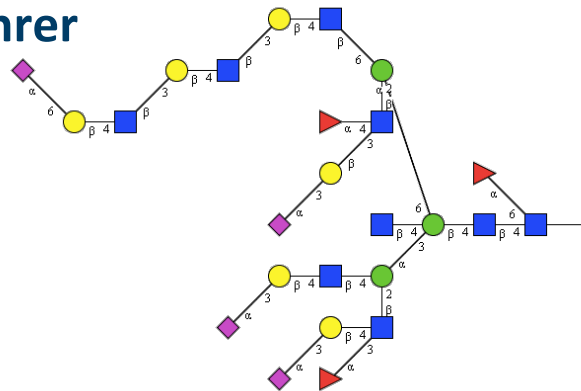


# Analytical Glycomics and Glycoproteomics Methods

**Manfred Wuhrer**



Center for Proteomics and Metabolomics, Leiden University Medical Center  
Leiden, The Netherlands



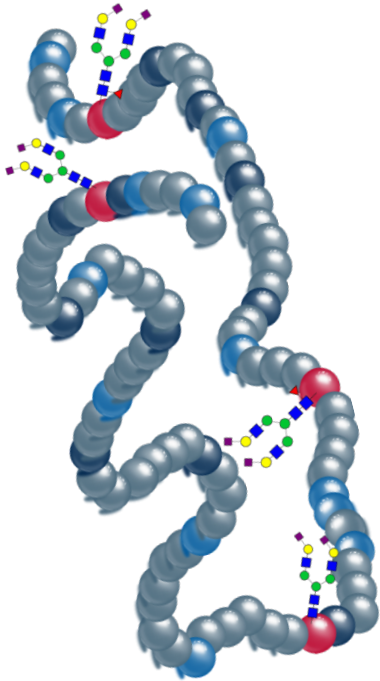
27 MARCH 2018

**IM FOR FUTURE**

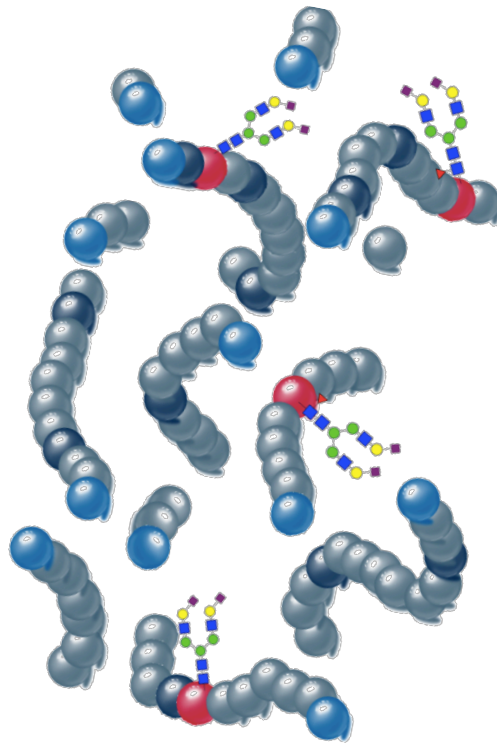


1ST ANNUAL MEETING, ZAGREB

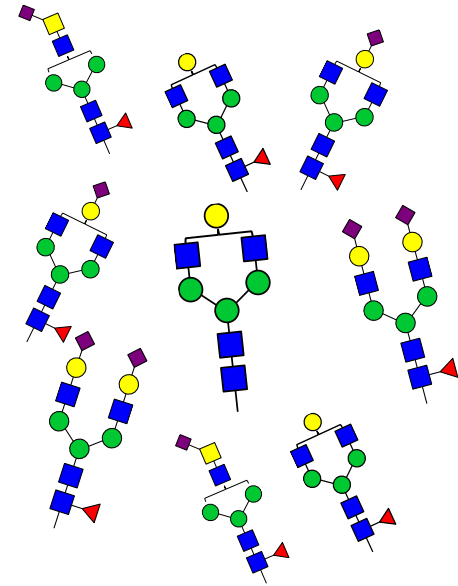
# Glycosylation analysis approaches



Intact glycoproteins

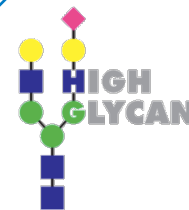


Glycopeptides



Released glycans

# Methods introduced



## **HILIC-UHPLC-FLD (Genos)**

- Hydrophilic interaction liquid chromatography – ultrahigh performance liquid chromatography – fluorescence detection
- Genos
- 2-AB labeling, 2-AA labeling
- GHP; hydrazide; LudgerClean HILIC-SPE
- Separation by hydrophilic interactions (proxy for glycan size) on BEH column
- Structural annotation by database

## **xCGE-LIF (MPI / glyXera)**

- Multiplexed capillary gel electrophoresis – laser-induced fluorescence detection
- APTS labeling
- BioGel HILIC-SPE
- Separation by charge and POP-7 polymer
- Structural annotation by database

## **MALDI-TOF-MS (LUMC / MPI)**

- Matrix-assisted laser desorption/ionization – time-of-flight – mass spectrometry
- Ethyl esterification
- GHP HILIC-SPE
- Reflectron positive MALDI-TOF-MS
- Compositional annotation by  $m/z$  and QC

## **+ LC-MS (LUMC / Genos / glyXera)**

Liquid chromatography – electrospray ionization – mass spectrometry

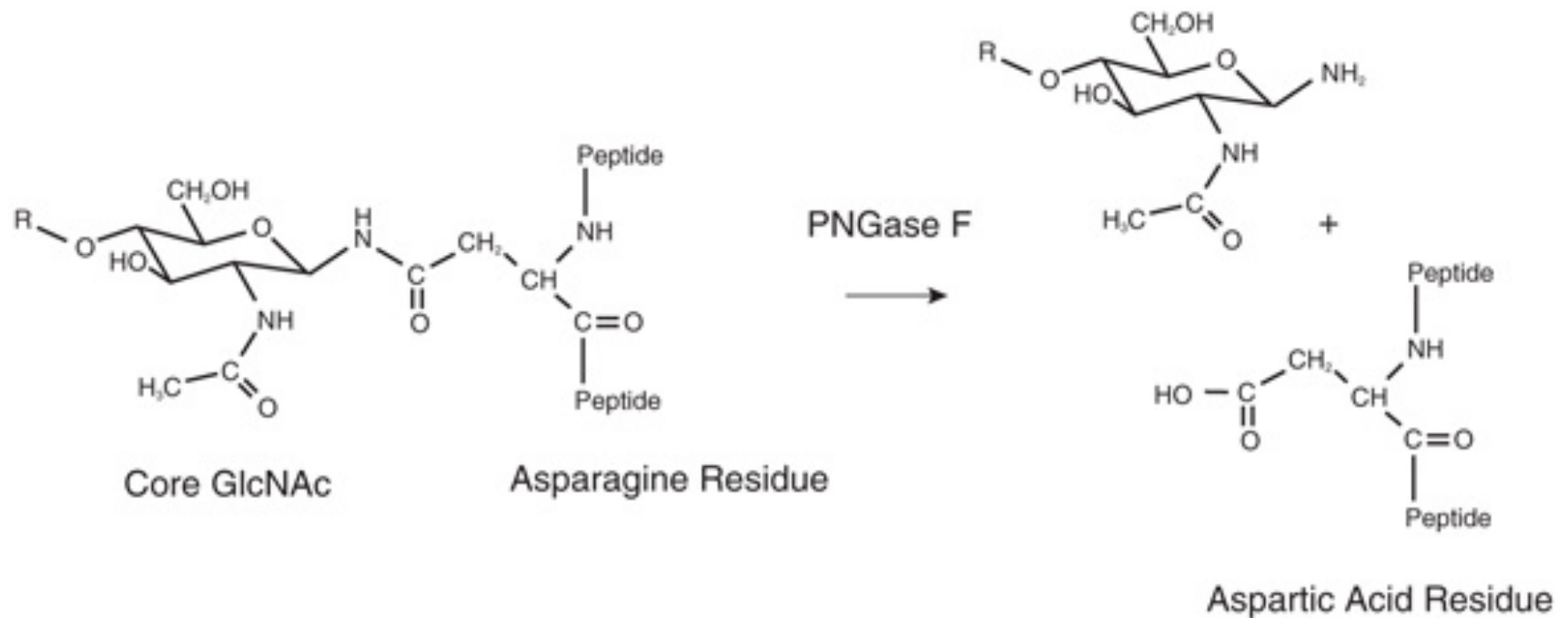
# Characteristics of HTP glycoanalytical methods



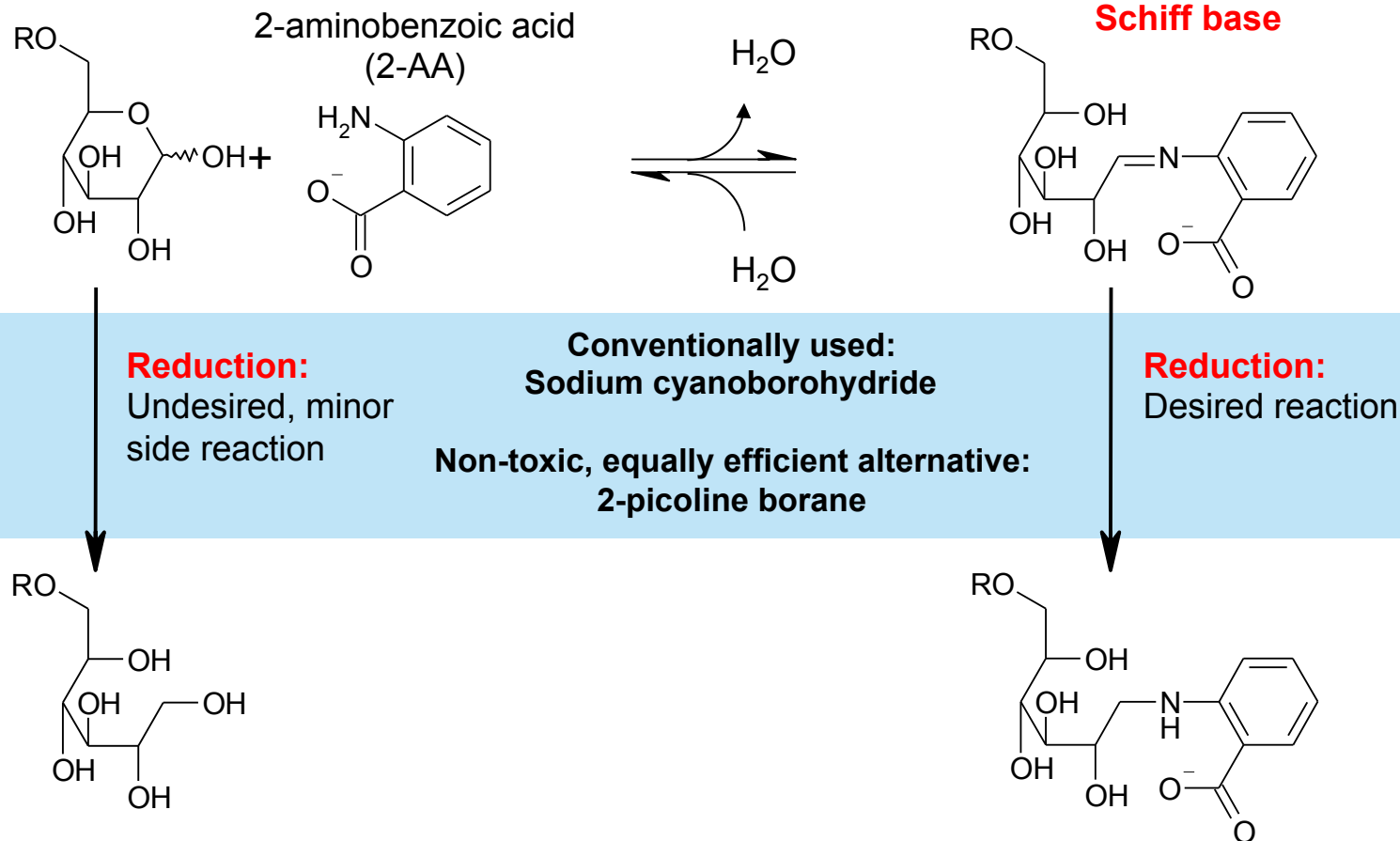
	1. MALDI-MS	2. HILIC-UPLC	3. CGE-LIF
<i>Acceptance / usage</i>	++	+++	+
<i>Throughput</i>	+++	+	+++
<i>Required expertise</i>	--	-	-
<i>Resolution</i>	+++	+	+
<i>Isomer separation</i>	(++)	+++	+++
<i>Quantification</i>	+	+++	++
<i>Costs of equipment</i>	---	--	--
<i>Costs per sample in HTP mode</i>	-	--	-



