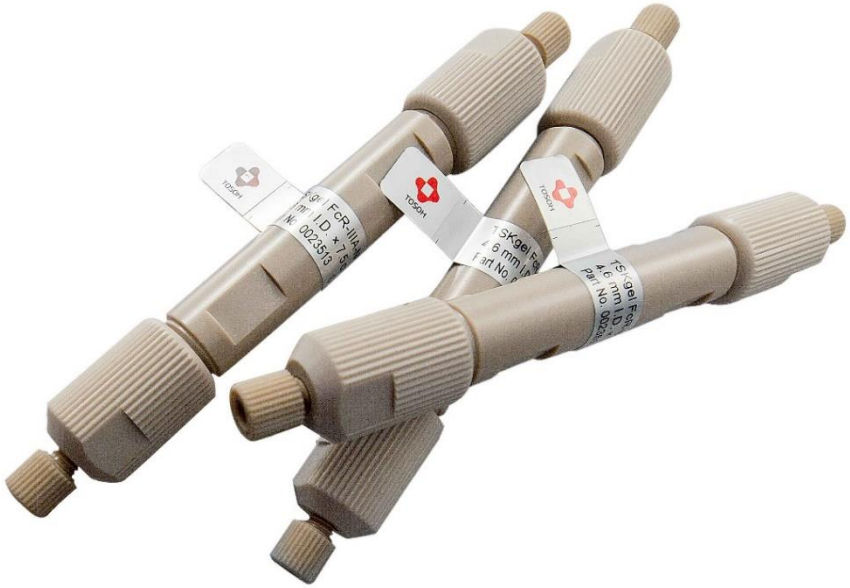
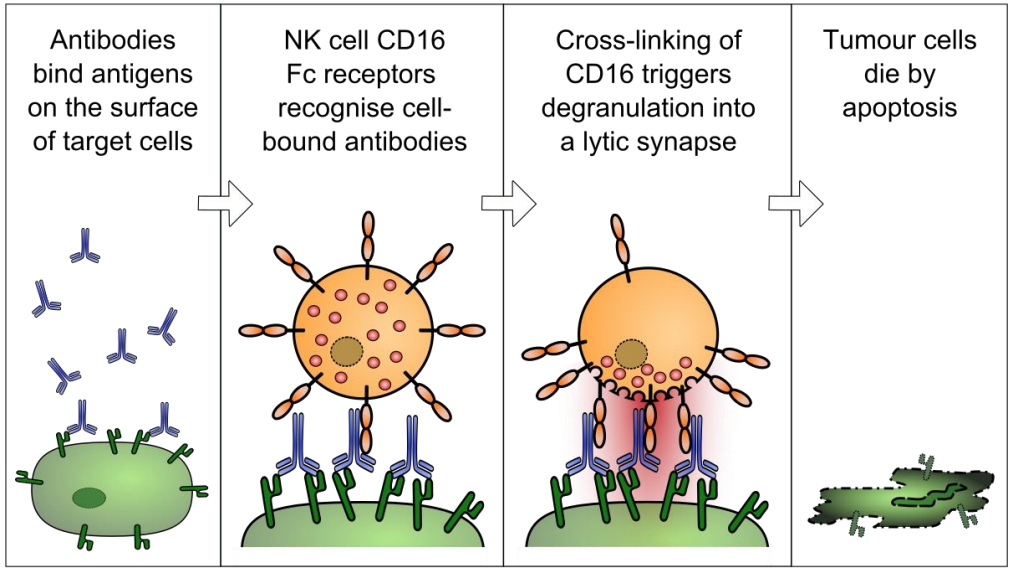
TSKgel FcR-IIIA-NPR Affinity Column for ADCC Evaluation



Antibody-dependent cell-mediated cytotoxicity (ADCC)

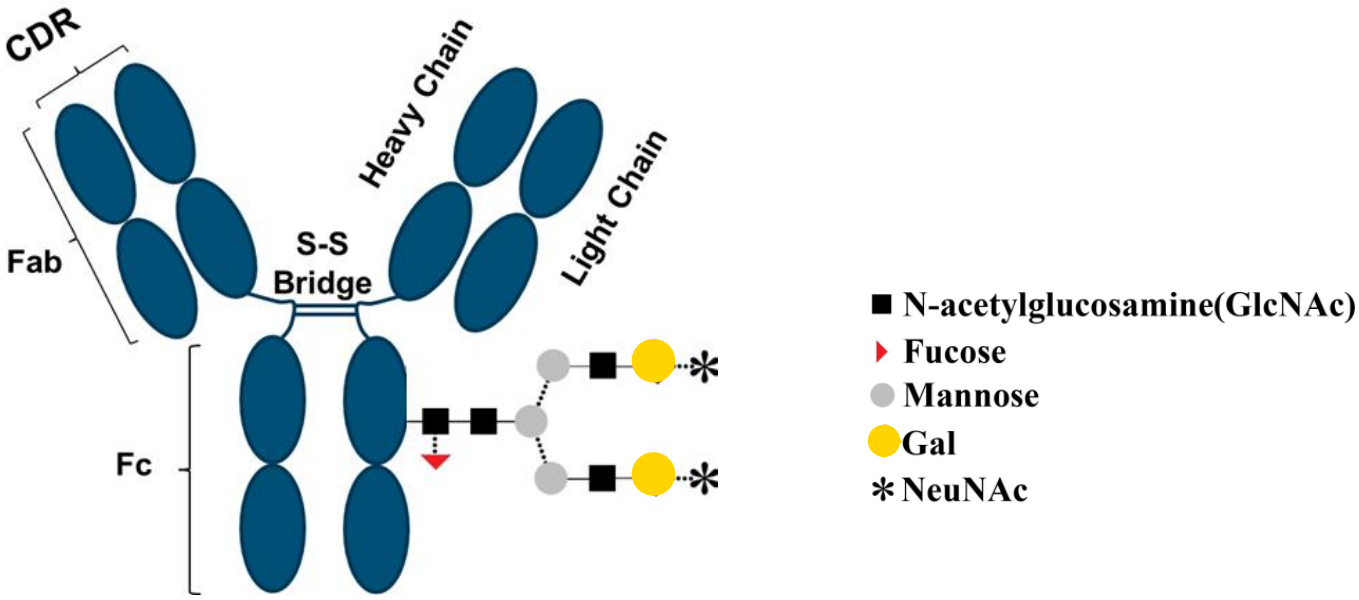
ADCC is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies. ADCC requires an effector cell (natural killer (NK) cells) that typically interact with IgG antibodies.



