Therapeutic antibody glycoforms: structures & function

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School of Immunity & Infection, University of Birmingham

The stability of biopharmaceuticals – getting the chemistry right

RSC - MIBio 2014: Cambridge, September 30th 2014

Antibody therapeutics: Glycoform structures & function

Antibody classes & isotypes

Antibody effector activities

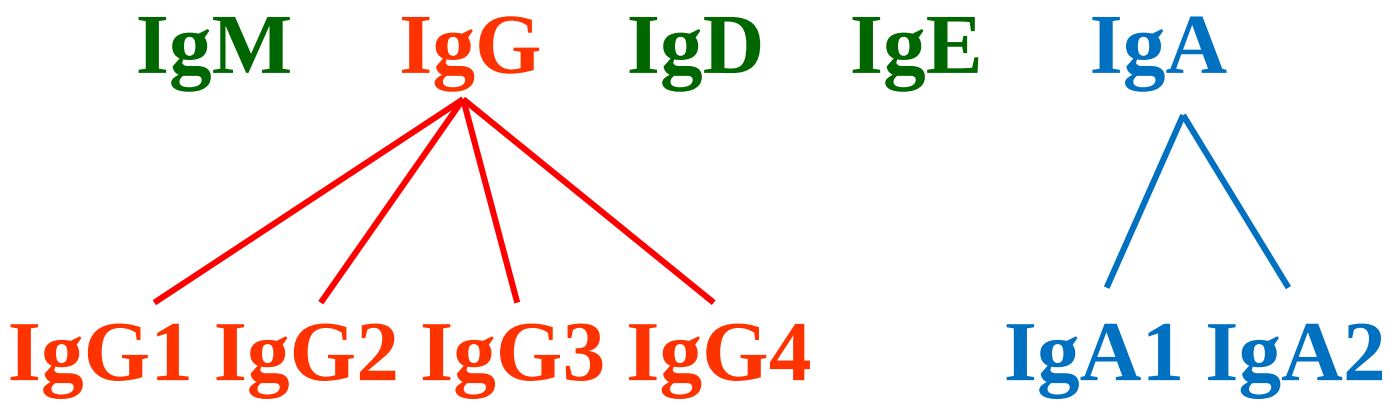
IgG-Fc glycoforms

Ligand binding sites

Engineering “biobetters”

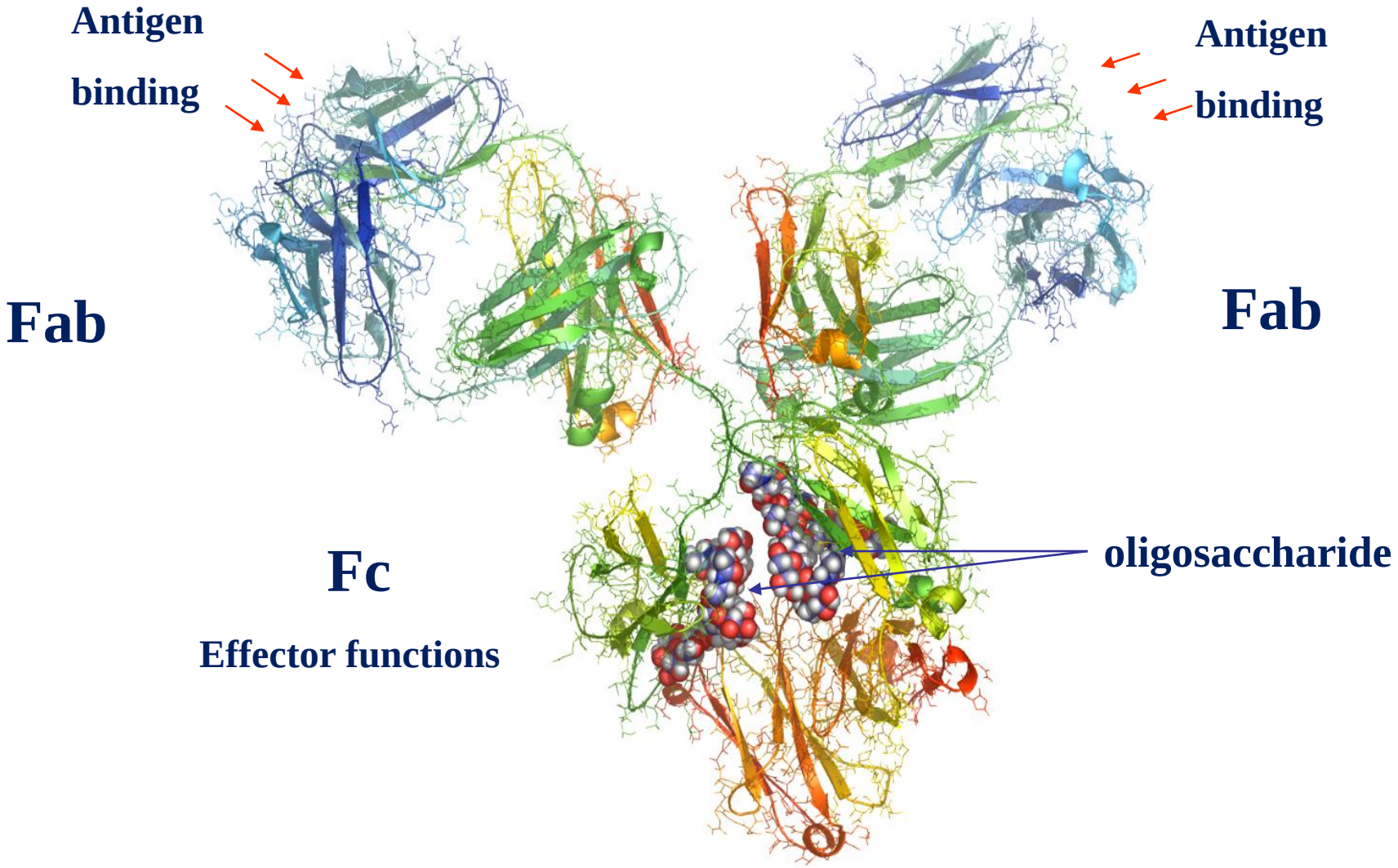
Human immunoglobulin classes & subclasses

The result of gene duplication, mutation & selection



The humoral immune response is “orchestrated” to provide optimal protection to a given “insult”

Domain structure of IgG



Ludger

The human IgG subclasses

IgG1

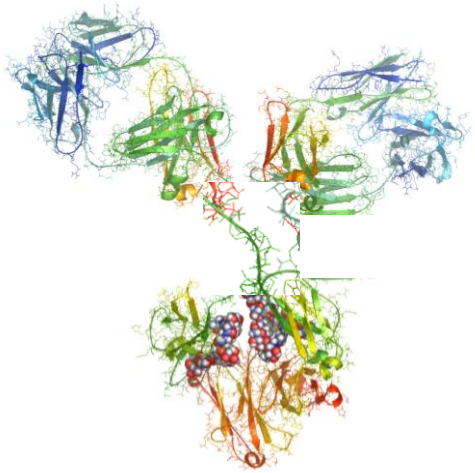
~ 60 %

IgG2

~ 25 %

IgG3

~ 10 %



IgG4

~ 5 %

Jefferis R. Arch. Biochem. Biophys. (2012)

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Ligands mediating IgG-Fc effector activities

FcγRI (1,3,4) **FcγRIIa/b****(1,2,3) **FcγRIIIa/b****(1,3,4)

phagocytosis, antibody dependent cellular cytotoxicity (ADCC), apoptosis, generation of superoxide, release of enzymes

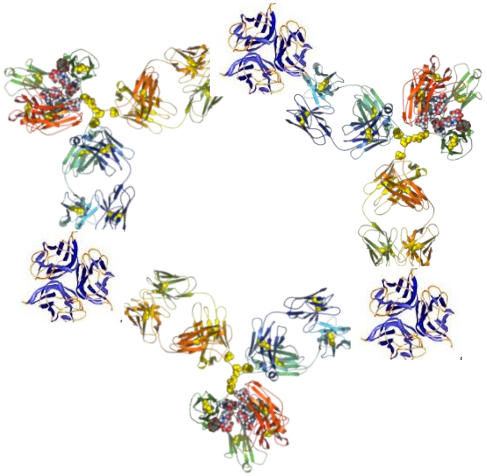
* dependent on FcγR polymorphisms; * dependent on glycoform

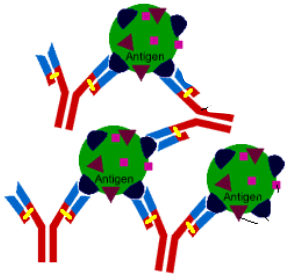
C1q: (IgG1,3) complement dependent cytotoxicity (CDC)

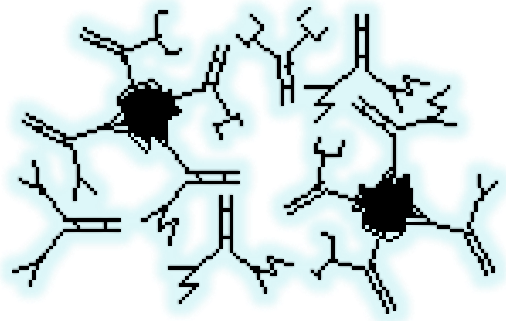
MBL (IgG1,2,3,4) Lectin pathway (CDC)

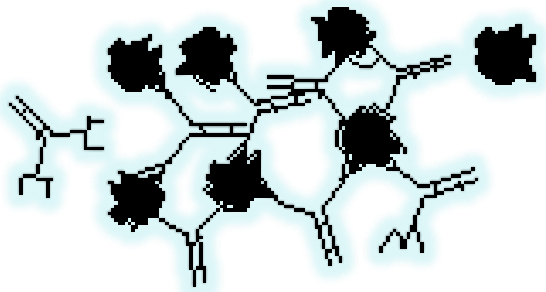
FcRn (1,2,3,4) Catabolism & placental transport

