INTERNATIONAL SCIENTIFIC CONFERENCE
"EARTH BIORESOURCES AND ENVIRONMENTAL BIOSAFETY:
CHALLENGES AND OPPORTUNITIES"
Kyiv, Ukraine, November 4-7, 2013

# Plants as Expression System for Recombinant Therapeutic Glycoproteins

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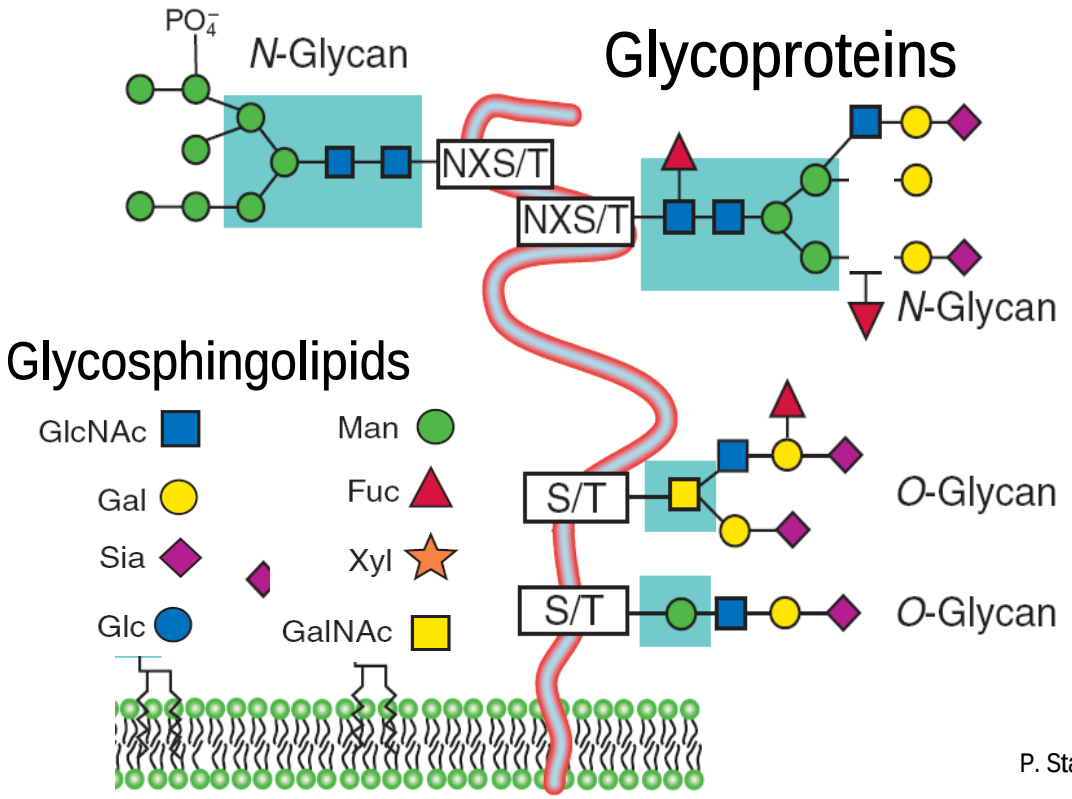
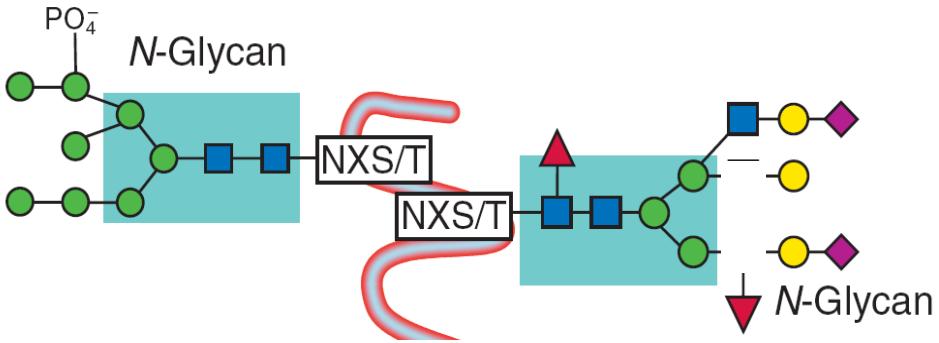
# Glycosylation of proteins

