IgG core a-fucosylation and its impact on FcγRIIIa binding

MipTec 21.09.2011

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Roche Glycart AG

Introduction

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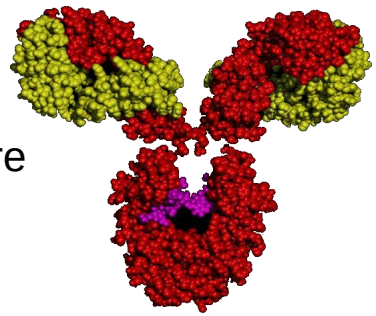
Introduction
Antibody therapies



- Monoclonal antibodies represent a growing class of therapeutics with more than 20 molecules licenced for the treatment of cancer and chronic diseases.
- Major indications: oncology, infectious diseases and autoimmunity.
- Efficacy results from their specificity to the antigen target as well as the activation of effector functions.
- In oncology one relevant mechanism of action is antibody-mediated cellular cytotoxicity (ADCC):
  - This was shown for various antibody therapeutics such as rituximab (anti-CD20) and trastuzumab (anti-Her2).
  - The mechanism underlying ADCC is the binding of FcγRIIIa on natural killer cells (NK cells) to the Fc portion of an IgG and killing of the target cell

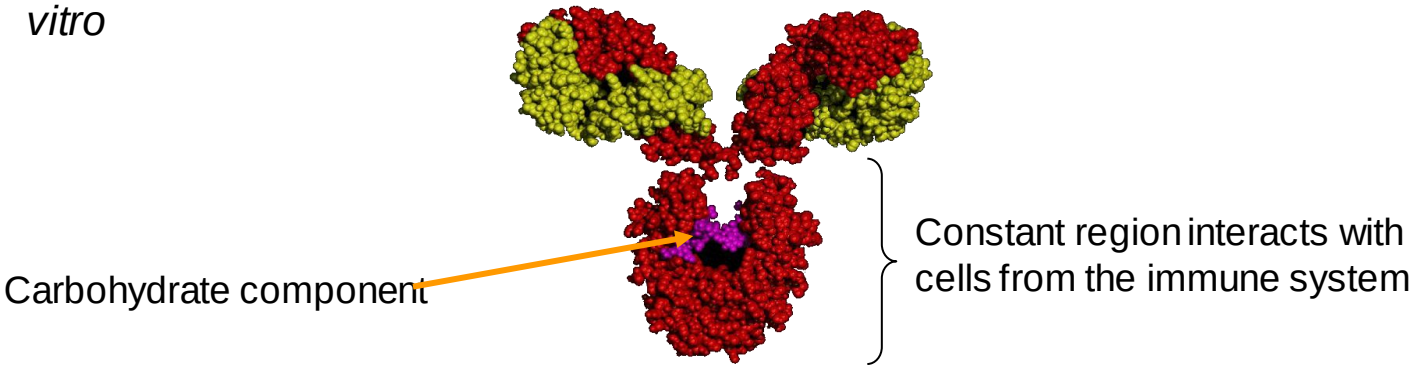
Introduction
Glycoengineering

Main goal for next generation therapeutic antibodies was:

Increase the binding of the antibody to activating FcγRs (FcγRIIIa).

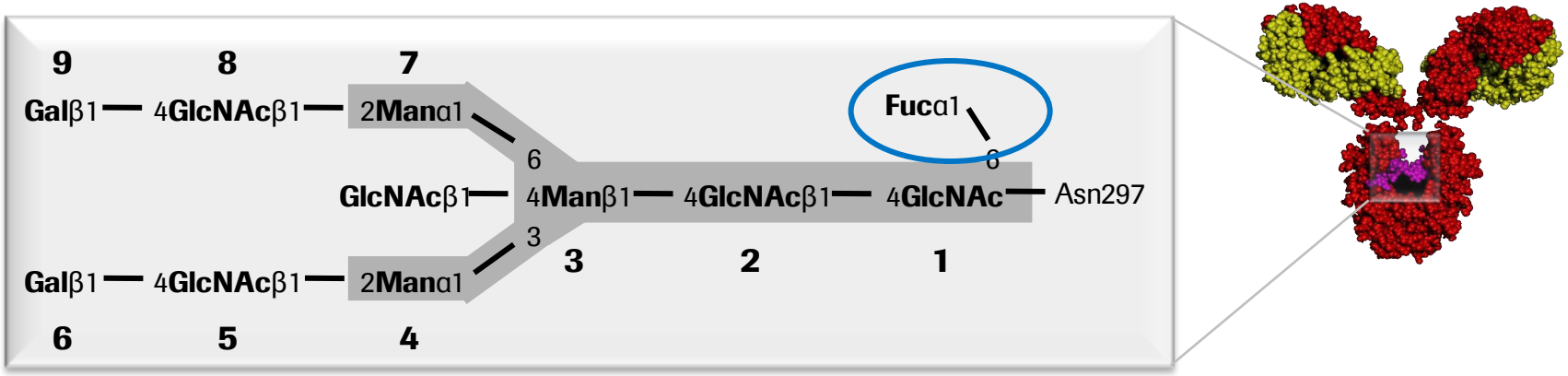
- *Two different strategies*:
  - Amino acid mutations in the Fc part of the antibody
  - Changing the carbohydrate moieties in the Fc portion of the antibody