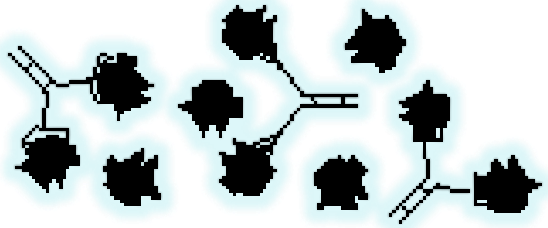
Formation of antigen/antibody complexes











Jefferis R., Steensgaard, J. Immunology. 46:751-60 (1982)

Activities of aglycosylated IgG-Fc

FcγRI	activation	reduced x 100
FcγRII	binding	abolished
FcγRIII	activation	abolished
C1	“ “	abolished
MBL	“ “	abolished
FcRn	binding	unaffected

Jefferis R. Arch. Biochem. Biophys. (2012)

Aglycosylated antibody products

UCB — **Cimzia (Fab-Peg) [TNF– licensed]**

Genentech, A Member of the Roche Group — **Lucentis (Fab) [VEGFA-licensed]**

TOLERX, Immune resolve. — **Otelixizumab (CD3)** (Asn_{297} – Ala)

Bristol-Myers Squibb — **BMS-945429 (IL-6)** (Asn_{297} – Ala)

Take home message:

Glycosylation is a Critical quality Attribute (CQA)

Therapeutic recombinant antibody production processes must deliver 100 % occupancy or 0 % occupancy

The glycoform profile of an antibody is achieved as a:

Quality by Design parameter (QbD)

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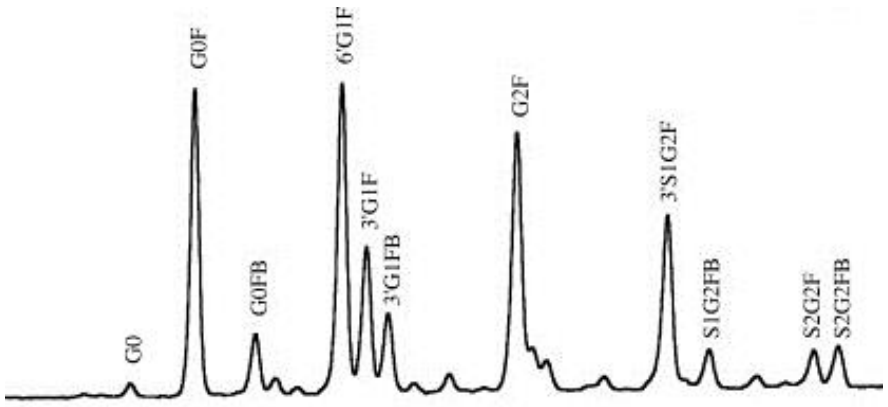
Oligosaccharides released from IgG

Polyclonal IgG

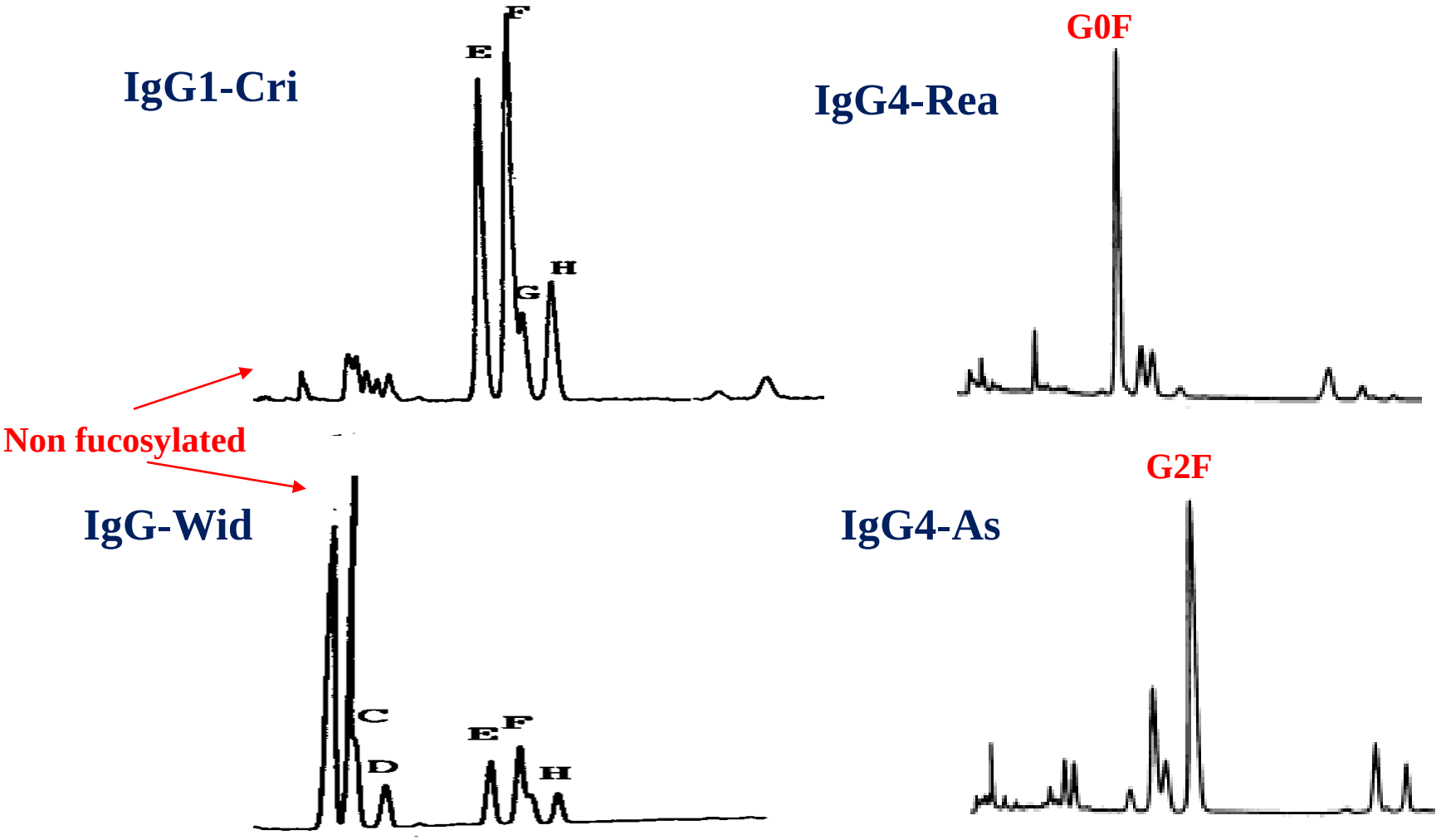


Anumula KR J Immunol Meth 382:167–176 (2012)

Adalimumab: CHO cells

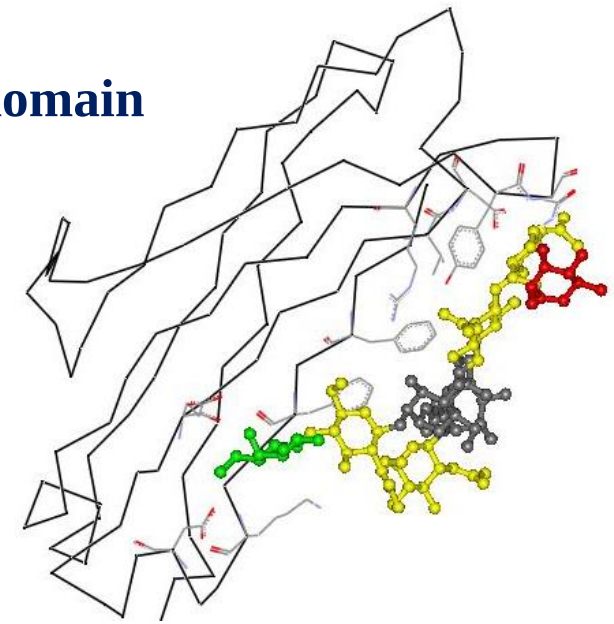
Mimura, Y. et al. Therapeutic antibodies: (Ed) An, Z., Wiley, 2009

Oligosaccharides released from monoclonal IgG1 & IgG4

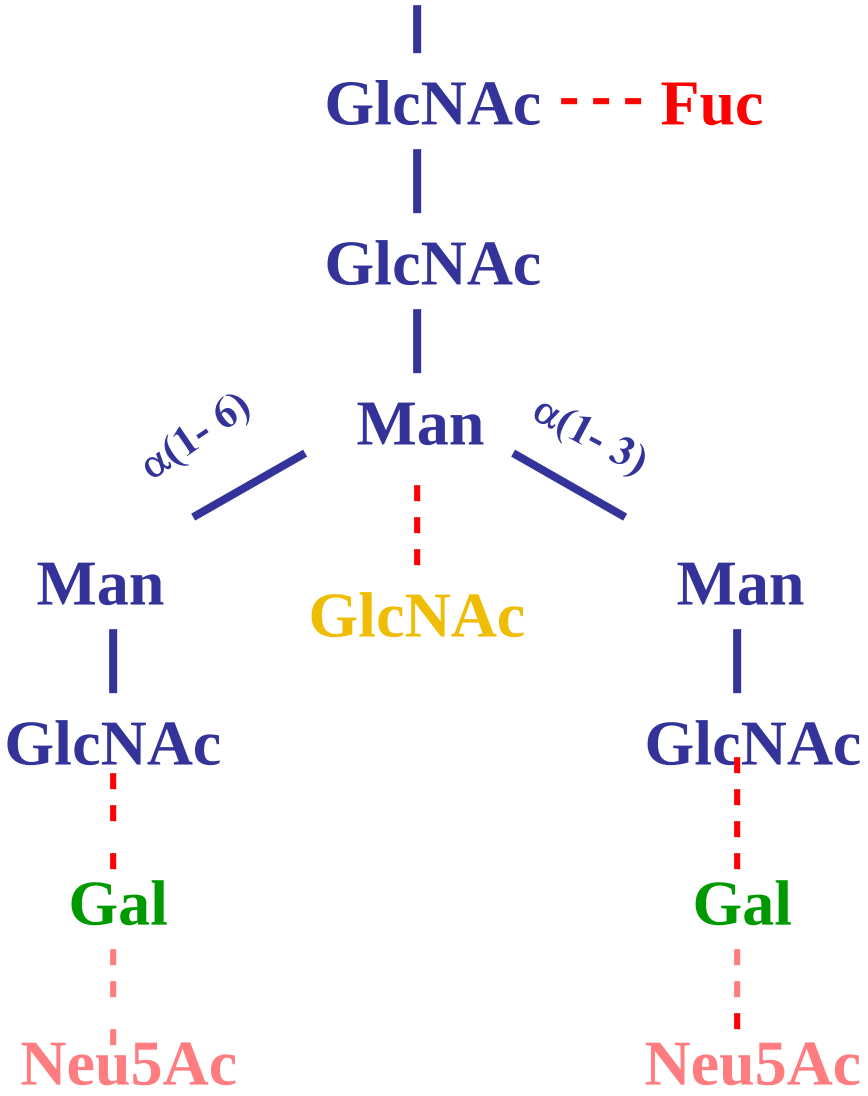


Jefferis, R. Takahashi N., et al. Biochem. J. 268: 529-537 (1990)