- N-Glycosylation: Asn-linked glycosylation (Asn-X-Ser/Thr) → N-glycans
- O-Glycosylation: Ser/Thr-linked glycosylation → O-glycans




P. Stanley, 2011 Cold Spring Harbor Laboratory Press

# N-glycosylation

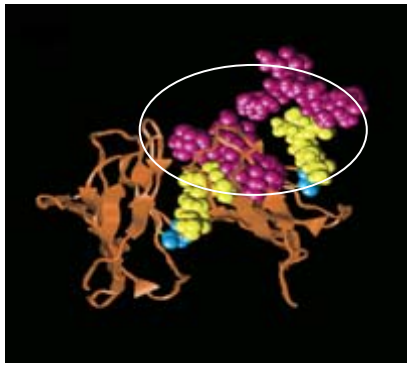



Function of N-glycans:

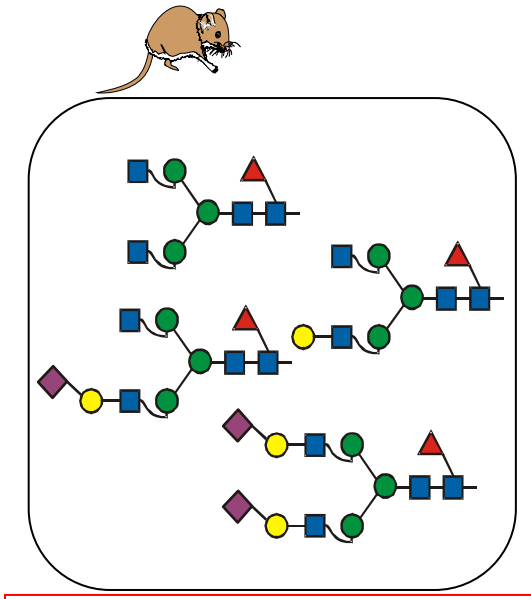
- Protein folding and stability
- Protein targeting (e.g. for lysosomal enzymes)
- Protein-protein or protein-carbohydrate interactions (lectins)
- Biological activity of proteins (e.g. effector functions of IgGs)
- Control of protein half-life (e.g. erythropoietin)

# Why glyco-engineering ?

- Changes in N-glycan structure may significantly affect protein confirmation and pharmacokinetic behaviour, thus influencing e.g. antigen binding and effector functions in case of monoclonal antibodies



- Current expression systems generate a mixture of glycoforms
- Substantial deficits exist in understanding the role of specific glycosylation patterns on therapeutic proteins like monoclonal antibodies
  - **Well defined glycoforms on recombinant proteins are urgently needed**
- **Expression systems are needed which produce "tailor-made" N-glycan structures**



**In CHO expression system:**
**A mixture of glycoforms**

# Why glyco-engineering ?

General goals:

- **Production of recombinant glycoproteins with a defined, homogeneous glycosylation profile in order to study**
  - **the impact of glycosylation**
  - **the therapeutic potency of various glycoforms**

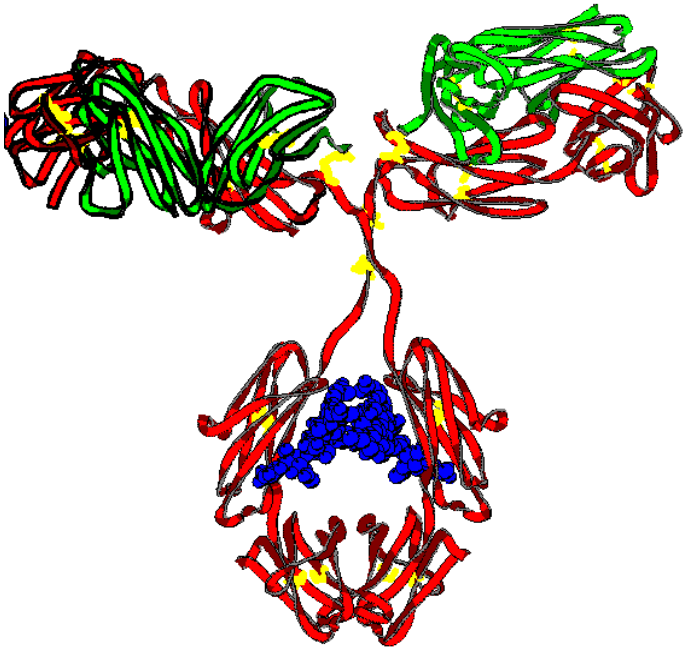
- **Further development of plant expression systems for therapeutically relevant glycoproteins**:

