Glycoengineering For therapeutic Proteins

Technical Journal Club, 15th July 2014

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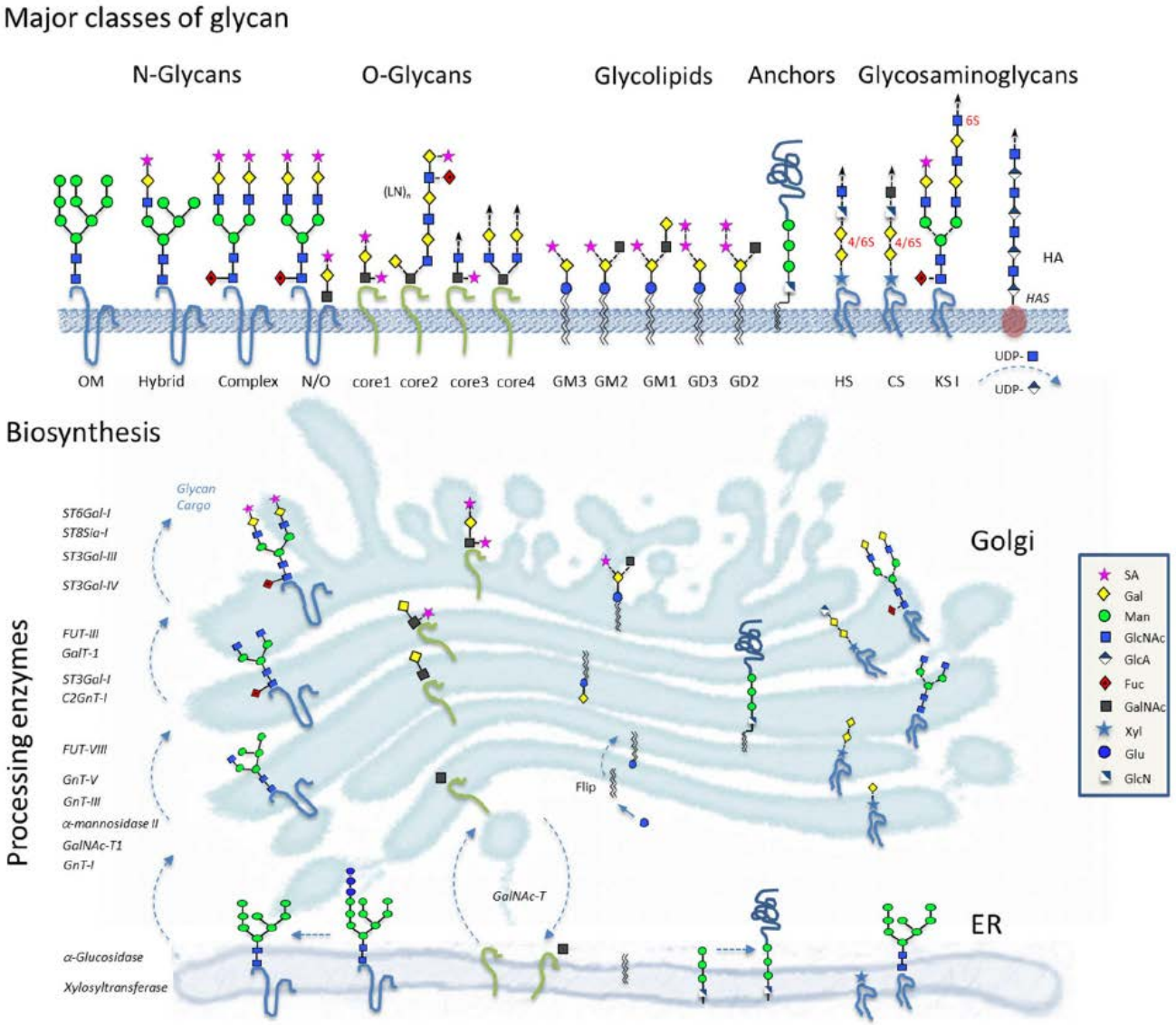
Mammalian gylcan biosynthetic pathway

Consensus sequence for N-Glycans:

N-X-S/T

No consensus sequence found yet for O-Glycans



Adapted from Dalziel et al. 2014; Science 343

Glycan have many biological propertis

1. Stabilization of portein folds

2. Targets of glycan-binding proteins (GBPs)
   1. Embryo development (e.g. Notch – Fringe)
   2. Immunology (e.g. siglecs and selectins)

3. Modulation of the properties of the proteins/lipids they are attached to
   1. E.g. serum glycoproteins like EPO → $T_{1/2}$
   2. Antibody interaction with immune receptors (FγRs)

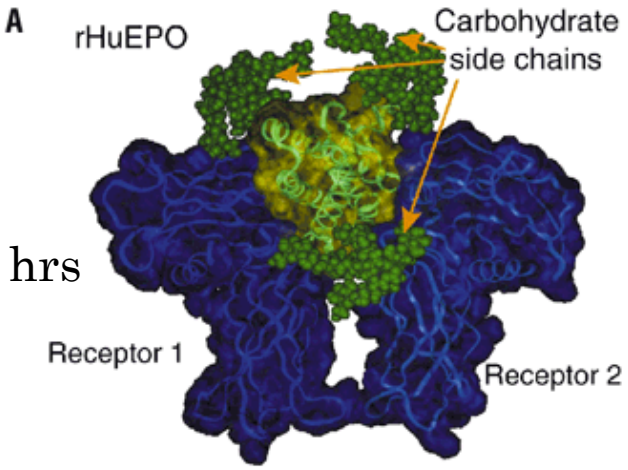
Glycosylation can affect the properties of therapeutic proteins

