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

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

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MAX-PLANCK-INSTITUT
DYNAMIK KOMPLEXER
TECHNISCHER SYSTEME
MAGDEBURG

Bioprocess Engineering at MPI

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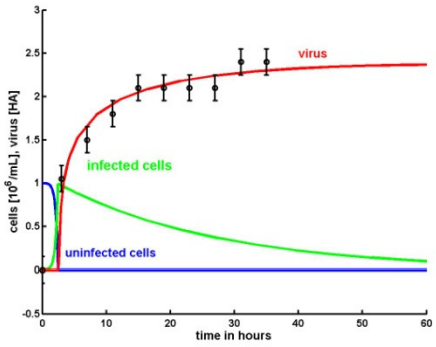
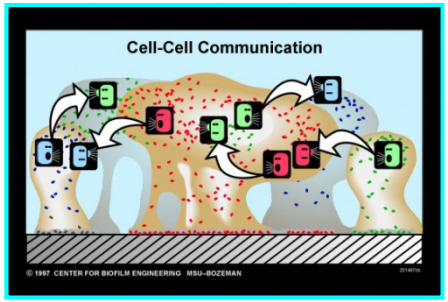
Fundamental research on cell culture based Influenza vaccine production:

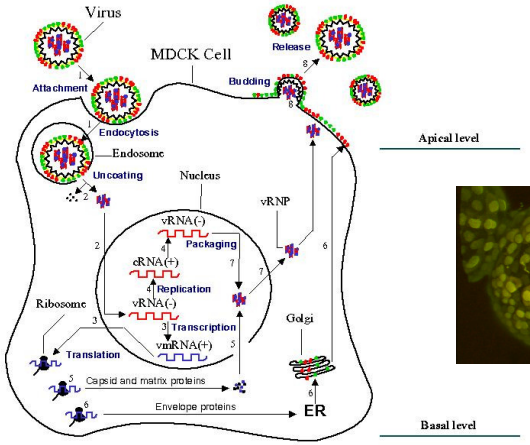
- **Process optimization**
  (esp.: cell growth and virus yield)
- **Mathematical modeling**

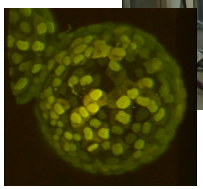
⇨ **Needed:**

Understanding of production systems and parameters on molecular level
(e.g.: virus ⇔ host cell interaction)

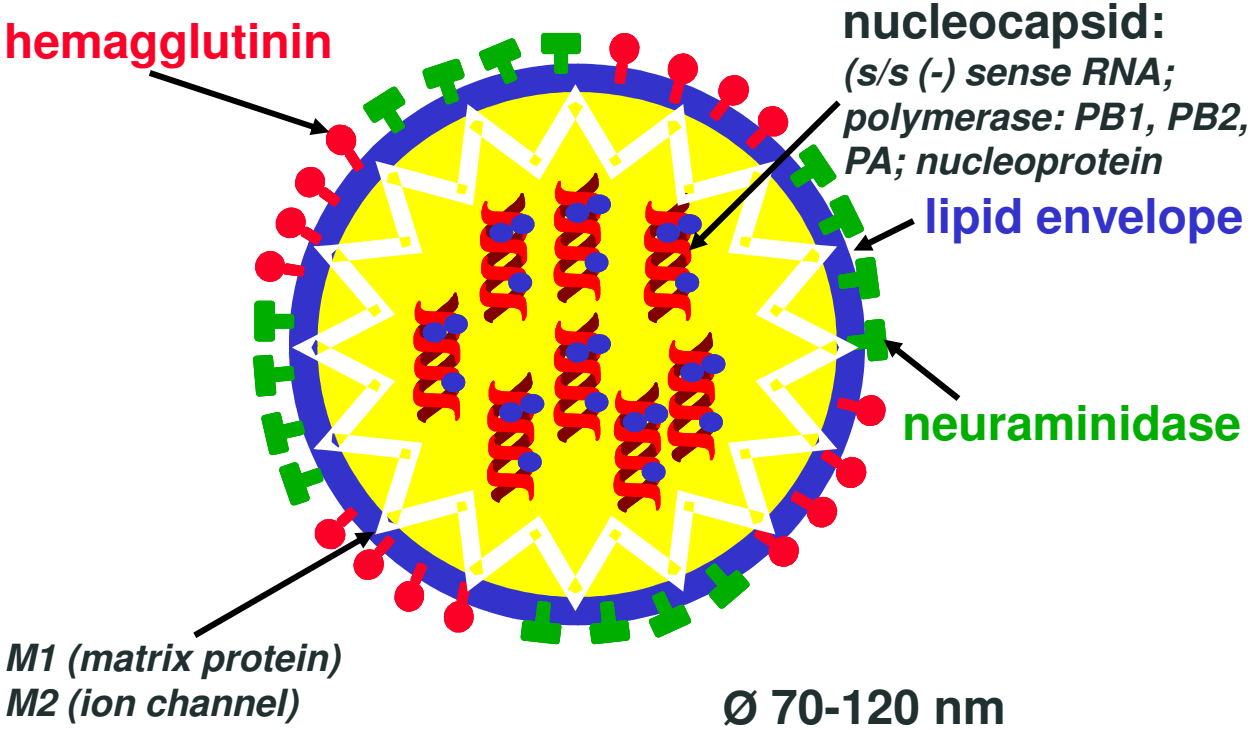






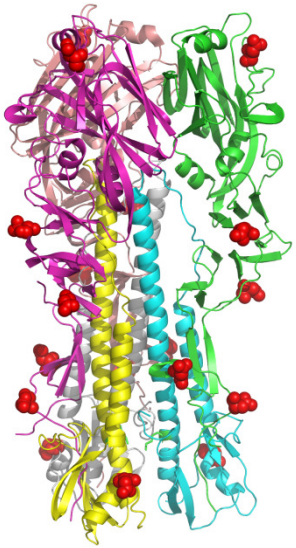


Influenza Virus



Ø 70-120 nm

Hemagglutinin Trimer



3-9 N-Glycosylation Sites

Edited from PDB: 1RU7F: molecule 1RU7, chain 70;
Gamblin et al., Science, 2004, Vol 303, p. 1838-1842

human Influenza A Puerto Rico 8/34 H1N1

host

virus type

geographic origin

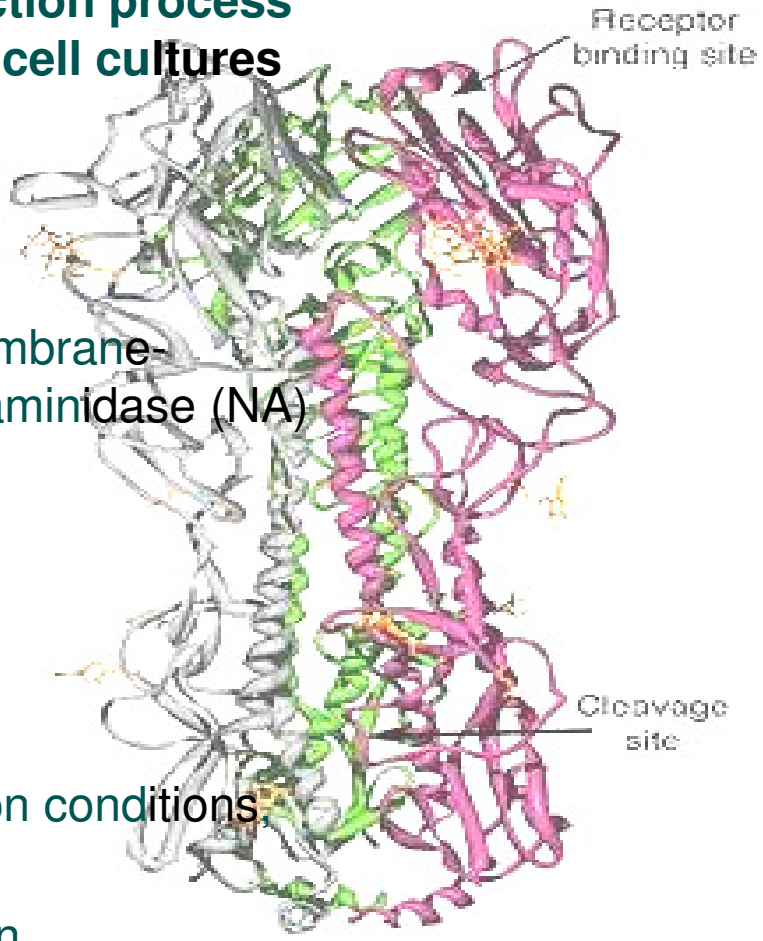
strain number

year of isolation

virus subtype

Our Motivation for Digging into Glycosylation

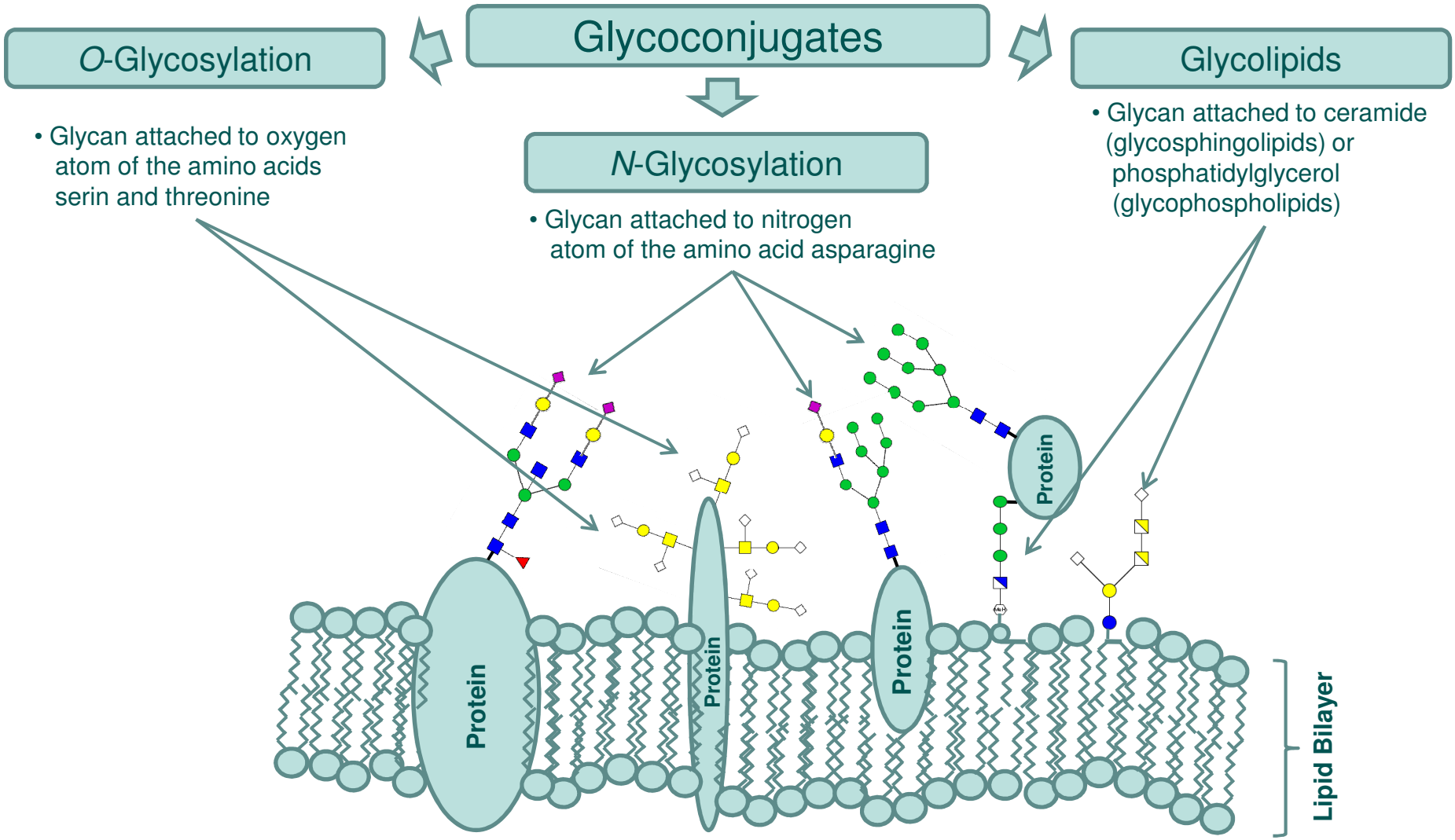
- **Change of common influenza vaccine production process in chicken eggs to production in mammalian cell cultures**
- **Influenza vaccine production process**
  (understanding & optimization)
- **One important aspect:**
  N-glycosylation pattern of the major viral membrane-glycoproteins Hemagglutinin (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_0 trimer from the 1918 influenza A virus

Source: http://www.accessexcellence.org/WN/SU/avianflufeb04.htm 20.10.05

Glycoconjugates / Glycosylation



Typical N-Glycans of Mammalian Cells

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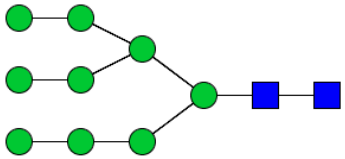
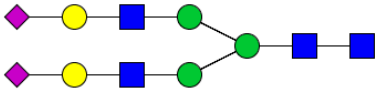
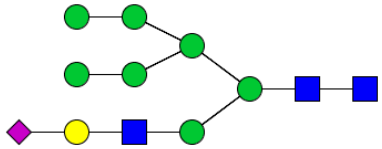
High-Mannose Type

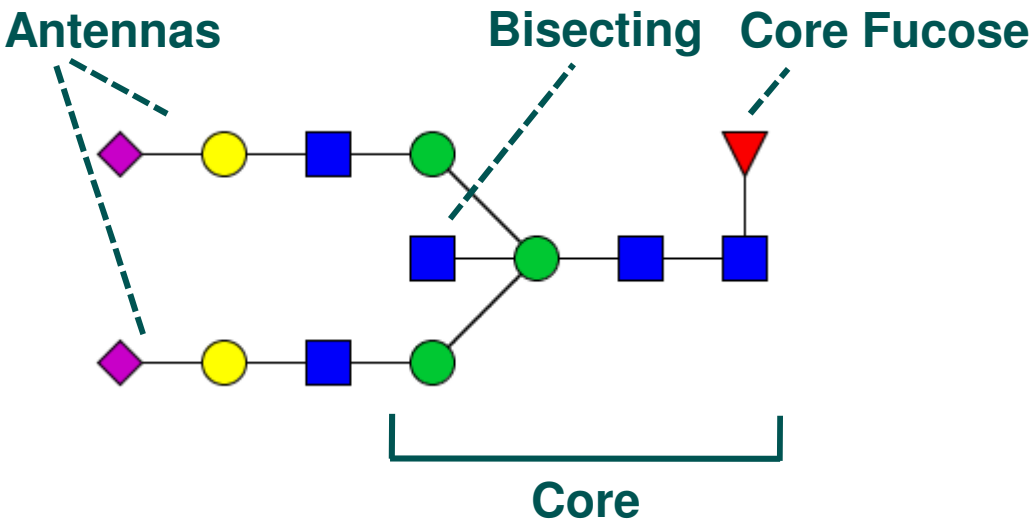


Complex Type



Hybrid Type



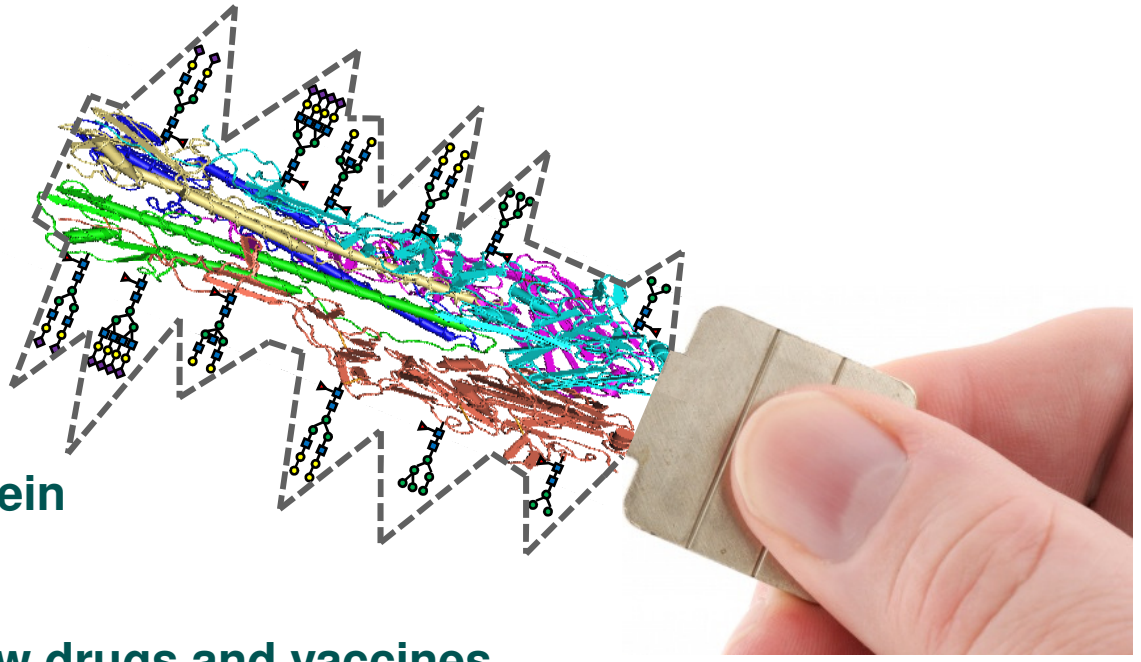


- N-Acetylglucosamine (GlcNAc)
- Mannose (Man)
- Galactose (Gal)
- Sialic Acid (SA)
- Fucose (Fuc)

