Merck Serono

MERCK
Living Innovation

Analytik von Biopharmazeutika – Vom Bioreaktor bis zum finalen Produkt

Gemeinsames Symposium des PVZ und der DPhG Fachgruppe Arzneimittelkontrolle/Pharmazeutische Analytik

Braunschweig, 12. März 2015

Christian Hunzinger

Agenda

1 Introduction

- Analytics of Biopharmaceuticals
- Process Analytics as a Specific Segment

2 Application Examples for Process Analytics

- Cell Culture & mAb Analysis by Mid-infrared Spectroscopy (MIR)
- Glycosylation Analysis by CGE-LIF
- Host Cell Protein Analysis by Simple Western™ Technology

3 Outlook

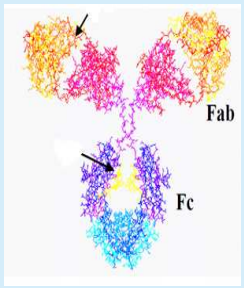
Biopharma Analytics Segments Have Different Requirements

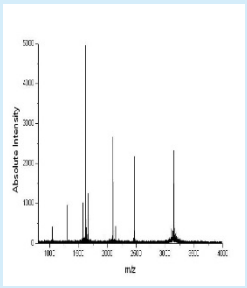
Characterization

Product focus

High-end & detailed methods

Low throughput



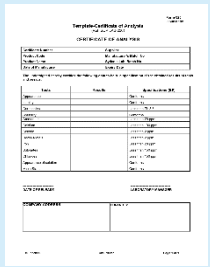


QC Testing

Product focus

Robust & precise methods

Medium throughput



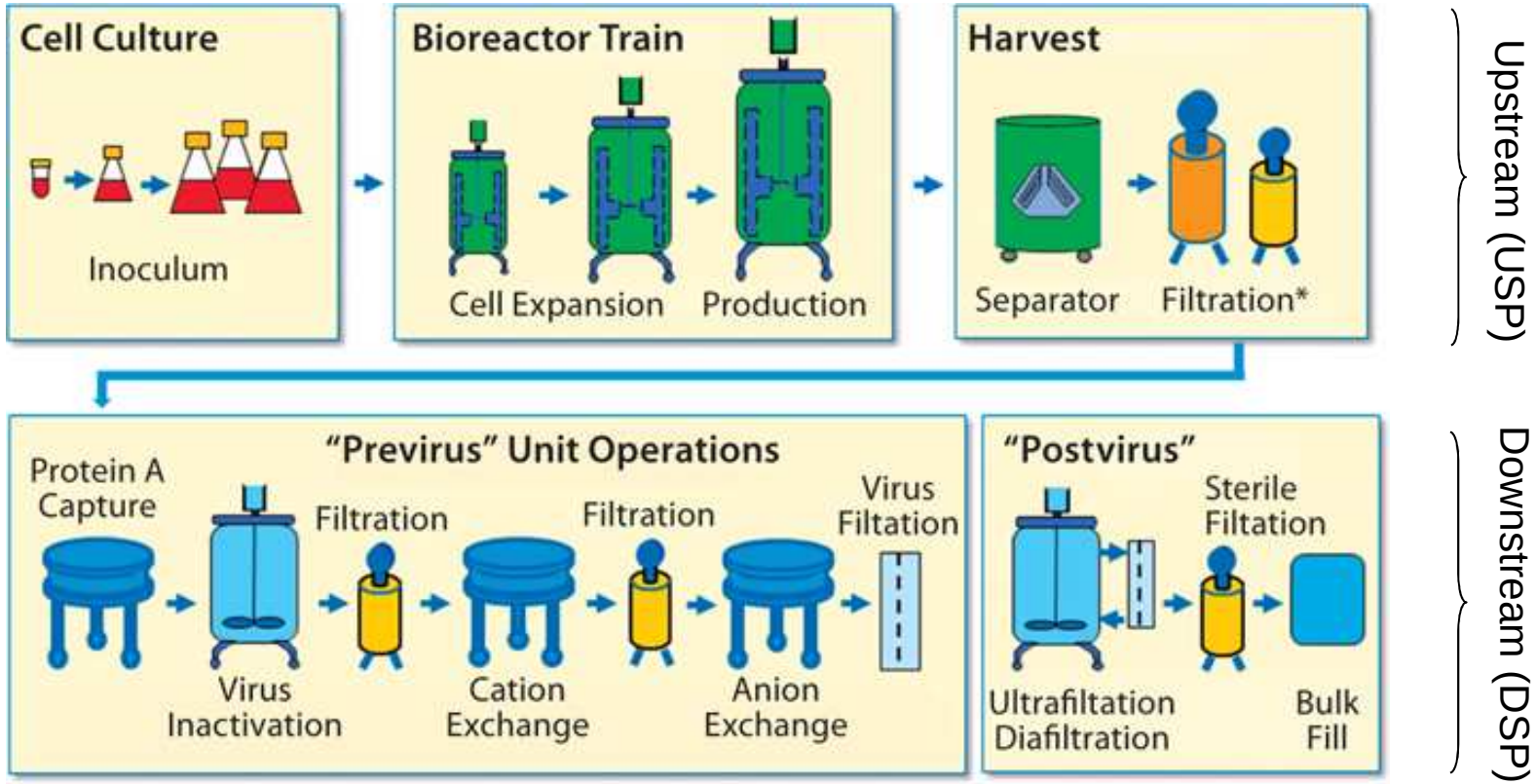
Process Analytics

Process & Product focus

Fast & simple methods

High throughput

The Classical mAb Manufacturing Process



BioProcess International, Vol. 10, No. 6, June 2012, pp. 48–57

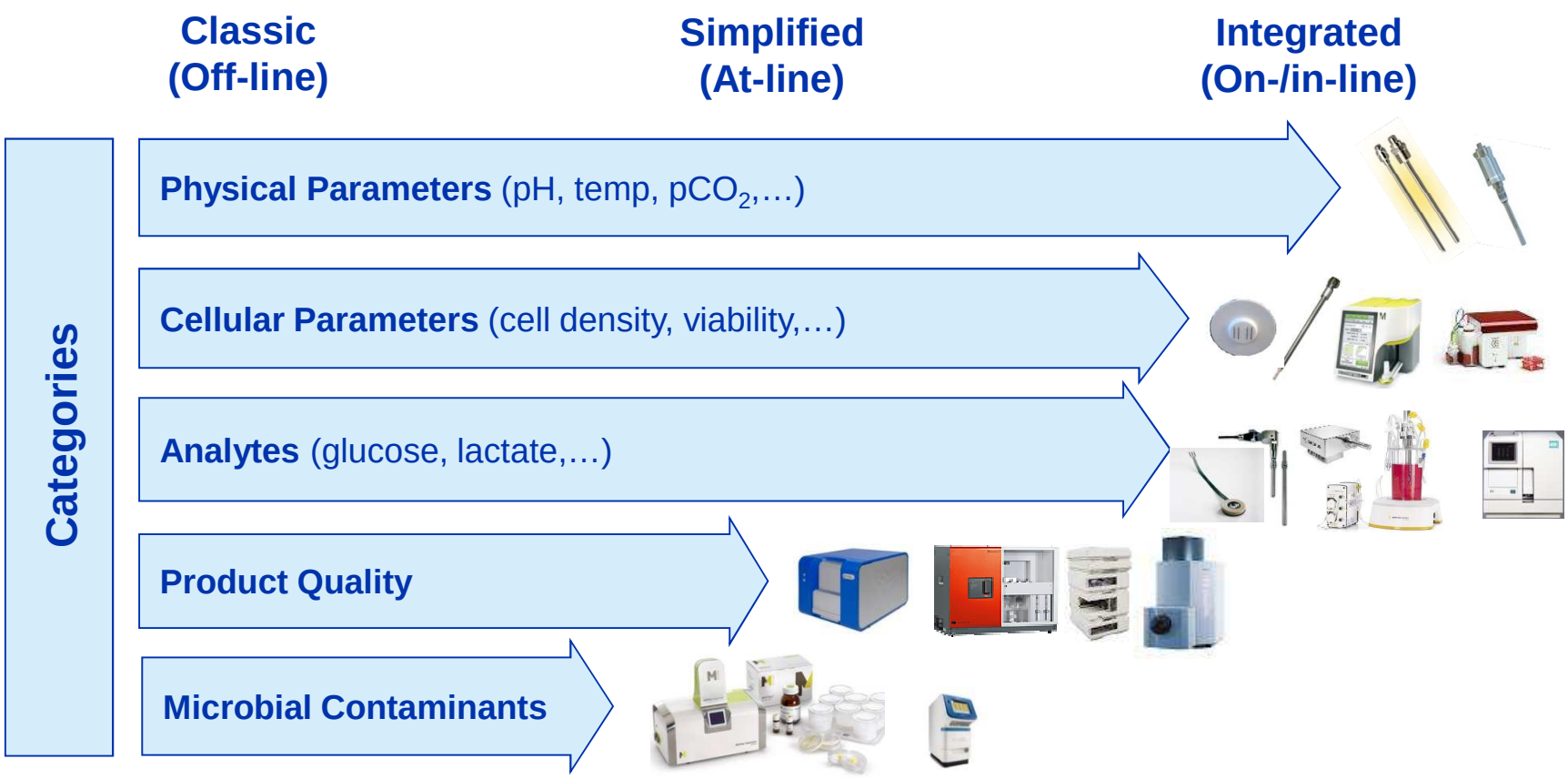
Quality by Design (QbD) Concept Builds on Process Analytics (PAT initiative)