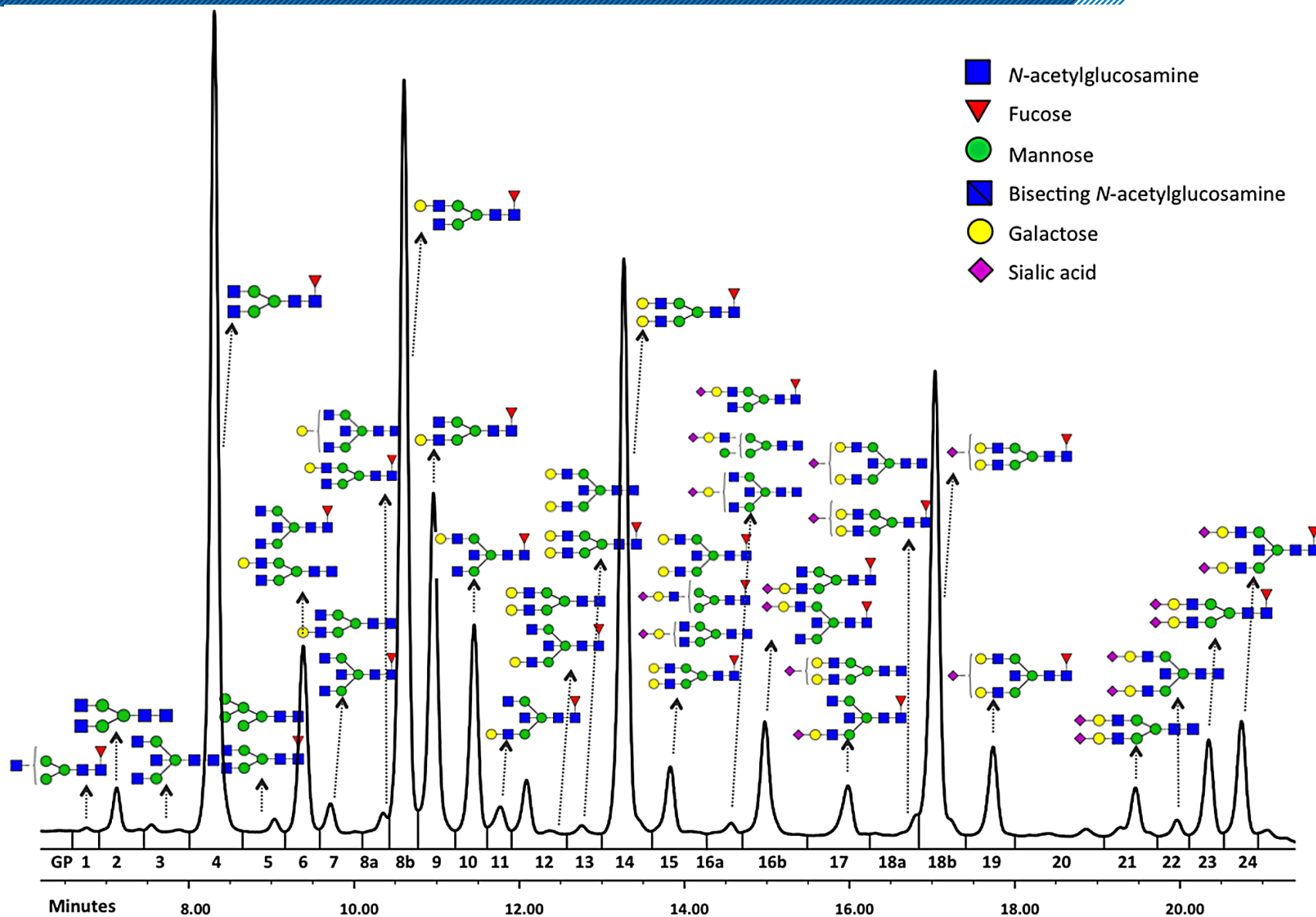
# Enzymatic N-glycan release



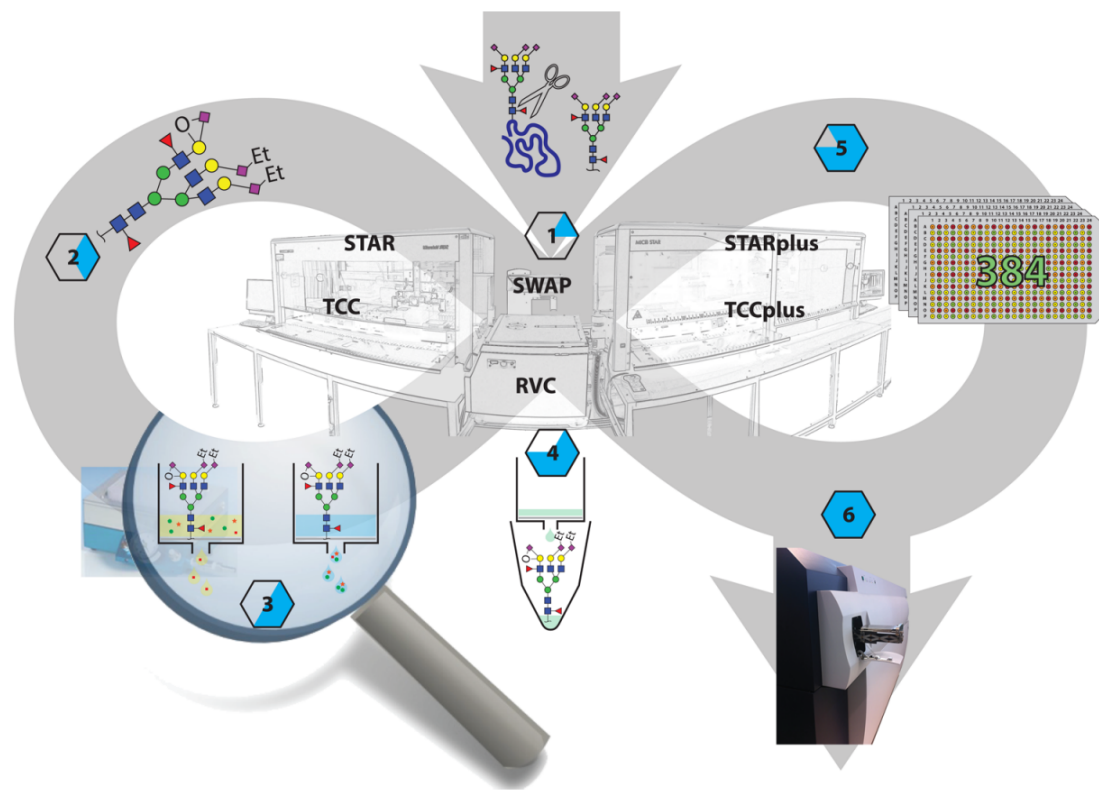
# Glycan labeling by reductive amination



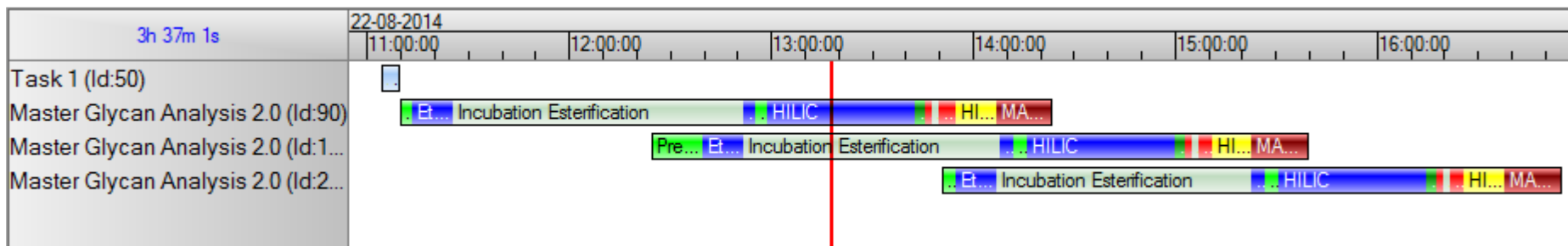
# HILIC-UPLC of IgG N-glycans



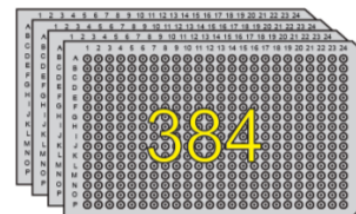
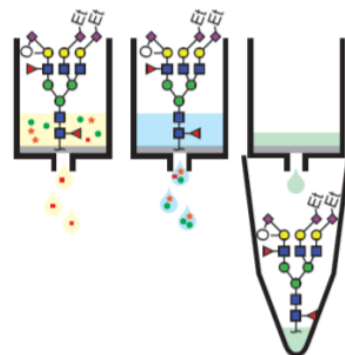
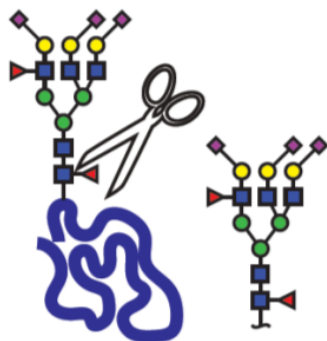
# Automation of sample preparation



- 1 – PNGase F release
- 2 – Ethyl esterification
- 3 – HILIC-SPE
- 4 – Elution
- 5 – MALDI target spotting
- 6 – MALDI-TOF-MS



