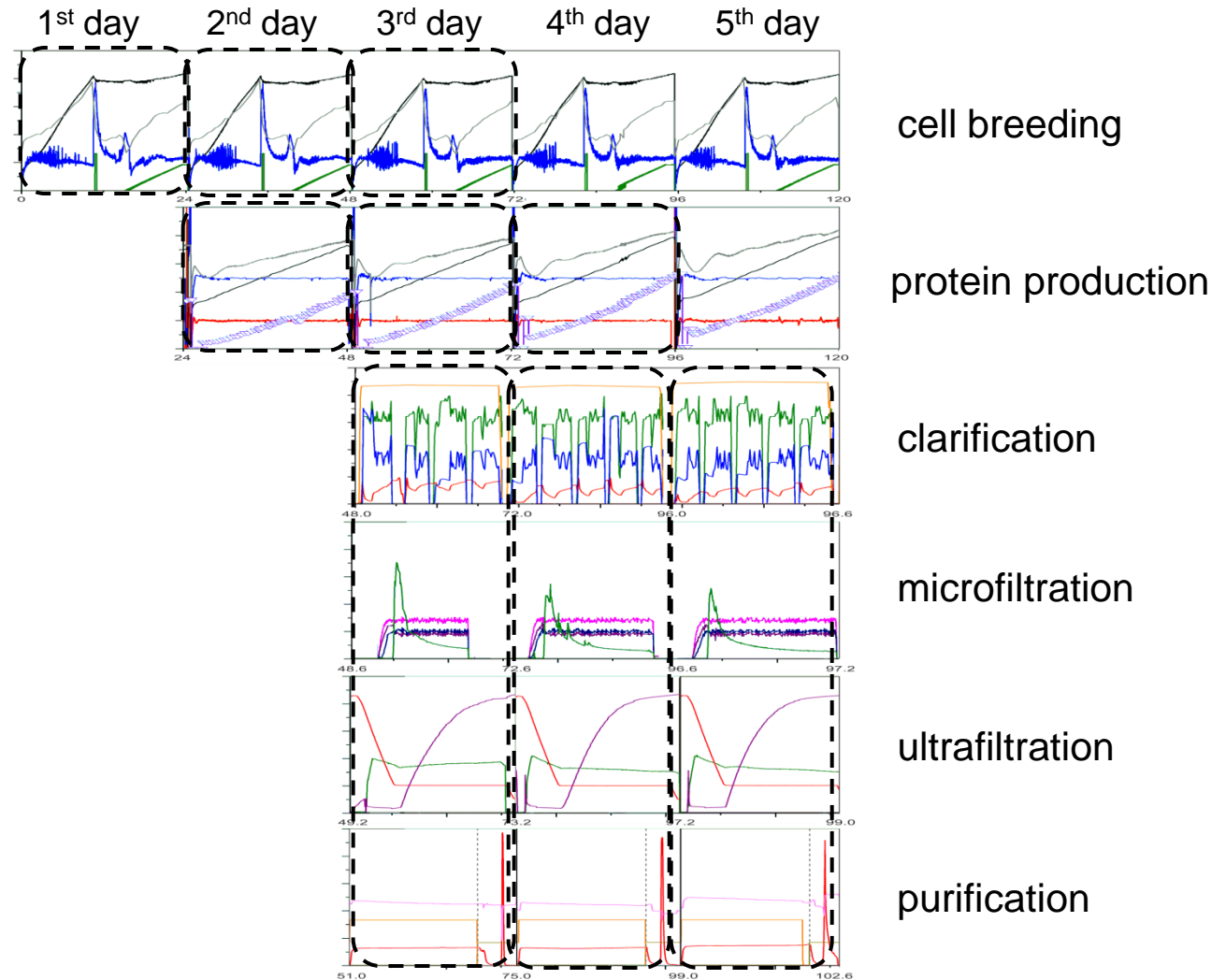


Fully automated *integrated production* of Malaria vaccine D1M1H

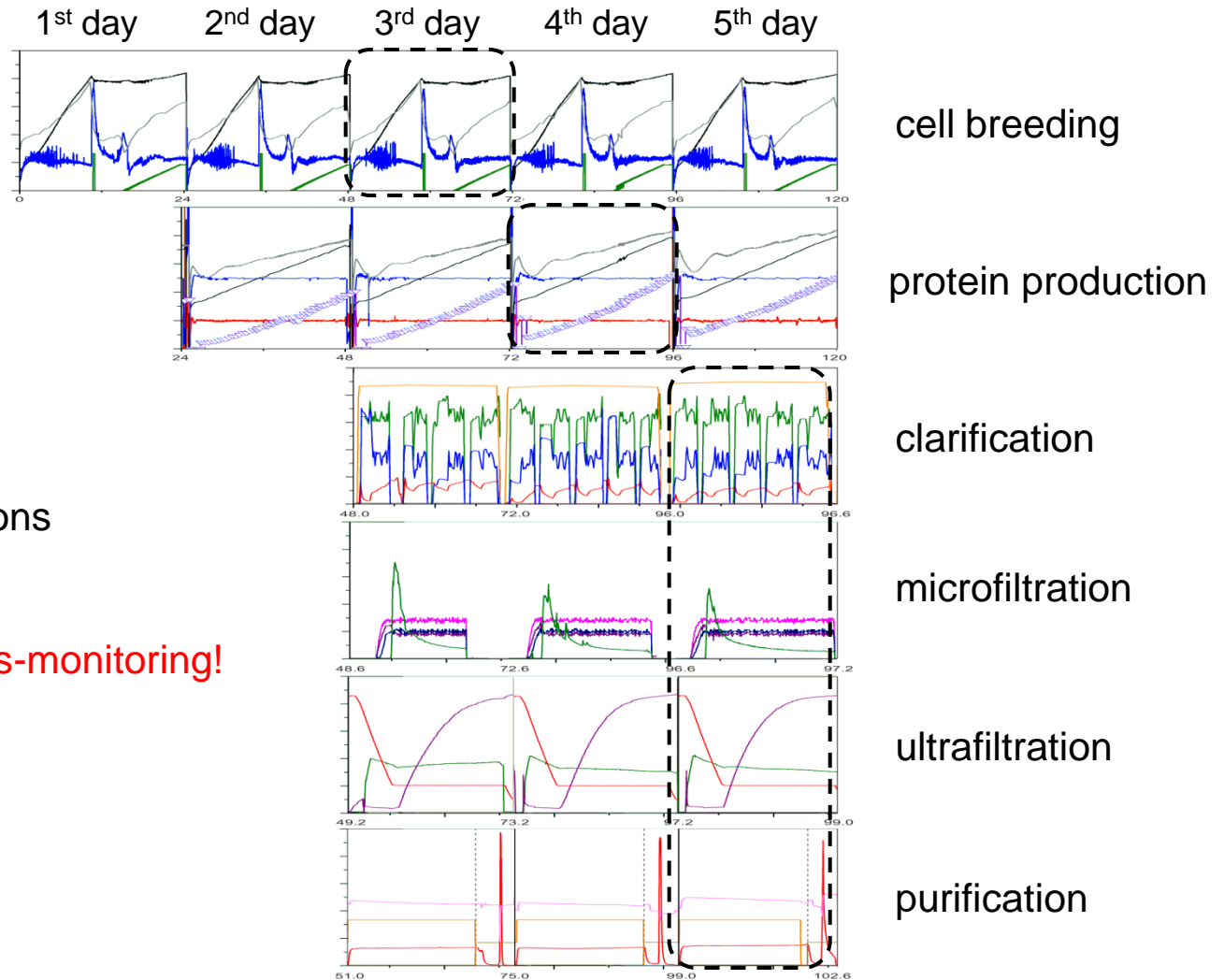


$c_{P1M2} = 80 \text{ mg l}^{-1}$	$c_{P1N} = 80 \text{ mg l}^{-1}$	$c_{P1P} = 60 \text{ mg l}^{-1}$	$c_{P1P} = 320 \text{ mg l}^{-1}$	$c_{P1C} = 27.1 \text{ g l}^{-1}$
$m_{P1M2} = 1040 \text{ mg}$	$m_{P1N} = 988 \text{ mg}$	$m_{P1P} = 780 \text{ mg}$	$m_{P1P} = 544 \text{ mg}$	$m_{P1C} = 463 \text{ mg}$
	$R_{P1sep} = 0.95$	$R_{P1mf} = 0.79$	$R_{P1uf} = 0.70$	$R_{P1pur} = 0.85$
			$R_{P1int} = 0.45$	$P_{P1int} = 0.98$

Quasi-continuous process with *sequential/parallel production*



Process quality verification with test of conditions for reproducibility



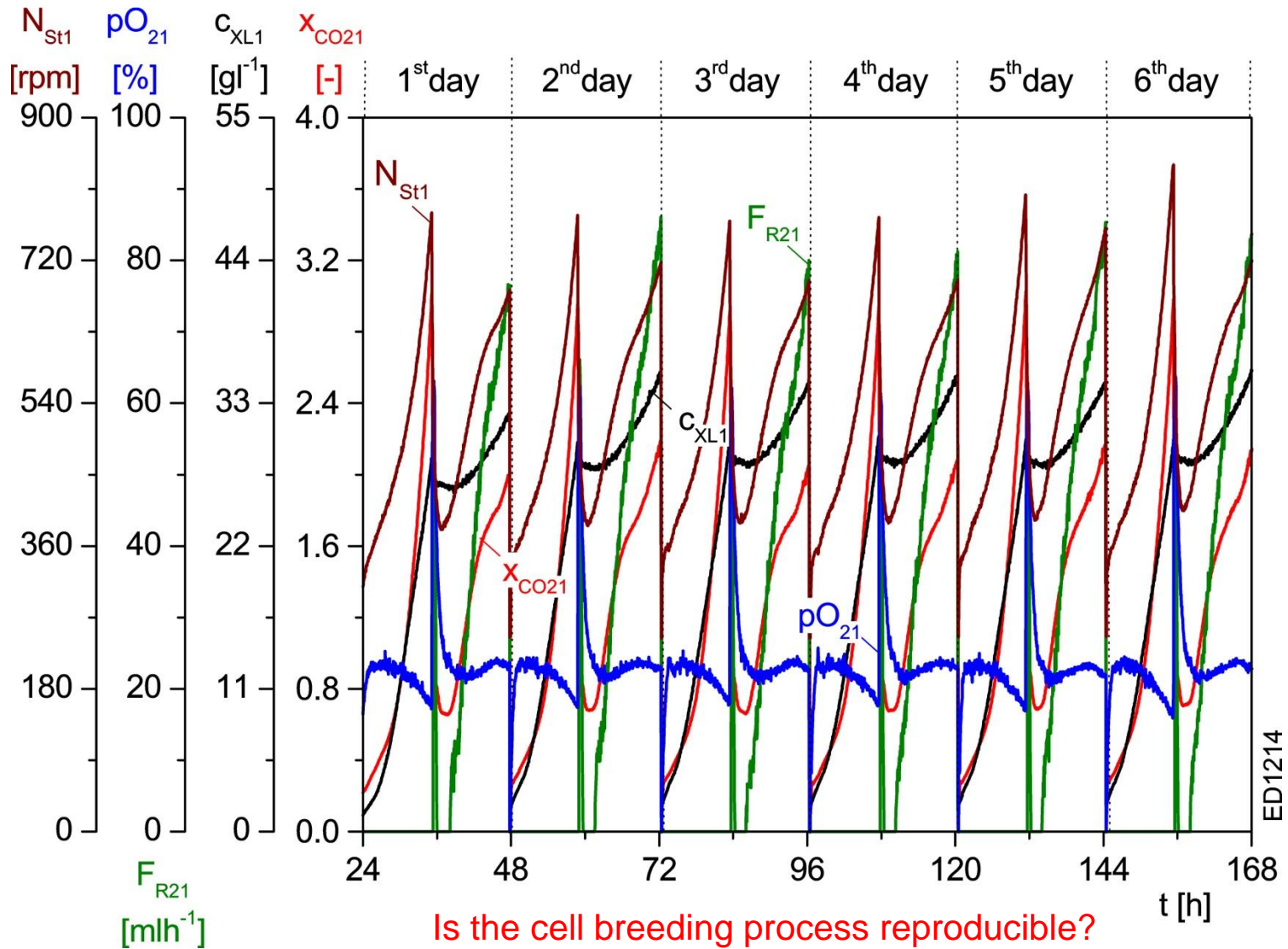
process quality test?

are the single unit operations
reproducible?

Multi-Variate Data Analysis-monitoring!



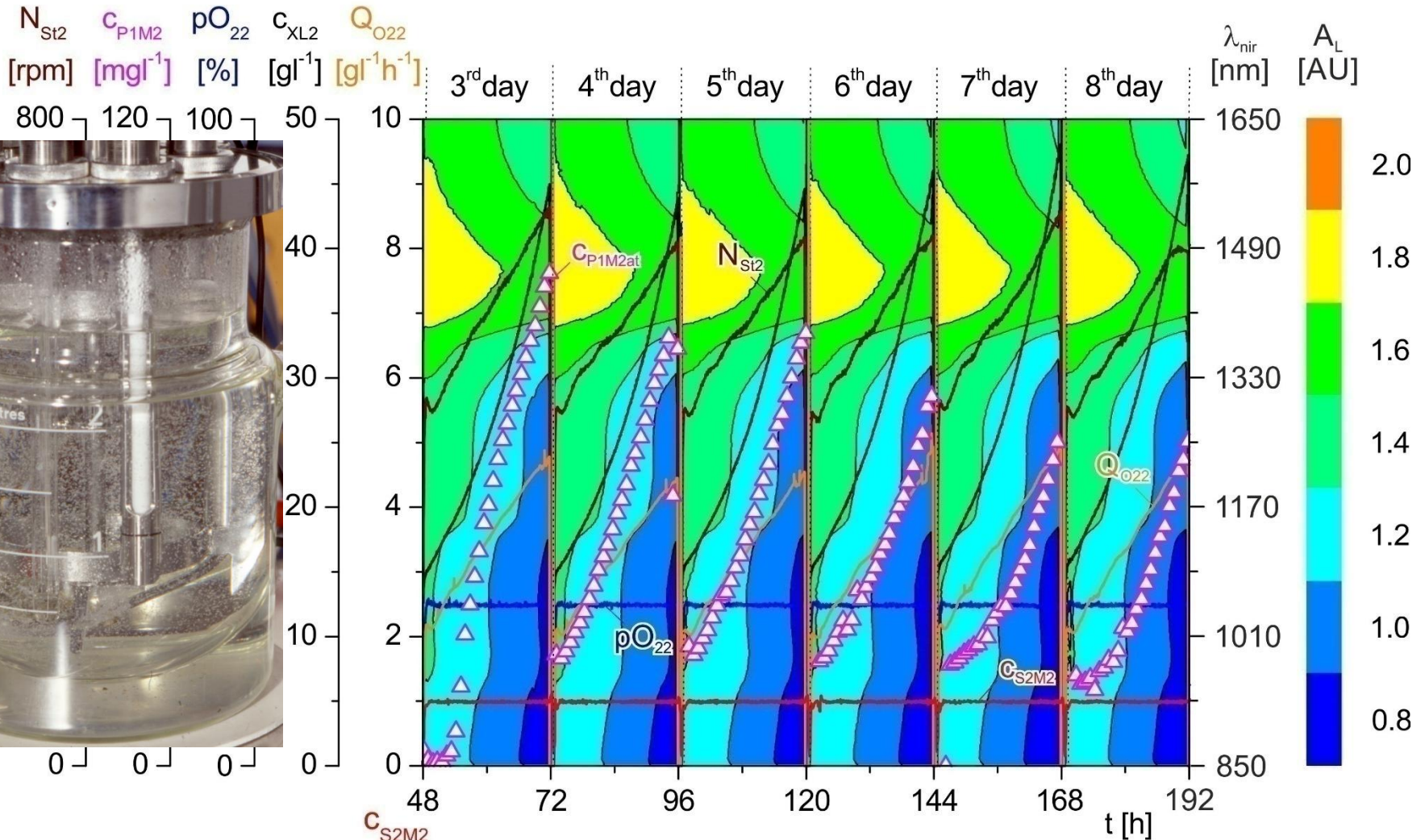
Time course of *six circular reproduced* cell breeding cultivations



Is the cell breeding process reproducible?