	Char	PAT	QC
Testing parameter			
Testing volume			

QbD concept

Nature Biotechnology 27, 26 - 34 (2009)

Process Analytics Categories Differ in Technological Maturity



The instruments shown are for illustrative purposes only.

Holistic Process Analytics Concept Demands Differentiated & Tailored Solutions

Upstream Example

Process Development

Production

Preference for at-line setups

- High Testing Volume
- Flexibility
- More CQAs

- More Detail
- Focus on „Understanding“

Preference for in/on-line setups

- Validated Systems
- Minimal Approach
- Focus on Control

The instruments shown are for illustrative purposes only.

Selected Process Analytics Tools Used at Merck

Process contaminants

qPCR

Process analytes,
Product titer

MIR

CGE-LIF

Product glycosylation

Product purity

SDS-CGE

SPR

Product efficacy

Product charge
variants

Autom. ELISA

cIEF

Process contaminant
profile

Process
contaminants

Mass
Spectrometry

Product sequence
integrity, PTMs

UPLC

Product Purity

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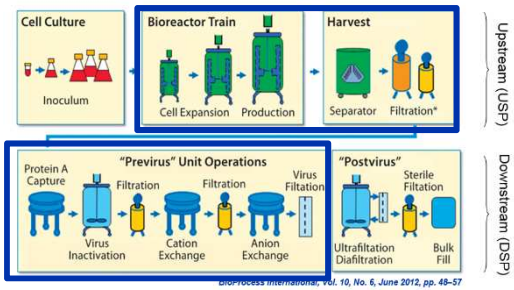

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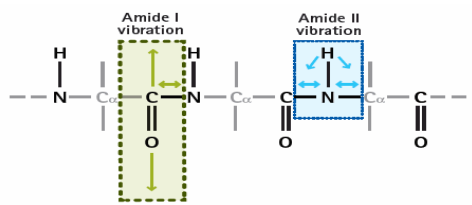
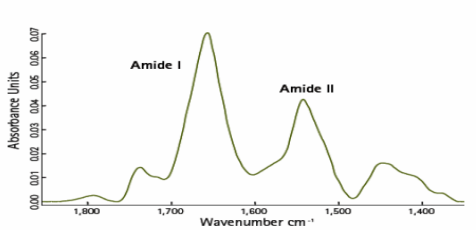
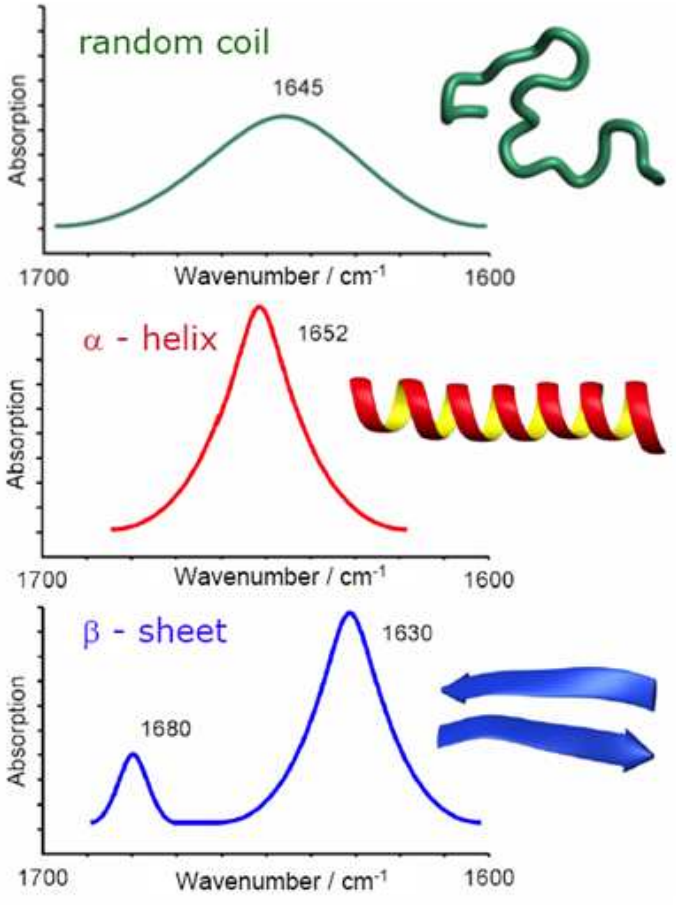
Mid-Infrared (MIR) Based Protein Analysis and Quantification





Fabian et al., Encyclopedia of Analytical Chemistry, Wiley & Sons, pp. 5779-5803.

wavenumber area in cm^{-1}	**assigned protein secondary structure**
~ 1615	aggregated strands
1620- 1635	beta-sheet
1640- 1650	irregular
~ 1640	3_{10}- helix
1650- 1658	alpha-helix
~ 1660	3_{10}- helix
1655- 1685	turns+ loops
1675- 1695	antiparallel beta-sheet
~ 1685	aggregated strands

