Mammalian and Insect Cell Metabolic Engineering for Biotechnology Applications

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NSF Metabolic Engineering Working Group
February 6 , 2004

Biotechnology & Pharmaceuticals

REMICADE®
INFLIXIMAB
recombinant

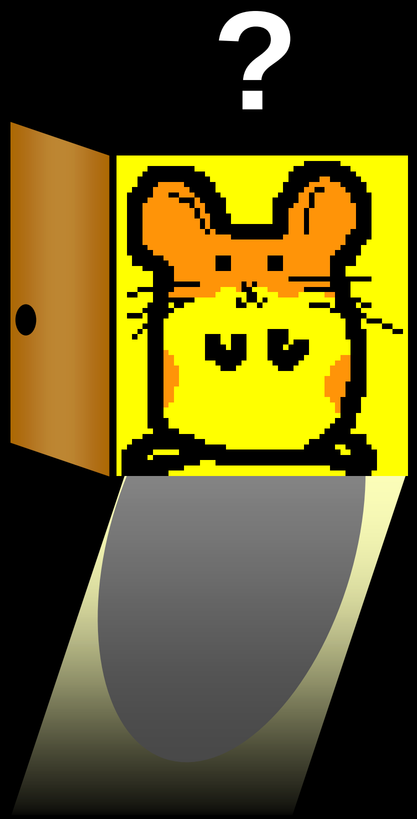
ACTIVASE®
ALTEPLASE
A RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR

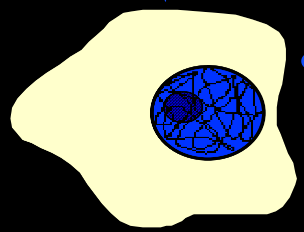
Genentech, Inc.

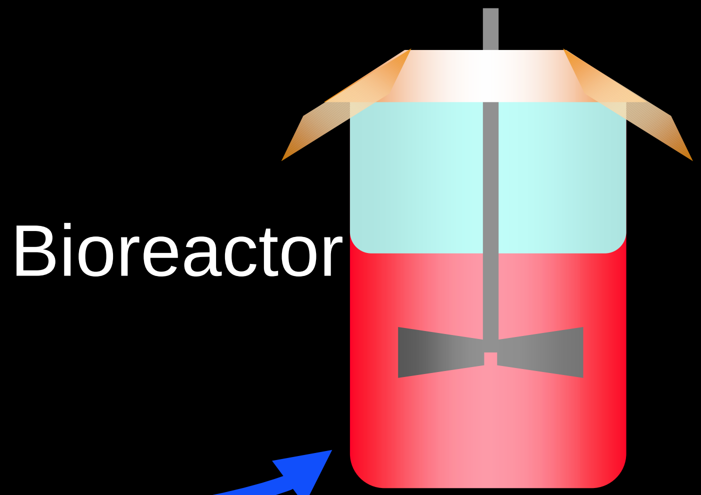
Humulin®
human insulin
(rDNA origin)

Lilly

Mammalian cell culture





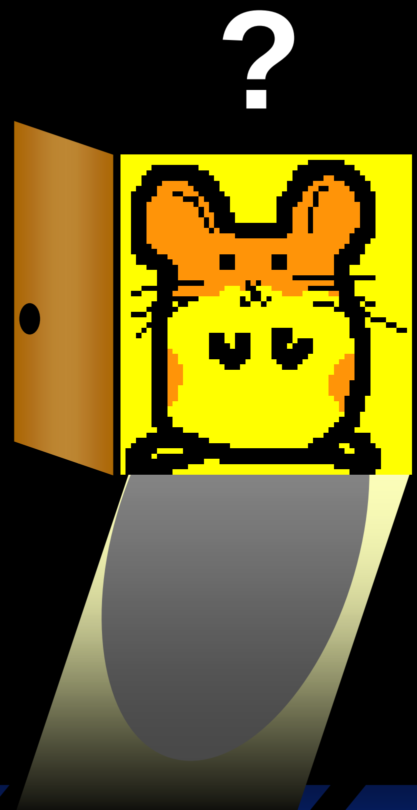


Immortal Cell Lines:
Chinese Hamster Ovary (CHO)
NS0 Mouse Myeloma
Human Embryonic Kidney: HEK 293
Baby Hamster Kidney (BHK)
SP2/0 Mouse Myeloma
Hybridomas

Therapeutic Proteins from Mammalian Cell Biotechnology

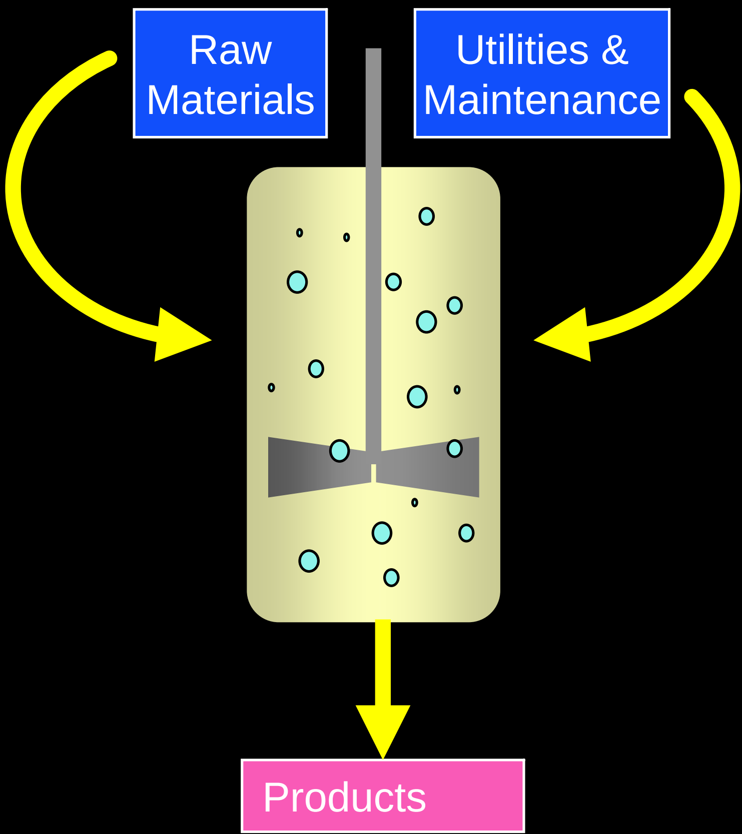
- 38 Commercial Proteins
  - Cancers
  - Arthritis
  - Anemia
  - Stroke and Heart Attack
  - Genetic Disorders
  - Infertility
- Sales in excess of 20 billion dollars (2001)
- Projected annual growth: 15%
- Nearly 50% of FDA Pipeline
  - With Vaccines and Gene Therapy Products

Why are mammalian cells the biotherapeutic champions?

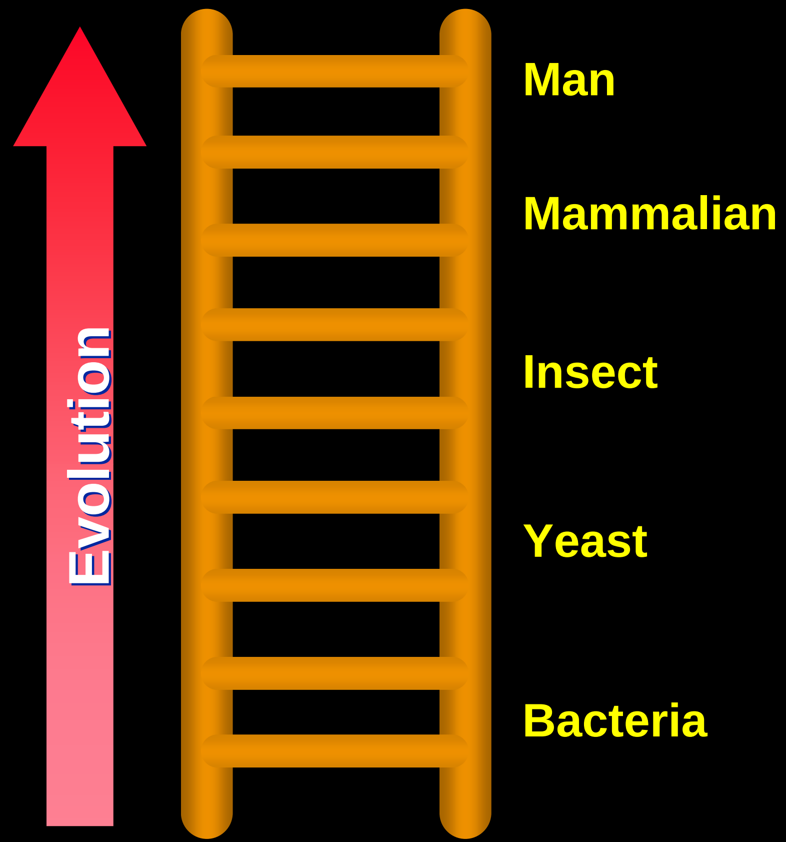


Factors in Choosing a Production System

Yield

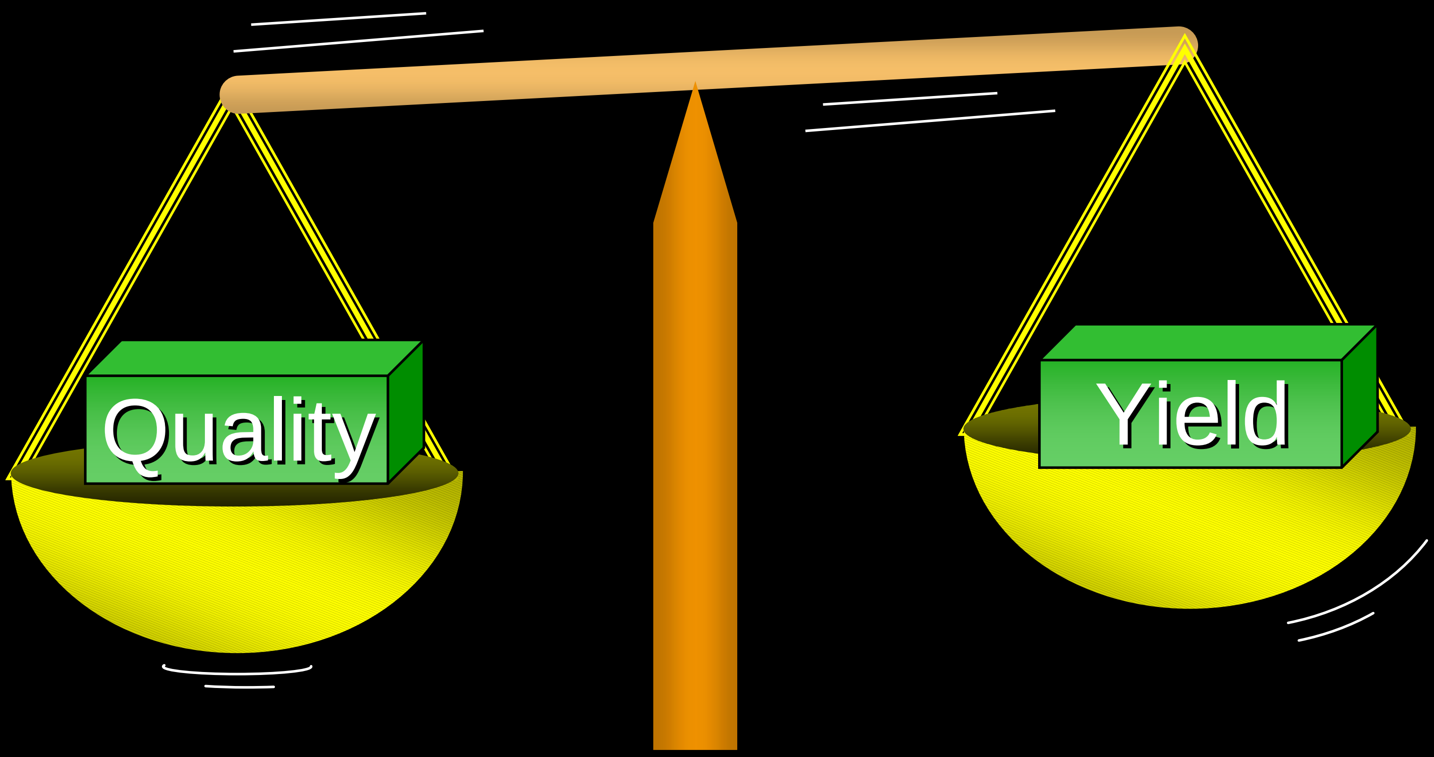


Quality



▯▯▯▯▯ **Bacteria (*E. coli*)**
▯▯▯▯ **Yeast**
▯▯ **Insect Cells**
▯ **Mammalian**

