Application of chemometrics for advanced bioprocess monitoring and simulation in view of the FDA´s PAT initiative

Markus Luchner

Department of Biotechnology

University of Natural Resources and Applied Life Sciences, Vienna

Outline

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Introduction to the kinetics of microbial recombinant protein expression
- Process monitoring: an overview
- Case studies: Prediction of complex process variables by chemometric modelling
- Process Analytical Technology (PAT)
- Conclusions

Outline

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

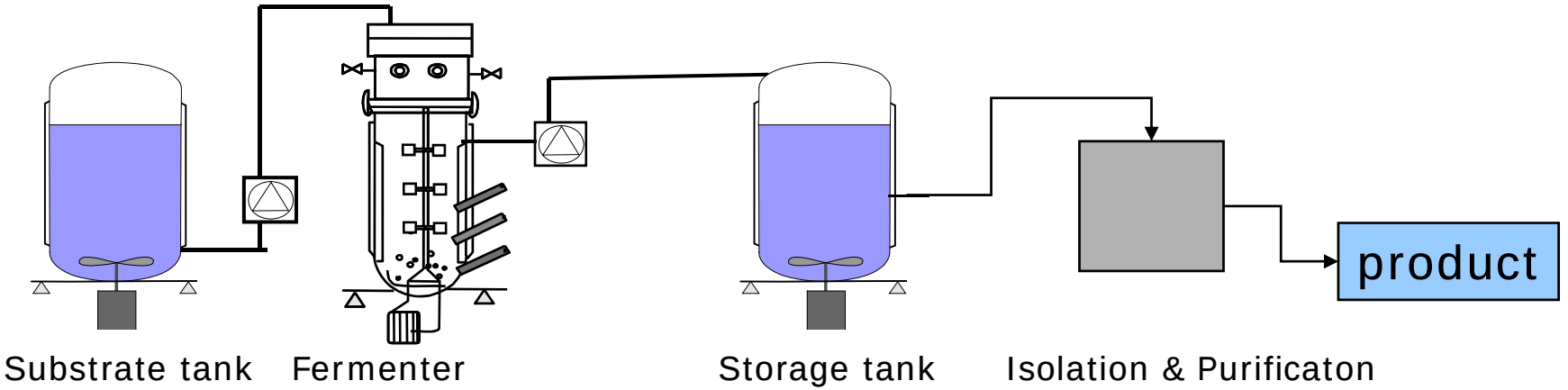
- Introduction to the kinetics of microbial recombinant protein expression
- Process monitoring: an overview
- Case studies: Prediction of complex process variables by chemometric modelling
- Process Analytical Technology (PAT)
- Conclusions

Principle configuration of a bioprocess

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

BIOPROCESS

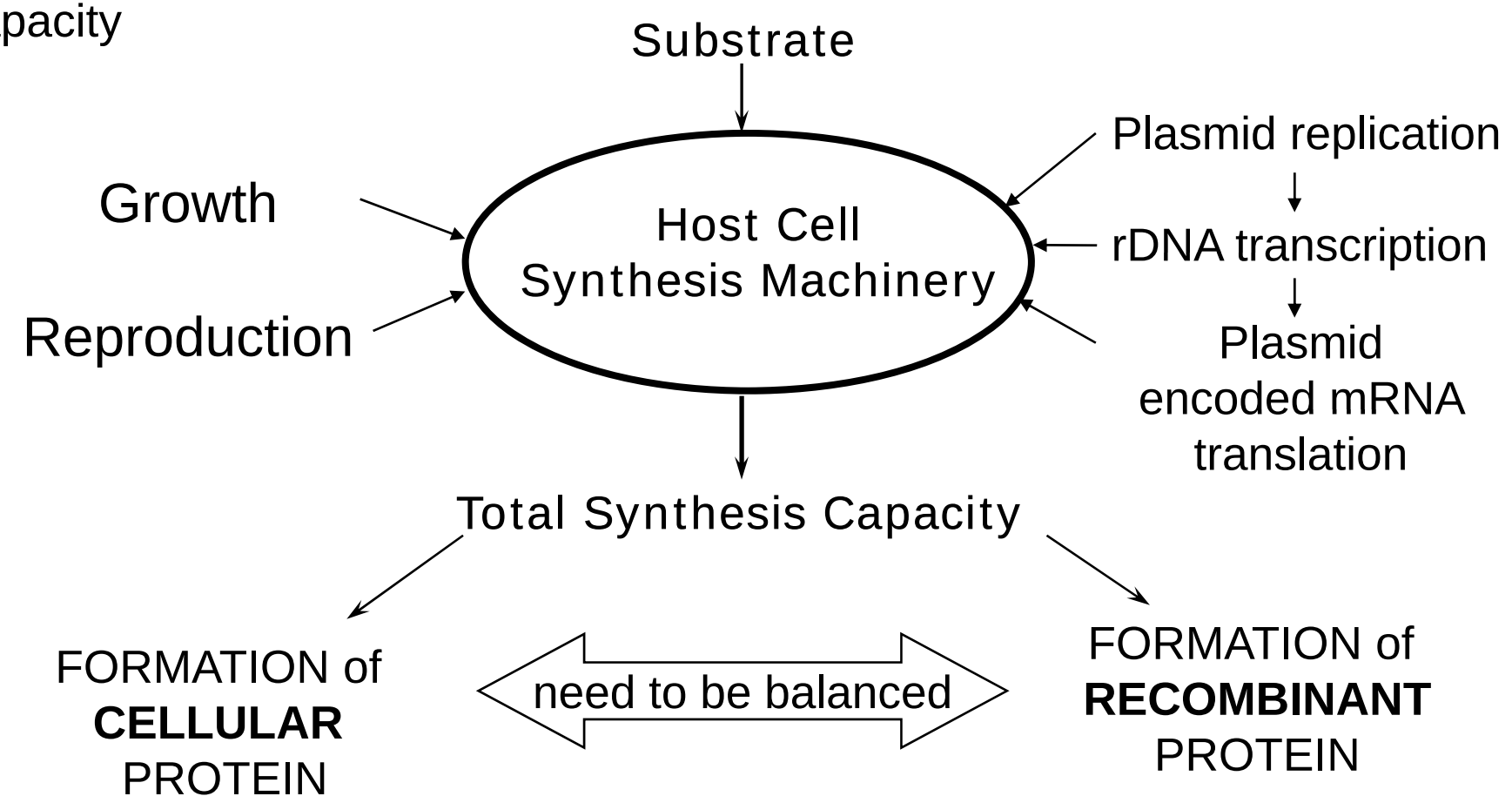
UPSTREAM PROCESSING → DOWNSTREAM PROCESSING