Spectroscopic NIR-investigation in production cycles reproducibility



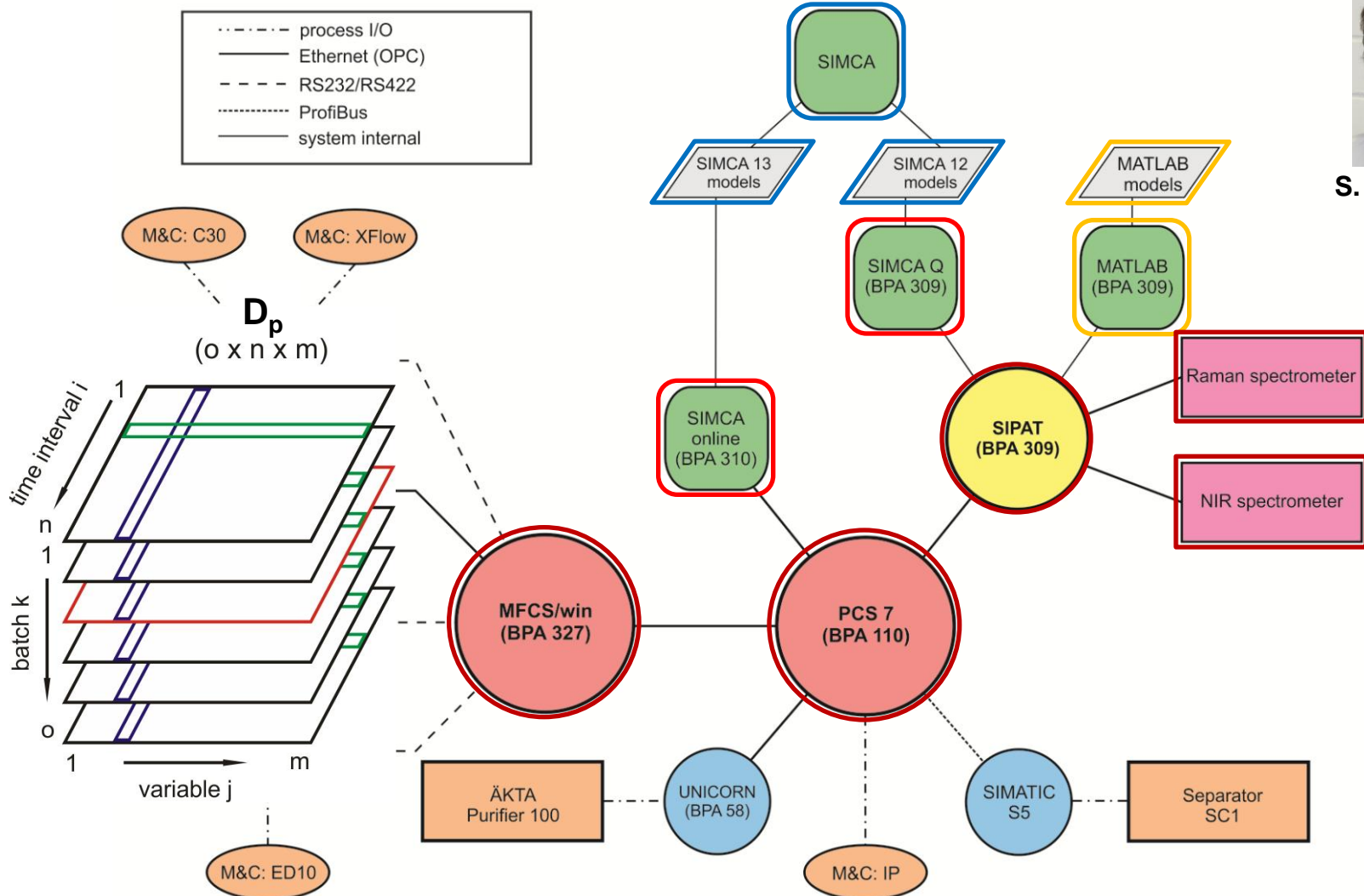
Is the protein production reproducible, optimal and QbD-compliant?



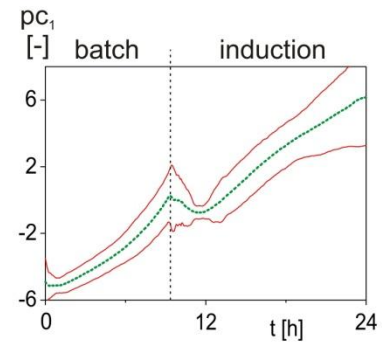
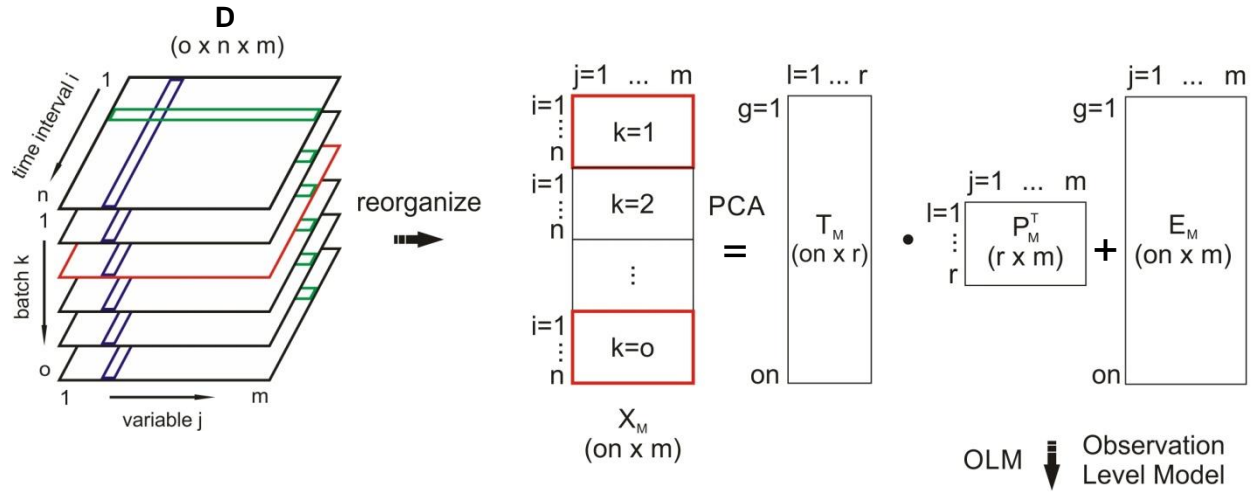
Expansion of data processing with SIPAT®, MATLAB® and SIMCA®



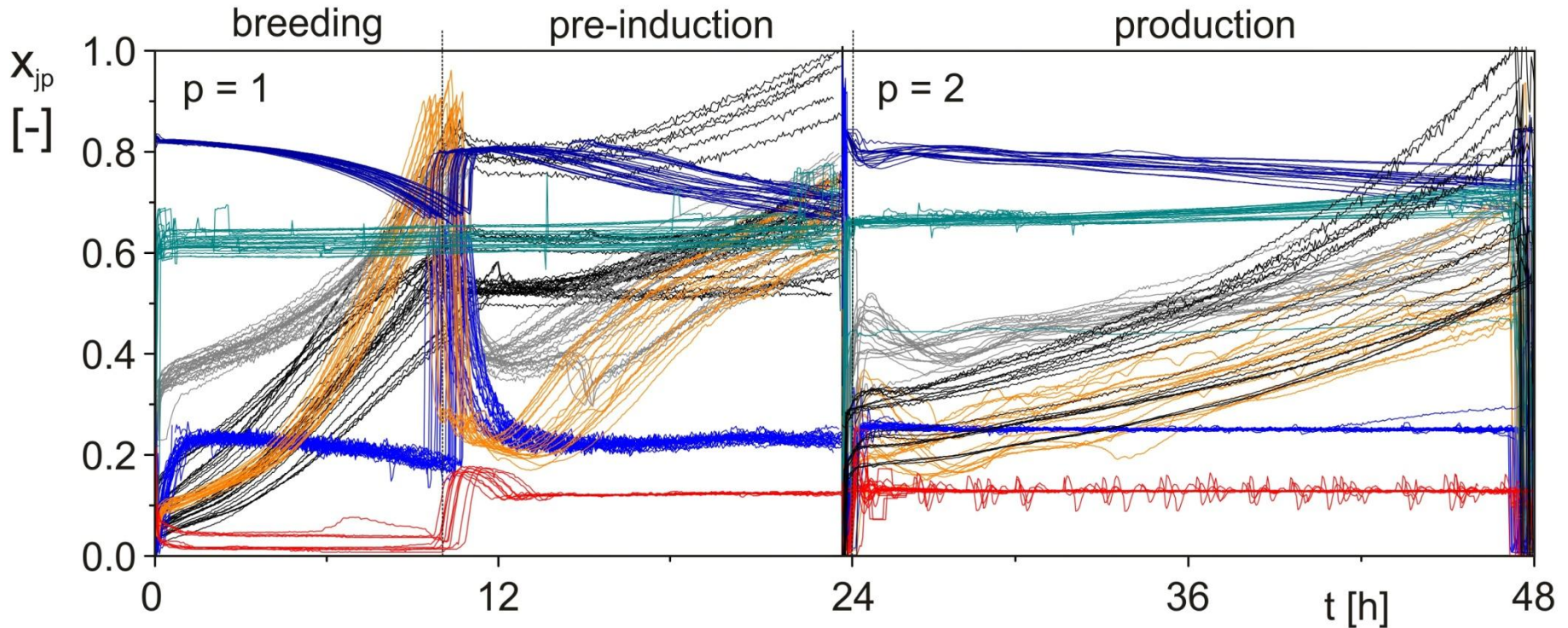
S. O. Borchert



MPCA – Multiway Principal Component Analysis: *Observation Level M*



1st task: *Autoscaling of Matrix D into X* for principal component analysis

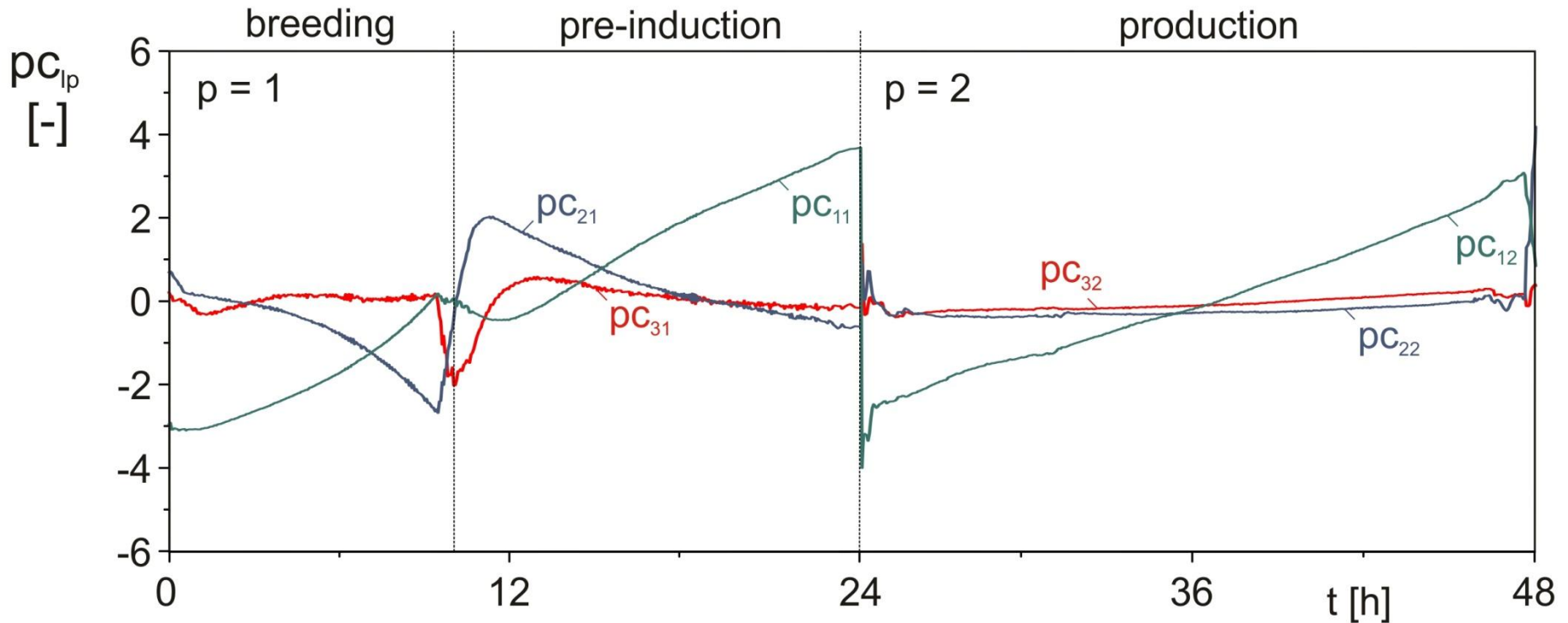


$$\begin{array}{c} m \\ \boxed{X} \\ n \end{array} = \begin{array}{c} r \\ \boxed{T} \\ n \end{array} \cdot \begin{array}{c} m \\ \boxed{P^T} \\ r \\ \text{loadings} \end{array} + \begin{array}{c} m \\ \boxed{E} \\ n \\ \text{error} \end{array}$$

data
scores
loadings
error

- transform *process data matrix X* into *score matrix T* and *loading matrix P*
- summarize *m (22) informations* in a few *variables r (2 or 3) only*

2nd task: *Principal Component Analysis* of autoscaled Data Matrix X

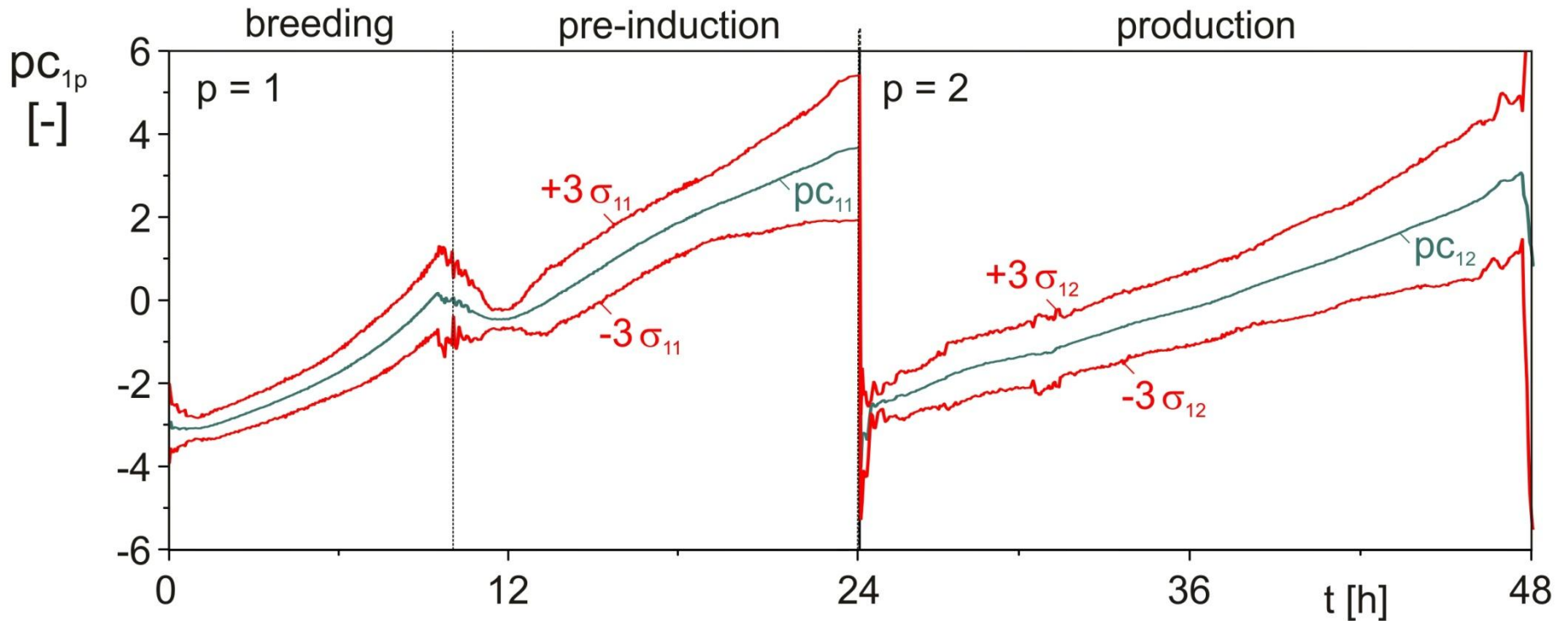


$$\begin{array}{c} r \\ \boxed{T} \\ n \end{array} = \begin{array}{c} m \\ \boxed{X} \\ n \end{array} \cdot \begin{array}{c} r \\ \boxed{P} \\ m \end{array} \cdot \left[\begin{array}{c} m \\ \boxed{P^T} \\ r \end{array} \cdot \begin{array}{c} r \\ \boxed{P} \\ m \end{array} \right]^{-1}$$

scores
data
loadings

- transformed m process data x_j into r (3) time dependent scores t_i (principle components pc_i) with $m \cdot r$ loadings p_{ij}