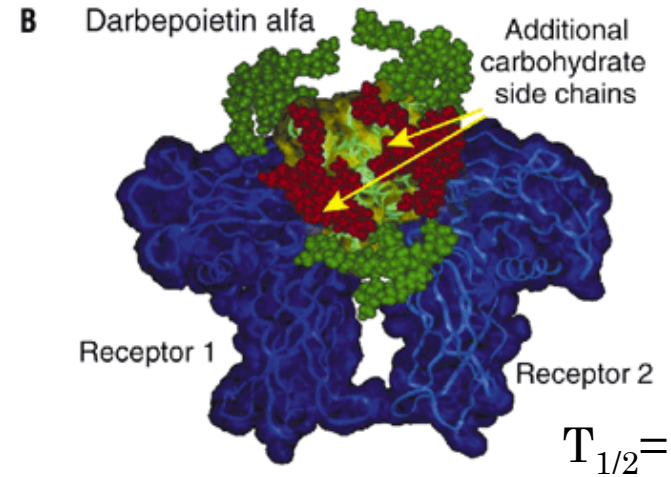
It affects:

- Molecular stability
- Solubility
- In vivo activity
- Serum half-life
- immunogenicity

Glycosylation patterns of Erythropoeitn(EPO) correlates with $T_{1/2}$

$T_{1/2}$= 19 hrs





$T_{1/2}$= 32 hrs

- Epoetins with different glycoforms are on the market, due to production in different cell lines
  - Sialic acid content is critical
- Darbepoeting alfa was the first Erythropoetin-Analog to be glycoengineered (addition of three N-glycosylations sites)

Adapted from Elliott et al. 2003; Nat Biotechnol. 2003 Apr;21(4):414-21

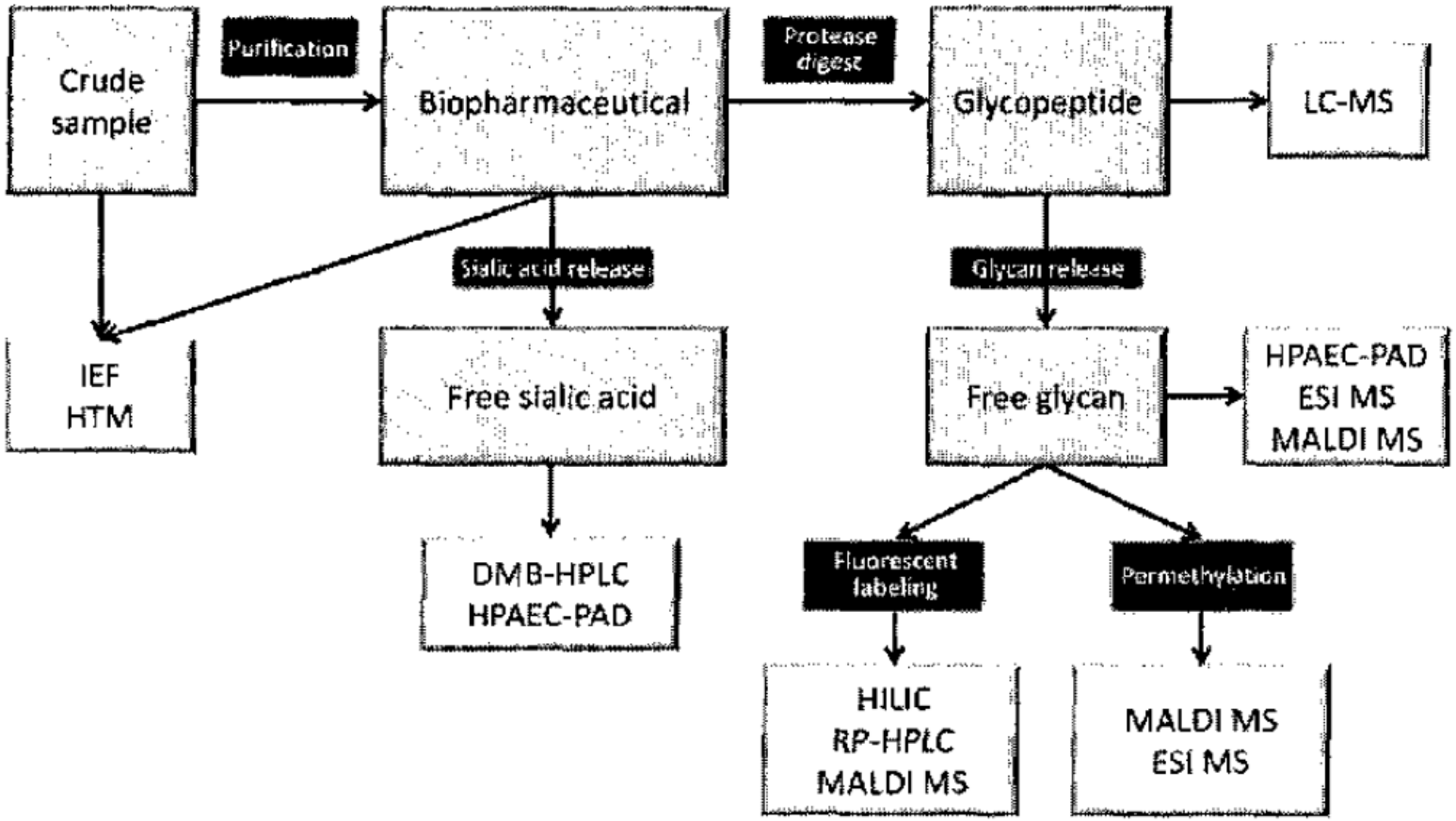
Different host organisms can be engineered to decorate Proteins with glycans