- ADCC is one of the most important mechanisms of action (MoA) of therapeutic antibodies applied in cancer therapy
- Fc-Glycans involved in interaction
- ADCC activity is typically analyzed by cell based ADCC bioassays or surface plasmon resonance on immobilized FcγRIIIa

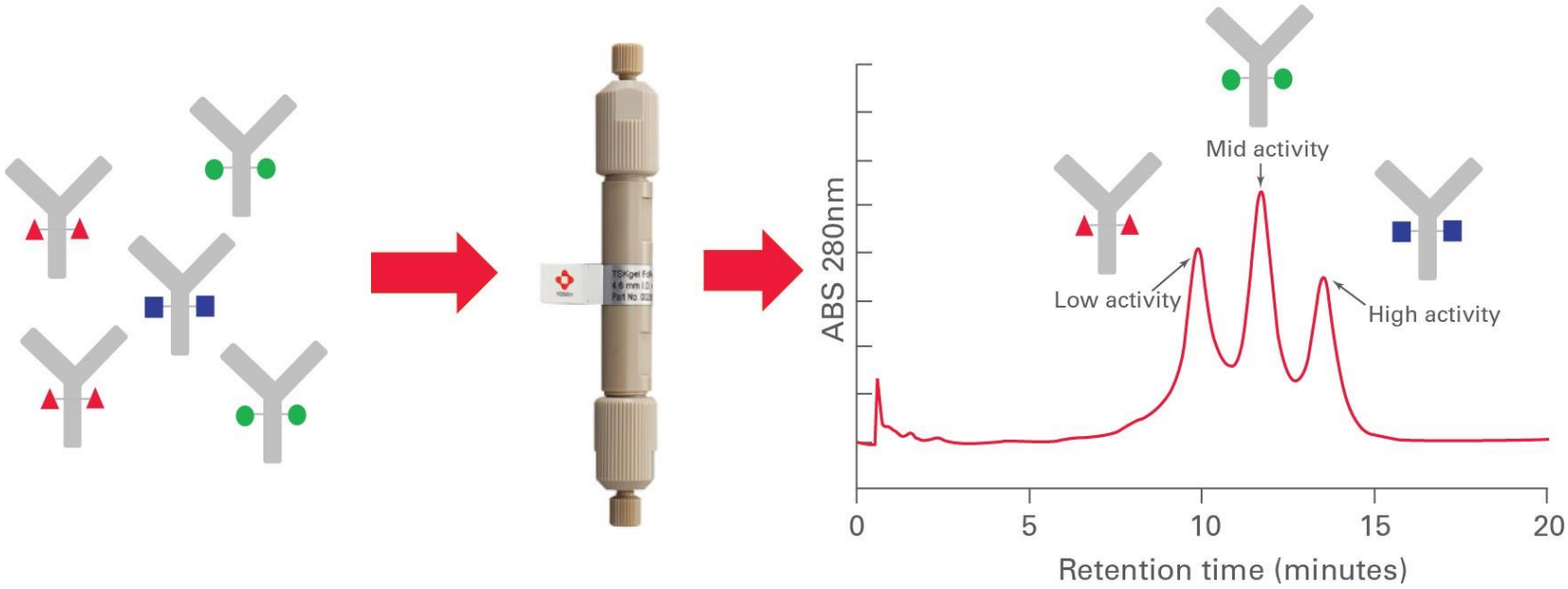
What influences ADCC?

- Fc receptor needs to bind Fc part of the antibody to start destruction of the respective cell by ADCC
- Oligosaccharide chains – N-glycans – of the Fc domain influence this interaction



WHY NOT USING FcγR, ONE OF THE KEY PLAYERS IN ADCC, FOR AFFINITY CHROMATOGRAPHY?

Fcγ IIIa Receptor Affinity Chromatography



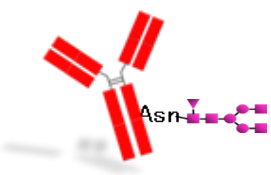
N-glycoforms of therapeutic antibody

FcR Affinity Chromatography
Recombinant ligand (E.coli)
4.6 x 75 mm; 5 µm, NPR

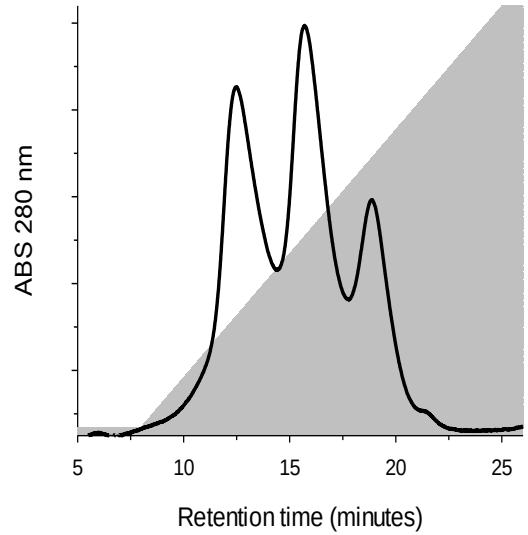
Glycoforms are separated based on affinity to Fcγ RIIIa which is influenced by Fc-glycans and corresponds to ADCC activity

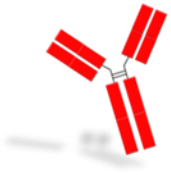
FcγR IIIA Affinity – Glycosylation*

Interaction of Fc receptor and IgG Fc region needs N-glycans - > deglycosylated mAbs do not bind to FcR column!