Golden Batch tunnels with three sigma limits for process evaluation



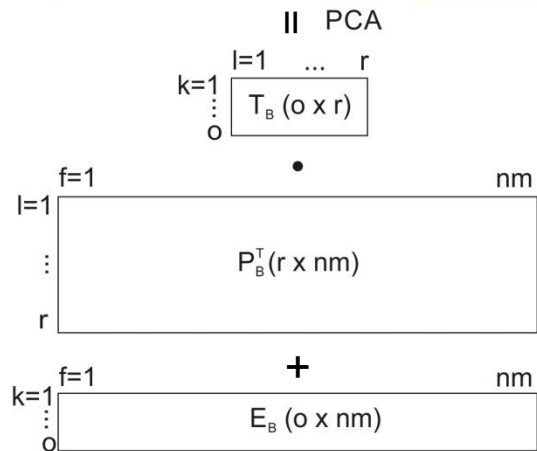
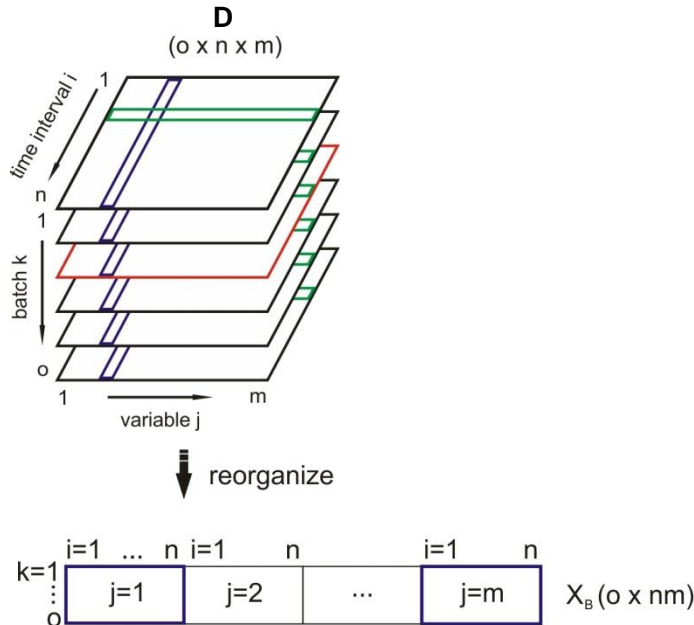
$$\begin{matrix} r \\ \boxed{T} \\ n \end{matrix} = \begin{matrix} m \\ \boxed{X} \\ n \end{matrix} \cdot \begin{matrix} r \\ \boxed{P} \\ m \end{matrix} \cdot \left[\begin{matrix} m \\ \boxed{P^T} \\ r \end{matrix} \cdot \begin{matrix} r \\ \boxed{P} \\ m \end{matrix} \right]^{-1}$$

scores
data
loadings

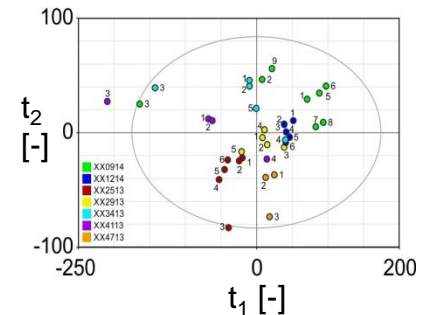
- **Golden Batch tunnel:**
Take *principle component* pc_{1p}
and add $\pm 3 \sigma_{1p}$ -*standard deviation*



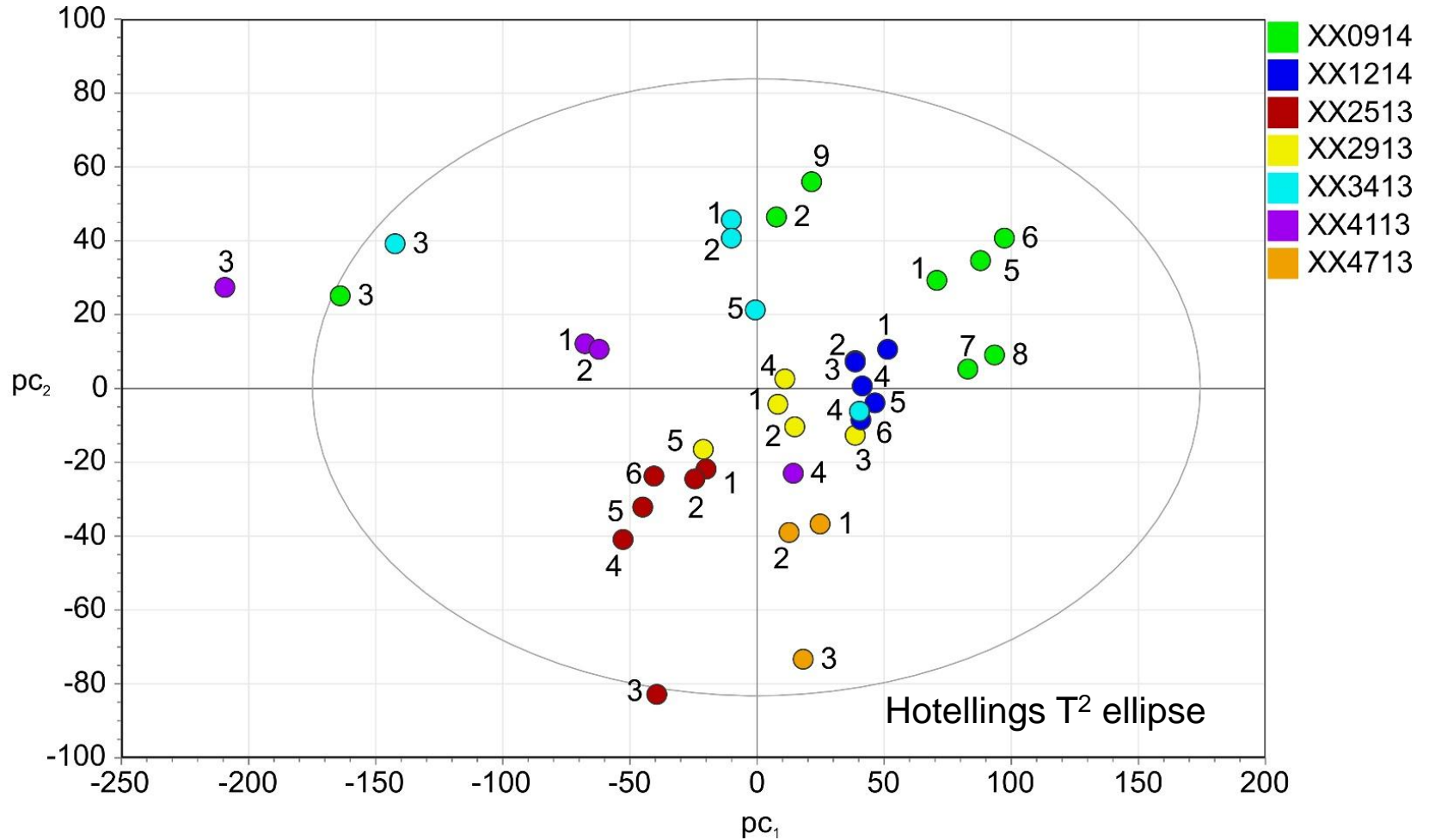
MPCA – Multiway Principal Component Analysis: *Batch Level B*



BLM
 Batch Level Model



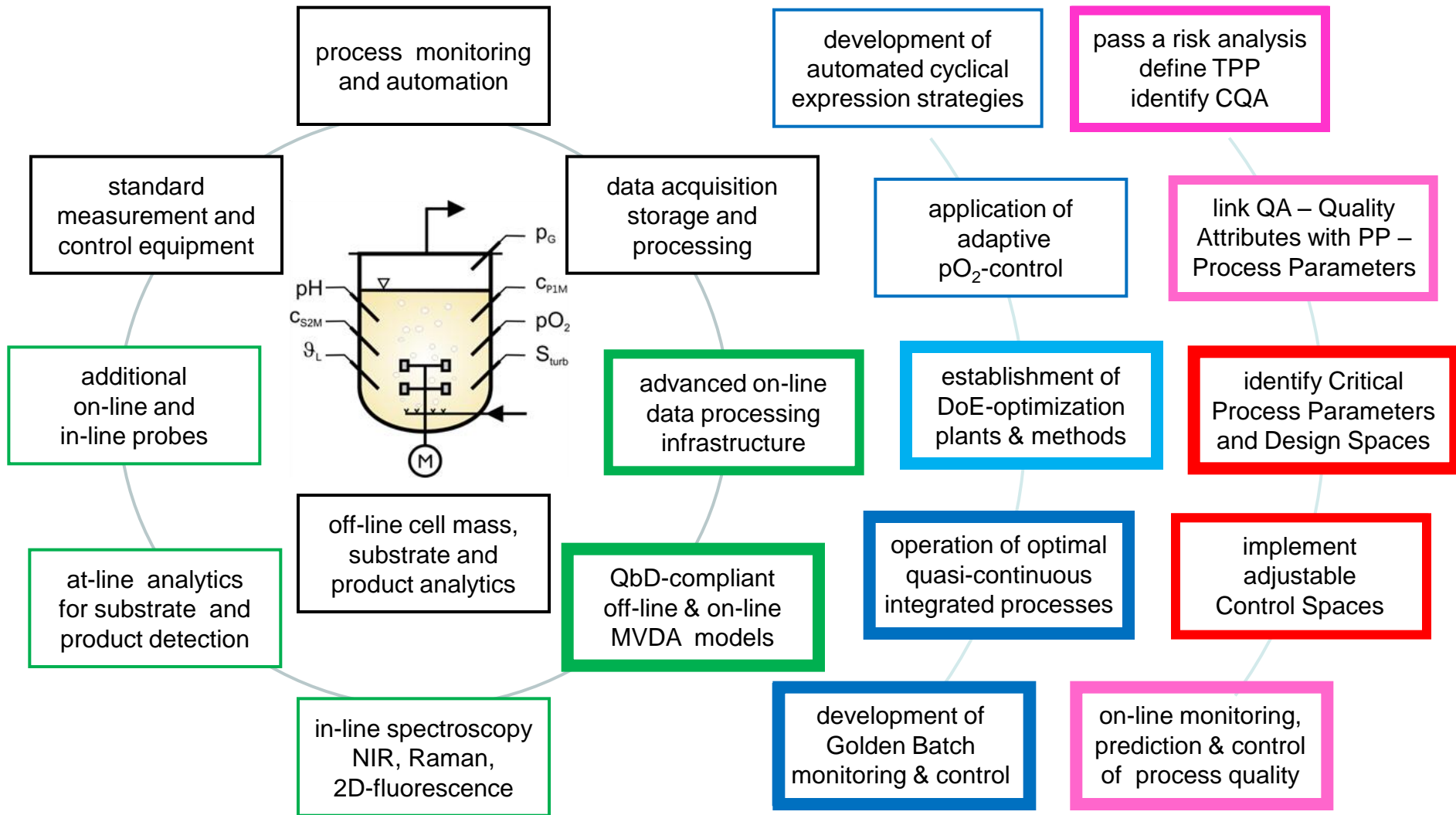
Batch level score scatter plot (pc_2 vs pc_1) for different campaigns XX_{ic1y}



Are these models QbD-compliant?



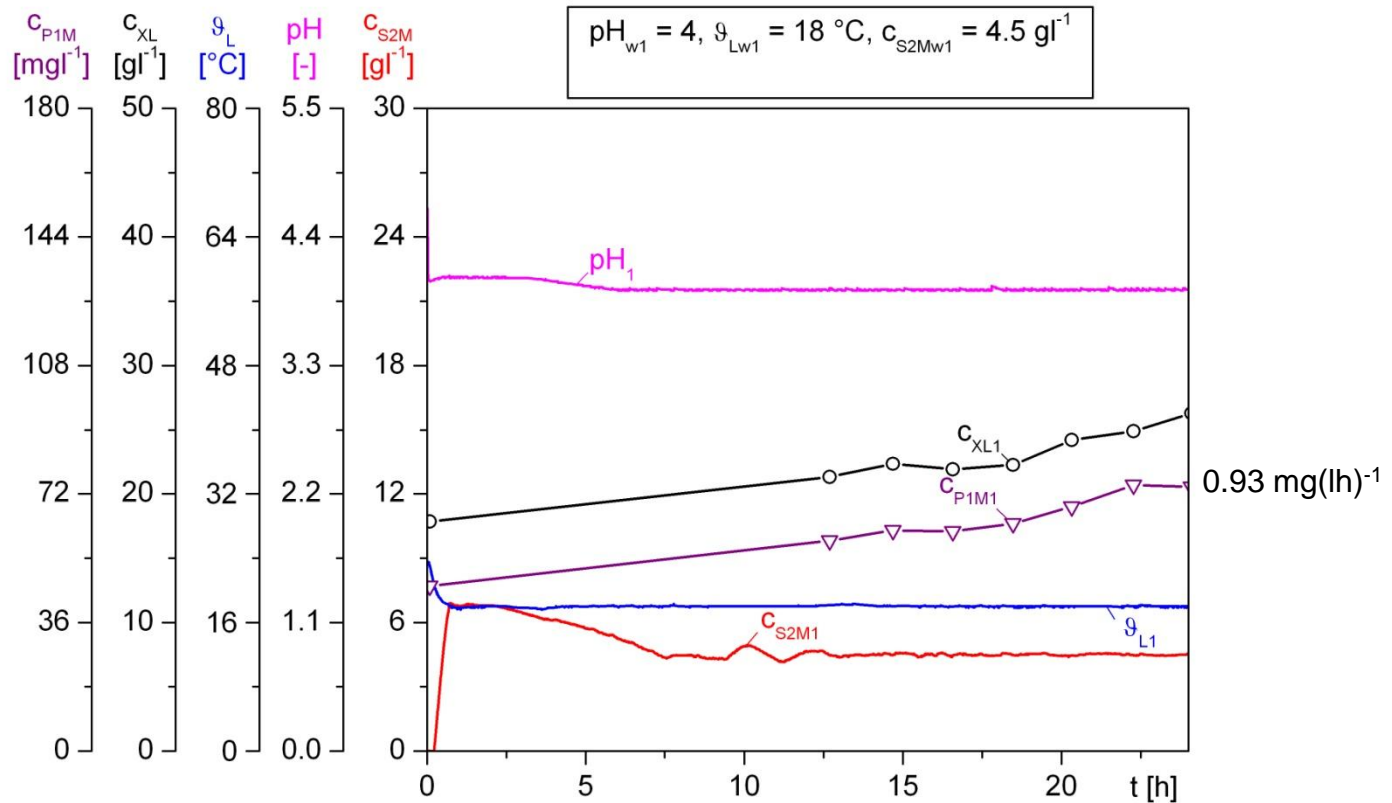
Enhanced process development – *The QbD-compliance level of PAT*