The Engineering Problem: Quality versus Yield

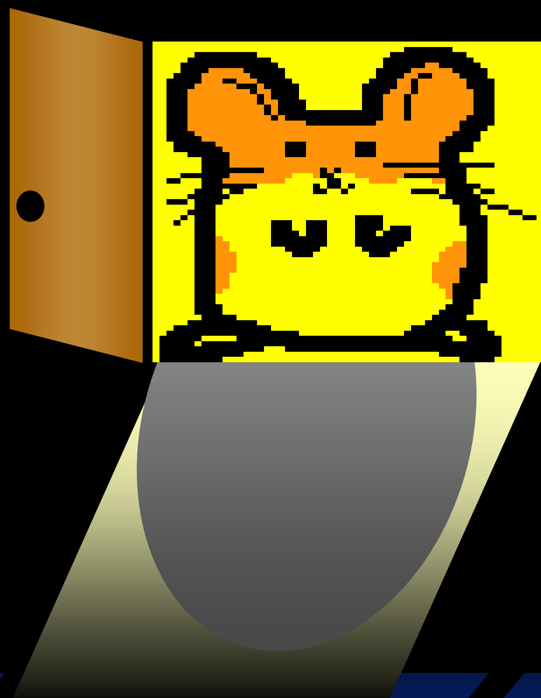


Essential pharmacological properties

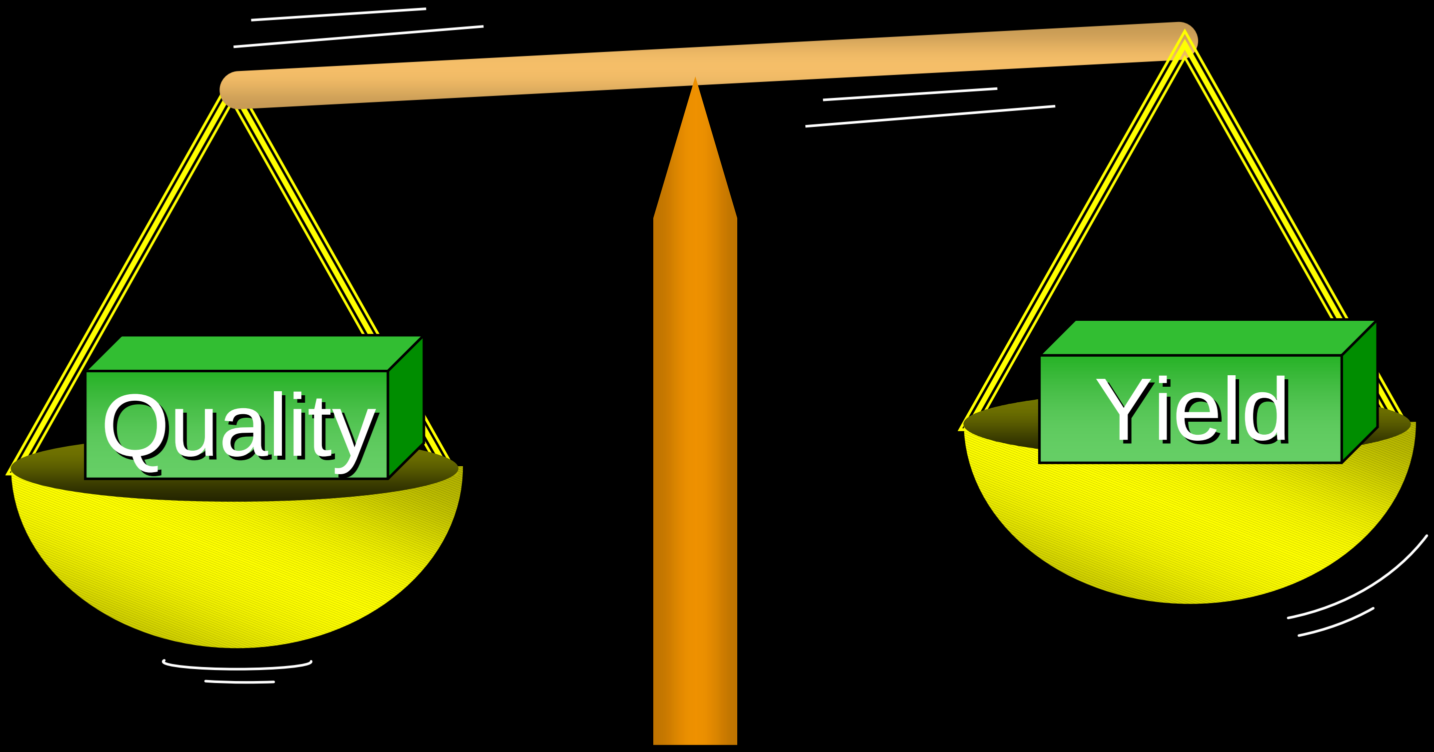
Efficient and inexpensive production scheme

Why are mammalian cells the biotherapeutic champions?

High Quality in spite of Lower Yields



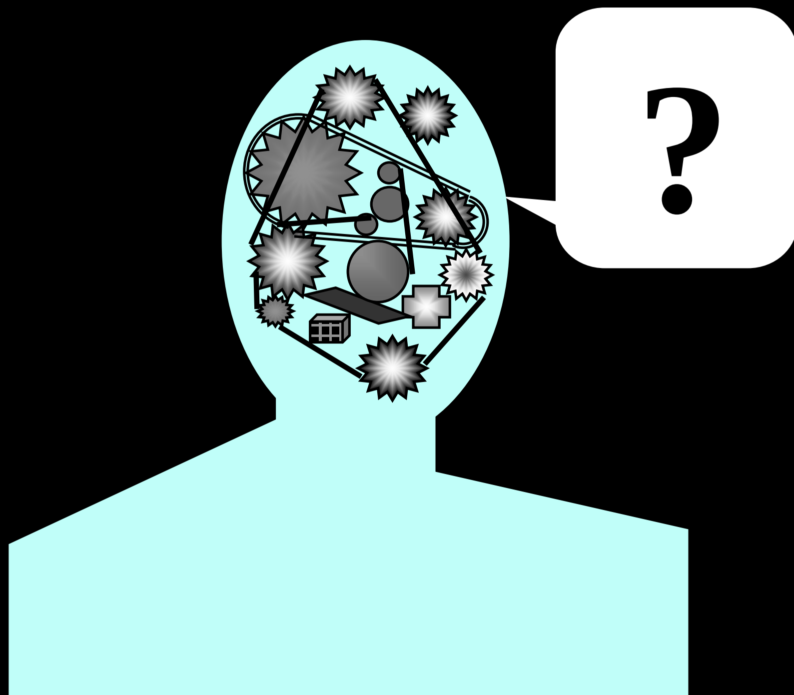
Current Approach : Quality trumps Yield



Essential pharmacological properties

Efficient and inexpensive production scheme

Question?...

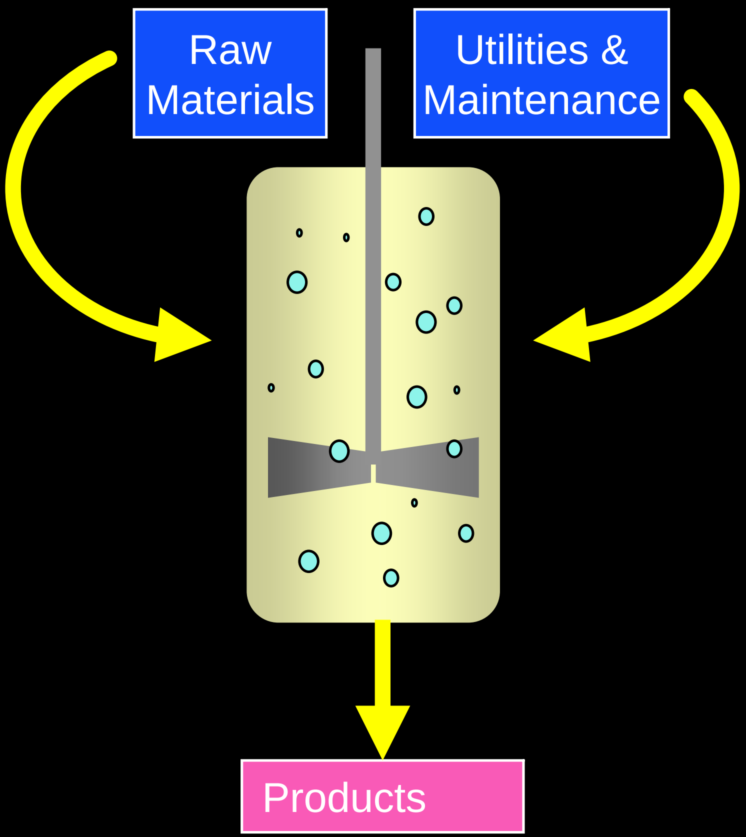


Can we solve the engineering problem?

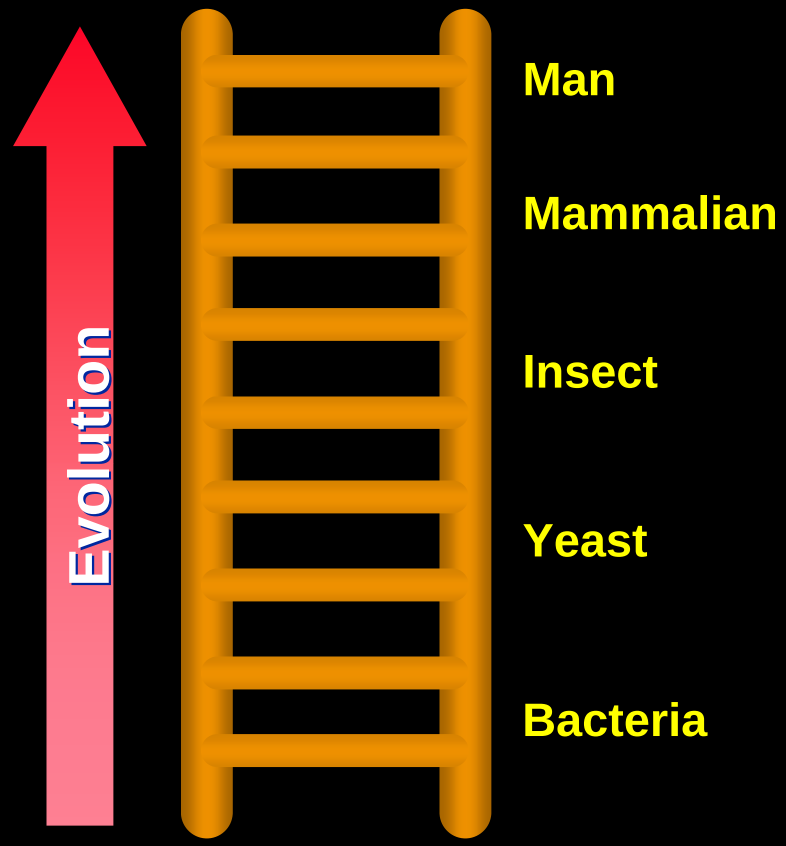
Can metabolic engineering provide high yields and high quality?

Factors in Choosing a Production System

Yield

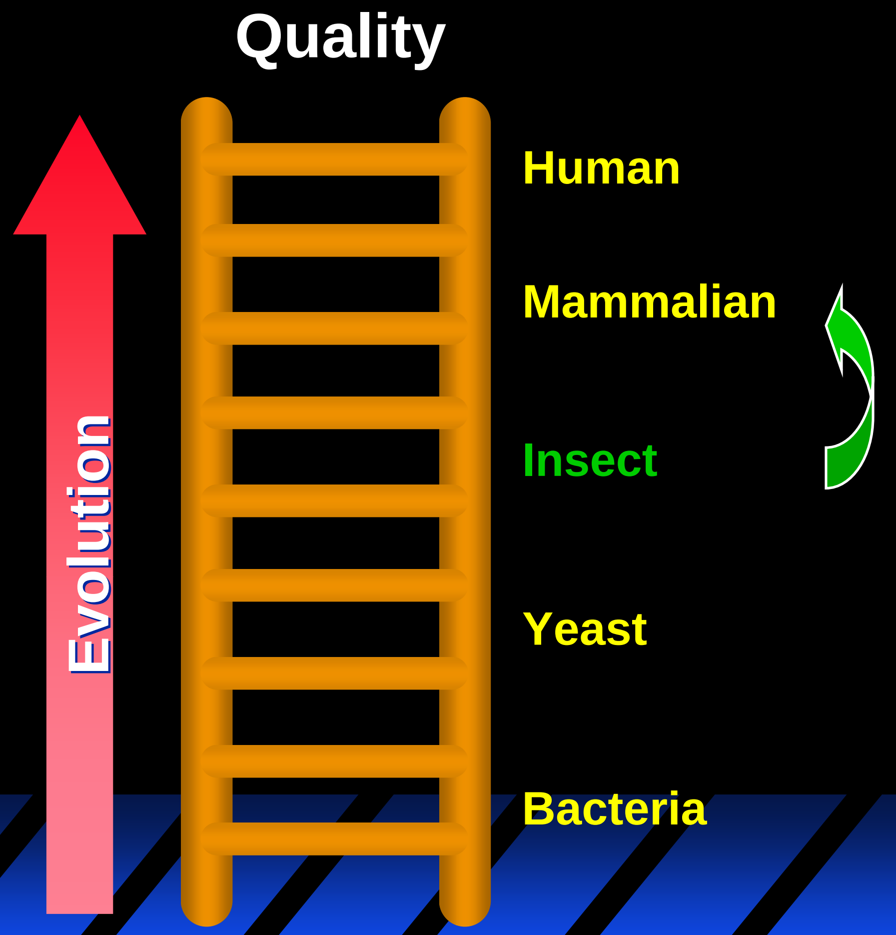


Quality

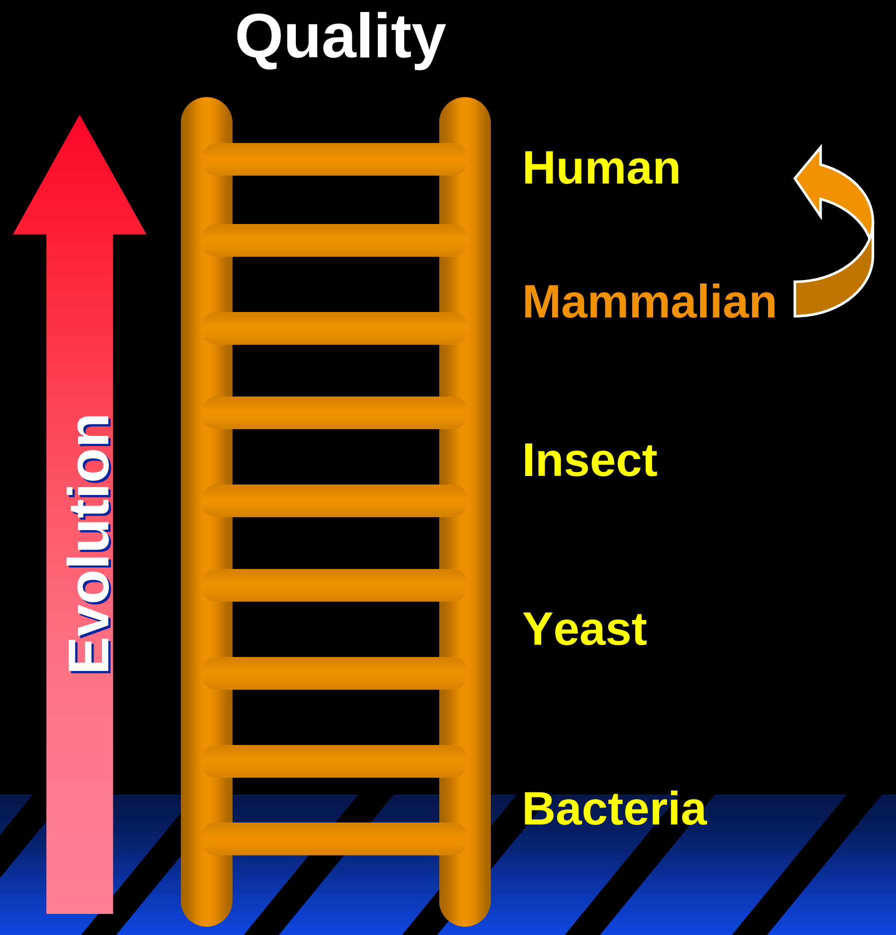


- **Bacteria (*E. coli*)**
- **Yeast**
- **Insect Cells**
- **Mammalian**

(1) Can metabolic engineering improve quality of biotherapeutics from insect cells?