→ a-fucosylated antibodies show increased binding to FcγRIIIa and enhanced ADCC *in vitro*



Introduction
Glycoengineering

➢ *Glycosylation of IgGs*



Removal of fucose → increased affinity for FcγRIIIa

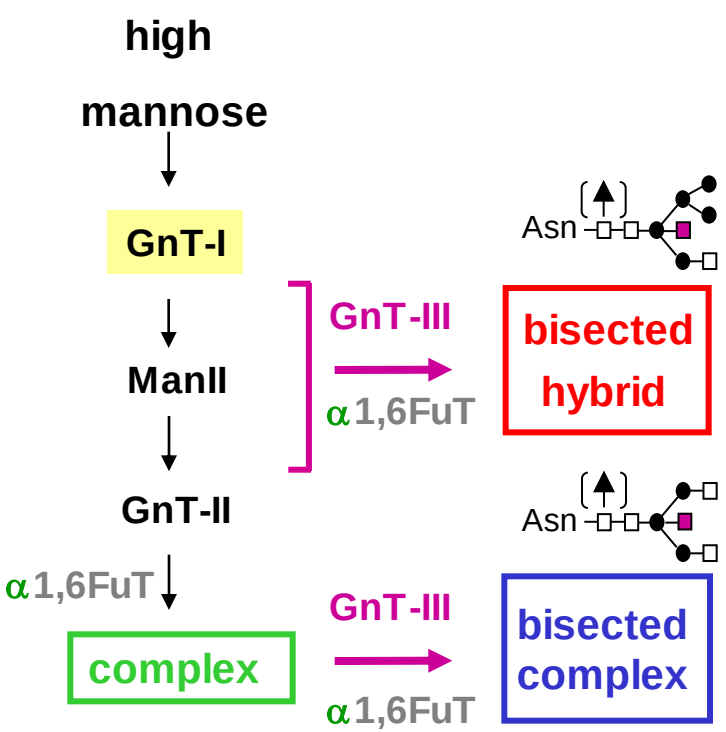
How can one generate antibodies lacking the fucose?

Picture taken from Claudia Ferrara, Roche Glycart AG

Introduction
Glycoengineering

→ **Engineering cell lines overexpressing GnTIII**

N-linked glycosylation



GnT-III → **bisected**

competes against

α1,6-FuT → non-fucosylated

ManII and **GnT-II** → **hybrid**

Why has the presence of the fucose such a great impact on binding?

□ N-acetylglucosamine (GlcNAc) ▲ Fucose

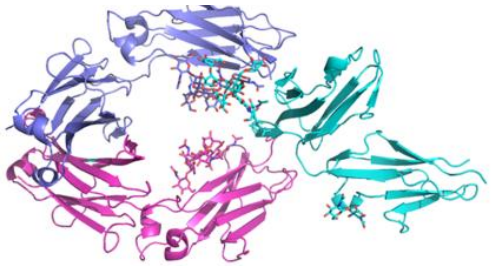
● Mannose

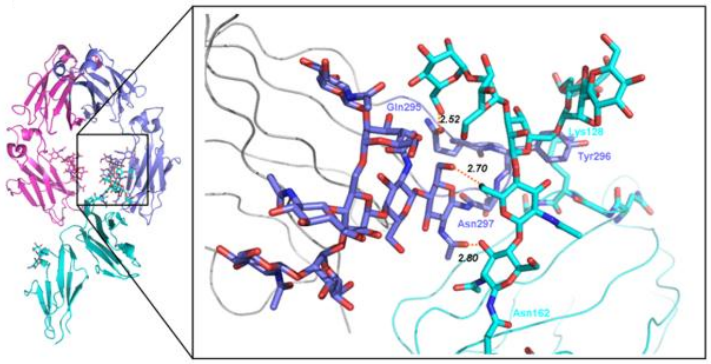
Picture taken from Claudia Ferrara, Roche Glycart AG

Introduction

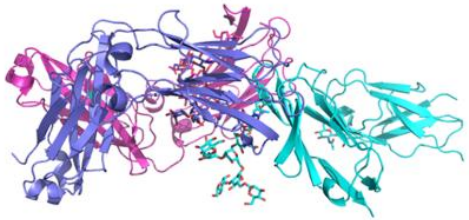
Crystal structure of FcγRIIIa complexed with either a-fucosylated or fucosylated Fc