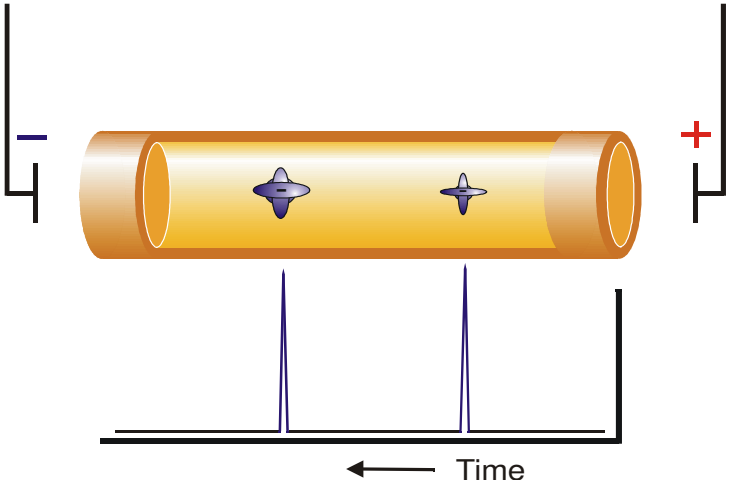
Impact of Complex Carbohydrates

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.**

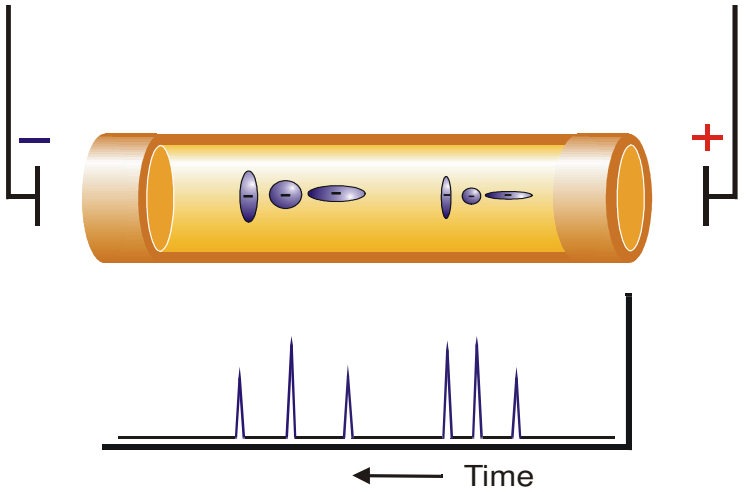


xCGE-LIF for Glycoanalysis
Principles and Advantages



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

System (Method, Software & Database) for Automated HT Glycoanalysis via xCGE-LIF

(Ongoing project of M. Borowiak (glyXera), PhD thesis of R. Hennig & PhD thesis of T. Muth)

First powerful "real" HT glycoanalysis-tool (method, software with GUI & database):

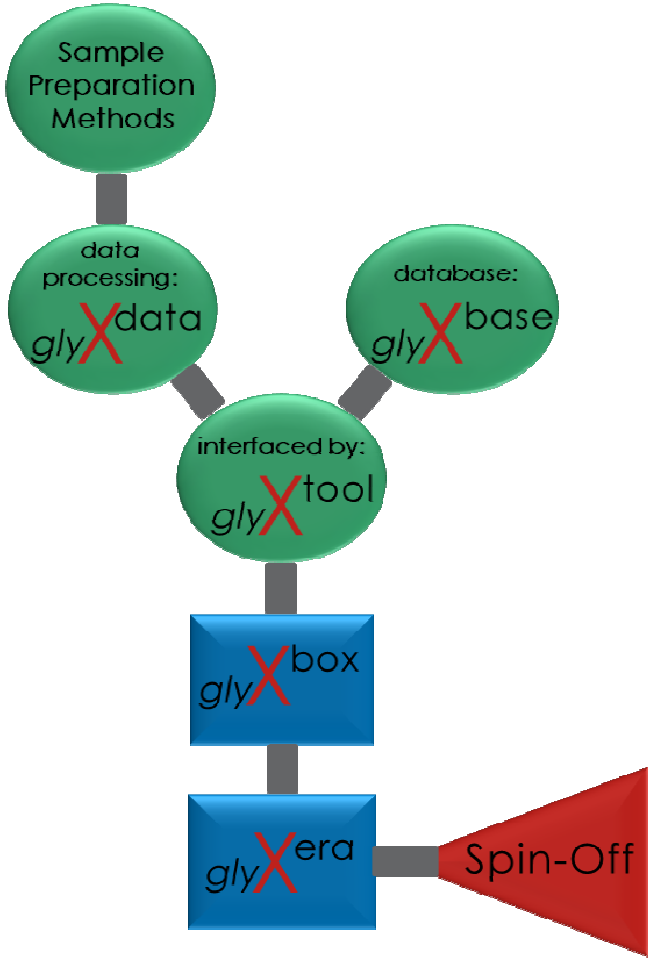
the "glyXbox"

⇒ **Sample preparation methods**

⇒ **Glycoanalysis with:**
- Automated parallel separation and sensitive detection with **xCGE-LIF** systems
- Automated data-processing (**glyXdata**)
- Automated data-analysis (**glyXtool**)

⇒ **Glycodatabase:**
an oligosaccharide / glycan database (**glyXbase**)

⇒ **The system is ready for take-off !**

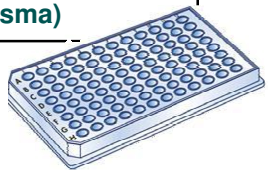


www.glyxera.com

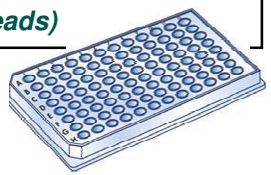
Using glyXbox for Automated HT-Glycoanalysis via xCGE-LIF

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

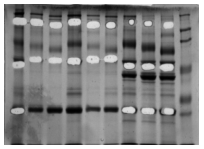
1a Protein Precipitation (e.g. to Extract Proteins from Citrate Blood Plasma)



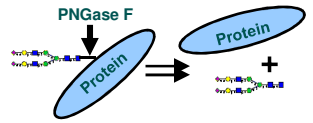
1b Protein Purification & Concentration by Affinity-SPE *(e.g.: Protein A Beads)*



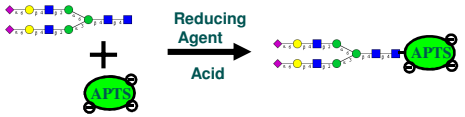
1c Protein Separation by 1D/2D-GE & Cut out of Bands/Spots:



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

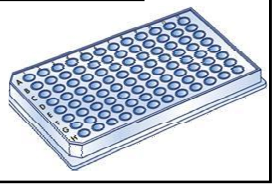


3 Fluorescent Labeling of Released *N*-Glycans with APTS

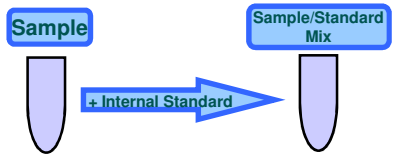


4 Post Labeling Clean-Up

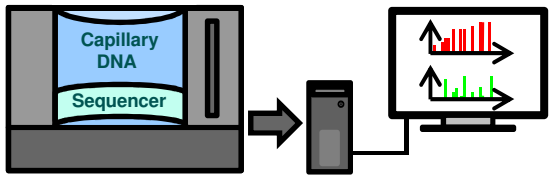
- SEC
- HILIC-SPE
- NONE



5 Addition of Internal Standards



6 xCGE-LIF Analysis, Normalization & Data Evaluation



glyXbox

Schwarzer J, Rapp E, Reichl U, Electrophoresis 2008, 29, 4203-4214.

Ruhaak L, Hennig R, Huhn C, Borowiak M, Dolhain R, Deelder A, Rapp E, Wuhrer M, J Proteome Res 2010, 9, 6655-6664.

Rapp E, Schwarzer J, Reichl U, Bohne C: European patent: EP 08007887.6 (24.4.2008).

Rapp E, Schwarzer J, Reichl U, Bohne C: US patent: US 12/428,003 (22.4.2009).

Software-Development for Automated HT Glycoanalysis via xCGE-LIF

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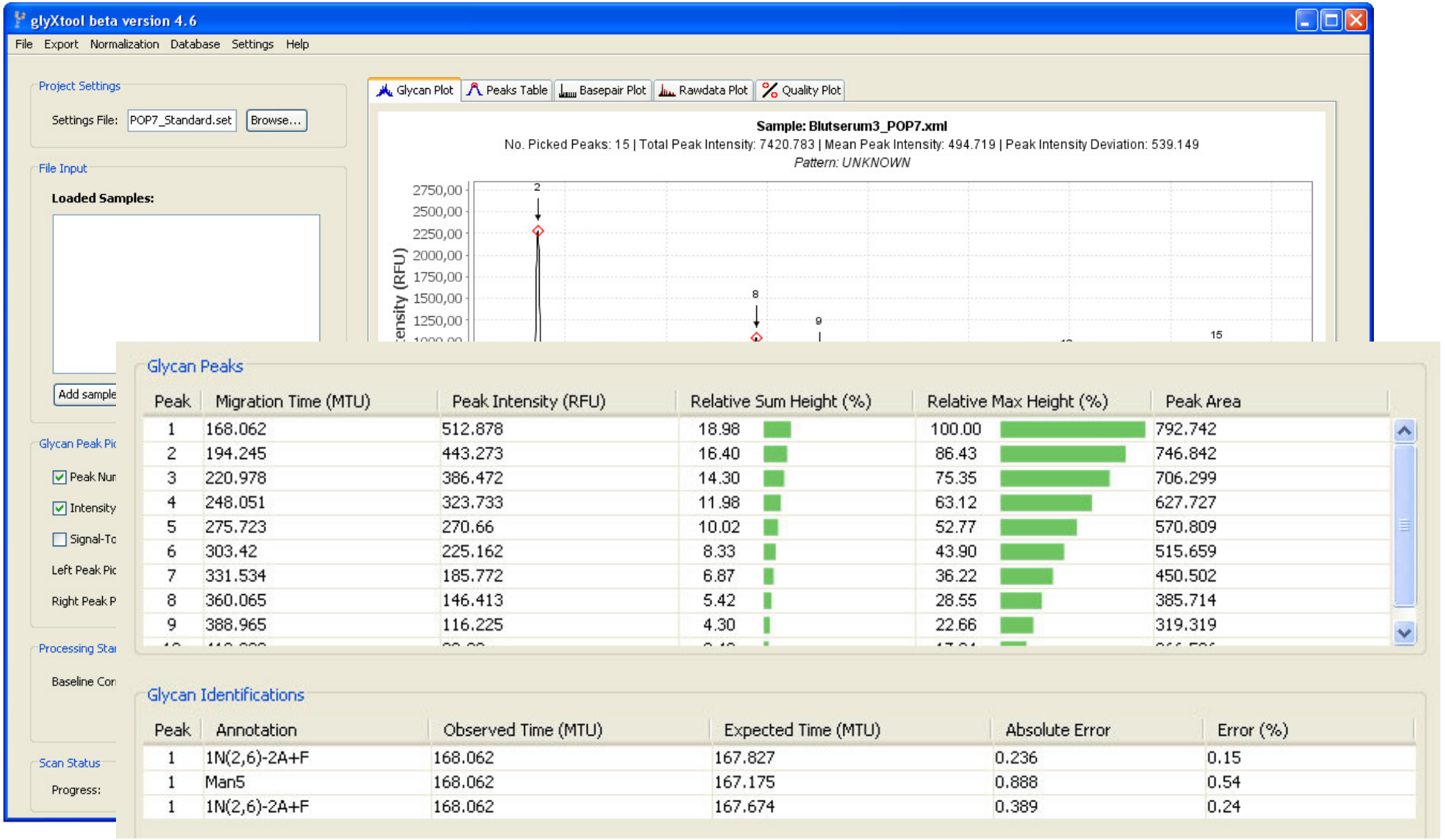
(Ongoing project @ glyXera, PhD thesis of R. Hennig & PhD thesis of T. Muth)



Glycan Peaks

Peak	Migration Time (MTU)	Peak Intensity (RFU)	Relative Sum Height (%)	Relative Max Height (%)	Peak Area
1	168.062	512.878	18.98	100.00	792.742
2	194.245	443.273	16.40	86.43	746.842
3	220.978	386.472	14.30	75.35	706.299
4	248.051	323.733	11.98	63.12	627.727
5	275.723	270.66	10.02	52.77	570.809
6	303.42	225.162	8.33	43.90	515.659
7	331.534	185.772	6.87	36.22	450.502
8	360.065	146.413	5.42	28.55	385.714
9	388.965	116.225	4.30	22.66	319.319

Glycan Identifications

Peak	Annotation	Observed Time (MTU)	Expected Time (MTU)	Absolute Error	Error (%)
1	1N(2,6)-2A+F	168.062	167.827	0.236	0.15
1	Man5	168.062	167.175	0.888	0.54
1	1N(2,6)-2A+F	168.062	167.674	0.389	0.24

Growing Glycan / Oligosaccharide Library

for Structural Elucidation via "Migration-Time-Matching"

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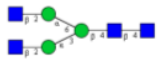
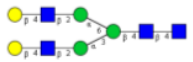
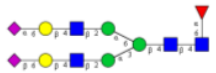
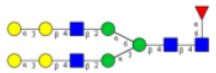
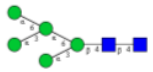
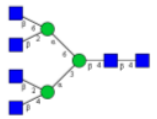
(Ongoing project of R. Hennig & R. Kottler)

Excerpt from in-house library:

No.	N-Glycan Standard Name			Simplified Structure	t_{mig} in MTU*
	TheraProteins Nomenclature	Merck Nomenclature	Dextra Nomenclature / Alternative Nomenclature		Major Peak
3	0N-2A-2G	A2G0	NGA2		252,45 (+/- 0,50)
5	0N-2A	A2G2	NA2		332,55 (+/- 0,65)
19	2N(2,6)-2A+F	A2FG2S2(2,6)	A2F		180,40 (+/-1,30)
22	0N-2A+2α(13)Gal+F	A2FG2αG2	-		435,75 (+/-0,60)
27	Man5	Man5	Man5		248,35 (+/-0,40)
34	0N-4A-4G	A4G0	NGA4		322,80 (+/-0,10)

At present:

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

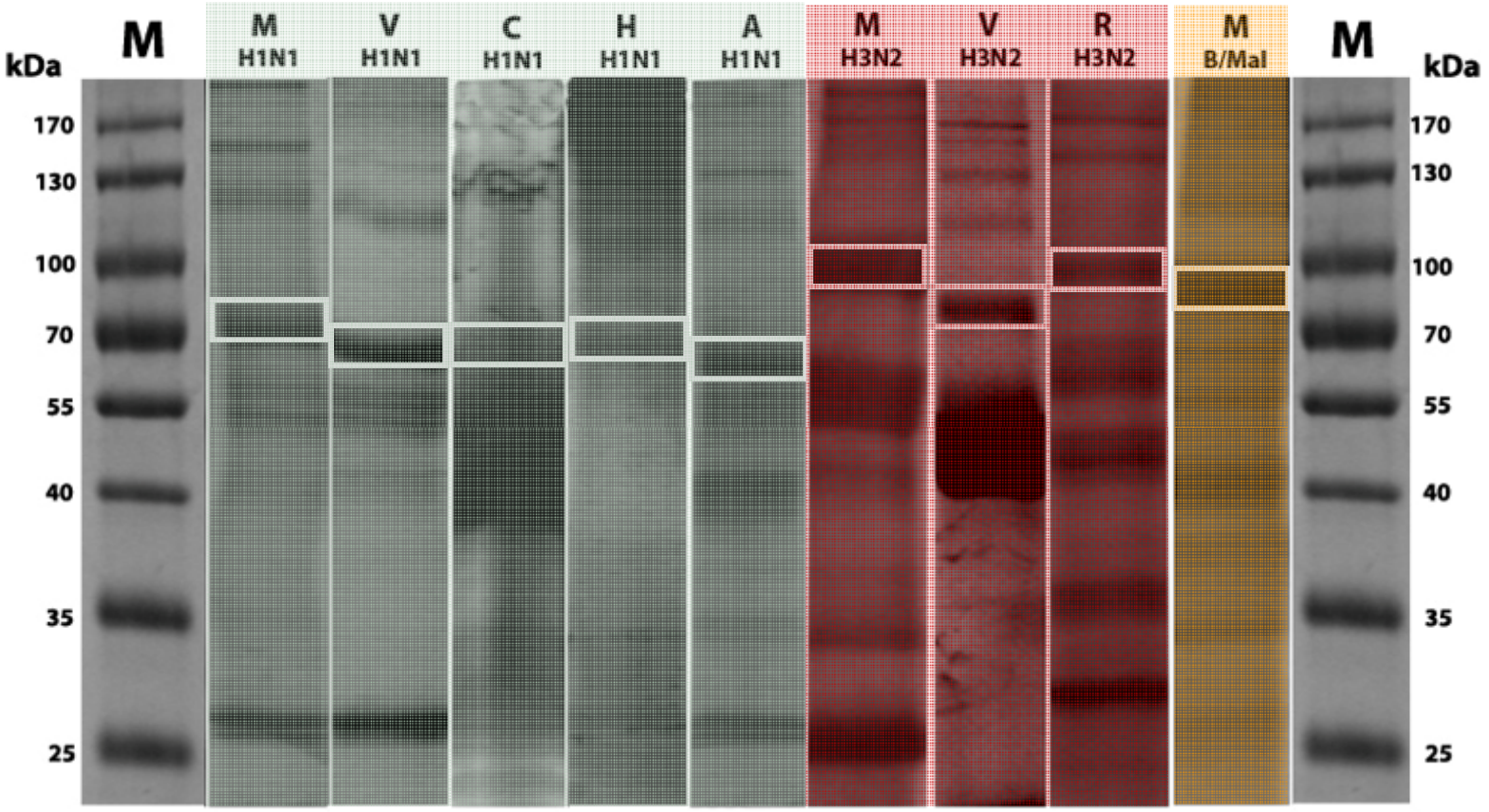
Summary I

- 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*

SDS-PAGE of Variants of Human Influenza Virus Type A (H1N1, H3N2) and Type B (B/Mal)



⇨ **Higher molecular weights MW for all variants of the H3N2 virus, compared to the H1N1 variants.**

Schwarzer J, Rapp E, Hennig R, Genzel Y, Reichl U, Vaccine 2009, 27, 4325–4336.