3 PAT levels: *instrumentation* *process development* *QbD-compliance*



Evaluation of optimization potential in secretion productivity PRD of D1

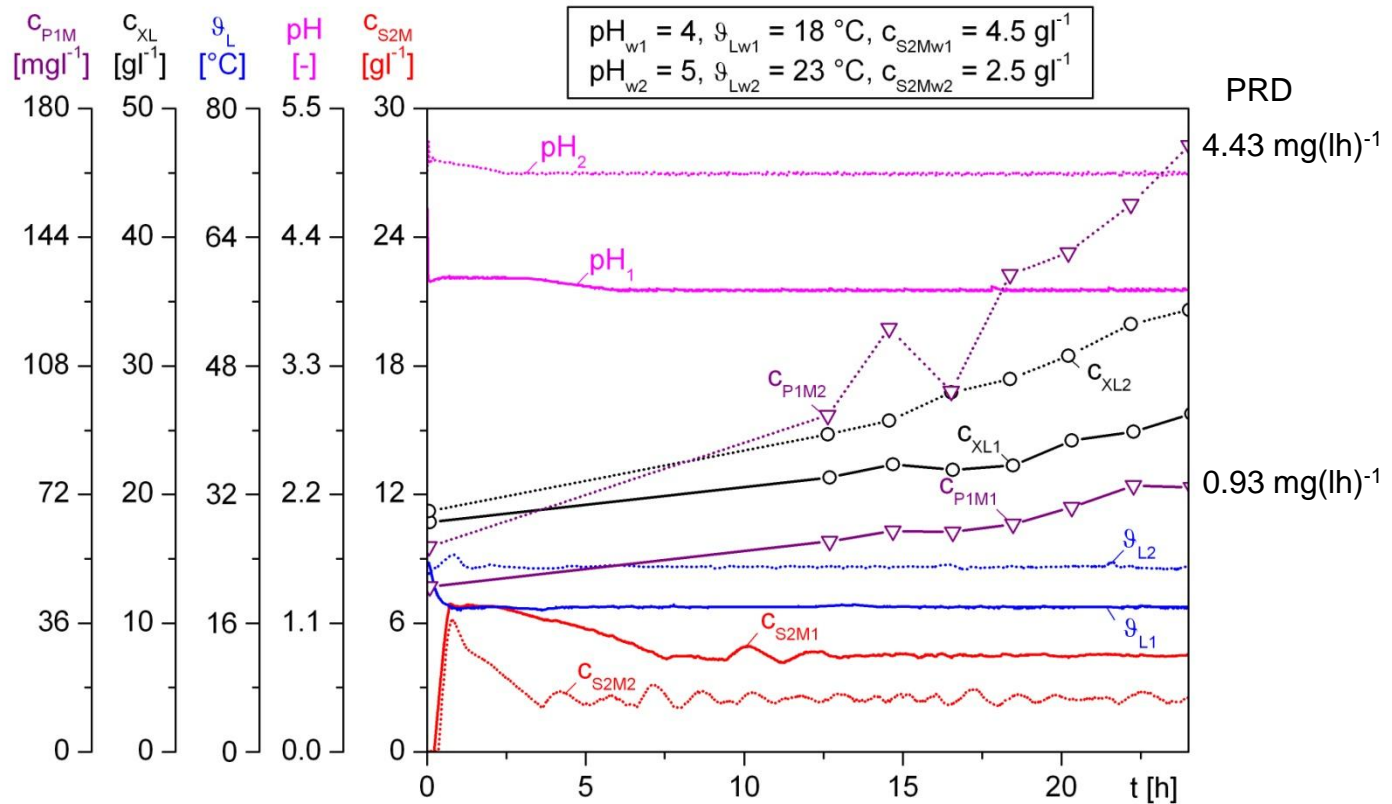


Volumetric secretion productivity PRD – process evaluation performance criterion

$$y_k = \text{PRD}_k = \left[c_{P1Mkn} \cdot V_{Lkn} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLkn}) - c_{P1Mk0} \cdot V_{Lk0} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLk0}) \right. \\ \left. + \sum_{j=1}^n c_{P1Mkj} \cdot \Delta V_{Skj} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLkj}) \right] \cdot (\rho_Z \cdot (t_{kn} - t_{k0}) \cdot V_{Lkn})^{-1}$$



Define Critical (Process) Quality Attribute: *Product secretion productivity*

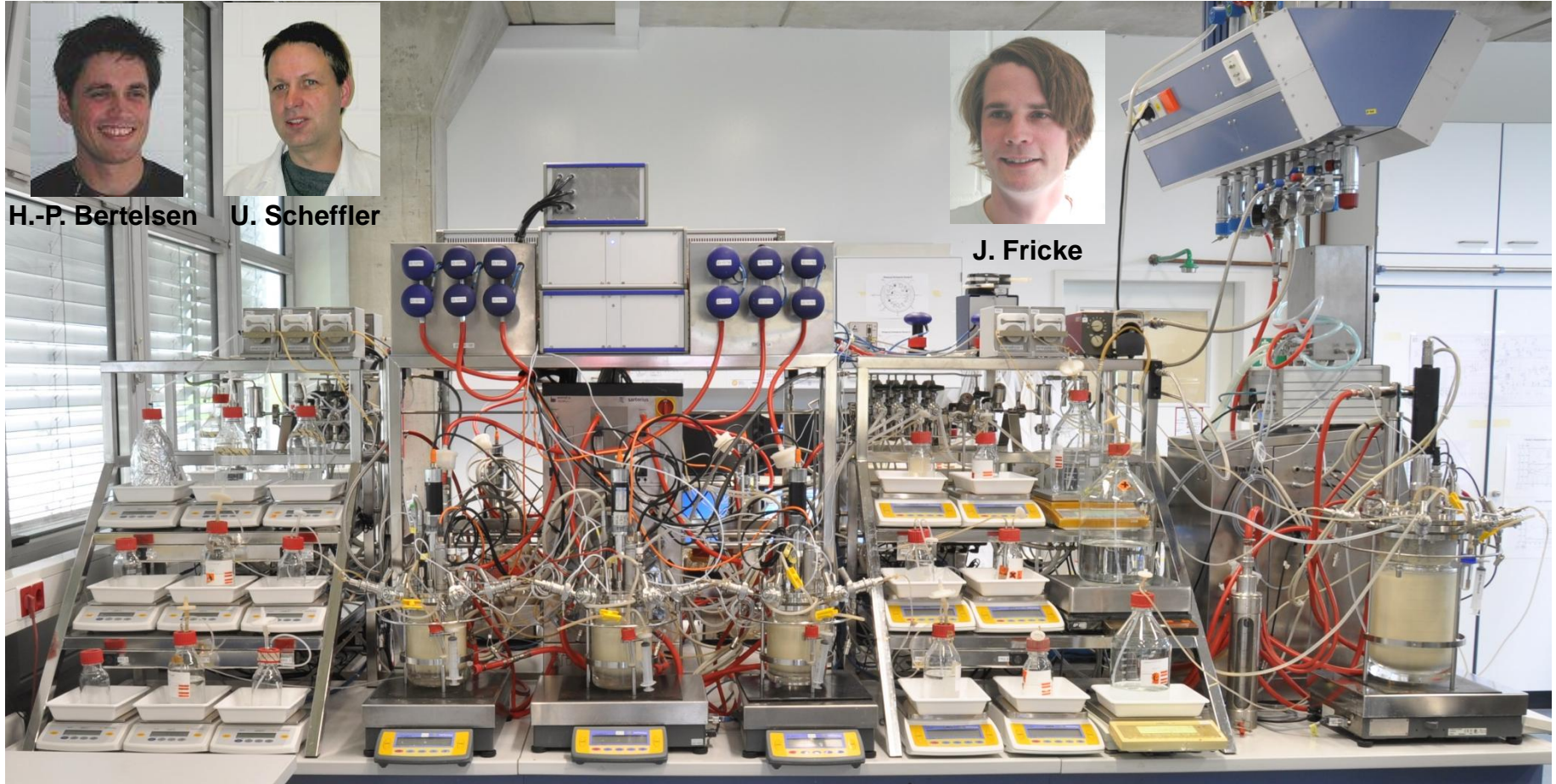


Volumetric secretion productivity PRD – process evaluation performance criterion

$$y_k = \text{PRD}_k = \left[c_{P1Mkn} \cdot V_{Lkn} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLkn}) - c_{P1Mk0} \cdot V_{Lk0} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLk0}) \right. \\ \left. + \sum_{j=1}^n c_{P1Mkj} \cdot \Delta V_{Skj} \cdot (\rho_Z - \alpha_{Z/X} \cdot c_{XLkj}) \right] \cdot (\rho_Z \cdot (t_{kn} - t_{k0}) \cdot V_{Lkn})^{-1}$$

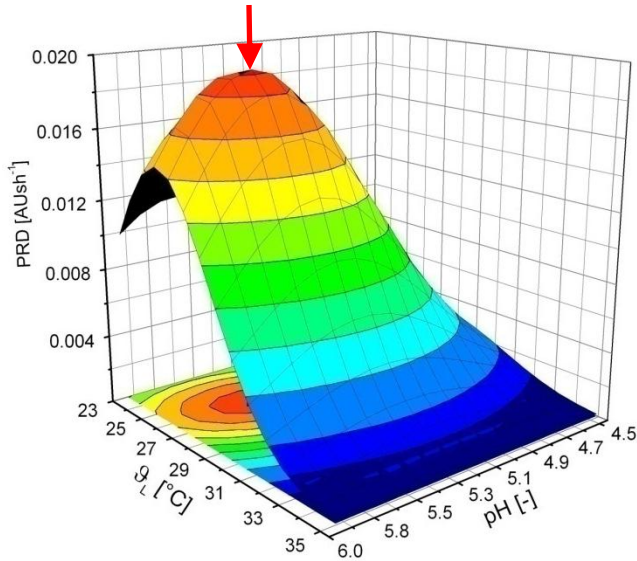


Multi-bioreactor DoE-plant: BIOSTAT® Bplus with a BIOSTAT® Qplus 6



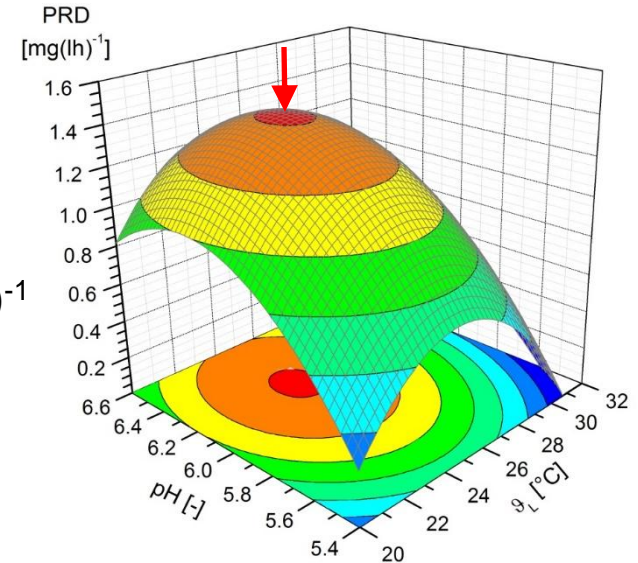
Optimal Critical Process Parameters in Malaria vaccine productions

D1: $PRD_{max} = 0.019 \text{ AUsh}^{-1}$



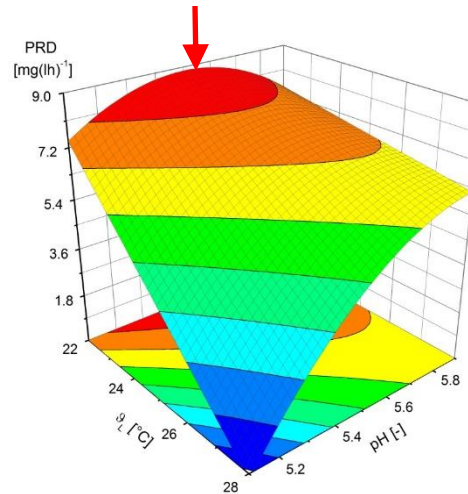
$\vartheta_{Lopt} = 25.8 \text{ }^{\circ}\text{C}$
 $pH_{opt} = 5.55$
 $C_{S2Mopt} = 1.0 \text{ gl}^{-1}$

D2M2D3H: $PRD_{max} = 1.3 \text{ mg(lh)}^{-1}$



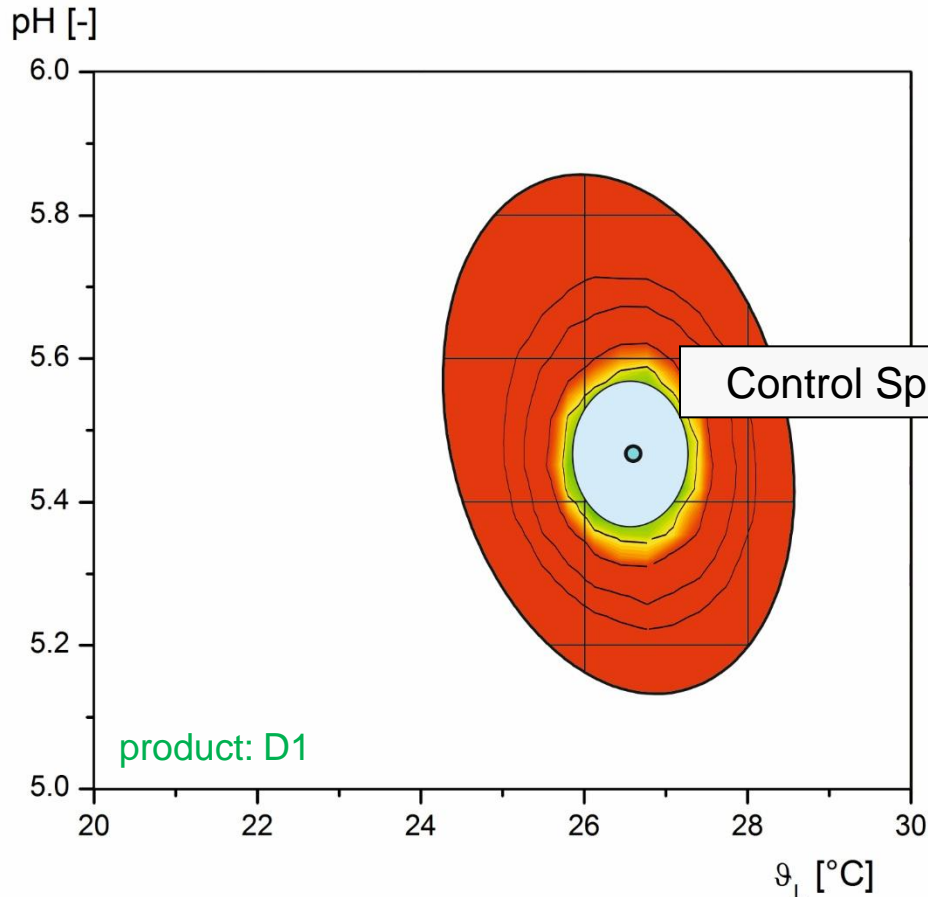
$\vartheta_{Lopt} = 24.7 \text{ }^{\circ}\text{C}$
 $pH_{opt} = 6.2$
 $C_{S2Mopt} = 1.0 \text{ gl}^{-1}$

D1M1H: $PRD_{max} = 9.0 \text{ mg(lh)}^{-1}$



$\vartheta_{Lopt} = 22.0 \text{ }^{\circ}\text{C}$
 $pH_{opt} = 5.55$
 $C_{S2Mopt} = 1.0 \text{ gl}^{-1}$

Implementation of Control Space – *Mathematically fixed adjustment*



Characterisation of an ellipsoid shape

$$\left(\frac{\vartheta_L - \vartheta_{Lcen}}{\Delta\vartheta_L} \right)^2 + \left(\frac{pH - pH_{cen}}{\Delta pH} \right)^2 \leq 1$$

$$\vartheta_{Losp} = 26.6 \text{ °C} \quad \Delta\vartheta_{Losp} = 0.7 \text{ °C}$$