CH2 domain



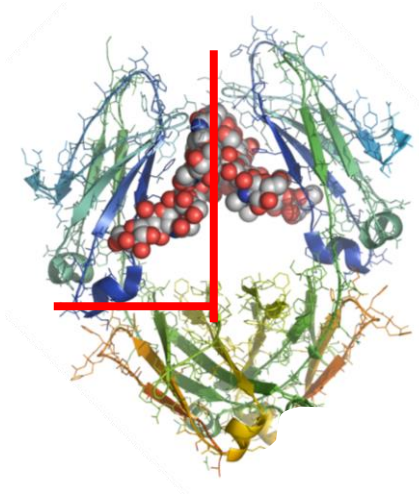
-Gln-Tyr-**Asn**$_{297}$-Ser-Thr-Tyr-Arg-

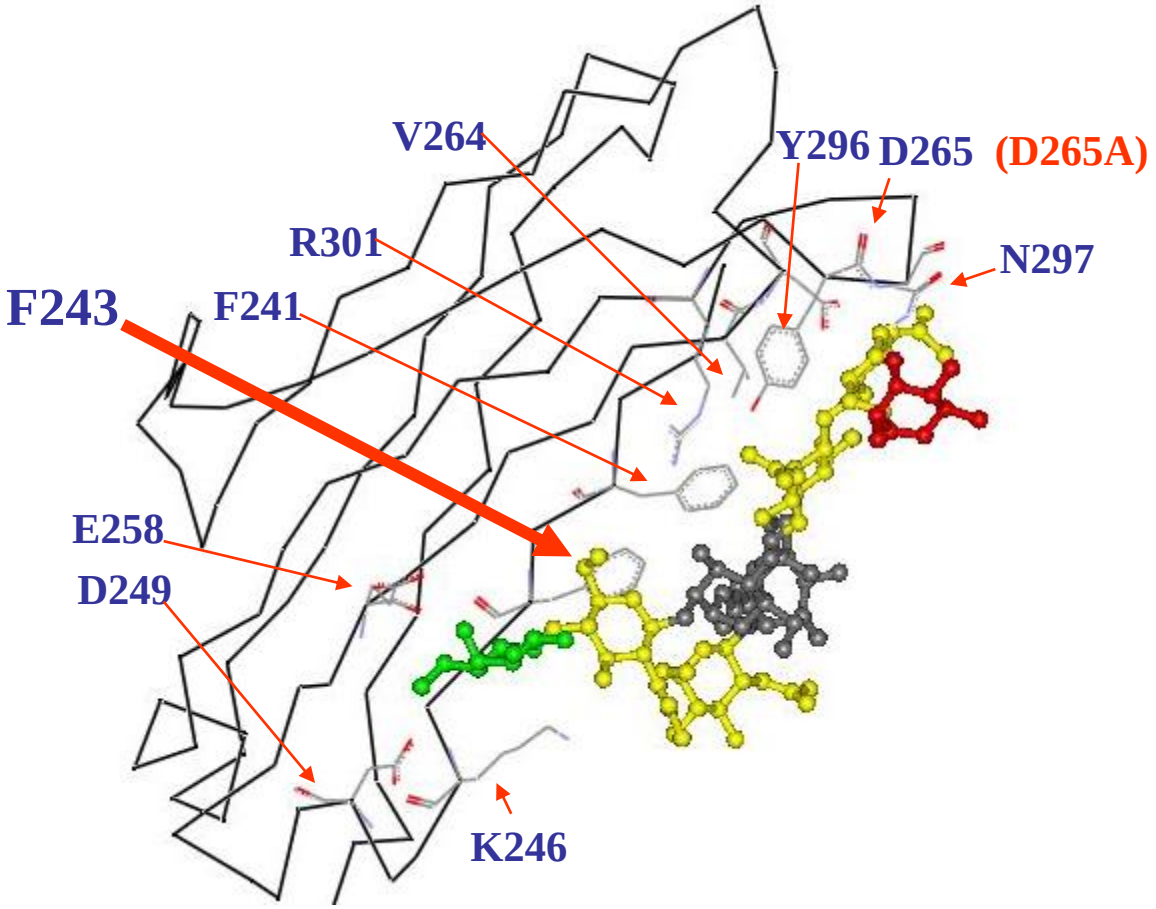


648/128 possible glycoforms

Jefferis R. Arch. Biochem. Biophys. 526:159-662 (2012)

Alanine replacement of amino acids residues forming non-covalent contacts with the oligosaccharide





Fuc

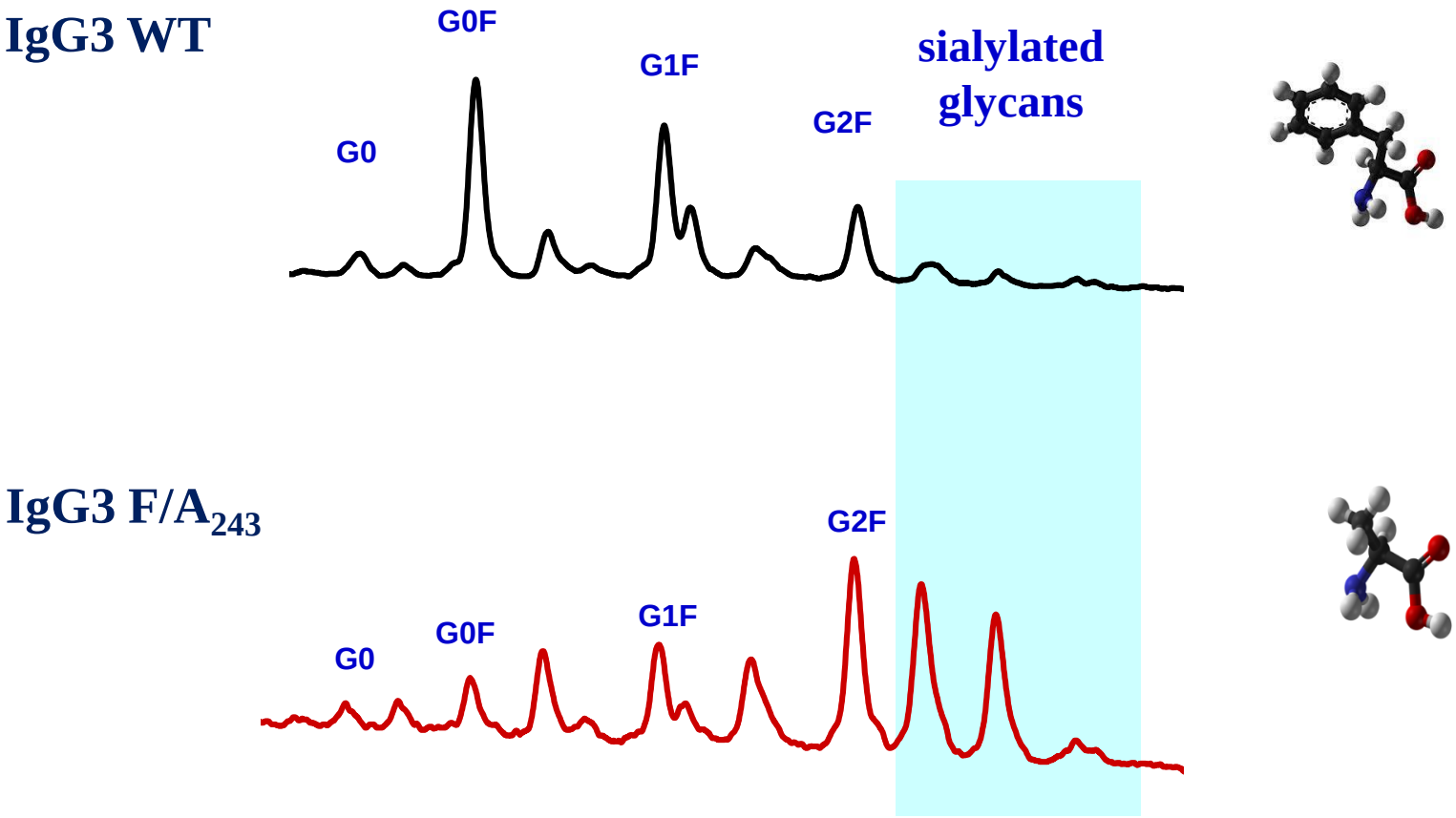
Gal-GlcNAc-Man

Man-GlcNAc-GlcNAc-N297

GlcNAc-Man

Lund J. et al. J,Immunol. 157: 4963-9 (1996)