- Bacteria
  - No glycosylation possible
- Yeasts
  - Recombinant yeast available able to produce human glycoproteins (e.g. *Pichia pastoris*)
- Filamentous fungi
- Insect cells
- Mammalian cells
  - Chines hamster ovary cells (CHO), HEK293
- Transgenic plants
- Transgenic animals

How to detect and analyze glycosylation in recombinant proteins - 1

- Isoelectric focusing (IEF)
  - Separation based on isoelectric point
  - Represents differentially charged glycoforms
- Sialic assay
  - Involves chemical reduction of interfering molecules, then enzymatic release of sialic acid and derivatization of it with malononitrile for fluorescent detection
- HPLC profiling
  - Either by reversed phase (after tagging) or normal phase
  - Can be conneceted to MS
- Mass spectrometry
- LC-MS

How to detect and analyze glycosylation in recombinant proteins - 2



Adapted from Lingg et al. 2012; Biotechnol. J. 2012, 7, 1462-1472

nature biotechnology

GlycoDelete engineering of mammalian cells simplifies N-glycosylation of recombinant proteins

Leander Meuris[1,2,4], Francis Santens[1,2,4], Greg Elson[3,4], Nele Festjens[1,2], Morgane Boone[1,2], Anaëlle Dos Santos[3], Simon Devos[2], François Rousseau[3], Evelyn Plets[1,2], Erica Houthuys[1,2], Pauline Malinge[3], Giovanni Magistrelli[3], Laura Cons[3], Laurence Chatel[3], Bart Devreese[2] & Nico Callewaert[1,2]

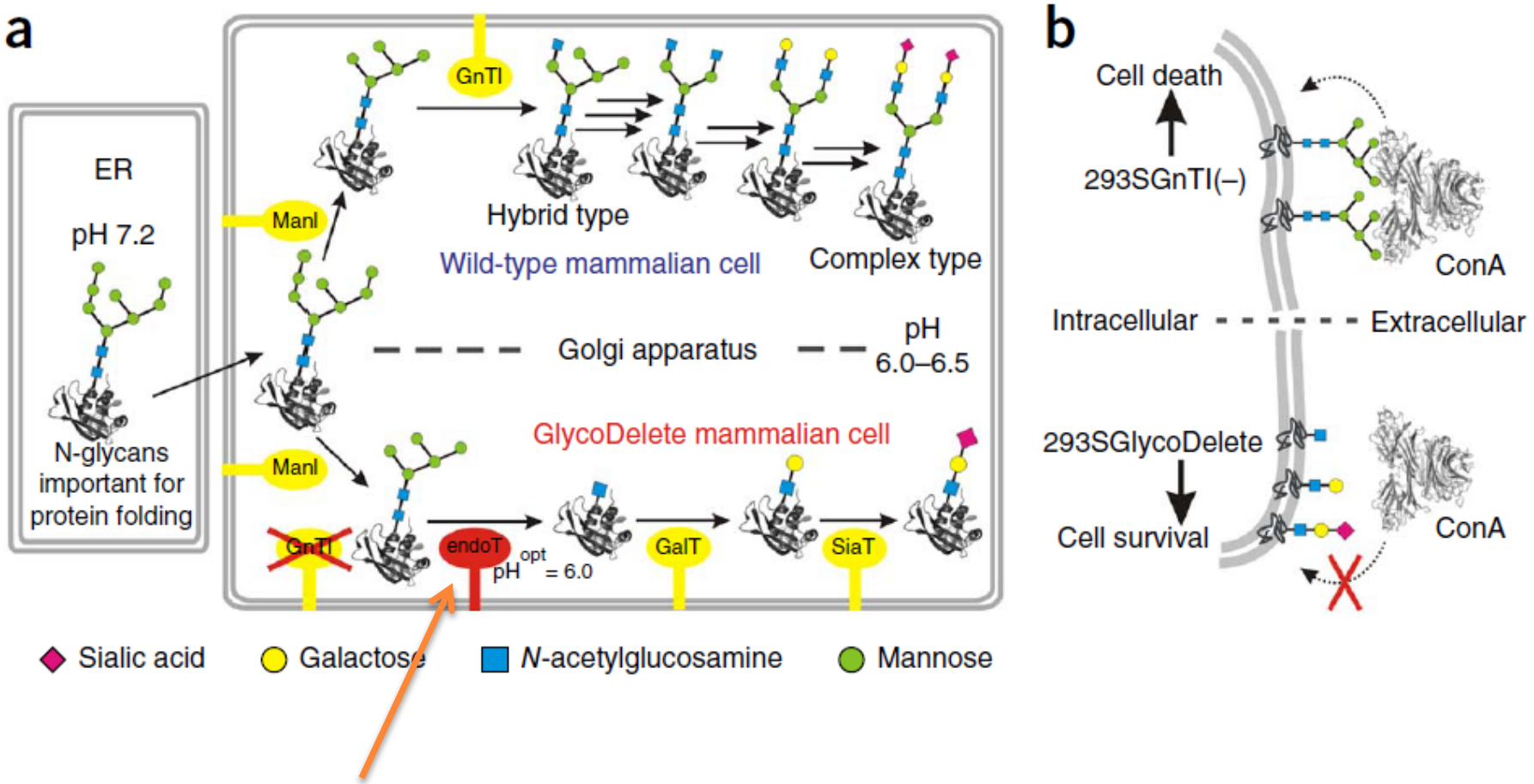
AIM

Hetereogenous glycosylation causes problems during manufacturing:

- e.g. different pharmakokintics and biologic activity
- Batch to batch variation

No glycoengineering technology is available in order to produce glycoproteins with reduced small structures to simplify manufacturing

Method



EndoT: deglycosylating enzyme from the fungus *Hypocrea jecorina*, works best at pH-optimum of 6.00 (present in the golgi)

Concanavalin A and HEK293T GnTi-

- Concanavalin A is a mannose/glucose-binding lectin firstly isolated from Jack Beans in 1916
- Used in chromatography for glycoprotein purification
- Used in preclinical trials as anti-neoplastic drug (tumours highly glycosylated)

HEK293S GnTI⁻ (ATCC® CRL-3022™)

Organism: ***Homo sapiens*, human** / Cell Type: **transformed with adenovirus 5 DNA** / Tissue

GENERAL INFORMATION | CHARACTERISTICS | CULTURE METHOD | HISTORY | DOCUMENTA

Genes Expressed	N-acetylglucosaminyltransferase I (GnTI), not expressed Ref
Comments	The HEK293S GnTI⁻ cell line was established by methanesulfonate mutagenesis followed by Ricin selection. HEK293S GnTI⁻ cells do not have N-acetyl-glucosaminyltransferase I (GnTI) activity and therefore lack complex N-glycans.Ref This cell line has been successfully used to overexpress a wide variety of mammalian membrane proteins. There were two versions of HEK293S GnTI⁻ cells in the original publication. One was HEK293S GnTI⁻ (ATCC CRL-3022), and the other version was HEK293S GnTI⁻ variant constructed with tetracycline-inducible system. CRL-3022 does not contain tetracycline-inducible system or pcDNA6-TR vector.

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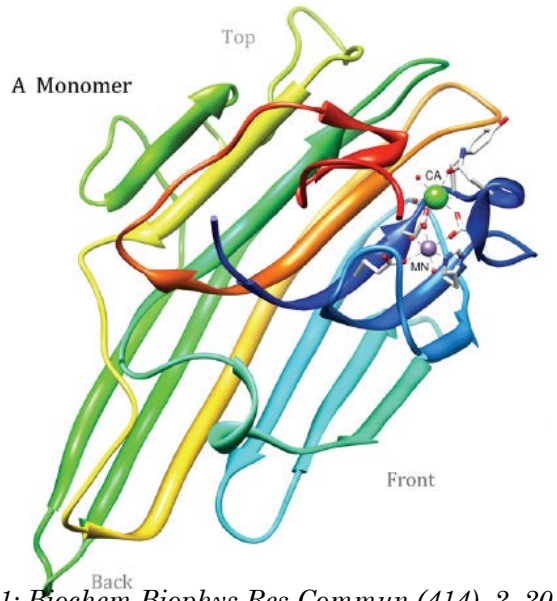
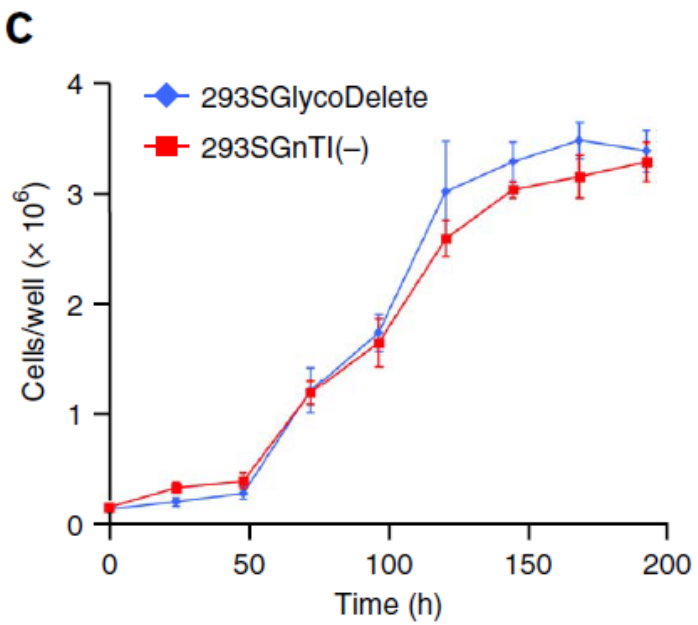
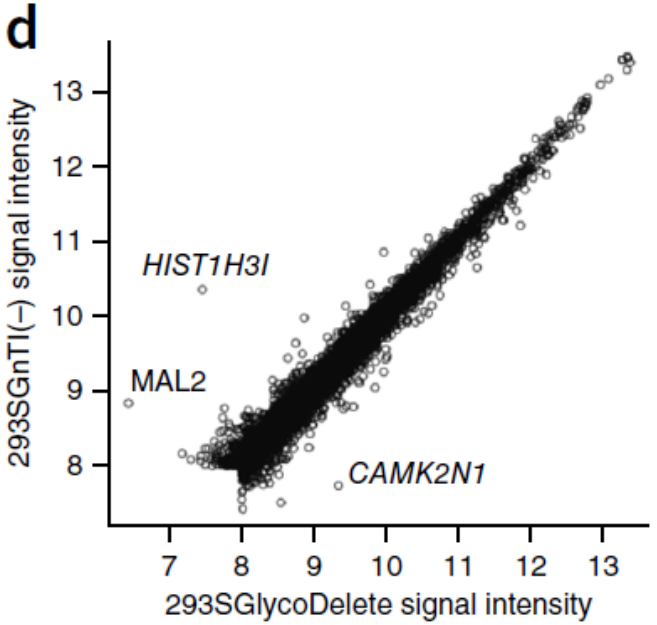