# MALDI-TOF-MS analysis

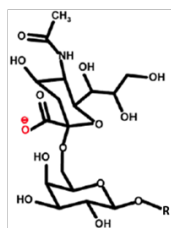


Glycan Release

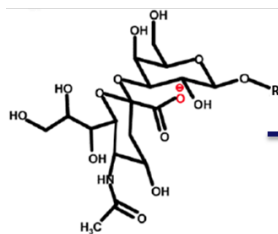
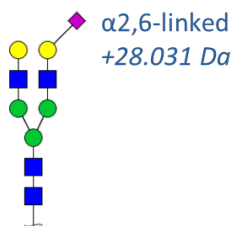
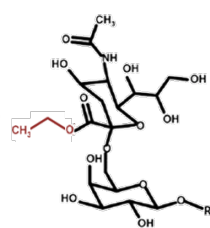
Glycan Derivatization

Glycan Purification

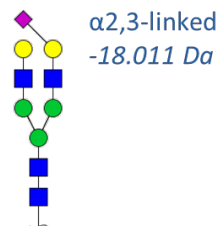
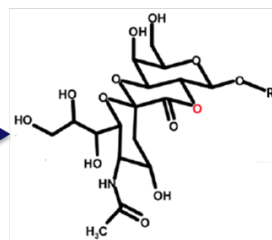
MALDI spotting and  
MS-Analysis



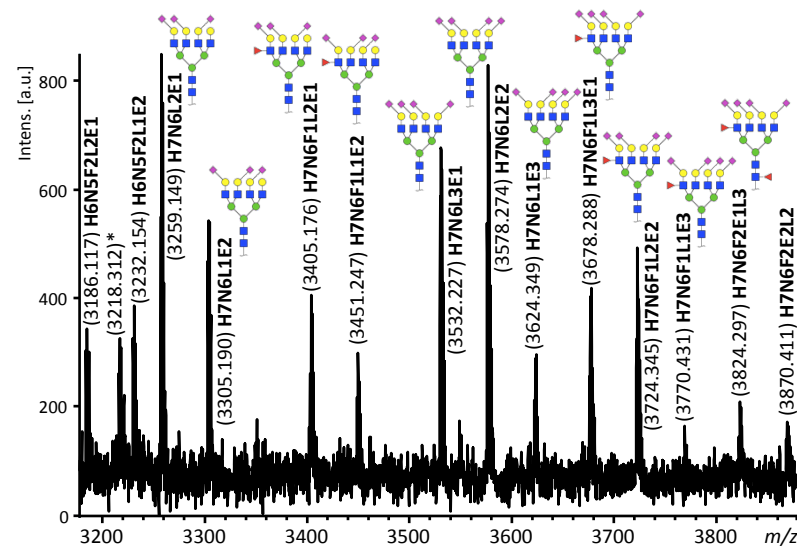
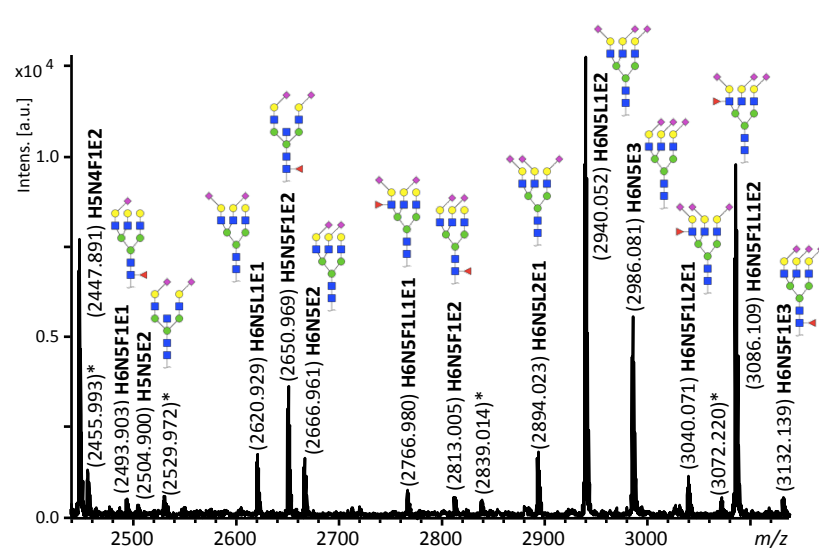
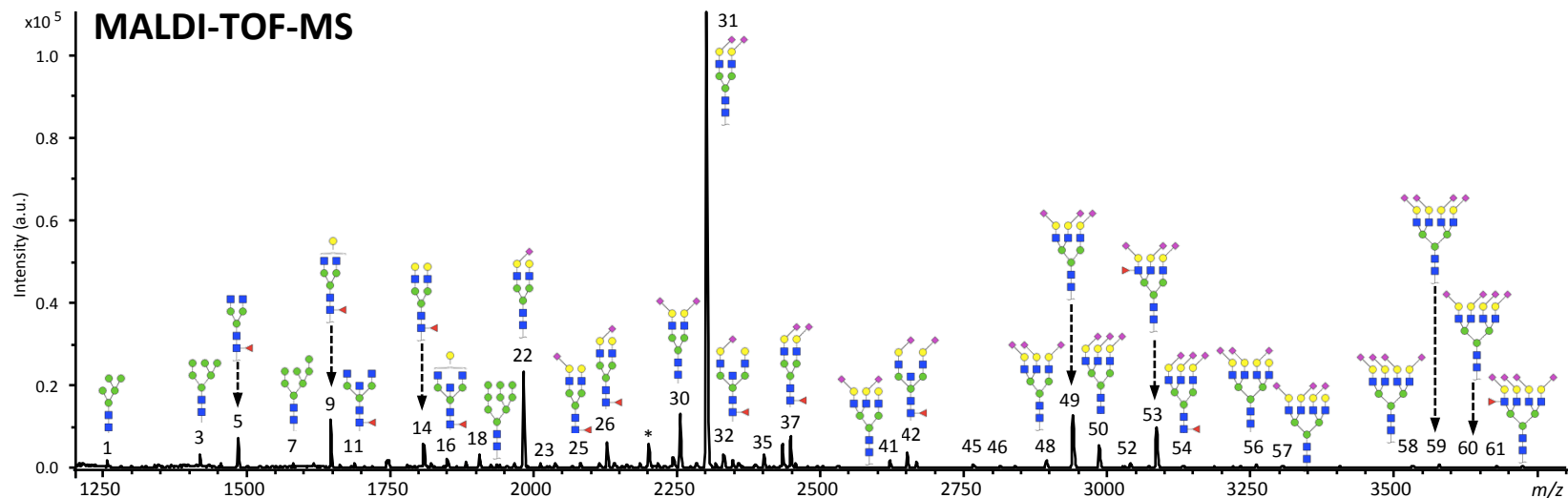
EDC/HOBt  
Ethanol  
1h 37 °C



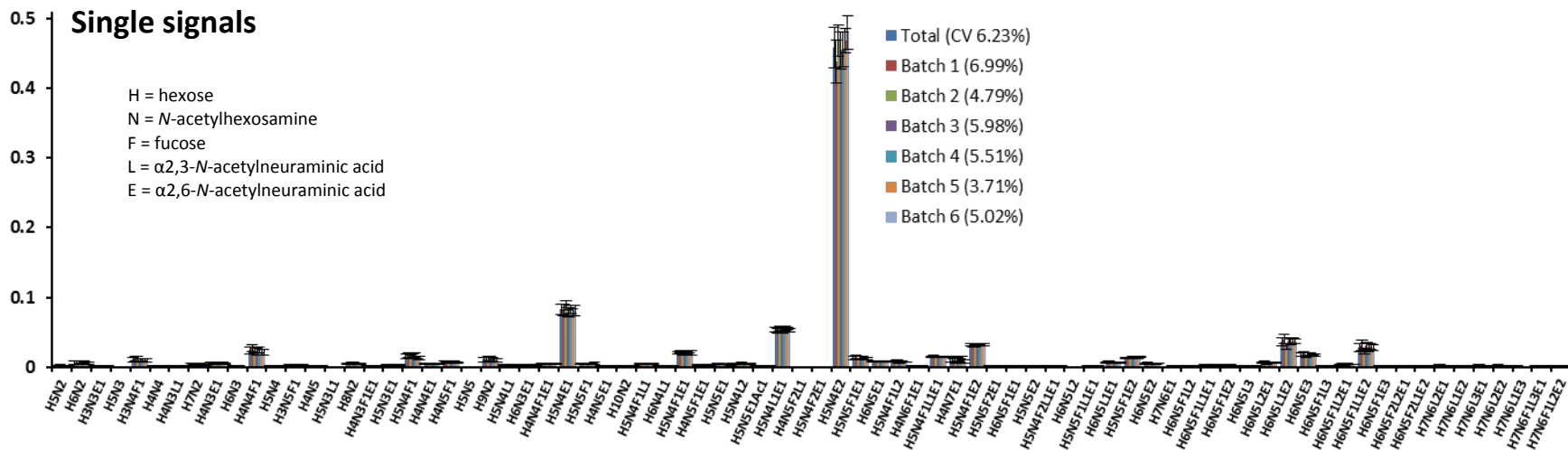
EDC/HOBt  
Ethanol  
1h 37 °C



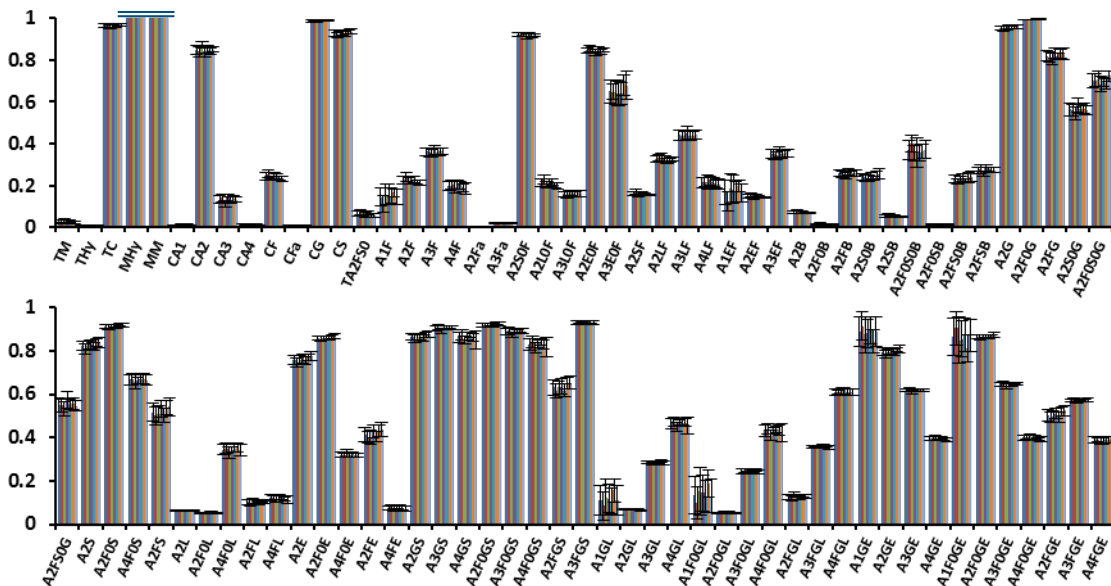
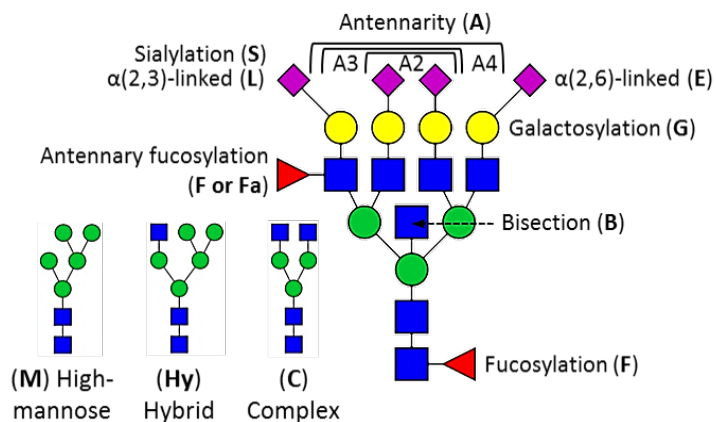
# MS serum *N*-glycome profile