Principles of recombinant protein production strategy

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

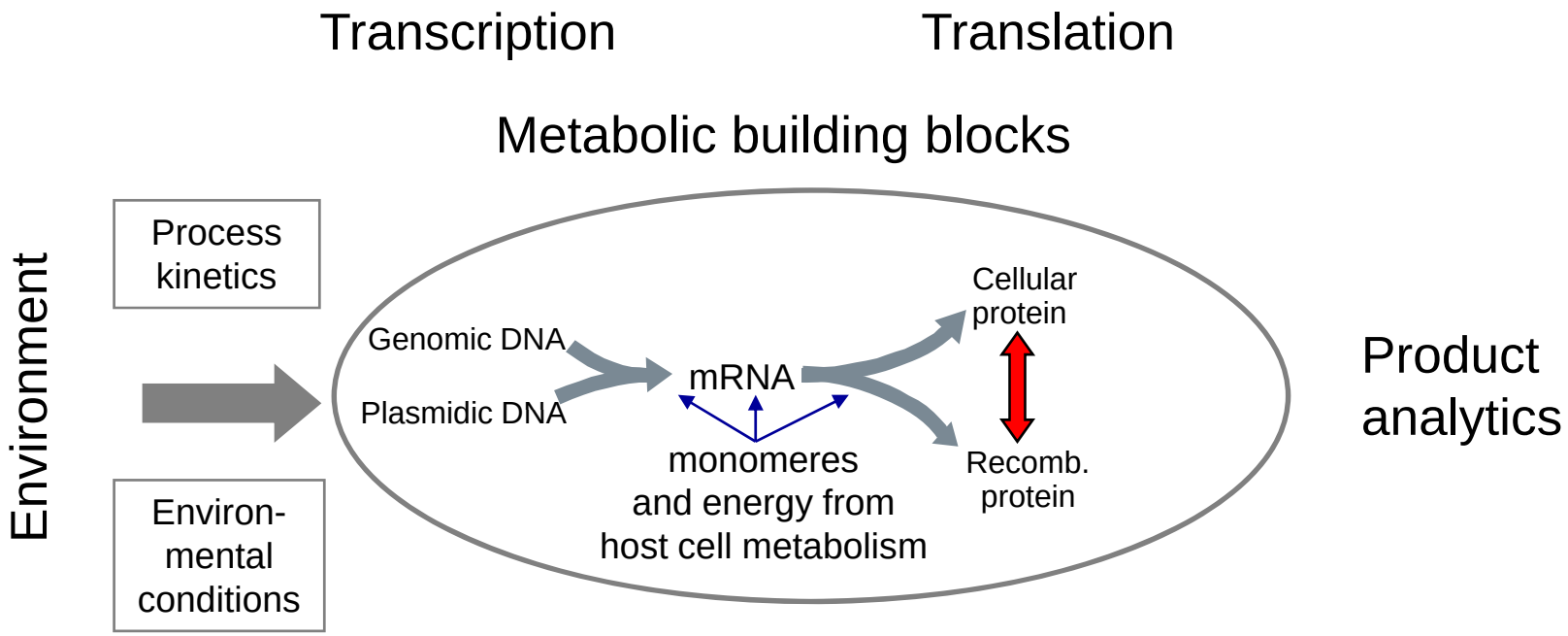
Objective: adaptation of recombinant protein production to host cell metabolic capacity

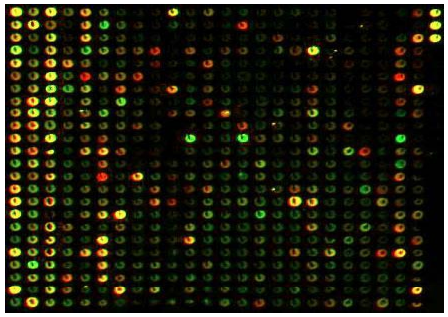


Priority TASK: control → need of specific monitoring

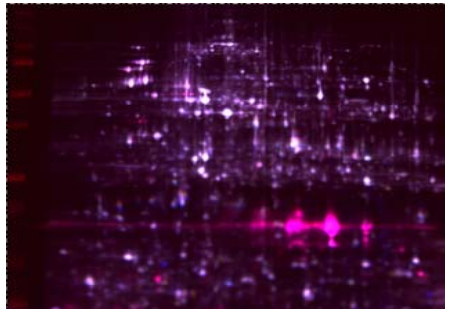
Identification of key variables

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology





DNA microarrays



2-D differential gel electrophoresis (DIGE)

Limits for process optimisation

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Inadiquate understanding of biological system and observability in real-time

- Complexity
- Lack of on- and in-line sensors
- Unpredictable interaction of recombinant protein with host metabolism

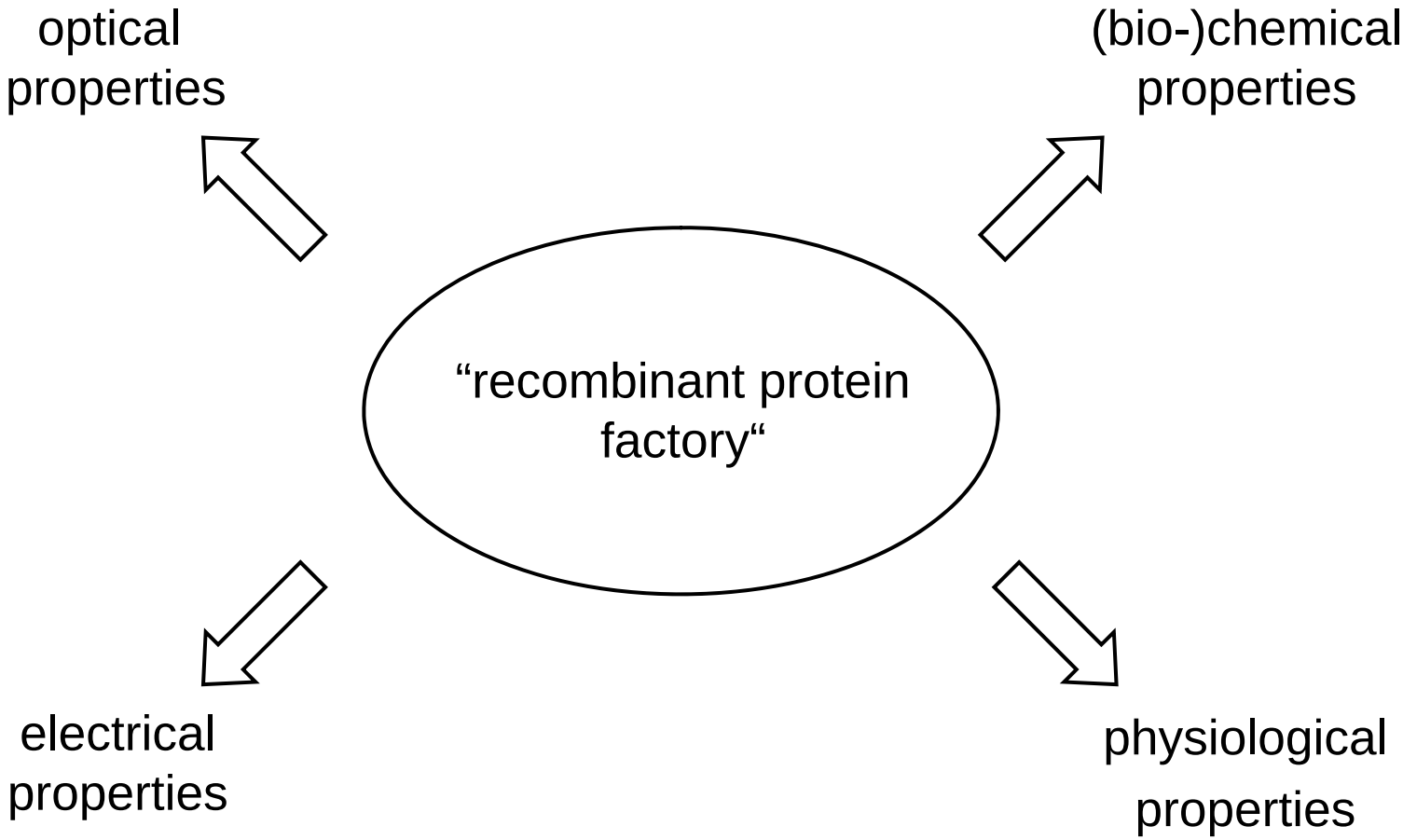
Outline

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Introduction to the kinetics of microbial recombinant protein expression
- Process monitoring: an overview
- Case studies: Prediction of complex process variables by chemometric modelling
- Process Analytical Technology (PAT)
- Conclusions

Information content of the cell

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology



Only few sensors for direct measurement of key process variables available!