# Why glyco-engineering in plants?

- Plant expression systems are rapidly evolving as expression systems for therapeutically relevant proteins, since they are
  - convenient,
  - biologically safe
  - cost-effective

- However, since plants differ in certain aspects of their N-glycosylation pathway from that in human, **"humanization"** of the N-glycan biosynthetic pathway is needed in order to
  - **avoid immunological problems**
  - **get authentic N-glycan structures**

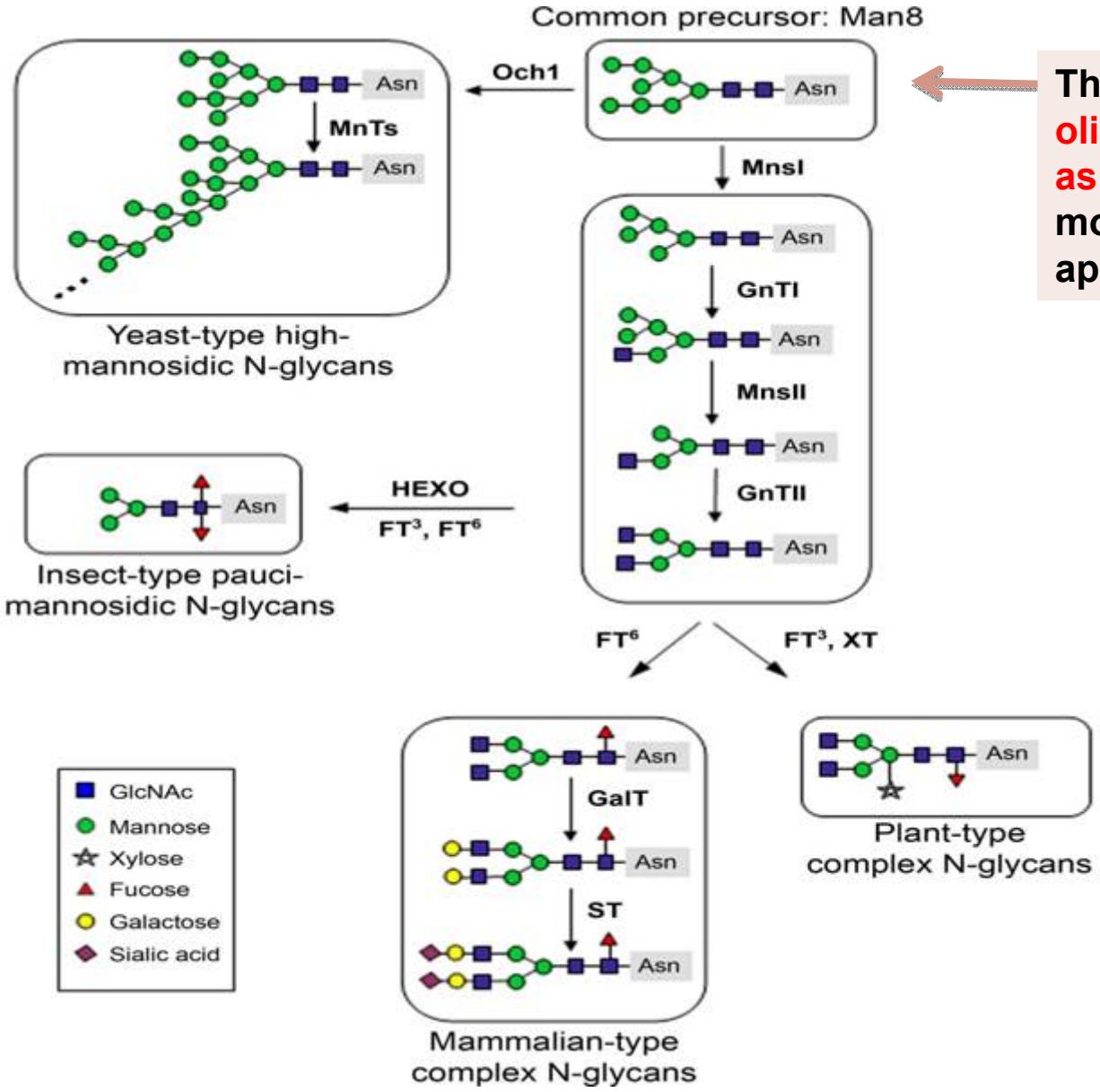
# N-glycosylation in mammalian cells



Prominent example:

- monoclonal antibodies (mAbs):
  - IgG1 heavy chain has conserved N-glycosylation site (Asn 297)
  - → N-glycan structure influences biological activity of antibodies

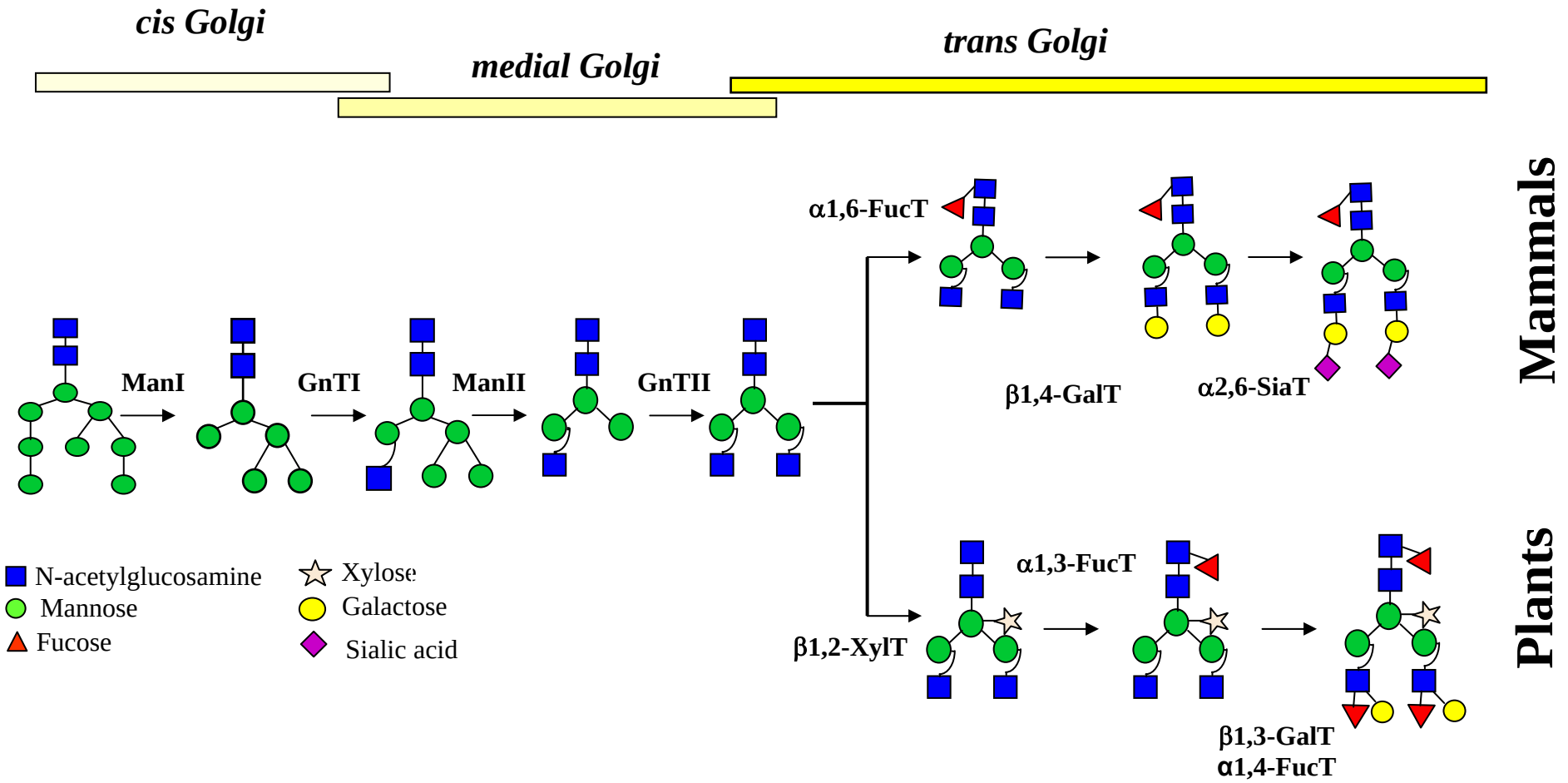
# Schematic presentation of the N-glycosylation pathways in humans, yeast, insect cells and plants