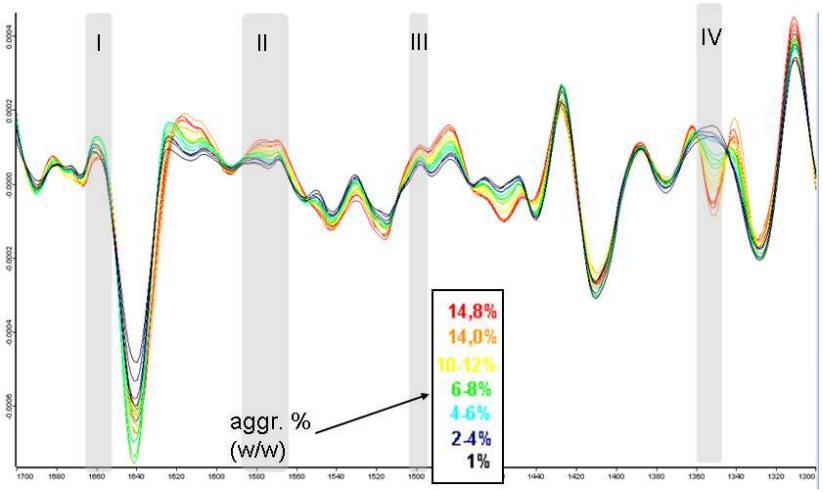


MIR - Technical Approach

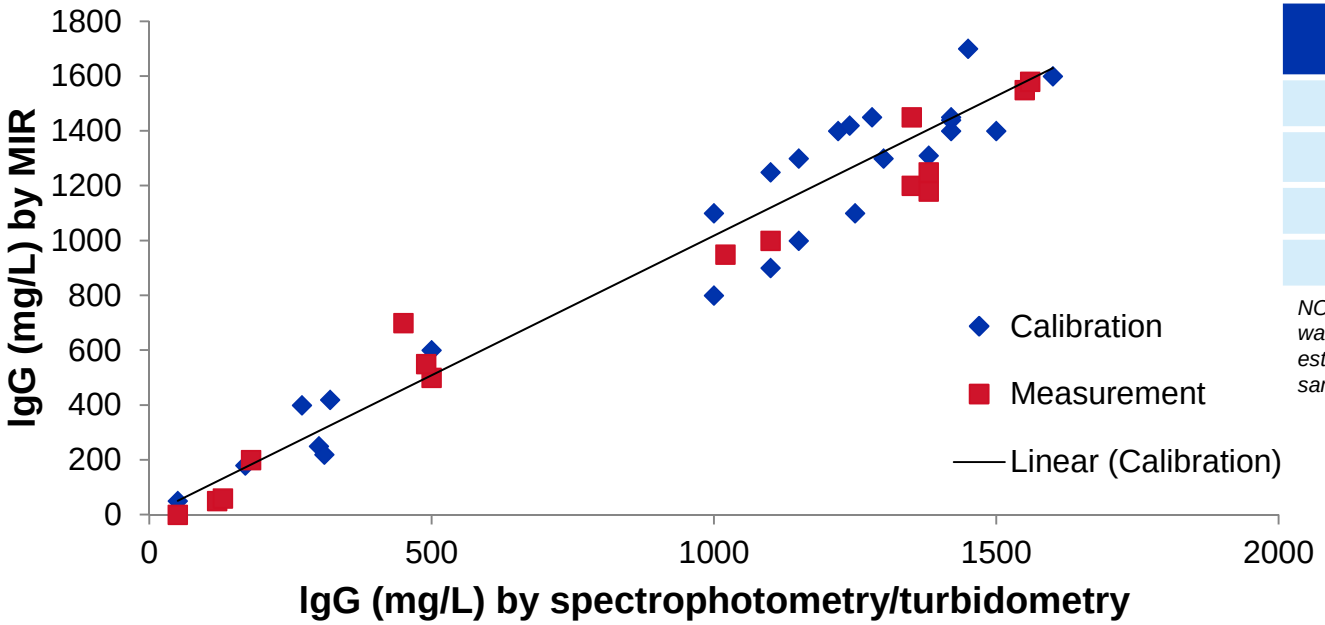
IgG Aggregation Example

Zone	Wavenumber range (cm^{-1})	Assigned
1	1665 - 1654	Amide I
2	1579 - 1567	Amide II
3	1502 - 1496	Amide II
4	1360 - 1355	C-O Carboxy

- Prepare samples with known parameter concentration
- Identify wavenumber ranges showing correlation of band intensity with parameter
- Optimize wavenumber ranges by automated processing (Bruker OPUS software)
- Design quantification models based on optimized wavenumber ranges



mAb Quantification in Cell Culture by MIR



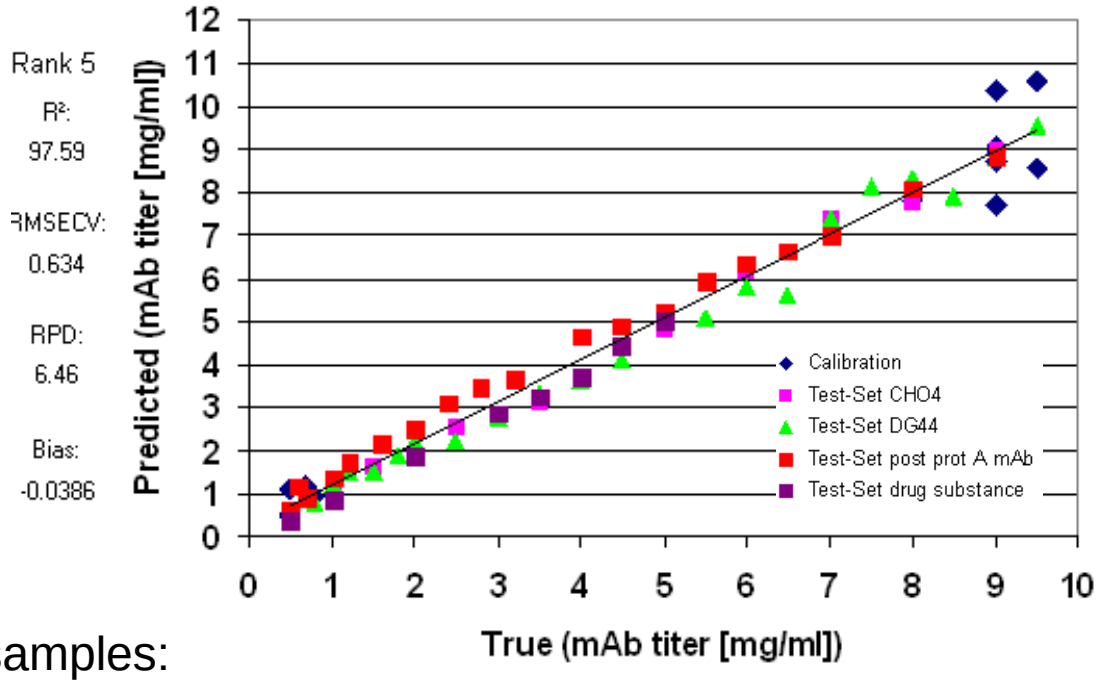
Zone	Wavenumber range (cm^{-1})
1	1690 - 1680
2	1642 - 1618
3	1519 - 1515
4	1454 - 1450

NOTE: These are just some example wavenumber ranges that were used to estimate the IgG amounts in cell culture samples

- Measured samples with known IgG concentration (e.g estimated by spectrophotometry);
- Identified wavenumber ranges showing correlation of band intensity with IgG;
- Obtained good agreement for the range >400 mg/L of IgG;
- 75% of all measured samples of >400mg/L IgG concentration are in 10% range of reference values.

mAb Quantification in Different Matrices by MIR

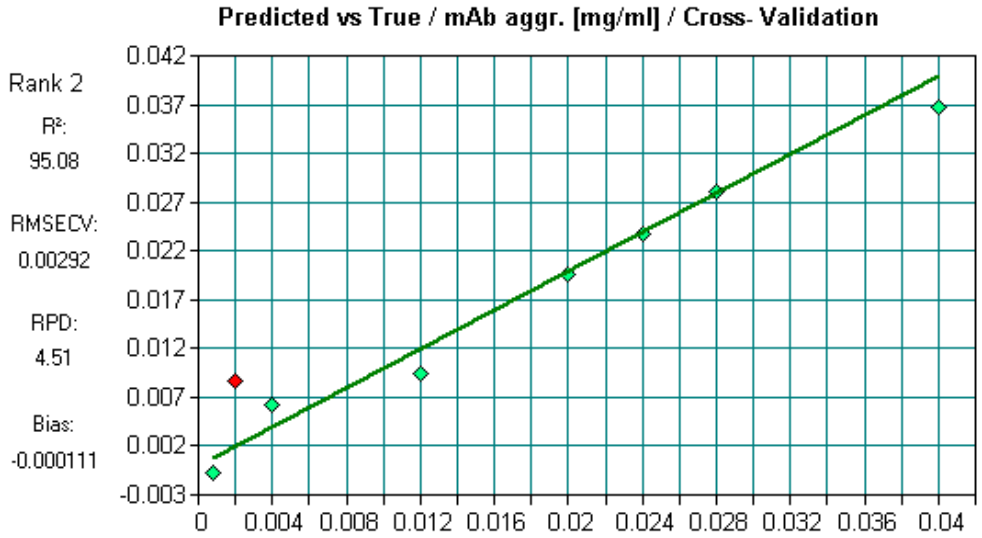
- 1 IgG
- Different IgG concentrations
- 4 matrices (2 cell culture fluids, post-prA pool, drug substance)



