Utilizing ‘omics tools to investigate the impact of process changes on product quality in cell culture-based influenza vaccine production

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Authors
Utilizing ‘Omics Tools to Investigate the Impact of Process Changes on Product Quality in Cell Culture-Based Influenza Vaccine Production

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Fundamental research on cell culture based Influenza vaccine production:

- **Process optimization** (esp.: cell growth and virus yield)
- **Mathematical modeling**

**Needed:** Understanding of production systems and parameters on molecular level (e.g.: virus ↔ host cell interaction)
Influenza Virus

- Hemagglutinin
- Nucleocapsid: (s/s (-) sense RNA; polymerase: PB1, PB2, PA; nucleoprotein)
- Lipid envelope
- Neuraminidase

Ø 70-120 nm

3-9 N-Glycosylation Sites


human Influenza A Puerto Rico 8/34 H1N1

- Host
- Virus type
- Geographic origin
- Strain number
- Year of isolation
- Virus subtype

Max Planck Institute Magdeburg

Oomics in Cell Culture-Based Influenza Vaccine Production

May 24th, 2012
Our Motivation for Digging into Glycosylation

- Change of common influenza vaccine production process in chicken eggs to production in mammalian cell cultures
- Influenza vaccine production process (understanding & optimization)
- One important aspect: 
  \(N\)-glycosylation pattern of the major viral membrane-glycoproteins Hemagglutinin (HA) and Neuraminidase (NA)

Glycosylation pattern may affect:
- Viral immunogenicity
- Virus attachment to host cells
- Viral replication dynamics

Glycosylation pattern of may be affected by:
- USP: Virus strain, host cell type, cultivation conditions, virus inactivation
- DSP: Each step of: filtration, centrifugation, & chromatography and the type of adjuvanting

Ribbon representation of the HA\(_6\) trimer from the 1918 influenza A virus

Source: http://www.accessexcellence.org/WS/SU/avianflube04.htm 20.10.05
Glycoconjugates / Glycosylation

**O-Glycosylation**
- Glycan attached to oxygen atom of the amino acids serin and threonine

**N-Glycosylation**
- Glycan attached to nitrogen atom of the amino acid asparagine

**Glycolipids**
- Glycan attached to ceramide (glycosphingolipids) or phosphatidyglycerol (glycophospholipids)

Lipid Bilayer

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Oomics in Cell Culture-Based Influenza Vaccine Production
May 24th, 2012
Typical N-Glycans of Mammalian Cells

High-Mannose Type

Complex Type

Hybrid Type

Antennas

Bisecting

Core Fucose

Core

- N-Acetylglucosamine (GlcNAc)
- Mannose (Man)
- Galactose (Gal)
- Sialic Acid (SA)
- Fucose (Fuc)
Impact of Complex Carbohydrates

Oligosaccharides, glycolipids, glycans etc. play a central role in many aspects of life:

• Key-and-Lock principle for receptors and ligands.

• Signal transduction / communication between cells and pathogens.

• Modification of enzyme / protein activities and specificities.

• Potency and specificity of new drugs and vaccines.

• Health-promoting / preventive functions in food, food additives and functional food.
**xCGE-LIF for Glycoanalysis**  
**Principles and Advantages**

- **separation by m/z**  
- **AND**  
- separation by molecular shape

- NO sample carryover  //  Only ion migration
- Extraordinary separation power and sensitivity
- High reproducibility of migration times (⇒ Longterm RSD for < 0.5%)
- Good reproducibility of relative peak heights (⇒ RSD < 5%)
- Fully automated multicapillary array systems enable “real” HT

⇒  xCGE-LIF with up to 96 capillaries
First powerful "real" HT glycoanalysis-tool (method, software with GUI & database):

the "glyXbox"

⇒ Sample preparation methods

⇒ Glycoanalysis with:
  • Automated parallel separation and sensitive detection with xCGE-LIF systems
  • Automated data-processing \( (\text{glyXdata}) \)
  • Automated data-analysis \( (\text{glyXtool}) \)

⇒ Glycodatabase:
  an oligosaccharide / glycan database \( (\text{glyXbase}) \)

⇒ The system is ready for take-off !

www.glyxera.com
Using glyXbox for Automated HT-Glycoanalysis via xCGE-LIF

(Project of R. Hennig, T. Muth & M. Borowiak)