State of the art of bioprocess monitoring – availability of off-, at-, on- and in-line measurements

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

At-, on- and in-line measurements:

Classical signals (exhaust gas, base/acid consumption)

Spectroscopic methods (Optical-, Infrared-Dielectric spectroscopy, Mass-spectrometry)

Biosensors

Electrochemical sensors

Flow injection analysis,.....

} Sterilisation!!

Off-line analysis:

Lab-on-a-chip (DNA / RNA / protein quantification)

Proteomics (DIGE)

DNA µ-arrays (transcription profiling)

Surface plasmon resonance (biomolecular interaction)

Chromatographic methods (GC, HPLC)

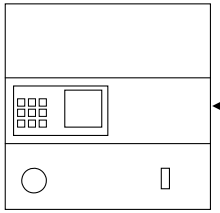
Real time data + highly significant off-line data sets

Solution: generate correlations

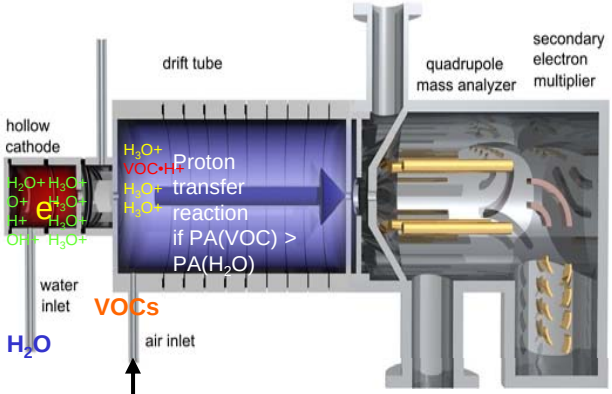
“on-line” monitoring of complex variables by simulation

Overview of our currently used in- and on-line sensor systems

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

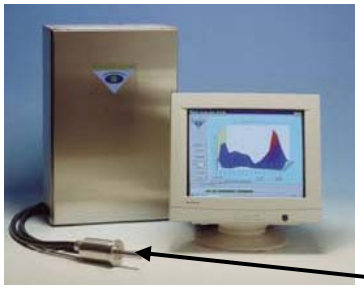


O_2 und CO_2 off-gas analyzer



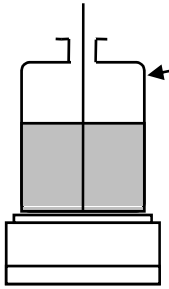
compact proton transfer reaction mass spectrometry

2-D multi-wavelength fluorescence spectroscopy



near infrared spectroscopy

dielectric spectroscopy

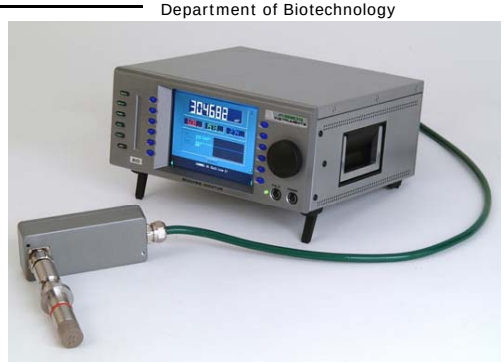


base consumption

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Dielectric spectroscopy:

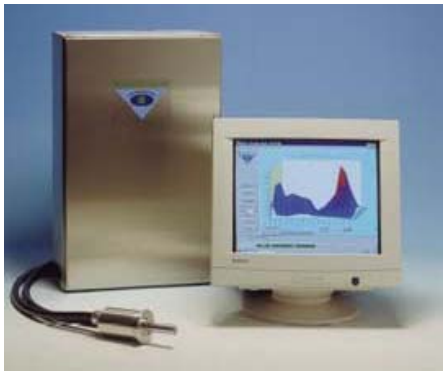
- Intact cells build up charge in electrical field (0.2 - 10 MHz) due to non-conducting nature of the cell
- plasma membrane act as capacitors
- resulting capacitance (pF) is proportional to number and cell size



Biomass Monitor BM214M®

Optical fluorescence spectroscopy:

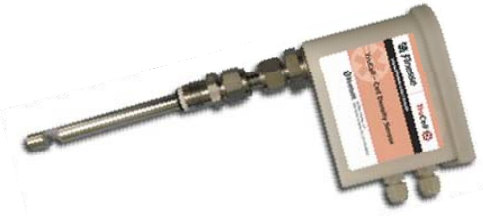
- Two-dimensional, multi-wavelength fluorescence spectroscopy
- excitation 270nm – 550nm / emission 310nm - 590nm → resulting in 150 excitation/emission wavelength combinations



DELTA BioView®

Near Infra Red spectroscopy:

- NIR 850 nm



TruCell™ www.finesse.com

Dielectric spectroscopy:

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Physical principle

- Application of a radio-frequency electrical field (0.2 - 10 MHz) to fermentation broth

- Intact cells build up charge due to non-conducting nature of the cell plasma membrane and therefore act as capacitors

- Measurement of resulting capacitance (pF), which is proportional to number and cell size (= measurement of membrane enclosed volume)

  - Pro's:
    - ➢ Good correlation to biomass

  - Con's:
    - ➢ No direct calibration possible due to changes in cell size
    - ➢ Additional measurement of conductivity (mS/cm) required

Biomass Monitor ABER Instruments BM214M®

Optical fluorescence spectroscopy:

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Two-dimensional, multi-wavelength fluorescence spectroscopy
- Fluorescent properties of biogenic substances are measured
- Wavelength range: excitation 270nm – 550nm / emission 310nm - 590nm in steps of 20 nm → resulting in 150 excitation/emission wavelength combinations

- Pro's:
  - Measurement of biogenic fluorophores which are directly involved in metabolic pathways and components
  - Multivariate data set
  - No fouling
  - Rapid measurement (interval for a full scan 90 sec.)

- Con's:
  - No direct correlation with variables of process operation
  - Interference of sample matrix

DELTA BioView®

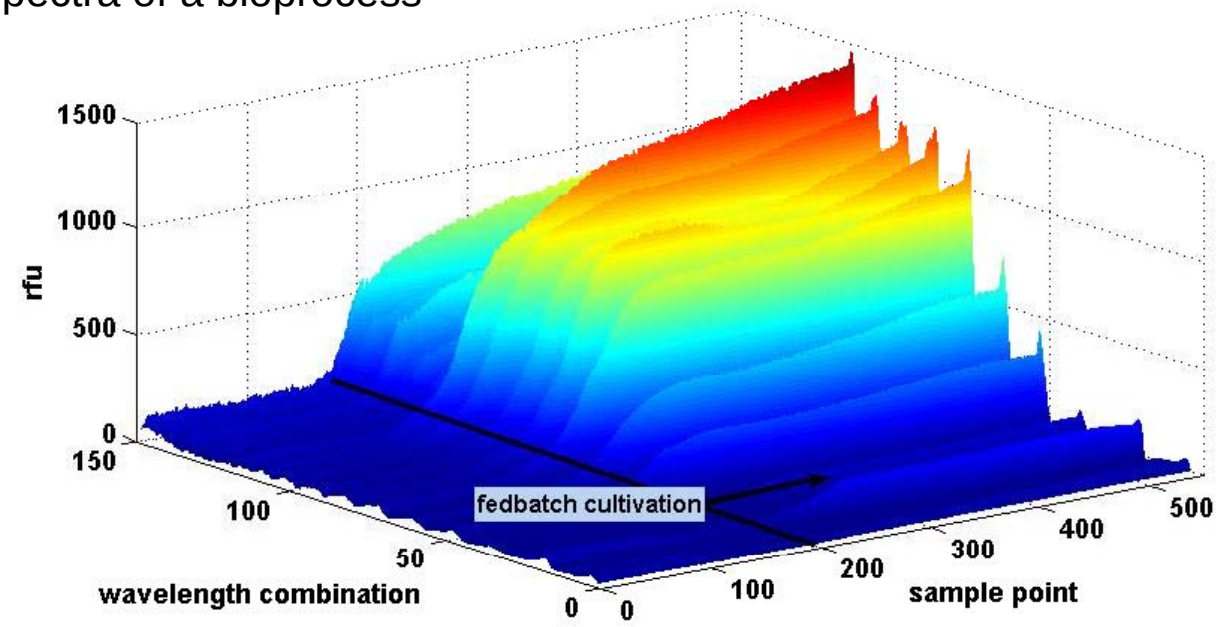
Physiological relevant wavelength combinations

