



Leiden University
Medical Center

Proteoform-resolved structure-function relationship studies of monoclonal antibodies by FcγRIIIa Affinity Liquid Chromatography-Native Mass Spectrometry

Analytical Technologies Europe 2020

Steffen Lippold, PhD student

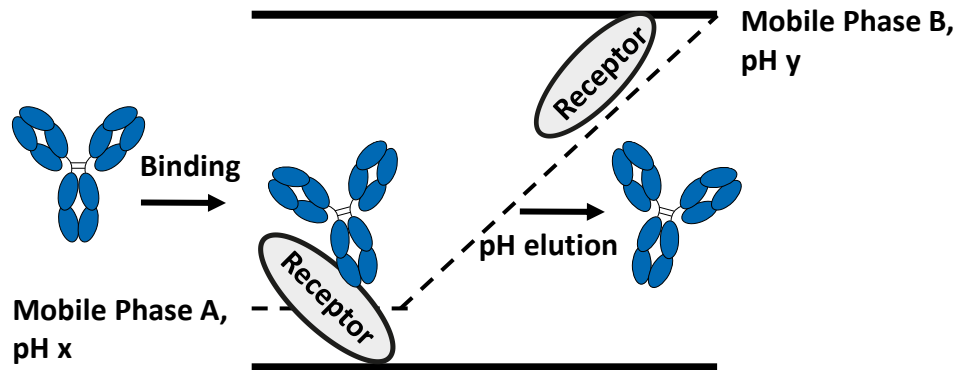
Leiden University Medical Center

04.11.2020



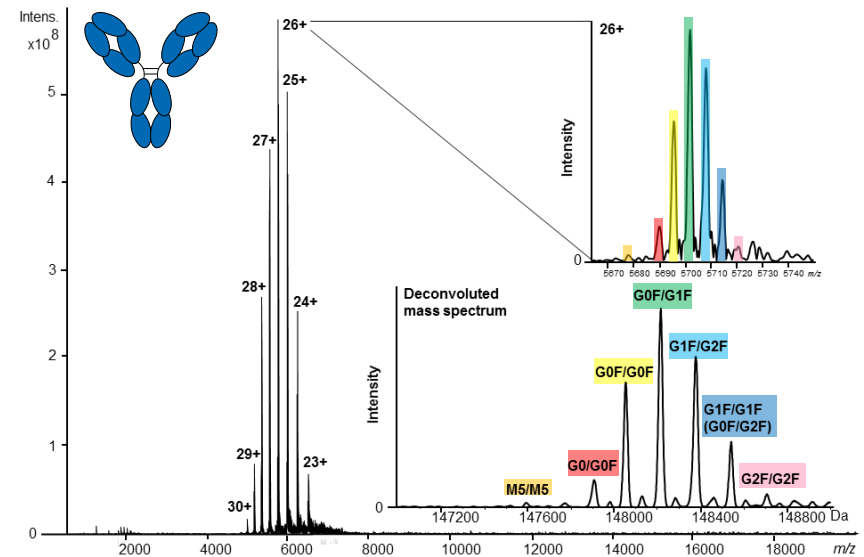
Affinity Chromatography for Affinity Separation – Mass Spectrometry for Proteoform Resolution

Affinity Chromatography



> Affinity Separation

Mass Spectrometry



> Proteoform Resolution

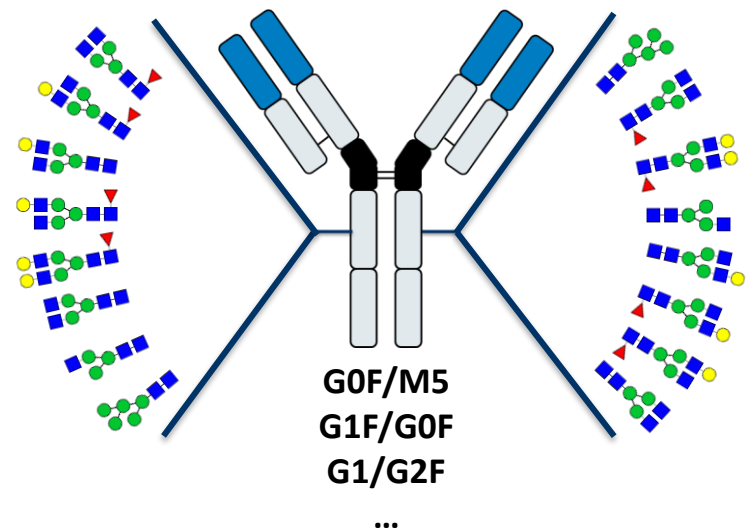
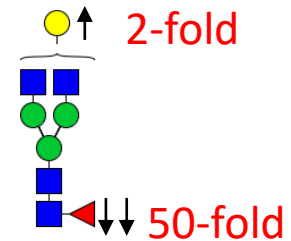
Fc Glycosylation Controls FcγRIIIa Affinity