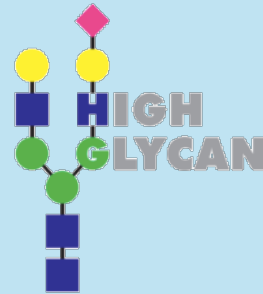
## Standard repeatability



## Derived traits





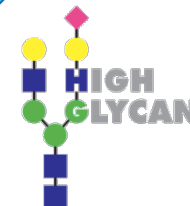


# Comparison of high-throughput *N*-glycomics methods





# Methods and collaborators



## HILIC-UHPLC-FLD

- Genos; NIBRT; Ludger
- 2-AB labeling
- GHP; hydrazide; LudgerClean HILIC-SPE
- Separation by BEH column
- Structural annotation by database

## xCGE-LIF

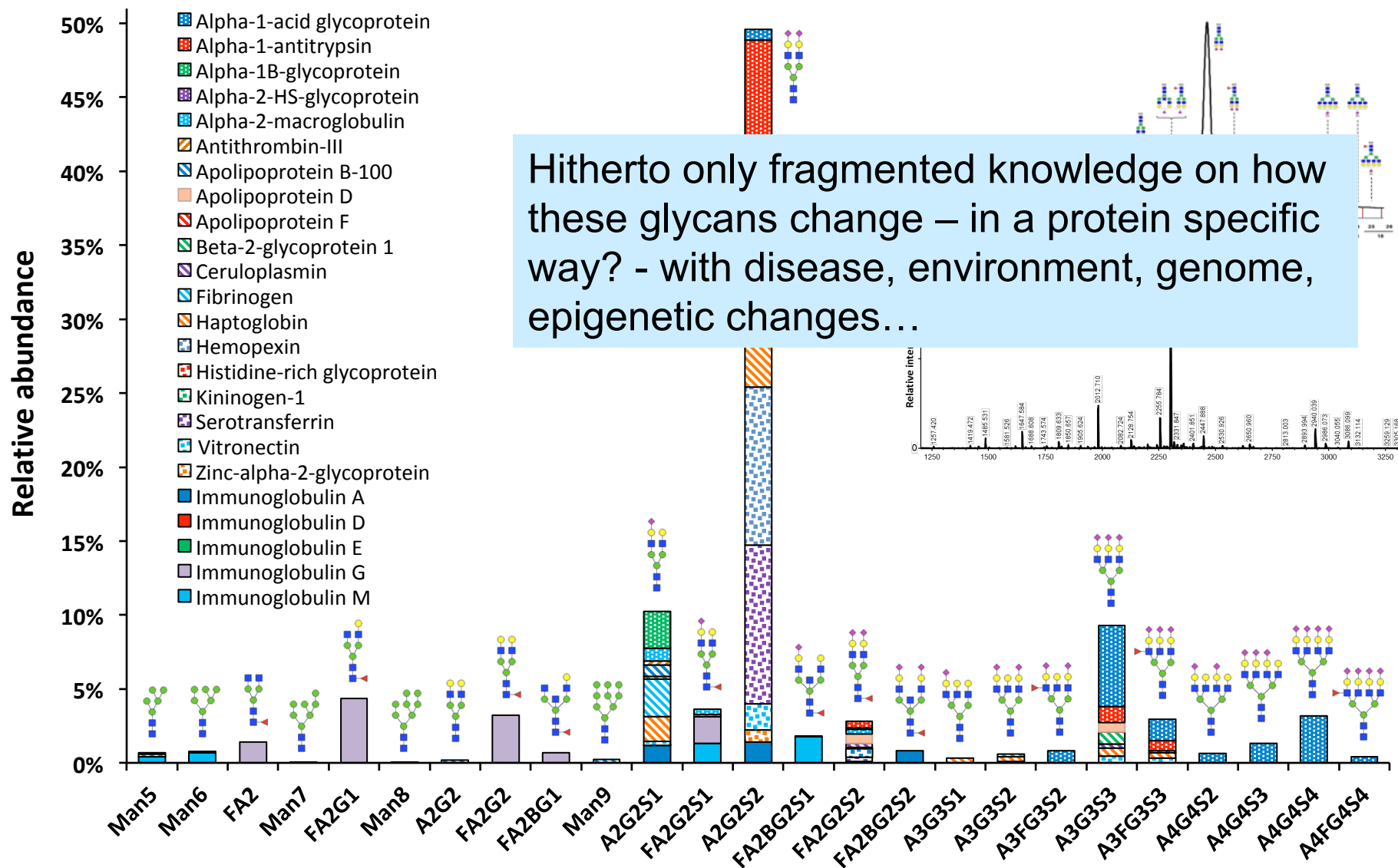
- MPI / glyXera
- APTS labeling
- BioGel HILIC-SPE
- Separation by charge and POP-7 polymer
- Structural annotation by database

## MALDI-TOF-MS

- LUMC
- Ethyl esterification
- GHP HILIC-SPE
- Reflectron positive MALDI-TOF-MS
- Compositional annotation by  $m/z$  and QC

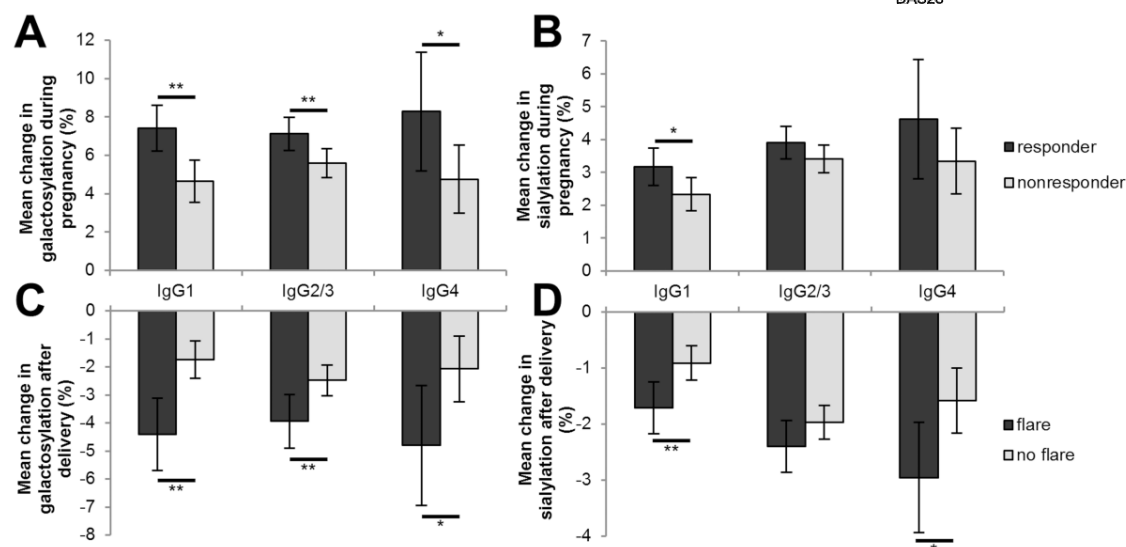
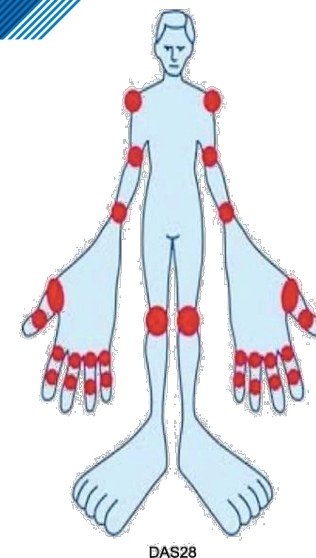
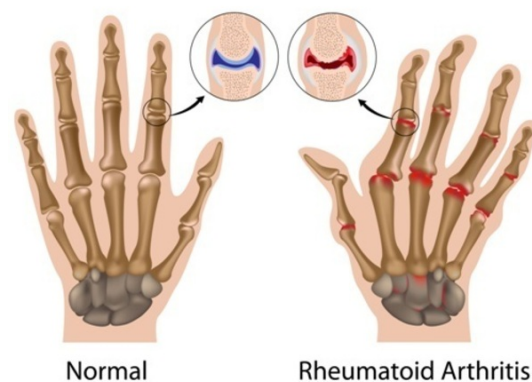


# Human plasma N-glycosylation



# Rheumatoid arthritis

- Most common type of autoimmune disease (1 – 2 % of population affected)
- Chronic pain and decreased quality of life
- Disease activity score (DAS28-CRP)
- Reported improvement of disease activity (score) during pregnancy
- IgG glycosylation shows association with changes in disease activity (response and flare)



Scott, et al., 2010, Lancet.

Figure 1: <https://ghr.nlm.nih.gov/condition/rheumatoid-arthritis>

Figure 2: Jeong, 2014, J. Rheum. Dis.

Bondt, et al., 2013, J. Proteome Res.