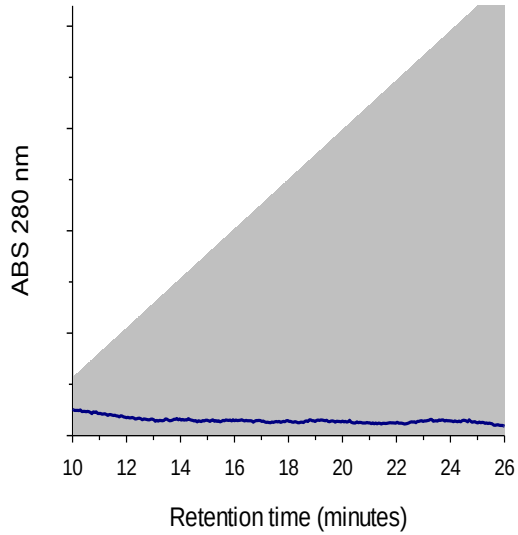


Rituximab





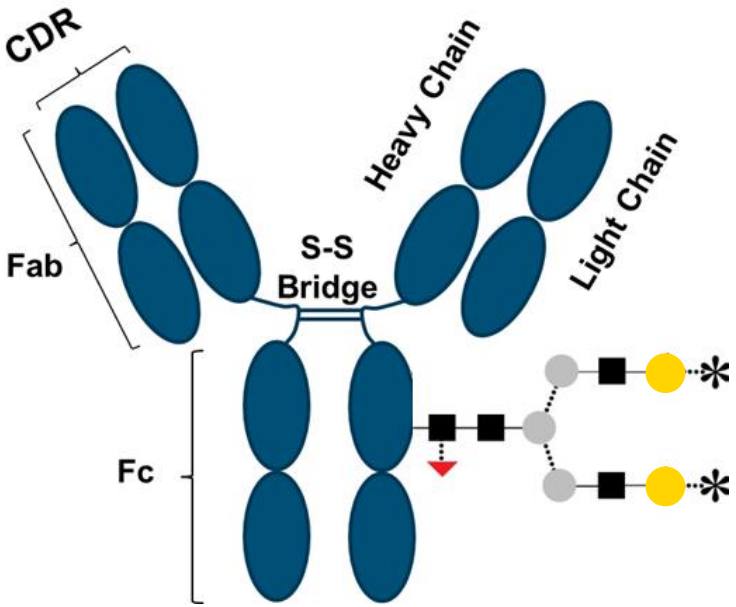
Deglycosylated Rituximab



Column:	TSKgel FcR-IIIa-NPR, 5 µm
Mobile Phase:	A: 50 mM Citrate, pH 6.5 ; B: 50mM Citrate, pH 4.5
Flow rate:	1 mL/min
Injection:	30 µL
Sample:	Rituximab (1µg/µl)

**Master Thesis Leila Ghaleh, TU Darmstadt*
Rituximab kindly provided by Rentschler Biopharma

FcγR IIIA Affinity – N-Glycosylation



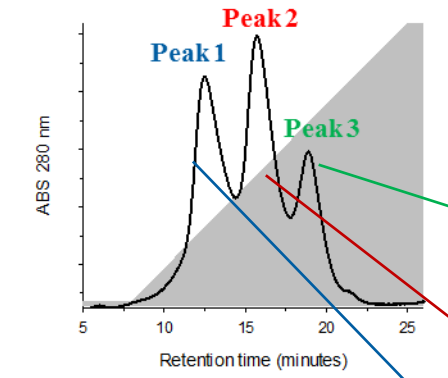
■ N-acetylglucosamine(GlcNAc)
▸ Fucose
● Mannose
● Gal
✻ NeuNAc

ADCC activity of glycovariants:

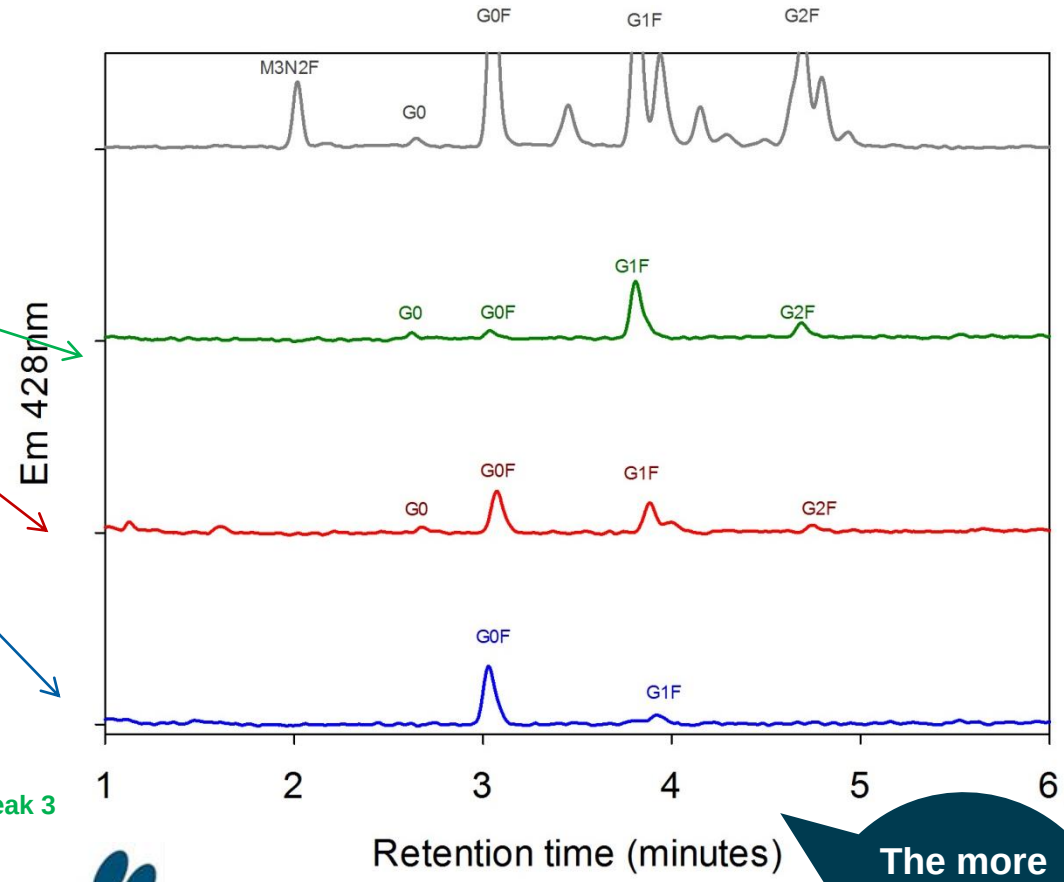
Terminal galactose (●) increases ADCC

Core fucose (▼) decreases ADCC

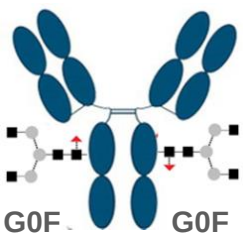
Rituximab – FcR Affinity / N-Glycans*



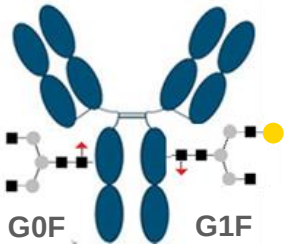
2-AB-Biantennary Library
Peak 1
Peak 2
Peak 3