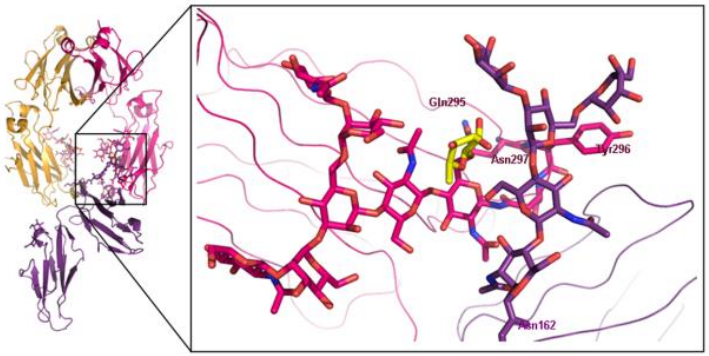
FcγRIIIa / a-fuc Fc





FcγRIIIa / fuc Fc





- Carbohydrate-carbohydrate mediated interactions are responsible for an up to 100-fold gain in binding affinity for a-fucosylated vs. fucosylated IgGs.

* Ferrara et al., PNAS, August 2, 2011, vol. 108, no. 31, 12671

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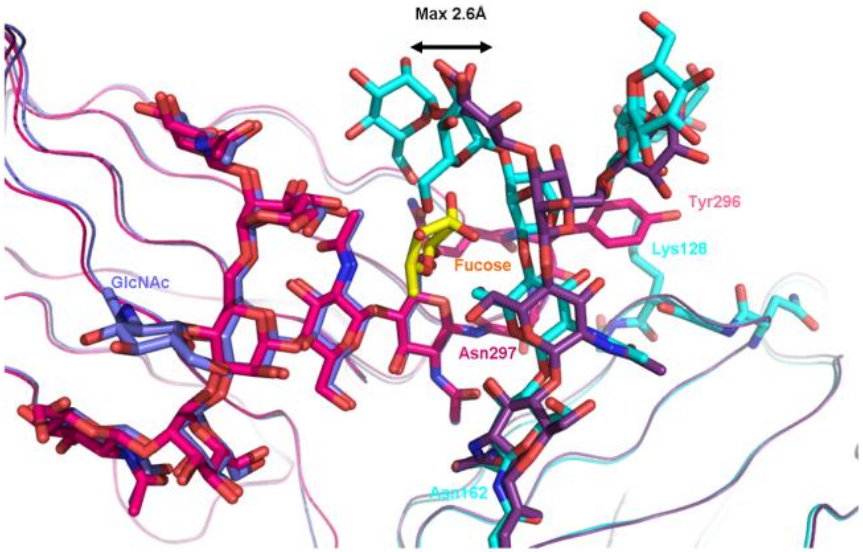
Introduction

Crystal structure of FcγRIIIa complexed with either a-fucosylated or fucosylated Fc



- The Fc-core fucose (highlighted in yellow) has to accomodate in the interface and the Asn162-receptor glycan has to move (↔).
- The result is a direct, steric inhibition caused by core fucose for the carbohydrate-mediated interaction with FcγRIIIa.
- The structures provide a molecular mechanism explaining the increased affinity for the receptor of a-fucosylated antibodies.

* Ferrara et al., PNAS, August 2, 2011, vol. 108, no. 31, 12671

Introduction

Characterization of monoclonal IgGs

Carbohydrate analysis

High throughput ProtA purification

↓

Endoglycosidase digest

↓

MALDI TOF MS analysis

Biological actitivity

ADCC

Affinities

Surface Plasmon Resonance

Could the TagLite technology combine some of these analysis?