Adapted from Li et al. 2011: Biochem Biophys Res Commun.(414), 2, 2011, 282–286

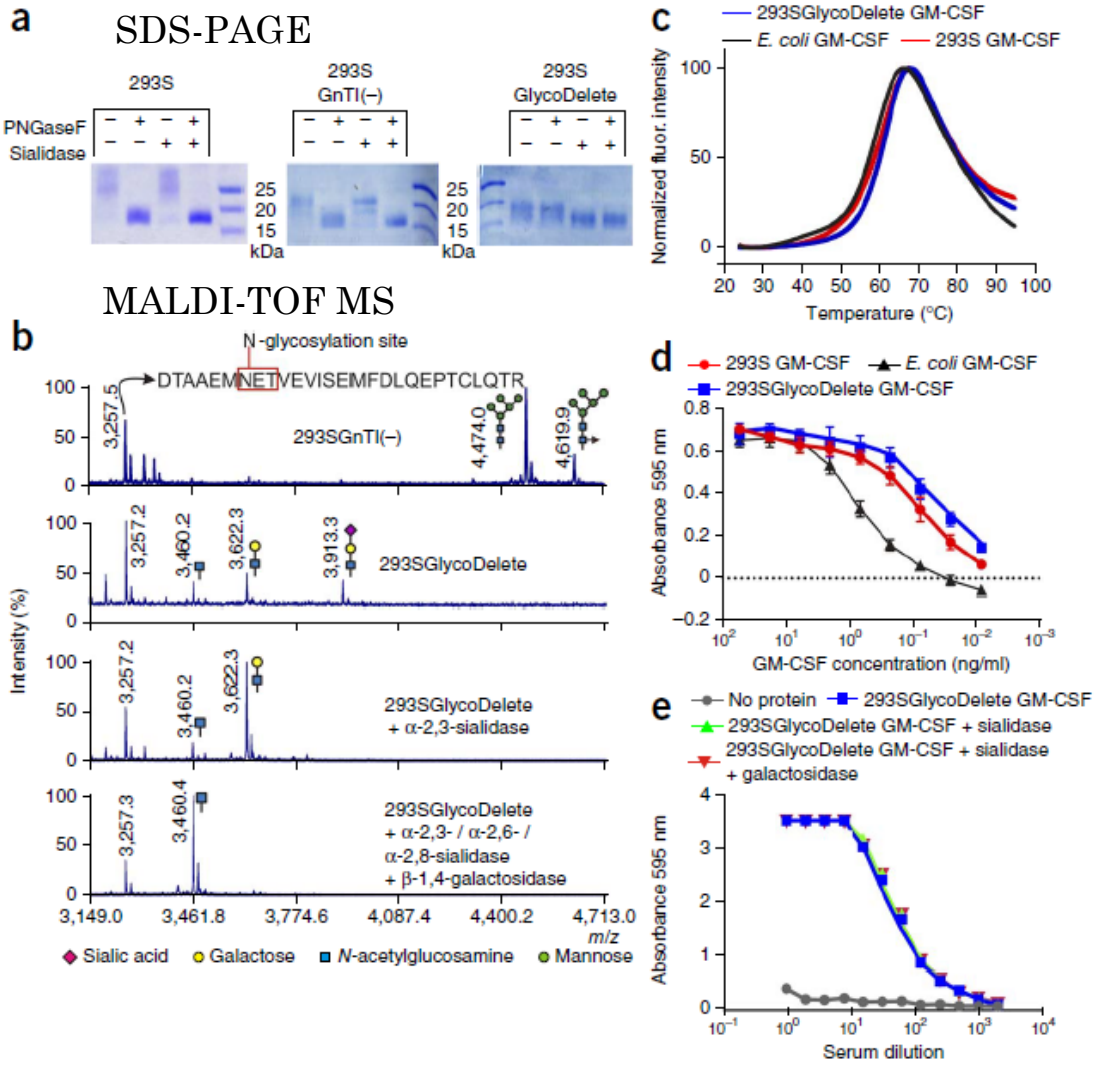
Validation of the new cell line

- 293SGlycoDelete cell line was compared to the parental one
  - Less adherent → suspension cultivation in biopharmaceutical industrie
- Profiling of the transcriptome was performed