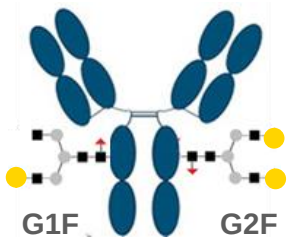
Peak 1



Peak 2



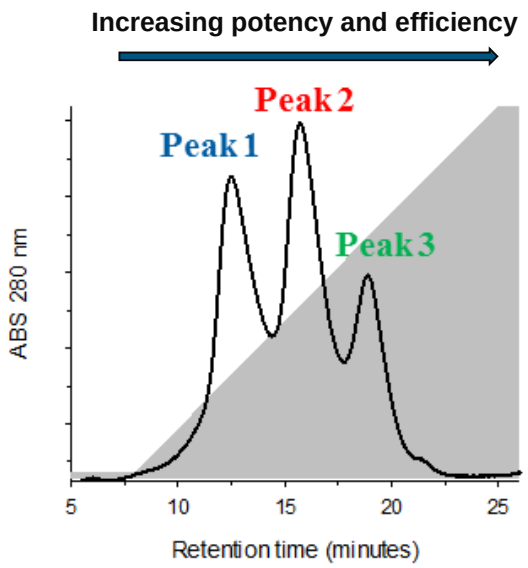
Peak 3



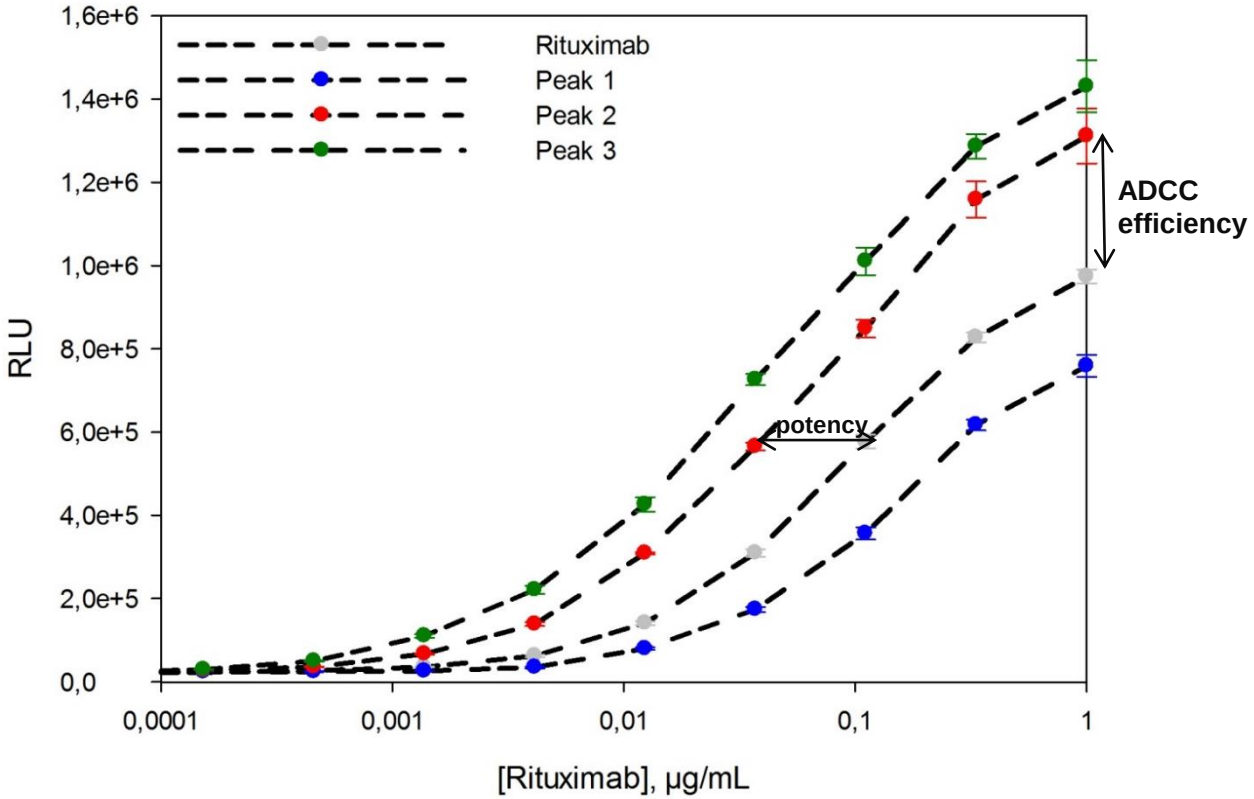
The more galactose the higher is the affinity!

**Data: Master Thesis of Leila Ghaleh, TU Darmstadt*
Rituximab kindly provided by Rentschler Biopharma

Rituximab – FcR Affinity / ADCC*



Antibody	EC_{50} (µg/ mL)
Rituximab	0,098
Peak 1	0,153
Peak 2	0,072
Peak 3	0,049

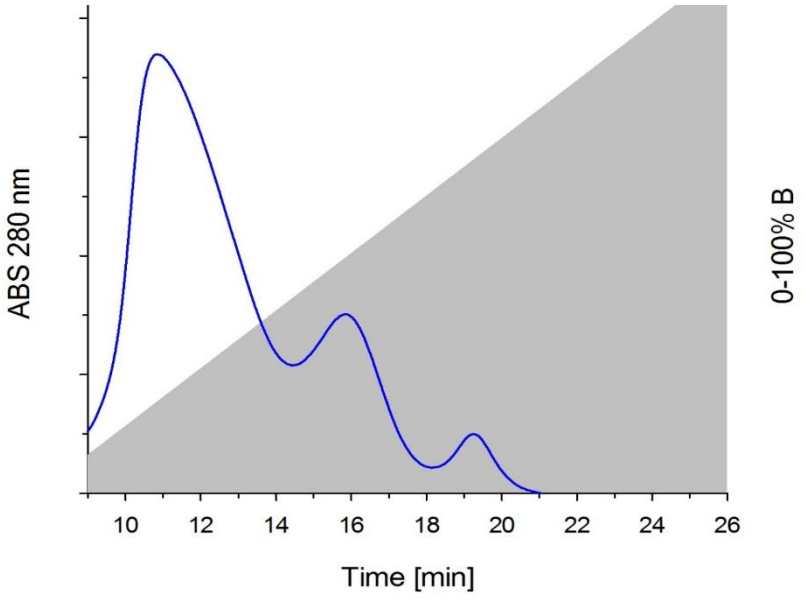


ADCC reporter bioassay (Promega)