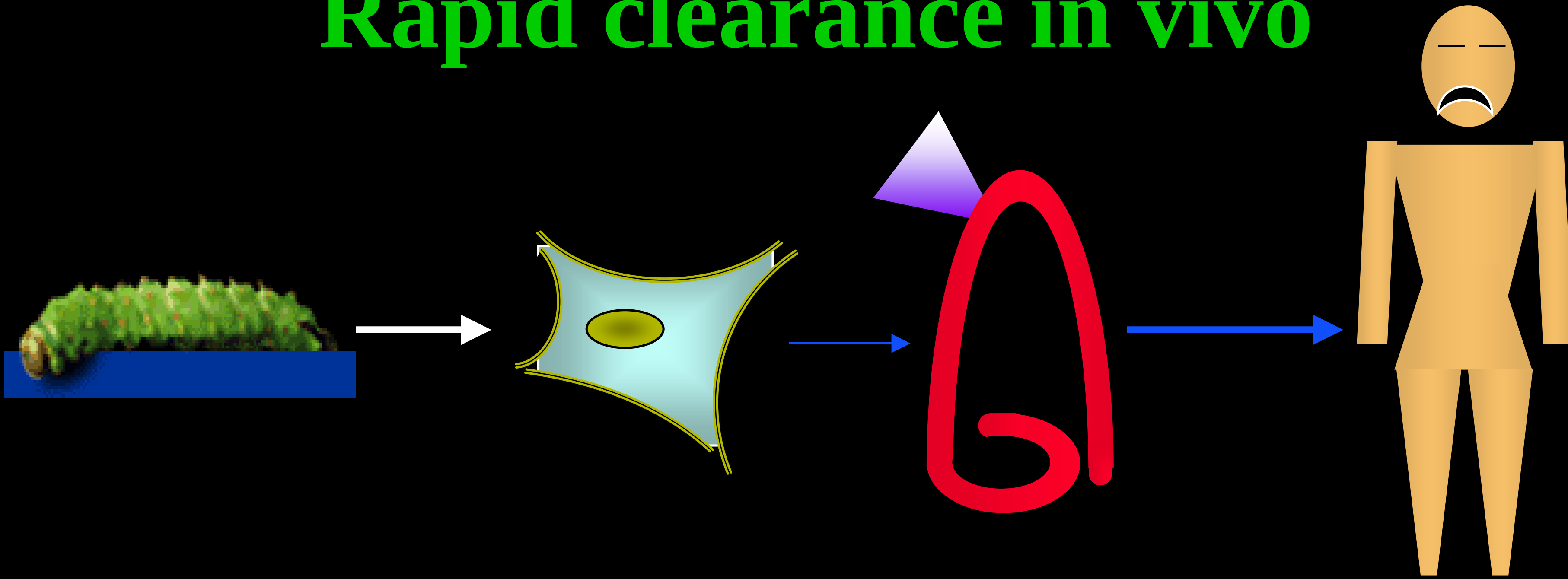


(2) Can metabolic engineering “humanize” biotherapeutic quality from mammalian cells?



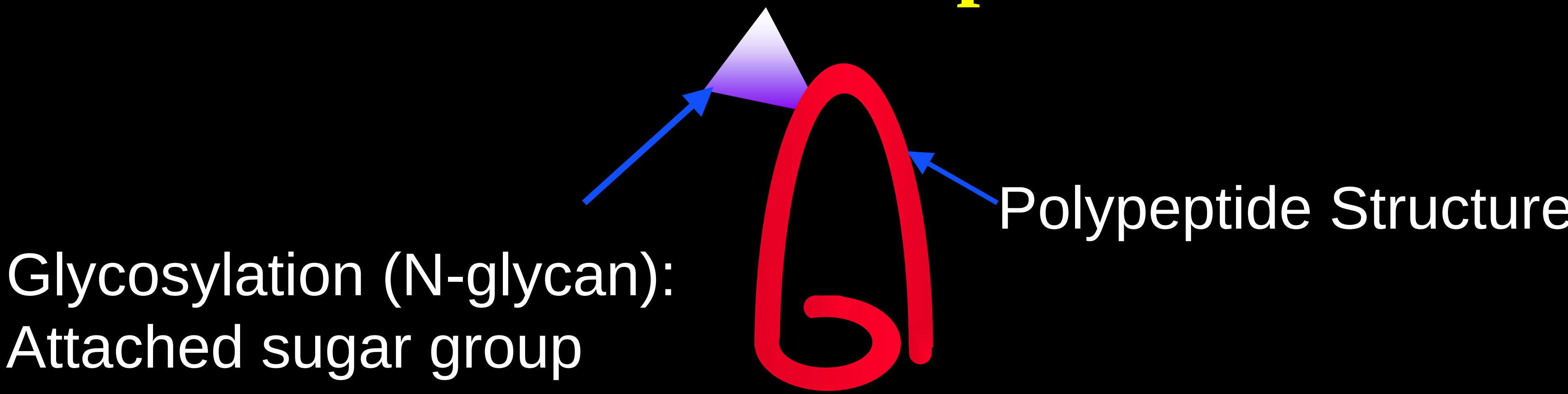
What is “quality” and why are mammalian cells champs over insects?

Why is Insect Quality Low?
Rapid clearance in vivo



- Insect-derived proteins are cleared rapidly from patients “in vivo”
- Mammalian-derived proteins have long “in vivo” circulatory lifetimes

What is the difference between insect and and mammalian products?



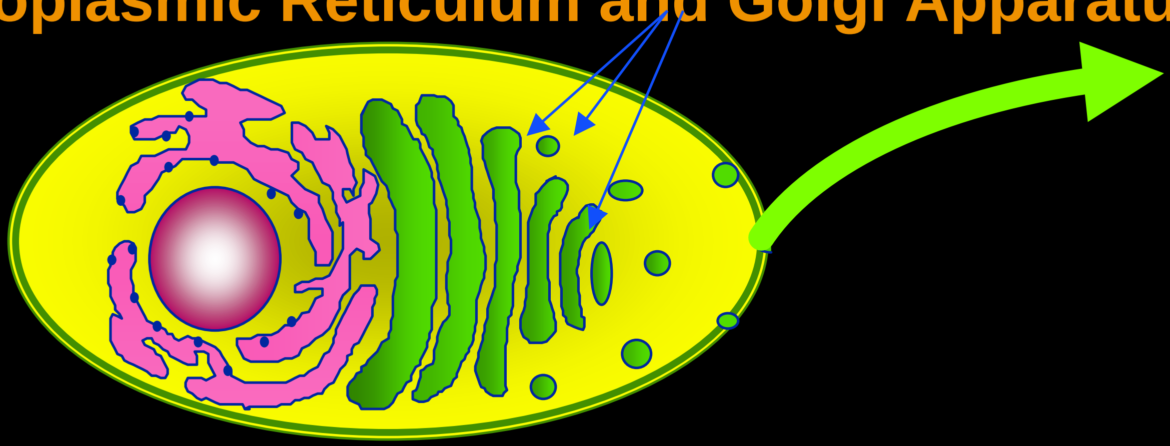
- Identical Polypetide Structures-Insect vs. Mammals
- Different Glycosylation Patterns: Insects vs. Mammals

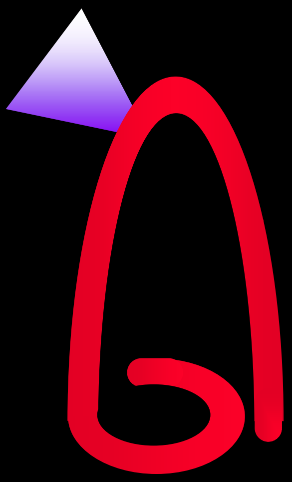
Glycosylation differences are the reason for lower quality of Insect-derived product versus mammalian product

Glycosylation Processing in the cell

Secretory Compartments:

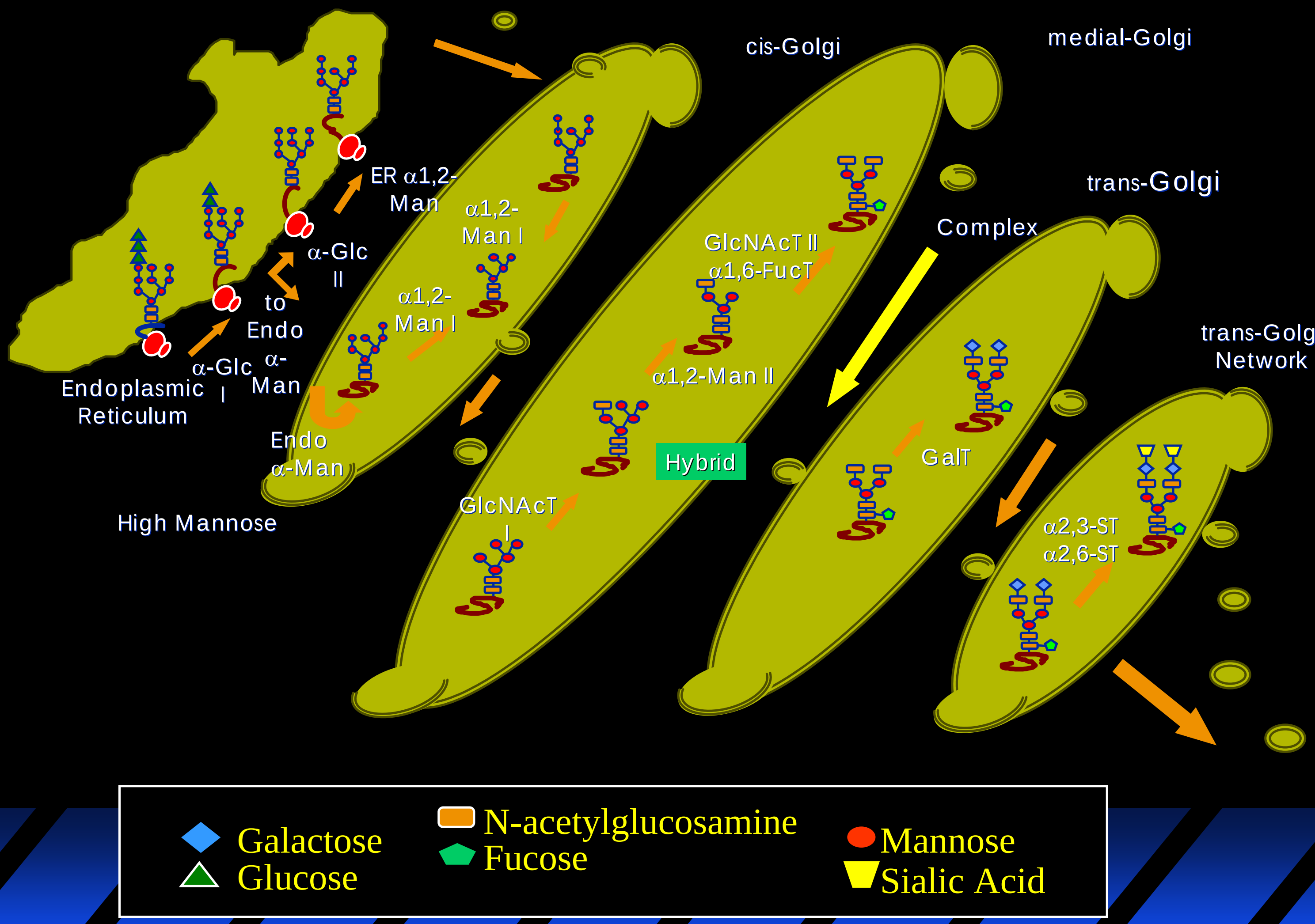
Endoplasmic Reticulum and Golgi Apparatus