**The common ER-resident oligosaccharide precursor acts as starting point for further modifications along the Golgi apparatus**

Legend:

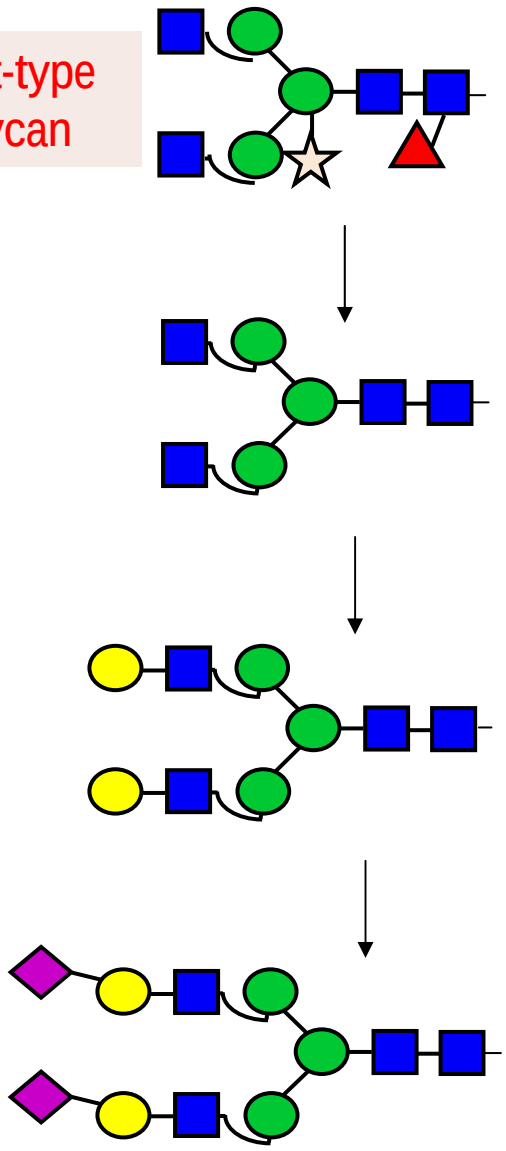
# N-Glycan Processing in Plants *vs.* Mammals




**Plant and mammalian N-glycan processing steps differ in the late Golgi steps**

# Engineering of the N-Glycan Processing Pathway in Plants

Plant-type N-glycan



„Humanized“ N-glycan

**Goal: „Humanised“ N-glycan structures**

**Strategy:**

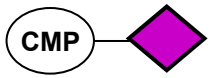
**Removal** of
- β1,2-xylosyltransferase
- core α1,3-fucosyltransferase

by
- **knock out** (*A. thaliana*)
- **RNAi** (*N. benthamiana*)