# Study design

## PARA study

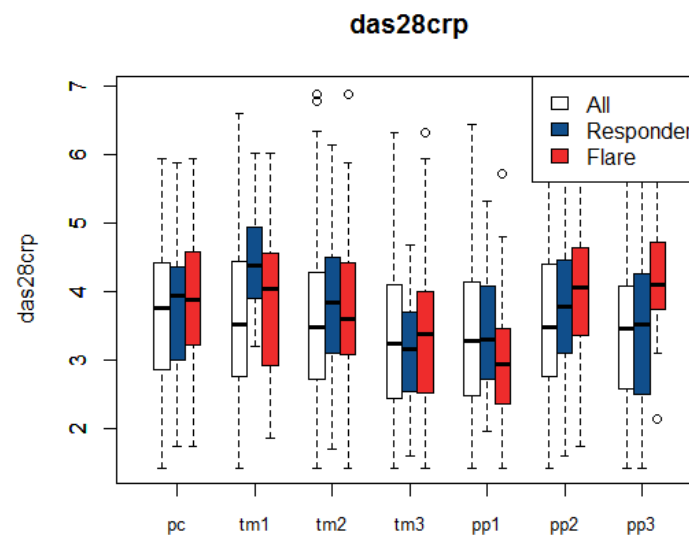
### *Pregnancy-induced Amelioration of Rheumatoid Arthritis*



- 219 RA patients (253 pregnancies)
- 32 healthy volunteers (32 pregnancies)
- Serum samples at multiple time points
  - Preconception (**pc**)
  - 1<sup>st</sup> trimester (**tm1**)
  - 2<sup>nd</sup> trimester (**tm2**)
  - 3<sup>rd</sup> trimester (**tm3**)
  - 6 weeks postpartum (**pp1**)
  - 12 weeks postpartum (**pp2**)
  - 26+ weeks postpartum (**pp3**)
- Additional information on:
  - DAS28-CRP, ACPA, RF, medication use, responder and flare status, ...
- Total number of samples: 1944

**Table 1. Cohort characteristics**

	control pregnancies (n = 32)	patient pregnancies (n = 253)
Age at delivery in years, mean (SD)	32.1 (4.4)	32.8 (3.7)
Duration of pregnancy in weeks, mean (SD)	40.1 (1.4)	39.2 (1.9)
Disease duration at first visit in years, mean (SD)	-	7.7 (6.3)
ACPA positive patients, n (%)	-	153/253 (60.5)
RF positive patients, n (%)	-	161/240 (67.1)
ACPA and/or RF positive patients, n (%)	-	181/248 (73.0)
Erosive disease, n (%)	-	149/229 (65.1)
Response during pregnancy, n (%)	-	72/137 (52.6)
Flare during postpartum period, n (%)	-	72/232 (31.0)



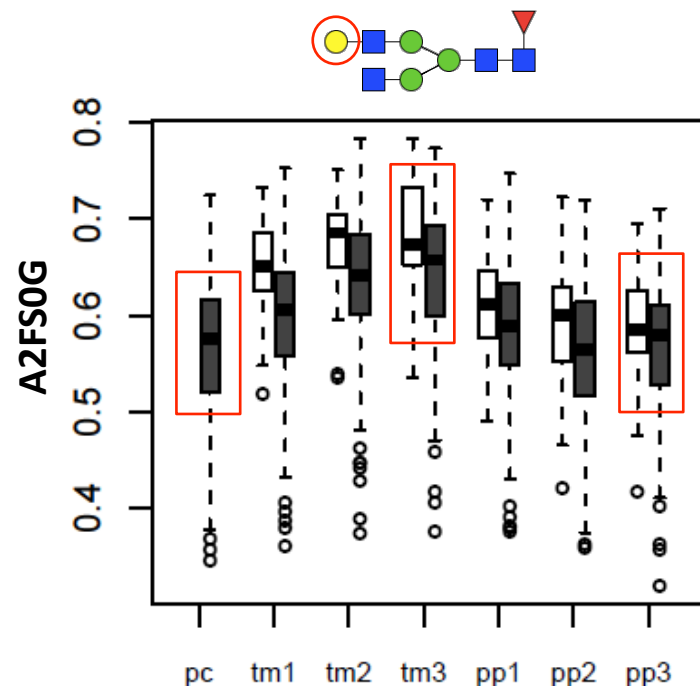
# Method comparison design

## High-throughput glycomics methods

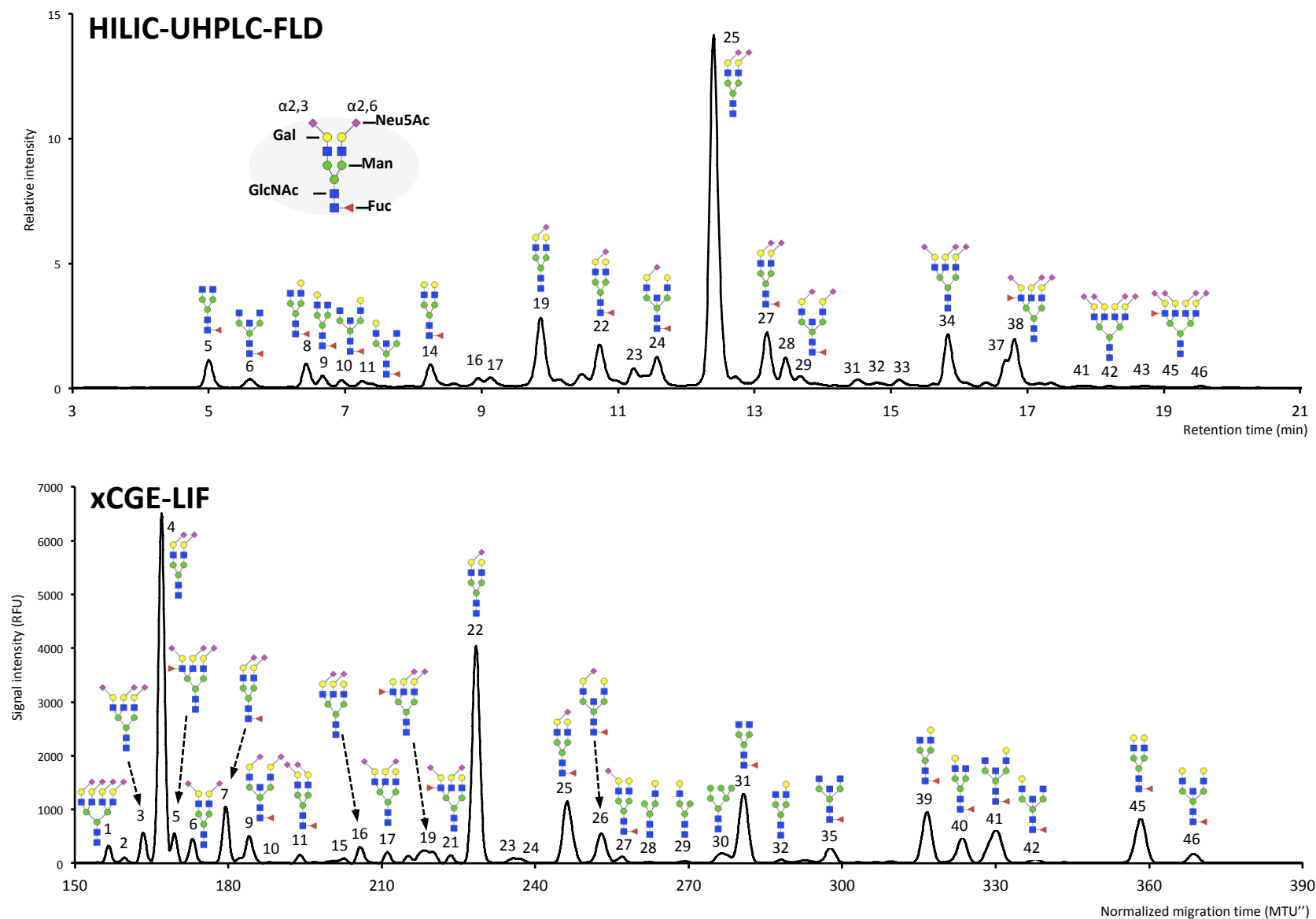
- HILIC-UHPLC-FLD
- xCGE-LIF
- MALDI-TOF-MS

## PARA cohort subselection

- RA samples
  - 36 x preconception (**pc**)
  - 36 x 3<sup>rd</sup> trimester pregnancy (**tm3**)
  - 36 x 26+ weeks postpartum (**pp3**)
- Healthy control samples
  - 32 x 3<sup>rd</sup> trimester (**tm3**)
  - 32 x 26 weeks+ postpartum (**pp3**)
- 10 common standards (commercially available pooled plasma)
- 5 local standards
- 4 blanks

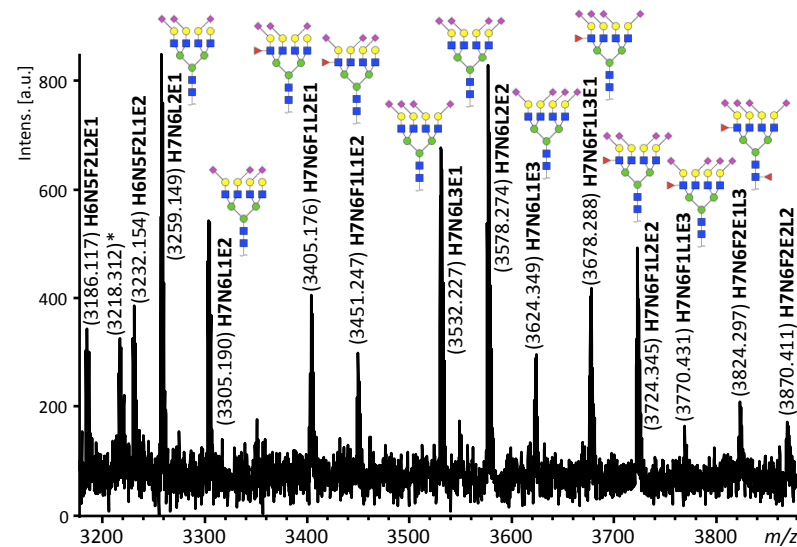
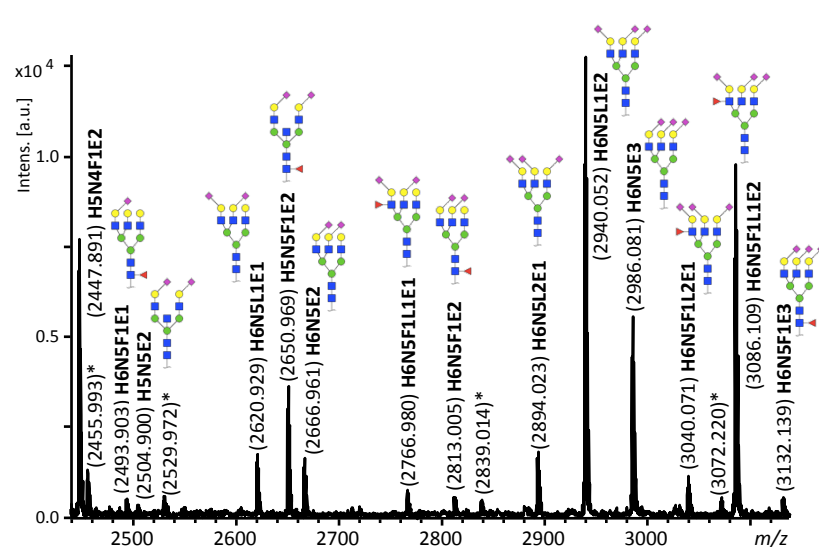
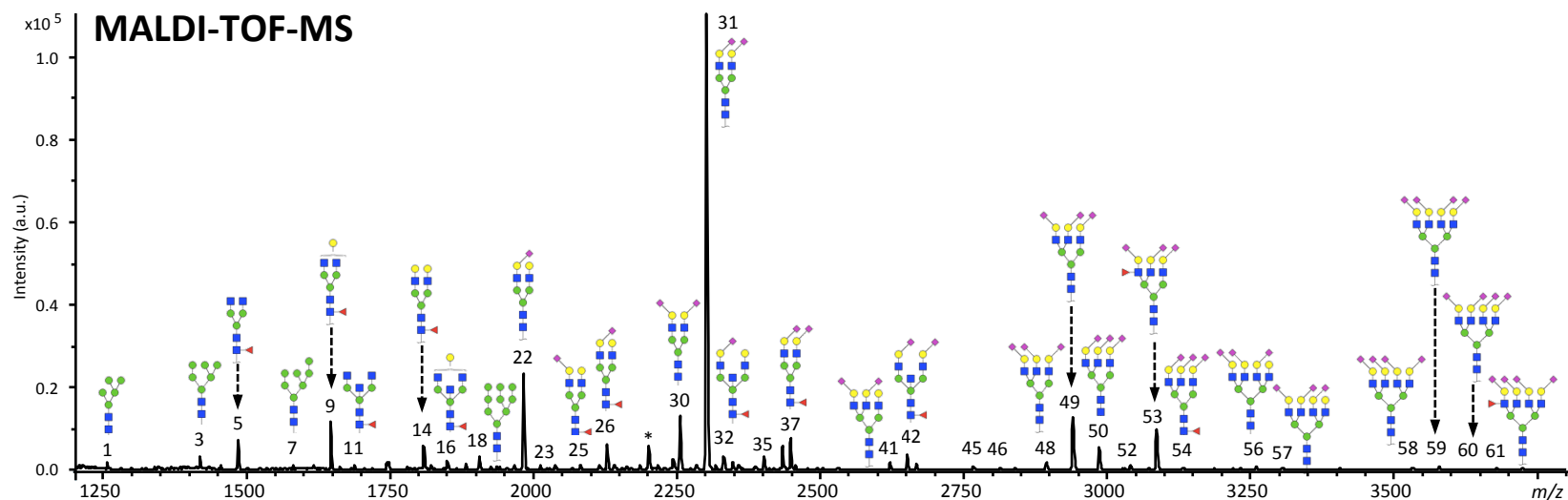