1a Protein Precipitation (e.g. to Extract Proteins from Citrate Blood Plasma)

1b Protein Purification & Concentration by Affinity-SPE (e.g.: Protein A Beads)

1c Protein Separation by 1D/2D-GE & Cut out of Bands/Spots:

2 On-Membrane, In-solution or In-Gel, Deglycosylation (by PNGase F):

3 Fluorescent Labeling of Released N-Glycans with APTS

4 Post Labeling Clean-Up
- SEC
- HILIC-SPE
- NONE

5 Addition of Internal Standards

6 xCGE-LIF Analysis, Normalization & Data Evaluation


Software-Development for Automated HT Glycoanalysis via xCGE-LIF

(Ongoing project @ glyXera, PhD thesis of R. Hennig & PhD thesis of T. Muth)
Growing Glycan / Oligosaccharide Library for Structural Elucidation via “Migration-Time-Matching”

(Ongoing project of R. Hennig & R. Kottler)

Excerpt from in-house library:

<table>
<thead>
<tr>
<th>No.</th>
<th>N-Glycan Standard Name</th>
<th>Simplified Structure</th>
<th>( t_{mig} ) in MTU$^*$</th>
<th>Major Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0N-2A-2G</td>
<td></td>
<td>252.45 (+/- 0.50)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0N-2A</td>
<td></td>
<td>332.55 (+/- 0.65)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>2N(2,6)-2A+F</td>
<td></td>
<td>180.40 (+/- 1.30)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>0N-2A+2o(13)GalF</td>
<td></td>
<td>436.75 (+/- 0.60)</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Man5</td>
<td></td>
<td>248.35 (+/- 0.40)</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>0N-4A-4G</td>
<td></td>
<td>322.80 (+/- 0.10)</td>
<td></td>
</tr>
</tbody>
</table>

At present:
- Over 70 entries for N-glycans.
- Normalized migration times for two different gel matrices.
- Human milk oligosaccharide database started (about 30 entries).
Summary I

- Systems allows fast and easy characterization of N-glycosylation patterns (qualitative & quantitative) and other carbohydrate pools.

- Highly sensitive - high resolution - “real” high throughput system & method for profiling glycoproteins and other carbohydrate mixtures.

- N-Glycans and other carbohydrates can be analyzed on three levels:
  - Fingerprint Analysis
  - Glycoprofiling
  - Extended Structural Analysis
Higher molecular weights MW for all variants of the H3N2 virus, compared to the H1N1 variants.

Differences in Molecular Weight of HA Due to Differences in N-Glycosylation

<table>
<thead>
<tr>
<th>variant and virus</th>
<th>overall MW of HA (estimated via SDS-PAGE) [kDa]</th>
<th>calculated mass of HA AA (^1) sequence* [kDa]</th>
<th>Estimated mass of HA N-glycan pool [kDa]</th>
<th>TNP</th>
<th>(t_{\text{mig}}) range of HA N-glycans [bp]</th>
</tr>
</thead>
<tbody>
<tr>
<td>M H1N1</td>
<td>79±5</td>
<td>63.0</td>
<td>16±5</td>
<td>16</td>
<td>273.3-426.5</td>
</tr>
<tr>
<td>V H1N1</td>
<td>68±5</td>
<td>63.0</td>
<td>5(±5)</td>
<td>16</td>
<td>214.0-378.3</td>
</tr>
<tr>
<td>C H1N1</td>
<td>68±5</td>
<td>63.0</td>
<td>5(±5)</td>
<td>14</td>
<td>222.5-385.0</td>
</tr>
<tr>
<td>H H1N1</td>
<td>68±5</td>
<td>63.0</td>
<td>5(±5)</td>
<td>14</td>
<td>243.0-406.9</td>
</tr>
<tr>
<td>A H1N1</td>
<td>65±5</td>
<td>63.0</td>
<td>2(±5)</td>
<td>11</td>
<td>214.0-406.9</td>
</tr>
<tr>
<td>M H3N2</td>
<td>95±5</td>
<td>62.1</td>
<td>31±5</td>
<td>34</td>
<td>57.9-418.0</td>
</tr>
<tr>
<td>V H3N2</td>
<td>81±5</td>
<td>62.1</td>
<td>17±5</td>
<td>29</td>
<td>51.8-372.5</td>
</tr>
<tr>
<td>R H3N2</td>
<td>93±5</td>
<td>62.1</td>
<td>29±5</td>
<td>19</td>
<td>64.0-308.9</td>
</tr>
<tr>
<td>M B/Mal</td>
<td>86±5</td>
<td>65.6</td>
<td>22±5</td>
<td>37</td>
<td>171.5-418.0</td>
</tr>
</tbody>
</table>