**Transformation with β1,4-galactosyltransferase**

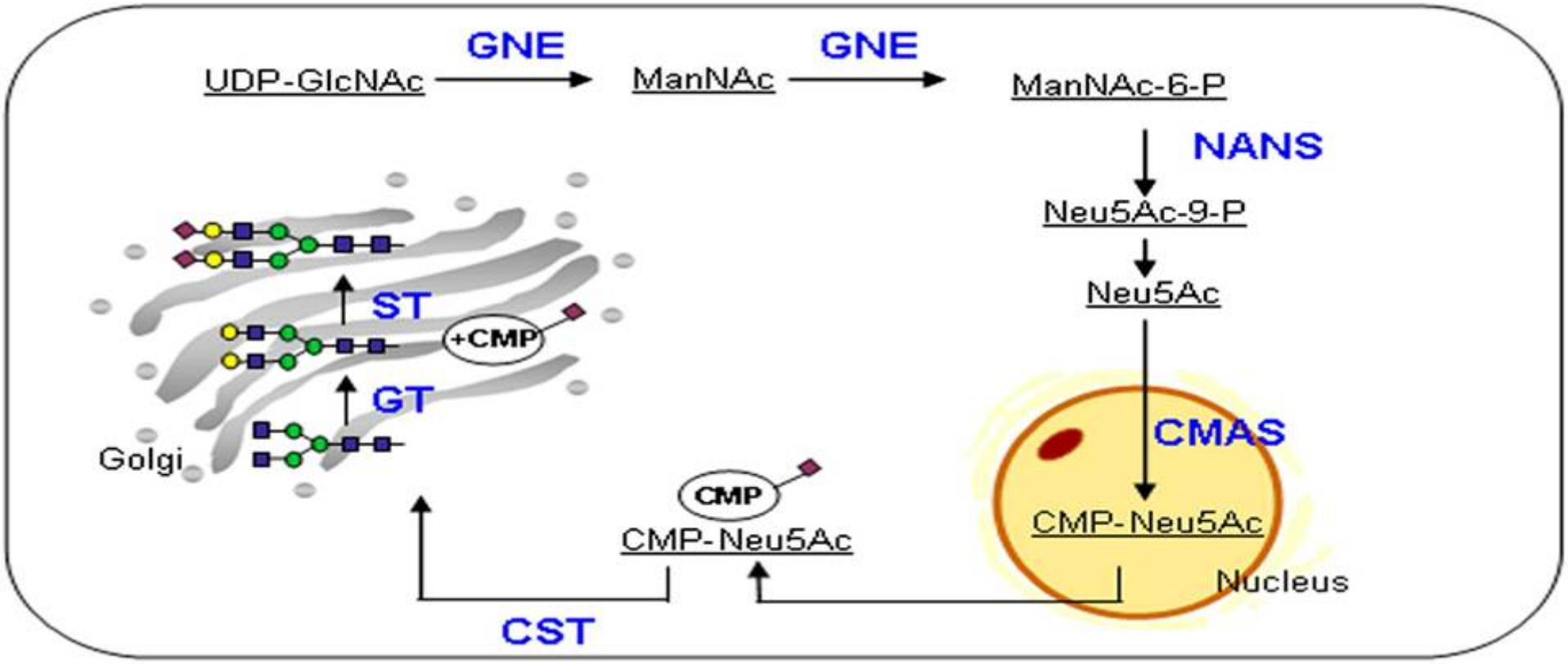
**Engineering of the sialic acid pathway** by:

- **Transformation of six genes** required for the biosynthesis of CMP-Neu5Ac and transport into the Golgi lumen




- **Transformation with α2,6-sialyltransferase**

# Reconstruction of the human sialylation pathway in plants




The genes for 6 proteins had to be introduced into plant cell, permitting the biosynthesis of sialic acid (Neu5Ac) , its activation, transport into the Golgi, and transfer onto terminal galactose

A. Castilho et al., J. Biol. Chem.285, 15923, 2010

# Different functional activities of monoclonal antibody (mAb) N-glycoforms

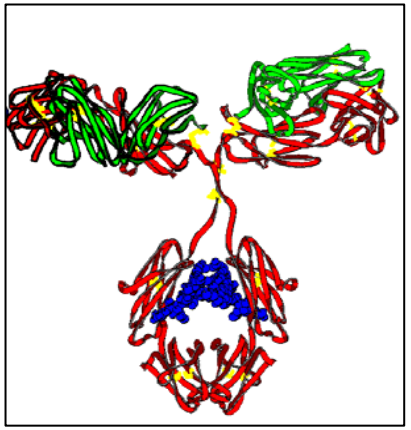
2 Examples of mAbs against viruses:

- anti HIV antibdy 2G12:

  (Polymun, Dept. Biotechnology, BOKU, Vienna)