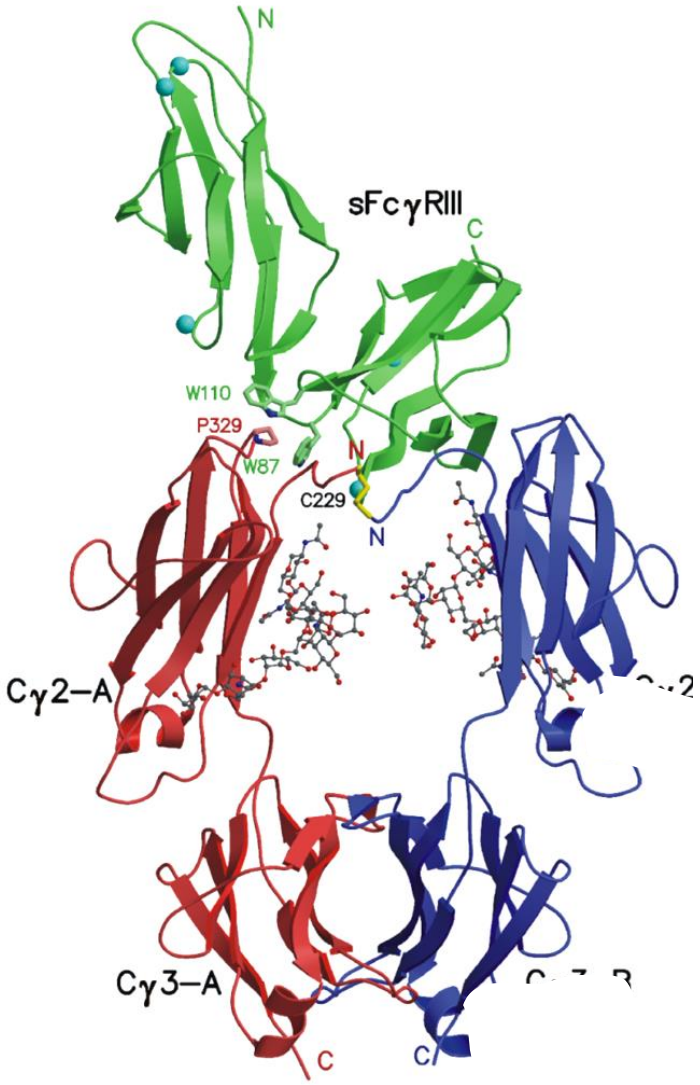
The F/A_{243} mutant yields high levels of sialylation



Mimura Y., Jefferis R., Rudd P. et al. unpublished

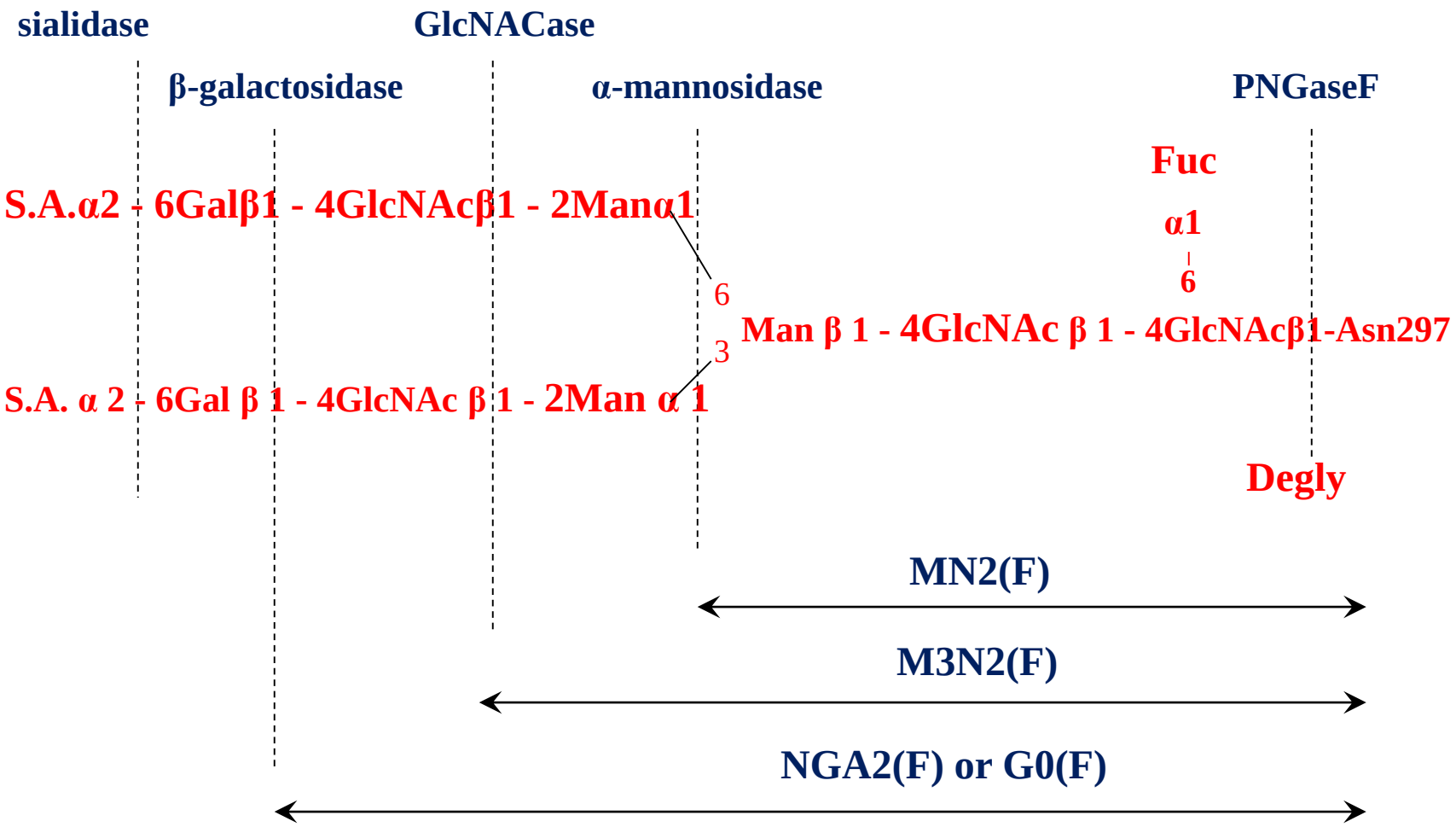
Lund J, Jefferis R., et al. J Immunol. 157:4963-4969 (1996).

IgG1-Fc in complex with aglycosylated FcγRIII



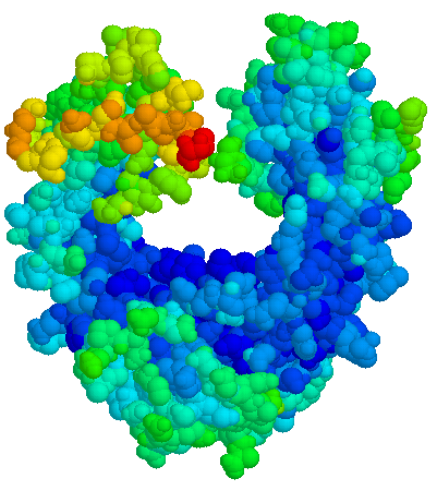
Sondermann P et al. Nature 406:267-273 (2000)
Radaev S et al. Mol Immunol. 38:1073-1083 (2002)

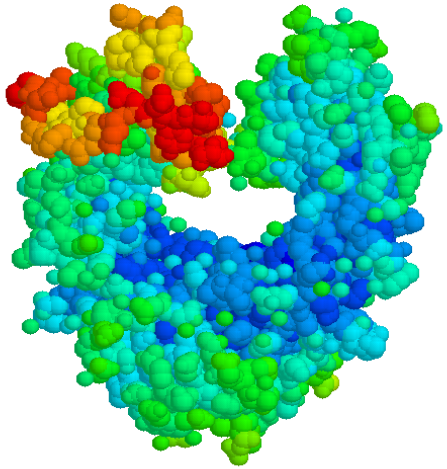
Preparation of homogeneous glycoforms of IgG

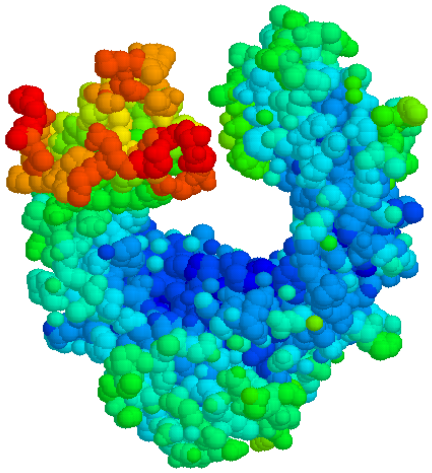


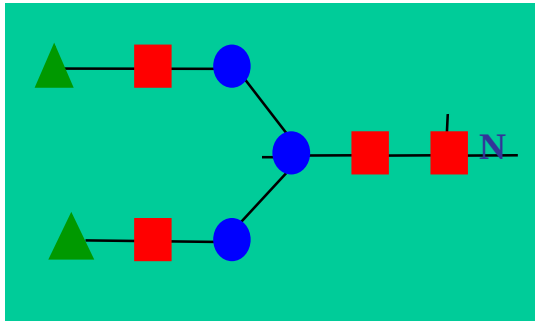
Mimura Y. et al. J Biol Chem. 276:45539-45547.(2001)