- Results from 61 test-set samples:
  - 72% with CV < 10%
  - 86% with CV < 20%
- A single mAb in various matrices can be quantified with one model

mAb Aggregate Quantification by MIR

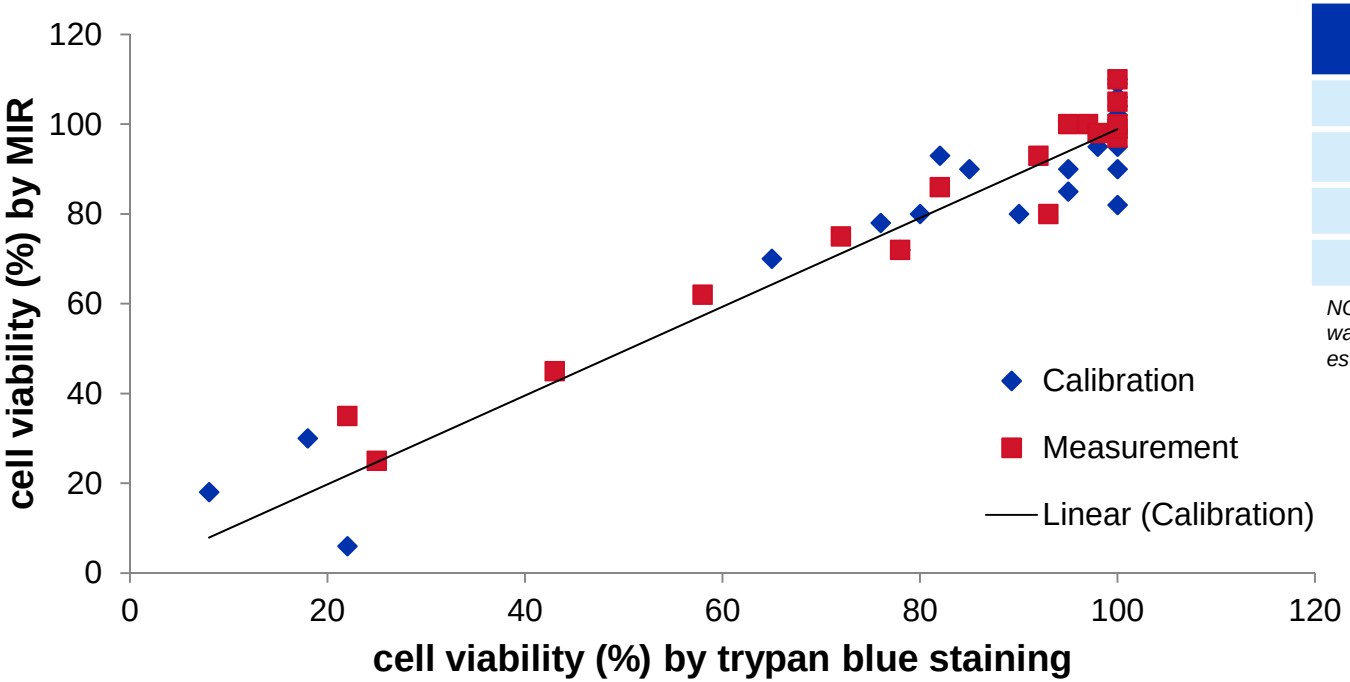
- 1 mAb
- Different mAb conc.
- Different mAb aggr. Conc.
- 1 matrix (cell culture fluid)



mAb A in CHO-DG44 CCF		
True [mg/ml]	Predicted [mg/ml]	CV %
0,0008	-0,0032	355,0
0,0020	0,0096	270,1
0,0040	0,0068	48,7
0,0080	0,0078	2,0
0,0120	0,0098	13,0
0,0160	0,0231	31,5
0,0200	0,0183	6,0
0,0240	0,0230	2,8
0,0280	0,0288	2,1
0,0400	0,0348	9,1

- Using models for small quantification windows, mAb aggregates down to 0,02 mg/ml can be quantified
- Corresponds to 0.2 – 2 % of mAb aggregates (10-1 g/L titer)

Relative Cell Viability in Cell Culture by MIR

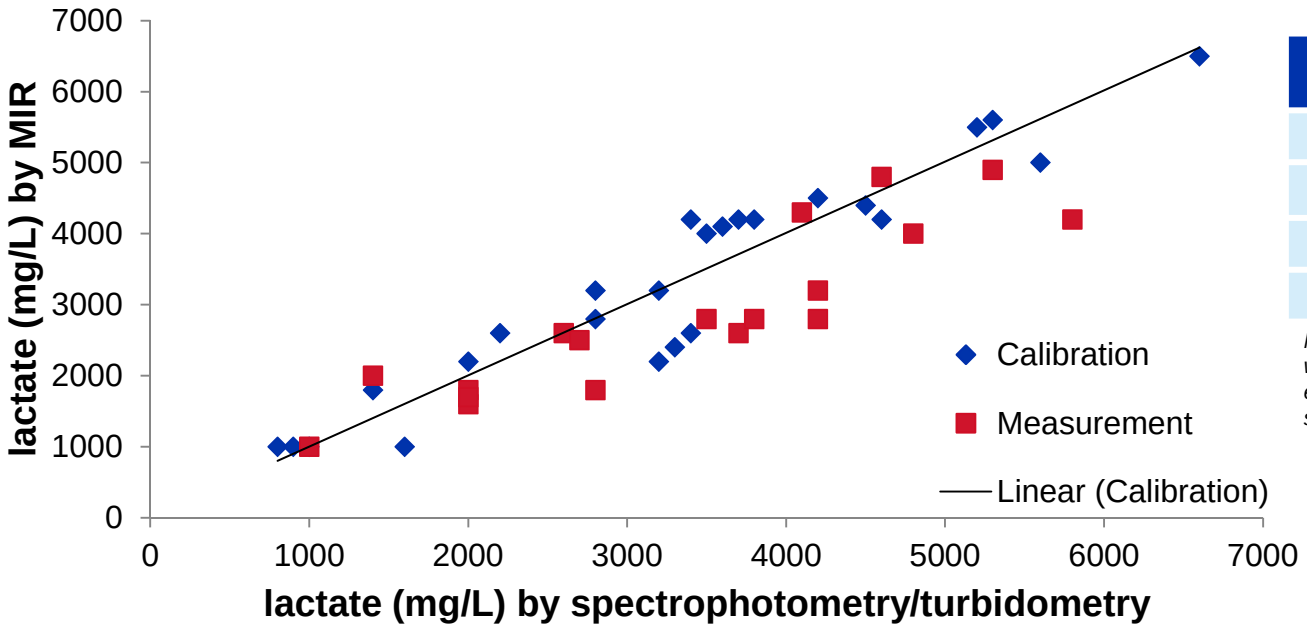


Zone	Wavenumber range (cm^{-1})
1	3538 - 3536
2	2360 - 2350
3	1491 - 1487
4	776 - 770

NOTE: These are just some example wavenumber ranges that were used to estimate the relative cell viability

- Measured samples with known cell viability (%) (e.g estimated by the trypan blue staining);
- Identified wavenumber ranges showing correlation of band intensity with cell viability;
- Obtained good agreement for the range between 20 and 95% of relative cell viability;
- If cell viability was 100% according staining method, MIR-based values deviated up to 20%.

Lactate Quantification in Cell Culture by MIR



Zone	Wavenumber range (cm^{-1})
1	2050 - 2010
2	1917 - 1910
3	1726 - 1712
4	830 - 820

NOTE: These are just some example wavenumber ranges that were used to estimate the lactate amount in cell culture samples

- Measured samples with known lactate amount (e.g estimated by spectrophotometry);
- Identified wavenumber ranges showing correlation of band intensity with lactate amount;
- Obtained larger deviations between reference values and predicted values, indicating a potentially indirect calibration. More calibration samples are necessary for better correlation.