- Almost identical MW comparing only AA-sequences
- Significant differences in MW for glycosylated forms

\((\text{virus \& host cell related})\)

Variations in the MW of the HA proteins of the different virus variants correlate with their HA N-glycan amount (TNP) and size distribution.
Application of glyXbox to Generate HA N-Glycan Fingerprints of Influenza Viruses Produced in Different Cell Lines

*PhD thesis of S. Schwarzer & J. Rödig – partly in coop. with ProBioGen (Berlin/D)*

---

**Variants of H1N1**

**Differences host cell related**

**Differences virus related**

**Variants of H3N2**
# Structural Investigation of HA N-glycan Pools of the Different Influenza Viruses

<table>
<thead>
<tr>
<th>virus and variant</th>
<th>complex type; with terminal ...</th>
<th>core fucosylation</th>
<th>high mannose type</th>
<th>hybrid type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/PR/8/34 H1N1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-variant</td>
<td>all; α- (8; 10-16)² and β-galactose (2-7; 9)²</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>V-variant</td>
<td>most; β-galactose (6-16)²</td>
<td>yes</td>
<td>some</td>
<td>no</td>
</tr>
<tr>
<td>C-variant</td>
<td>most; β-galactose (5-14)²</td>
<td>yes</td>
<td>some</td>
<td>some</td>
</tr>
<tr>
<td>A-variant</td>
<td>all; β-galactose (5-11)²</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>H-variant</td>
<td>all; β-galactose (4-14)²</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A/WSN/67/2005 H3N2</th>
<th></th>
<th></th>
<th>major peaks (16, some &gt;16)²</th>
<th>no</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-variant</td>
<td>few; α- and β-galactose (some &gt; 16)²</td>
<td>ND¹</td>
<td>major peaks (9-16, 19-22, some &gt; 23)²</td>
<td>no</td>
</tr>
<tr>
<td>V-variant</td>
<td>some; β-galactose (17,18,23, some &gt; 23)²</td>
<td>ND¹</td>
<td>major peaks (12,13,16,18)²</td>
<td>no</td>
</tr>
<tr>
<td>R-variant</td>
<td>no</td>
<td>ND¹</td>
<td>few (11,14,15,17,19)²</td>
<td>major peaks</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B/Mal/2506/2004</th>
<th></th>
<th></th>
<th>major peaks (all &lt; 13, some &gt; 15)²</th>
<th>no</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-variant</td>
<td>some; β-galactose (13-15, some &gt; 15)²</td>
<td>ND¹</td>
<td>major peaks (all &lt; 13, some &gt; 15)²</td>
<td>no</td>
</tr>
</tbody>
</table>

¹ ND, not determined
² numbers of the peaks (corresponding to glyXdata peaklists of the normalized EPGs) related to the particular N-glycan types
• Virus and host cell type are determining the HA N-glycosylation pattern.

• Both seem to impact the principal N-glycan type attached.

• Virus mainly determines the number of different N-glycans attached.

• Host cell mainly causes:  
  - Variations of (monomeric) constitution of single N-glycans.
  - Shifts of N-glycan pool composition.  
    (percentage of different N-glycan types)
Virus Adaptation to Host Cells

Influenza Virus A PR/8/34 (H1N1) from RKI
Influenza Virus A PR/8/34 (H1N1) from NIBSC

Sequence Analysis of Virus Genomes

N-Glycosylation Pattern Analysis

1st 2nd 3rd 4th 5th 6th 7th 8th 9th 10th 11th

MDCK Vero MDCK
Virus Titer Improves During Adaptation

[Graph showing the logarithm of HA titer (log HA units/100 µL) over time post infection (hpi) for different datasets labeled (2.), (3.), (4.), (5.), and (6.).]
### HA Quasispecies Composition