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

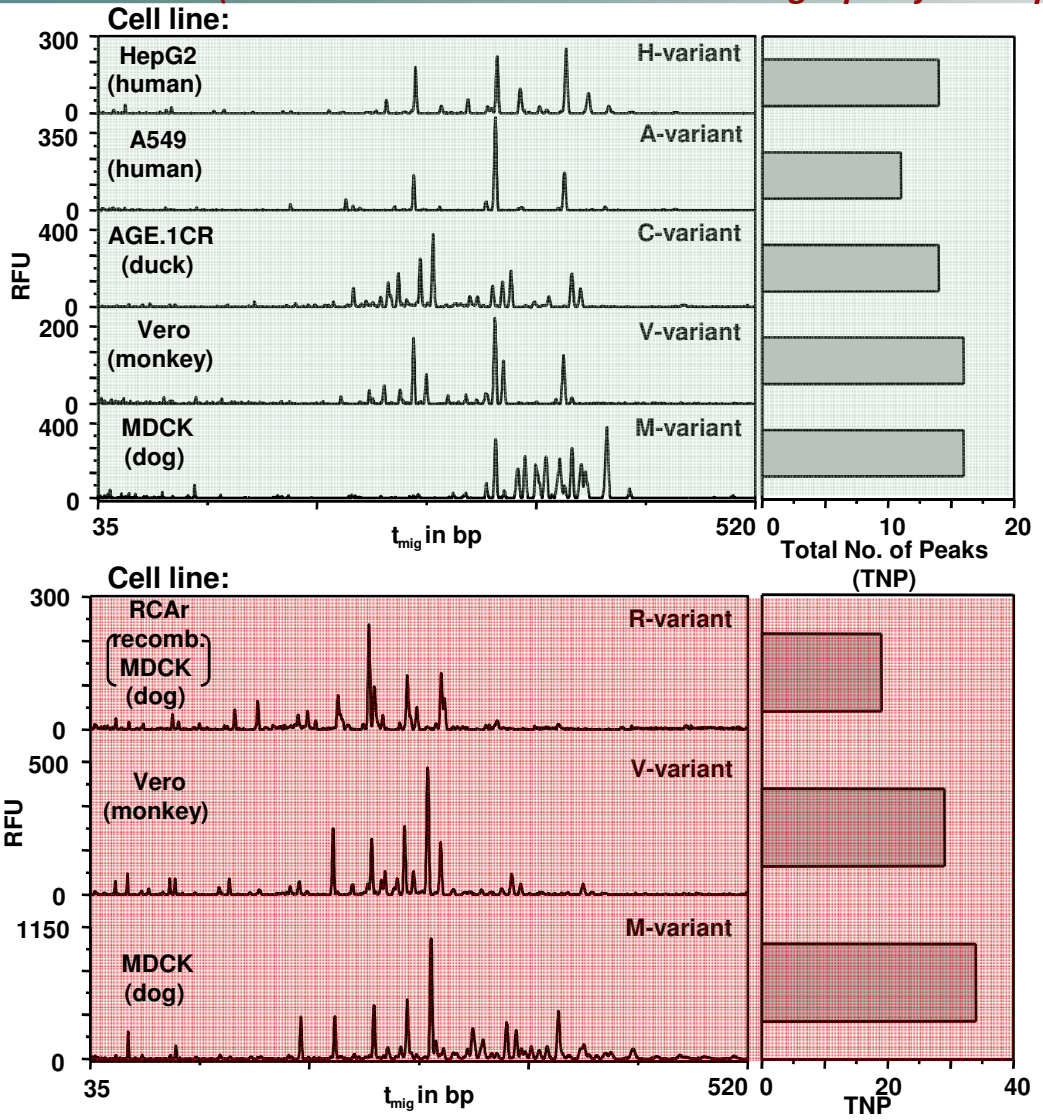
variant and virus		overall MW of HA (estimated via SDS-PAGE) [kDa]	calculated mass of HA AA[1] sequence* [kDa]	Estimated mass of HA N-glycan pool [kDa]	TNP	t_{mig} range of HA N-glycans [bp]
M	H1N1	79±5	63.0	16±5	16	273.3-426.5
V	H1N1	68±5	63.0	5(±5)	16	214.0-378.3
C	H1N1	68±5	63.0	5(±5)	14	222.5-385.0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

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

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=> detailed structural information via SED and "in-house" database matching

virus and variant	*complex type; with terminal …*	*core fucosylation*	*high mannose type*	*hybrid type*
A/PR/8/34 H1N1				
M-variant	all; α- (8; 10-16)[2] and β-galactose (2-7; 9)[2]	yes	no	no
V-variant	most; β-galactose (6-16)[2]	yes	some	no
C-variant	most; β-galactose (5-14)[2]	yes	some	some
A-variant	all; β-galactose (5-11)[2]	yes	no	no
H-variant	all; β-galactose (4-14)[2]	yes	no	no
A/WSN/67/2005 H3N2				
M-variant	few; α- and β-galactose (some > 16)[2]	ND[1]	major peaks (< 16, some >16)[2]	no
V-variant	some; β-galactose (17,18,23, some > 23)[2]	ND[1]	major peaks (9-16, 19-22, some > 23)[2]	no
R-variant	no	ND[1]	few (11,14,15,17,19)[2]	major peaks (12,13,16,18)[2]
B/Mal/2506/2004				
M-variant	some; β-galactose (13-15, some > 15)[2]	ND[1]	major peaks (all < 13, some > 15)[2]	no

[1] ND, *not determined*

[2] *numbers of the peaks (corresponding to glyXdata peaklists of the normalized EPGs) related to the particular N-glycan types*

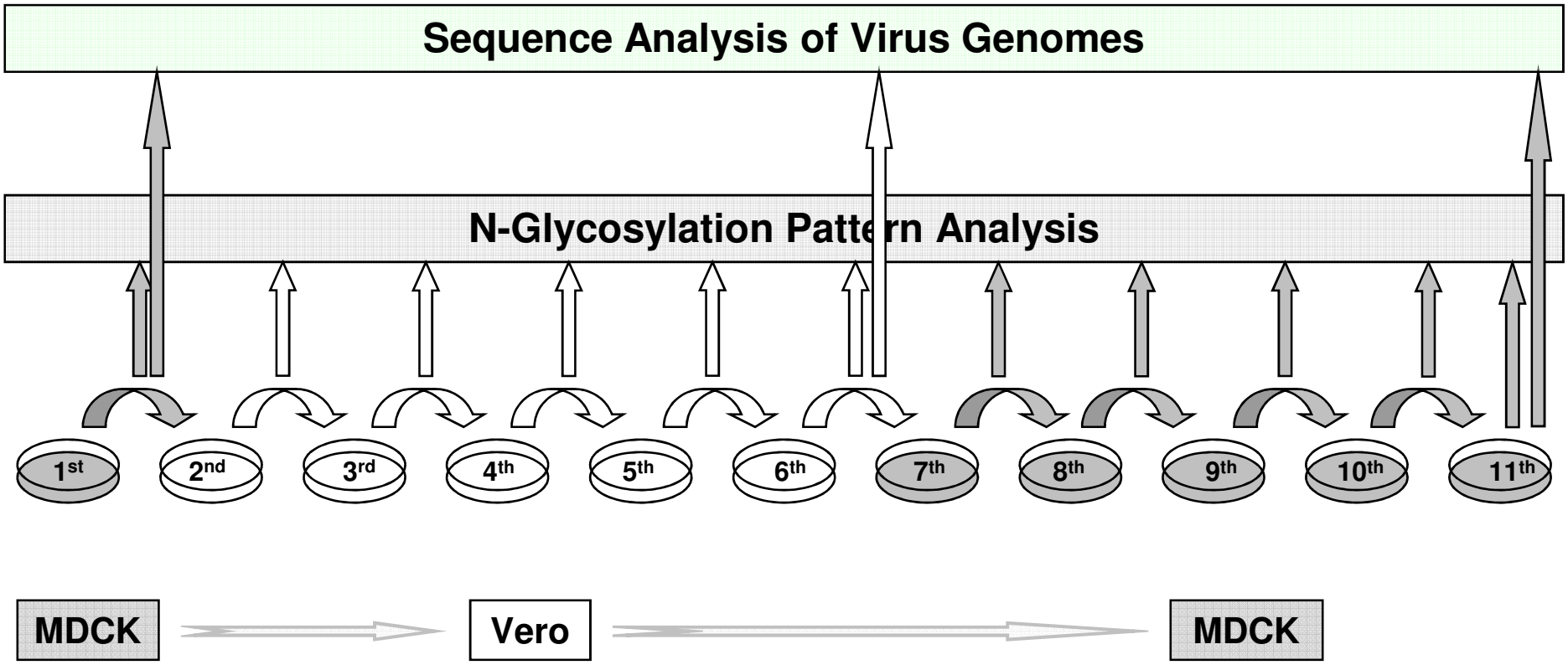
Summary II

- 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.

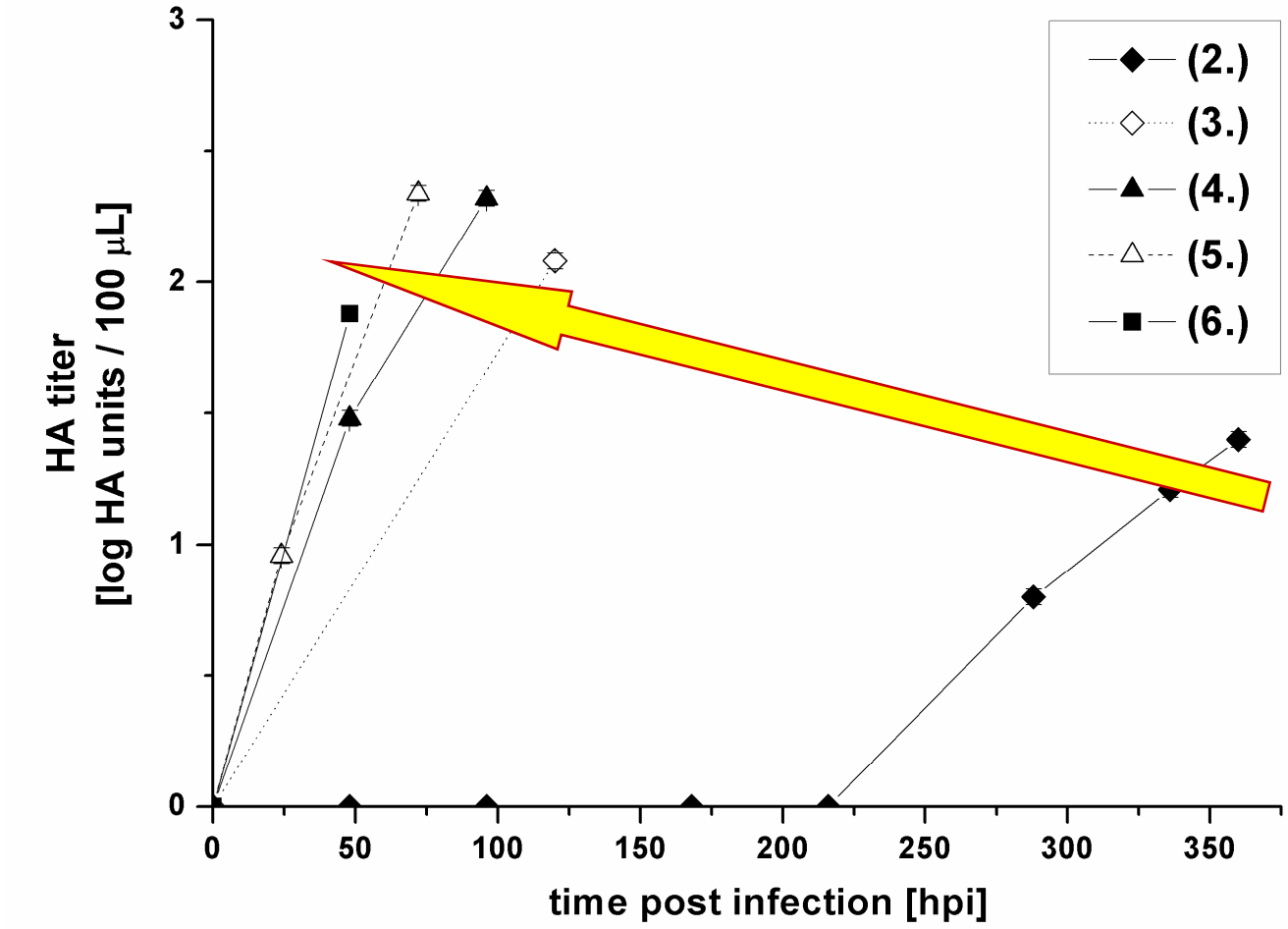
  (percentage of different N-glycan types)

Virus Adaptation to Host Cells

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

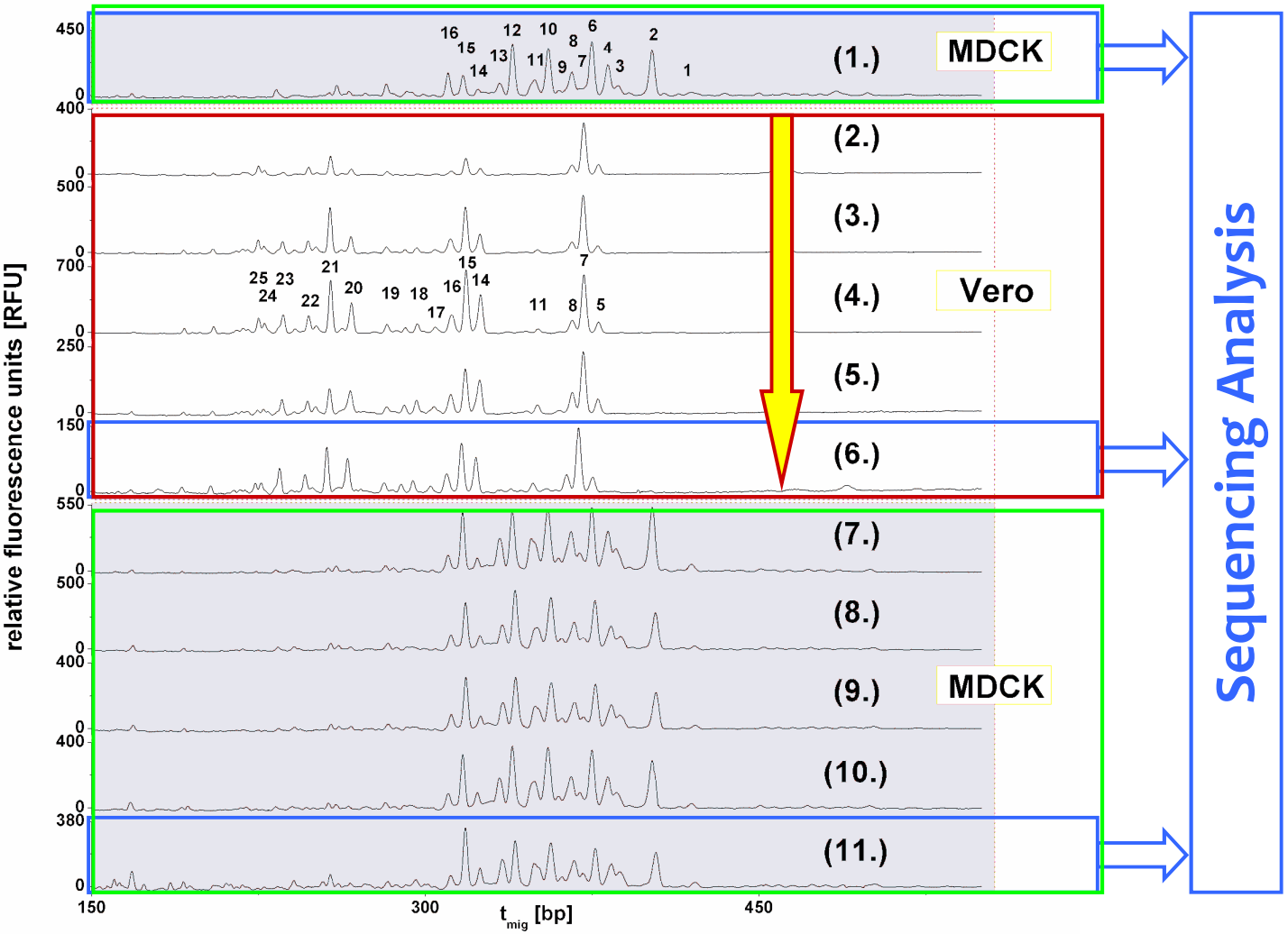


Virus Titer Improves During Adaptation



Host Cell Specificity of HA N-Glycosylation

(Changes during Adaptation)



HA Quasispecies Composition
RKI ↔ NIBSC

MAX-PLANCK-GESELLSCHAFT

table S3: Changes of quasispecies' composition on HA level of Influenza A/PR/8/34 (*H1N1*) (RKI, Amp. 3138) during virus ... ve virus ... k-adaptation. In passage 6 and 11 the two substitutions at amino acid positions 457 and 460 are uncoupled.

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

DNA Level	Protein Level	Passage 1	Passage 6	Passage 11
C 1370 T	S 457 L	0	19	9
A 1378 G	K 460 E	0	80	81
initial seed virus	no AA-substitutions	100	few reads	10

table S4: Changes of quasispecies' composition on HA level of Influenza A/PR/8/34 (*H1N1*) (NIBSC, #06/114) during virus adaptation. Passage 1 represents the MDCK cell adapted seed virus. Passage 6 represents the last of five successive virus passages in Vero c... age the two substitutions D455Y and N460D are uncoupled.