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

– Riboflavin, FAD, FMN	460/520, 380/520
– NAD(P)H	340/460
– Pyridoxine, Pyridoxamine, Pyridoxal-5-P	330/400, 400/500
– Tryptophane	290/350
– Tyrosine	280/310
– Phenylalanine	270/290

(Marose et al., 1998)

Spectra of a bioprocess



Near Infra Red spectroscopy:

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Principle: NIR 850 nm
- range: 0 – 4 AU (Absorbance Units)
- until OD_{600} >350

- Pro's:
  - ➢ Good correlation to biomass
- Con's:
  - ➢ No direct calibration possible

TruCell™ www.finesse.com

In- and on-line signals

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Sensor device	Number of signals
O_2 off gas	1
CO_2 off gas	1
Base consumption	1
Dielectric spectroscopy (capacity, conductivity)	2
Multi-wavelength fluorescence	150
NIR	1
total	**156**

Large data sets

→ Data mining – screening of relevant variables

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- No direct measurement of physiological meaningful variables possible
- Variety of on- and in-line signals available
- Highly developed off-line analytics

Needs:

- Mathematical (chemometric) methods to extract meaningful, yet hidden information and find correlations to off-line variables

Goal:

- Real-time estimation of complex biological variables utilising available on-line sensor signals

Outline

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Introduction to the kinetics of microbial recombinant protein expression
- Process monitoring: an overview
- Case studies: Prediction of complex process variables by chemometric modelling
- Process Analytical Technology (PAT)
- Conclusions

Data flow and tools for pre-processing and modelling of data

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Pre-processing of data

Filtering and interpolation (Matlab)

Data mining - screening of relevant on-line data

Kohonen Self Organzing Maps based on Ward distance Cluster analysis

(Viscovery® Profiler (Eudaptics GmbH Vienna))

Modelling of data - selection of model type

Partial Least Squares (PLS)

Artificial Neuronal Network (ANN)

Data mining - screening of relevant on-line data

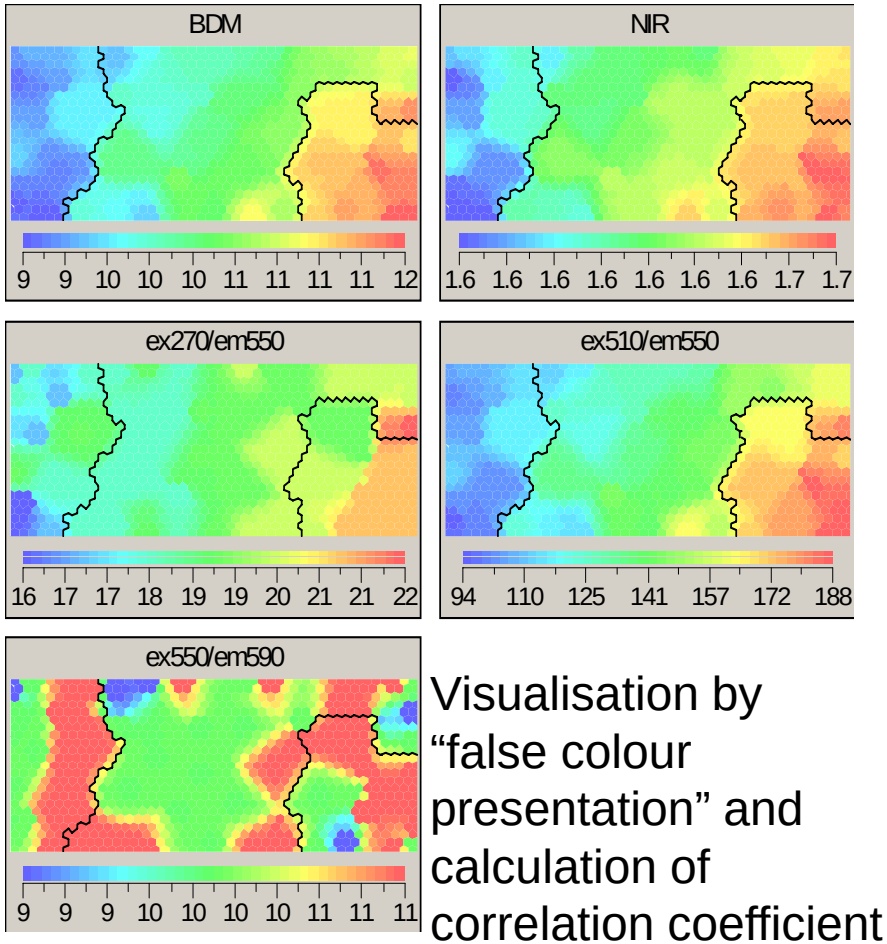
University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

SOM´s (Self organising maps – Kohonen* algorithm)

$$E = \int \sum h_{ci} \left| \vec{w}_i - \vec{x}_i \right|^2 g(\vec{x}) d^n x$$

in-line signal	NIR	ex510/ em550	ex270/ em550	ex550/ em590
correlation-coefficient	0.9845	0.9982	0.8894	0.1403

approx. 60 % of fluorescence signals: correlation coeffitient > 0.75



Visualisation by “false colour presentation” and calculation of correlation coefficient

*T. Kohonen, Springer, 3rd editon, 2001

Model types

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Non-linear model:
  - Partial least squares (PLS)
    - reduction of multidimensional data sets to lower dimensions for analysis
  - Radial Basis Function Neural Network (RBF): Neural networks are better suited for non-linear data
    - supervised learning method
    - non-linear transfer function
    - training by vector weighting

Quality of estimation:

- Root Mean Square Error of Prediction (RMSEP): RMSEP represents the overall error of the modelled data