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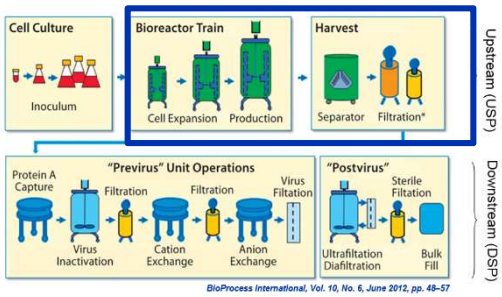

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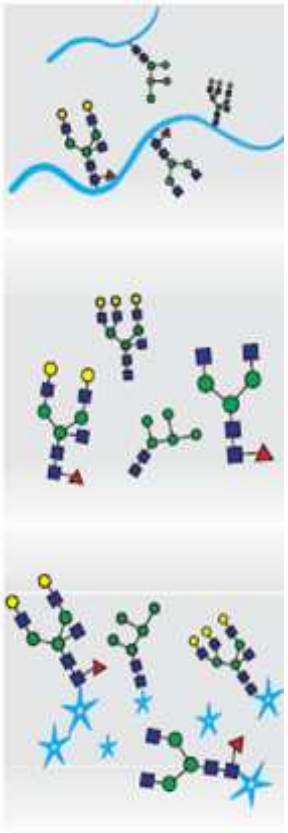
3 Outlook

CGE-LIF N-Glycan Analysis Workflow



Protein purification (prA)

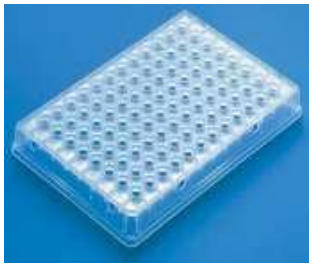
Protein reduction (DDT and IAA)

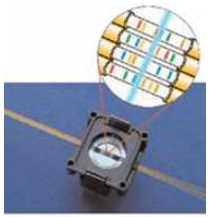
Glycan release with **PNGase F**

Glycan extraction and reconcentration

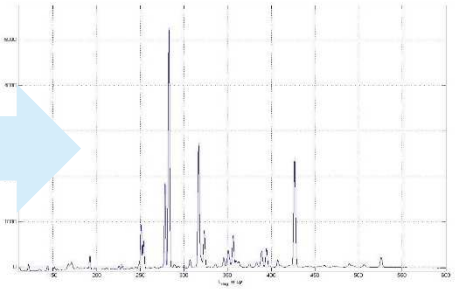
Glycan labeling with **APTS**

Removal of excess APTS optional





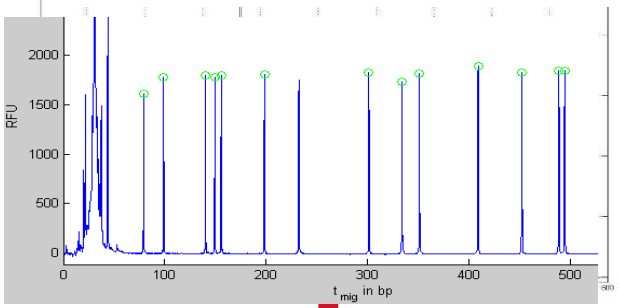
DNA Sequencer

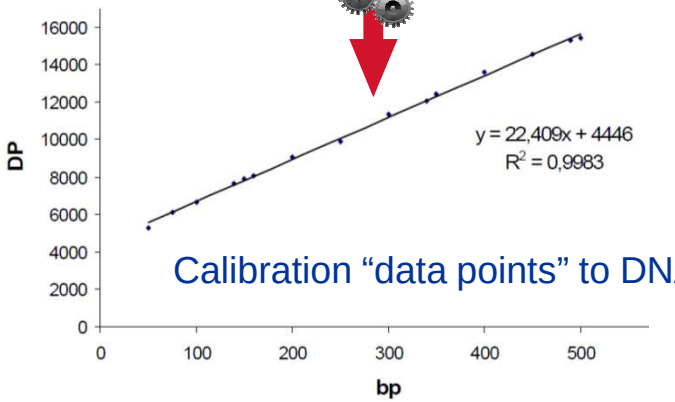


Method adapted from Papac et al., Glycobiology , 1998 and Laroy et al. Nature Method, 2006

CGE-LIF N-Glycan Data Processing

DNA ladder injected with each glycan preparation

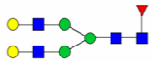




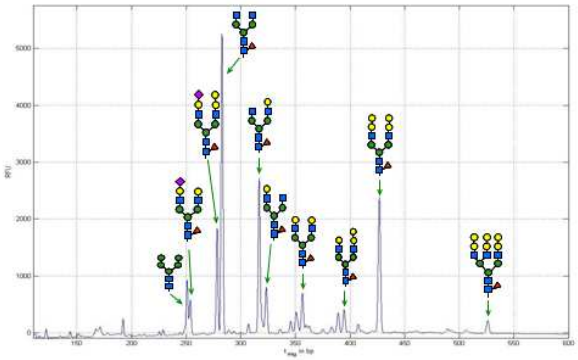
Calibration “data points” to DNA bp

“glyXtool” by glyXera*

Glycan to DNA bp “**conversion**”

standard *N*-glycan		t_{mig} (bp)
	FA2 / (NGA2F)	200
	FA2G2 / (NA2F)	264

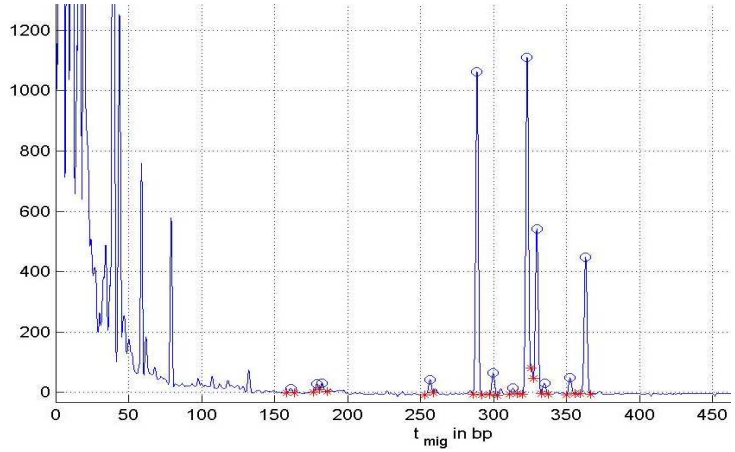
N-oligosaccharides identification



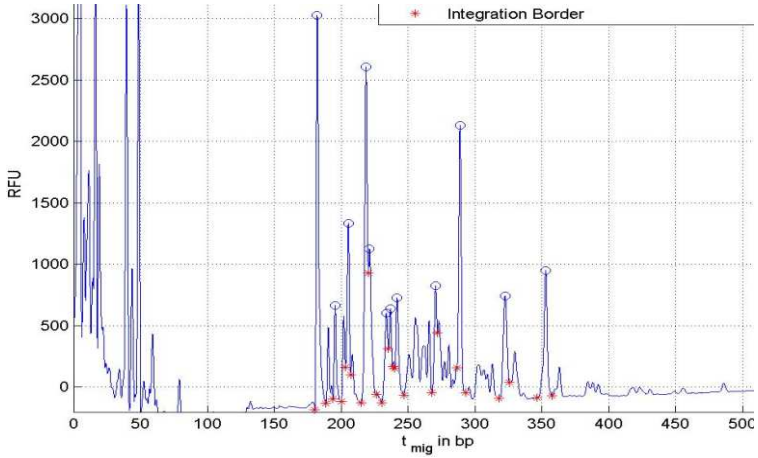
Access to “glyXtool” granted within the framework of a collaboration with E. RAPP, Max Planck Institute, Magdeburg, Germany