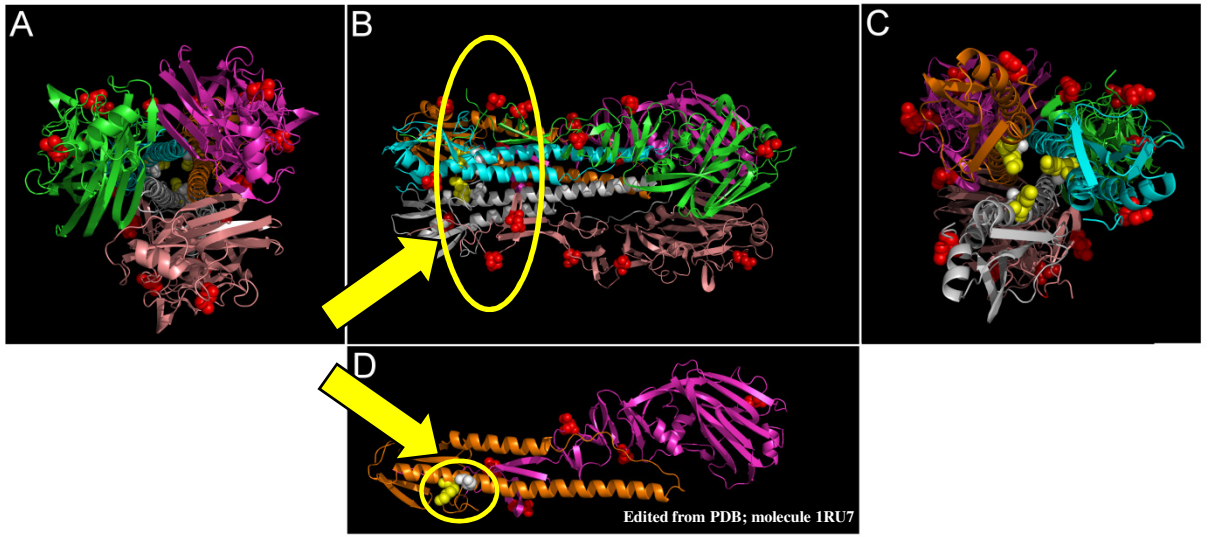
Influenza Virus A PR/8/34 (*H1N1*) from NIBSC

DNA Level	Protein Level	Passage 1	Passage 6	Passage 11
Base Substitution	Amino Acid Substitution	Population Ratio [%]	Population Ratio [%]	Population Ratio [%]
T 70 C	Y 24 H	22	0	0
G 1183 A	V 395 M	0	41.5	11.3
A 1189 G	T 397 A	1.3	0	0
A 1189 T	T 397 S	0.6	0	5.4
G 1363 T	D 455 Y	21.4	6.1	3.2
G 1363 C	D 455 H	0	52	44.1
A 1375 G	K 459 E	0	0	44.2
A 1378 G	N 460 D	12.2	41.1	10.5

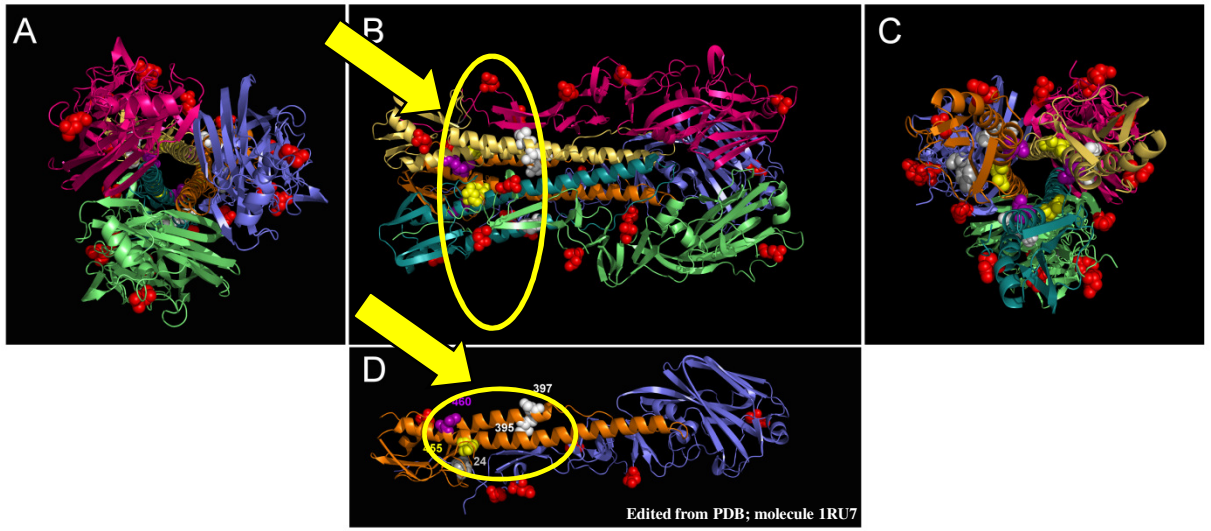
Substitution Sites within the HA-Molecule

MAX-PLANCK-GESELLSCHAFT

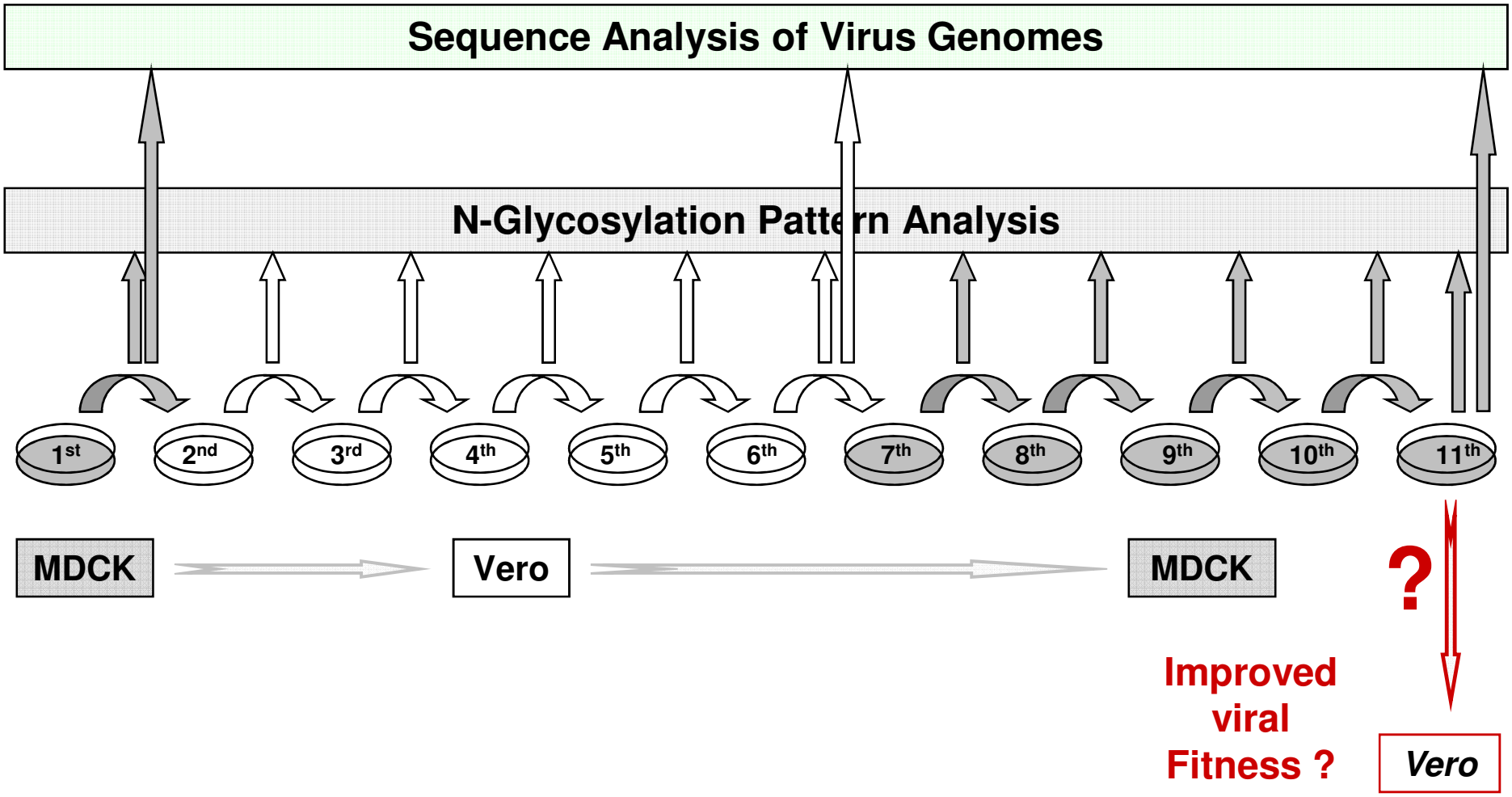
A/PR/8/34 from RKI



A/PR/8/34 from NIBSC

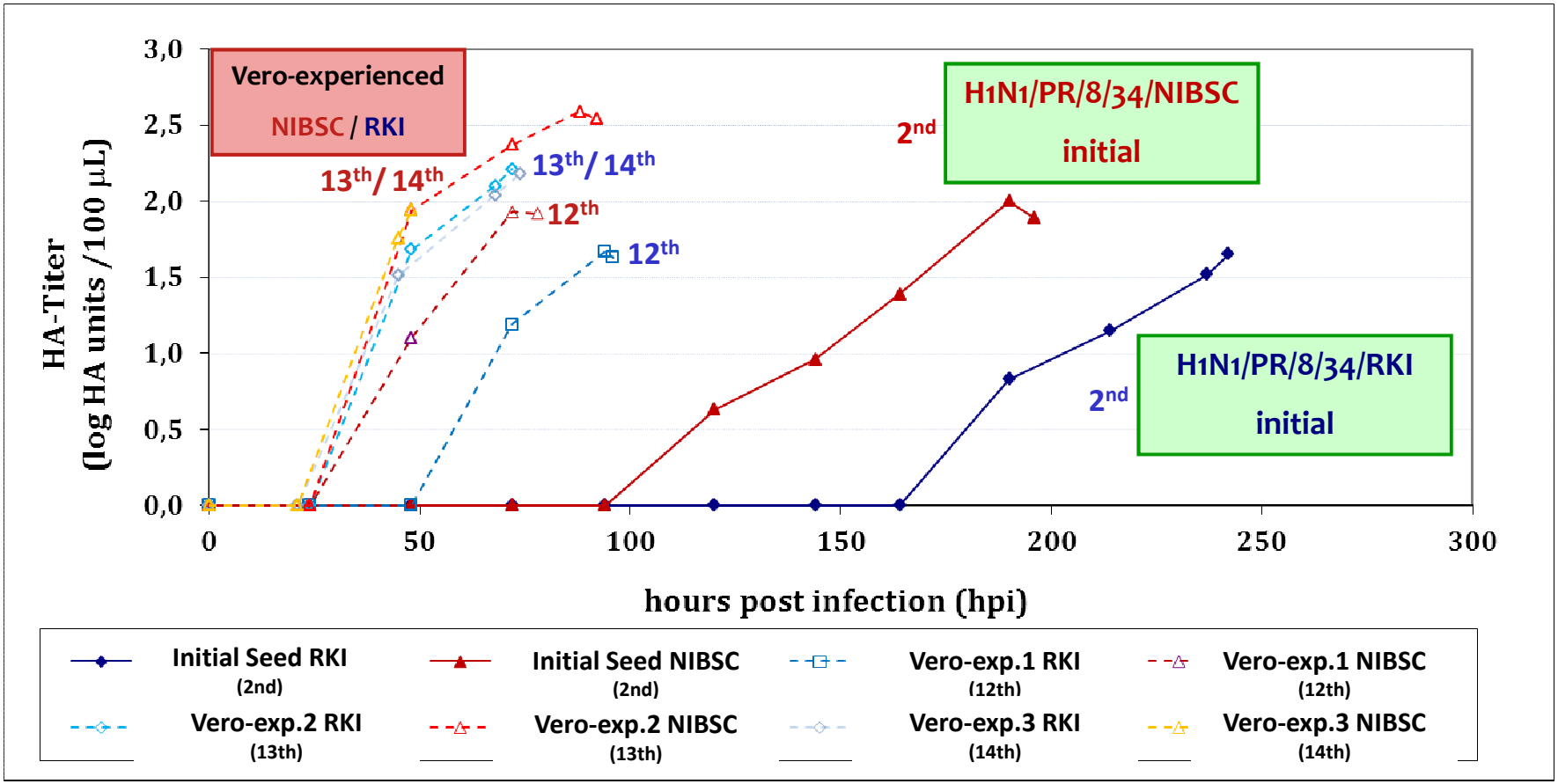


Do Substitutions Increase Viral Fitness in Vero?



Improved Viral Fitness also in Vero !

MDCK → Vero → MDCK → Vero



Helene Kaffka

Summary III

- „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)*

TCR-HA transgenic mouse
(TCRαβ specific for $HA_{110-120}$)

Isolation of spleen cells

$HA_{110-120}$ (synthetic peptide)
✚ MDCK-variant (glycos./ deglycos.)
Vero-variant (glycos./ deglycos.)

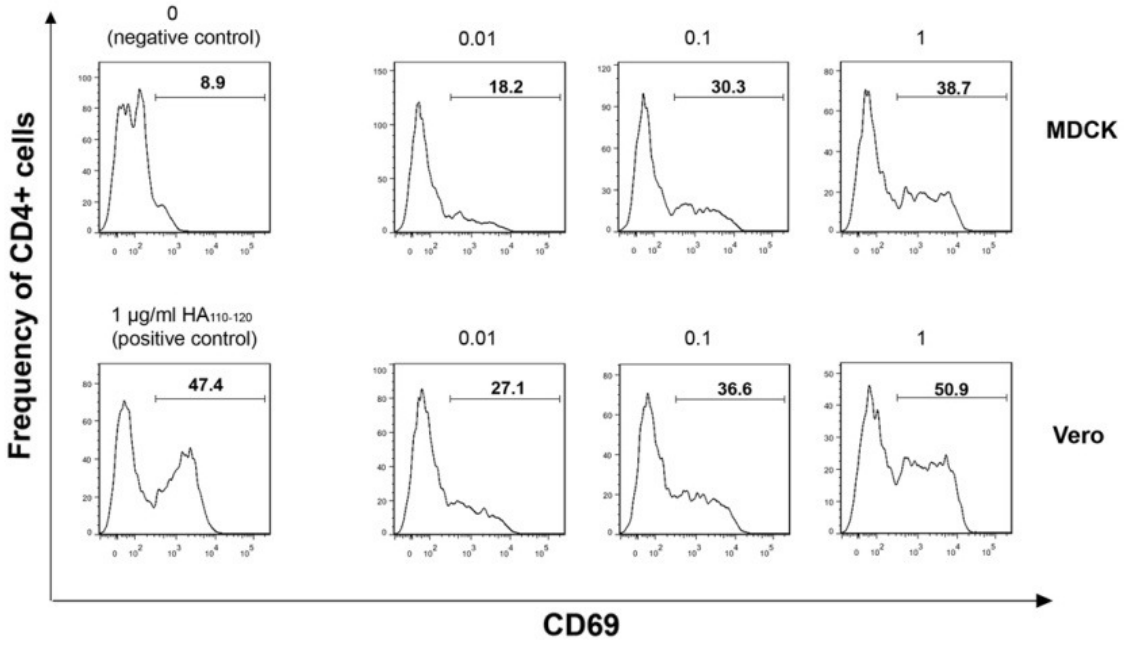
Incubation at 37 °C for 48 h

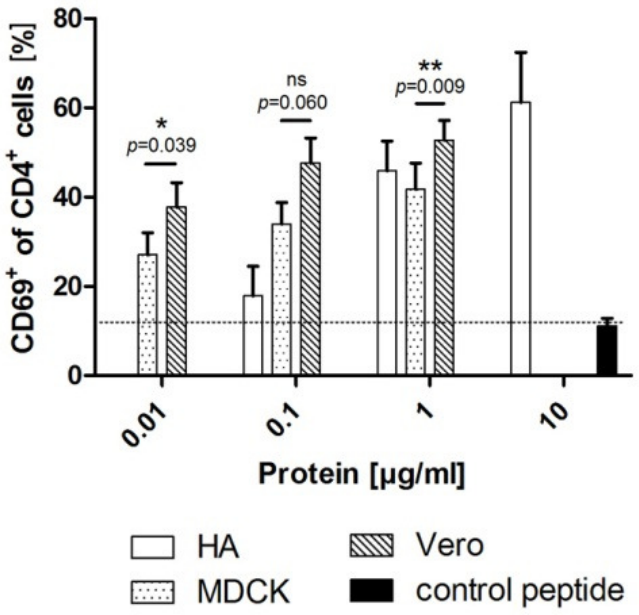
<u>Supernatant:</u>
Cytokine production
(IL-2, IFN-γ, IL-4)

<u>Cells:</u>
T cell activation markers
(CD69, CD25)

(performed by Julia Hütter @ MPI of Colloids and Interfaces)

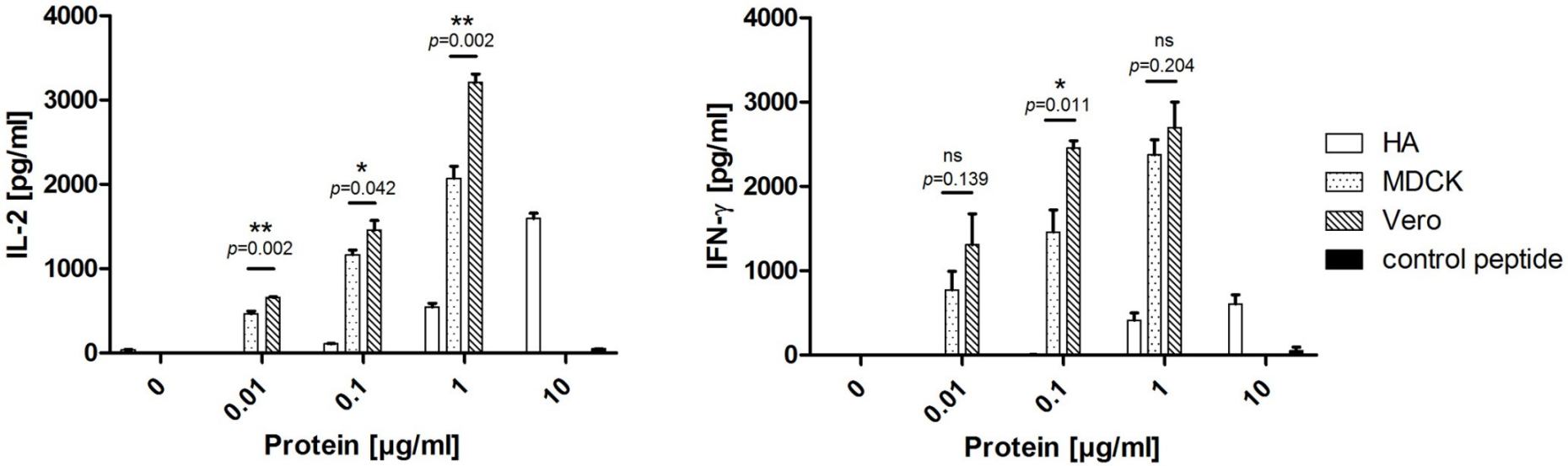
HA N-Glycosylation Impacts T Cell Activation