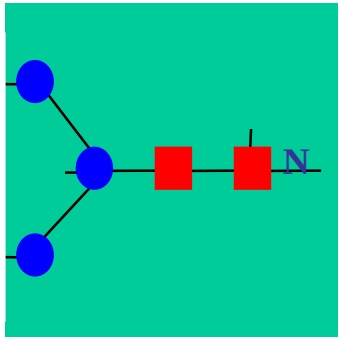
Truncated glycoforms of IgG-Fc

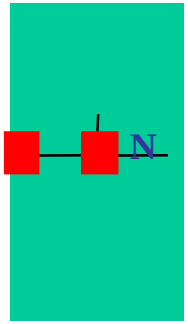






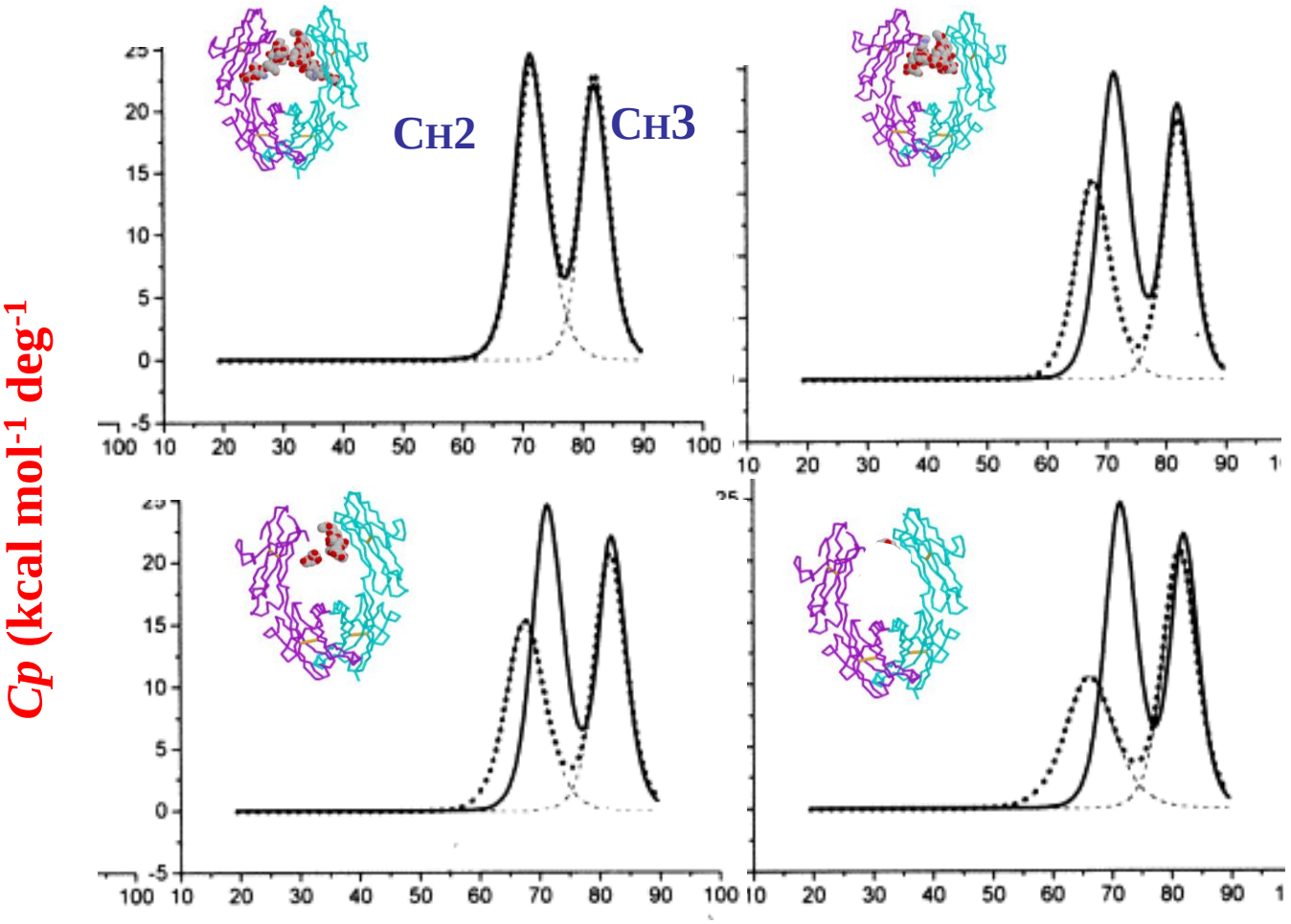






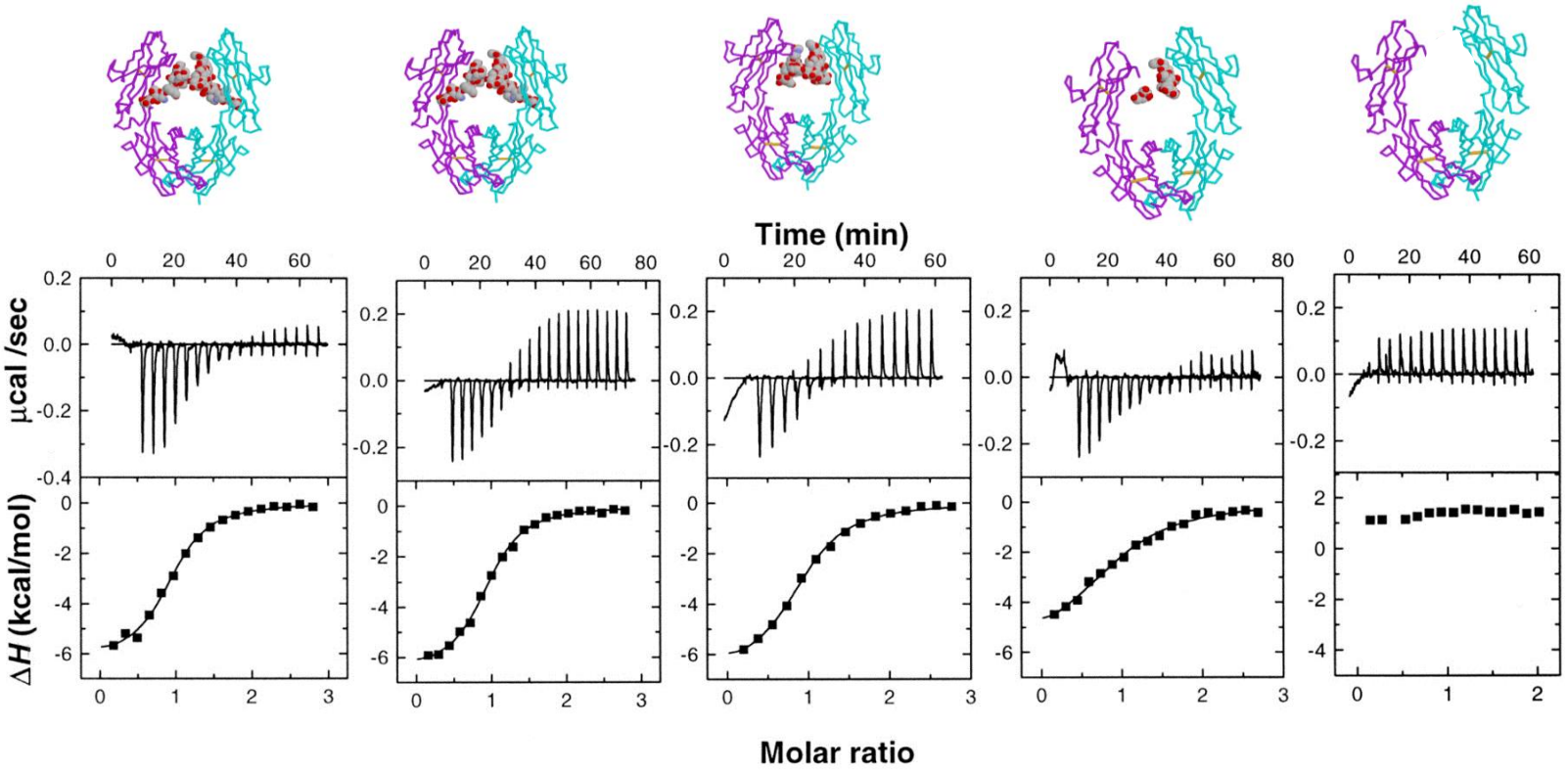
Krapp, S., Mimura, Y. et al. J.Mol.Biol. 325:979-989 (2003)

Differential scanning micro calorimetry of IgG-Fc



Mimura Y. et al. Mol Immunol. 37:697-706 (2000)

Binding isotherms for FcγRIIb/IgG1-Fc glycoforms

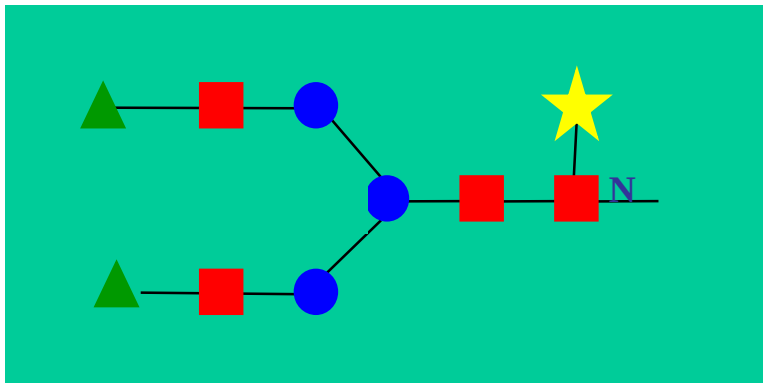


Mimura, Y., Jefferis, R. et al. J.Biol.Chem. 276:45539-45547 (2001).

Fully galactosylated glycoforms: G2

exhibit enhanced:

C1 complement activation; FcRn placental passage

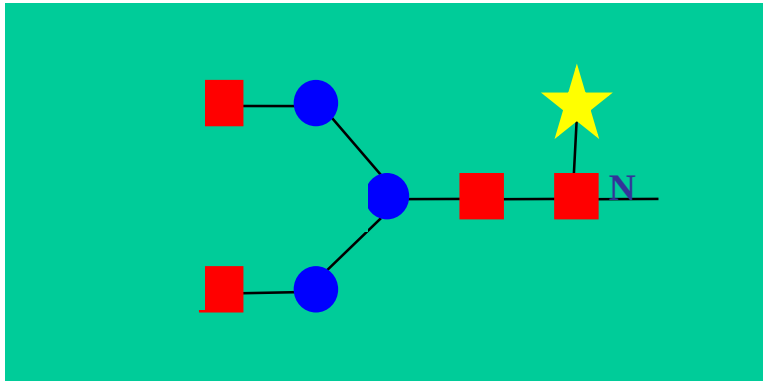


Jefferis, R. Nature Reviews: Drug Discovery. 8: 226-234 (2009)
Hodoniczky J, et al.. Biotechnol Prog. 21:1644-52 (2005)

Absence of galactose: G0

Terminal N-acetyl glucosamine residues may bind/activate pattern recognition receptors, e.g.