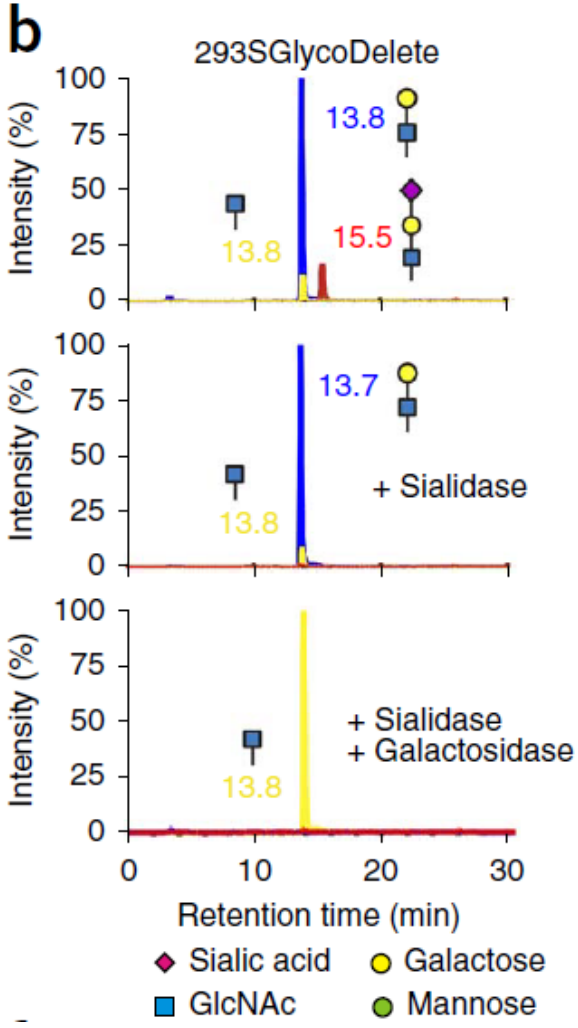
Transient Expression of GM-CSF

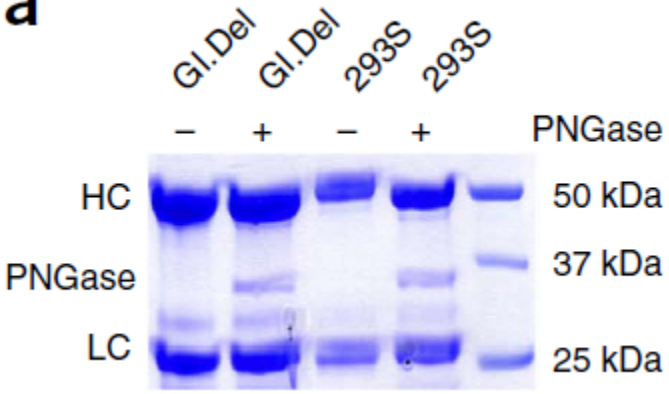


- T_m analysis (ThermoFluorAssay)
- Functional analysis (TF-1 proliferation assay)
- Testing of immunogenicity in rabbits

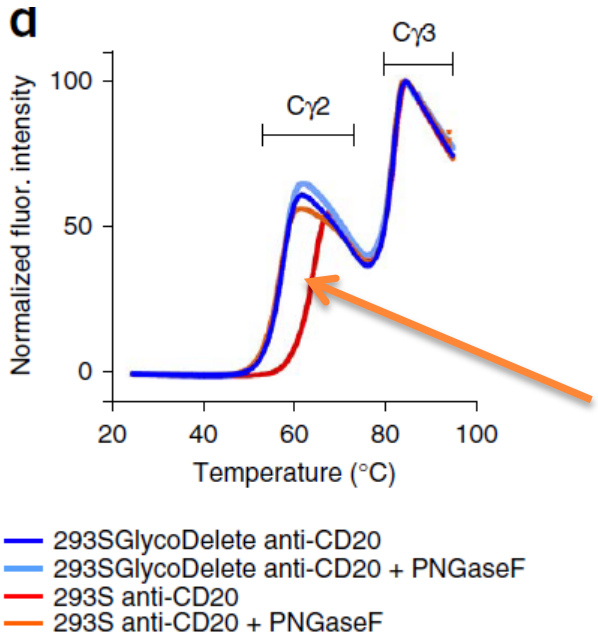
Transient Expression of anti-CD20 – structural analysis