- Significantly higher frequency of T cells expressing the activation marker CD69 upon stimulation with the Vero cell-derived influenza virus glycovariant

HA N-Glycosylation Affects Cytokine Production

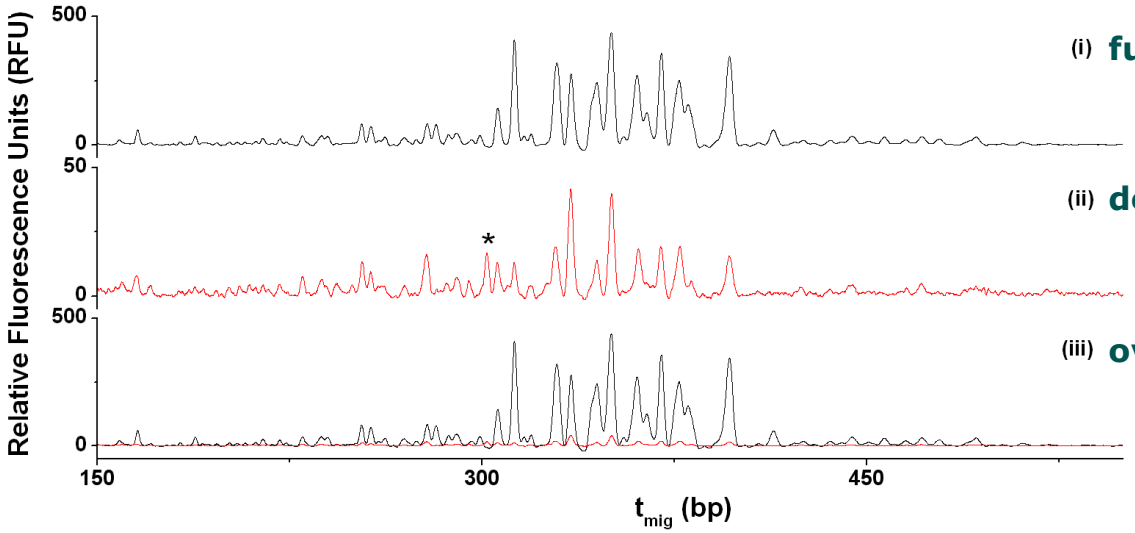


- IL-2 and IFN-γ are produced in significantly higher levels by splenocytes stimulated with the Vero cell-derived influenza virus glycovariant

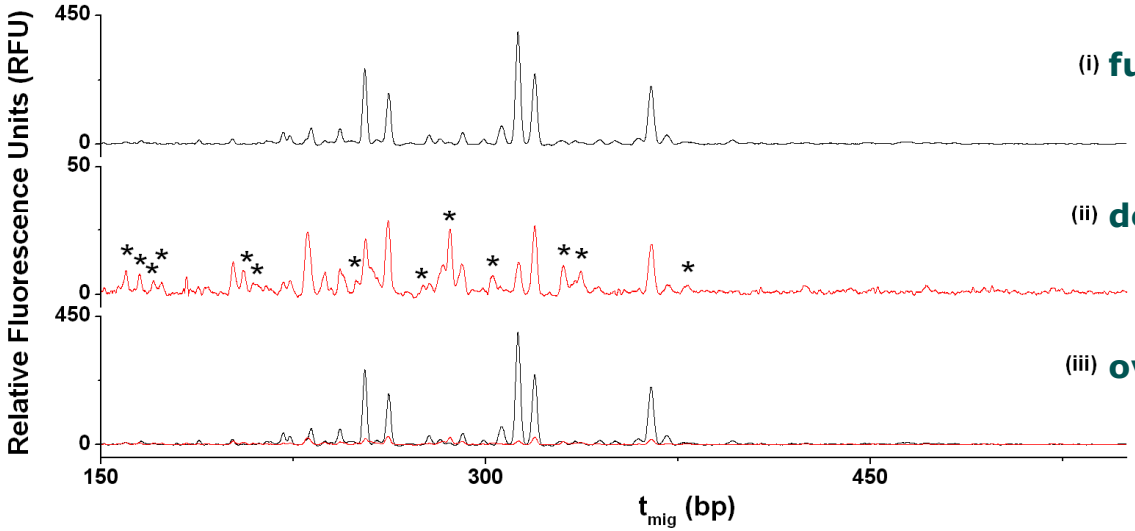
Hemagglutinin - Derived from Glycosylated & Deglycosylated Virus

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

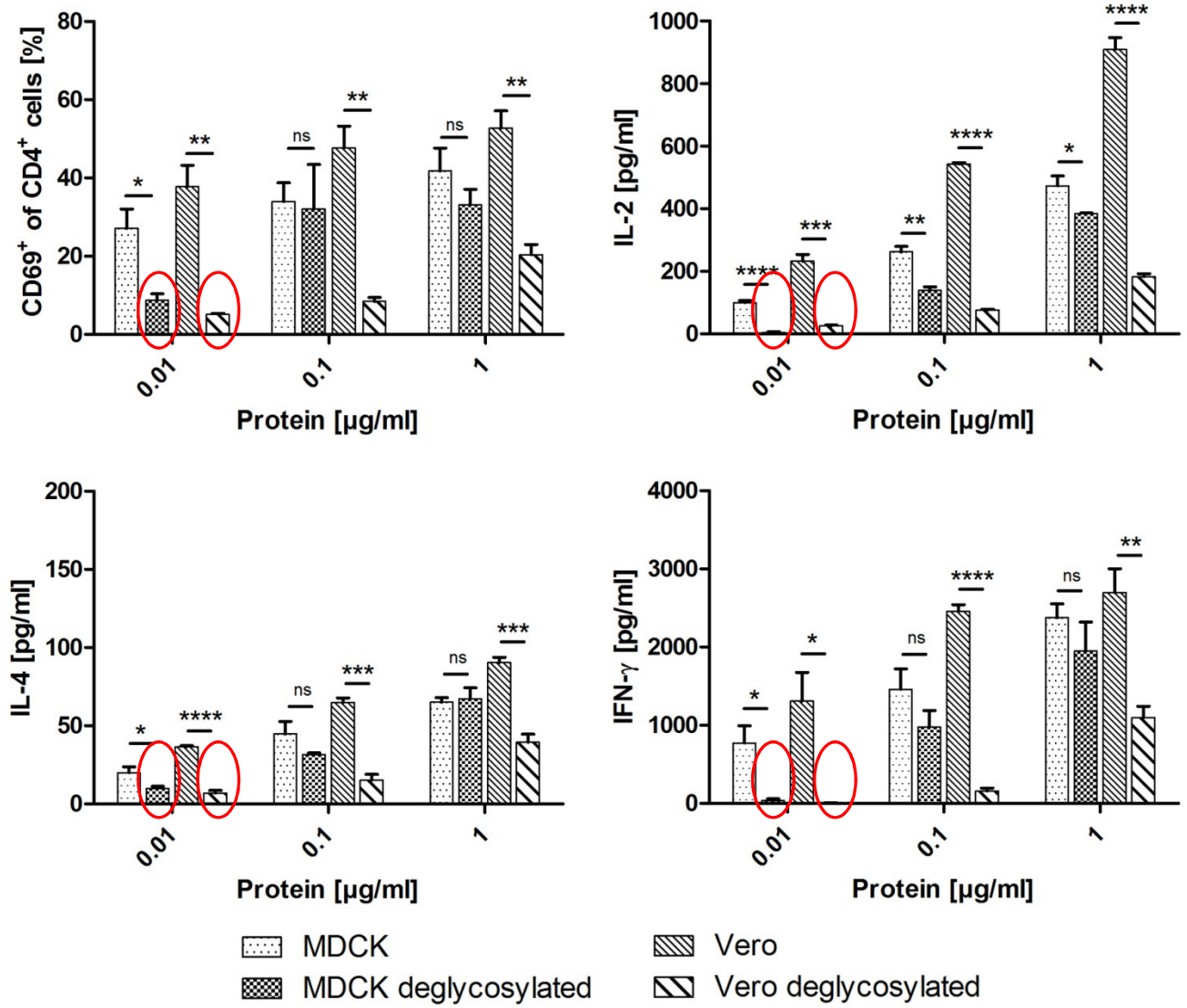
MDCK cell-derived Influenza Virus A PR/8/34 (H1N1)



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



Virus Deglycosylation Abolishes Cytokine Production



Summary IV

- 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:** glyXera GmbH **www.glyxera.com**

Thanks to ...

... YOU

... the BPE-Group

esp.:
- Yvonne Genzel
- Udo Reichl

... and the A-Team !!!

esp.:
- Jana Schwarzer
  ... now J.Bohne
  @ Novartis Vaccines
- Jana Rödig
- René Hennig

... our cooperation partners:

DANONE RESEARCH
CENTRE FOR SPECIALISED NUTRITION

Merck Serono
Living science, transforming lives

IDT

NOVARTIS VACCINES

HIGH GLYCAN

LUMC

Bayer HealthCare

ProBioGen
Intelligent Biopharmaceutical Solutions

Influenza Vaccine Production

Classical production:

Chicken eggs

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

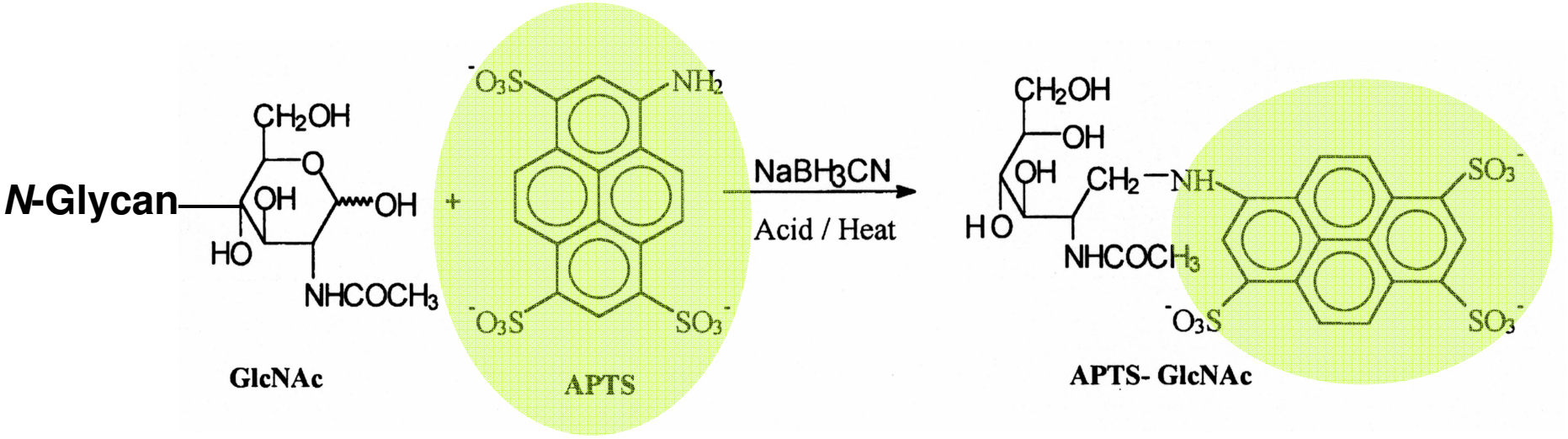
Trend:

Mammalian cell culture

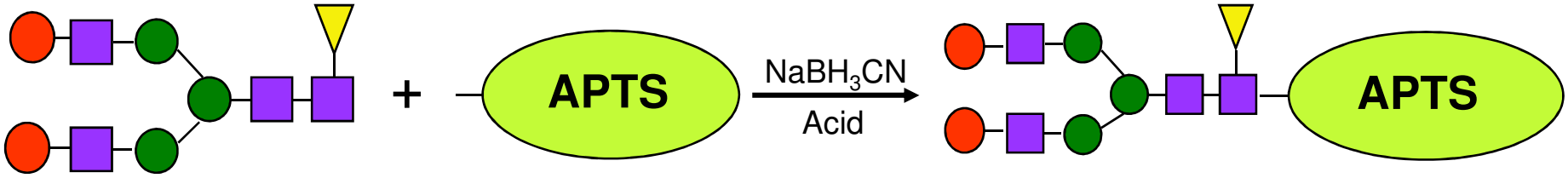
Advantages:

- 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)

Labeling

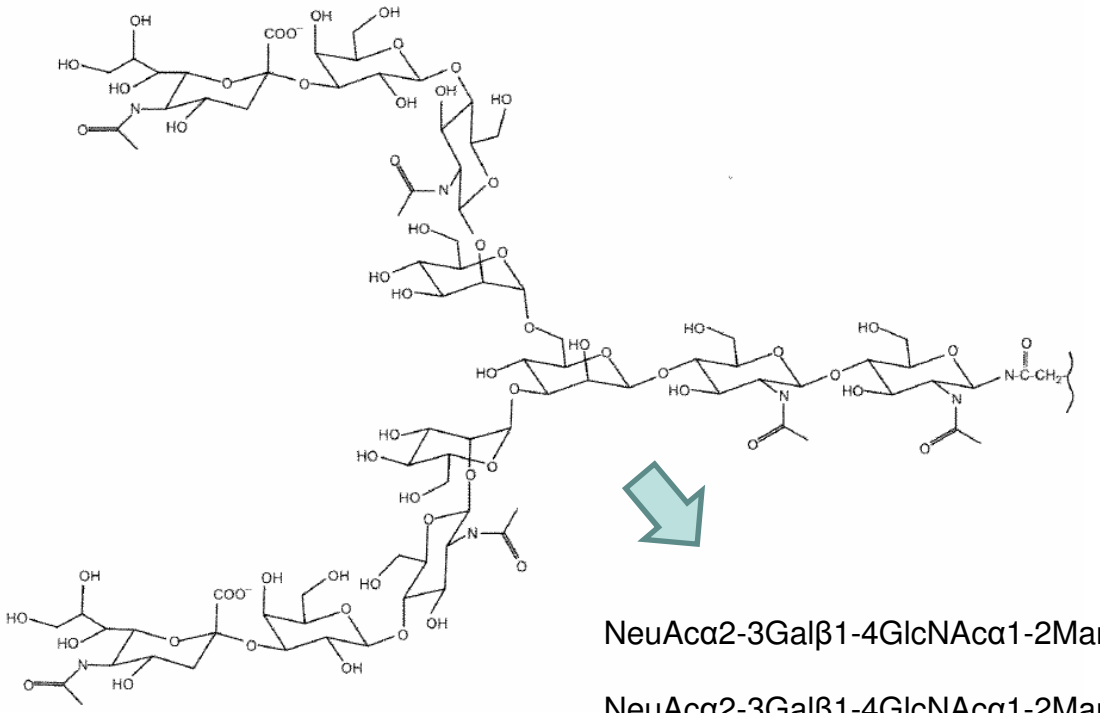


Modified from: Chen et al. (1998) Glycobiology (8): 1045-1052

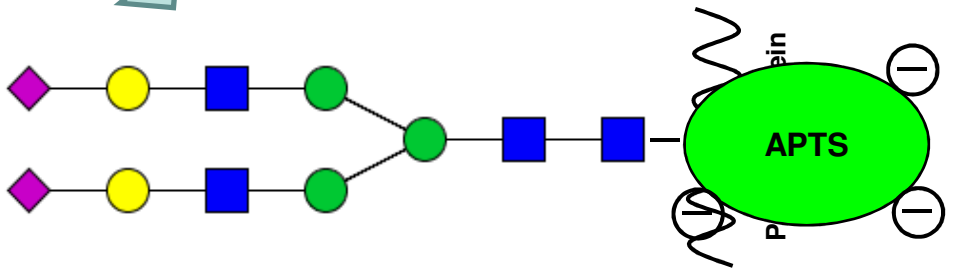


APTS = 8-amino-l,3,6-pyrenetrisulfonic acid

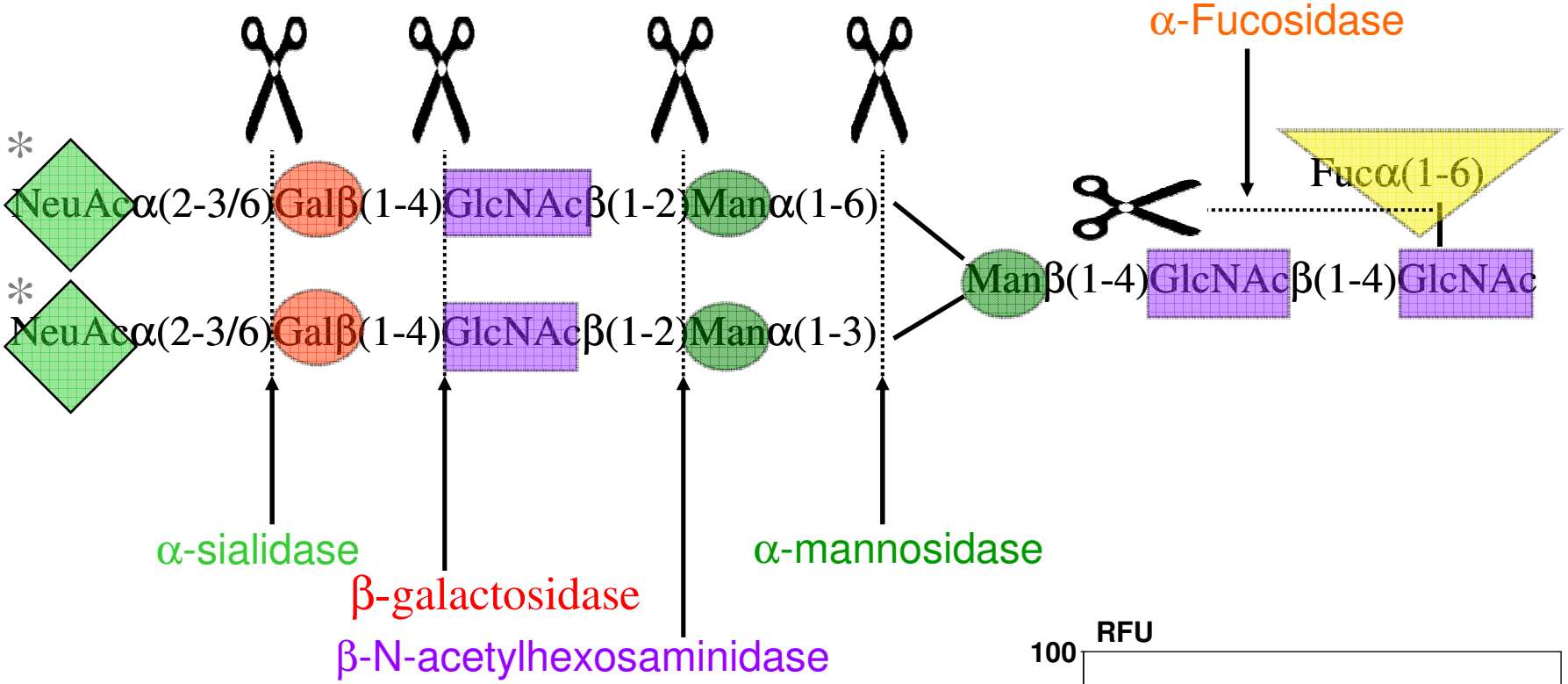
Schematic Representation of *N*-Glycans

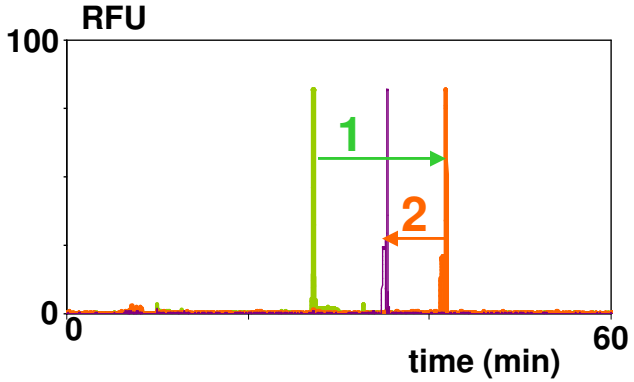


NeuAcα2-3Galβ1-4GlcNAcα1-2Manα1
NeuAcα2-3Galβ1-4GlcNAcα1-2Manα1
6
3
Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn
Peptide / Protein