Mannan binding lectin (MBL); Mannose receptor (MR)



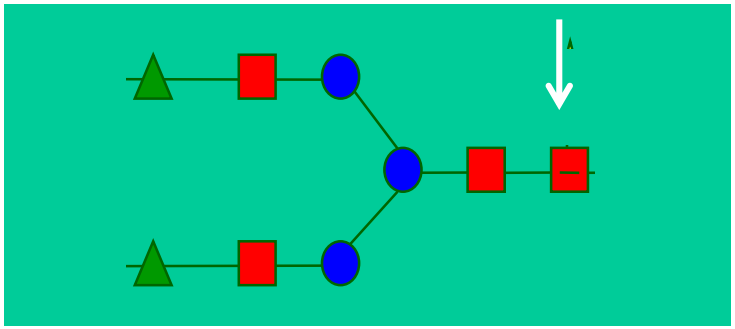
Jefferis, R. Nature Reviews: Drug Discovery. 8: 226-234 (2009)
Hodoniczky J, et al.. Biotechnol Prog. 21:1644-52 (2005)

Non fucosylated IgG has enhanced NK cell (FcγRIII) mediated ADCC

α1,6 fucosyltransferase “knockout” CHO cells

Potelligent technology

BIOWA



Lonza

Mogamulizumab anti-CCR4
Anti Ebola virus h-13F

GlycoFi

MERCK

BIOLEX THERAPEUTICS

Igeneon AG

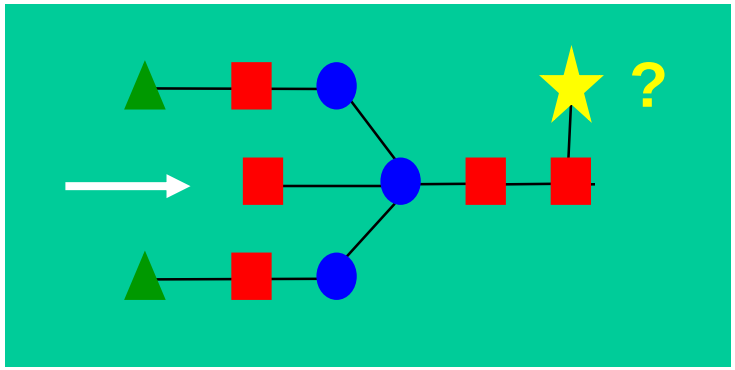
ProBioGen
Supporting Biopharmaceutical Visions

Kanazawa T. et al. Clin Cancer Res. 2014 Aug 12

The presence of bisecting N-acetylglucosamine enhances NK cell mediated ADCC (x 100)

"Knock-in" of GNTIII transferase in CHO cells

GLYCART biotechnology



Roche

GlycoMAb

Obinutuzumab anti-CD20 (Gazyva); GA201 anti-EGFR

http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm373263.htm

Gerdes CA. & Umana P. Clin Cancer Res. 20:1055 (2014)

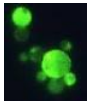
Glycoprotein production platforms:

Mammalian: CHO, Sp2/0; NSO; Per.C6 etc

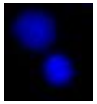


Transgenics: goat; sheep; cows; rabbits; pigs etc

Aves: chickens (eggs)



Yeasts: Pichia pastoris; Saccharomyces cerevisiae



Insect cells: Sf9 (baculovirus infected)

Plants: tobacco; corn; tomato; potato; moss

Bacteria: Escherichia coli; Bacillus subtilis

??????????????????????????

The immune system “orchestrates” the antibody isotype response to be optimal for resolution of an “insult”, i.e. infection by a pathogen.

Can the immune system “orchestrate” the glycoform profile of an IgG response to be optimal for resolution of and insult?

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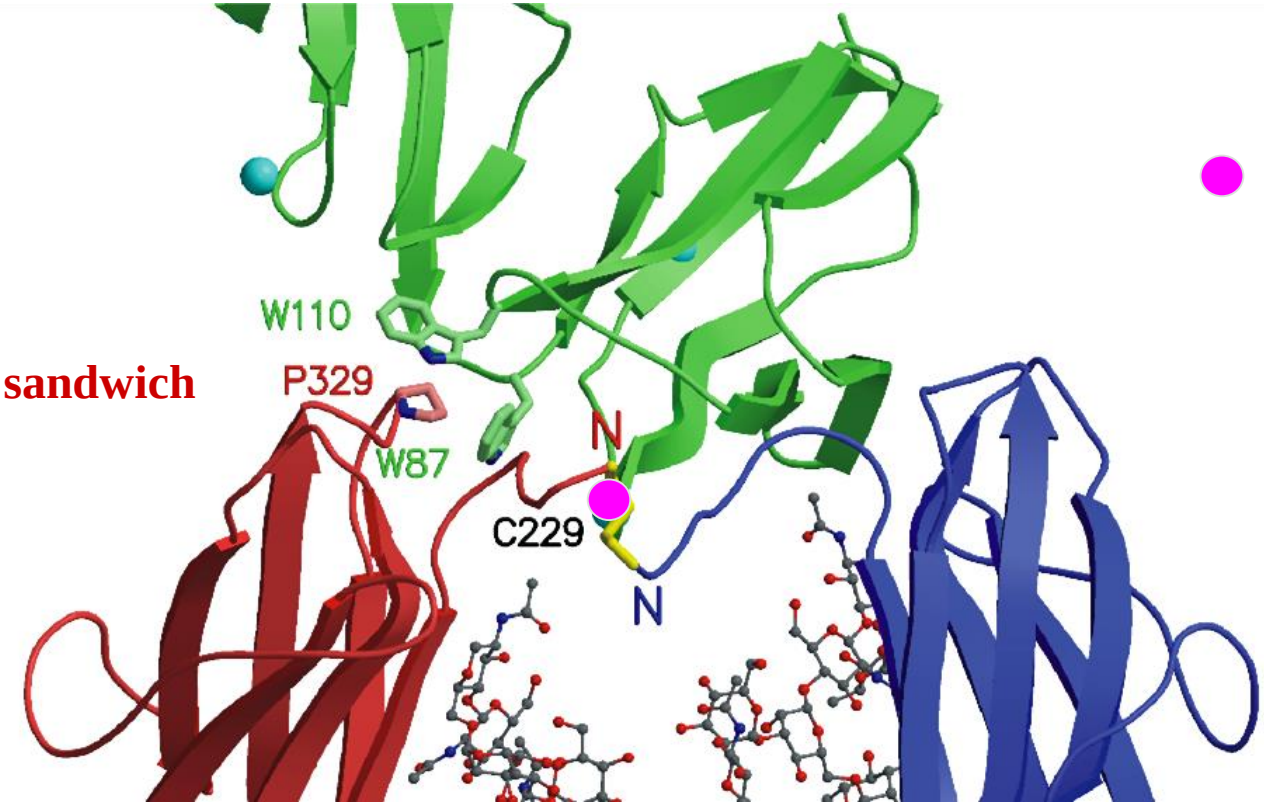
IgG-Fc/FcγRIIIa complex

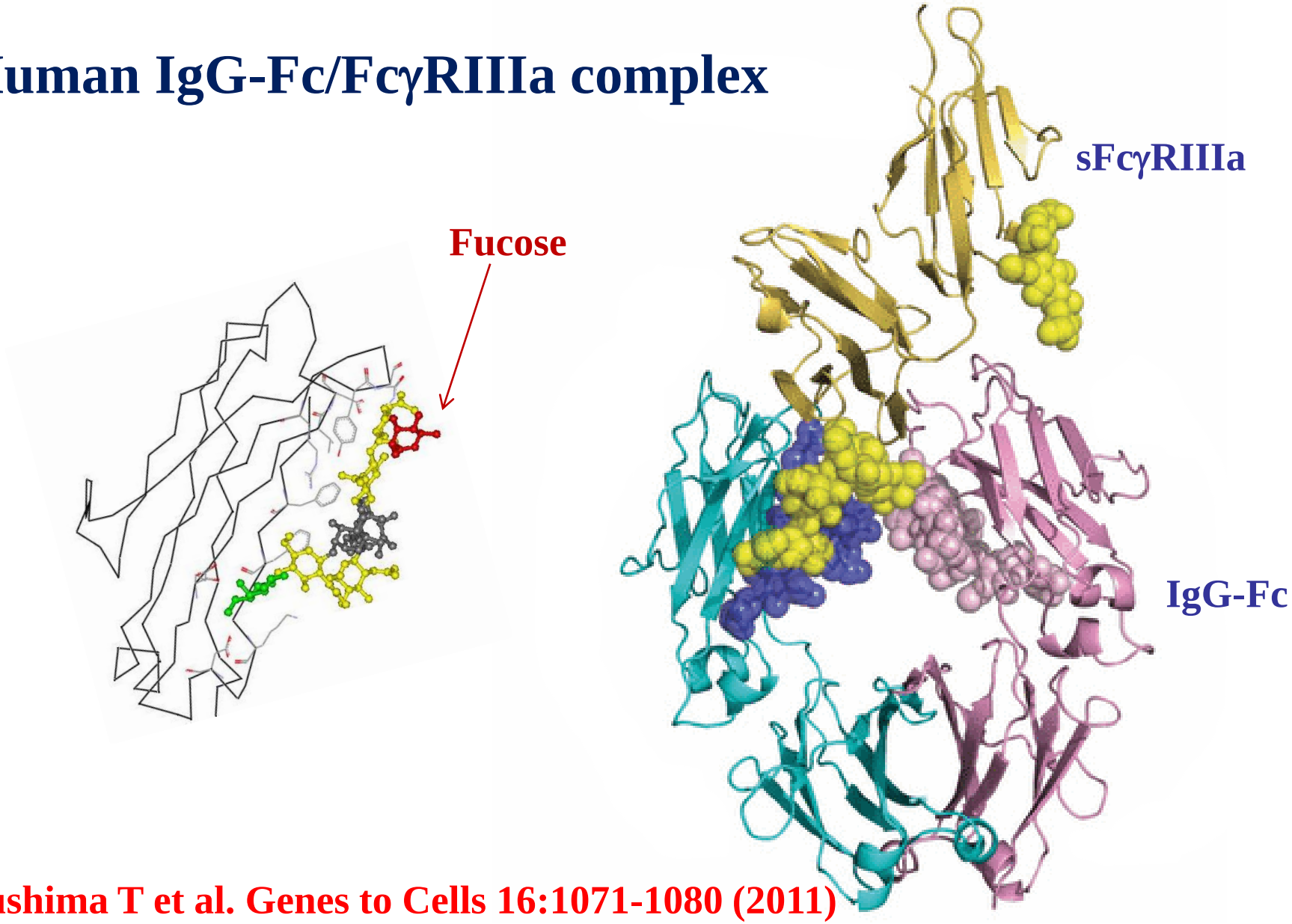


Drescher B. et al., Immunol 110: 335-40 (2003)

Sondermann P et al. Nature 406:267-273 (2000)