**RKI ↔ NIBSC**

#### Influenza Virus A PR/8/34 (H1N1) from RKI

<table>
<thead>
<tr>
<th>DNA Level</th>
<th>Protein Level</th>
<th>Passage 1</th>
<th>Passage 6</th>
<th>Passage 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 1370 T</td>
<td>S 457 T</td>
<td>0</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>A 1378 G</td>
<td>K 460 E</td>
<td>0</td>
<td>80</td>
<td>81</td>
</tr>
</tbody>
</table>

Initial seed virus: no AA-substitutions

#### Influenza Virus A PR/8/34 (H1N1) from NIBSC

<table>
<thead>
<tr>
<th>DNA Level</th>
<th>Protein Level</th>
<th>Population Ratio [%]</th>
<th>Population Ratio [%]</th>
<th>Population Ratio [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 170 C</td>
<td>Y 24 H</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G 1183 A</td>
<td>V 395 M</td>
<td>41.5</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>A 1189 G</td>
<td>T 397 A</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A 1189 T</td>
<td>T 397 S</td>
<td>0.6</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>G 1333 T</td>
<td>D 455 Y</td>
<td>21.4</td>
<td>6.1</td>
<td>3.2</td>
</tr>
<tr>
<td>G 1333 C</td>
<td>D 455 H</td>
<td>0</td>
<td>52</td>
<td>44.1</td>
</tr>
<tr>
<td>A 1375 G</td>
<td>K 459 E</td>
<td>0</td>
<td>0</td>
<td>44.2</td>
</tr>
<tr>
<td>A 1376 G</td>
<td>N 460 D</td>
<td>12.2</td>
<td>41.1</td>
<td>10.5</td>
</tr>
</tbody>
</table>
Substitution Sites within the HA-Molecule

A/PR/8/34 from RKI

A/PR/8/34 from NIBSC
Do Substitutions Increase Viral Fitness in Vero?

Sequence Analysis of Virus Genomes

N-Glycosylation Pattern Analysis

1st 2nd 3rd 4th 5th 6th 7th 8th 9th 10th 11th

MDCK Vero MDCK

Improved viral Fitness?
Improved Viral Fitness also in Vero!

MDCK → Vero → MDCK → Vero

- Vero-experienced NIBSC / RKI
- H1N1/PR/8/34/NIBSC initial
- H1N1/PR/8/34/RKI initial

- 2nd Initial Seed RKI
- 2nd Initial Seed NIBSC
- Vero-exp.1 RKI (2nd)
- Vero-exp.1 NIBSC (12th)
- Vero-exp.2 RKI (13th)
- Vero-exp.2 NIBSC (13th)
- Vero-exp.3 RKI (14th)
- Vero-exp.3 NIBSC (14th)

Helene Kaffka

Max Planck Institute Magdeburg
Oomics in Cell Culture-Based Influenza Vaccine Production

May 24th, 2012
• „Unexperienced“ Influenza viruses being adapted to new host cells need 2-3 passages to stabilize their glycosylation pattern.

• NIBSC derived seed virus shows much more heterogeneous quasispezies composition than RKI derived.

• Challenging Influenza viruses with new host cells results in “rescue mutations” and quasispezies diversification.

• “Experienced” Influenza viruses show improved viral fitness.
Impact of HA N-Glycosylation on Immunogenicity of Influenza Viruses Produced in Different Cell Lines

(PhD thesis of J. Rödig - in coop. with Dr. Bernd Lepenies @ MPI of Colloids and Interfaces)

**Whole spleen cell stimulation assay (in vitro)**

- **Isolation of spleen cells**
- **TCR-HA transgenic mouse** (TCRαβ specific for HA$_{110-120}$)
- **HA$_{110-120}$ MDCK-variant** (synthetic peptide) (glycos./ deglycos.)
- **Vero-variant** (glycos./ deglycos.)
- **Supernatant:** Cytokine production (IL-2, IFN-γ, IL-4)
- **Cells:** T cell activation markers (CD69, CD25)
- **Incubation at 37 °C for 48 h**