CGE-LIF Shows Wide Application Range

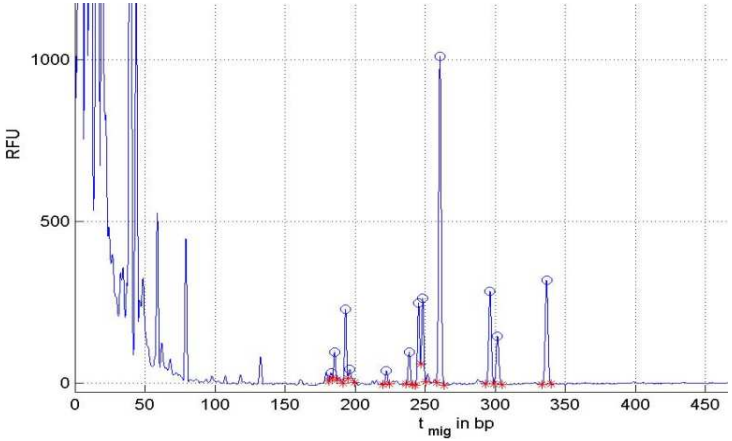
Classical h-mAb



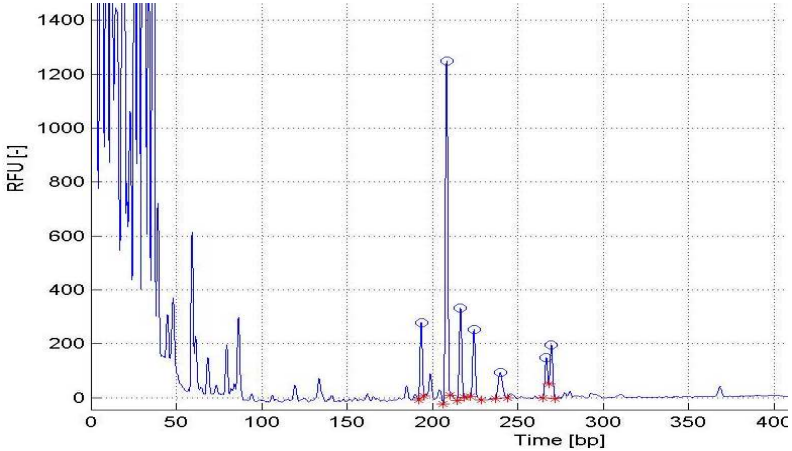
Highly glycosylated Fc-fusion



Highly glycosylated h-mAb



Small glycoprotein

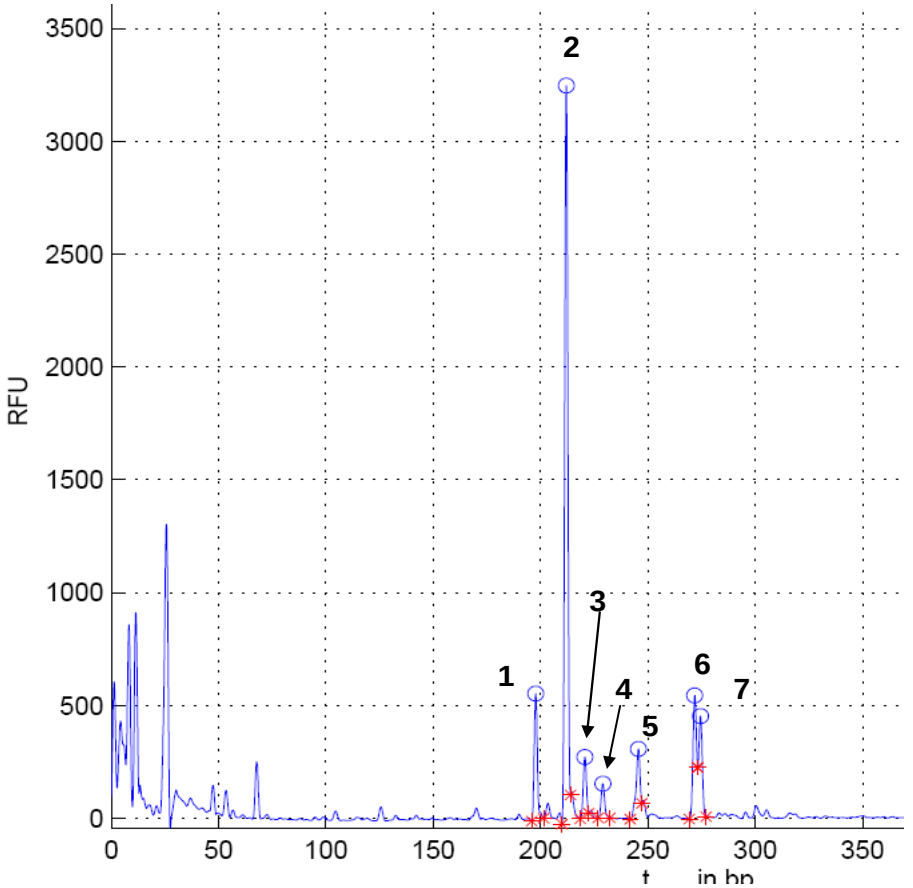


CGE-LIF Yields Excellent Reproducibility

Glycoprotein with 7 main N-oligosaccharides, 12 preparations per run, 3 independent runs.

Migration Time		
Peak	AVG	STDEV%
1	198.16	0.17
2	212.44	0.17
3	221.11	0.17
4	229.49	0.17
5	246.13	0.17
6	272.44	0.17
7	275.10	0.17

Relative Peak Height %		
Peak	AVG	STDEV%
1	10.18	1.82
2	58.79	0.35
3	4.89	1.06
4	2.79	2.62
5	5.55	1.26
6	9.73	1.66
7	8.08	1.47



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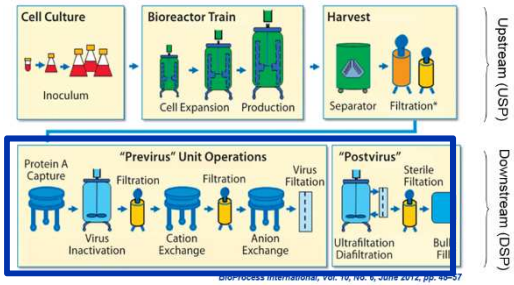

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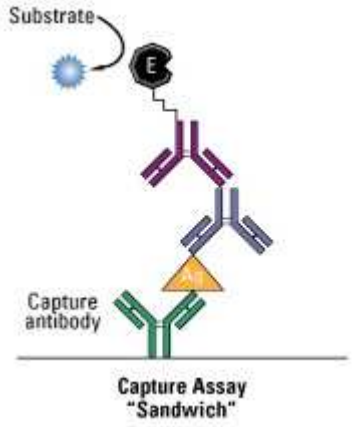
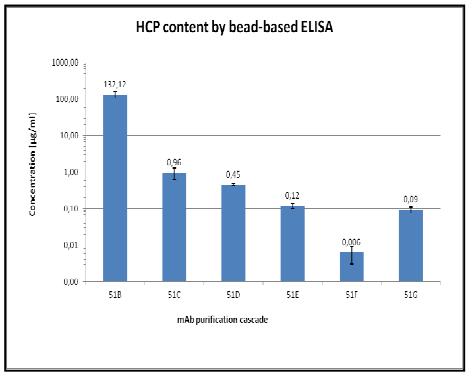
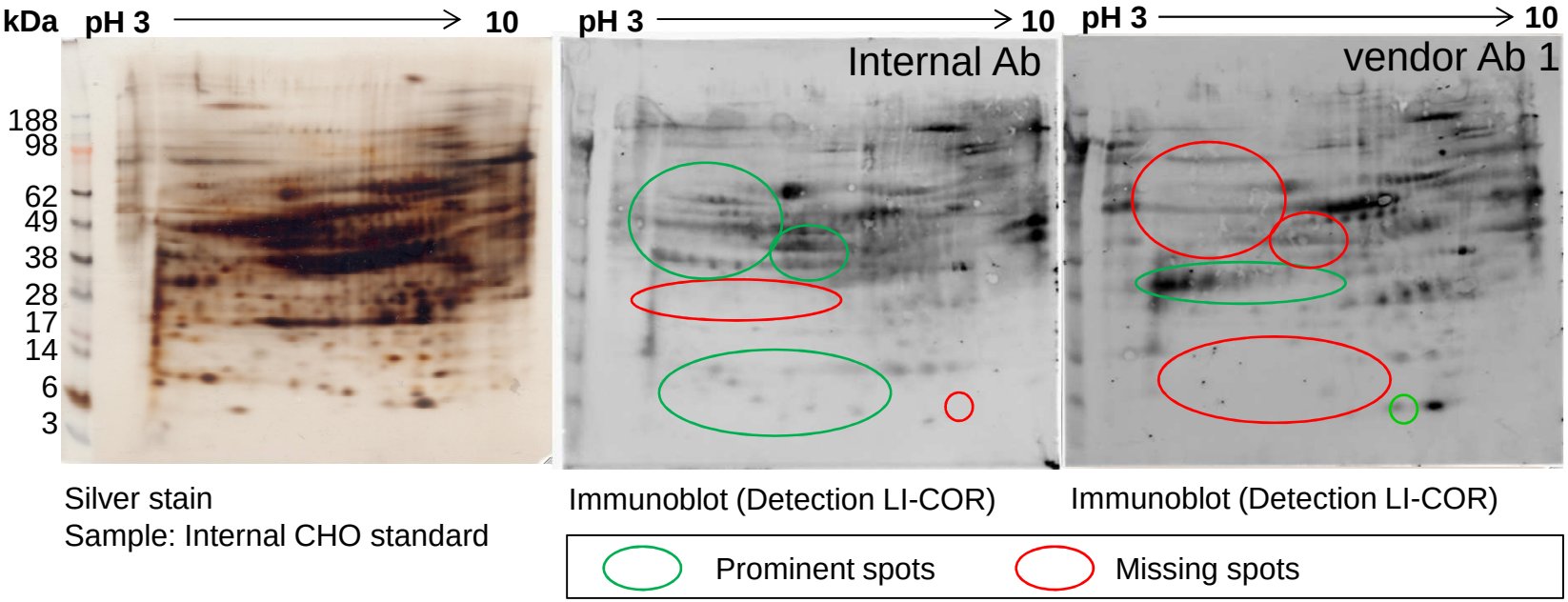
Current Standard Host Cell Protein (HCP) Analysis is Limited