- anti EBOLA virus antibody 13F6

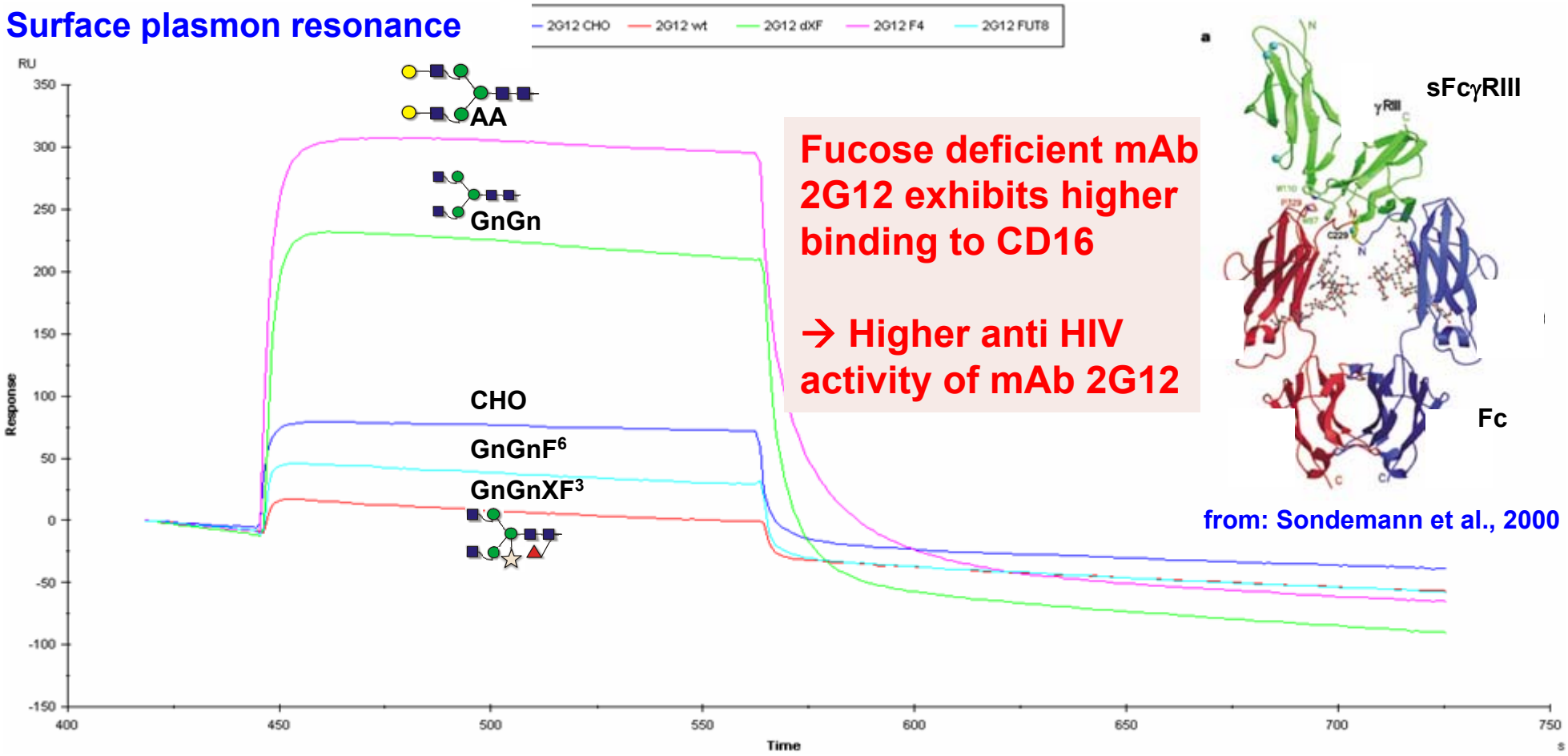
  (Mapp Biopharmaceutical, CA, USA)