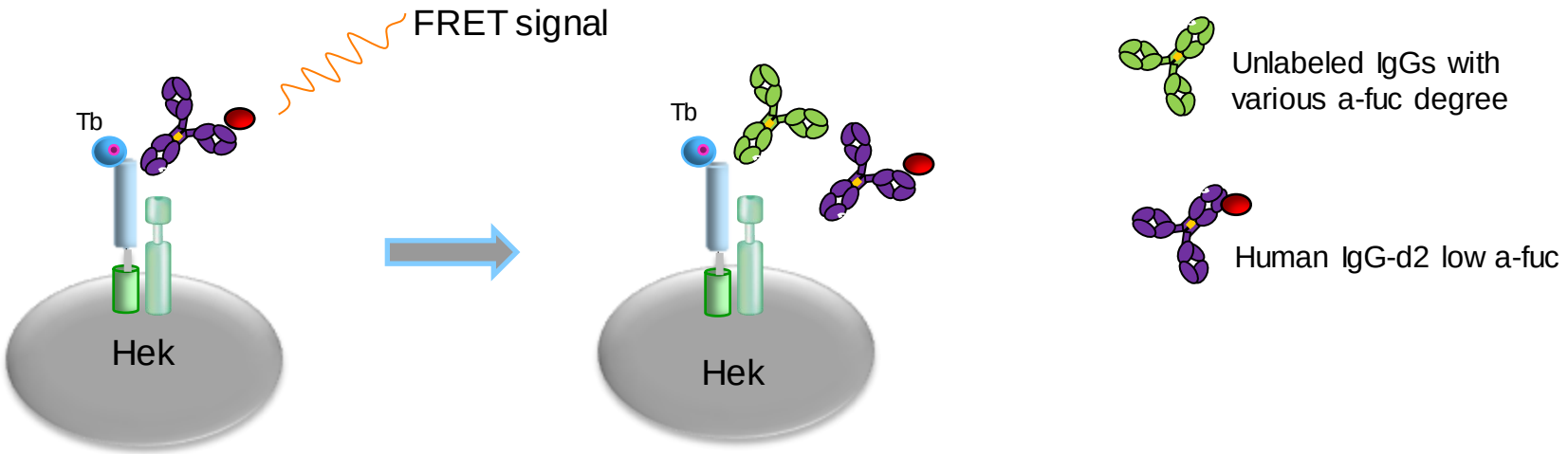
Introduction

TagLite Results

Outlook

TagLite – results

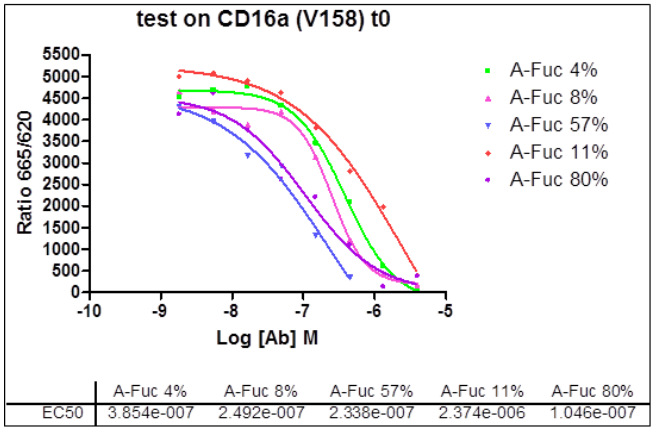
FcγRIIIaV158 and F158 competition assay



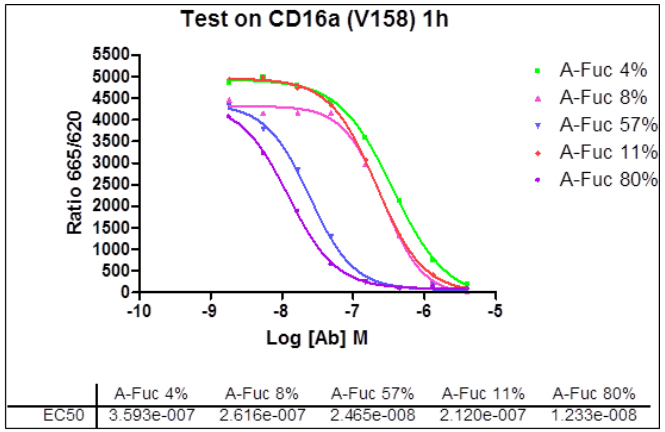
Assay setup:

- 10.000 cells per well (expressing huFcγRIIIaV158 or F158 labeled with Tb)
- IgGs with various a-fucosylation degrees (4, 8, 11, 57 and 80 %) conc. 4000 to 1.8 nM final in well (1:3 dilutions).
- Labeled IgGs (human IgG-d2); conc. 50 nM final in well
- Incubation time: 0h, 1h, 3h, 5h @ RT

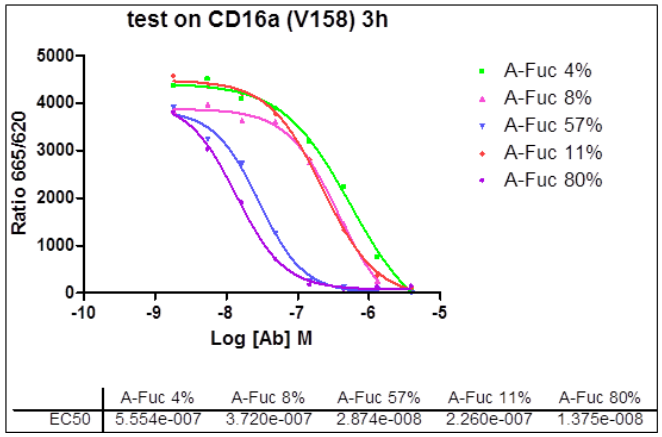
TagLite – results
FcγRIIIa(V158)