# Non-MS serum N-glycome profiles



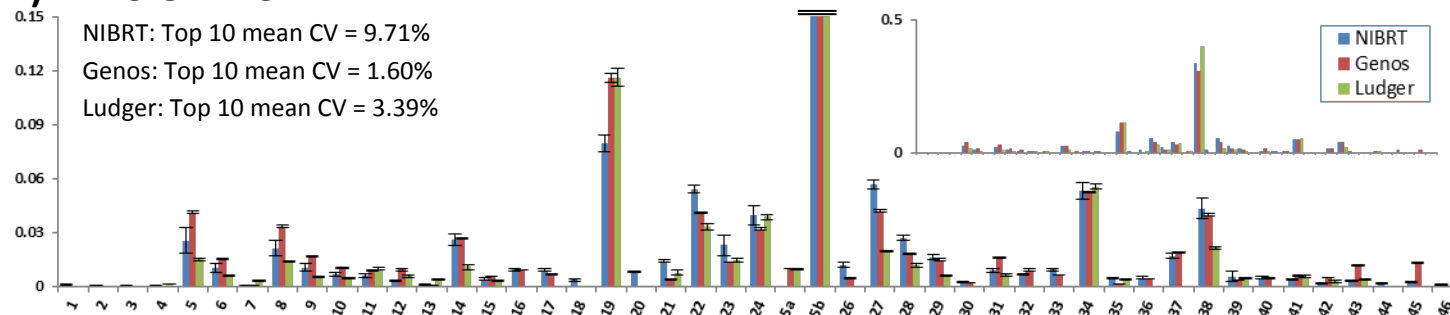


# MS serum N-glycome profile

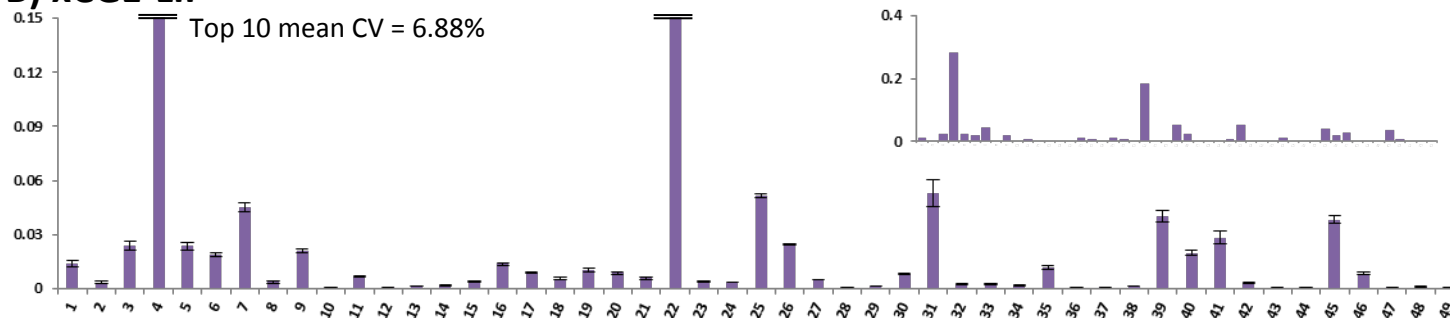


# Method repeatability

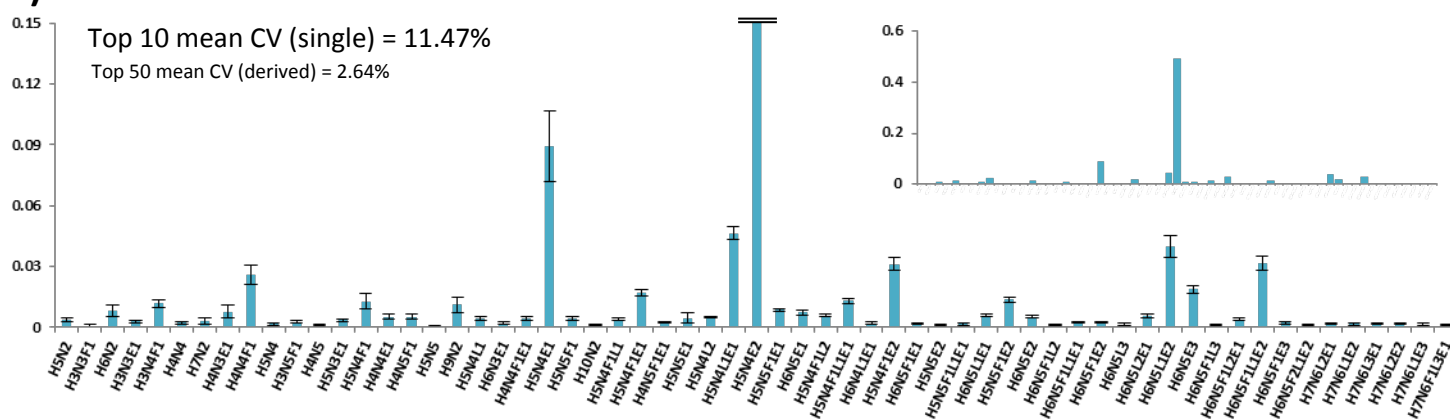
## A) HILIC-UHPLC



## B) xCGE-LIF

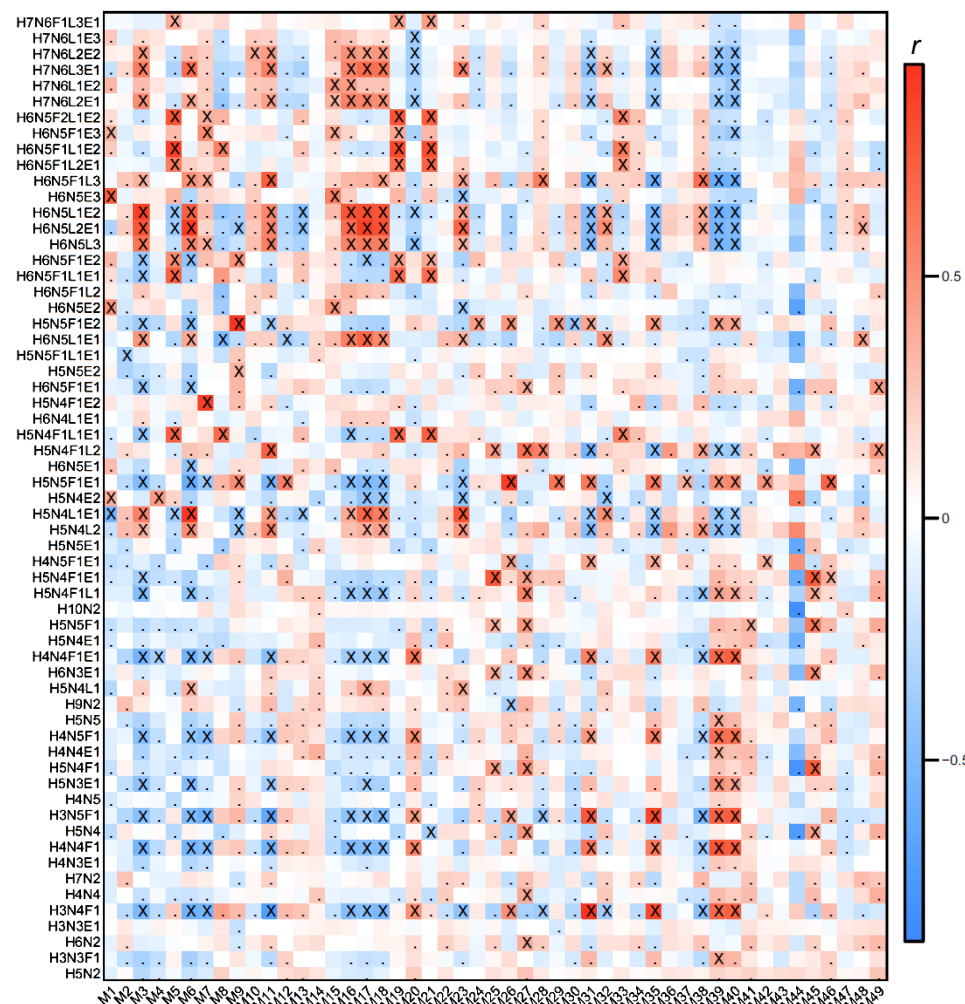
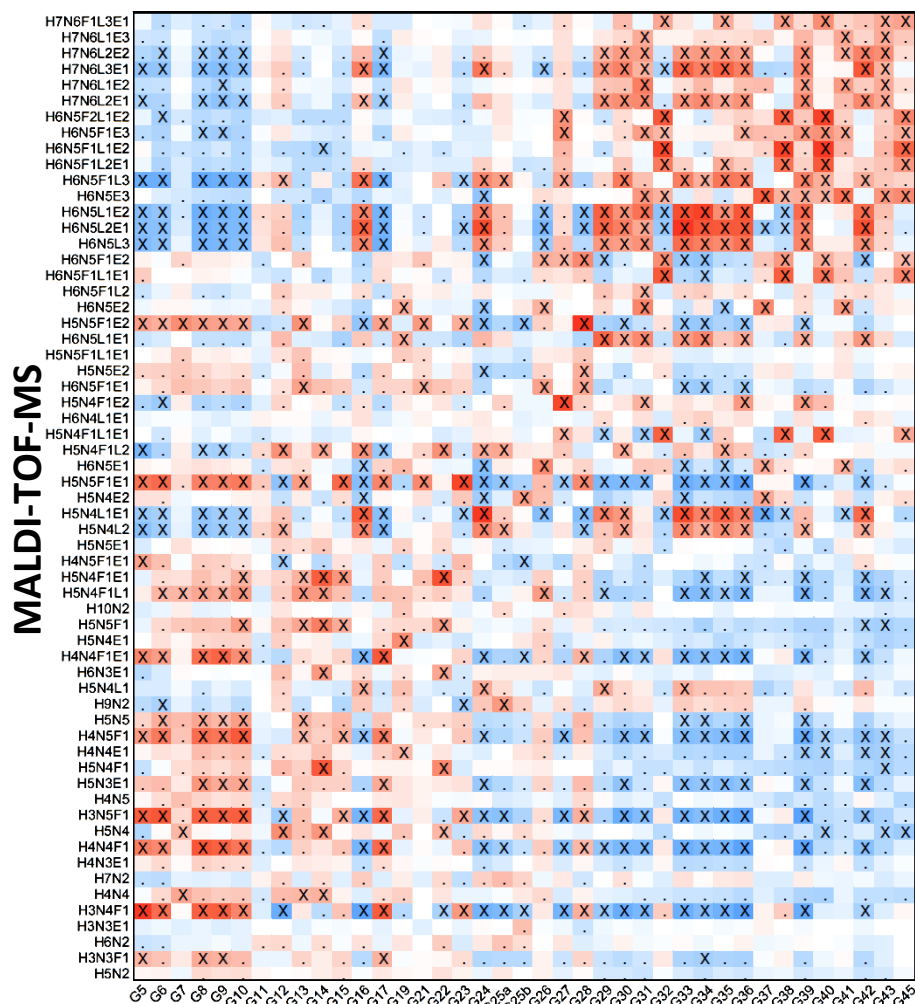