Radaev S et al. Mol Immunol. 38:1073-1083 (2002)

Human IgG-Fc/FcγRIIIa complex



Mizushima T et al. Genes to Cells 16:1071-1080 (2011)

Ferrara C. et al. Proc Natl Acad Sci U S A. 108:12669-12674 (2011)

Features of IgG-Fc/FcγRIIIa interactions:

Both heavy chains are involved in the formation of an asymmetric binding site

Affinity of binding is dependent on glycoform profile of the Fc

Affinity of binding is dependent on glycoform profile of the FcγRIII

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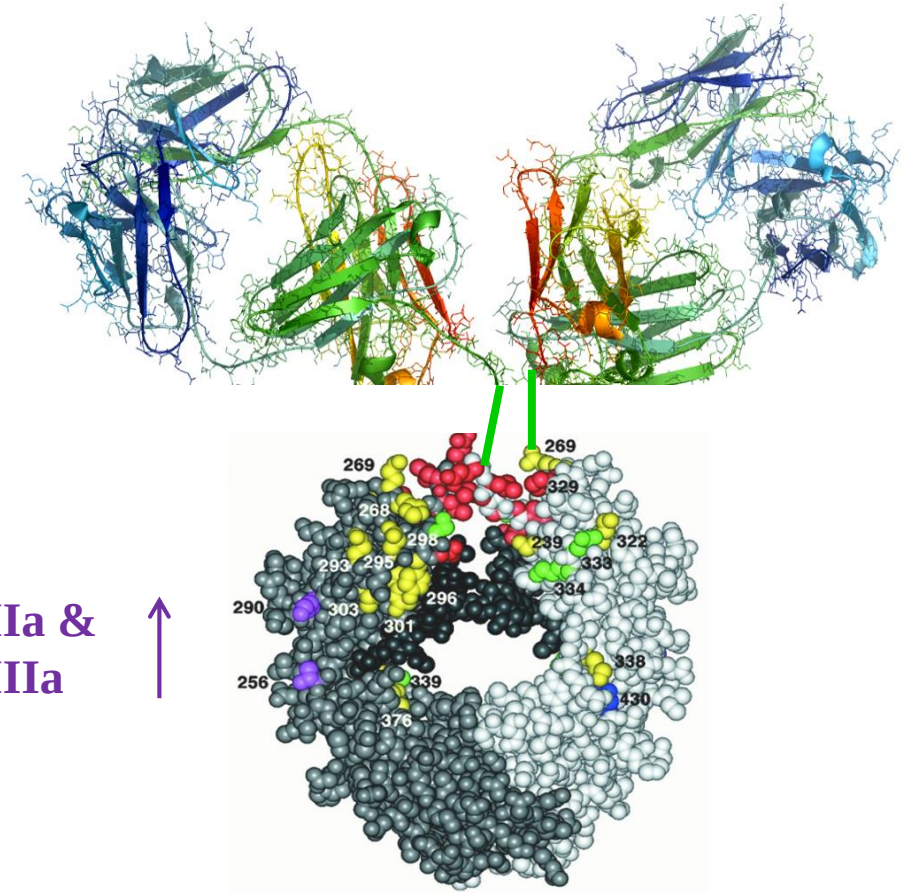
Alanine “scanning” identifies residues impacting FcγR & FcRn binding



FcγRIIa & FcγRIIIa ↑

Red:

FcγRI, FcγRII & FcγRIIIa ↓

Green:

FcγRIIIa ↑

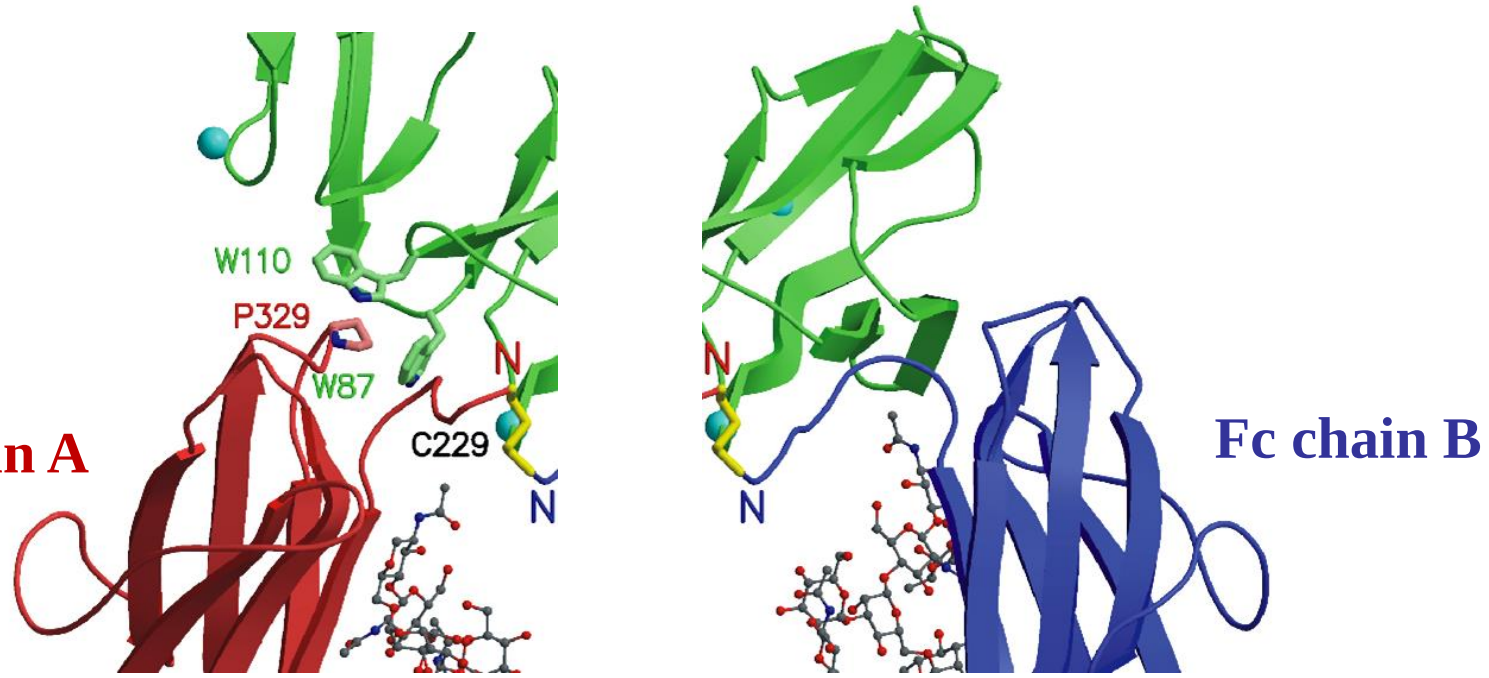
Yellow

FcγRIIIa ↓

Lund J. et al. (1991). J.Immunol. 147 2657 - 2662 (1991)

Shields RL. et al., JBC 276:6591-6604 (2001)

Generation of asymmetrically engineered IgG1-Fc



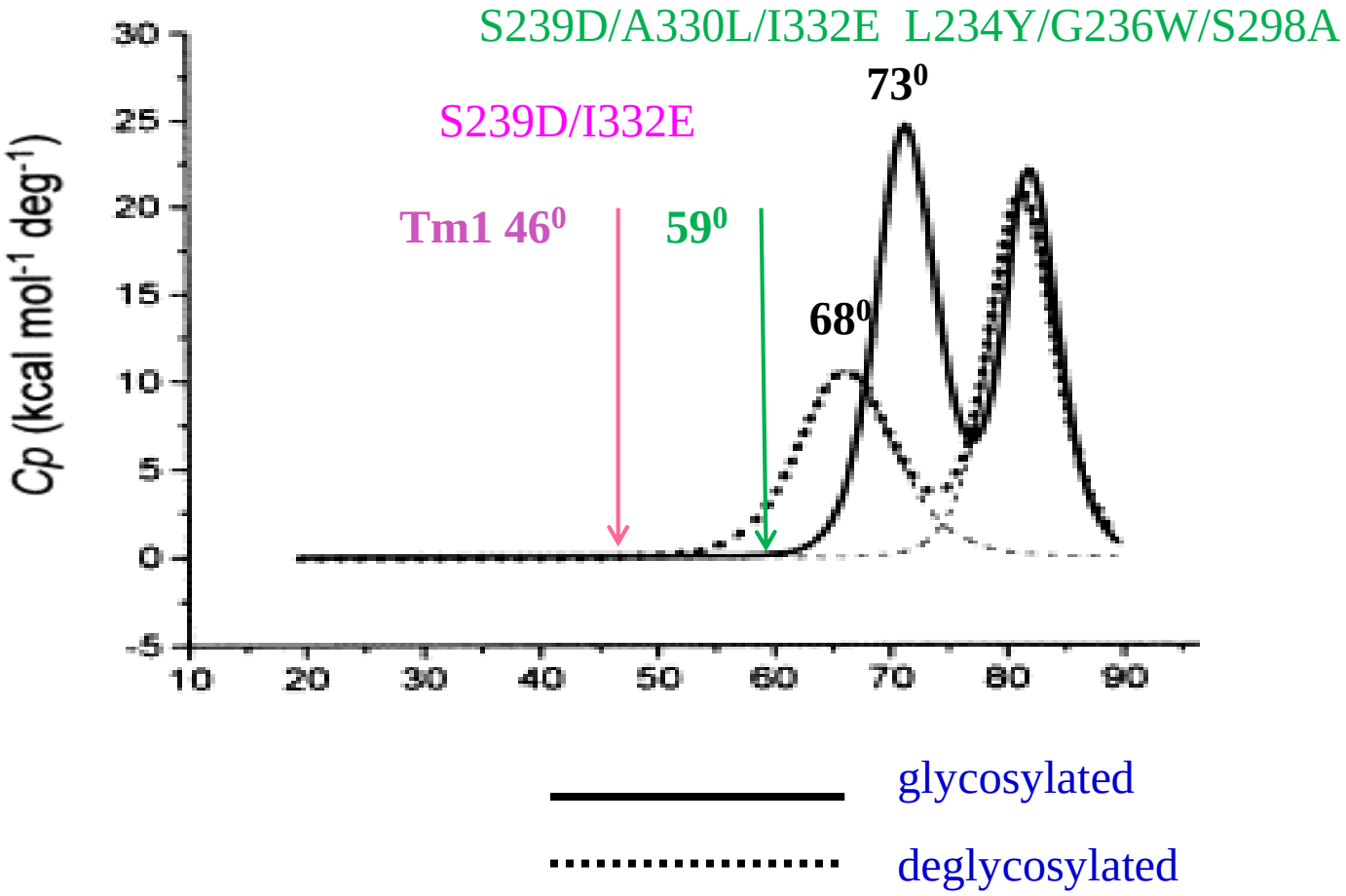
S239D/A330L/I332E

L234Y/G236W/S298A

Mimoto F. et al. MAbs 5:229–236 (2013)