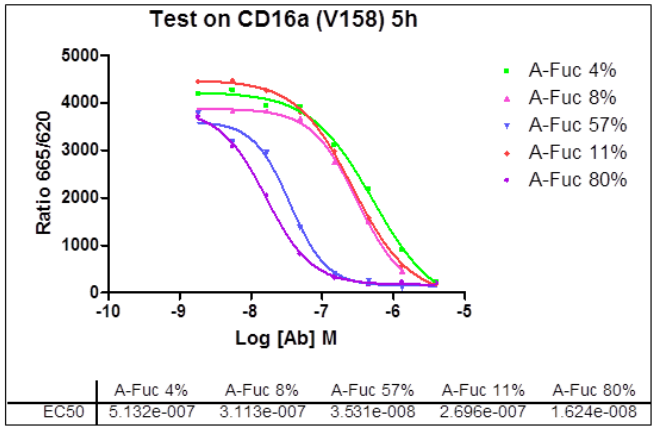
	A-Fuc 4%	A-Fuc 8%	A-Fuc 57%	A-Fuc 11%	A-Fuc 80%
EC50	3.854e-007	2.492e-007	2.338e-007	2.374e-006	1.046e-007



	A-Fuc 4%	A-Fuc 8%	A-Fuc 57%	A-Fuc 11%	A-Fuc 80%
EC50	3.593e-007	2.616e-007	2.465e-008	2.120e-007	1.233e-008



	A-Fuc 4%	A-Fuc 8%	A-Fuc 57%	A-Fuc 11%	A-Fuc 80%
EC50	5.554e-007	3.720e-007	2.874e-008	2.260e-007	1.375e-008



	A-Fuc 4%	A-Fuc 8%	A-Fuc 57%	A-Fuc 11%	A-Fuc 80%
EC50	5.132e-007	3.113e-007	3.531e-008	2.696e-007	1.624e-008

- The assay works nicely with the high affinity receptor.
- Already after one hour incubation ranking of the abs can be detected.
- The 2 IgGs with high a-fuc degree compete much better than the 3 IgGs with lower a-fuc degree.

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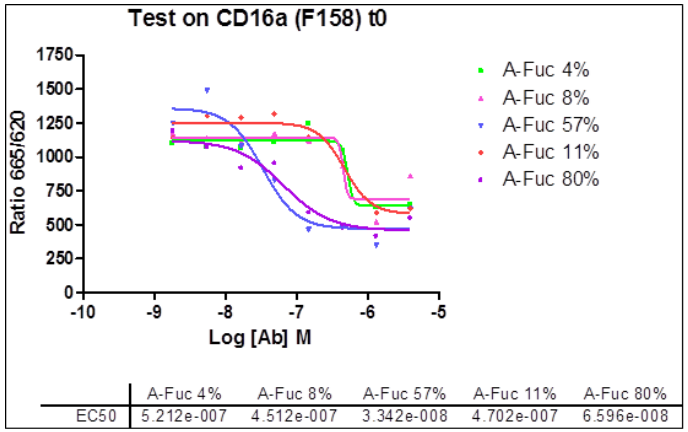
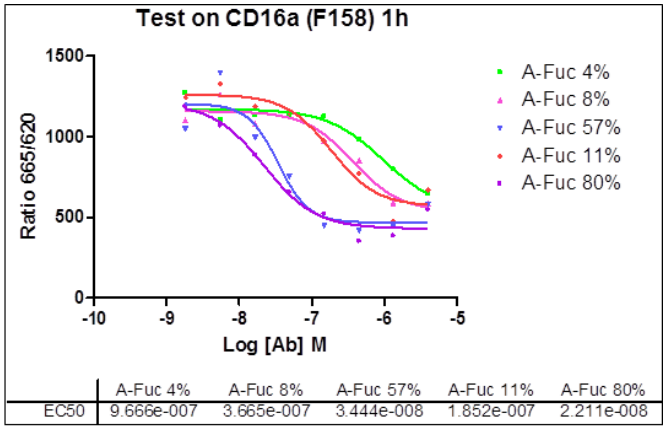
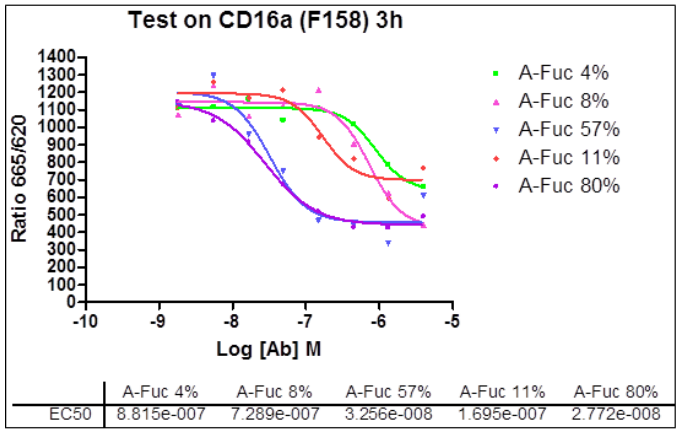
TagLite – results
FcγRIIIa(F158)