## C) MALDI-TOF-MS



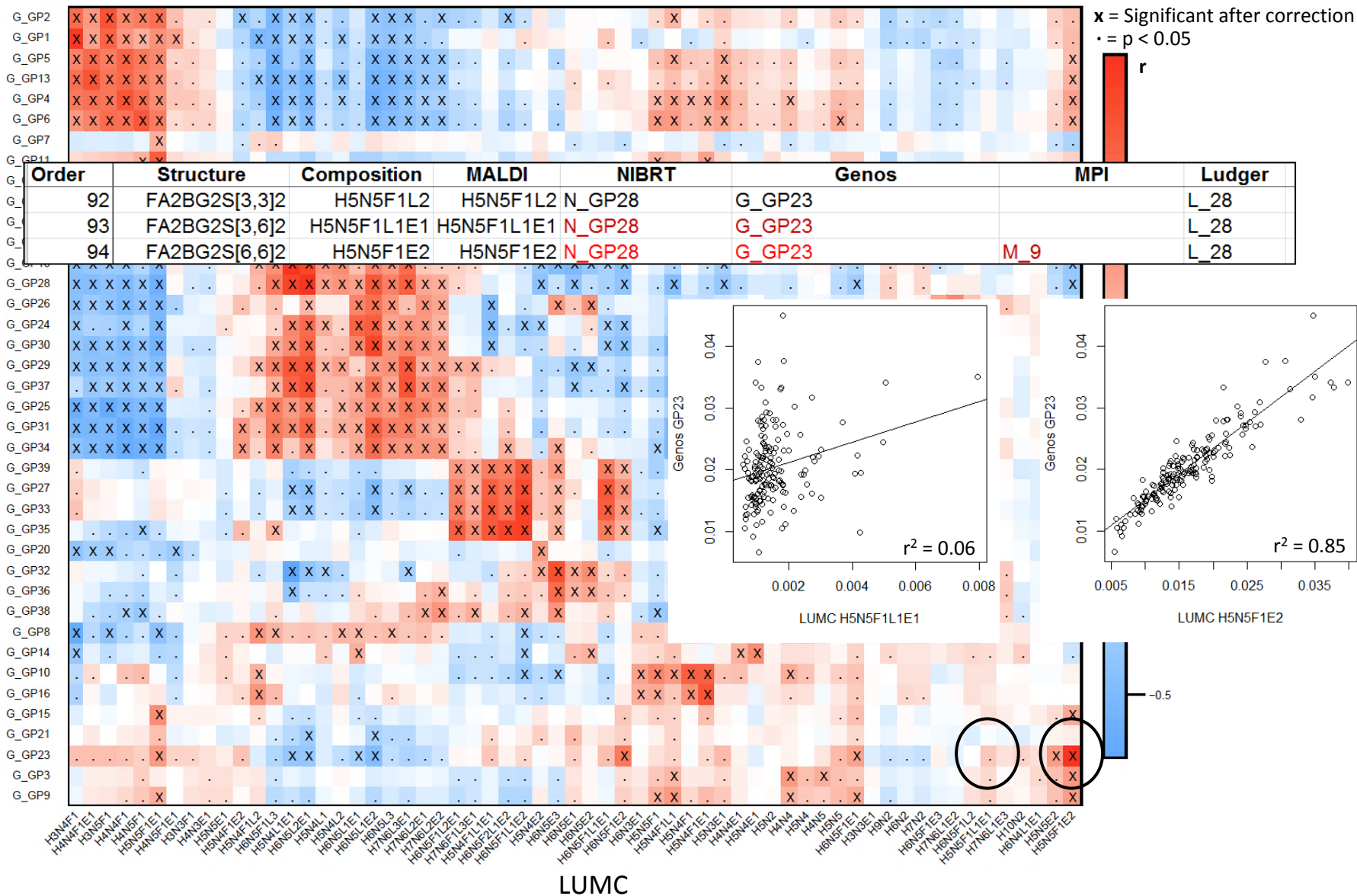


# Signal correlation

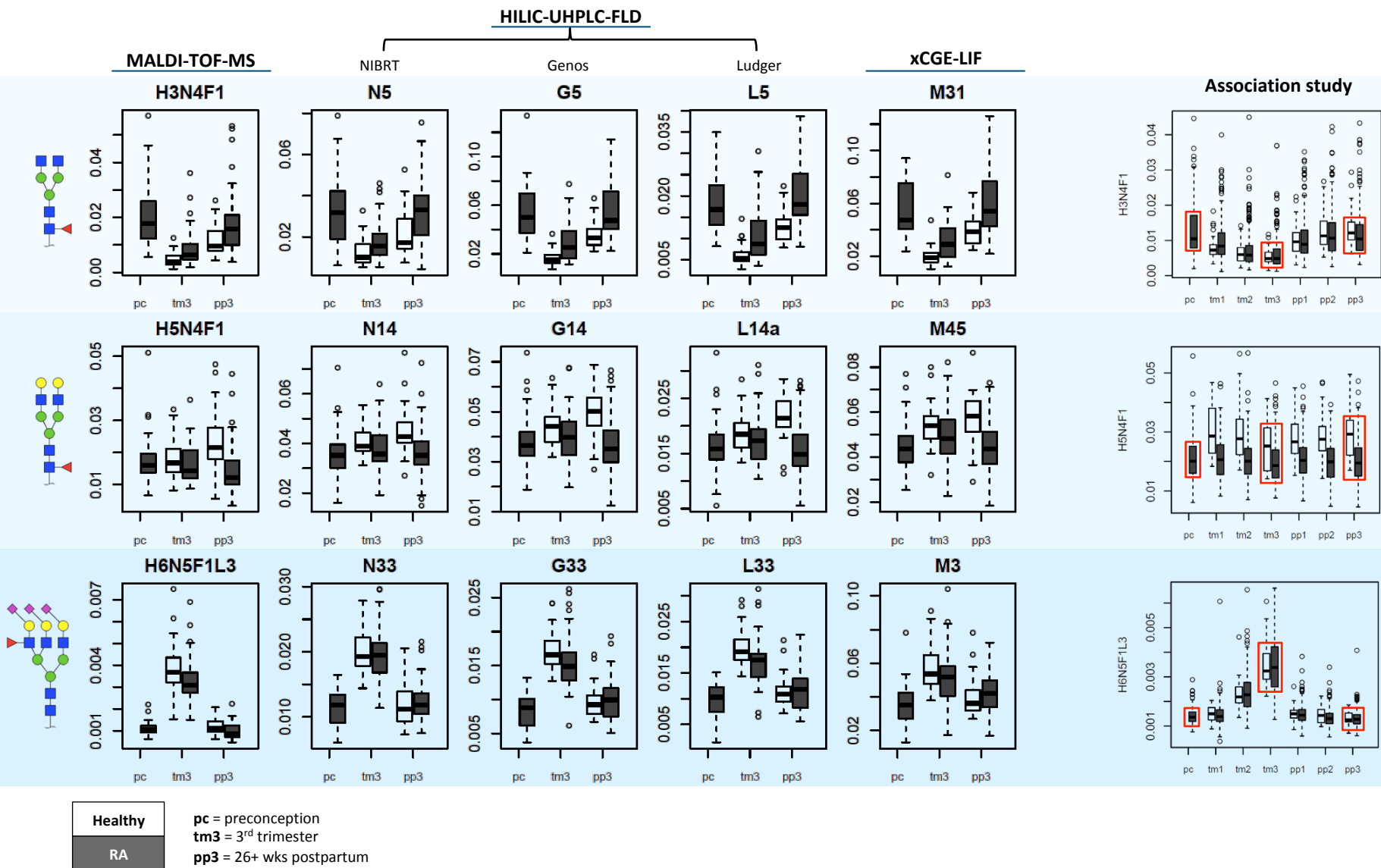


Pearson correlation ( $r$ )  
**X** = study-wide significant  
 . =  $p$ -value < 0.05

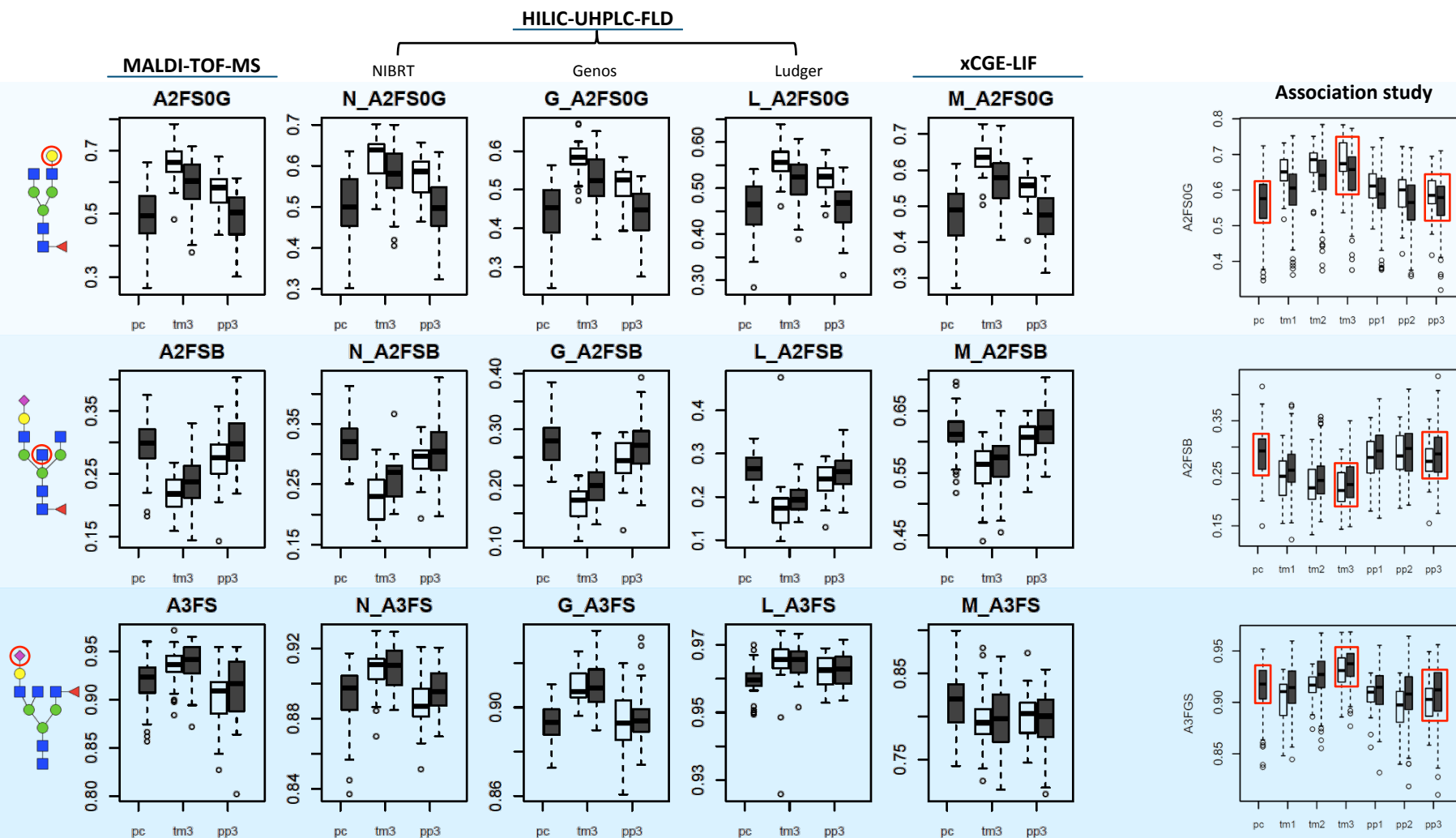
# Signal correlation (clustered)