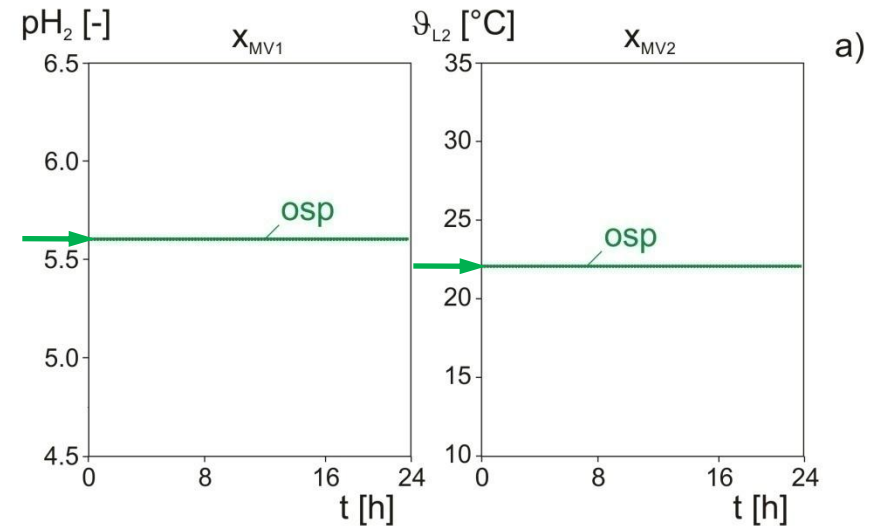
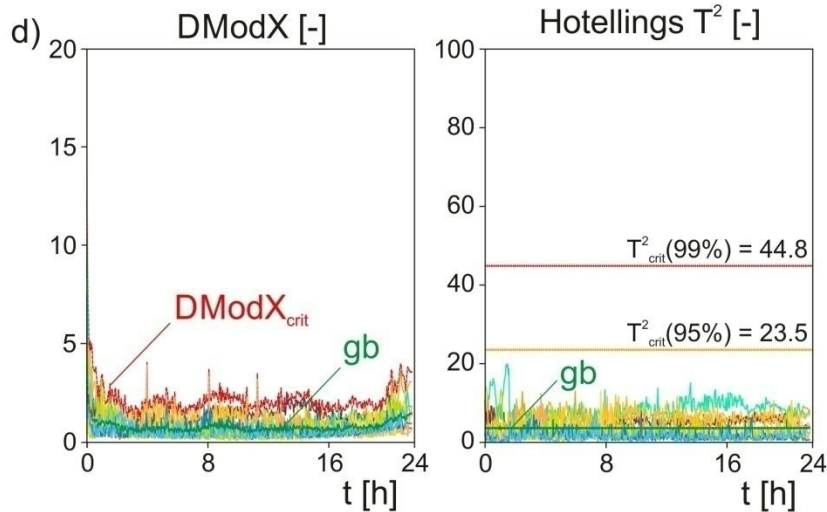
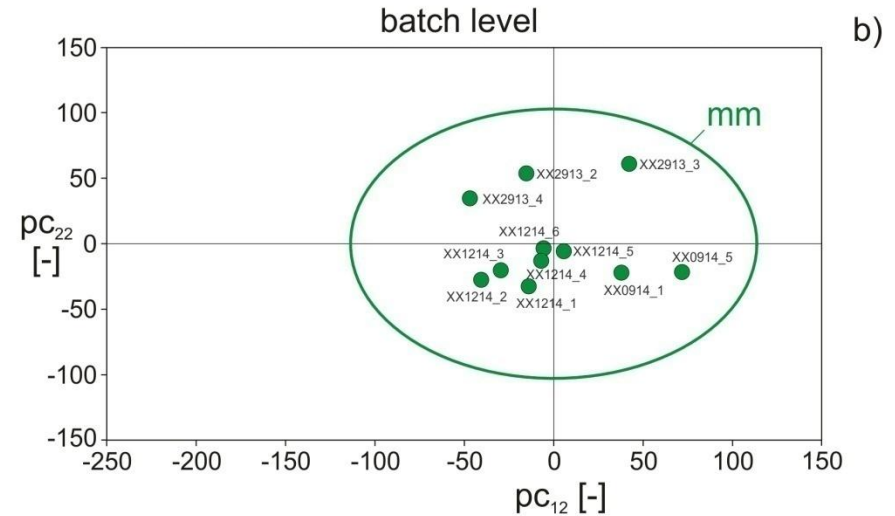
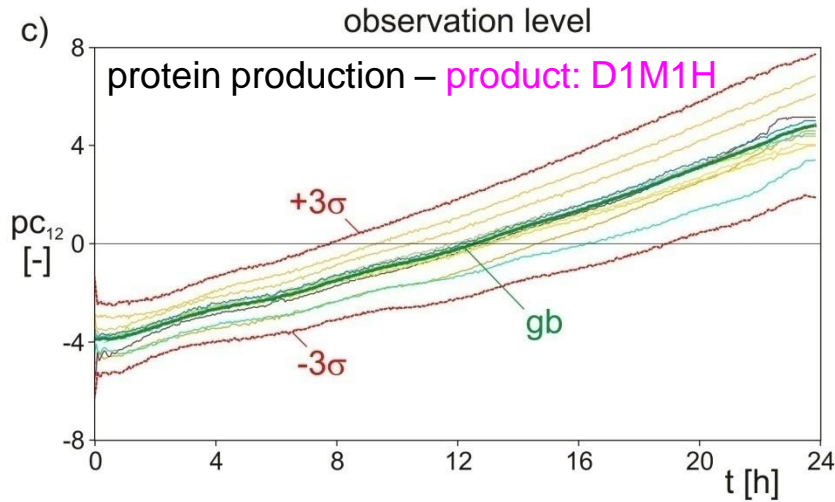
$$pH_{osp} = 5.47 \quad \Delta pH_{osp} = 0.1$$

product: D1M1H

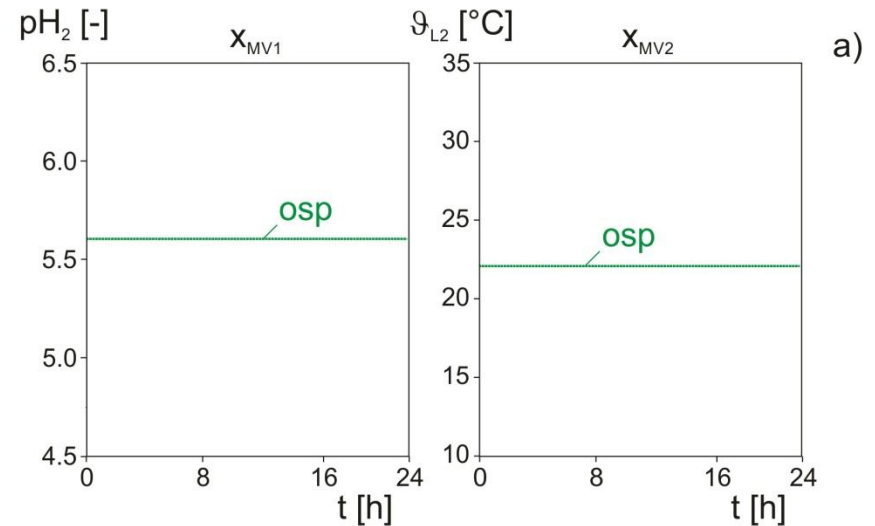
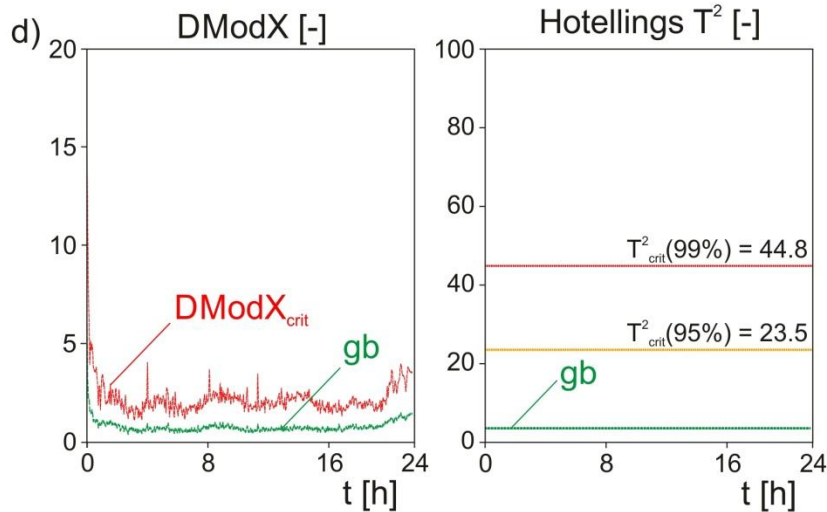
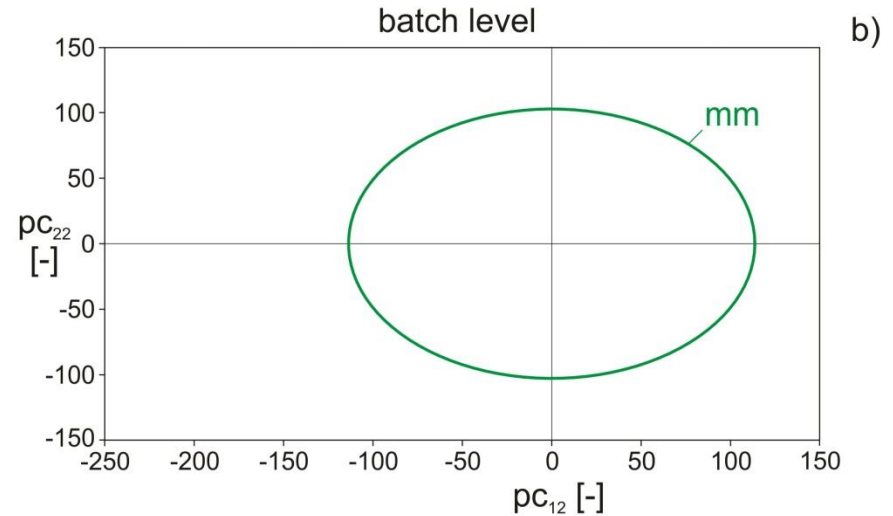
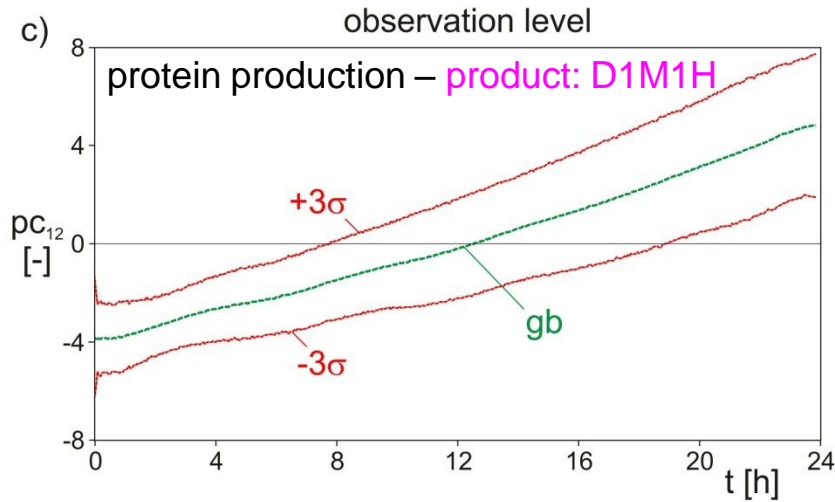
$$\vartheta_{Losp} = 22.0 \text{ °C}$$

$$pH_{osp} = 5.55$$

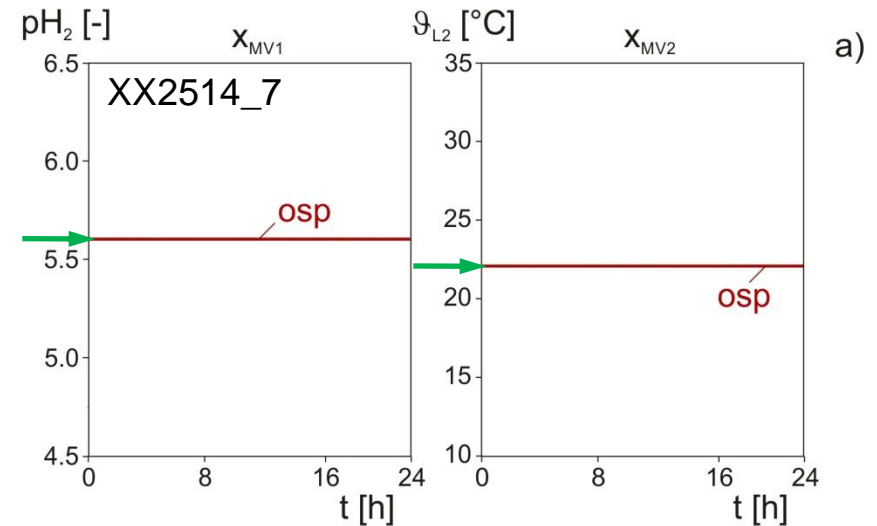
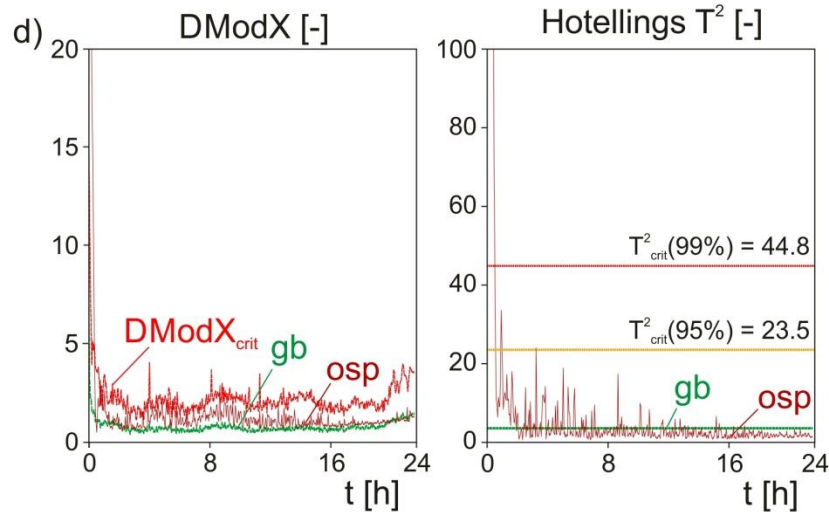
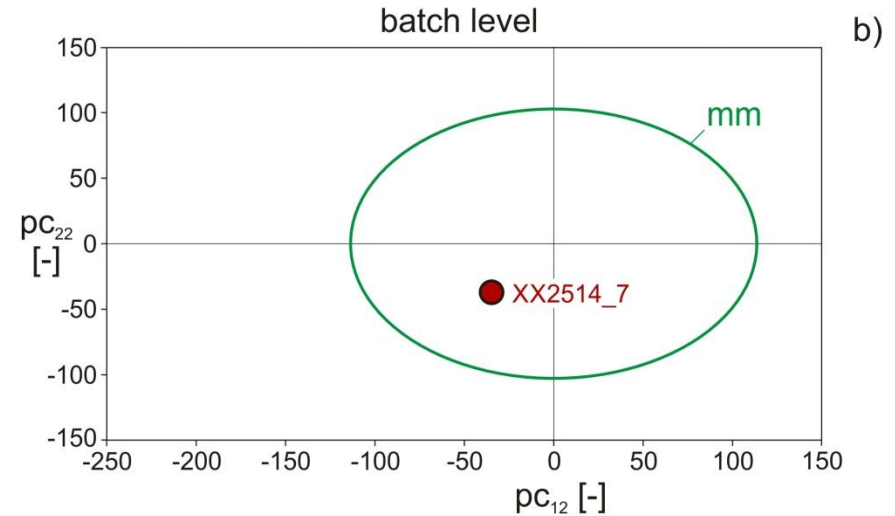
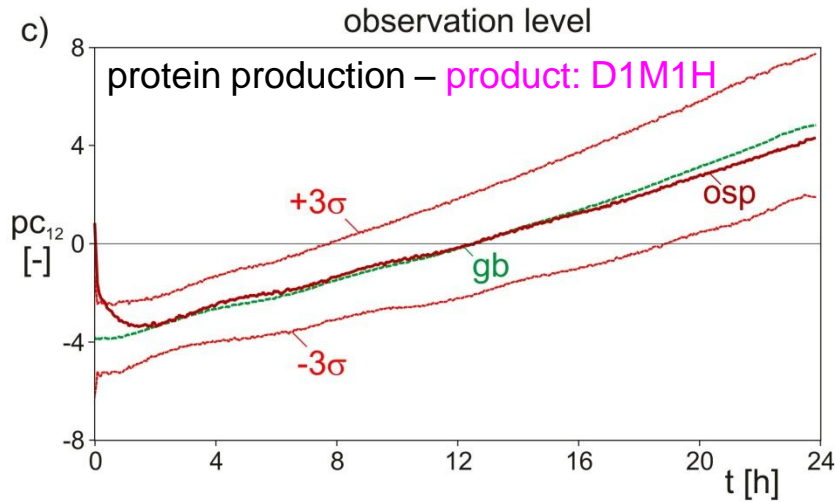
QbD-evaluation – Golden Batch models of *Design Space* production