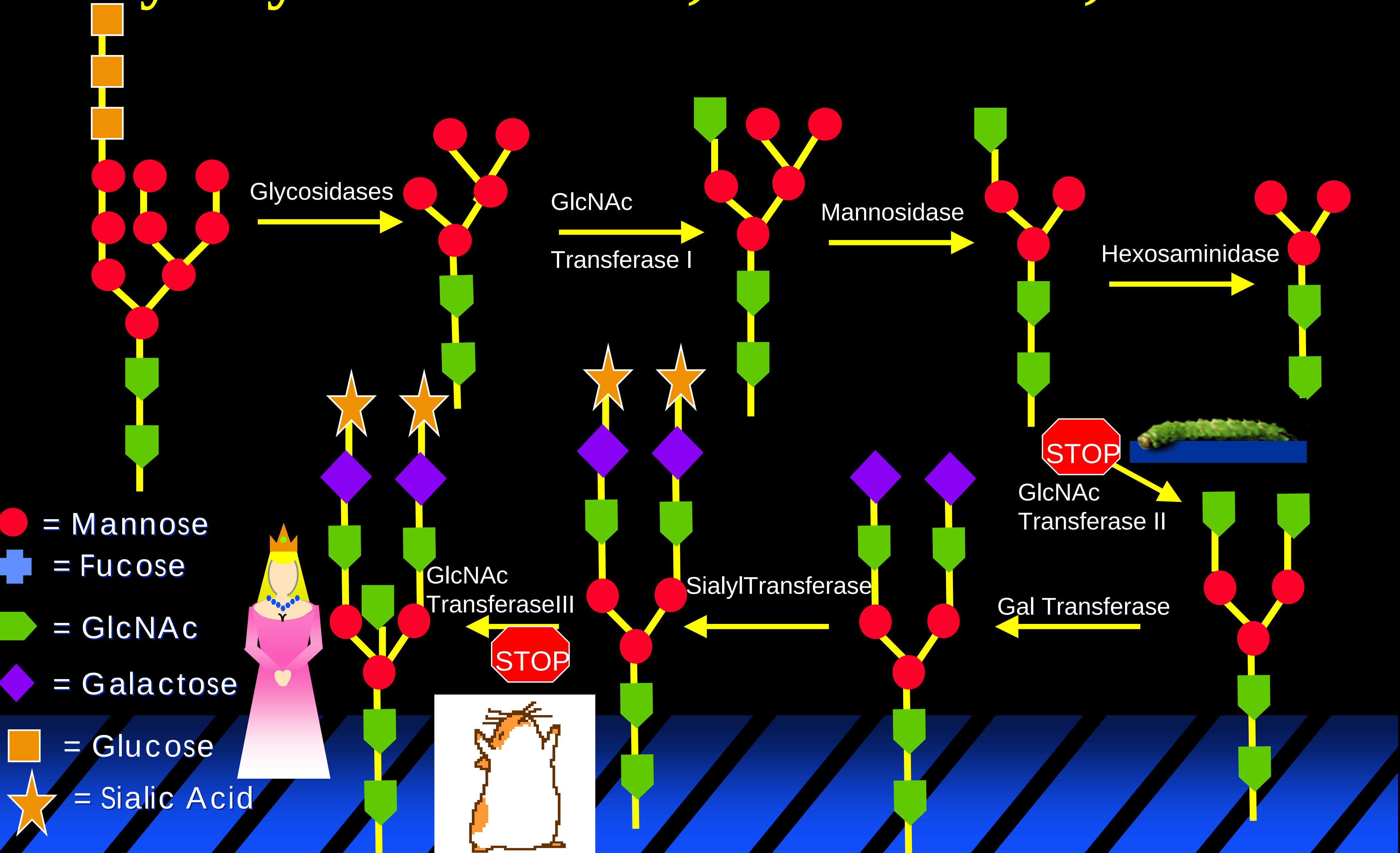


secreted product

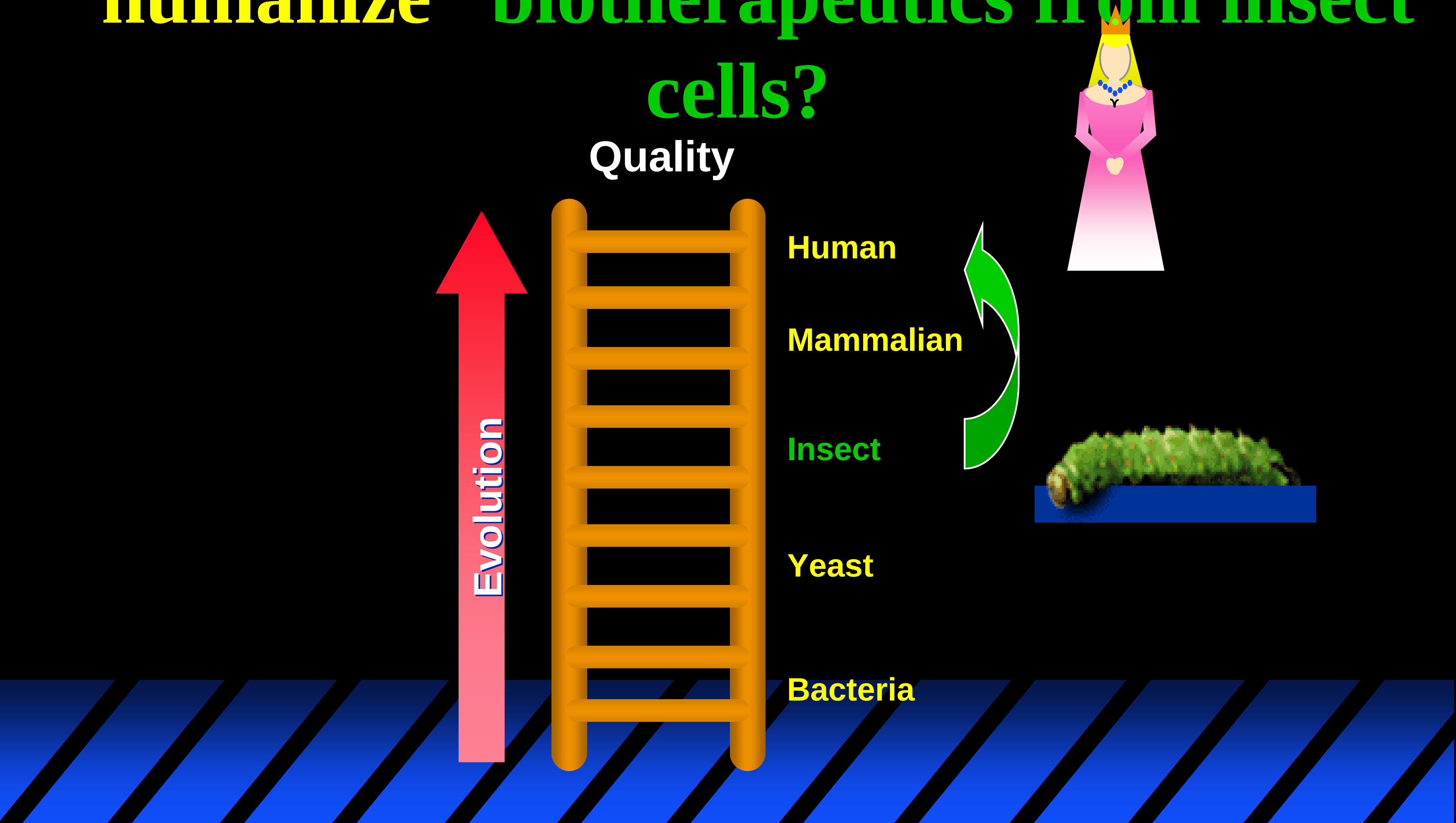
Glycosylation Processing in the Cell



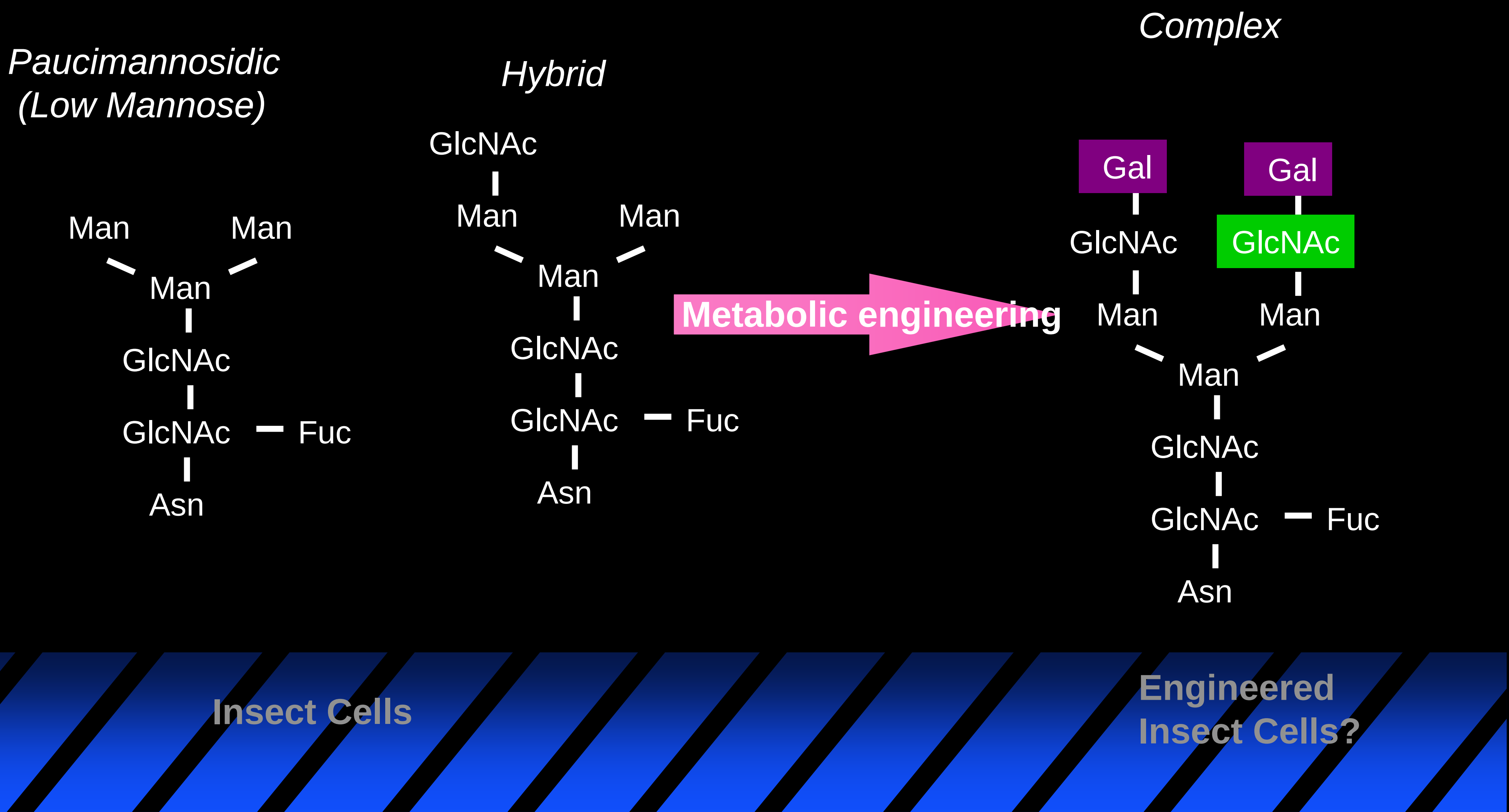
Glycosylation: Insect, Mammalian, Human



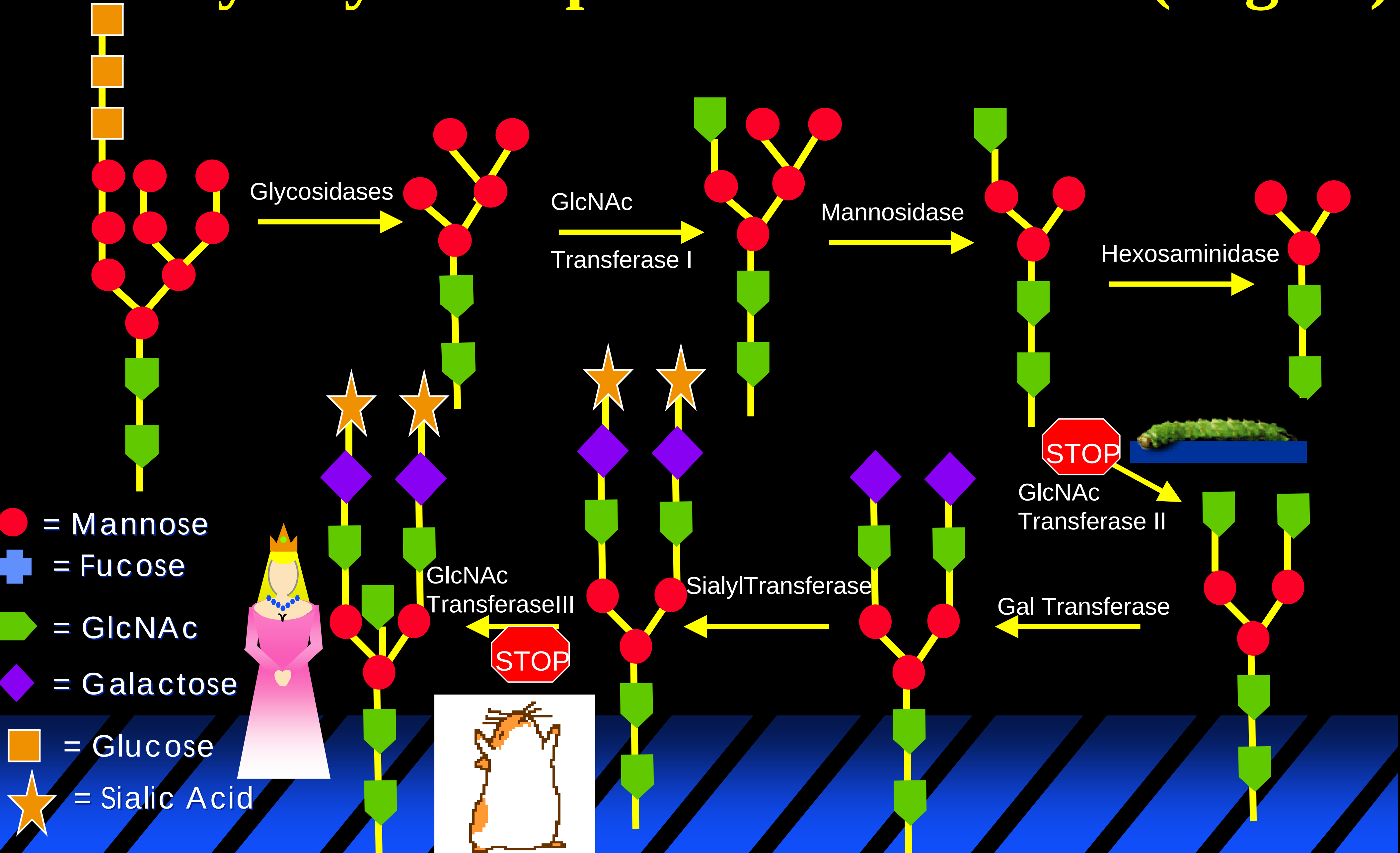
(1) Can metabolic engineering "humanize" biotherapeutics from insect cells?