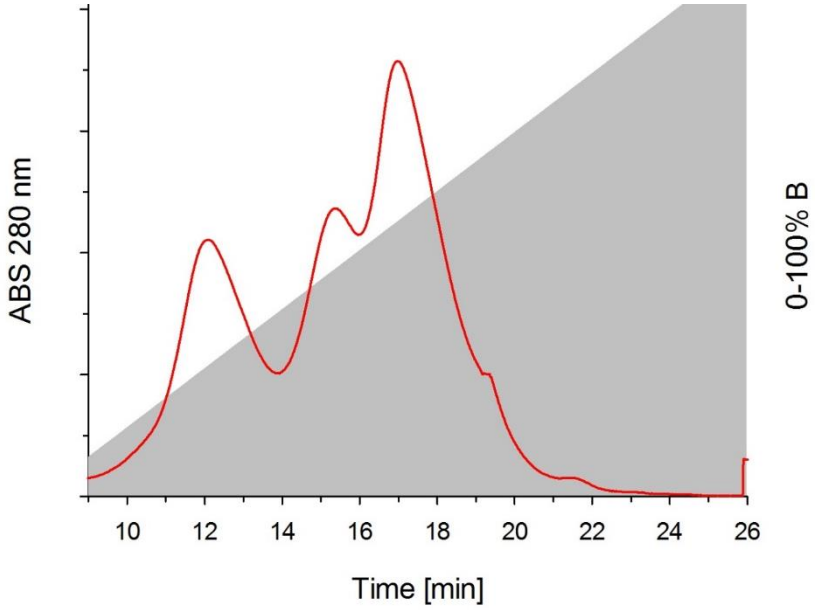
**Data: Master Thesis Leila Ghaleh, TU Darmstadt*
Rituximab kindly provided by Rentschler Biopharma

Impact of Fucosylation (Trastuzumab)*

Fucosylated Trastuzumab**



Non-fucosylated Trastuzumab

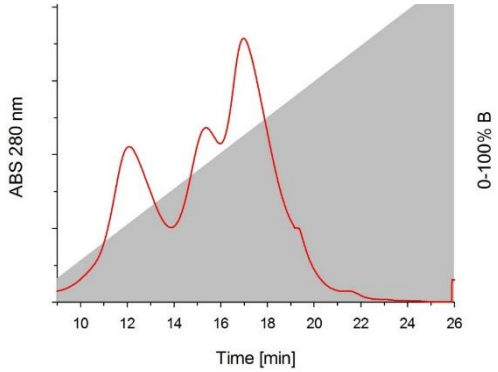


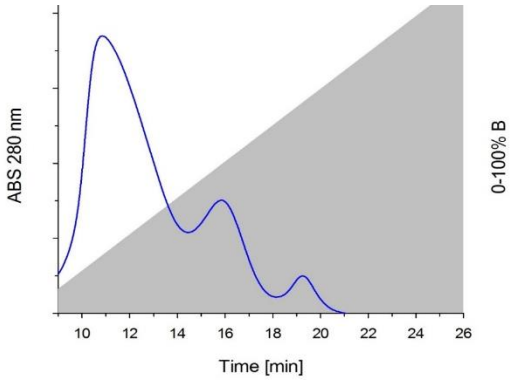
**Data: Master Thesis Leila Ghaleh, TU Darmstadt*

***Fucosylated and non-fucosylated Trastuzumab from Bioceros*

Impact of Fucosylation (Trastuzumab)*

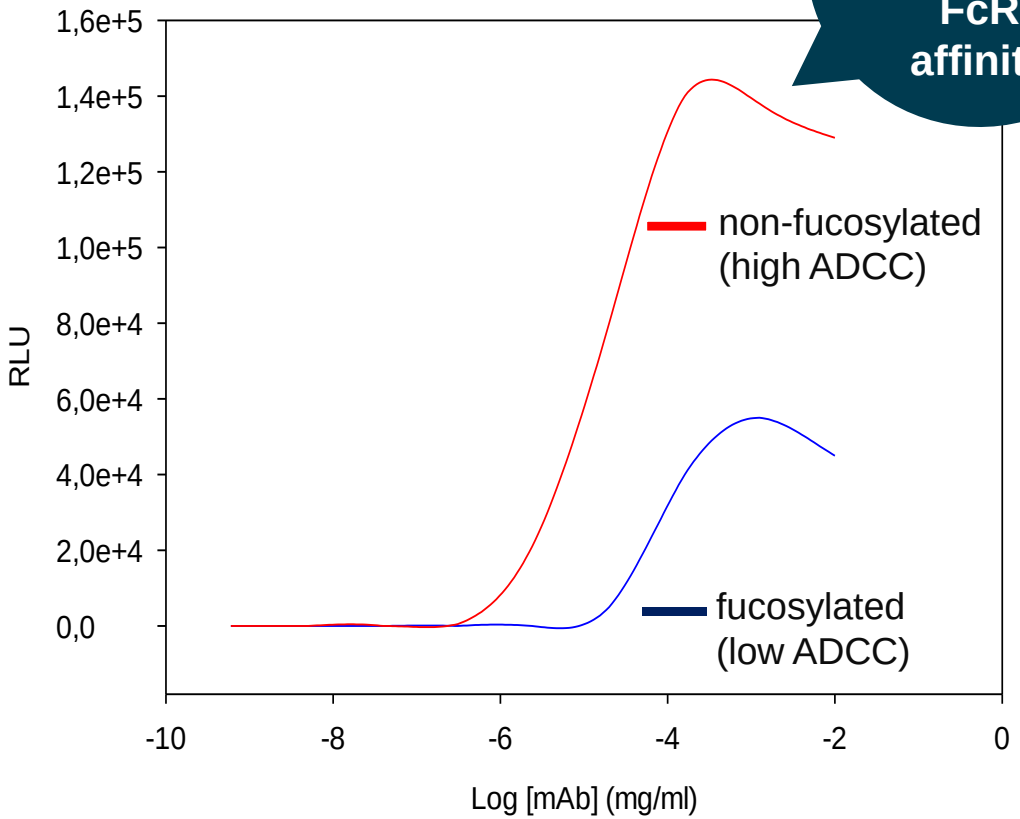
Non-fucosylated Trastuzumab





Fucosylated Trastuzumab**

ADCC matches observed FcR affinity



ADCC reporter bioassay (Promega, data Bioceros)