Sondermann P et al. Nature 406:267-273 (2000)

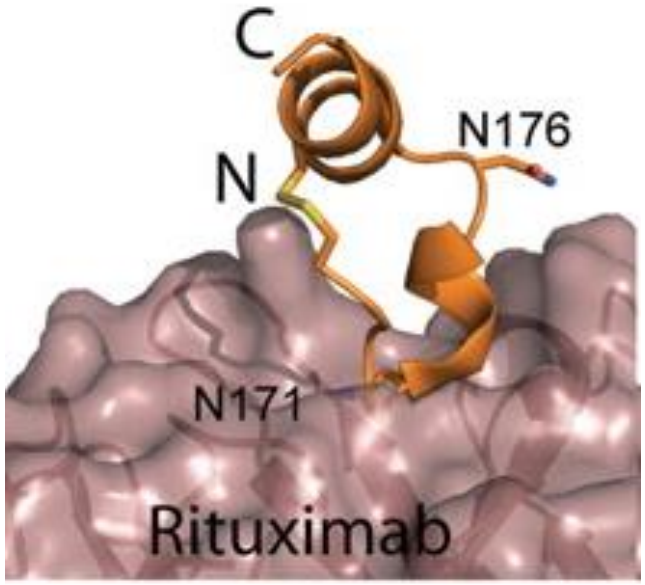
DSC of IgG1 Fc proteins



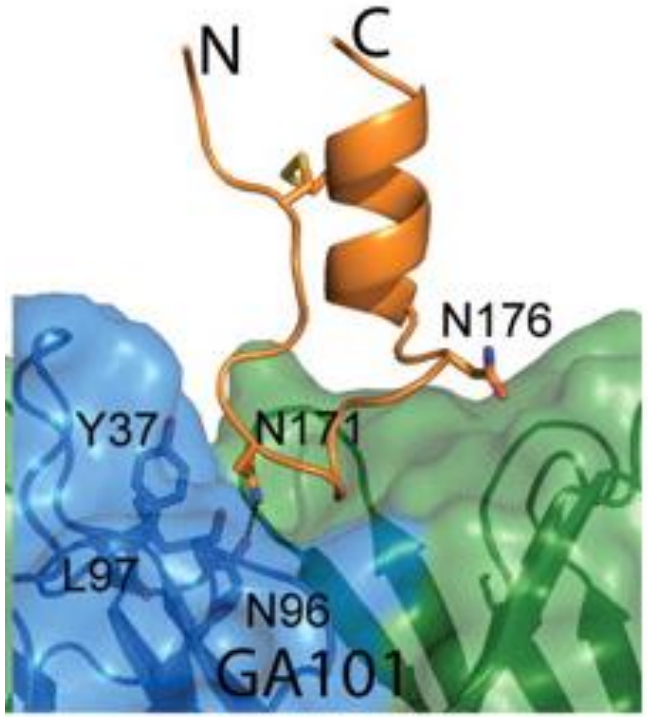
Mimoto F. et al. MAbs 5:229–236 (2013)

Mimura Y., et al., Molec Immunol 37:697 – 706 (2000)

Paratope/epitope orientations for rituximab (Type I) & obinutuzumab (Type II) anti-CD20 antibodies



Rituximab



Obinutuzumab (GA101)

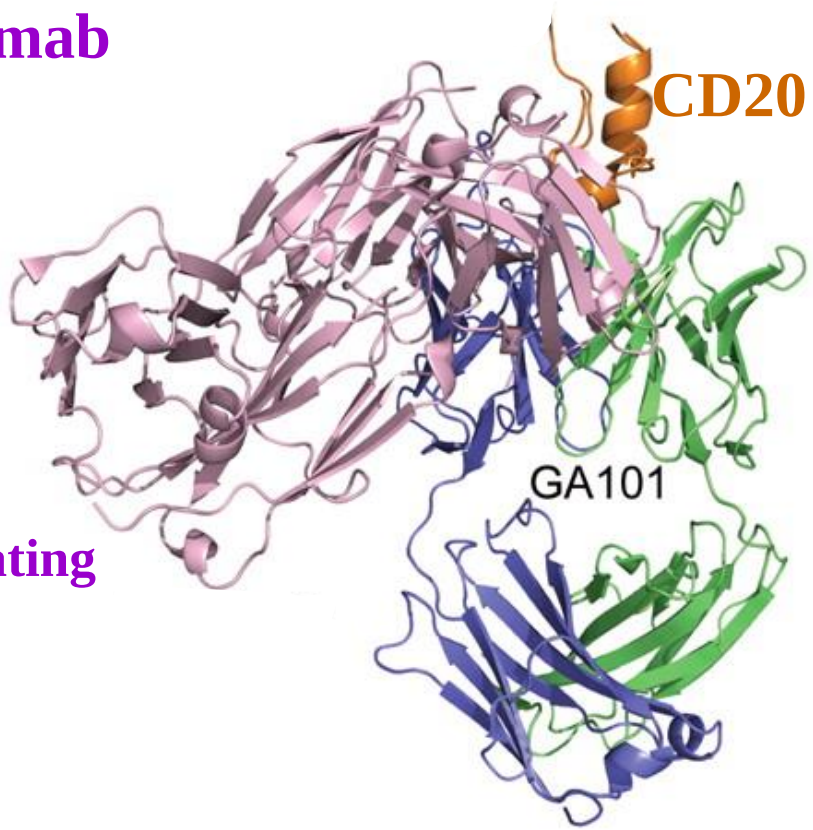
Genmab

Niederfellner G et al. Blood 118:358-367 (2011)

Anti-CD20 Type I (Rituximab) **& Type II** (GA101) **mAbs**

Rituximab

Obinutuzumab (GA101)



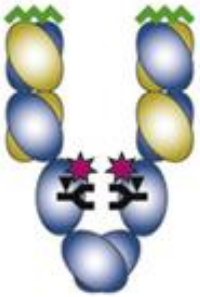
Complement activating

Direct cell killing

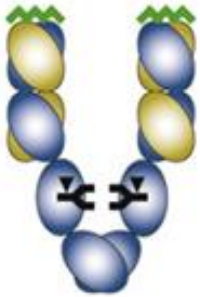
Genmab

Niederfellner G et al. Blood 118:358-367 (2011)

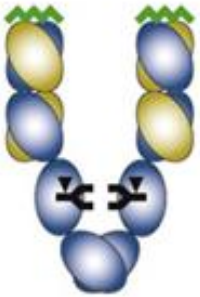
Next generation anti-CD20 antibodies



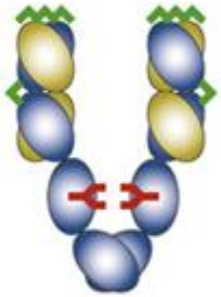
AME-133v
AME/Lilly
↑ADCC
NHL II



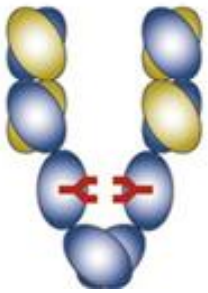
Ocrelizumab
Genentech/Roche
↑ADCC, ↓CDC
MS, RA II S *



Arzerra/Ofatumumab
Genmab/GSK
↑CDC
CLL A



GA101 **Obinutuzumab**
Glycart/Roche
↑ADCC, other
NHL III



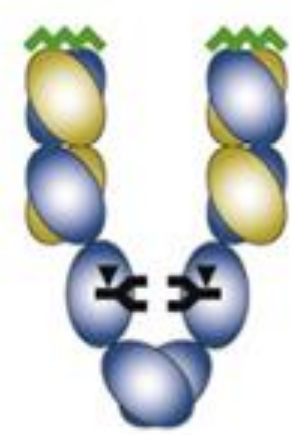
LFB-R603 Ublituximab
LFB
↑ADCC
CLL I

Xencor

Desjarlais JR. Lazar G. Exp Cell Res. 3171278-85. (2011)

Next generation anti-CD20 antibodies: Adverse effects

Risk/Benefit



Ocrelizumab
Genentech/Roche
↑ADCC, ↓CDC
MS, RA II S *

In March 2010, Roche announced the suspension of clinical trials in rheumatoid arthritis and lupus erythematosus. This step followed excess deaths due to opportunistic infection

Phase III development for multiple sclerosis continues

Xencor

Desjarlais JR. Lazar G. Exp Cell Res. 3171278-85. (2011)

Reconstitution of antibody therapeutics for administration

Instructions for Herceptin® states:

1) the stream of the diluent should be directed into the lyophilized cake

2) the vial should be swirled gently, without shaking

3) the vial should stand for five minutes undisturbed

4) drawing into a syringe should be performed slowly.

Peters BJM et al. mAbs 5:162–170 (2013)

Arvinte T. et al. MAbs. 5:491-500 (2013)

Davies AM, Jefferis, Sutton BJ. ***Structural determinants of unique properties of human IgG4-Fc***. *J Mol Biol. 426:630-644 (2014)*

Davies AM., Jefferis R., Sutton BJ. ***Crystal structure of deglycosylated IgG4-Fc*** *.Mol Immunol. 62:46-53 (2014)*

Jefferis R. ***Monoclonal Antibodies: Mechanisms of action***. *In: Current state of the art for the characterisation of mAbs. Eds: D. Davis, J. Schiel & Borisov O. ACS 2014*

Jefferis R. ***Isotype & glycoform selection for antibody therapeutics.*** *Arch Biochem Biophys 526:159-166 (2012)*