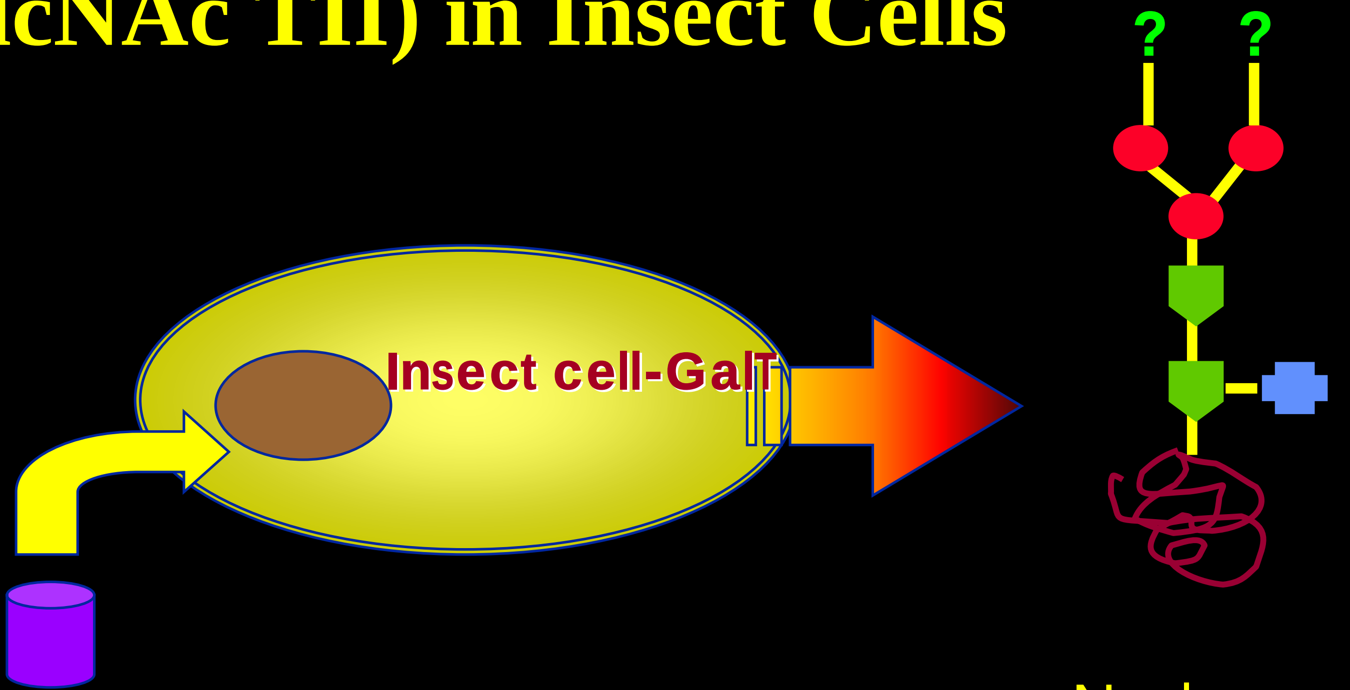
Humanizing Insect Cell Glycosylation: Add GlcNAc and Galactose (Gal)



Glycosylation patterns from T. ni (High 5)



Express Mammalian Galactose Tranferase (Gal T) and N-acetylglucosamine transferase II (GlcNAc TII) in Insect Cells

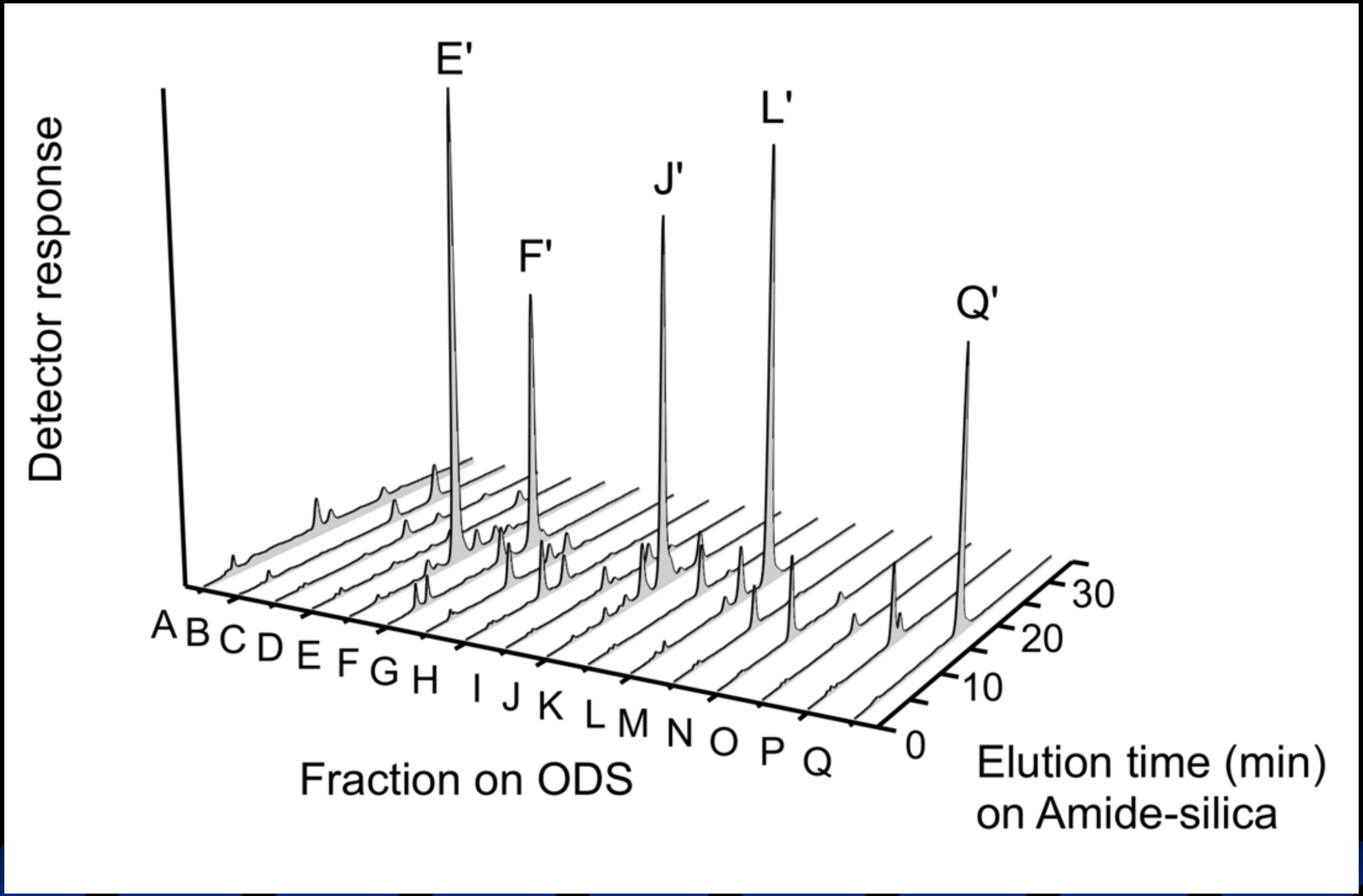


Baculovirus with Mammalian GlcNAc Transferase TII (GlcNAc TII)/ target protein

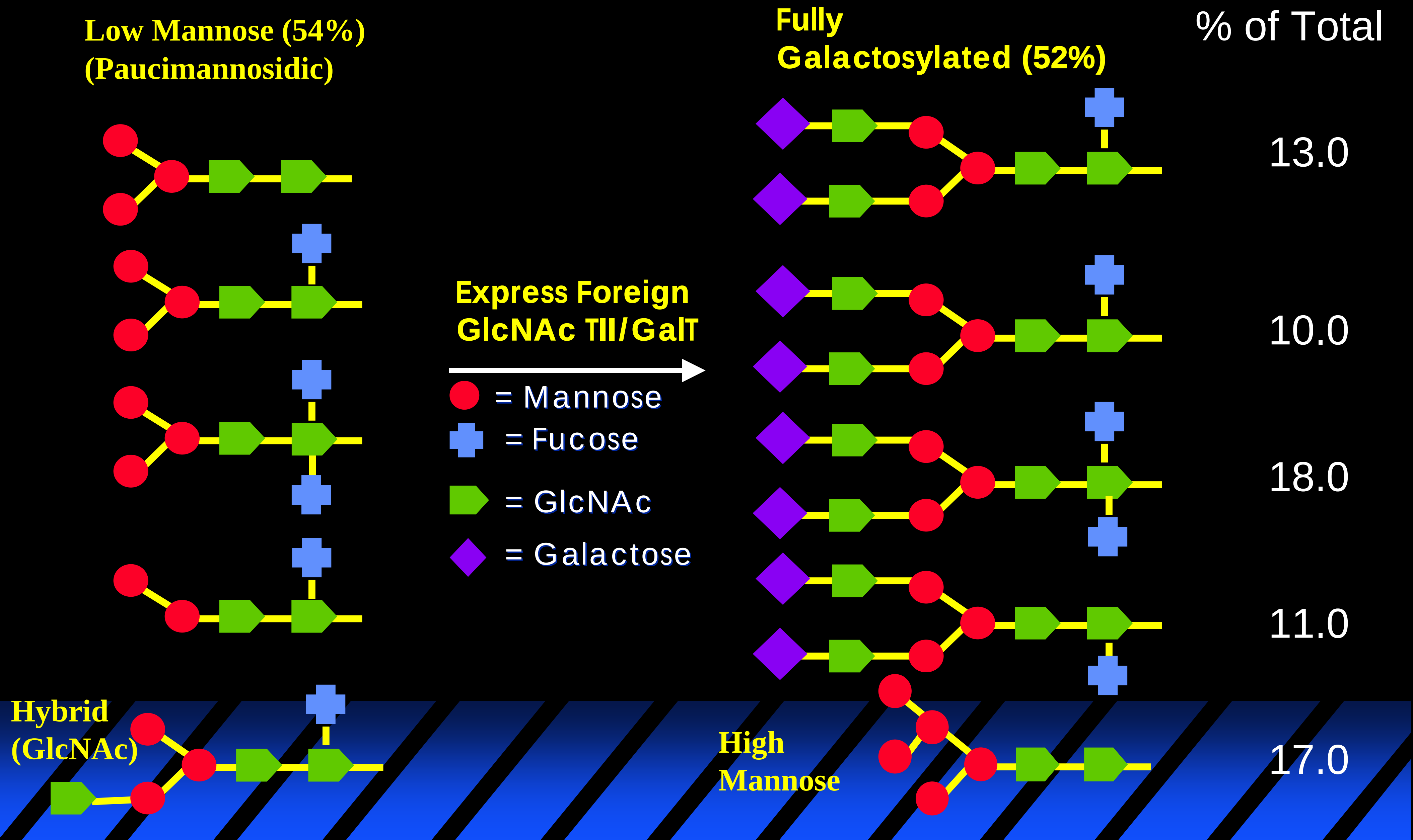
N-glycan patterns?