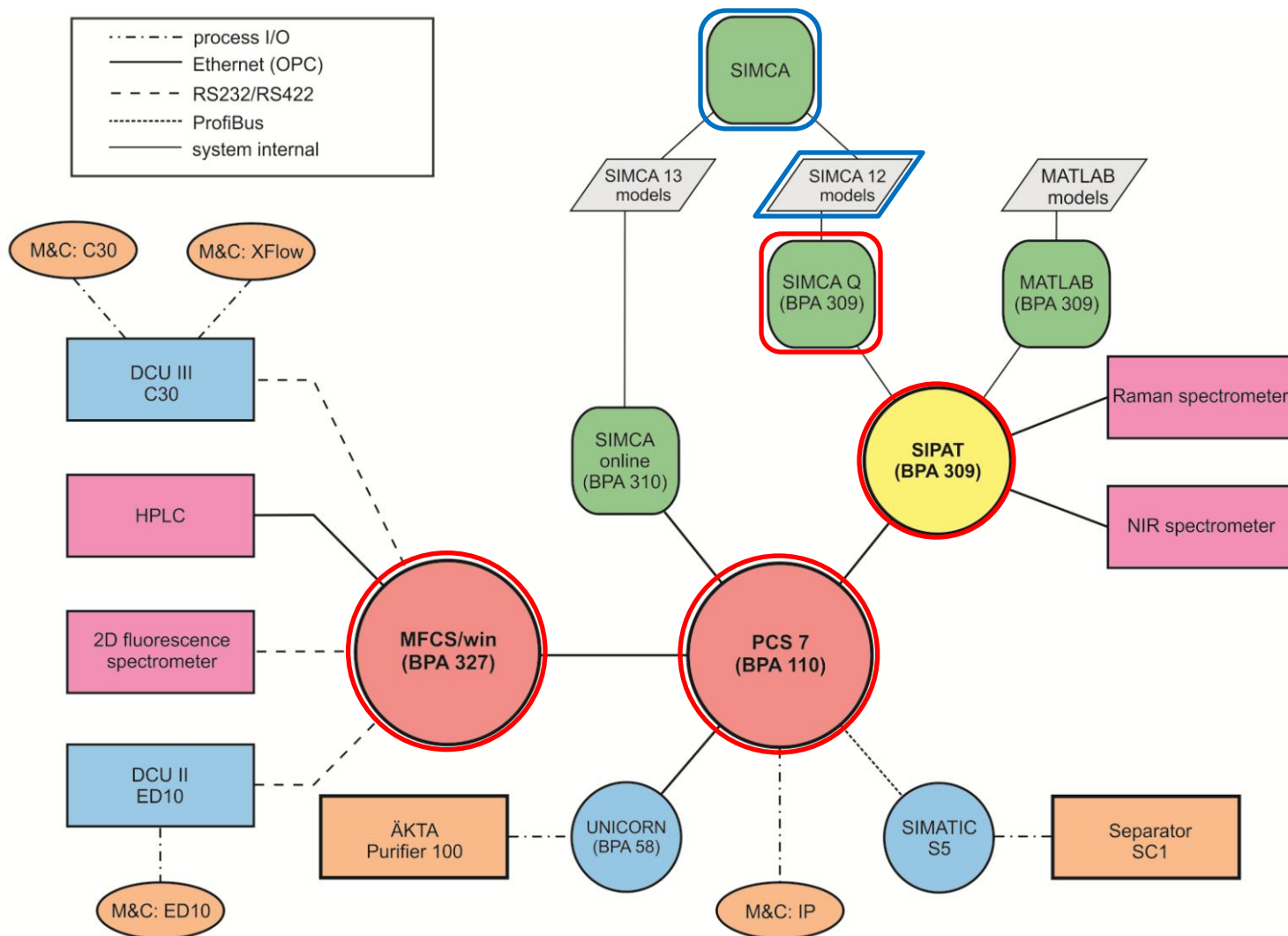
Resulting multivariate limits for future on-line process evaluation



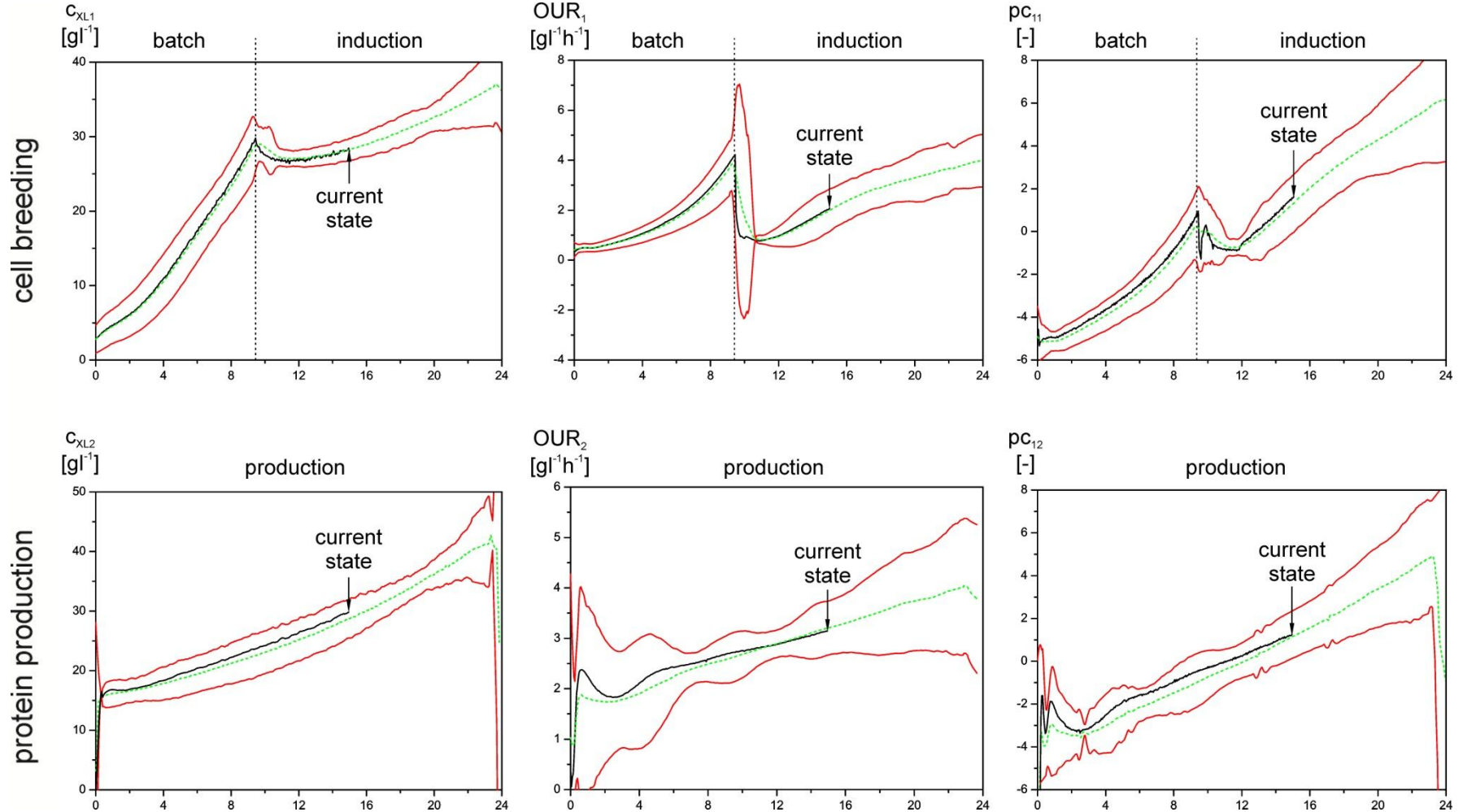
Final evaluation of a new production with „optimal“ settings for X_{MV}



On-line evaluation of MVDA models with SIPAT[®] and SIMCA[®] Q



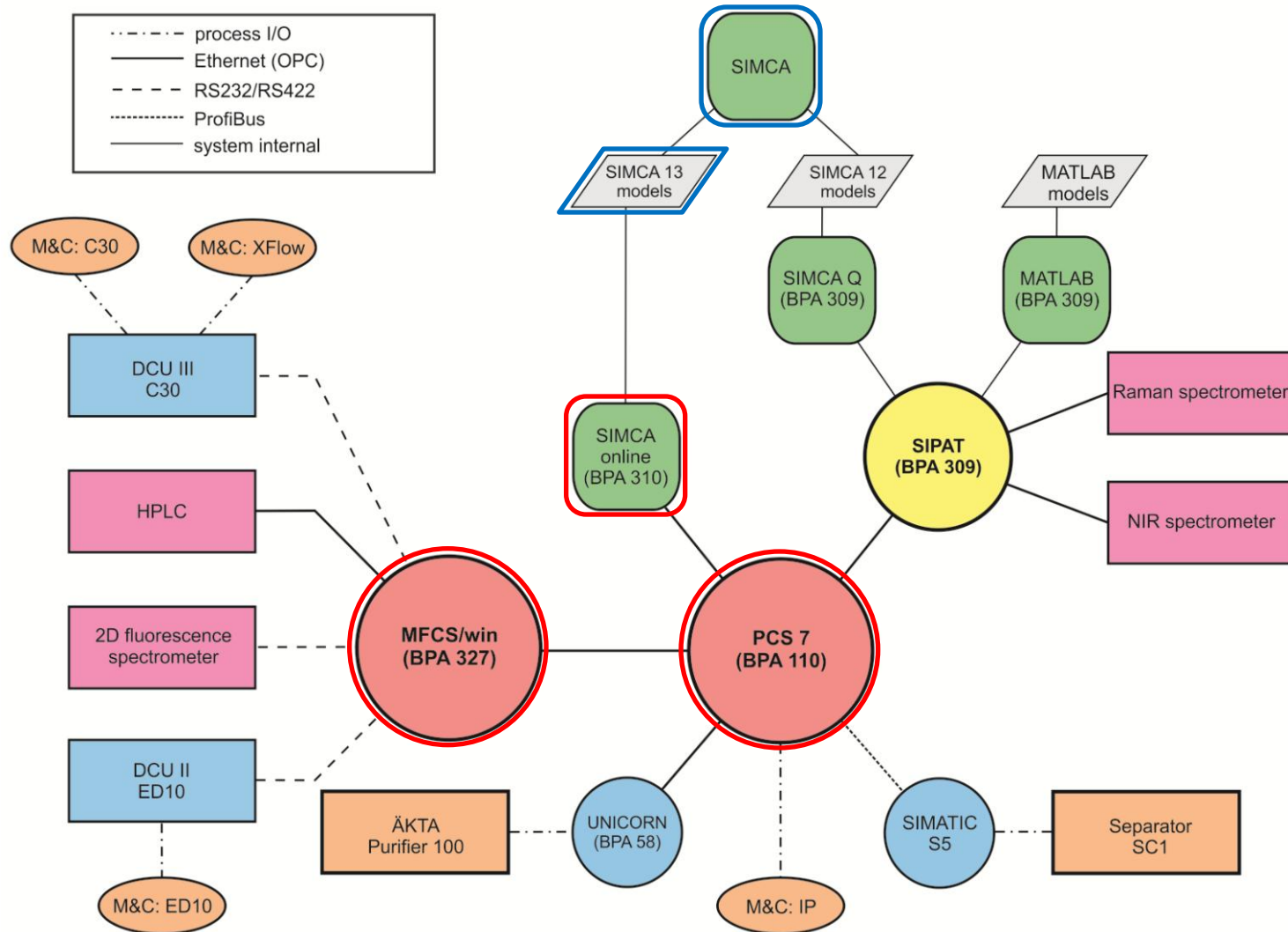
On-line monitoring of Golden Batch variables in both sub-processes



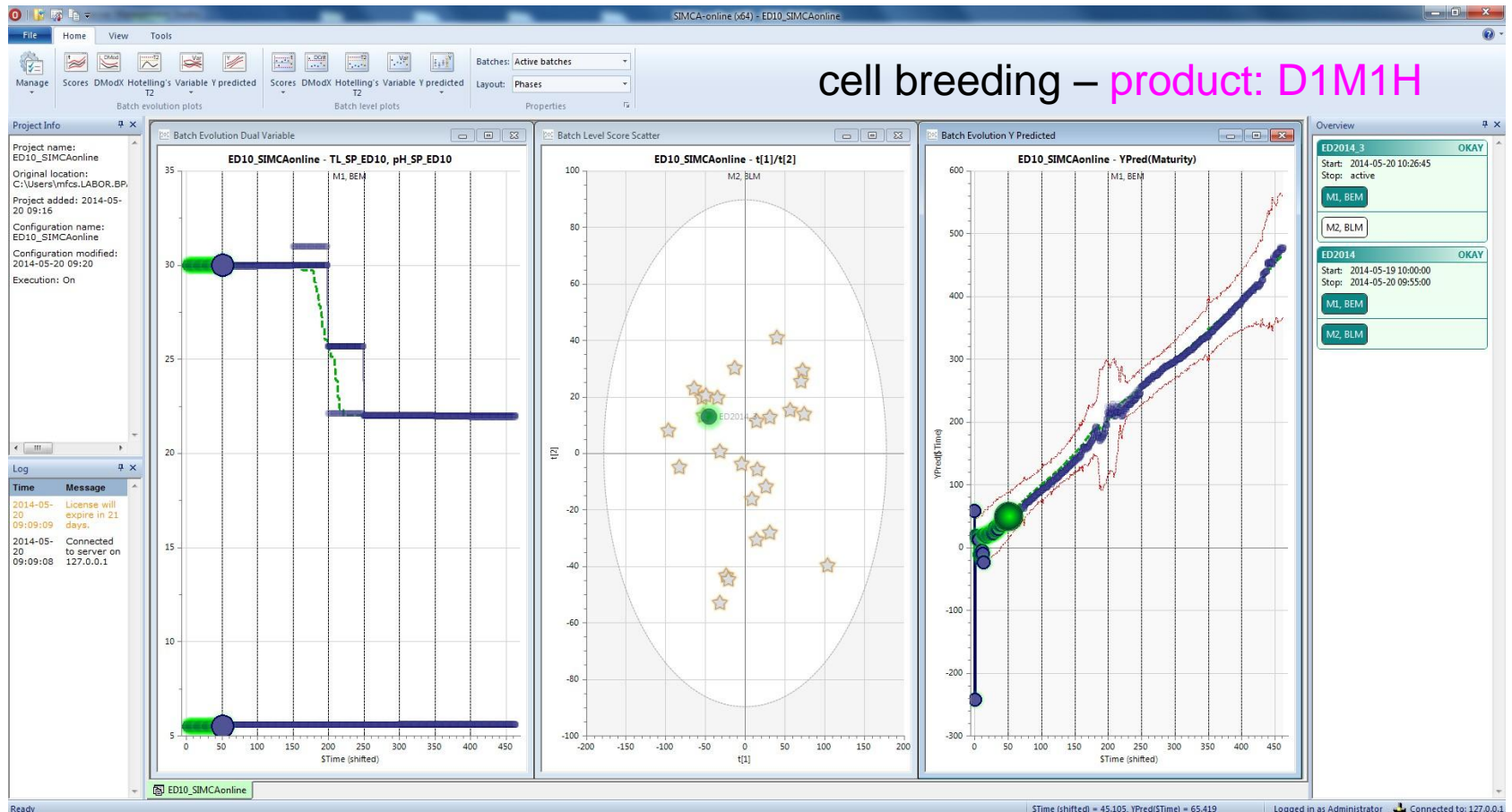
Is it possible to look into the future of process behaviour?