Case study: prediction of complex variables

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Used data sets

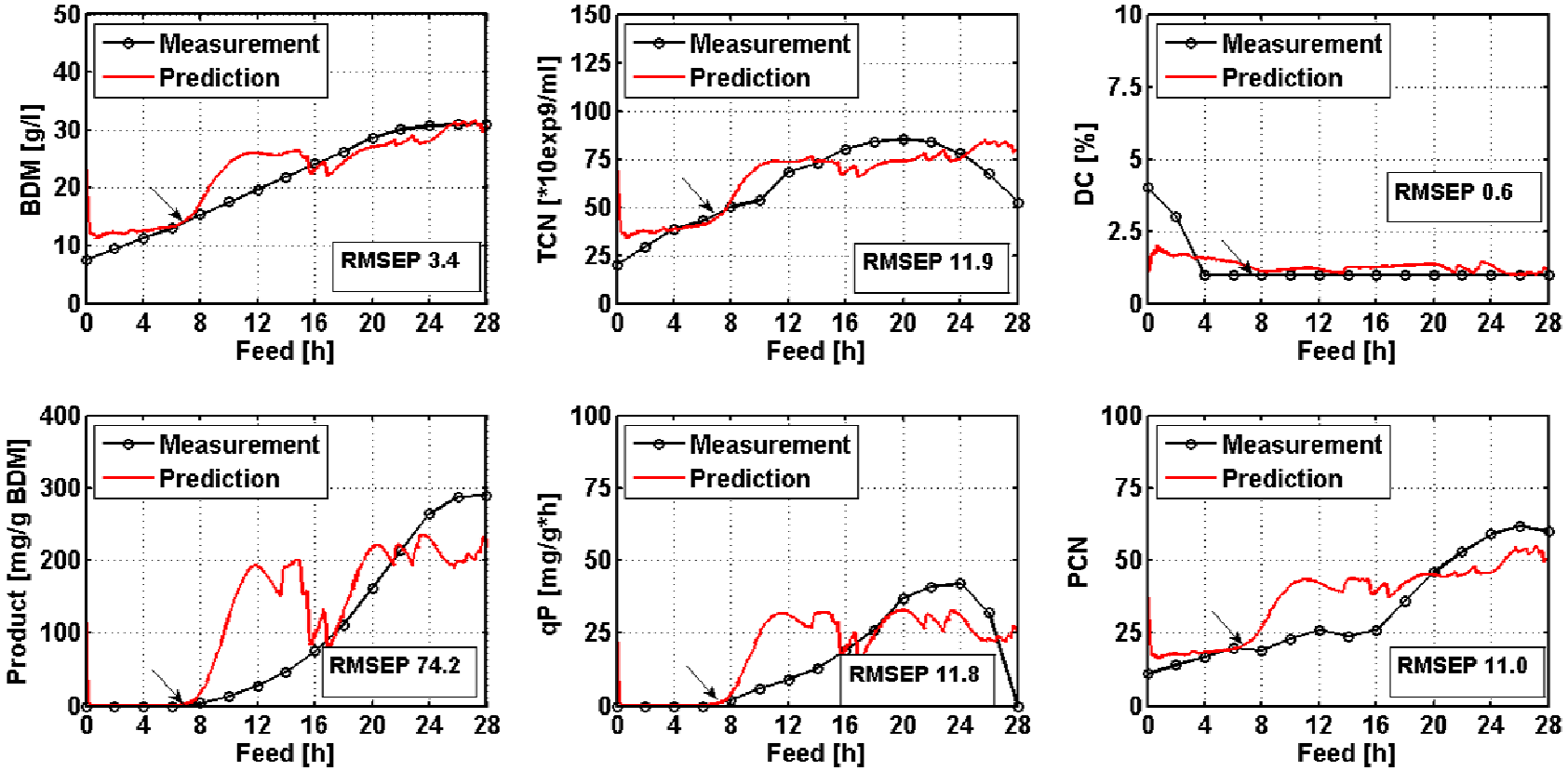
- On-line
  - Exhaust gas composition: O_2, CO_2
  - Base consumption rate
  - Fluorescence signals
  - Capacity, conductivity

- Off-line:
  - Bacterial Dry Matter (BDM) (gravimetric)
  - Total Cell Number (TCN) / Dead Cell Number (DC) (flow cytometry)
  - Product (mg/g BDM) (electrophoretic)
  - Plasmid Copy Number (PCN) (electrophoretic)

Prediction of key variables in fed-batch cultivation applying RBF-Network

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Input: Classical signals (base consumption, exhaust-gas analysis)



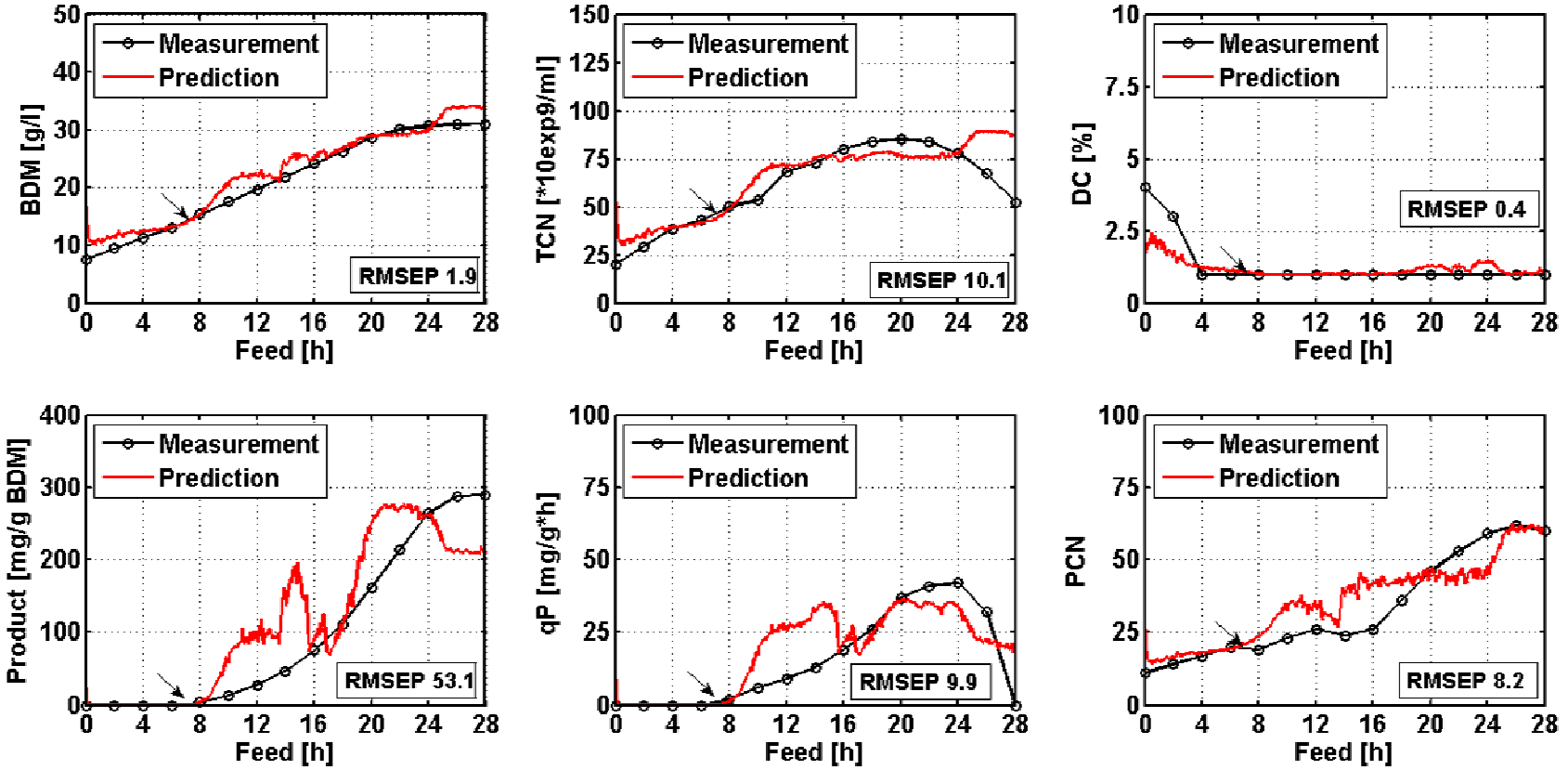
Arrows indicate induction of recombinant protein expression