Sequential Exoglycosidase Digestion





* can be substituted with α-galactose

Bio/Process-Analytics

MAX-PLANCK-GESELLSCHAFT

Hardware-, Software- & Method-Development

Genomics

Transcriptomics

Proteomics

Metabolomics

Qualitative Protein Analysis

Quantitative Protein Analysis

Analysis of Post-Translational Modifications

- **Phosphorylation**
- **Glycosylation**
- **…**

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

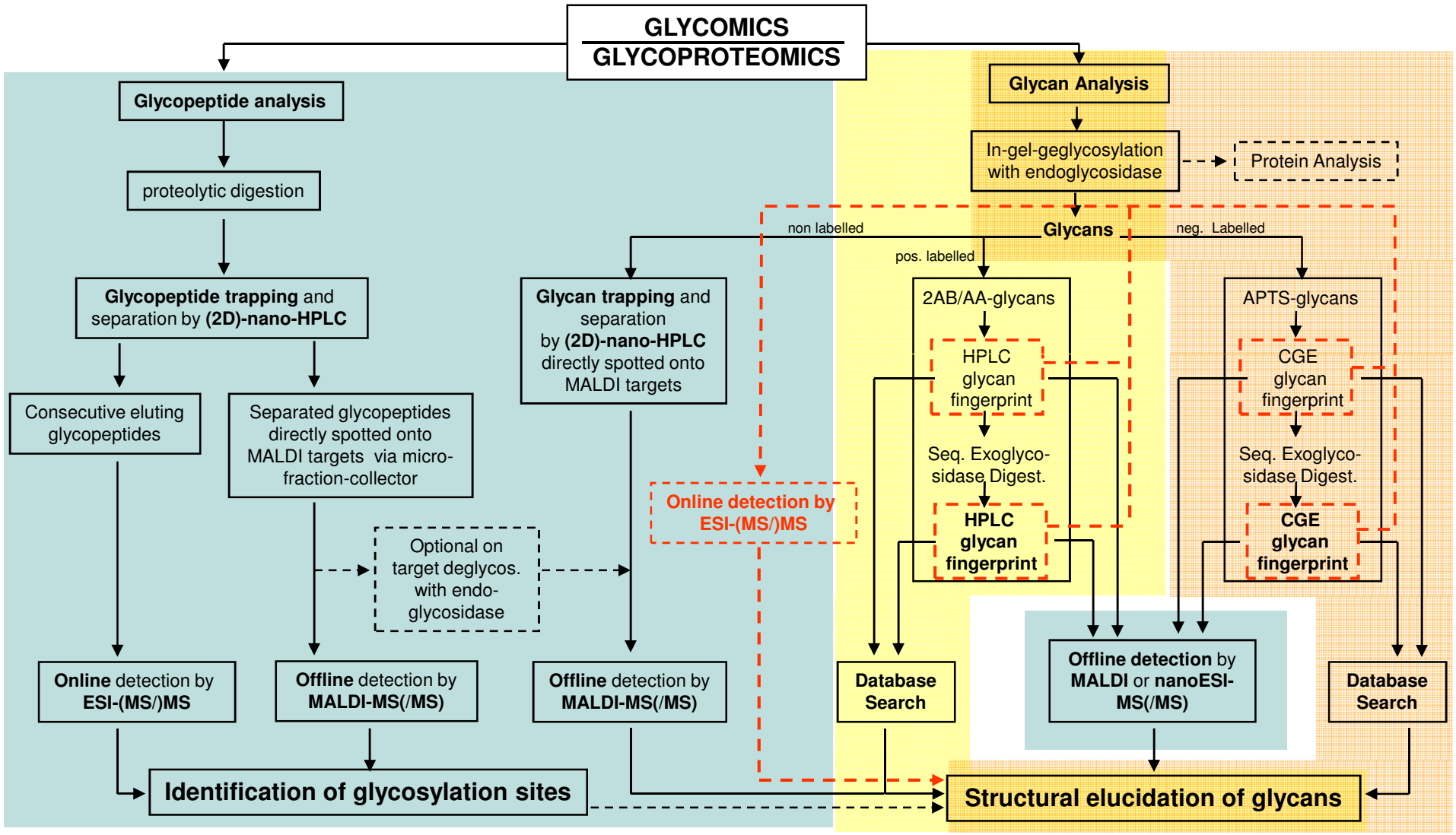
Instrumentation:

- **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**

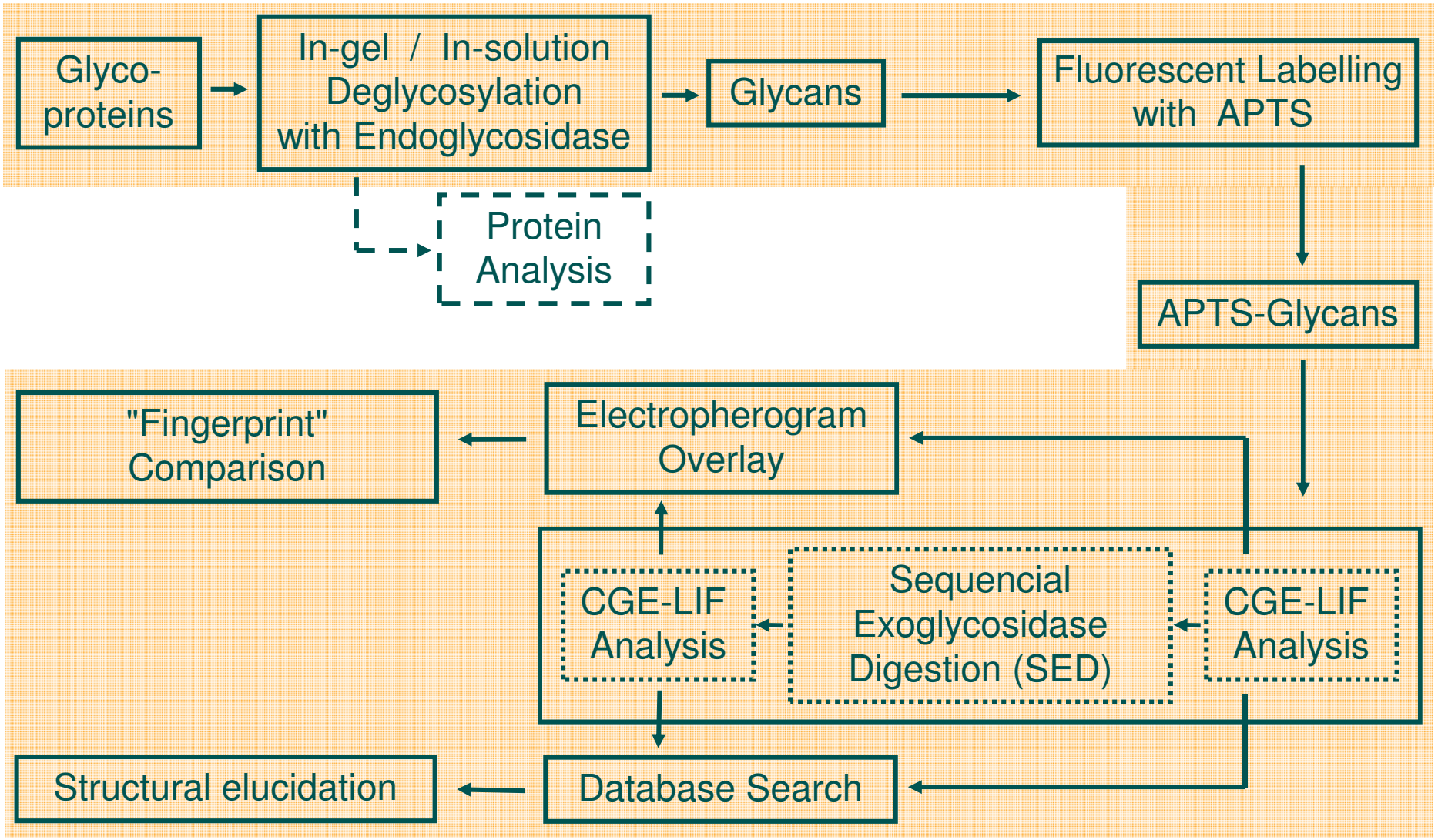
Glycomics, Glycoproteomics & Carbohydrate Analytics

- **Glycans**
- **Glycopeptides**
- **Glycoproteins and**
- **other Carbohydrates (e.g.: Milk oligosaccharides)**

Glycomics & Glycoproteomics Toolbox



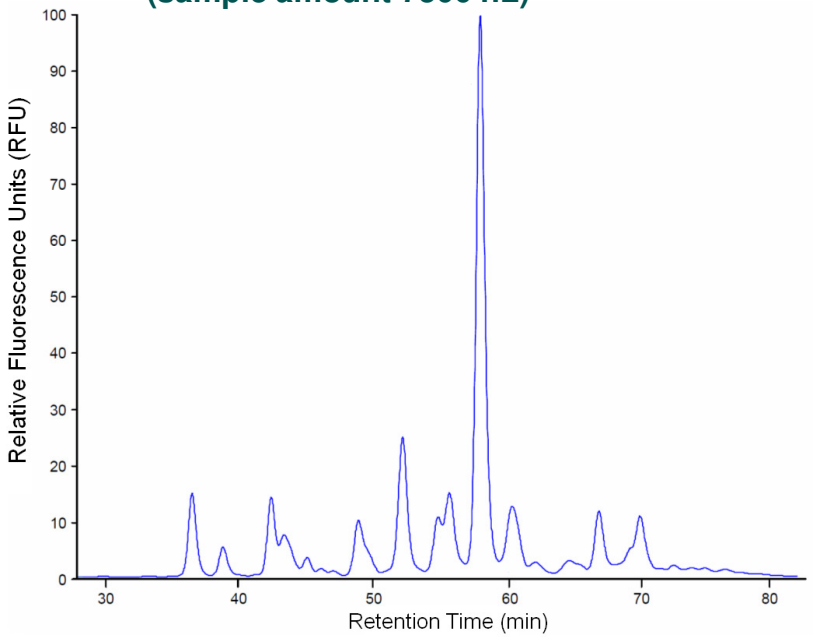
CGE-LIF Based Glycomics



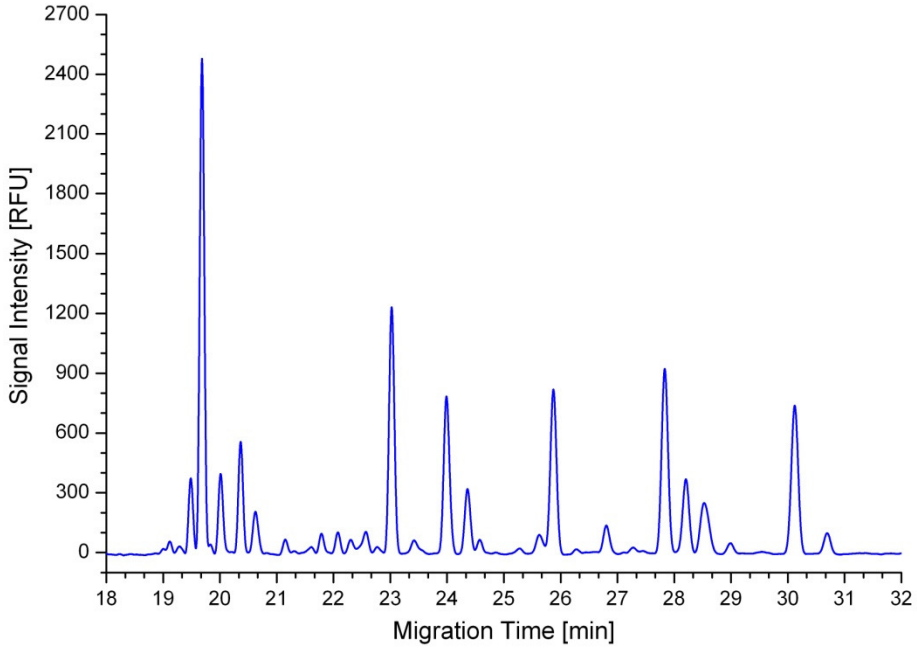
HILIC-FLR *vs.* xCGE-LIF

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 !**

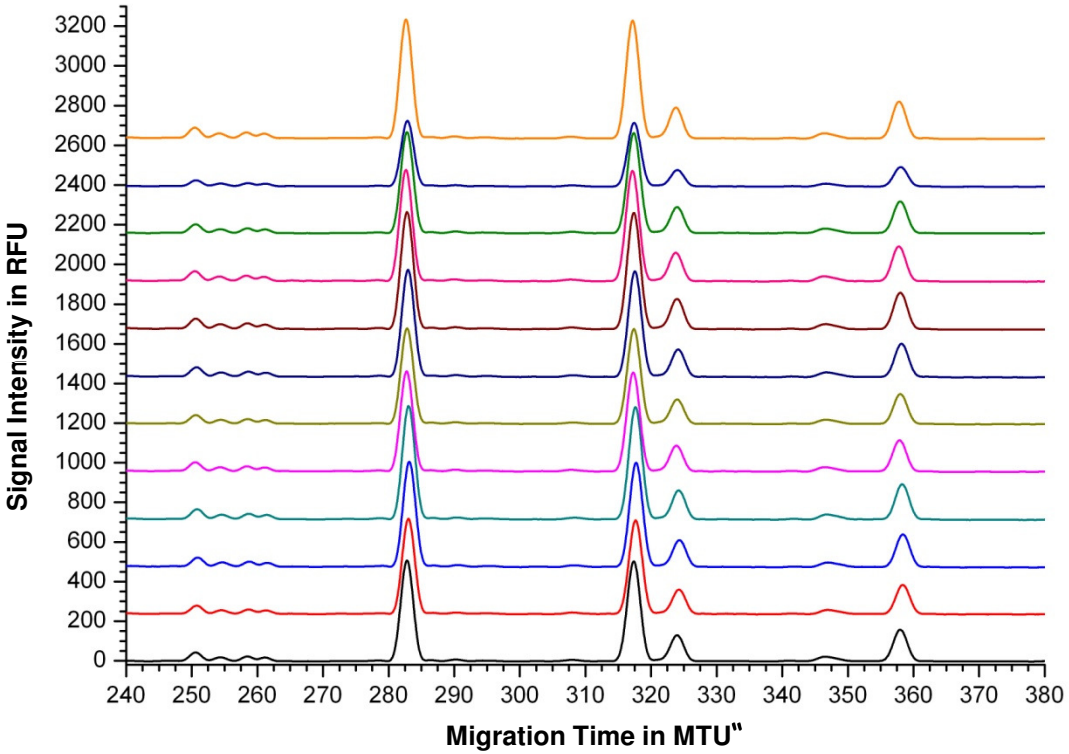
xCGE-LIF Analysis of *N*-Glycan Pools

(Normalized Electropherogram = " Fingerprint")

(LOD and reproducibility of xCGE-LIF)