Process prediction and model predictive control with SIMCA®online



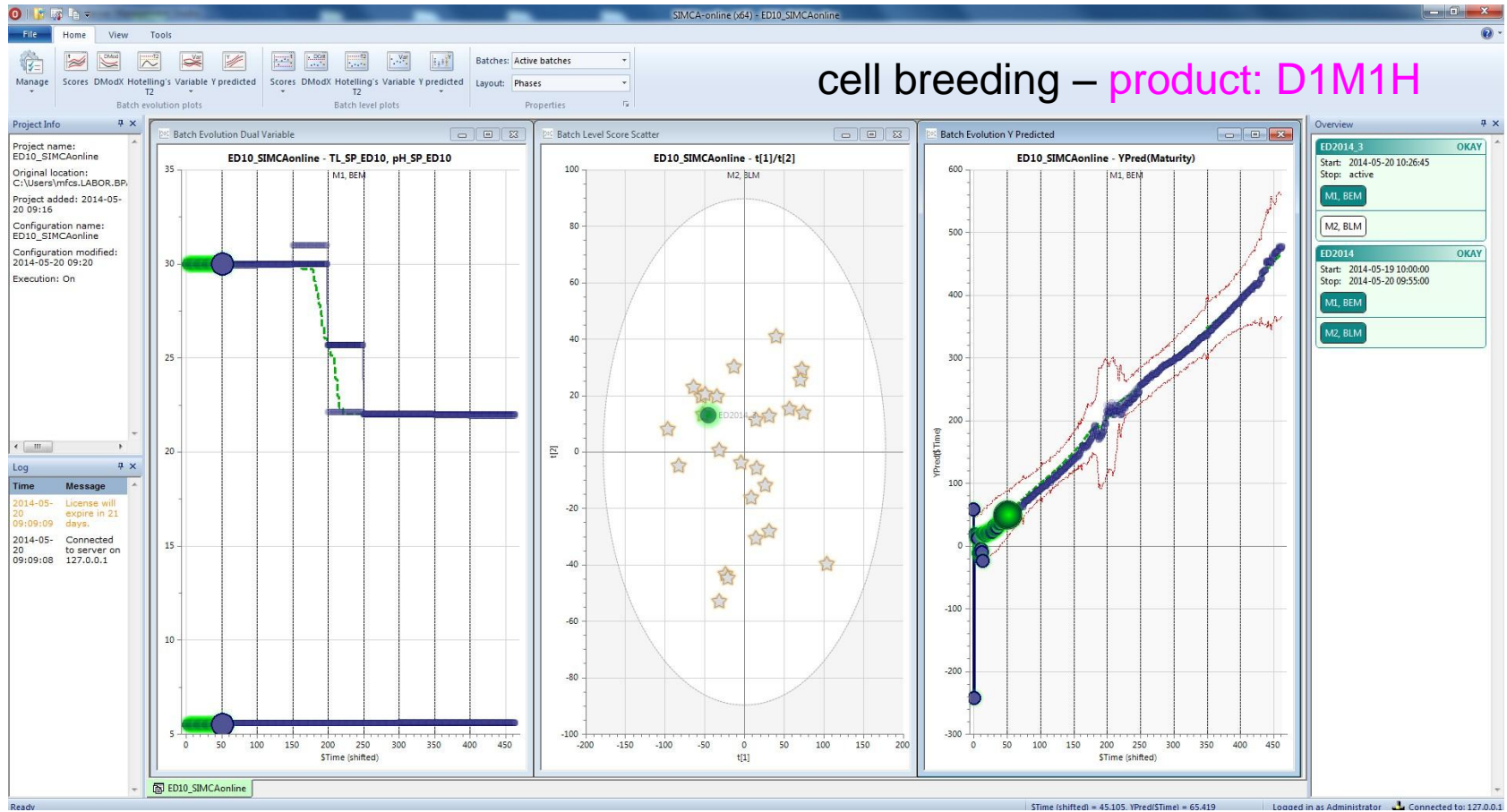
Golden Batch-prediction with IBR – Imputation by Regression



What is to do, if we are leaving the Golden Batch tunnel?



Quality control: *BOBYQA – Bound Optimization BY Quadratic Approximation*

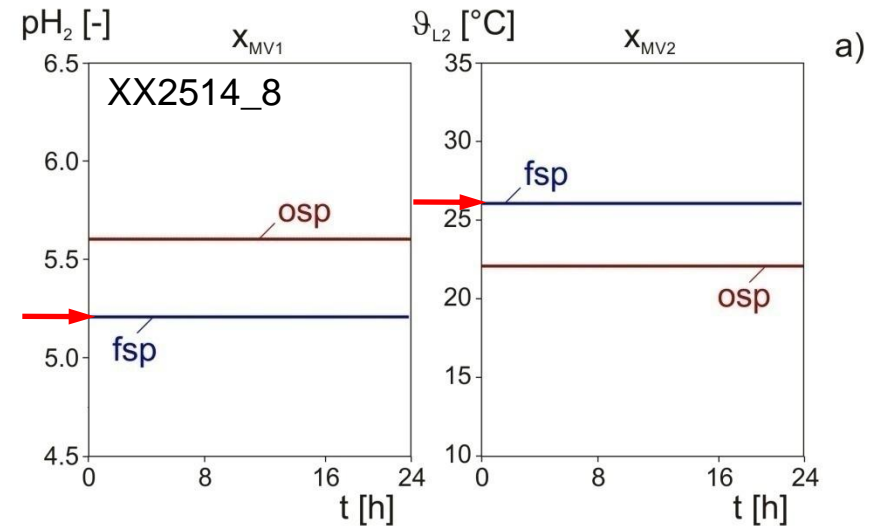
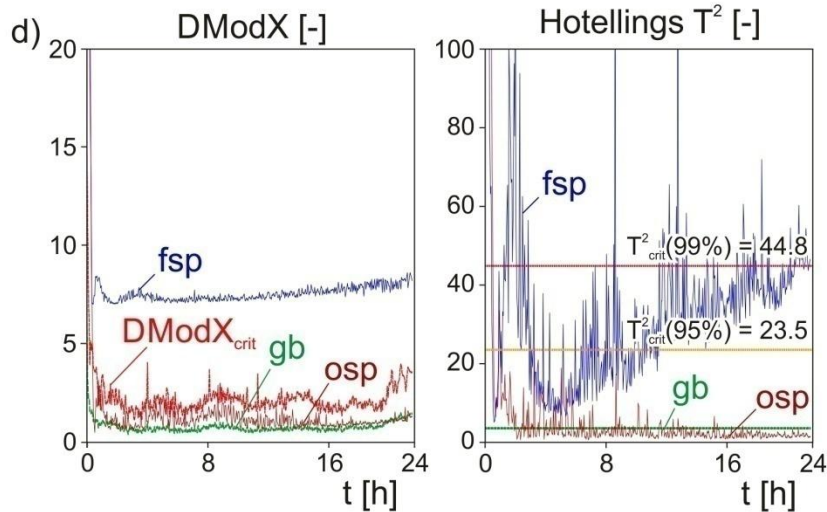
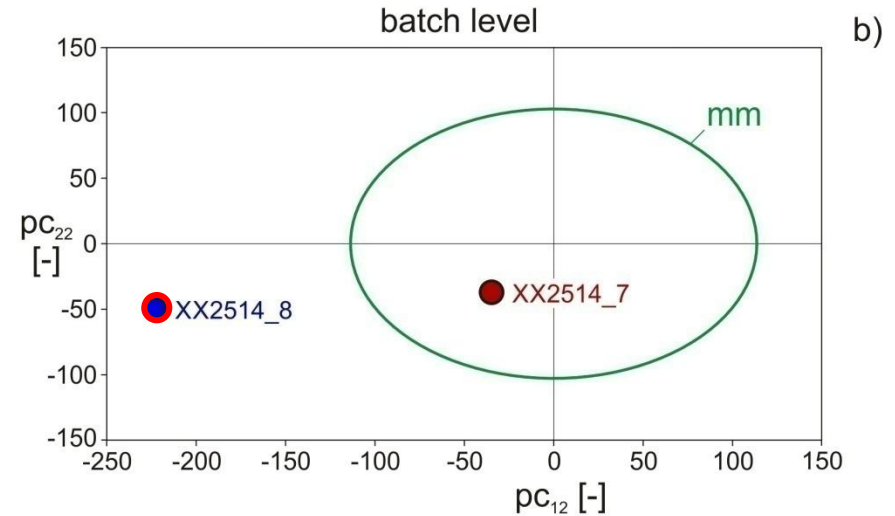
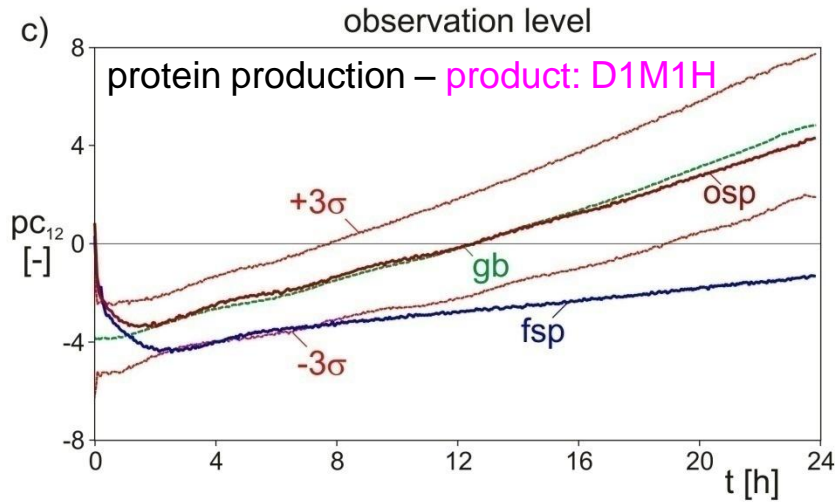


BOBYQA – Minimize the quadratic deviation from an *optimal process behaviour J*

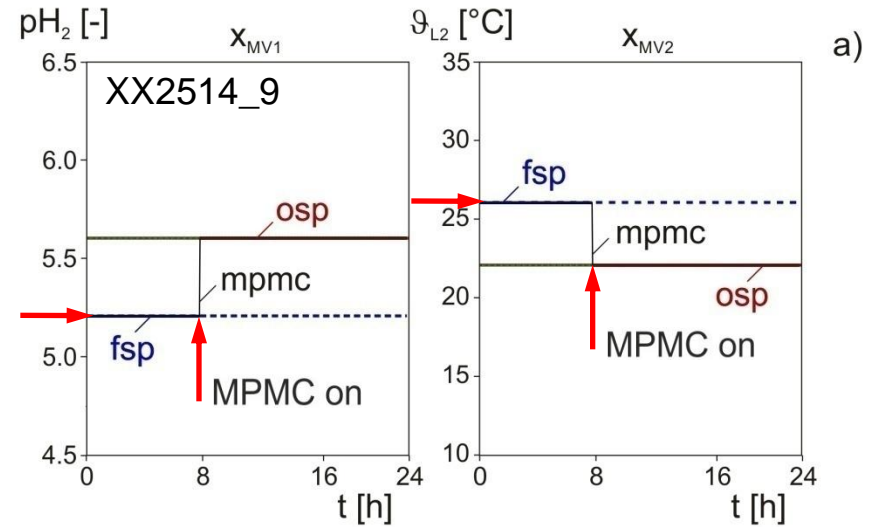
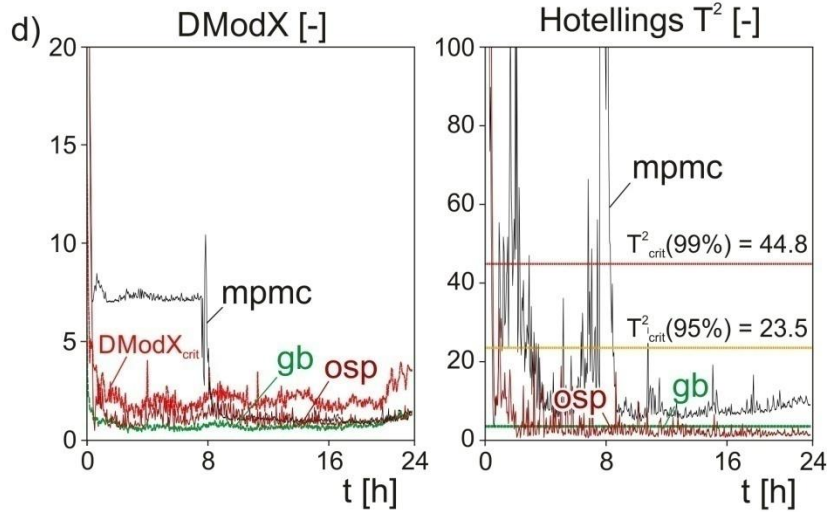
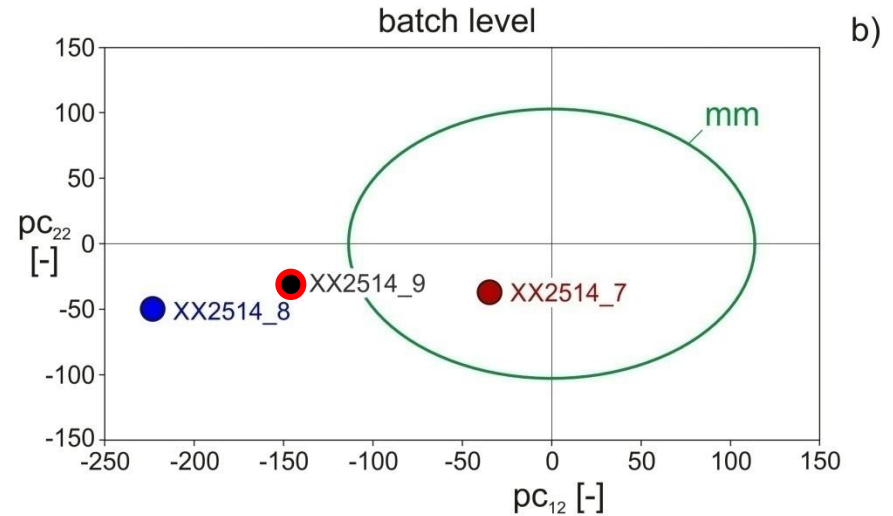
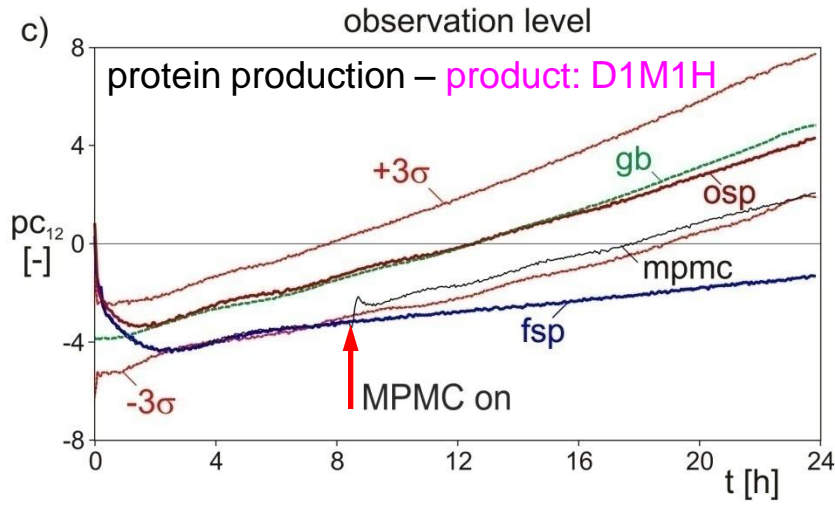
$$\min_{X_{MV}} J \Rightarrow J = \theta_{X_{MV}} \cdot (X_{MV_{gb}} - X_{MV})^2 + \theta_Y \cdot (Y_{SP} - Y_{pred})^2 + \theta_{DModX} \cdot (DModX)^2 + \theta_{T_2} \cdot (T^2)^2$$