- Fc glycosylation of immunoglobulin G (IgG) has tremendous effects on its FcγRIIIa interaction

- Fc:FcγRIIIa affinity strongly linked to ADCC activation

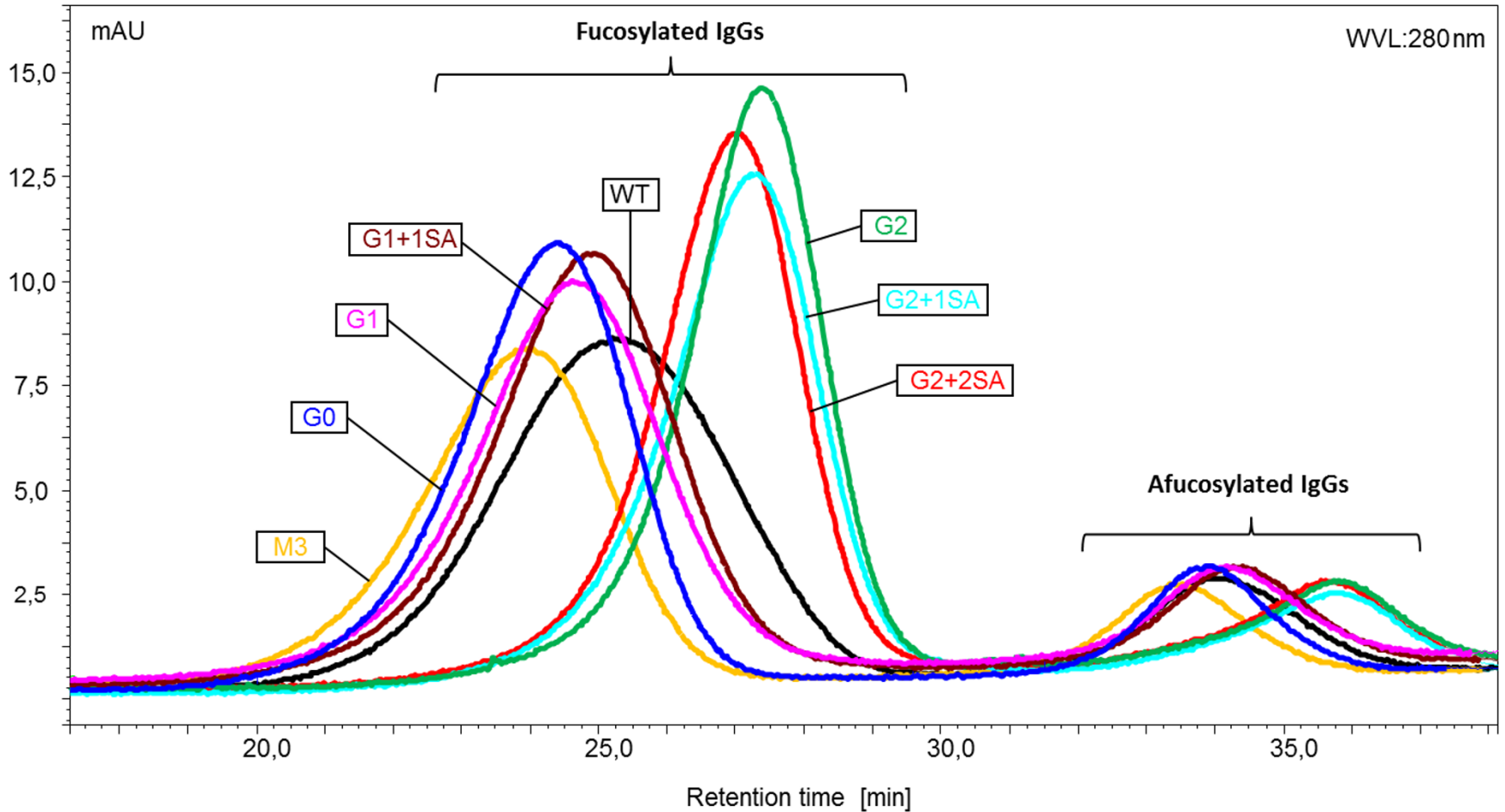
- Interaction in 1:1 stoichiometry (> pairing relevance)