(Performed by Julia Hütter @ MPI of Colloids and Interfaces)
Significantly higher frequency of T cells expressing the activation marker CD69 upon stimulation with the Vero cell-derived influenza virus glycovariant.
IL-2 and IFN-γ are produced in significantly higher levels by splenocytes stimulated with the Vero cell-derived influenza virus glycovariant.
Hemagglutinin - Derived from Glycosylated & Deglycosylated Virus

MDCK cell-derived Influenza Virus A PR/8/34 (H1N1)

Vero cell-derived Influenza Virus A PR/8/34 (H1N1)

(i) fully N-glycosylated
(ii) deglycosylated
(iii) overlay

(i) fully N-glycosylated
(ii) deglycosylated
(iii) overlay
Virus Deglycosylation Abolishes Cytokine Production

- CD69$^+$ of CD4$^+$ cells [%]
- IL-2 [pg/ml]
- IL-4 [pg/ml]
- IFN-γ [pg/ml]

Protein [µg/ml]: 0.01, 0.1, 1

MDCK, MDCK deglycosylated, Vero, Vero deglycosylated
The platform of pyrosequencing, glycoanalysis and immunogenicity assays allows to investigate immunogenic differences of influenza virus glycovariants.

Hemagglutinin N-glycosylation has a significant impact on immunogenicity.

The differential immune stimulatory effects mediated by the influenza virus glycovariants seem to be also relevant in vivo.

These findings might impact cell line-based influenza vaccine design.
Outlook

- Screening for the optimal influenza production system using pyrosequencing, glycoanalysis and immunogenicity assays => “Sweet” vaccine design
- Extension of N-glycan and HMOS libraries and generation of other oligosaccharide libraries (e.g. O-glycans)
- Applying this method to other fields: (e.g. in the context of the "HighGlycan" EU-consortium)
  - Glycome GWAS studies
  - Biopharmaceuticals like recombinant glycoproteins or vaccines
  - Functional food (e.g. infant nutrition) & food additives
  - Large scale clinical studies
  - Early diagnosis of diseases (e.g.: diabetes, cancer, …)
  - ...
- Commercialized via: www.glyxera.com
Thanks to ...

... YOU

... our cooperation partners:

... the BPE-Group

esp.:
  • Yvonne Genzel
  • Udo Reichl

... and the A-Team !!!

esp.:
  • Jana Schwarzer
  ... now J.Bohne
  @ Novartis Vaccines
  • Jana Rödig
  • René Hennig
Influenza Vaccine Production

Classical production:

- 1-3 eggs per vaccination
- 5 mio. vaccinations every year

Advantages:

- Cell culture derived viruses are closer to the lateron human host
- Alternative for patients showing allergic reactions against chicken proteins
- Enables faster vaccine production scale-up in case of epidemics or pandemics
- Enables vaccine production for protection against avian influenza (H5N1)

Trend:

- Mammalian cell culture
Labeling

\[ \text{GlcNAc} + \text{APTS} \xrightarrow{\text{NaBH}_3\text{CN}} \text{APTS- GlcNAc} \]

Modified from: Chen et al. (1998) Glycobiology (8): 1045-1052

APTS = 8-amino-1,3,6-pyrenetrisulfonic acid
Schematic Representation of N-Glycans

NeuAcα2-3Galβ1-4GlcNacα1-2Manα1
NeuAcα2-3Galβ1-4GlcNacα1-2Manα1

6^-Manβ1-4GlcNacβ1-4GlcNacβ1-Asn

APTS
Sequential Exoglycosidase Digestion

* NeuAcα(2-3/6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)

* NeuAcα(2-3/6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)

α-sialidase

β-galactosidase

α-mannosidase

β-N-acetylhexosaminidase

* can be substituted with α-galactose

α-Fucosidase

Max Planck Institute Magdeburg

Omics in Cell Culture-Based Influenza Vaccine Production

May 24th, 2012
Bio/Process-Analytics

Hardware-, Software- & Method-Development

Genomics

Poteomics

Transcriptomics

Metabolomics

Qualitative Protein Analysis

Quantitative Protein Analysis

Analysis of Post-Translational Modifications

• Phosphorylation
• Glycosylation
• …

Team:
9 PhD Students, 8 Students, 1 Techn. Assistant

Instrumentation:

• Capillary-(Gel-)Electrophoresis Systems:
  - CE System with UV-Detection
  - Multiplexing CGE-LIF Systems (xCGE-LIF)
• HPLC Systems:
  - HPLC-FLR
  - nanoHPLC-LIF
  - HPAEC-PAD
• Diverse Gelelectrophoresis Systems
  (1D, 2D & DIGE)
• LC-MS/MS Systems:
  - Online: nanoHPLC-QqTof & nanoHPLC-QIT
  - Offline: 2D-nanoHPLC & MALDI-Tof/Tof
• GC-MS/MS System

Glycomics, Glycoproteomics & Carbohydrate Analytics

• Glycans
• Glycopeptides
• Glycoproteins and
• other Carbohydrates (e.g.: Milk oligosaccharides)
Glycomics & Glycoproteomics Toolbox

**Glycomics & Glycoproteomics**

- **Glycopeptide analysis**
  - proteolytic digestion
  - Glycopeptide trapping and separation by (2D)-nano-HPLC
    - Consecutive eluting glycopeptides
      - Online detection by ESI-(MS)/MS
      - Identification of glycosylation sites
    - Separated glycopeptides directly spotted onto MALDI targets via micro-fraction-collector
      - Optional on target deglycos. with endoglycosidase
      - Offline detection by MALDI-MS/(MS)
      - Database Search
      - Structural elucidation of glycans

- **Glycan Analysis**
  - In-gel-glycosylation with endoglycosidase
    - Glycan trapping and separation by (2D)-nano-HPLC directly spotted onto MALDI targets
  - 2AB/AA-glycans
    - HPLC glycan fingerprint
      - Seq. Exoglycosidase Digest.
        - HPLC glycan fingerprint
          - Online detection by ESI-(MS)/MS
          - Structural elucidation of glycans
    - APTS-glycans
      - CGE glycan fingerprint
        - Seq. Exoglycosidase Digest.
          - CGE glycan fingerprint
            - Database Search
            - Structural elucidation of glycans

- **Protein Analysis**
  - Glycans
    - non-labelled
    - pos. labelled
    - neg. labelled
  - Offline detection by MALDI or nanoESI-MS/(MS)
  - Offline detection by MALDI-MS/(MS)
  - Database Search

Max Planck Institute Magdeburg | Omics in Cell Culture-Based Influenza Vaccine Production | May 24th, 2012
CGE-LIF Based Glycomics

Glycoproteins → In-gel / In-solution Deglycosylation with Endoglycosidase → Glycans → Fluorescent Labelling with APTS

Protein Analysis

"Fingerprint" Comparison

Electropherogram Overlay

CGE-LIF Analysis

Structural elucidation

Database Search

Sequencial Exoglycosidase Digestion (SED)

CGE-LIF Analysis

APTS-Glycans
HILIC-FLR vs. xCGE-LIF

Separation power, performance and sensitivity:

HILIC-FLR  
(sample amount 7500 nL)

xCGE-LIF (via DNA-Sequencer)  
(sample amount 2 nL)

Separation of two aliquots of the same sample: the "blood-plasma glycome"

⇒ Separation power more than one order of magnitude better !
⇒ Sensitivity more than three orders of magnitude higher !
Overlay of 12 "fingerprints" of the N-glycan pool of a mAB:

- Limit of detection: 50 attomole on column.
- Linear dynamic range: 4 orders of magnitude.
- Good reproducibility with respect to relative peak heights.
- RSD for migration times of more than 36 consecutive runs < 0.03%.

(xCGE-LIF analyses of 3 techn. replicates à 12 repeated runs)
- Longterm RSD (about two years) for migration times < 0.5%.
Application of glyXbox for Automated HT Blood Plasma Glycomics

(Project of R. Hennig & M. Borowiak - in coop. with Dr. Manfred Wuhrer @ LUMC (Leiden/NL))

⇒ Separation of more than 4500 samples in 48 hours!
⇒ Due to multiplexing with up to 96 capillaries in parallel, 90 - 450 times faster than comparable analysis methods!
Separation Power of N-Glycan Analysis via xCGE-LIF

A2FG2S2(2,6)

A2FG2S2(2,3)

Signal Intensity in RFU (Offset 2000 RFU)