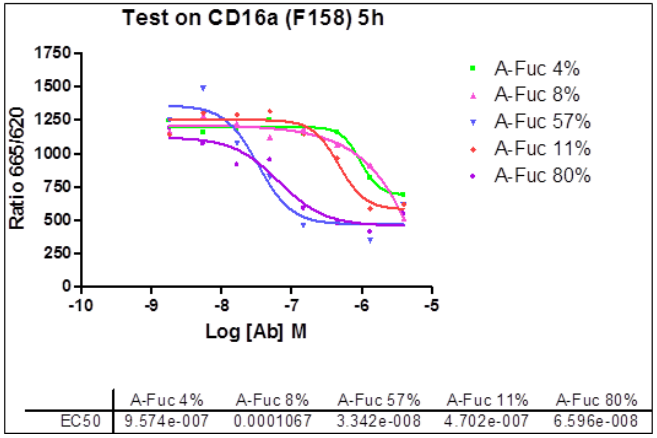
	A-Fuc 4%	A-Fuc 8%	A-Fuc 57%	A-Fuc 11%	A-Fuc 80%
EC50	5.212e-007	4.512e-007	3.342e-008	4.702e-007	6.596e-008



	A-Fuc 4%	A-Fuc 8%	A-Fuc 57%	A-Fuc 11%	A-Fuc 80%
EC50	9.666e-007	3.665e-007	3.444e-008	1.852e-007	2.211e-008



	A-Fuc 4%	A-Fuc 8%	A-Fuc 57%	A-Fuc 11%	A-Fuc 80%
EC50	8.815e-007	7.289e-007	3.256e-008	1.695e-007	2.772e-008

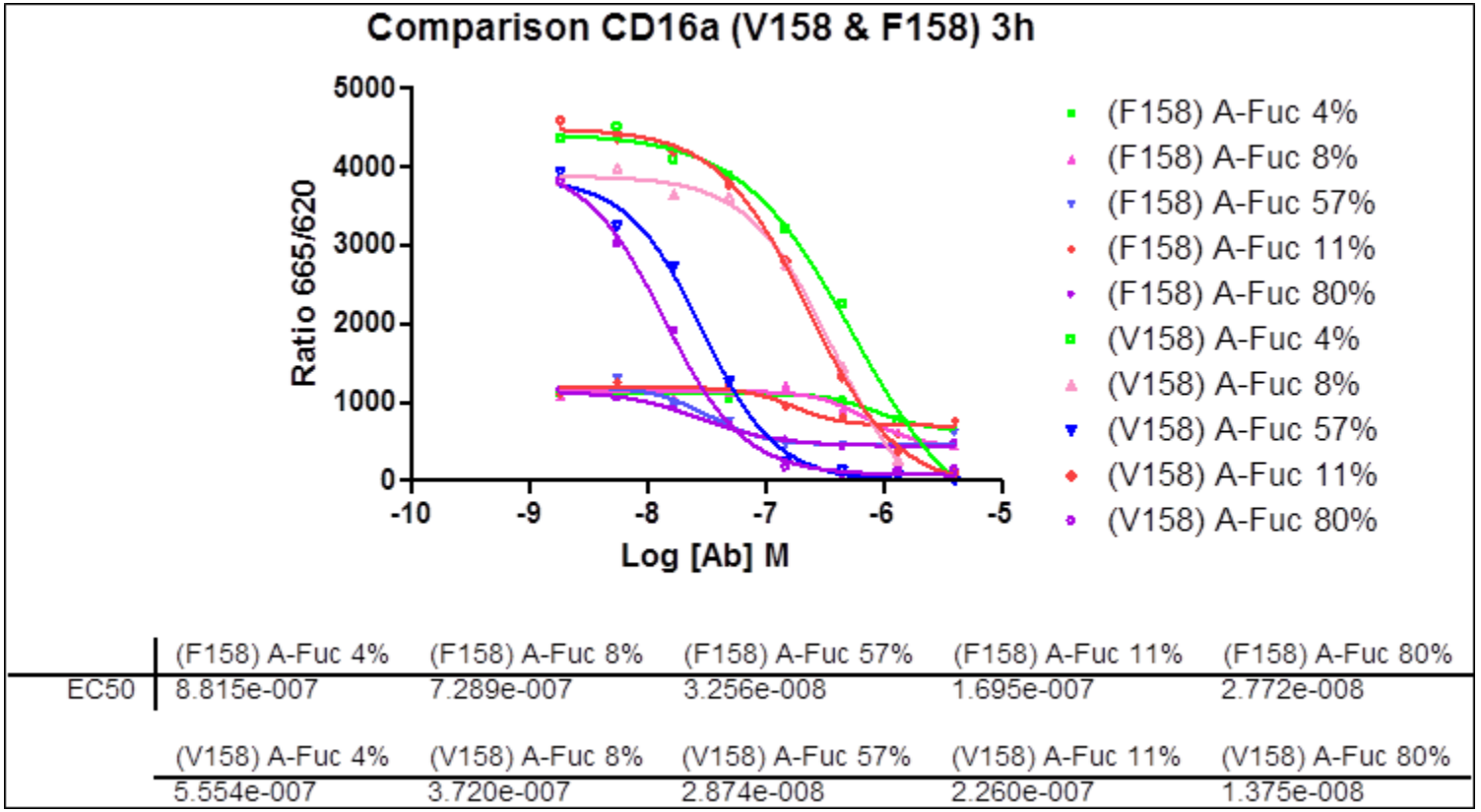


	A-Fuc 4%	A-Fuc 8%	A-Fuc 57%	A-Fuc 11%	A-Fuc 80%
EC50	9.574e-007	0.0001067	3.342e-008	4.702e-007	6.596e-008

- The assay also works with the low affinity receptor but the assay window is much smaller.
- Still also here the higher a-fucosylated IgGs compete better than the ones with lower a-fuc content.