Data: Master Thesis Leila Ghaleh, TU Darmstadt

***Fucosylated and non-fucosylated Trastuzumab provided by Bioceros*

Scientific Reports – Fc-Glycans

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OPEN

Assessing the Heterogeneity of the Fc-Glycan of a Therapeutic Antibody Using an engineered FcγReceptor IIIa-Immobilized Column

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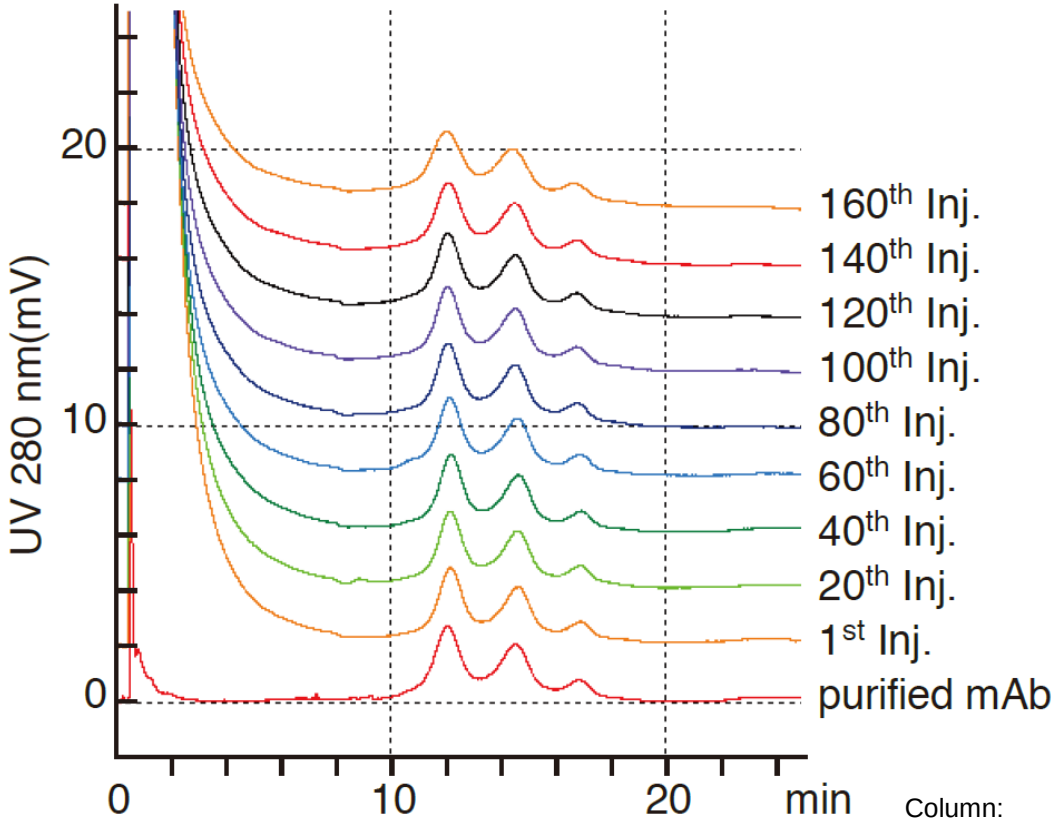
Masato Kiyoshi[1], Jose M. M. Caaveiro[2,3], Minoru Tada[1], Hiroko Tamura[2], Toru Tanaka[4], Yosuke Terao[4], Koldo Morante[2], Akira Harazono[1], Noritaka Hashii[1], Hiroko Shibata[1], Daisuke Kuroda[2], Satoru Nagatoishi[2], Seigo Oe[4], Teruhiko Ide[4], Kouhei Tsumoto[2,5,6] & Akiko Ishii-Watabe[1]

The N-glycan moiety of IgG-Fc has a significant impact on multifaceted properties of antibodies such as in their effector function, structure, and stability. Numerous studies have been devoted to understanding its biological effect since the exact composition of the Fc N-glycan modulates the magnitude of effector functions such as the antibody-dependent cell mediated cytotoxicity (ADCC), and the complement-dependent cytotoxicity (CDC). To date, systematic analyses of the properties and influence of glycan variants have been of great interest. Understanding the principles on how N-glycosylation modulates those properties is important for the molecular design, manufacturing, process optimization, and quality control of therapeutic antibodies. In this study, we have separated a model therapeutic antibody into three fractions according to the composition of the N-glycan by using a novel FcγRIIIa chromatography column. Notably, Fc galactosylation was a major factor influencing the affinity of IgG-Fc to the FcγRIIIa immobilized on the column. Each antibody fraction was employed for structural, biological, and physicochemical analysis, illustrating the mechanism by which galactose modulates the affinity to FcγRIIIa. In addition, we discuss the benefits of the FcγRIIIa chromatography column to assess the heterogeneity of the N-glycan.

SCIENTIFIC REPORTS | (2018) 8:3955 | DOI:10.1038/s41598-018-22199-8

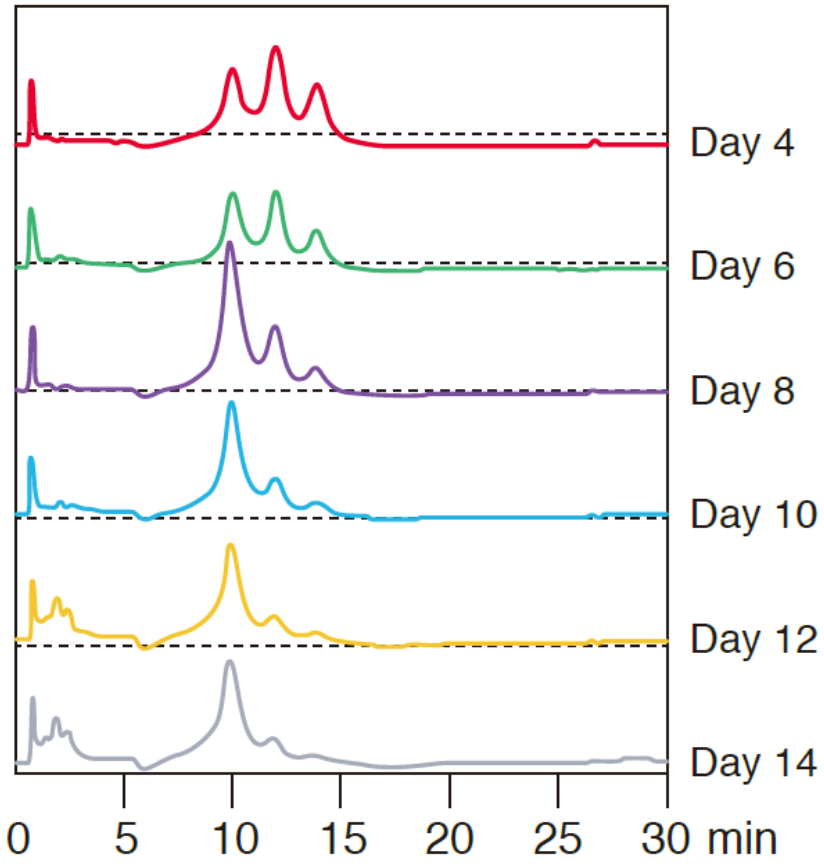
Robustness – CHO Supernatant



Ideal for cell line selection or upstream monitoring!