- Alternative methods (e.g. SPR) require highly pure glycoengineering for assessment of individual glycoforms

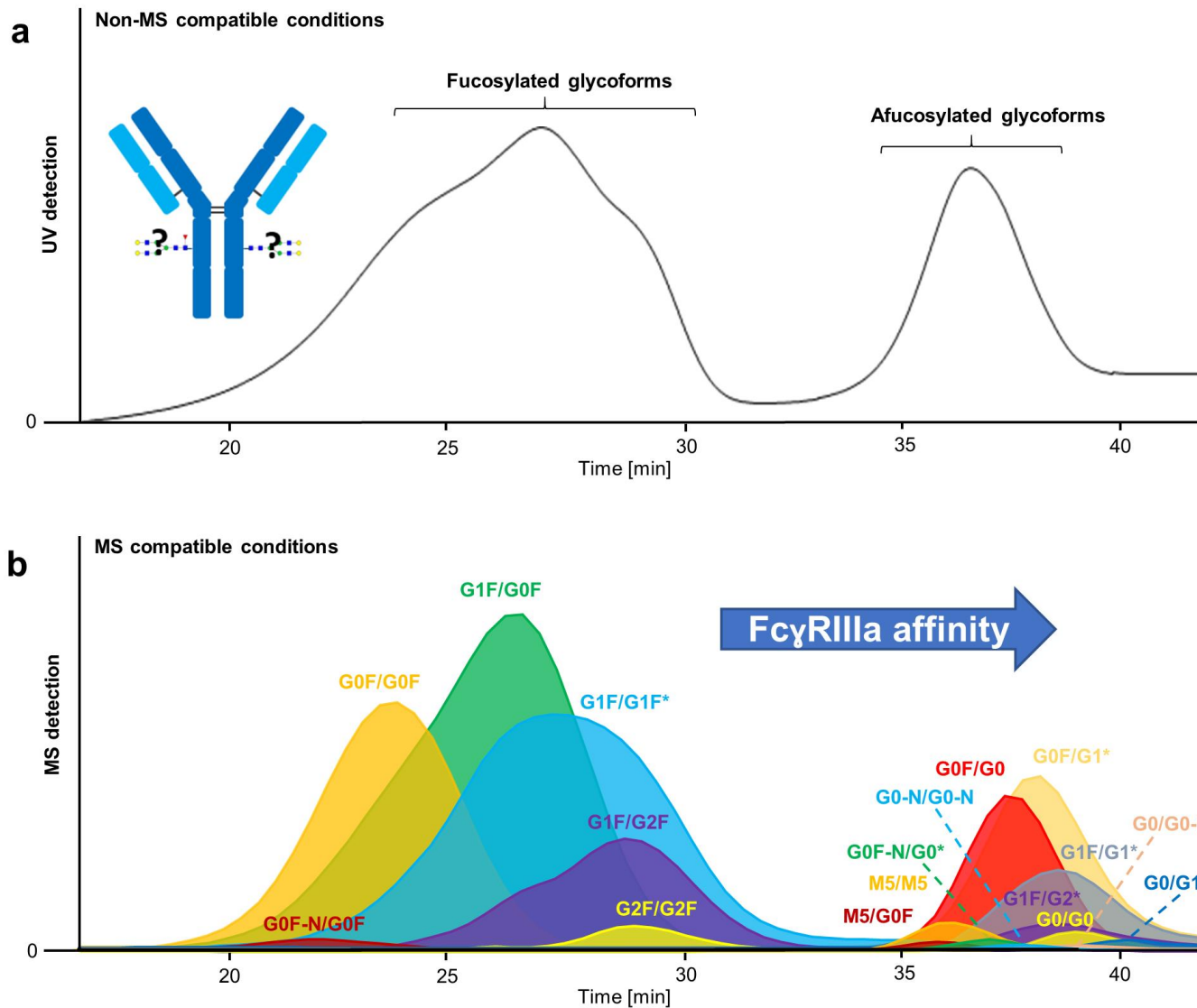


Previous studies: Affinity Chromatography with UV Detection and *in-vitro* Glycoengineered mAbs

Modified from Dashivets, T. *et al. PLoS One* 10, e0143520 (2015)