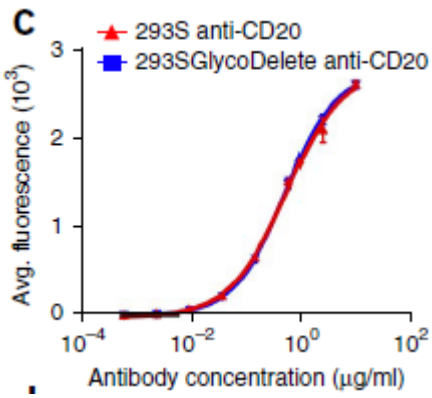
LC-MS/MS analysis





Average melting curves

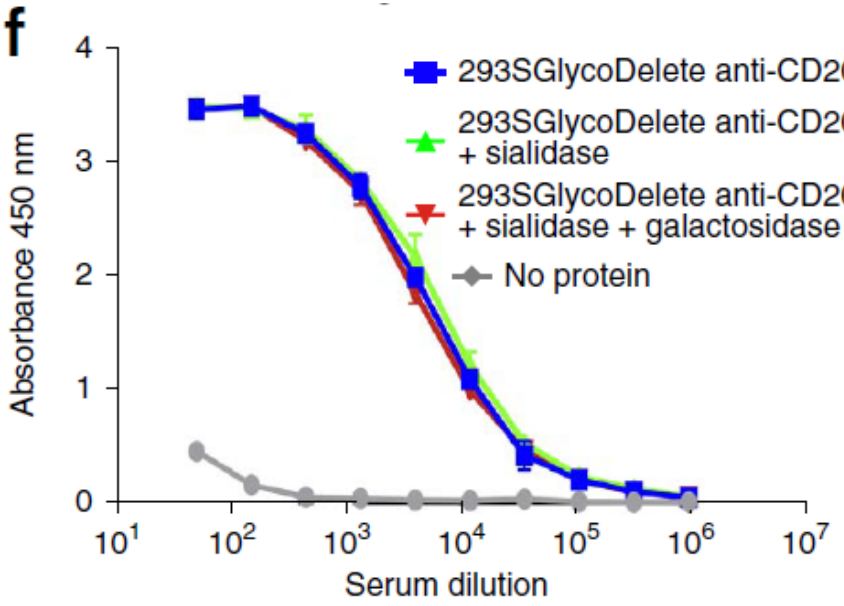




Expected drop

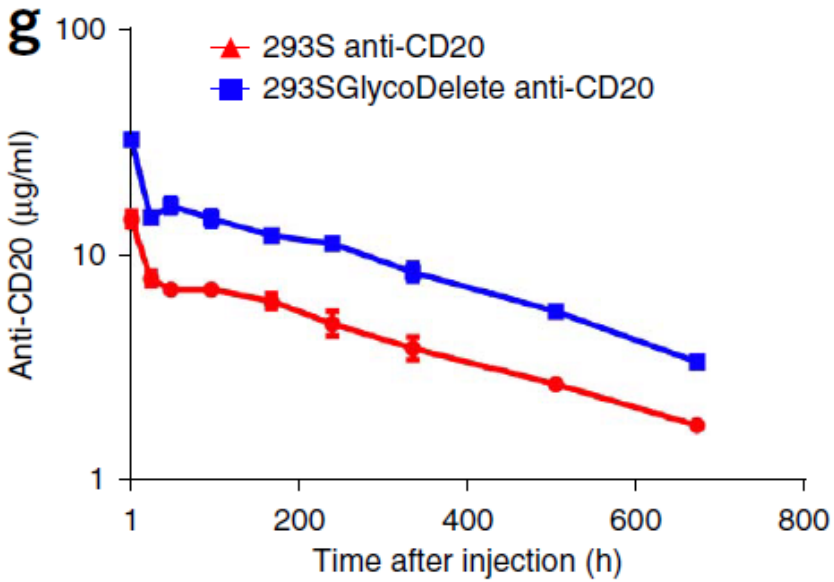
Transient Expression of anti-CD20 – Safety and Pharmakokinetics