Column:	TSKgel FcR-IIIa-NPR, 5 µm; 4.6 x 75 mm
Mobile Phase:	A: 50 mM Citrate+150 mM NaCl, pH 6.5 ; B: 50mM Citrate +150 mM NaCl, pH 4.5
Flow rate:	1 mL/min
Gradient:	0 – 100 % B (7-25 min)
Detection:	UV@280
Sample:	CHO cell culture supernatant + purified mAb (1 µg/µl) (filtered)

Upstream Monitoring of CHO Culture

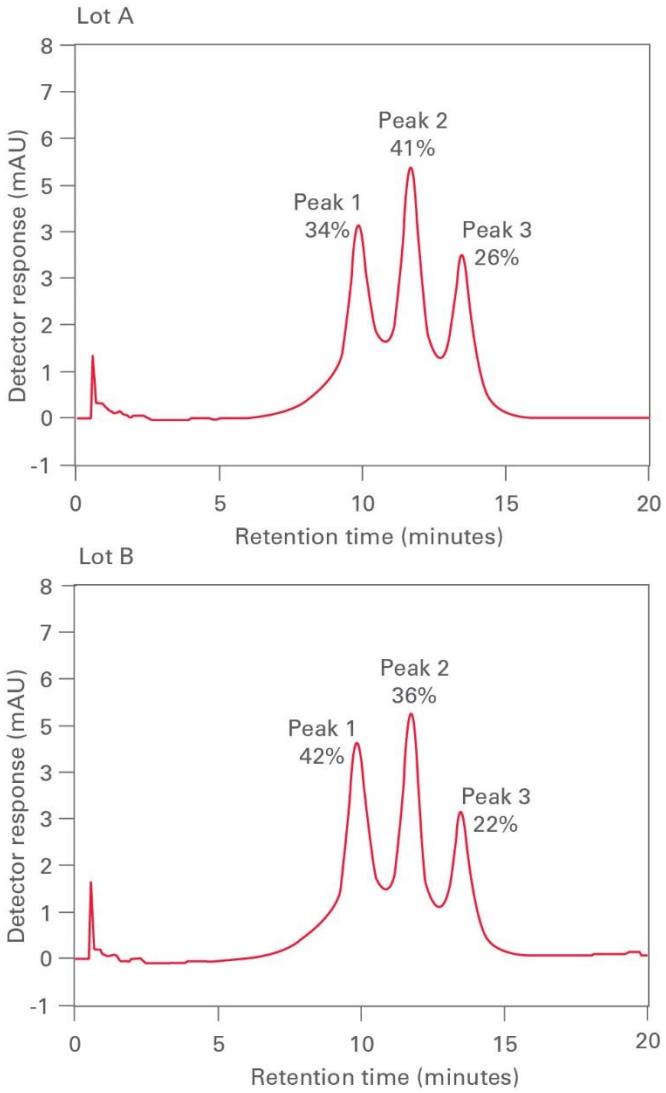


CHO cell culture was sampled periodically after starting culture, filtered, purified by protein A chromatography and separated by TSKgel FcR-IIIA-NPR

Column:	TSKgel FcR-IIIa-NPR, 5 µm; 4.6 x 75 mm
Mobile Phase:	A: 50 mM Citrate, pH 6.5 ; B: 50mM Citrate, pH 4.5
Flow rate:	1 mL/min
Gradient:	0 – 100 % B (2-20 min)
Detection:	UV@280
Sample:	Protein A eluted mAb (5 µg)