Information obtained from UV vs. MS detection



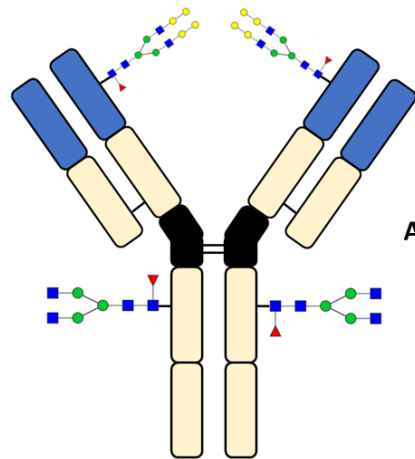
Lippold, Steffen, et al. "Glycoform-resolved Fc γ RIIIa affinity chromatography–mass spectrometry." *MAbs*. Vol. 11. No. 7. Taylor & Francis, 2019.

Limitations of Intact mAb Analysis – Proteoform Ambiguity of Fab Glycosylated Cetuximab

Intact cetuximab

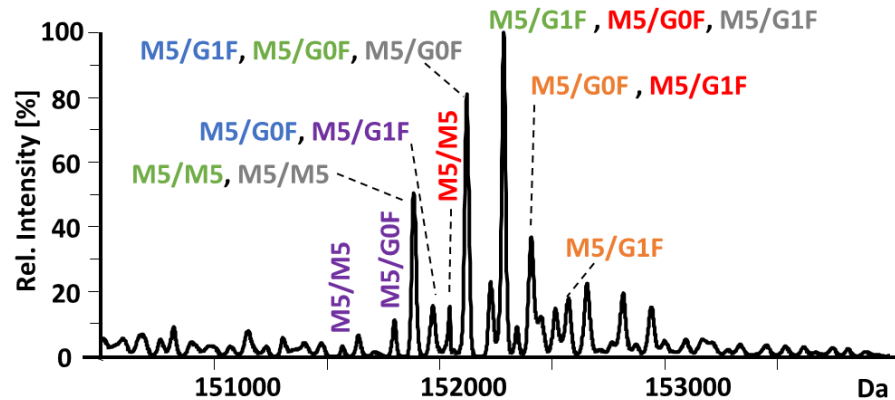
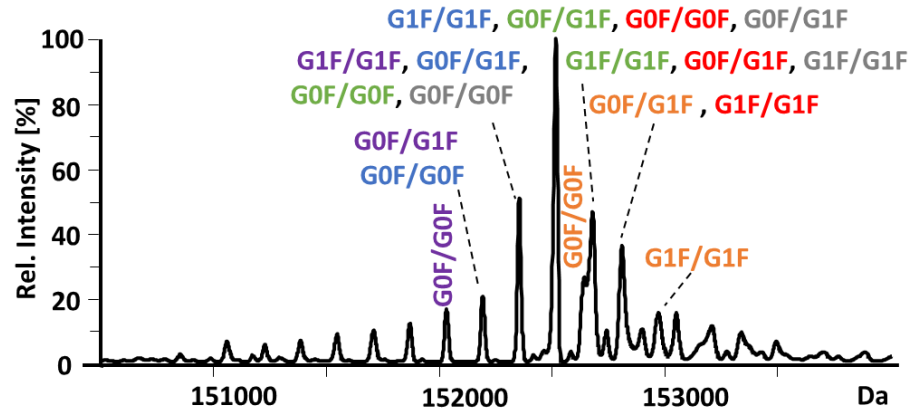
Fab:

- H7N4F1/H7N4F1
- H7N4F1/H6N4F1S1
- H7N4F1/H6N4F1
- H6N4F1S1/H6N4F1S1
- H6N4F1/H6N4F1S1
- H6N4F1/H6N4F1



Fucosylated glycoforms

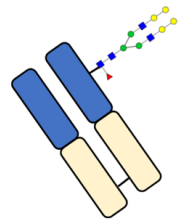
Afucosylated glycoforms



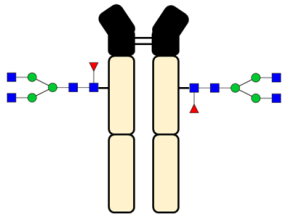
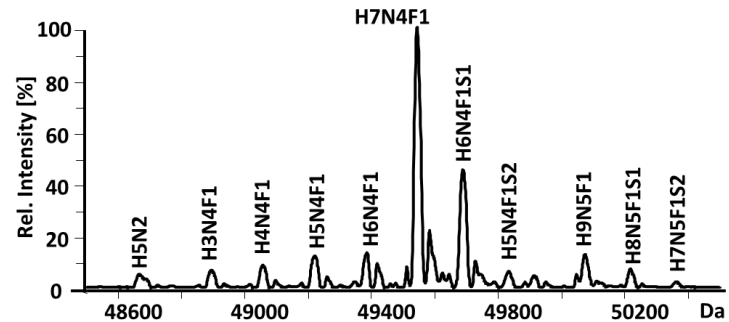
Lippold, Steffen, et al. "Proteoform-resolved FcγRIIIa binding assay for Fab glycosylated monoclonal antibodies achieved by affinity chromatography mass spectrometry of Fc moieties." *Frontiers in chemistry* 7 (2019): 698.