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.**

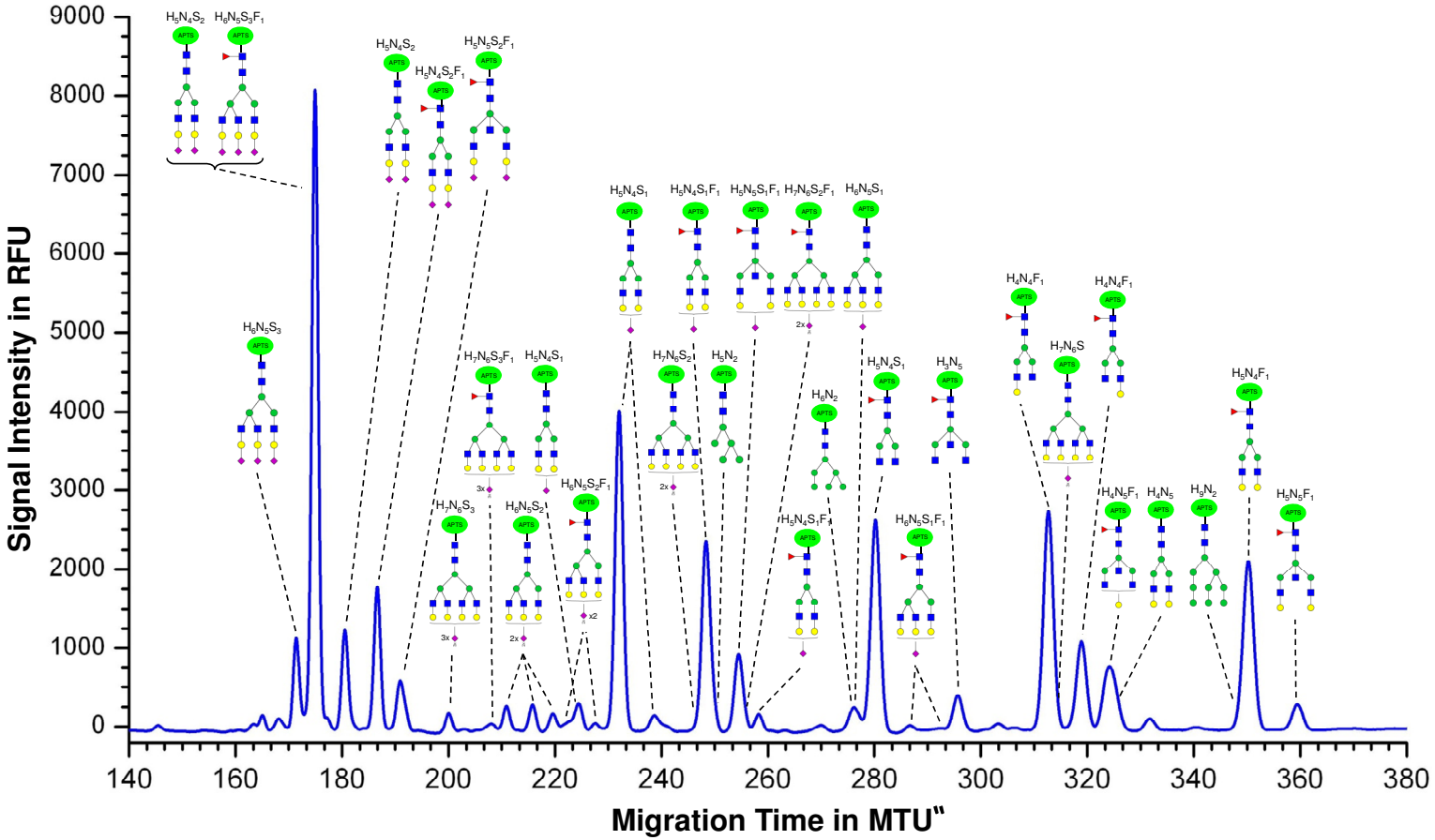


- **RSD for migration times of more than 36 consecutive runs < 0,03%.**
  (xCGE-LIF analyses of 3 techn. replicates à 12 repeated runs)
- **Longterm RSD (about two years) for migration times < 0,5%.**

Application of glyXbox for Automated HT Blood Plasma Glycomics

MAX-PLANCK-GESELLSCHAFT

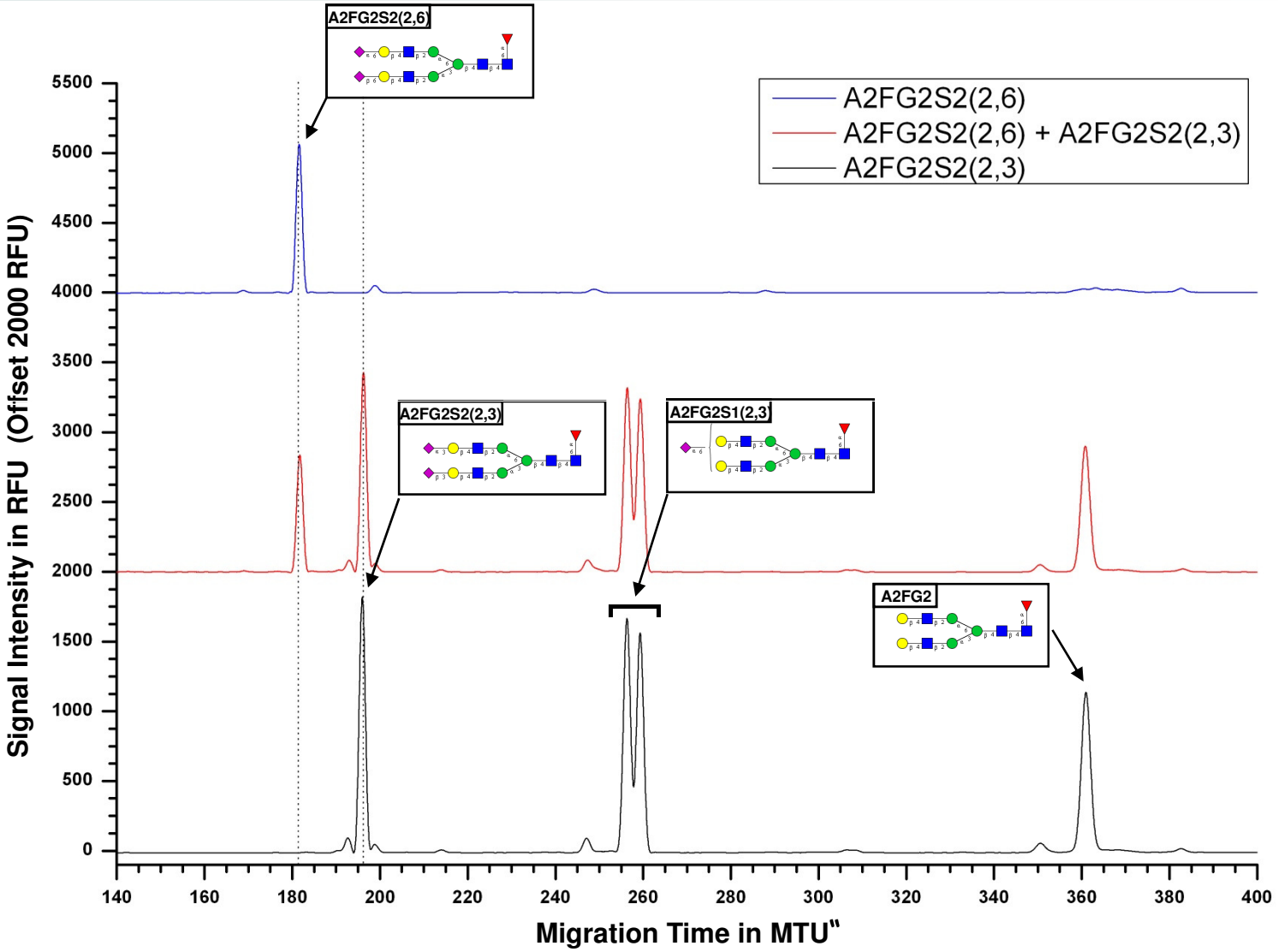
(Project of R. Hennig & M. Borowiak - in coop. with Dr. Manfred Wuhrer @ LUMC (Leiden/NL))

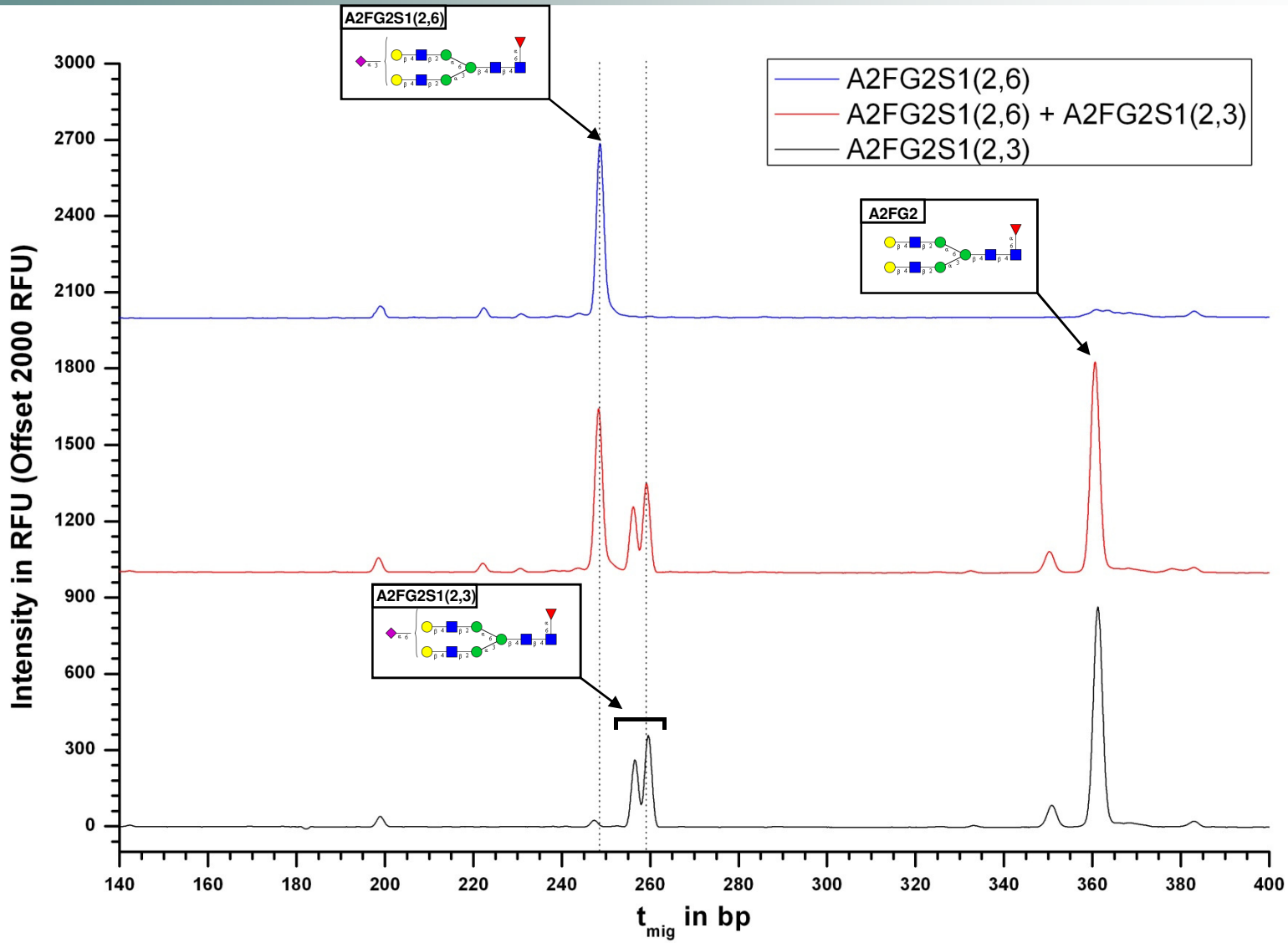


⇒ **Separation of more than 4500 samples in 48 hours !**

⇒ **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

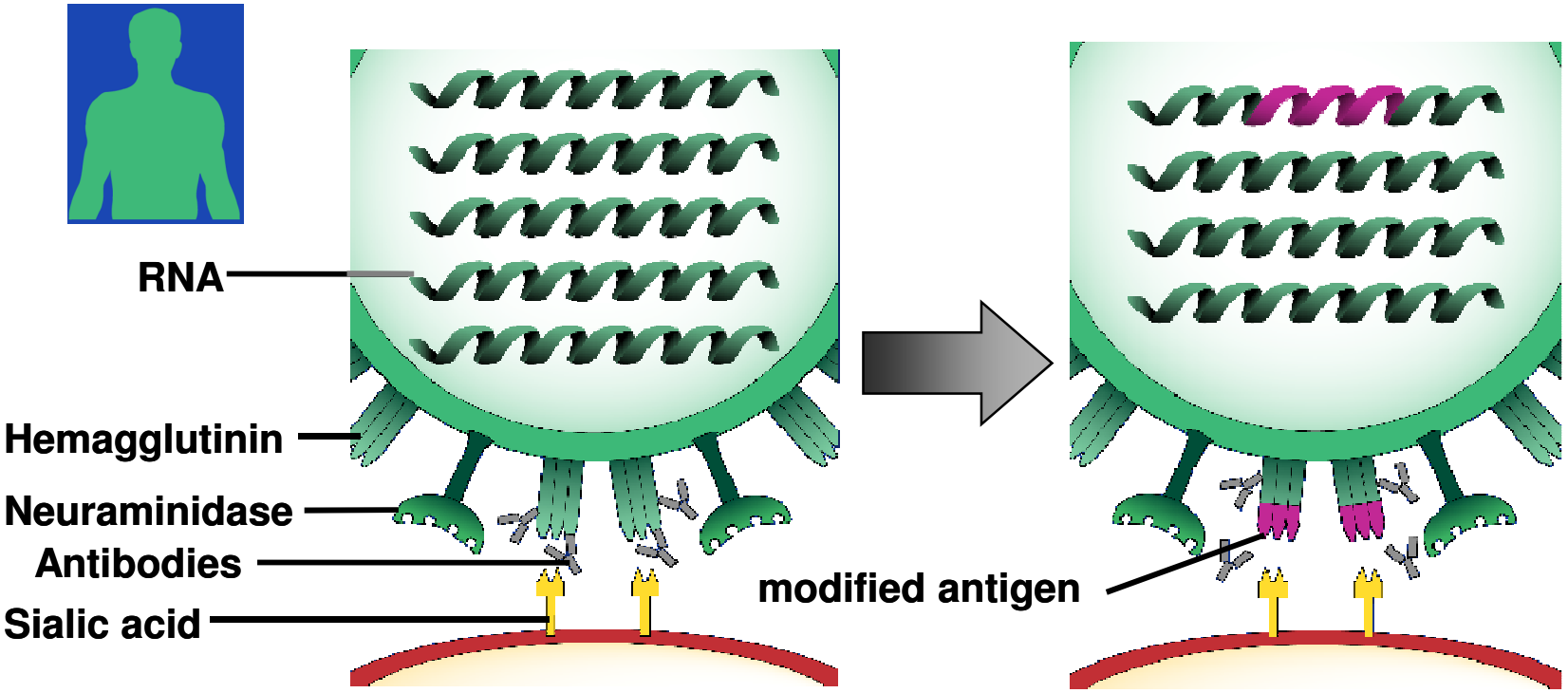



A2FG2S1(2,6)
A2FG2S1(2,6)
A2FG2S1(2,6) + A2FG2S1(2,3)
A2FG2S1(2,3)
A2FG2
A2FG2S1(2,3)
Intensity in RFU (Offset 2000 RFU)
3000
2700
2400
2100
1800
1500
1200
900
600
300
0
140
160
180
200
220
240
260
280
300
320
340
360
380
400
t mig in bp

Typical Cells

cell line	derivation	application
CHO	Chinese hamster ovary	good product glycosylation
CHO dhfr	Mutant for genetic amplification	recombinant products
BHK21	Syrian hamster	viruses/veterinary vaccines
HeLa	Human cervical adenocarcinoma	polio vaccine
Namalwa	Human B lymphoblastoid	Interferon
COS 1/COS 7	African green monkey kidney	recombinant products
293	Adenovirus transformed HEK	Gene therapy
Sf9	Spodoptera frugipeda	Baculoviruses
MDCK	Cocker spaniel kidney	viral vaccines
Vero	African green monkey, kidney	viral vaccines
AGE1.CR	Duck, retina	viral vaccines
A549	Human, lung epithel carcinoma	viral vaccines
HepG2	Human, hepatocell. epith. carcin.	viral vaccines
RCAr	modified MDCK	viral vaccines