- Standard approach for detection of HCP is still ELISA based detection using polyclonal antisera against the host cell proteome
- Limitations of this approach
  - Gives only overall estimate of HCP content
  - Coverage of HCPs strongly depends on the quality of the antiserum (especially true for commercial assays)
  - Low or non immunogenic proteins are not captured
- Consequently, Health Authorities are requesting development of cell-line specific HCP assays including extensive characterization
- In addition, mass spectrometric (LC-MS) approaches are increasingly implemented as orthogonal approaches





Standard HCP Characterization by 2D-PAGE/ Immunoblotting



- 2D Immunoblotting technique still state of the art for mAb characterization, but limited in throughput and reproducibility/quality of results.

Assessment of the Simple Western Technology for HCP Characterization

- Simple Western Technology developed for preclinical R&D to analyze individual target proteins in cell / tissue lysates

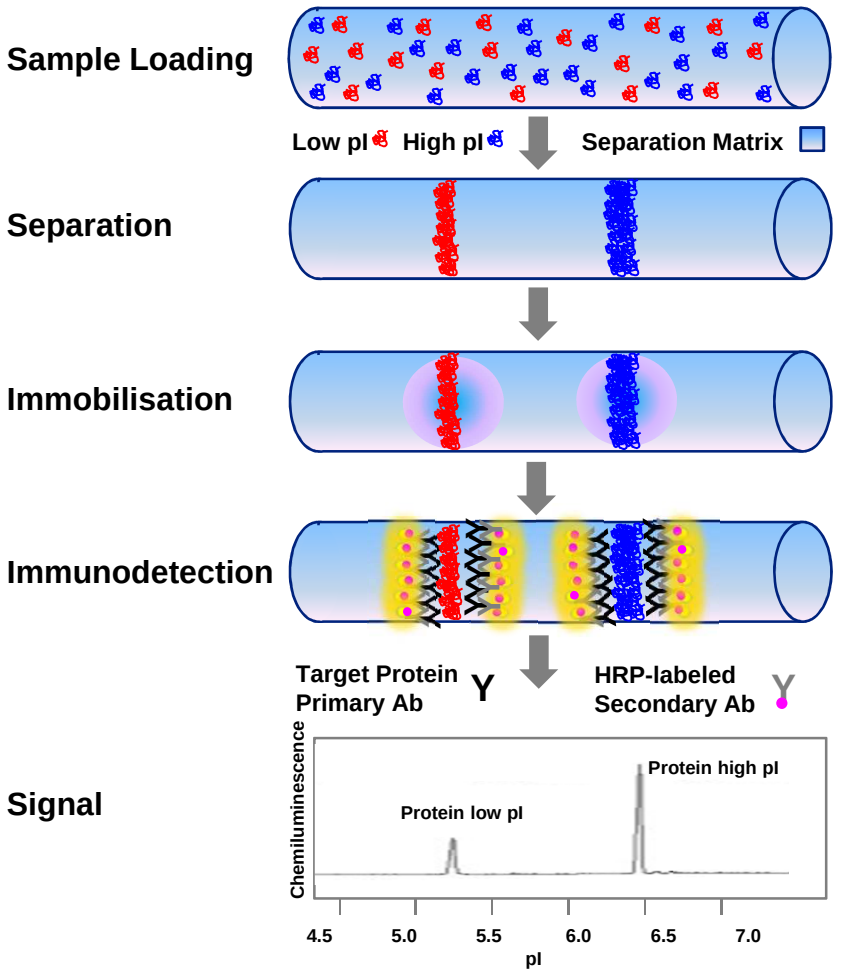
Can the CE-based, HT Simple Western technology add value to the HCP characterization and assay development?

Picture by courtesy of Protein Simple

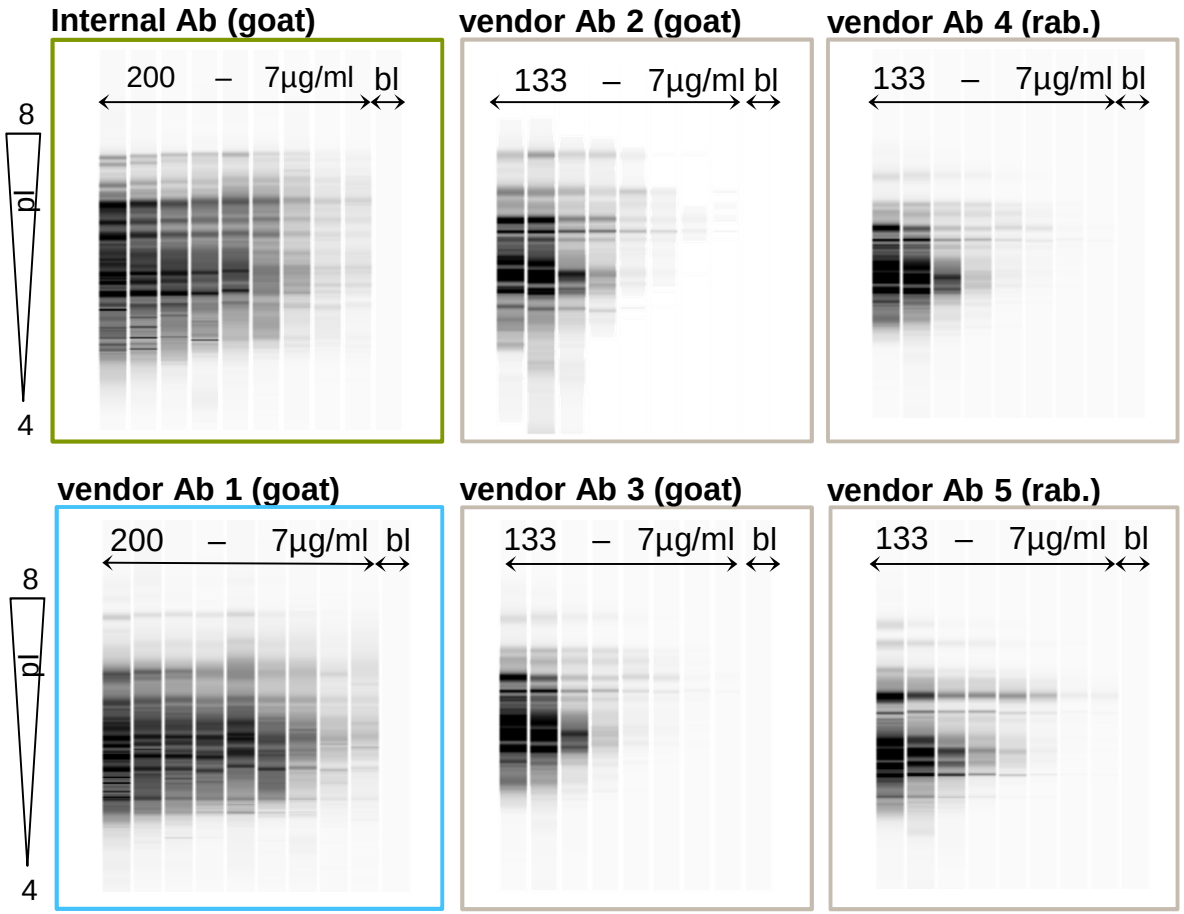
The Simple Western™ Technology (Protein Simple)