Middle-up FcγRIIIa AC-MS of Cetuximab – Proteoform Assignments

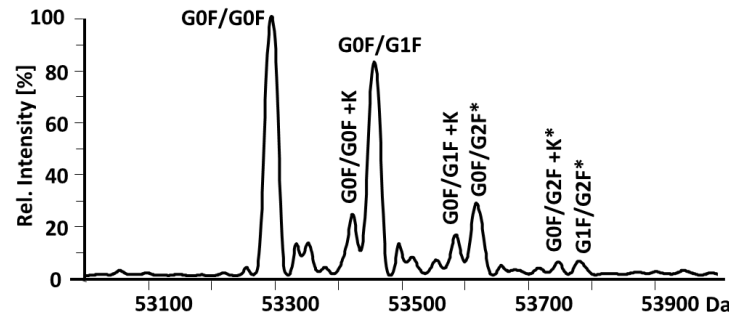
Kgp middle-up cetuximab



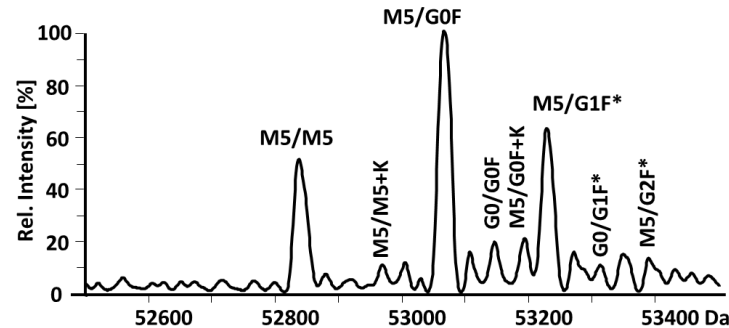
Fab glycoforms



Fucosylated Fc glycoforms

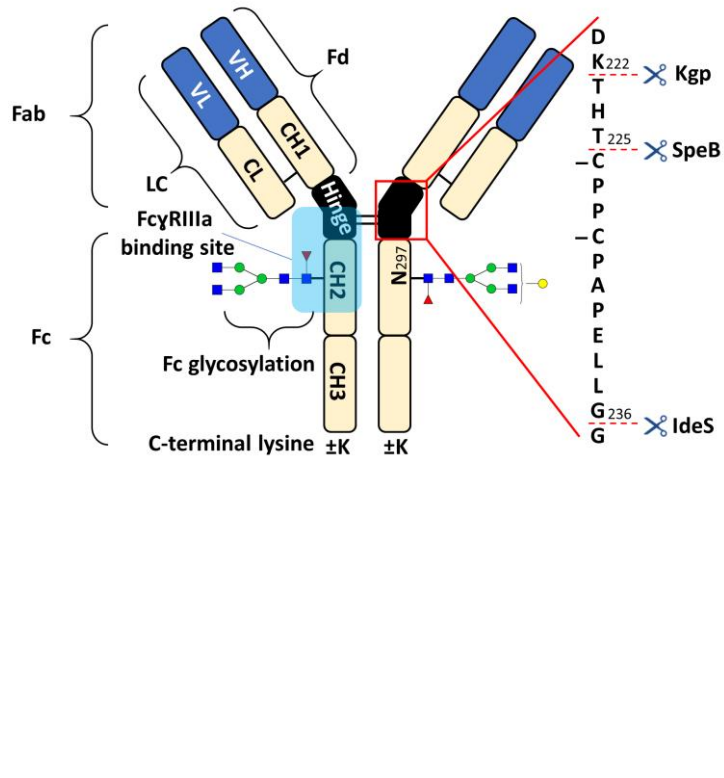


Afucosylated Fc glycoforms



Lippold, Steffen, et al. "Proteoform-resolved FcγRIIIa binding assay for Fab glycosylated monoclonal antibodies achieved by affinity chromatography mass spectrometry of Fc moieties." *Frontiers in chemistry* 7 (2019): 698.