Collaborator: Don Jarvis, Univ. of Wyoming

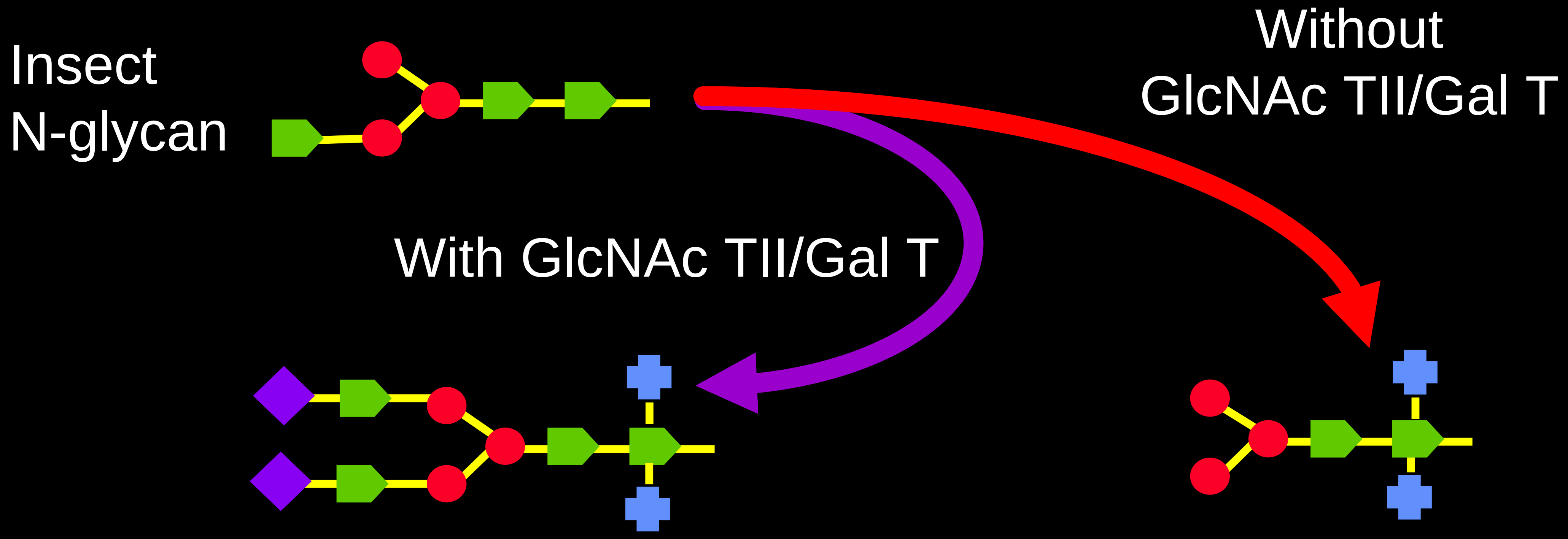
HPLC Analysis of Glycosylation



Glycosylation patterns from Insect Cells



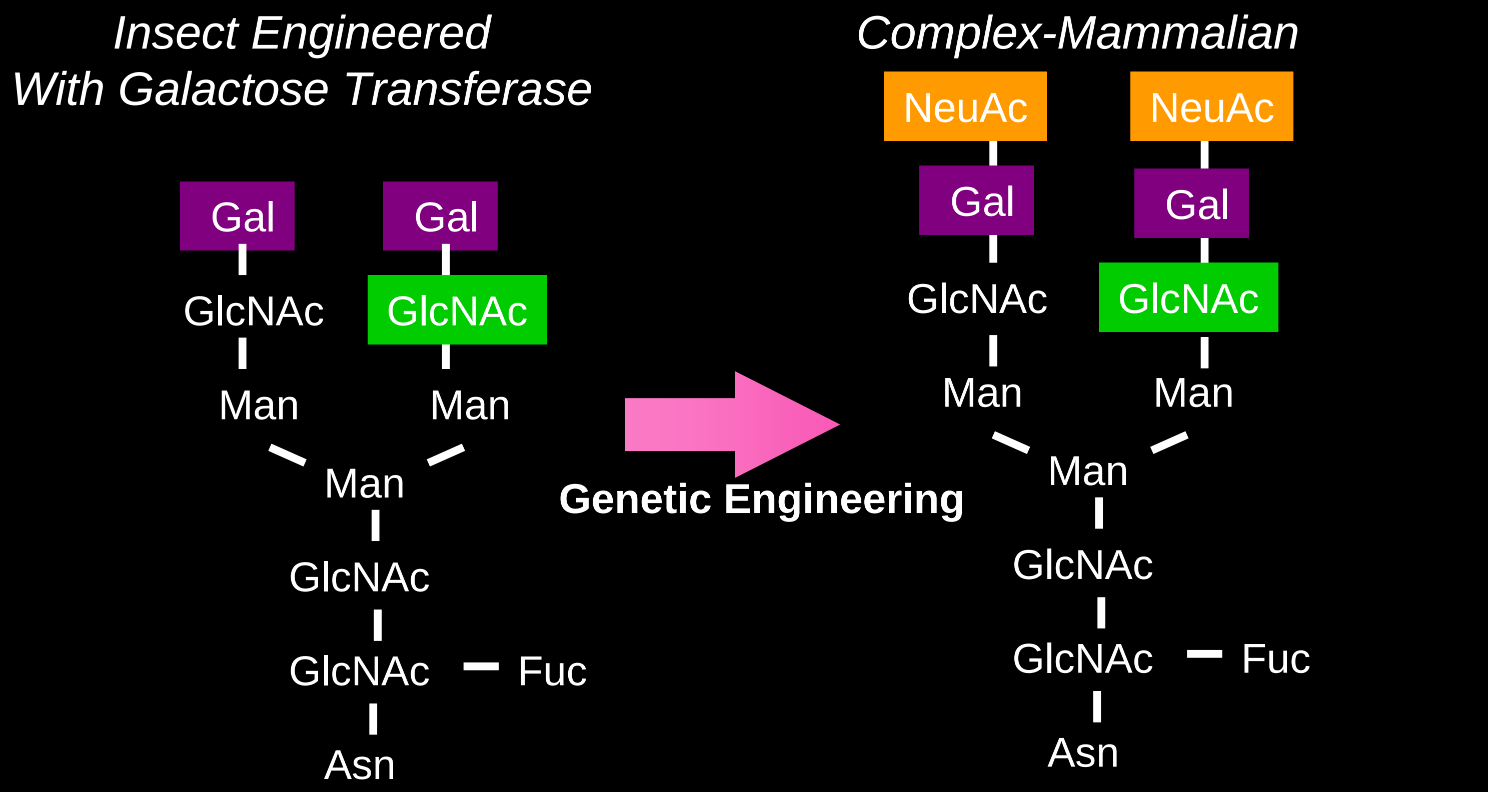
GlcNAc TII/Gal T makes N-glycans more "mammalian" –fully galactosylated



1. Adds GlcNAc and Terminal Galactose
2. Blocks Removal of GlcNAc

= Fucose; = Glucose; = Galactose
= N-acetylglucosamine; = Mannose

Next, we must add sialic acid to insect cell glycosylation

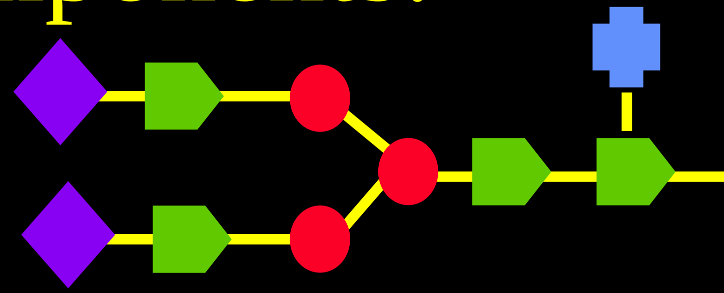


Engineered Insect Cells

Re-Engineered Insect Cells?

Sialylation reaction requires three components:

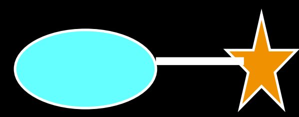
1. Galactose-terminated Structure



✓

+

2) CMP-Sialic acid-CMP-Neu5Ac (nucleotide sugar-Cytidine Monophospho-N-acetylneuraminic acid)

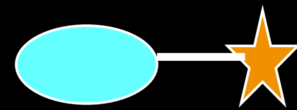


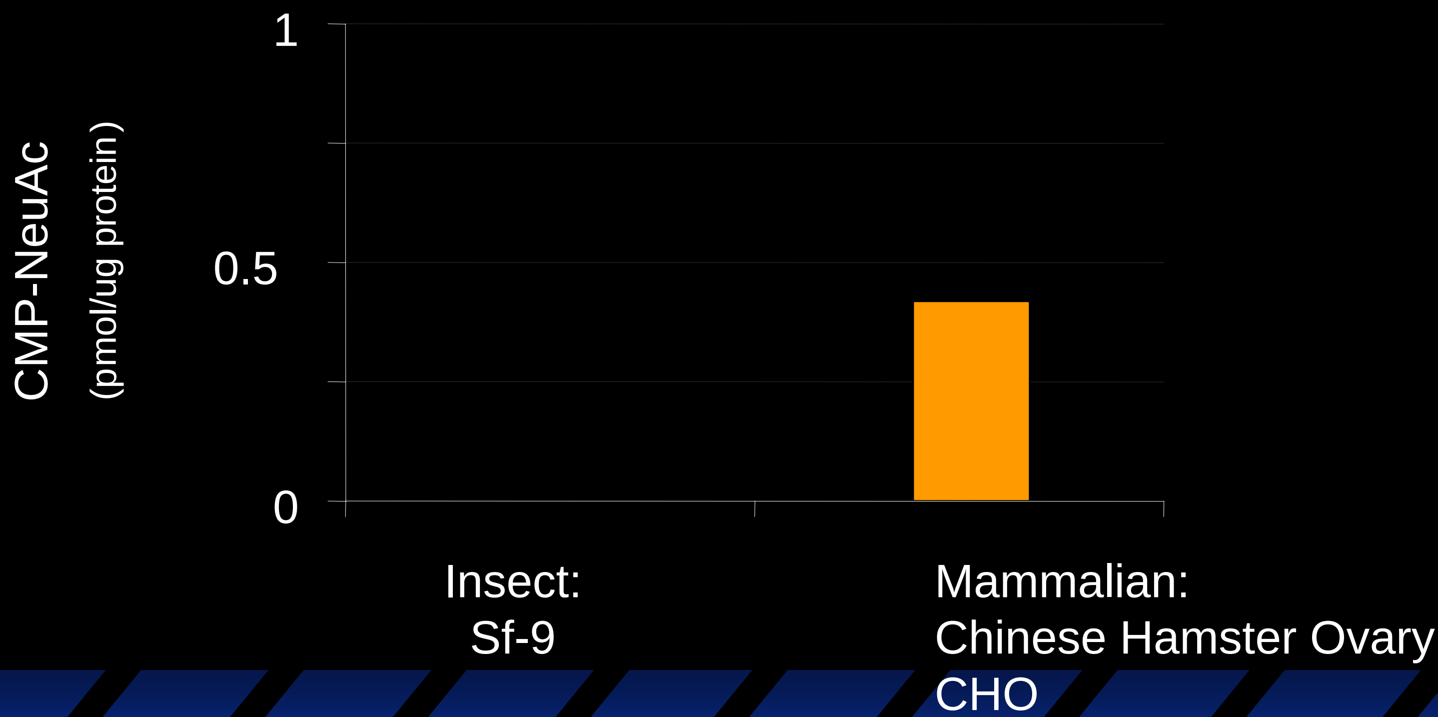
?

+

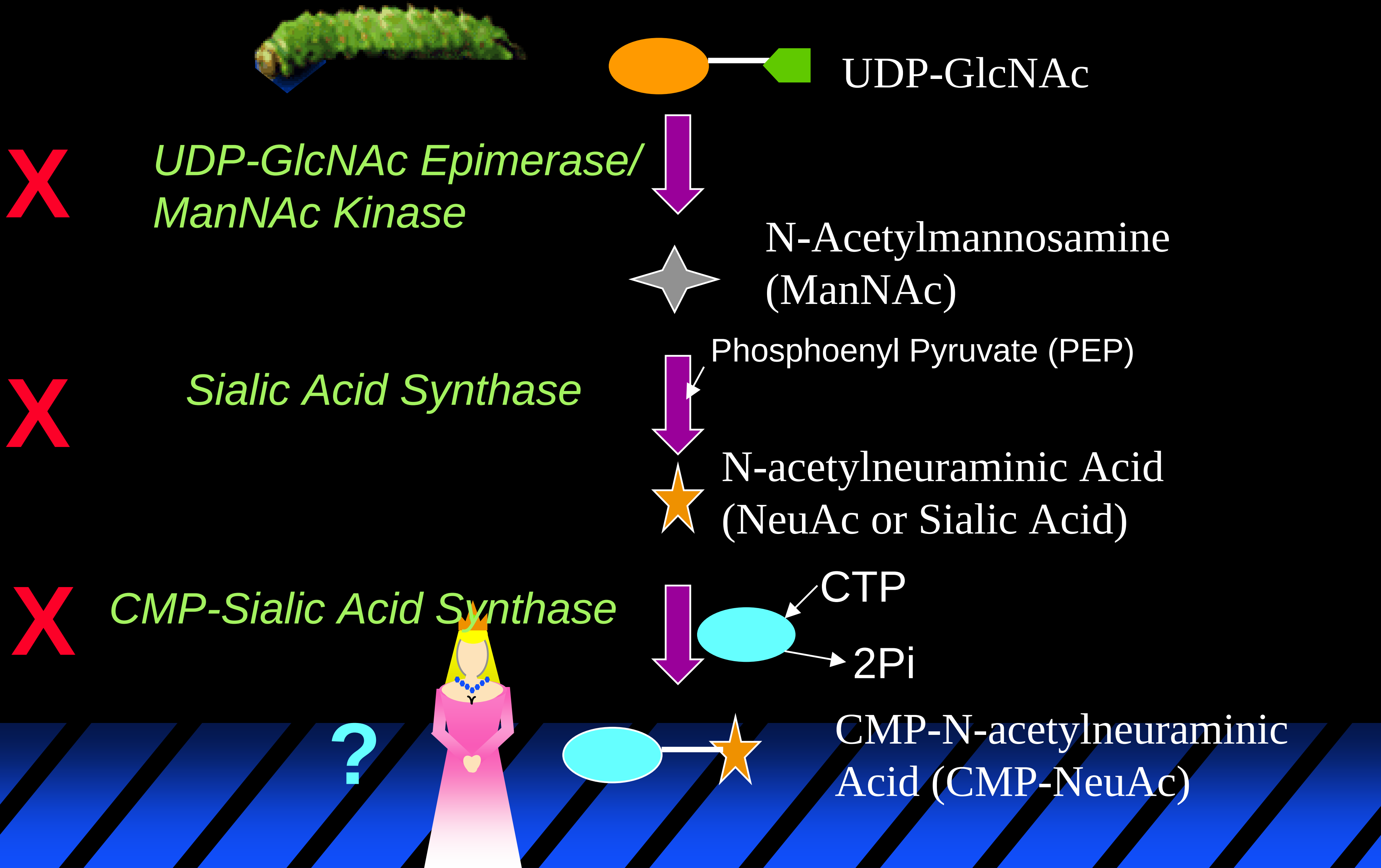
3) Sialyltransferase

?