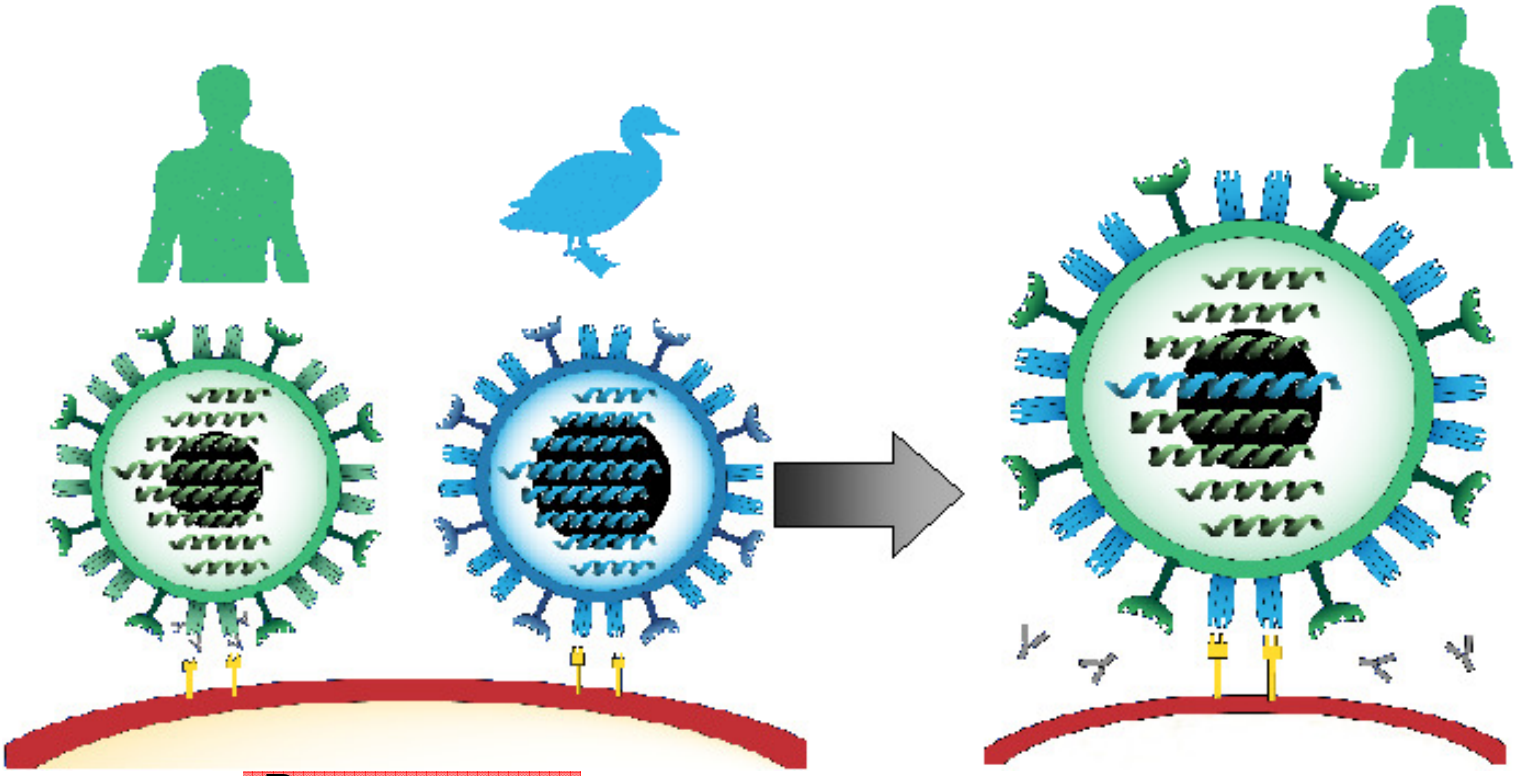
Antigenic Drift



back

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

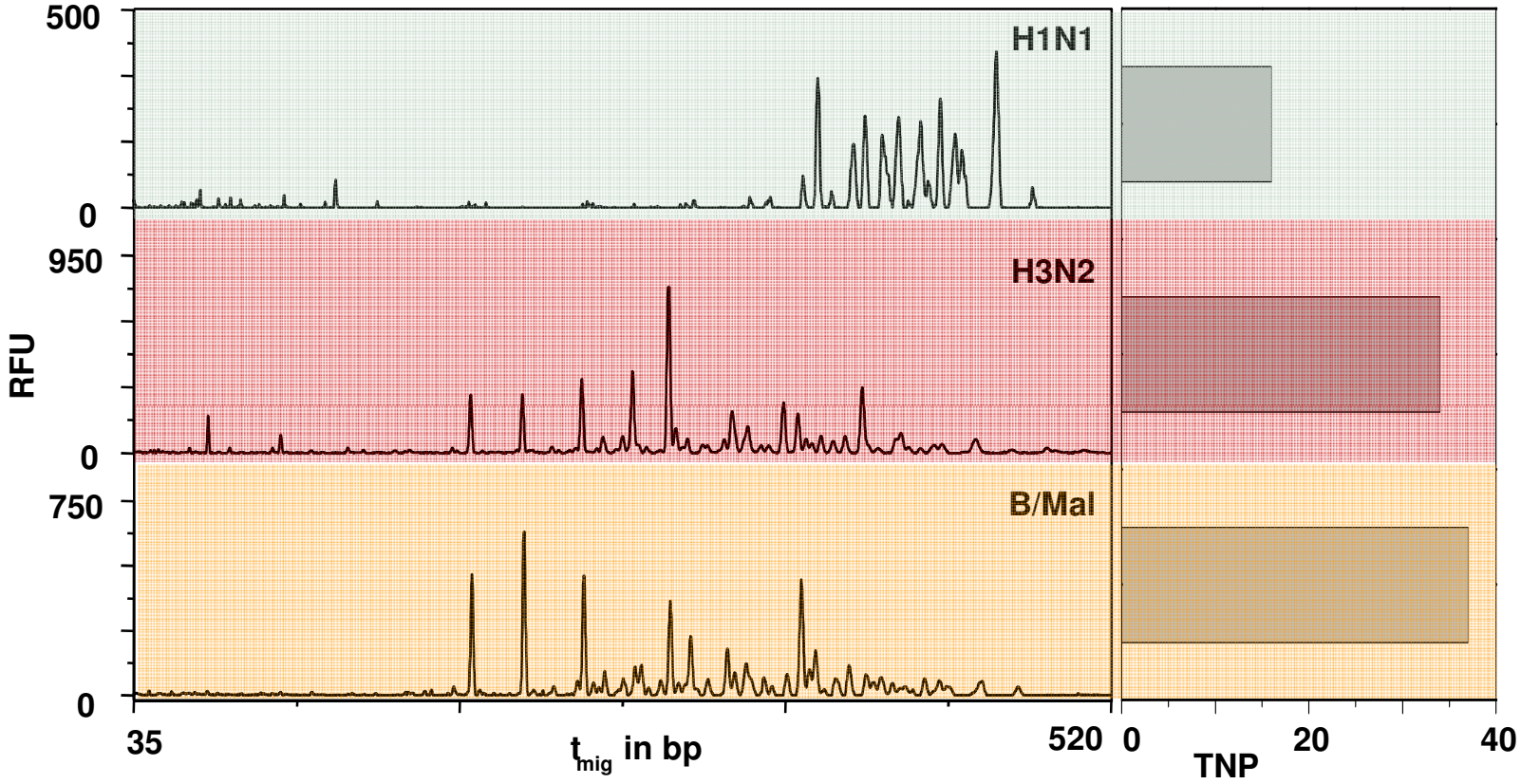
Antigenic Shift



back

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**

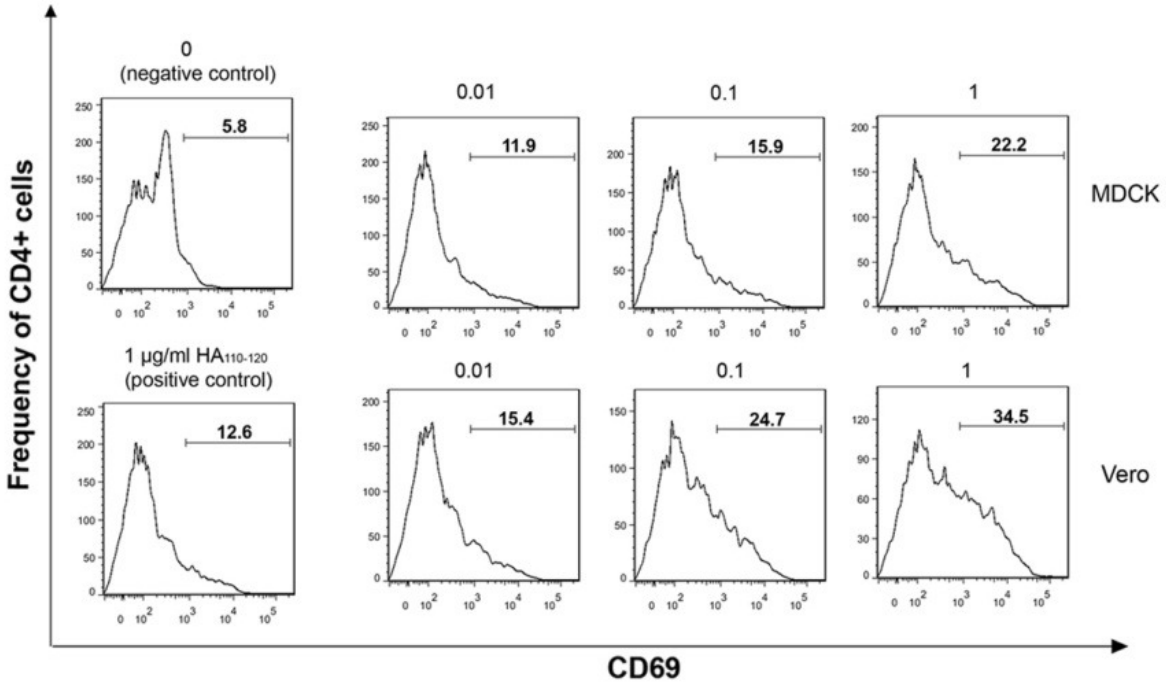
Quasispecies Composition - RKI

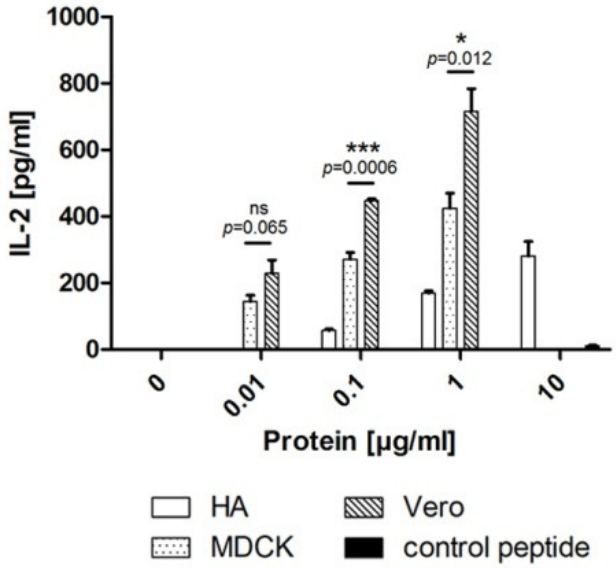
Multiple Substitutions in Consensus Sequence

Segment	Coded Protein	bp-Substitution			AA-substitution			Passage 1	Passage 6	Passage11
1	PB2	C	588	G	C	196	W			44
		G	1351	A	V	451	I		12	
2	PB1	ACAAAGA	524-530	CAAG	NKE	175-177	TR		16	
3	PA	T	150	C	D	50	D			24
		G	585	A	E	195	E			34
4	HA	A	189	G	G	63	G			<10
		C	1370	T	S	457	L		19*	9*
		A	1378	G	K	460	E		80*	81*
		initial seed virus			no AA-substitutions			100	few reads	10
5	NP	A	859	C	S	287	R		22	50
		G	882	T	E	294	D		45	
		A	926	G	N	309	S		19	
		G	1414	C	A	472	P		42	
		G	1418	A	S	473	N		31	
6	NA	A	21	G	I	7	M		<5	>95
8	NS1	T	307	T	S	103	P		100	100

=> Substitutions Improve Viral Fitness in Vero Host Cell System

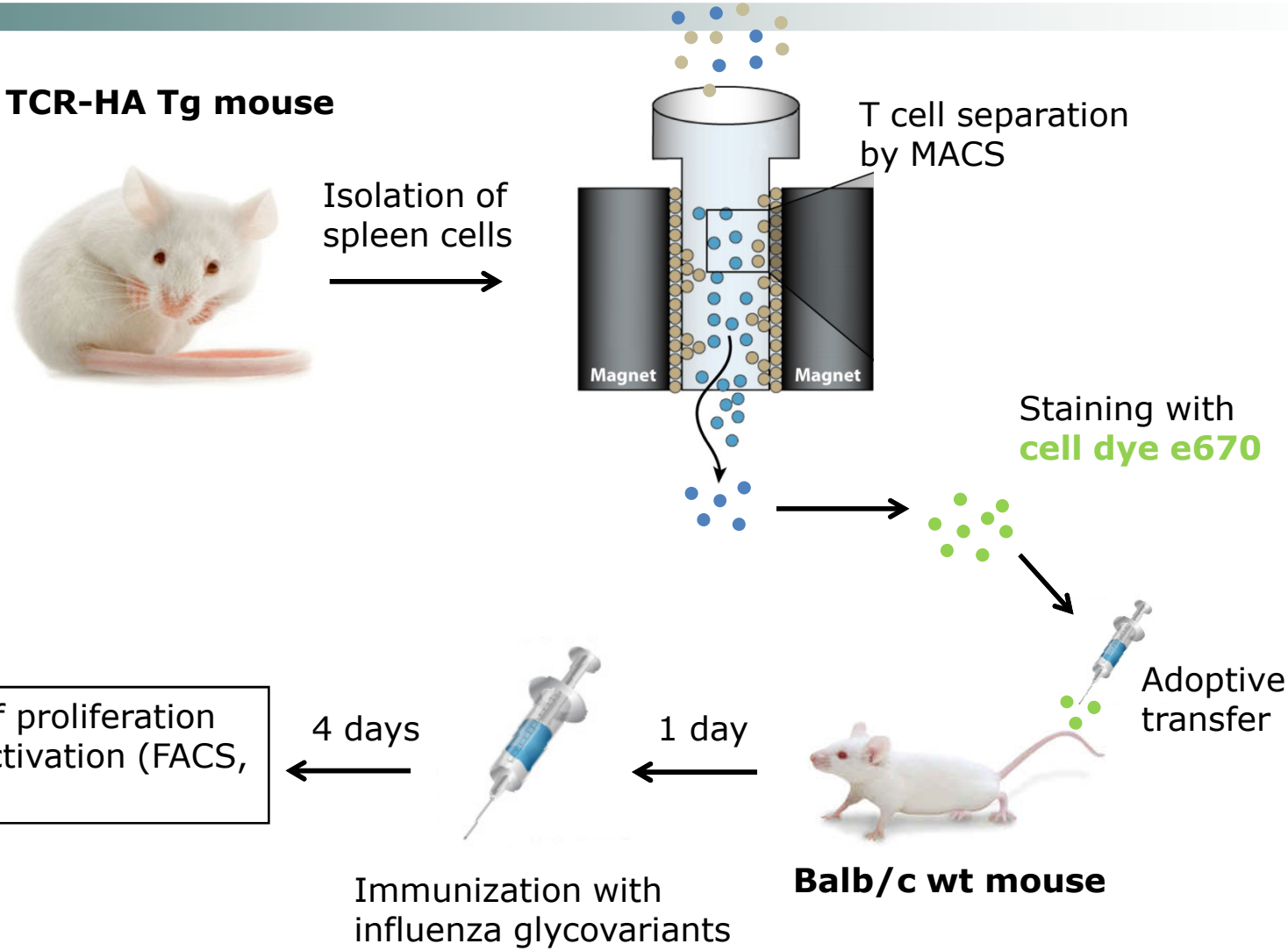
Crucial role of CD11c+ dendritic cells



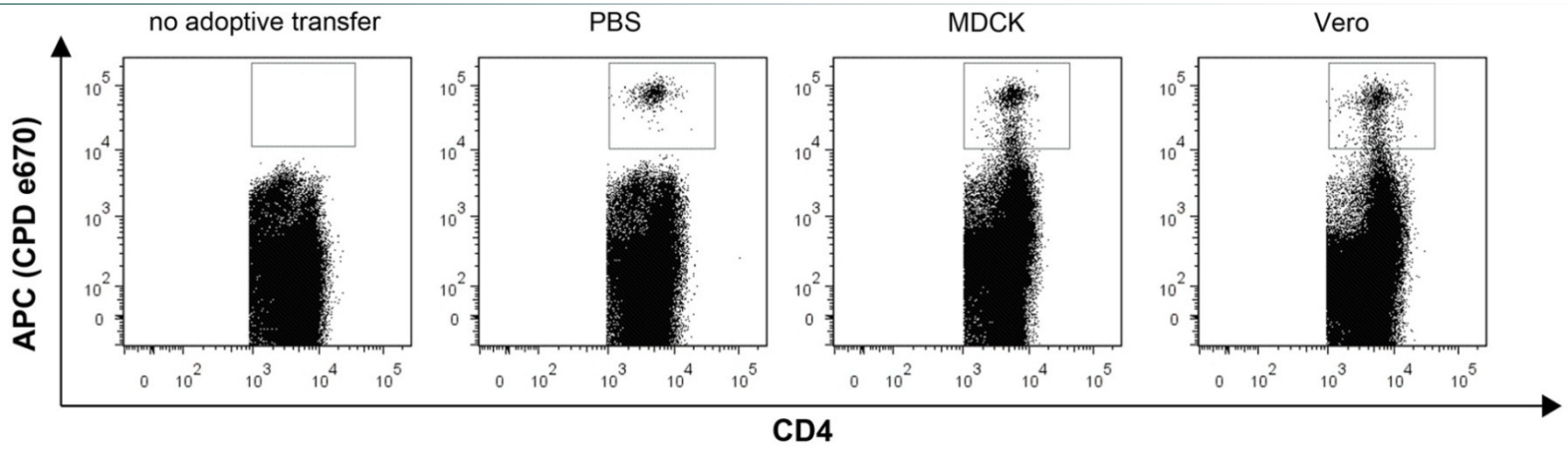


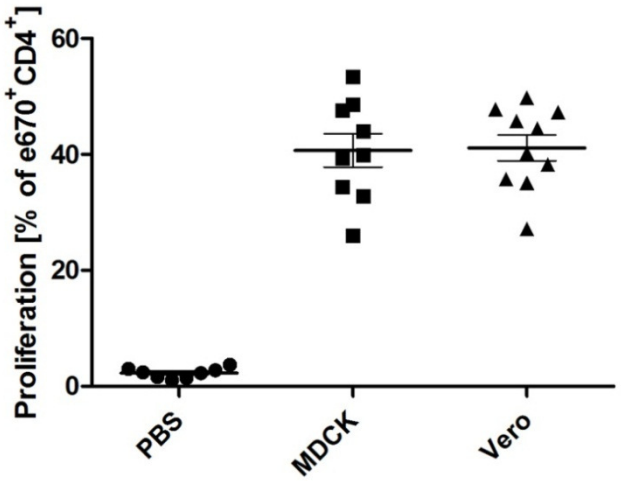
- CD11c+ dendritic cells were separated by magnetic cell separation (MACS) and co-cultivated with TCR-HA transgenic T cells
- Dendritic cells are responsible for the differential T cell activation, presumably by differential recognition and/or uptake of the glycovariants

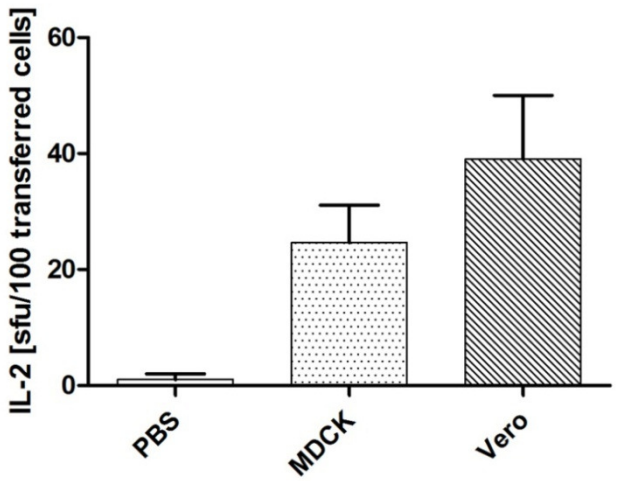
Adoptive transfer of transgenic T cells



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