Middle-up Protease Kgp enables FcγRIIIa AC-MS of Fc Moieties



FabRICATOR®

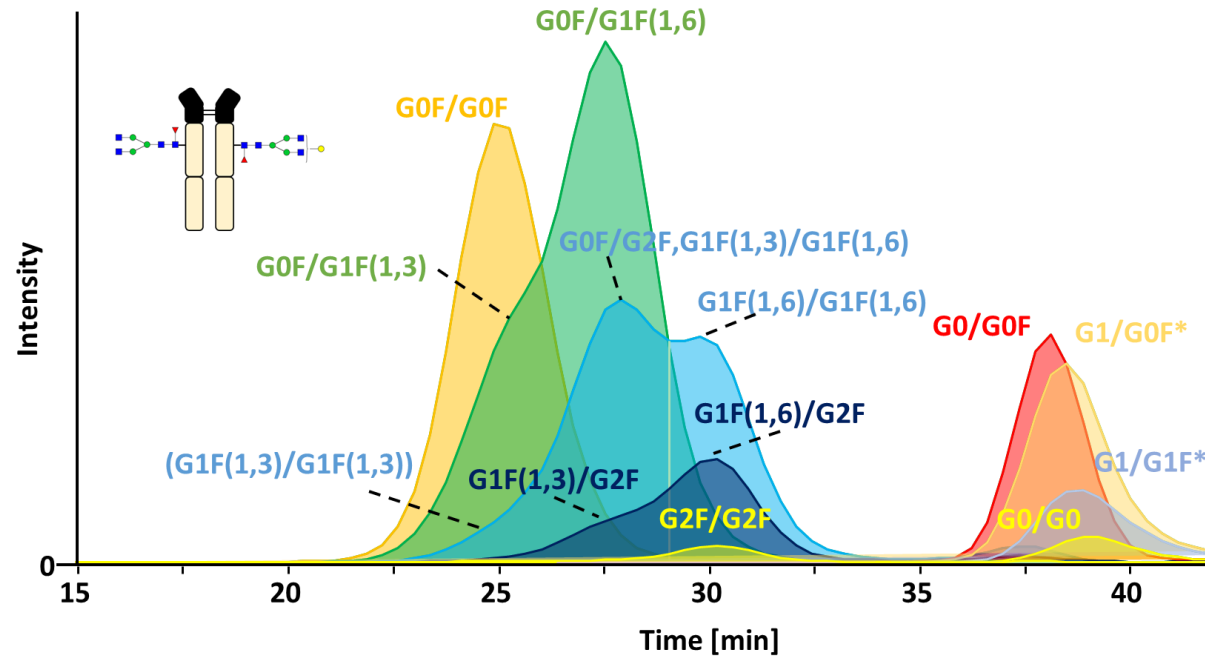
FabULOUS®

GingisKHAN®

<https://www.genovis.com/products/igg-proteases/>

Lippold, Steffen, et al. "Proteoform-resolved FcγRIIIa binding assay for Fab glycosylated monoclonal antibodies achieved by affinity chromatography mass spectrometry of Fc moieties." *Frontiers in chemistry* 7 (2019): 698.

Increased Chromatographic Resolution for Fc Moieties



Lippold, Steffen, et al. "Proteoform-resolved Fc γ R1IIa binding assay for Fab glycosylated monoclonal antibodies achieved by affinity chromatography mass spectrometry of Fc moieties." *Frontiers in chemistry* 7 (2019): 698.

Conclusions

Glycosylation features

- Increased affinity 2x afucosylated > 1x (a)fucosylated >> 2x fucosylated
- Positive effect of galactosylation on all fucosylation levels
- Decreased affinity of monoantennary structures (-N)

Intact mAb analysis

- Great advance in functional characterization of individual glycoforms
- Omits the need for high purity glycoengineering
- Limitations in proteoform heterogeneity

Middle-up approach

- Application for more complex formats (e.g. Fab glycosylated)
- IgG protease Kgp is suitable for middle-up FcγRIIIa AC-MS
- Increased chromatographic resolution of Fc moieties

Acknowledgements



David Falck
Manfred Wuhrer
Simone Nicolardi
Elena Domínguez-Vega



Dietmar Reusch
Markus Habegger
Tilman Schlothauer
Alexander Knaupp



Sanquin

Gestur Vidarsson