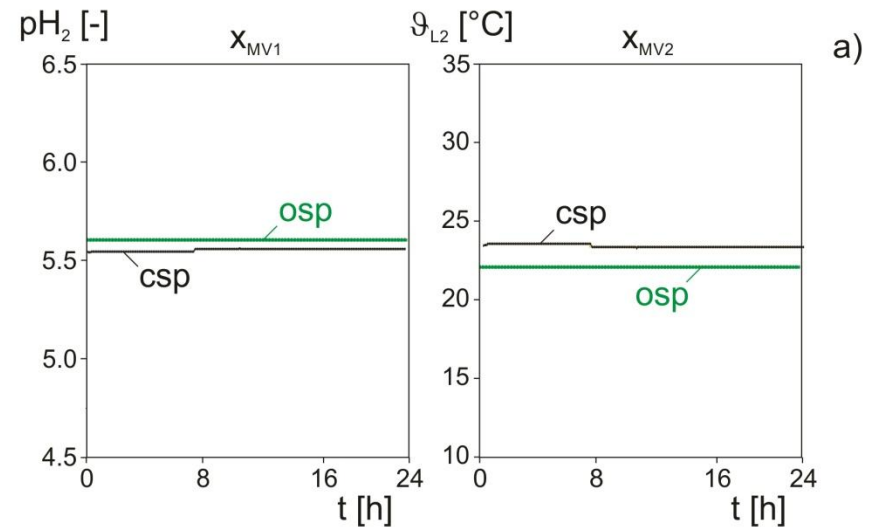
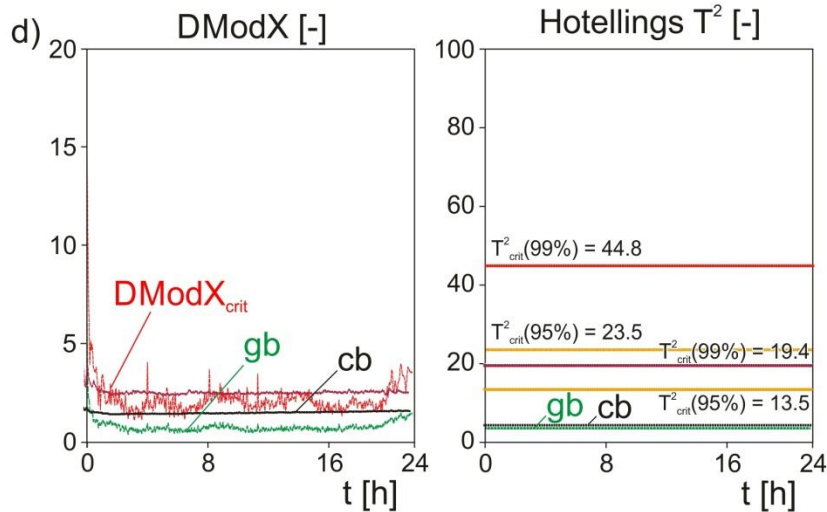
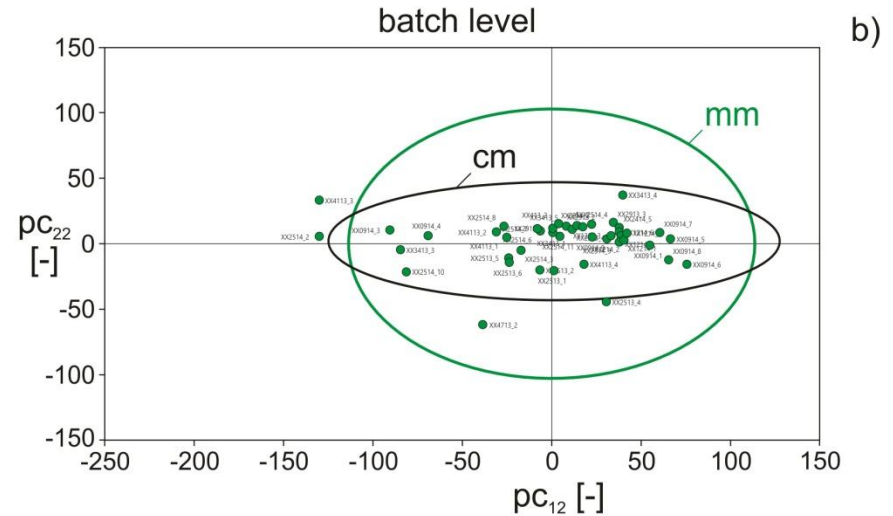
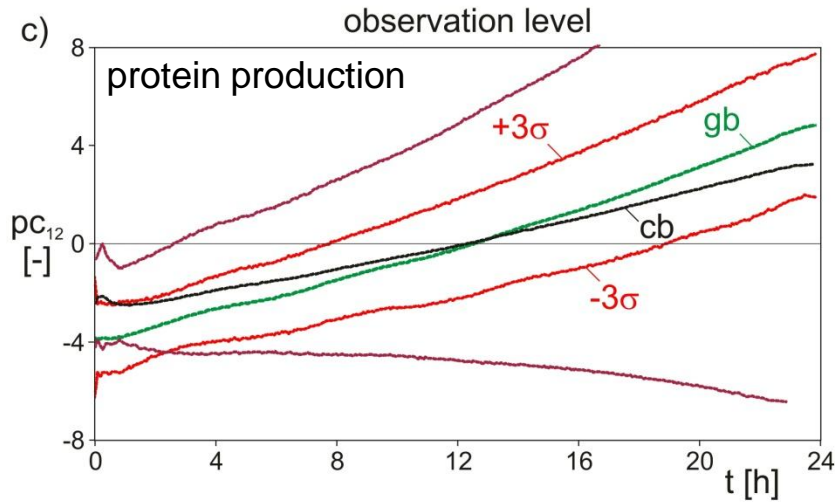
Conducting an experiment with „out of design space“ settings for X_{MV}



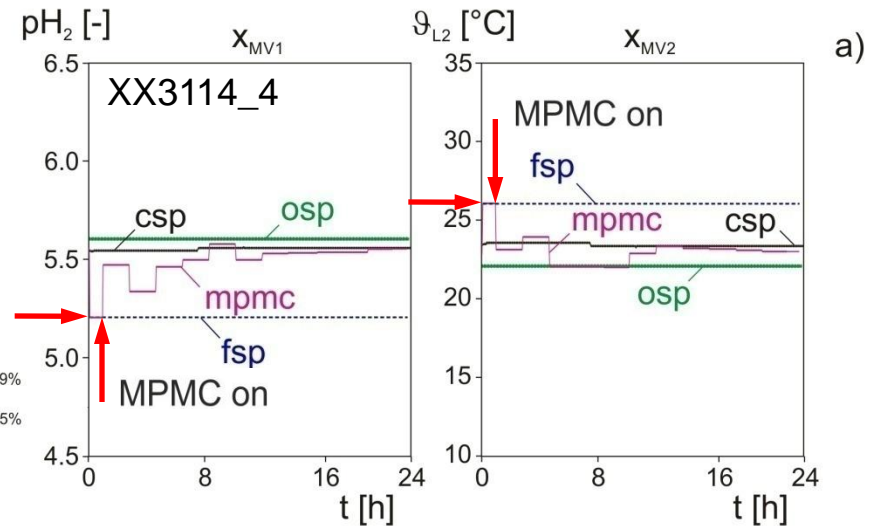
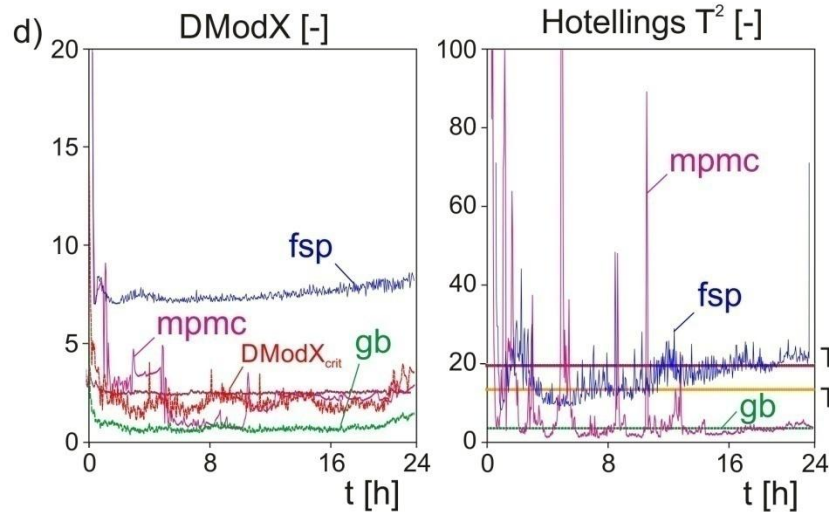
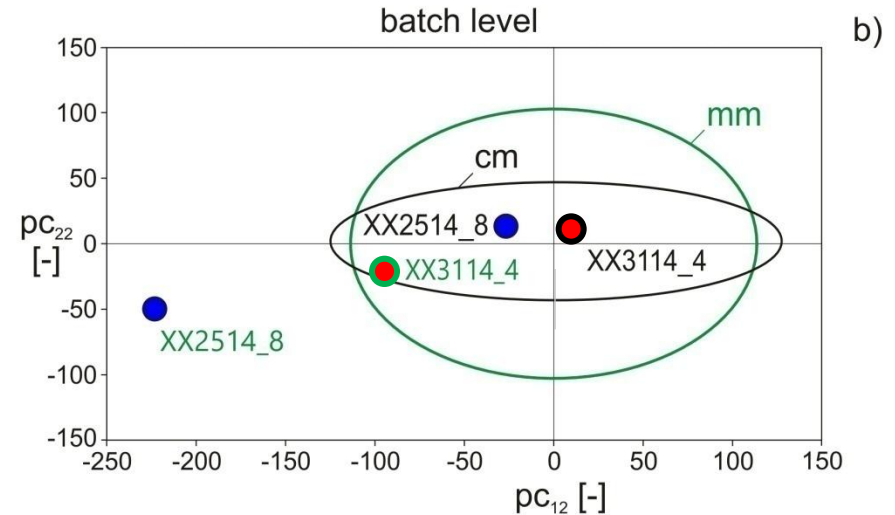
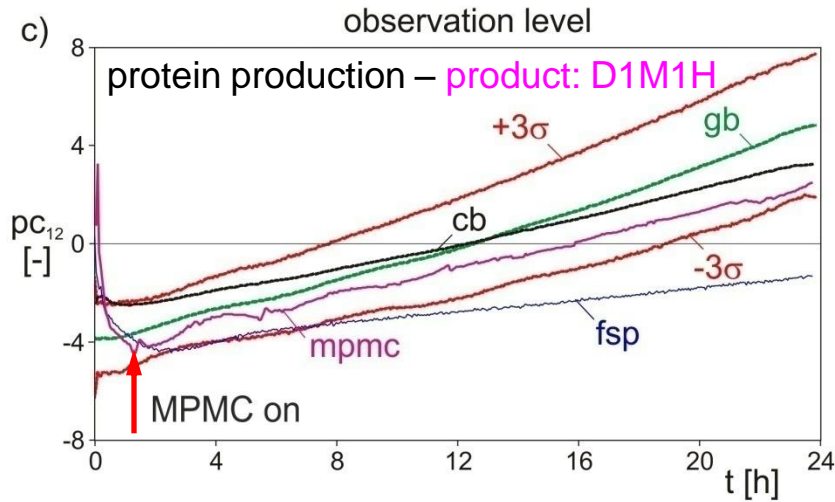
MPMC-Model Predictive Multivariate Control with the monitoring model



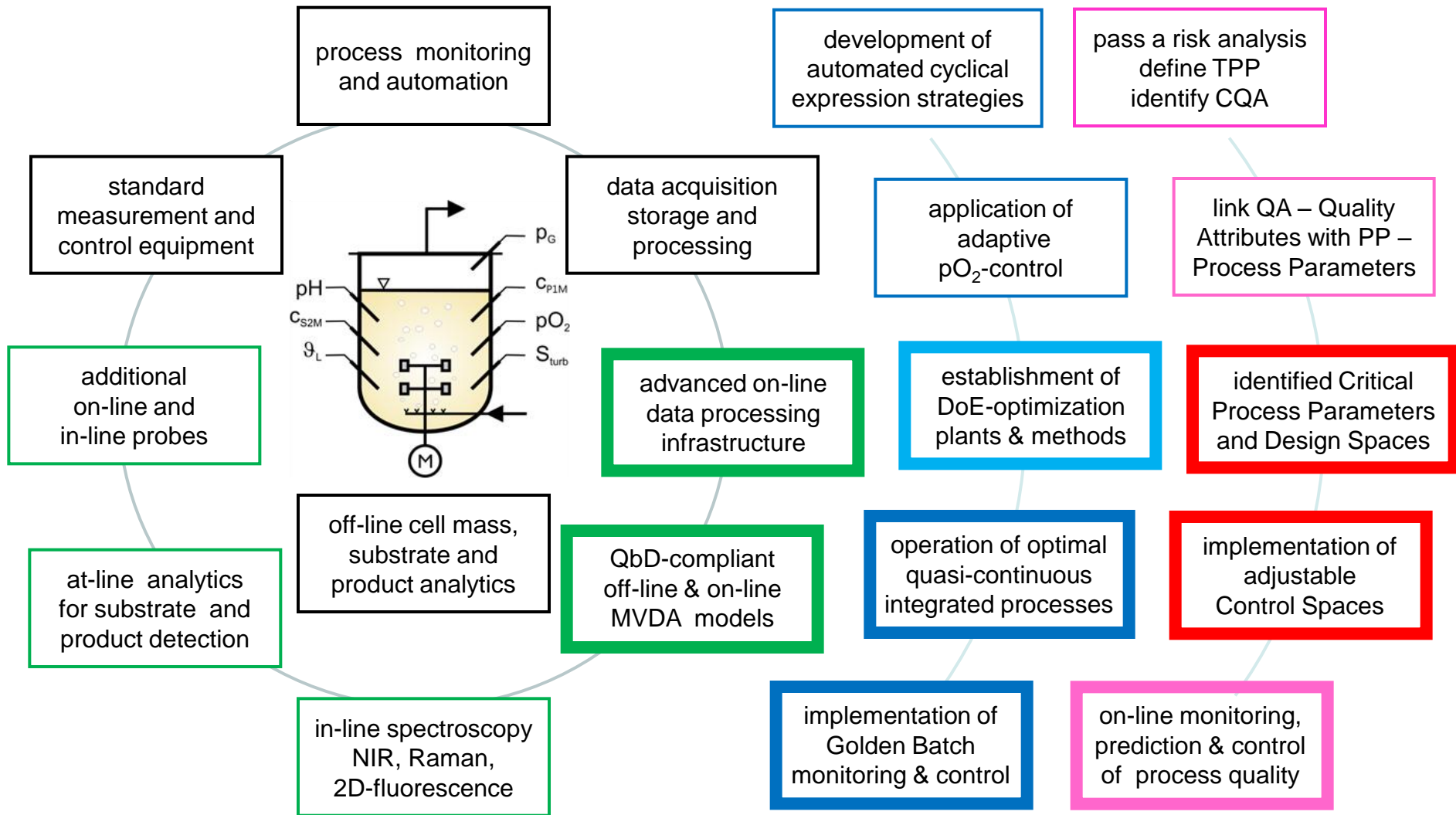
Control Model *cm* – Golden Batch with „playing around“ set points



MPMC – *Model Predictive Multivariate Control* with the control model



Conclusions: *Development of QbD-compliant quality-controlled ICB*



3 PAT levels: *instrumentation* *process development* *QbD-compliance*





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Sequential/parallel production of potential Malaria vaccines – A direct way from single batch to quasi-continuous integrated production

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ABSTRACT

An intensification of pharmaceutical protein production processes can be achieved by the integration of unit operations and application of recurring sequences of all biochemical process steps. Within optimization procedures each individual step as well as the overall process has to be in the focus of scientific interest. This paper includes a description of the development of a fully automated production plant, starting with a two step upstream followed by a four step downstream line, including cell clarification, broth cleaning with microfiltration, product concentration with ultrafiltration and purification with column chromatography. Recursive production strategies are developed where a cell breeding, the protein production and the whole downstream is operated in series but also in parallel, each main operation shifted by one day. The quality and reproducibility of the recursive protein expression is monitored on-line by Golden Batch and this is controlled by Model Predictive Multivariate Control (MPMC). As a demonstration process the production of potential Malaria vaccines with *Pichia pastoris* is under investigation.

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The presentation is now open for *applause and discussions*