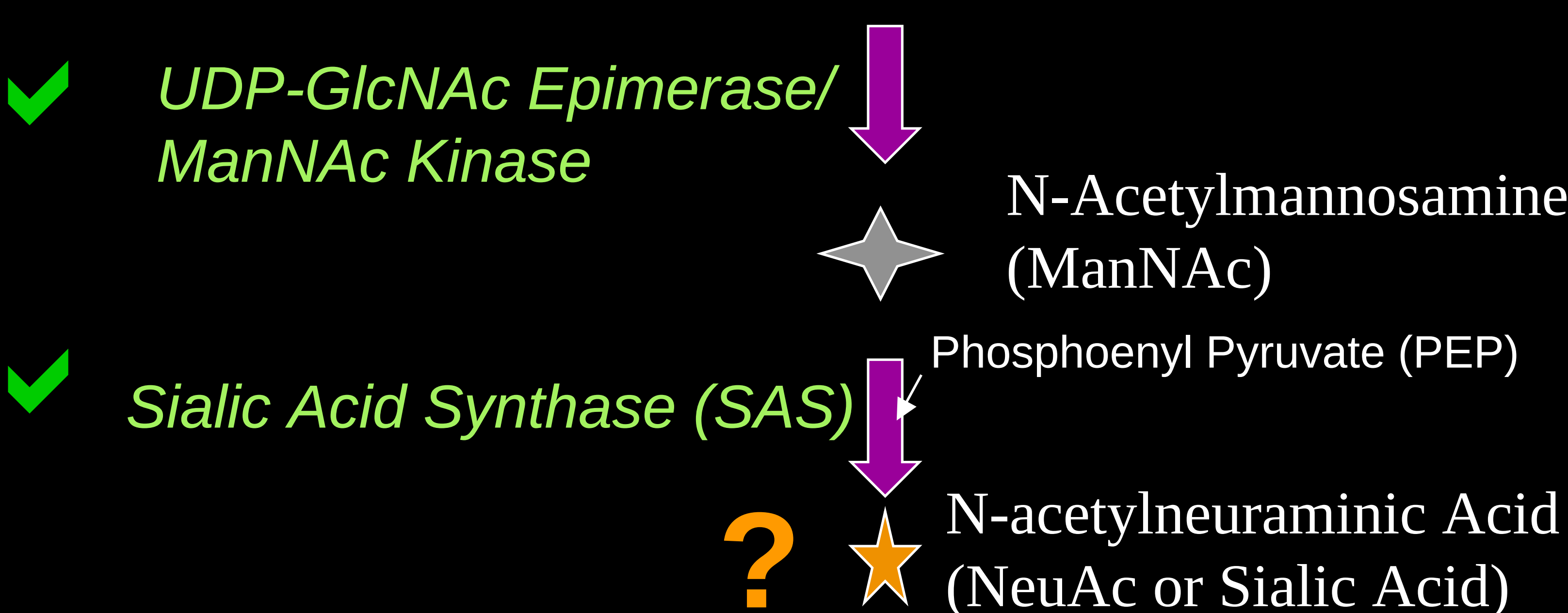
But CMP-Sialic Acid  is not present in Insect Cells



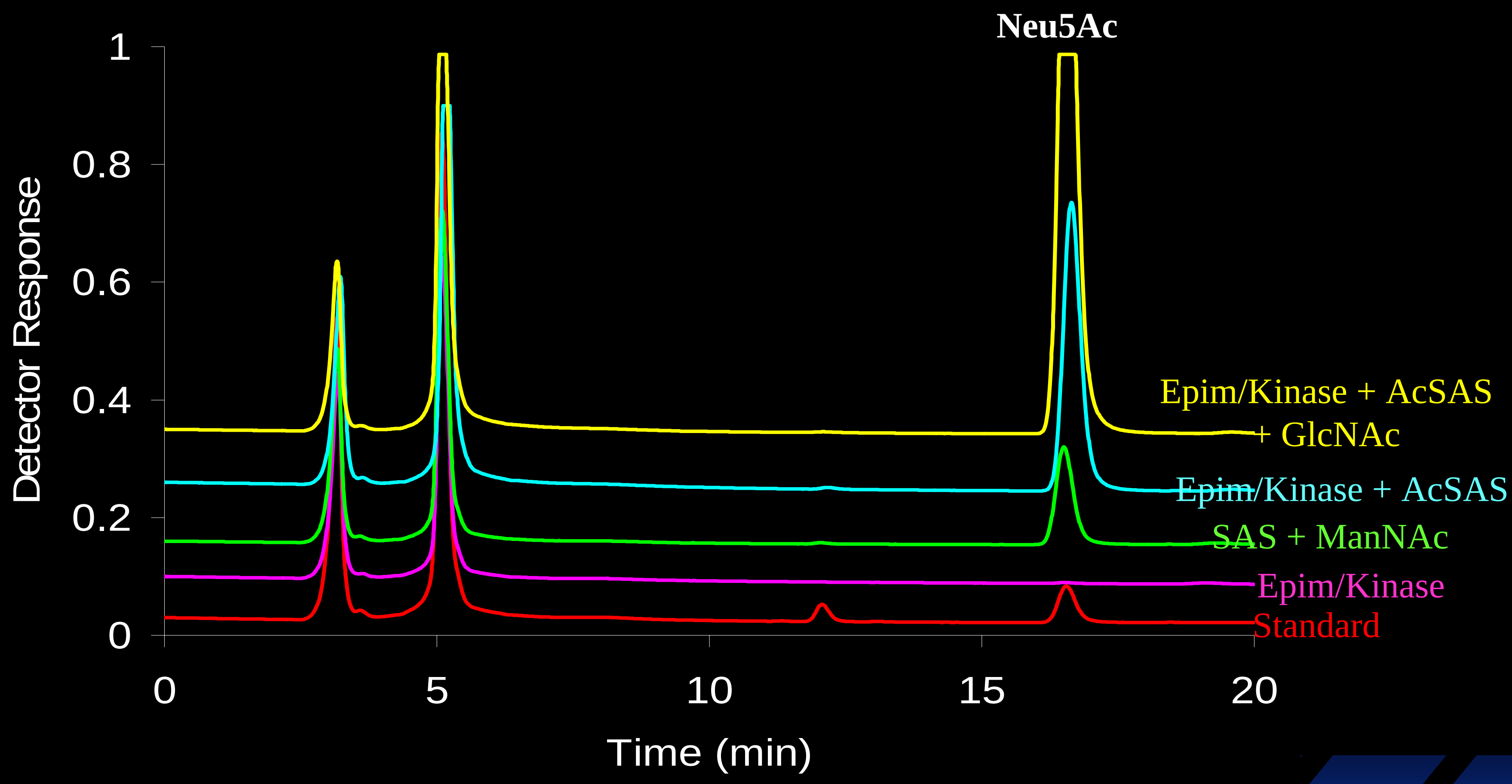
Can we make CMP-Sialic Acid?



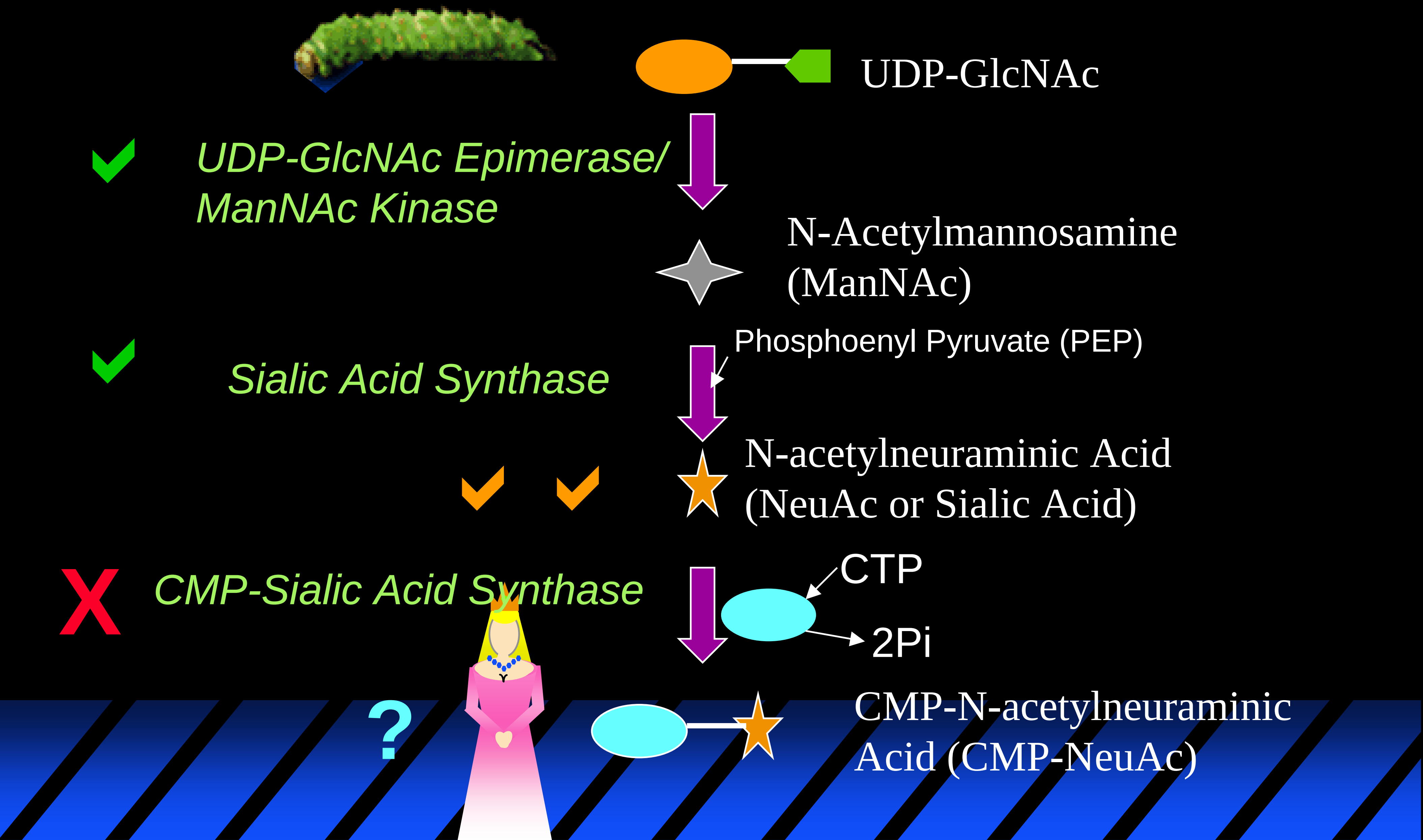
Mammalian Enzymes Engineered into Insect Cells



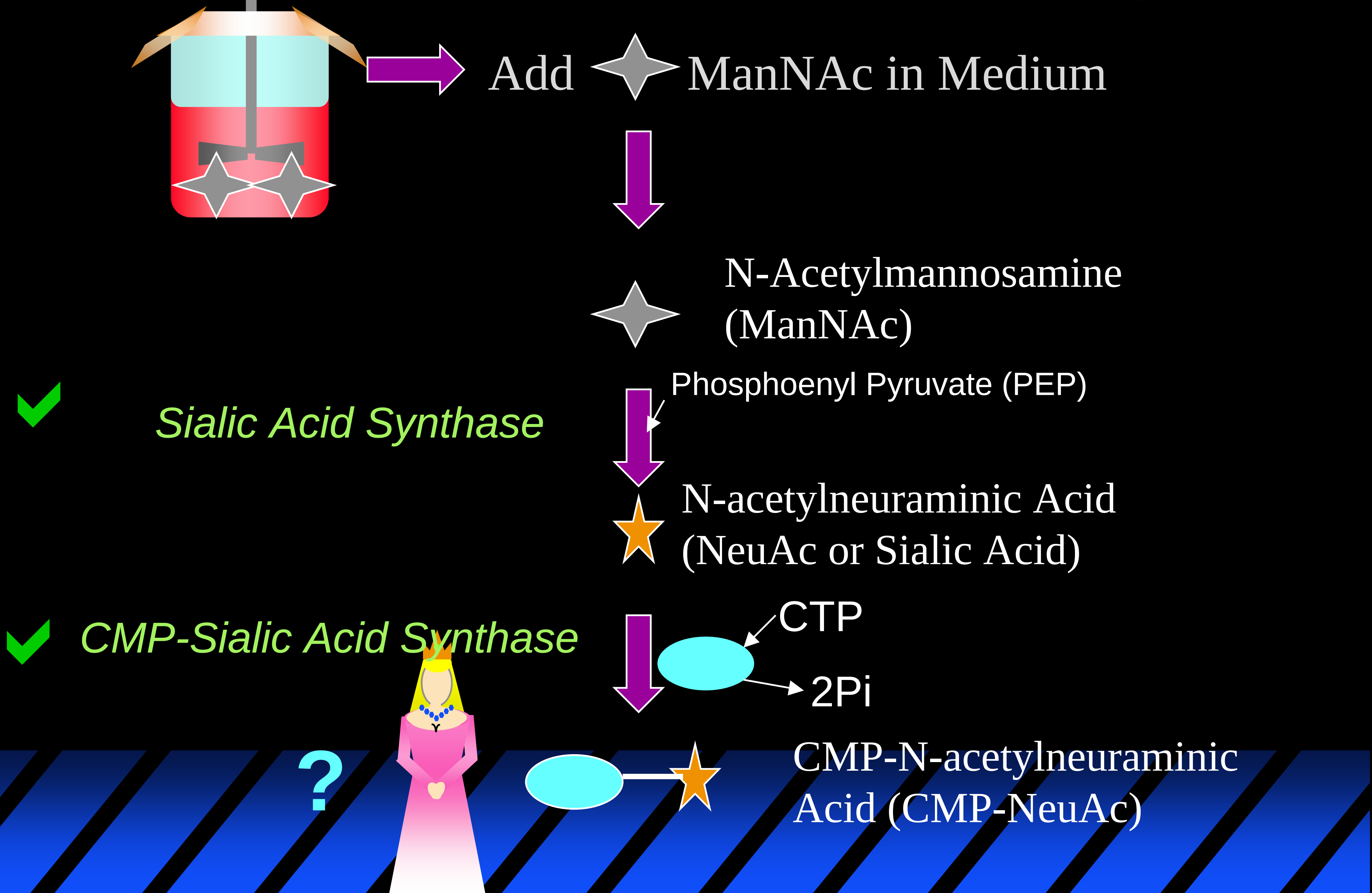
Recombinant Epimerase/Kinase + Sialic Acid Synthase (SAS) in Insect Cells



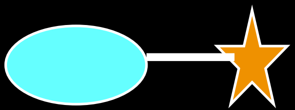
Can we make CMP-Sialic Acid?