Technology

- Size- or charge-based separation of proteins by capillary electrophoresis (SDS-CGE / **cIEF**)
- UV-fixation of separated proteins to capillary wall
- Chemiluminescence immunodetection by in-capillary incubation with 1st Ab and HRP-labeled 2nd Ab / Luminol
- Analysis using electropherograms or visualization as gel-like pictures
- Throughput 88 samples per run (12-24 h)



Multiple HCP Antibody Titrations can be Performed in one Experiment



◀ **Gel-like pictures**

Sample:
Internal CHO standard

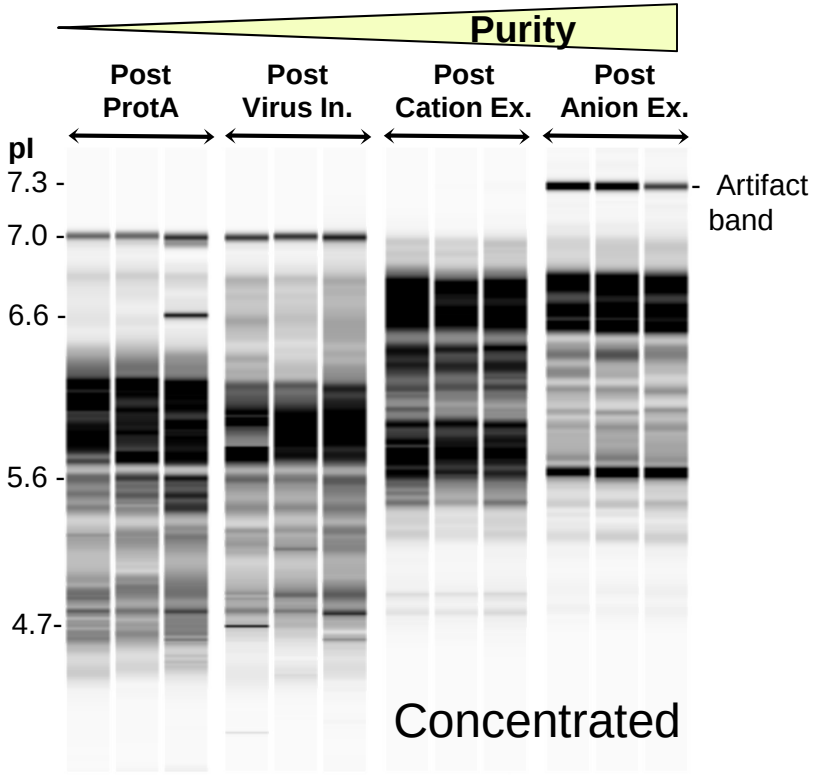
bl = blank (w/o 1st Ab)

Benefits:

- Increased throughput
- Improved resolution
- Improved consistency

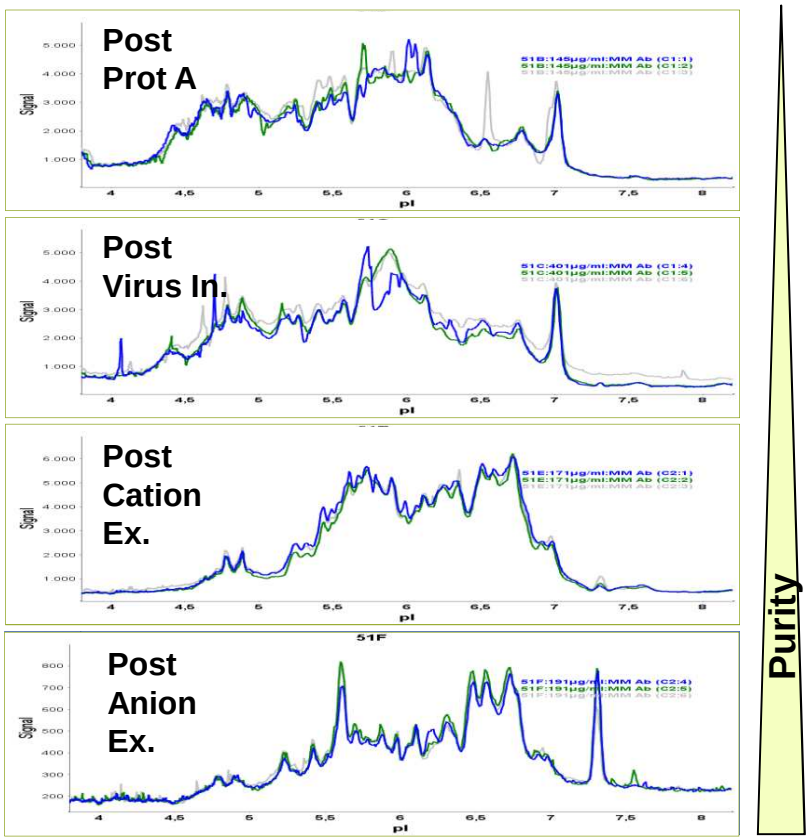
Analysis of HCP pI Distribution in a mAb Purification Cascade can Guide DSP Development

Gel-like picture



NOTE: Black-white intensities between different purification fractions do not represent quantitative relations, as the contrast was adjusted manually in order to obtain best possible qualitative data.

Electropherograms



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Agenda

1 Introduction

- Analytics of Biopharmaceuticals
- Process Analytics as a Specific Segment

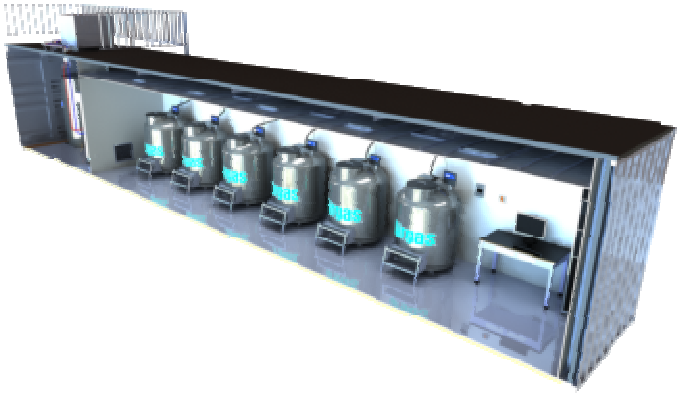
2 Application Examples for Process Analytics

- Cell Culture Analysis by Mid-infrared Spectroscopy (MIR)
- Product Titer and Aggregation Analysis by MIR
- Host Cell Protein Analysis by Simple Western™ Technology

3 Outlook

Future Dimensions of Biomanufacturing

- Modular & flexible manufacturing concepts
- Continuous & closed processes
- Increased use of single-use equipment



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In the Future Analytics and Bioprocess Systems Merge to Create Revolutionary Products

- Technological advancement has proven a clear trend in the direction of “Smart Systems”. These will have integrated sensors to provide complete process and product information in real-time

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In case of further questions, please contact me at

christian.hunzinger@merckgroup.com