Immunogenicity



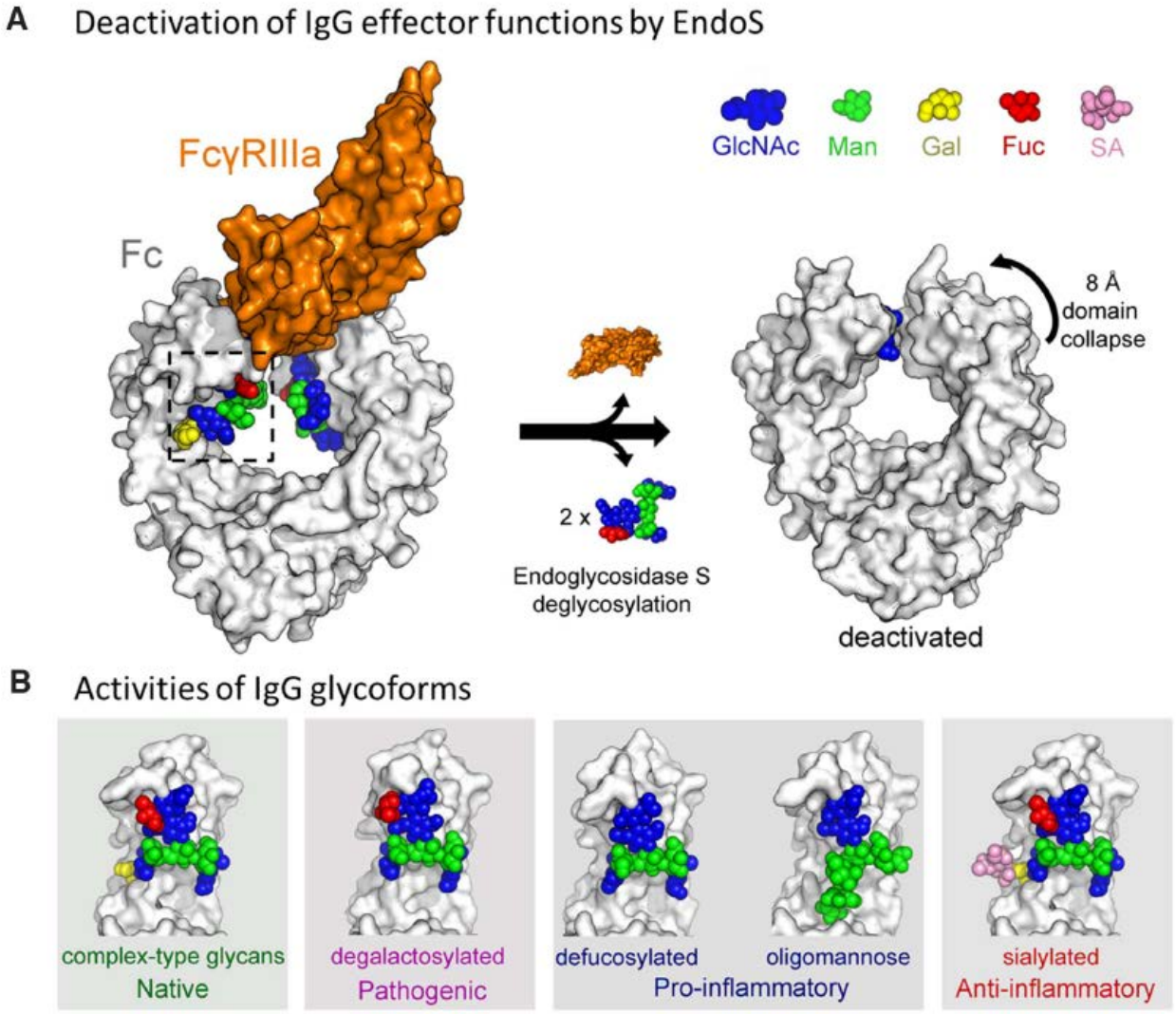
Immunization of rabbits

Pharmakokinetics



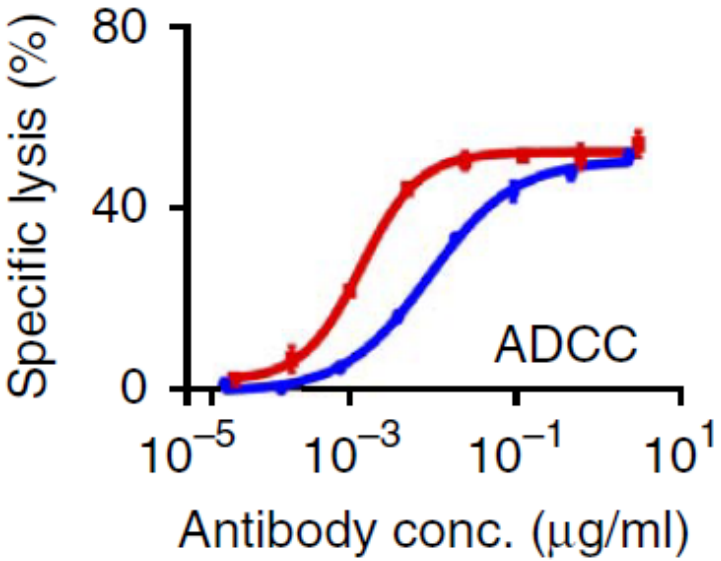
Injection of 1 mg/kg mAb to C57BL/6J followed by collection of blood at different time points

Glycosylation can affect effector function of antibodies

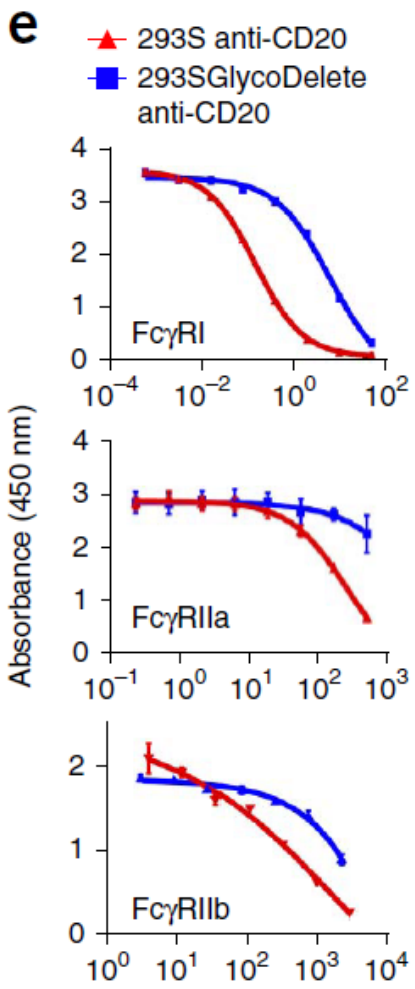


Adapted from Dalziel et al. 2014; Science 343

Transient Expression of anti-CD20 – Functionality



Incubation of Raji cells ($CD20^{+}$) with different antibody concentration followed by incubation with NK cells isolated from PBMCs
Read out: specific lysis by LDH measurement



Conclusion

- GlycoDelete glycoengineering strategy as an approach to solve the issue of N-glycosylation heterogeneity in mammalian cell–based glycoprotein production
- involves the inactivation of a single glycosyltransferase and overexpression of a deglycosylating enzyme, followed by lectin selection
- cells produce proteins with the Gal-GlcNAc disaccharide or its α-2,3-sialylated trisaccharide derivative and some of the monosaccharide intermediate
- retaining the folding-enhancing functions of N-glycans and avoiding the extensive heterogeneity introduced through mammalian Golgi N-glycan processing
- GlycoDelete engineering alters the characteristics of antibodies when the therapeutic goal is antigen neutralization with no need for additional effector function

THANK YOU!