# Functional activities of mAbs glycoforms

## mAb 2G12 binding to Fc γ receptor IIIa (CD16a)

**Surface plasmon resonance**

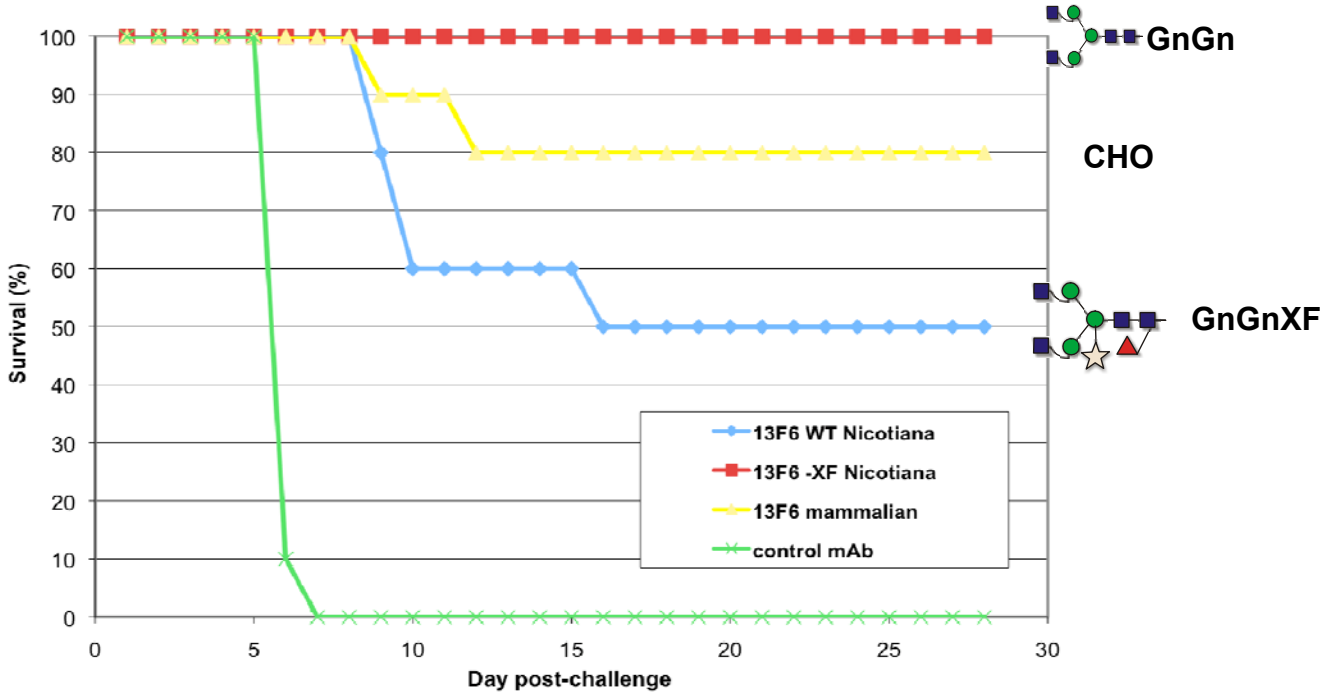



D.N. Forthal et al., J. Immunol. 185, 6876, 2010

# Functional activities of mAbs glycoforms

mAb 13F6 (anti Ebola virus)

## Virus protection assay in mice




Kevin Whaley

Mapp Biopharmaceutical

# Summary and Outlook

- It is now possible to produce complex human proteins for therapeutic purposes, largely correctly folded and *N*-glycosylated, in plants
- Plants have demonstrated a high degree of tolerance to changes in the *N*-glycosylation pathway, allowing recombinant proteins to be modified into human-like structures
- Glyco-engineering has paved the way to fully humanize the plant N-glycosylation pathway
- Frequently the results are a largely homogeneously glycosylated protein, enabling to study
  - the impact of glycosylation
  - the therapeutic potency of various glycoforms
- There is increasing evidence for the significance of proper *N*-glycosylation for the efficacy of biopharmaceuticals
- Glyco-engineering has become an important issue not only for academia but also for the biopharmaceutical industry.

# Acknowledgements

**BOKU - University of Natural Resources and Life Sciences, Vienna**

Department of Applied Genetics and Cell Biology
Herta Steinkellner
Richard Strasser
Alexandra Castilho
Pia Gattinger
Jakub Jez

Department of Chemistry
(N-glcosylation analyses)
Friedrich Altmann
Johannes Stadlmann

Department of Biotechnology
(HIV testing)
Renate Kunert
Heribert Quendler

**Supported by:**
Austrian Science Fund
European Commission

# University of Natural Resources and Life Sciences, Vienna (BOKU)

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## congratulates to the

## 115th Anniversary of NULES of Ukraine and the 15th Anniversary of GCHERA


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