**CHO cell culture was kindly provided by Manufacturing Technology Association of Biologics*

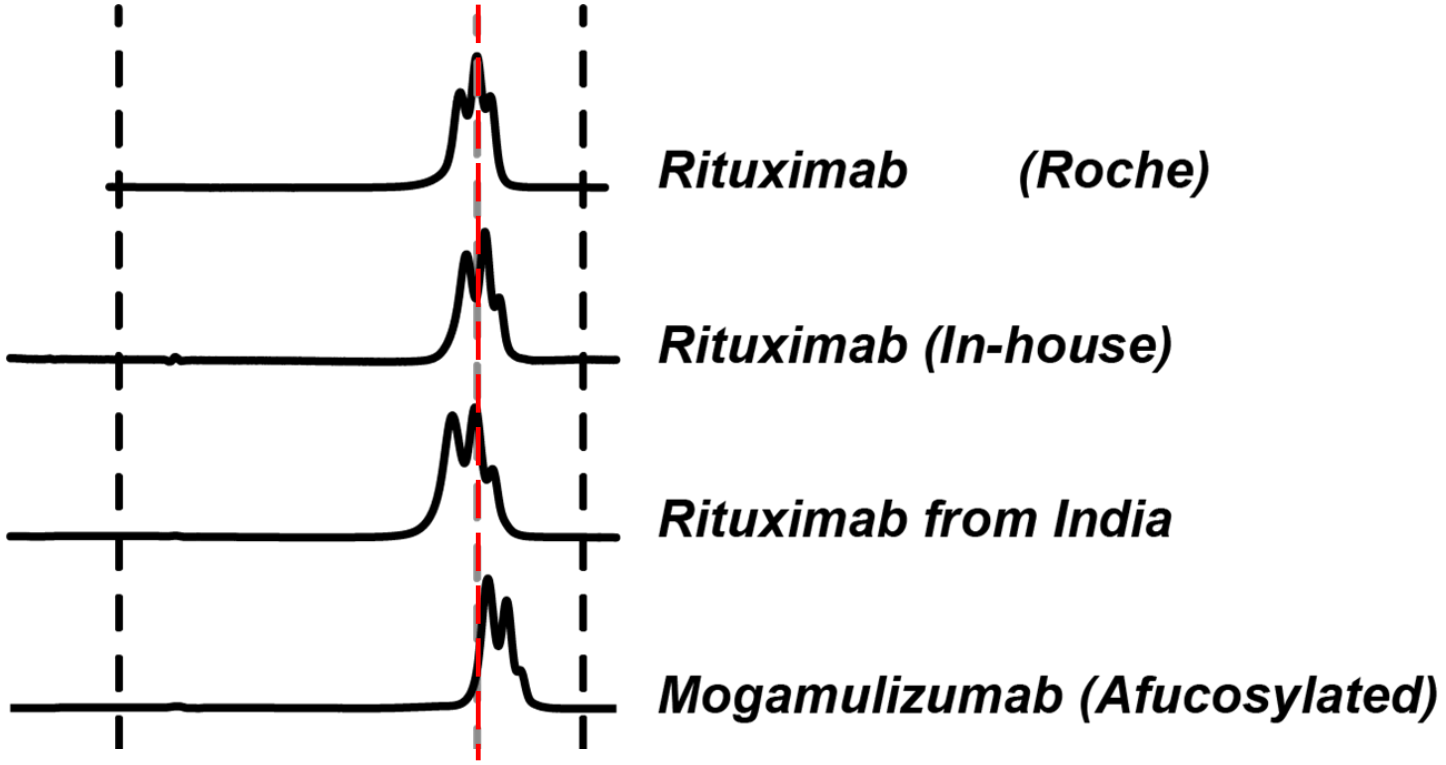
Lot-to-Lot Comparison of mAbs



FcR affinity pattern of therapeutic antibody lots vary.

Mobile phase:	A: 50 mmol/L Na-Citrate pH 6.5; B: 50 mmol/L Na-Citrate pH 4.5
Gradient:	0-100 % B (2-20 min, linear)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temp:	25° C

Originator versus Biosimilar



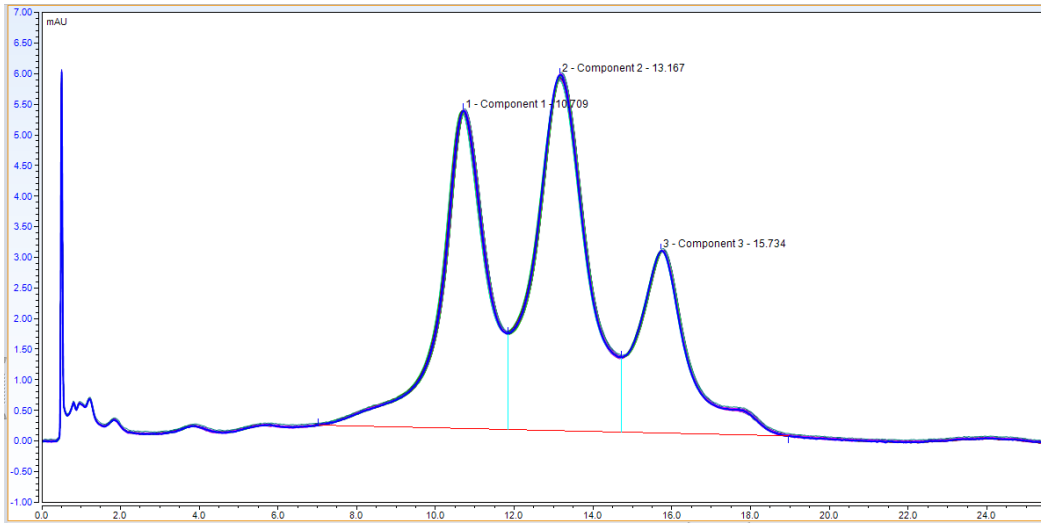
Ref.: M. Kiyoshi et al., Regulatory Science 2017, poster

Biosimilars show different chromatographic pattern from innovator
→ different glycan structure and activity

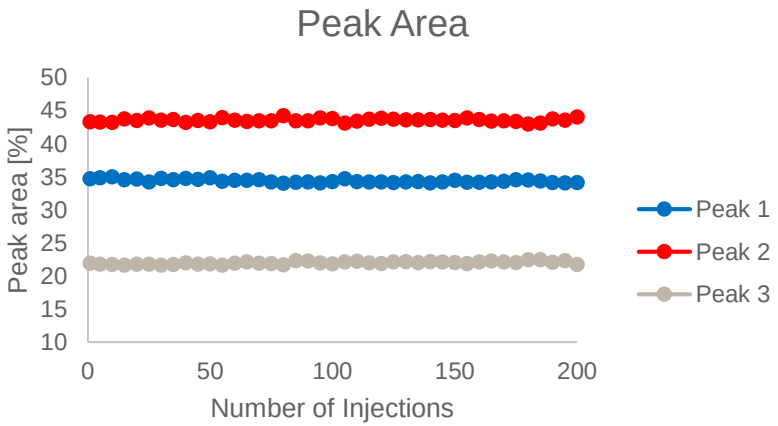
Gycoengineered „biobetter“ shows higher affinity /higer ADCC

Reproducibility of FcR-IIIA-NPR

Overlay of run1-25 of 200 (25 µg rituximab each)!



Better reproducibility than bioassays!



%CV (200 runs) <1%

Rituximab kindly provided by Rentschler Biopharma

FcR Affinity Column Characteristics

	TSKgel FcR-IIIA-NPR (P/N: 0023513)
Ligand	modified recombinant human FcγRIIIA (*E. coli*)
Base matrix	5 µm, hydrophilic, non-porous polymer
Dimension	4.6 x 75 mm, PEEK
Storage temperature	2 – 8° C; do not freeze!
Pressure limit	9.0 MPa
Sample mass	5 – 50 µg IgG
Operating temperature	15-25 ° C
Flow rate	Max. 1 mL/min
Recommended mobile phase	A: 50 mmol/L Citrate buffer, pH 6.5 B: 50 mmol/L Citrate buffer, pH 4.5

Summary – FcγRIIIa Affinity HPLC

- Fast (30 min) and reproducible method
- Ideal to get a clue about Fcγ receptor affinity/expected ADCC activity of antibodies especially in phases where ADCC bioassays are too expensive (e.g. large number of samples) or not fast enough (monitoring).
- Potential areas of application:
  - cell line screening
  - upstream optimization
  - monitoring of glycoengineering
  - lot comparison
  - originator/biosimilar comparison
  - stability testing