Migration Time in MTU

Max Planck Institute Magdeburg

Omics in Cell Culture-Based Influenza Vaccine Production

May 24th, 2012
## Typical Cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Derivation</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>Chinese hamster ovary</td>
<td>good product glycosylation</td>
</tr>
<tr>
<td>CHO dhfr</td>
<td>Mutant for genetic amplification</td>
<td>recombinant products</td>
</tr>
<tr>
<td>BHK21</td>
<td>Syrian hamster</td>
<td>viruses/veterinary vaccines</td>
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<tr>
<td>HeLa</td>
<td>Human cervical adenocarcinoma</td>
<td>polio vaccine</td>
</tr>
<tr>
<td>Namalwa</td>
<td>Human B lymphoblastoid</td>
<td>Interferon</td>
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<tr>
<td>COS 1/COS 7</td>
<td>African green monkey kidney</td>
<td>recombinant products</td>
</tr>
<tr>
<td>293</td>
<td>Adenovirus transformed HEK</td>
<td>Gene therapy</td>
</tr>
<tr>
<td>Sf9</td>
<td>Spodoptera frugipeda</td>
<td>Baculoviruses</td>
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<tr>
<td>MDCK</td>
<td>Cocker spaniel kidney</td>
<td>viral vaccines</td>
</tr>
<tr>
<td>Vero</td>
<td>African green monkey, kidney</td>
<td>viral vaccines</td>
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<tr>
<td>AGE1.CR</td>
<td>Duck, retina</td>
<td>viral vaccines</td>
</tr>
<tr>
<td>A549</td>
<td>Human, lung epithel carcinoma</td>
<td>viral vaccines</td>
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<tr>
<td>HepG2</td>
<td>Human, hepatocell. epith. carcin.</td>
<td>viral vaccines</td>
</tr>
<tr>
<td>RCAr</td>
<td>modified MDCK</td>
<td>viral vaccines</td>
</tr>
</tbody>
</table>
Antigenic Drift

RNA
Hemagglutinin
Neuraminidase
Antibodies
Sialic acid

modified antigen

Modified from: Influenza: Virus and Disease; Roche homepage; Oktober 2005
Antigenic Shift

Reassortment

Modified from: Influenza: Virus and Disease; Roche homepage; Oktober 2005
HA N-Glycan Fingerprints of Different Human Influenza Viruses Produced in MDCK cells

Differences virus related
**Multiple Substitutions in Consensus Sequence**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Coded Protein</th>
<th>bp-Substitution</th>
<th>AA-substitution</th>
<th>Passage 1</th>
<th>Passage 6</th>
<th>Passage 11</th>
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<tr>
<td>1</td>
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<td>C 588 G</td>
<td>C 196 W</td>
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<td>D 50 D</td>
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<td>G 585 A</td>
<td>E 195 E</td>
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<td></td>
<td>A 189 G</td>
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<tr>
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<td>C 1370 T</td>
<td>S 457 L</td>
<td>19*</td>
<td>9*</td>
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<td></td>
<td>A 1378 G</td>
<td>K 460 E</td>
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<td>81*</td>
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<td></td>
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<td>initial seed virus</td>
<td>no AA-substitutions</td>
<td>100</td>
<td>few reads</td>
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<td>5</td>
<td>NP</td>
<td>A 889 C</td>
<td>S 287 R</td>
<td>22</td>
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<td>G 882 T</td>
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<td>I 7 M</td>
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<td>T 397 T</td>
<td>S 103 P</td>
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</tbody>
</table>

=> Substitutions Improve Viral Fitness in Vero Host Cell System
Crucial role of CD11c+ dendritic cells

- CD11c+ dendritic cells were separated by magnetic cell separation (MACS) and co-cultivated with TCR-HA transgenic T cells
- Dendritic cells are responsible for the differential T cell activation, presumably by differential recognition and/or uptake of the glycovariants
Adoptive transfer of transgenic T cells

TCR-HA Tg mouse

Isolation of spleen cells

T cell separation by MACS

Staining with cell dye e670

Analysis of proliferation and cell activation (FACS, ELISpot)

Immunization with influenza glycovariants

Adoptive T cell transfer

Balb/c wt mouse

4 days

1 day
In vivo T cell activation and proliferation

Increased IL-2 production upon immunization with Vero cell-derived glycovariant
=> observed effects might also be relevant in vivo