F. Clementschitsch et al. (2005), Journal of Biotechnology, 2, 120, 183-196.
F. Clementschitsch et al. (2006), Microbial Cell Factories, 5,19-30

Prediction of key variables in fed-batch cultivation applying RBF-Network

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Input: Dielectric spectroscopy signals, classical signals (capacity, conducitvity, exhaust-gas analysis, base consumption)



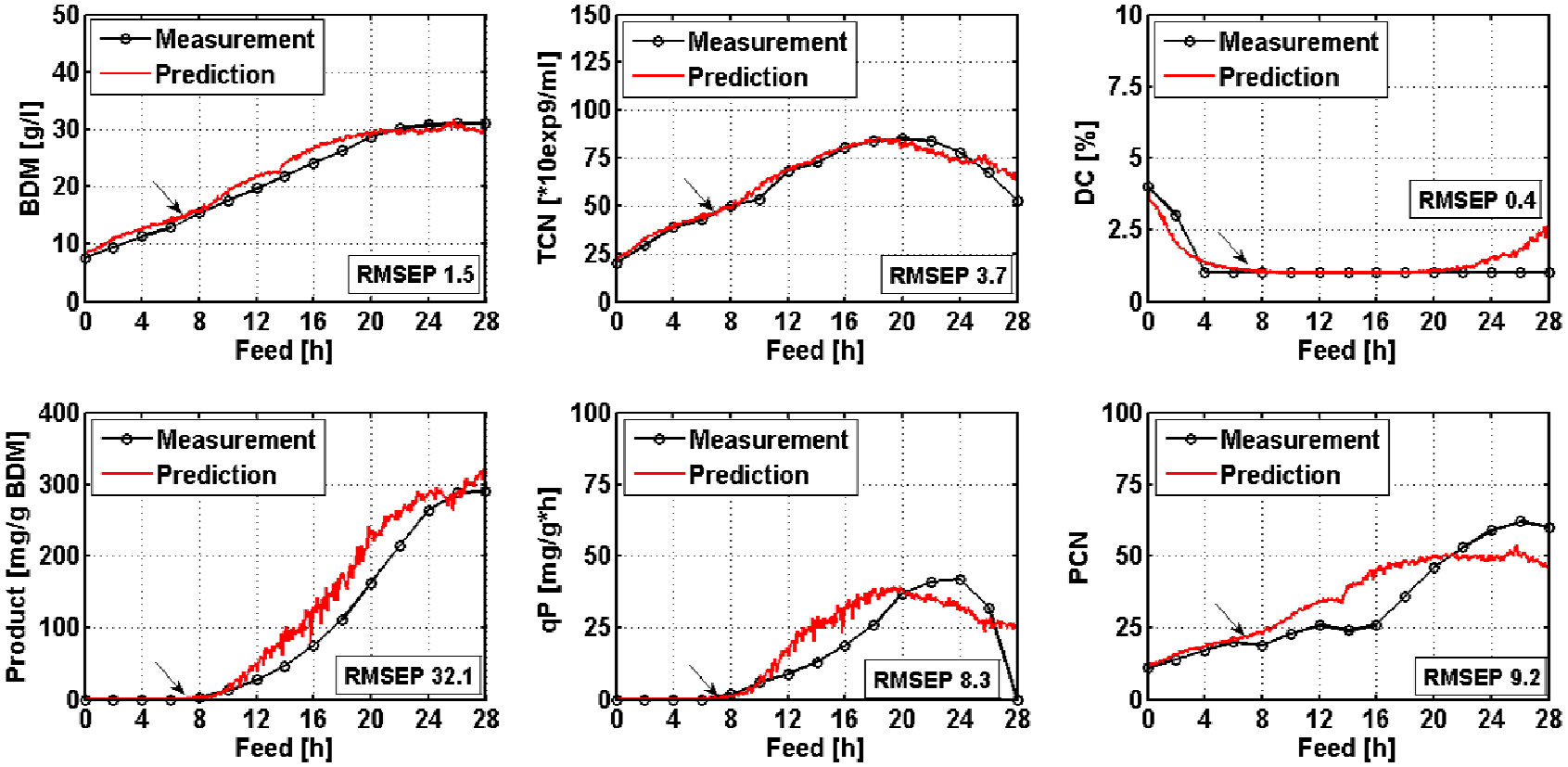
Arrows indicate induction of recombinant protein expression

F. Clementschitsch et al. (2005), Journal of Biotechnology, 2, 120, 183-196.
F. Clementschitsch et al. (2006), Microbial Cell Factories, 5,19-30

Prediction of key variables in fed-batch cultivation applying RBF-Network

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Input: Selected signals (capacity, conducitvity, selected fluorescence wavelength combinations, exhaust-gas analysis, base consumption)



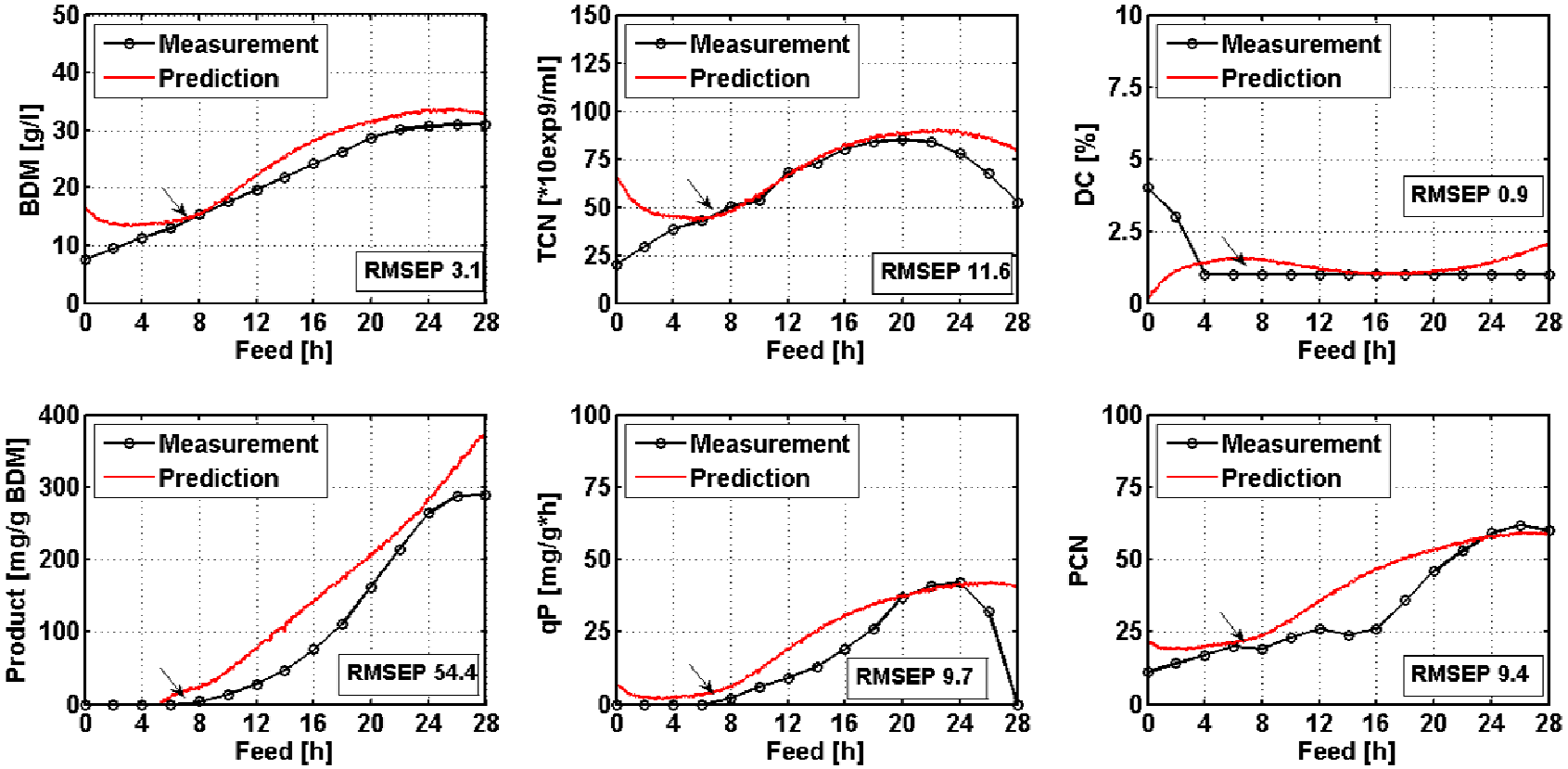
Arrows indicate induction of recombinant protein expression