TagLite – results

Comparison FcγRIIIa(V158) and FcγRIIIa(F158)



	(F158) A-Fuc 4%	(F158) A-Fuc 8%	(F158) A-Fuc 57%	(F158) A-Fuc 11%	(F158) A-Fuc 80%
EC50	8.815e-007	7.289e-007	3.256e-008	1.695e-007	2.772e-008

(V158) A-Fuc 4%	(V158) A-Fuc 8%	(V158) A-Fuc 57%	(V158) A-Fuc 11%	(V158) A-Fuc 80%
5.554e-007	3.720e-007	2.874e-008	2.260e-007	1.375e-008

TagLite – results

Correlation with other methods

huFcγRIIIaF158	MALDI	Biacore	TagLite
	a-fuc (%)	KD (nM)	EC50 (nM)
832	4	1300	881
834	8	940	728
835	11	845	170
836	57	68	32
842	80	39	27

a-fuc increase ↓

improve in KD/EC50 ↓

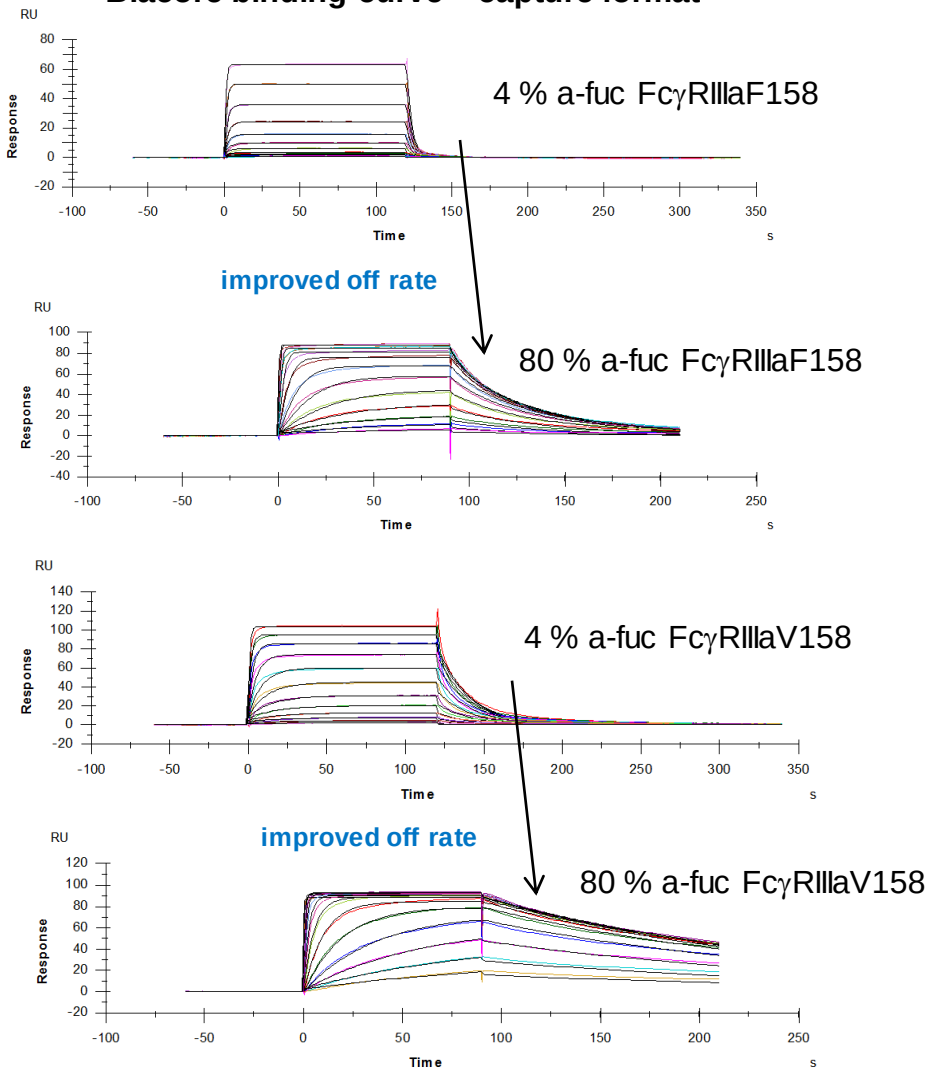
huFcγRIIIaV158	MALDI	Biacore	TagLite
	a-fuc (%)	KD (nM)	EC50 (nM)
832	4	200	555
834	8	201	370
835	11	111	226
836	57	17	28
842	80	7	13

a-fuc increase ↓

improve in KD/EC50 ↓

Note: KD values (Biacore) depend a lot on the format used.
In the present format KD values for a-fuc abs tend to be lower than with other formats. You can expect values ranging from 500-700 nM for FcgRIIIaV158 and low a-fuc abs.