# Pregnancy/RA single signal comparison



# Pregnancy/RA derived trait comparison



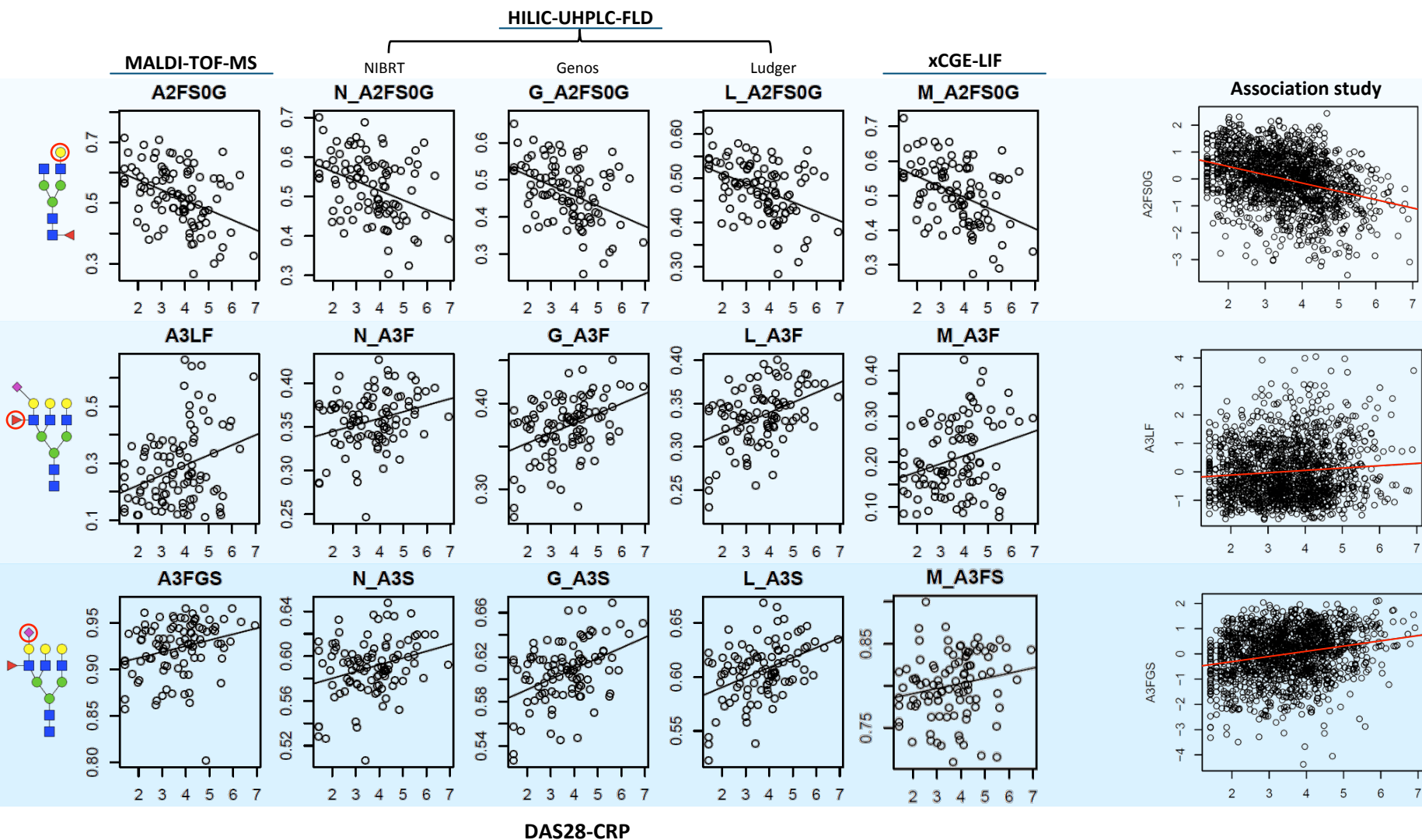
Healthy

RA

pc = preconception  
tm3 = 3<sup>rd</sup> trimester  
pp3 = 26+ wks postpartum

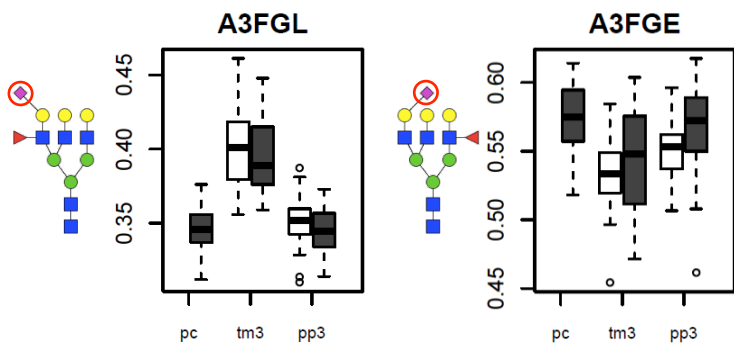


# DAS28-CRP comparison

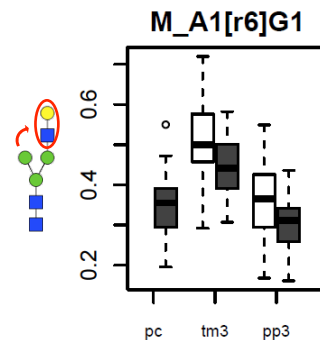


# Method-unique findings

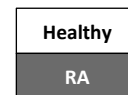
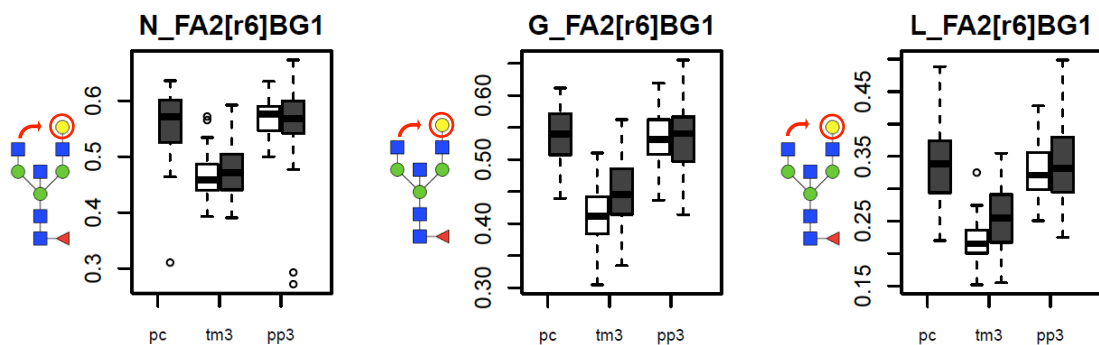
## MALDI-TOF-MS



## xCGE-LIF



## HILIC-UHPLC-FLD



pc = preconception  
tm3 = 3<sup>rd</sup> trimester  
pp3 = 26+ wks postpartum

# Summary

## **Glycosylation with pregnancy and RA disease activity**

- Pregnancy large effect on TSNG (e.g. new glycans appear)
- RA and healthy pregnancies show similar glycosylation changes
- Sialic acid linkage differentiates pregnancy and RA effect
- DAS28-CRP: replication of IgG-Fc findings (A2FS0G) and new detection of A3 sialylation (A3FGS)
- Findings reproducible across methods and laboratories

## **Comparative method performance**

- Throughput
- Repeatability
- Annotation
- Quality control
- Low complexity separation ( $\leq$  A2)
- Higher complexity separation ( $\geq$  A3; mixtures)

# PARA study acknowledgments



## Erasmus MC

Radboud J. Dolhain  
Johanna M. W. Hazes



## Genos

Irena T. Akmačić  
Maja Pučić-Baković  
Gordan Lauc



## NIBRT

Roisin O'Flaherty  
Pauline M. Rudd



## Ludger

Richard Gardner  
Archana Shubhakar  
Daniel I. Spencer  
Daryl L. Fernandes



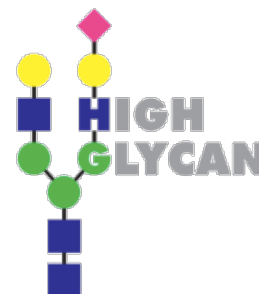
## MPI / glyXera

René Hennig  
Erdmann Rapp



## LUMC

Karli Reiding  
Albert Bondt



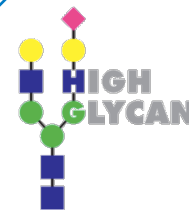


# Characteristics of HTP glycoanalytical methods



	1. MALDI-MS	2. HILIC-UPLC	3. CGE-LIF
<i>Acceptance / usage</i>	++	+++	+
<i>Throughput</i>	+++	+	+++
<i>Required expertise</i>	--	-	-
<i>Resolution</i>	+++	+	+
<i>Isomer separation</i>	(++)	+++	+++
<i>Quantification</i>	+	+++	++
<i>Costs of equipment</i>	---	--	--
<i>Costs per sample in HTP mode</i>	-	--	-

# Let's go for the labtour...



## **HILIC-UHPLC-FLD (Genos)**

- Hydrophilic interaction liquid chromatography – ultrahigh performance liquid chromatography – fluorescence detection
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- GHP HILIC-SPE
- Reflectron positive MALDI-TOF-MS
- Compositional annotation by  $m/z$  and QC

## **+ LC-MS (LUMC / Genos / glyXera)**

Liquid chromatography – electrospray ionization – mass spectrometry