F. Clementschitsch et al. (2005), Journal of Biotechnology, 2, 120, 183-196.
F. Clementschitsch et al. (2006), Microbial Cell Factories, 5,19-30

Prediction of key variables in fed-batch cultivation applying PLS

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Input: Selected signals (capacity, conducitvity, selected fluorescence wavelength combinations, exhaust-gas analysis, base consumption)

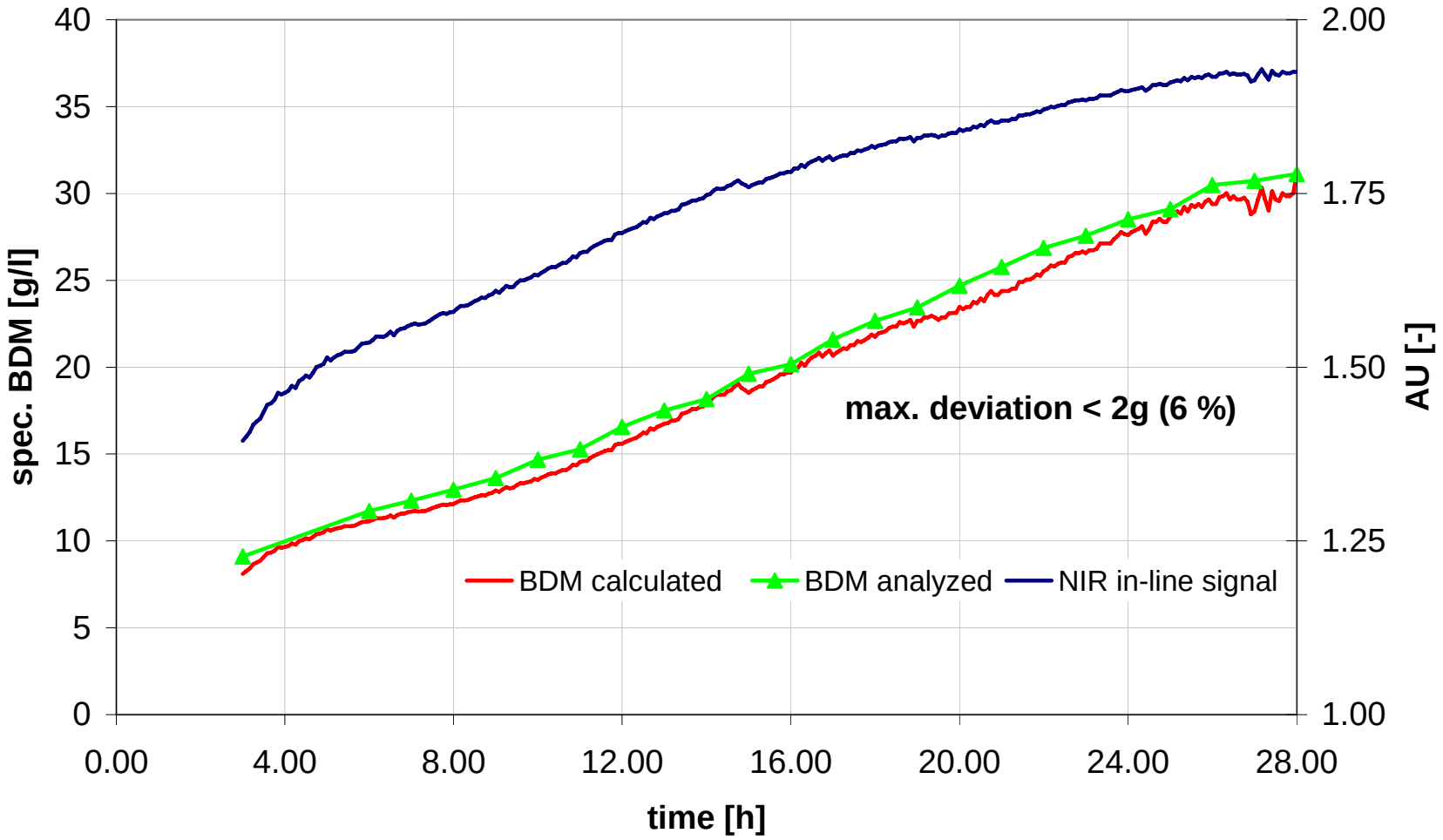


Arrows indicate induction of recombinant protein expression

F. Clementschitsch et al. (2005), Journal of Biotechnology, 2, 120, 183-196.
F. Clementschitsch et al. (2006), Microbial Cell Factories, 5,19-30

Example: application of NIR for monitoring

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology



f(x) = p1*x^4 + p2*x^3 + p3*x^2 + p4*x + p5
p1 = -81.13, p2 = 650.3, p3 = -1825, p4 = 2201, p5 = -968.9

- Achievements:
  - On-line prediction of key variables
  - Set up of control loops enabled

- Limitations
  - Monitoring of deviations on molecular level (e.g. stress response)
  - Validability of prediction by chemometric methods not fully accepted by regulatory authorities

⟹ Extension of on-line data base

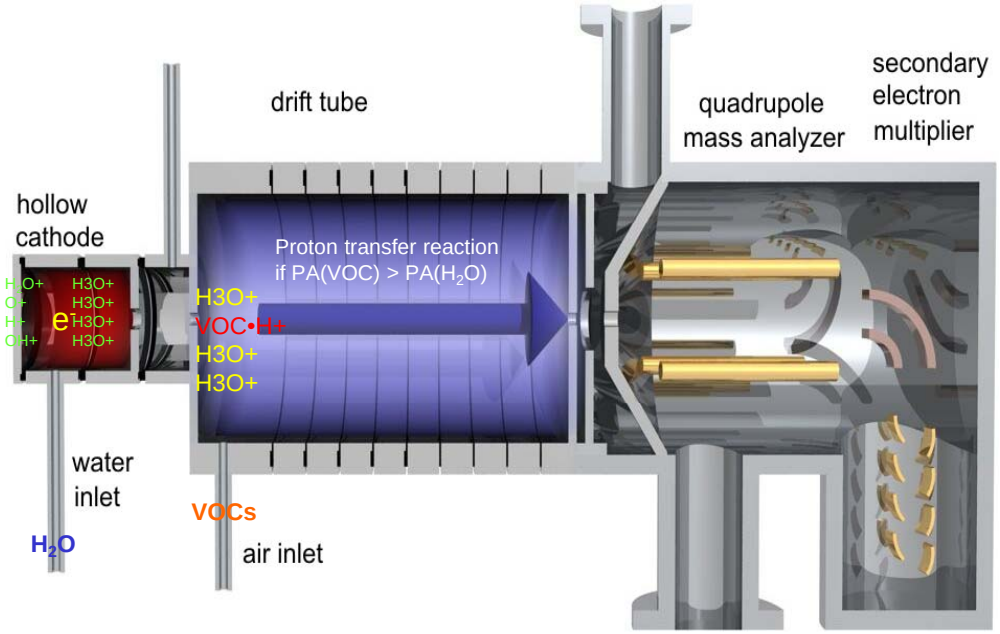
Proton Transfer Reaction Mass Spectrometry:

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Reaction: VOCs charged by

  $H_3O^+ + R \rightarrow RH^+ + H_2O$

- Detection limit: 500 pptv
- Mass range: 1 – 300 am



- Pro's:
  - ➢ Non invasive measurement
  - ➢ Measurement of metabolites
  - ➢ Rapid measurement (approximately 3 minutes per cycle)
  - ➢ Soft ionization – no fragmentation

- Con's:
  - ➢ Mass information but no structure information

www.ptrms.com
www.ionimed.com

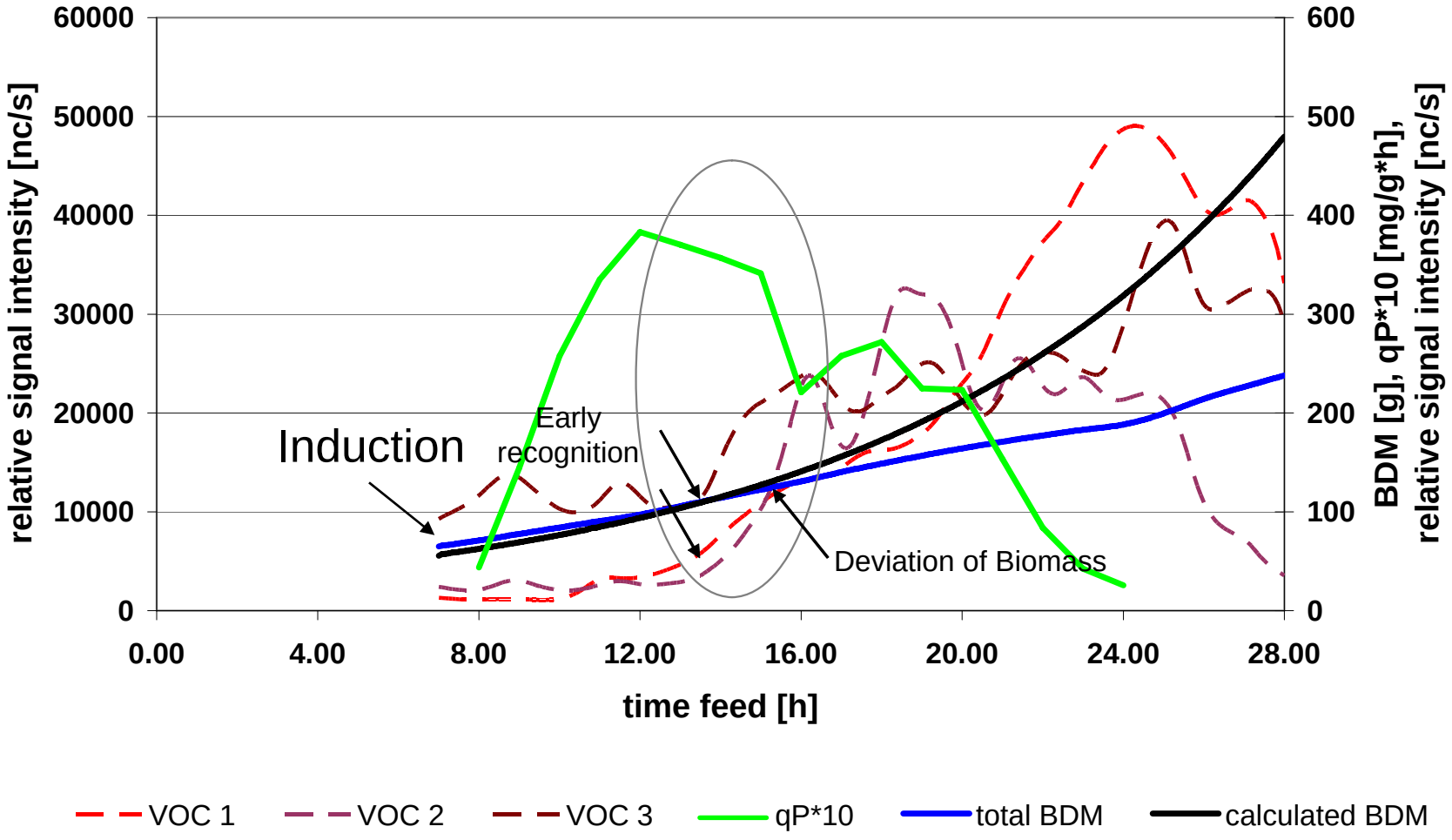
In- and on-line signals

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Sensor device	Number of signals
O_2 off gas	1
CO_2 off gas	1
Base consumption	1
Dielectric spectroscopy (capacity, conductivity)	2
Multi-wavelength fluorescence	150
NIR	1
PTR-MS	Up to 60
total	**Up to 216**

Application of PTR-MS for process monitoring

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology



PTR-MS enables the transition from pattern recognition to quantitative analysis of volatile metabolites

PTR-MS

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Non invasive sampling device
- Sensor for early detecting of different physiological states
  - e.g. growth and non growth associated recombinant protein production and overburden of the cell
- Real time availability of complex variables for process control

Outline

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Introduction to the kinetics of microbial recombinant protein expression
- Process monitoring: an overview
- Case studies: Prediction of complex process variables by chemometric modelling
- Process Analytical Technology (PAT)
- Conclusions

Process Analytical Technology (PAT) and Quality by Design (QbD)

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- **P**rocess **A**nalytical **T**echnology initiative:
  - a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e. during processing) of critical quality and performance attributes of raw- and in-process materials and processes with the goal of ensuring final product quality. (http://www.fda.gov/Cder/OPS/pat.htm)

- Required tools for the implementation of PAT :
  - Multivariate data acquisition and data analysis tools
  - Modern process analyzers or process analytical chemistry tools
  - Extension of process monitoring and control tools

GOAL: definition of the design space to gain more flexibility in operation

Outline

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Introduction to the kinetics of microbial recombinant protein expression
- Process monitoring: an overview
- Case studies: Prediction of complex process variables by chemometric modelling
- Process Analytical Technology (PAT)
- Conclusions

Conclusions I

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

- Chemometric modelling and prediction contribute to the improvement of process monitoring and control
- Contribution of individual sensor signals:
  - Classical signals do not contain enough information to allow the estimation of complex process variables
  - Monitoring of key variables achieved through signal combination
  - Selection of input signal improves quality of prediction
- PTR-MS technology enables
  - early detection of deviations and different physiological states
  - real-time quantification of specific process relevant compounds

Conclusions II

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Complex diagnostics platform comprising in-, on- and off-line data delivers a broad spectrum of information

→ basis for PAT and QbD compliance

→ enables the definition of the designs pace (ICH Q8)

Acknowledgements

University of Natural Resources and Applied Life Sciences, Vienna
Department of Biotechnology

Department of Biotechnology

Group of Microbial Fermentation

Funding by the Austrian Center of Biopharmaceutical Technology (www.acbt.at)