Biacore binding curve – capture format



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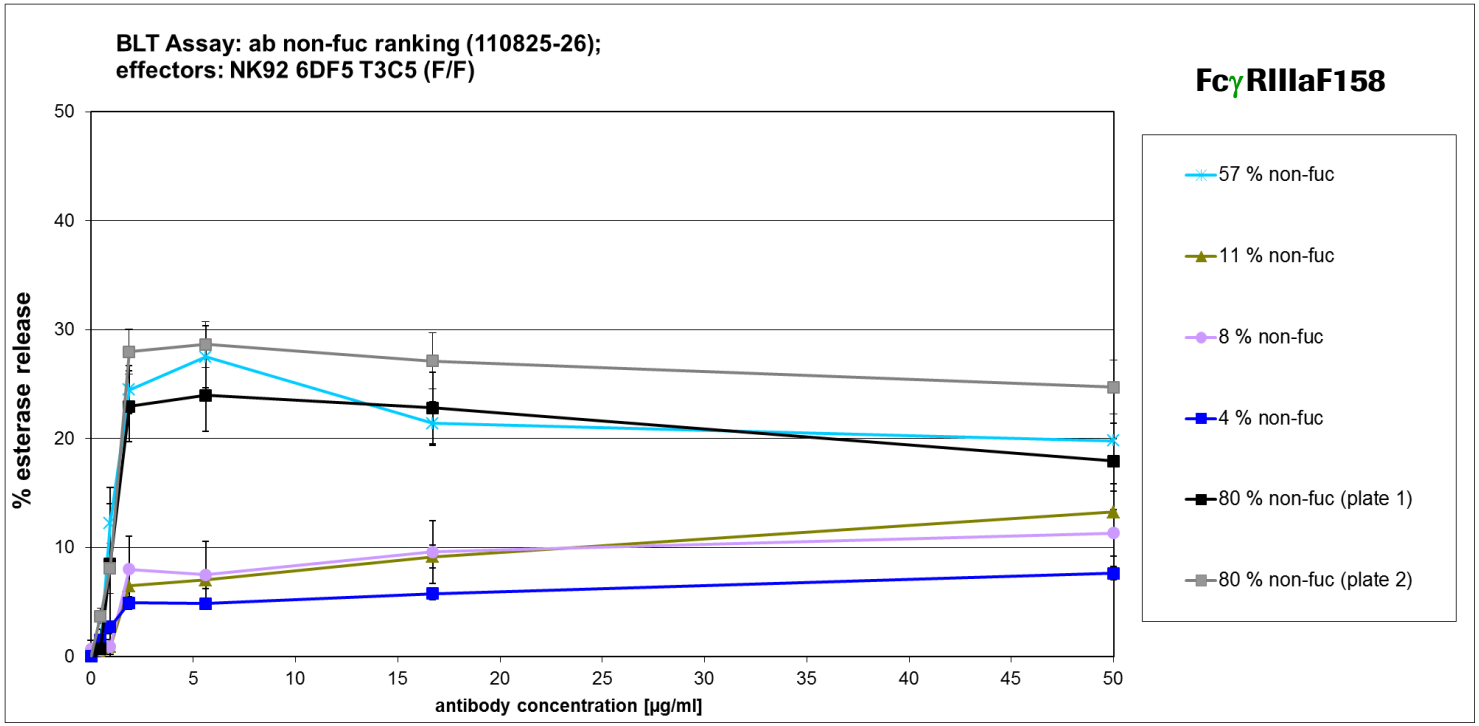
TagLite – results
Correlation with other methods

Biological activity - BLT assay



- The BLT assay measures the release of esterase upon activation of NK cells
- The graph shows that the 2 IgGs containing higher a-fuc degree lead to a 3 fold higher release of esterase than the ones with lower a-fuc degree.
- The release correlates well with killing of the target cell.

Introduction

TagLite Results

Outlook

Conclusion/Outlook

- The TagLite technology provides a nice, robust and easy to use tool to study binding of antibody Fc portions to FcγRIIIa on cells.
- The data correlate well with data from Biacore and cell based assays like ADCC
- An advantage compared to Biacore analysis is that binding occurs with native receptor embedded in the cell membrane rather than purified soluble forms which translates better into cell based assays.
- Interesting would be to test also other hu Fcγ receptors as well as muFcγR which might be of importance fo mouse models.
- Also to be checked: Could you determine a-fucosylation degree simply by using a standard curve of IgGs with various a-fucosylation degree and therefore use the TagLite assay for screening purposes?

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