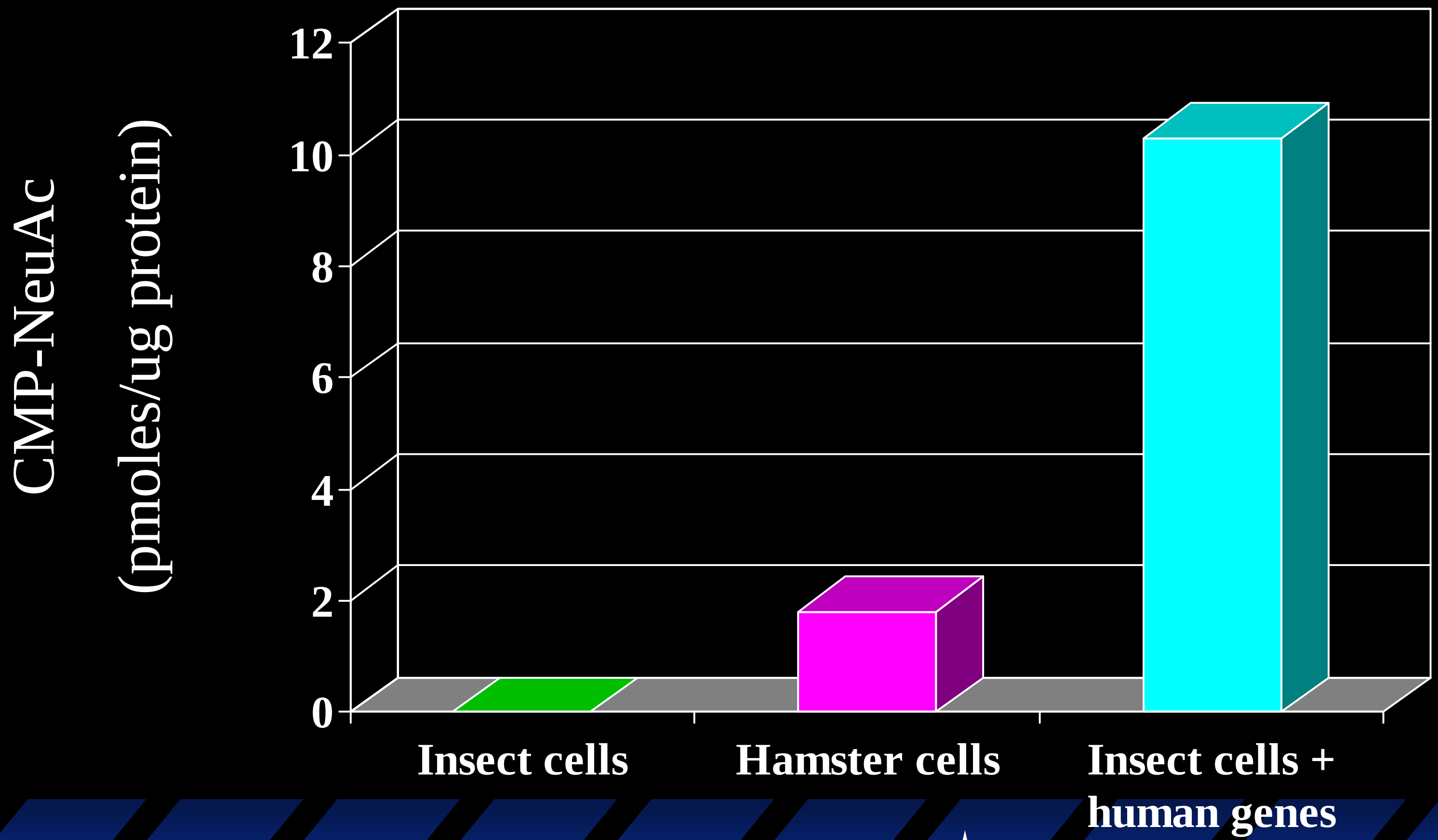


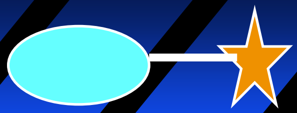
Can we make CMP-Sialic Acid?



Engineered Insect Cells make high levels of CMP-Sialic Acid:

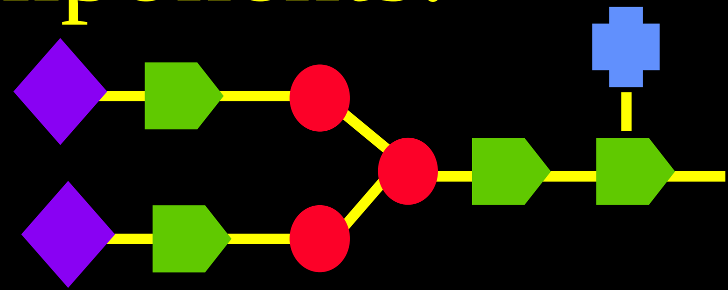






Sialylation reaction requires three components:

1. Galactose-terminated Acceptor



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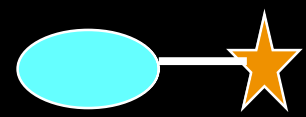
2) CMP-Neu5Ac (nucleotide sugar)
(Cytidine Monophospho-
N-acetylneuraminic acid/Sialic Acid)



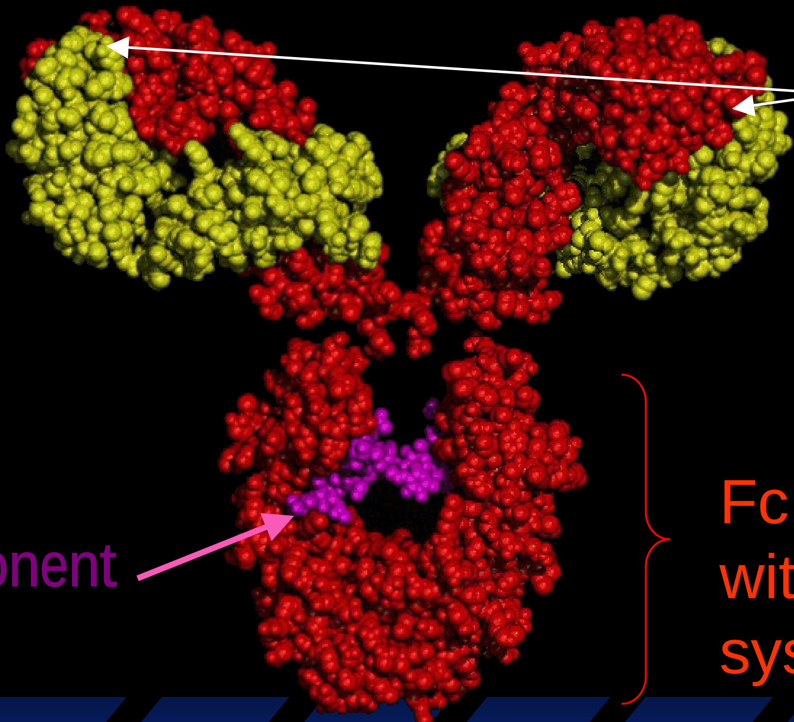
+

3) Sialyltransferase

Don Jarvis-Wyoming

But you have to put them all together too….

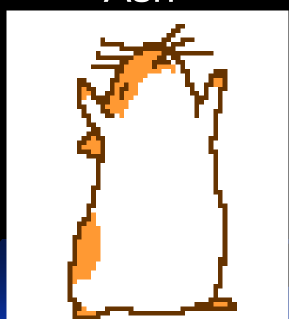
(2) Can metabolic engineering improve antibody glycosylation quality from mammalian cells?



Umana et al., Nature Biotechnol.

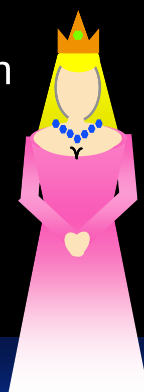
Humanizing Hamster&Mouse Glycosylation: Bisecting GlcNAc

Gal | Gal
GlcNAc | GlcNAc
Man | Man
Man
GlcNAc
GlcNAc
Asn



Metabolic engineering:
Add Bisecting N-acetylglucosamine (GlcNAc) By expressing N-acetylglucosamine Transferase III (GlcNAc TIII)

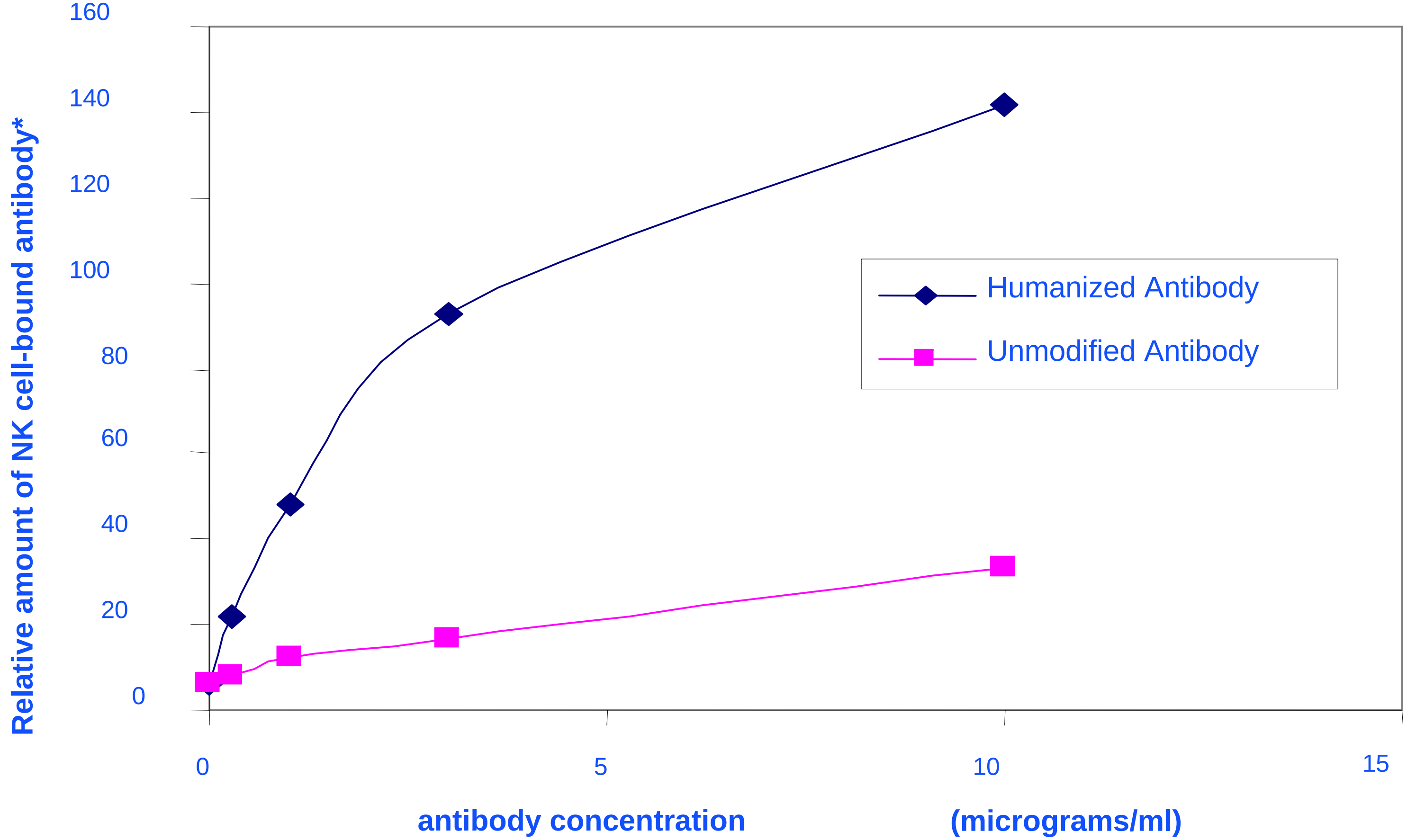
Gal | Gal
GlcNAc | GlcNAc
Man | GlcNAc | Man
Man
GlcNAc
GlcNAc
Asn



Chinese Hamster Ovary (CHO) and NSO Mouse Cells

Human cells and Humanized CHO&NSO

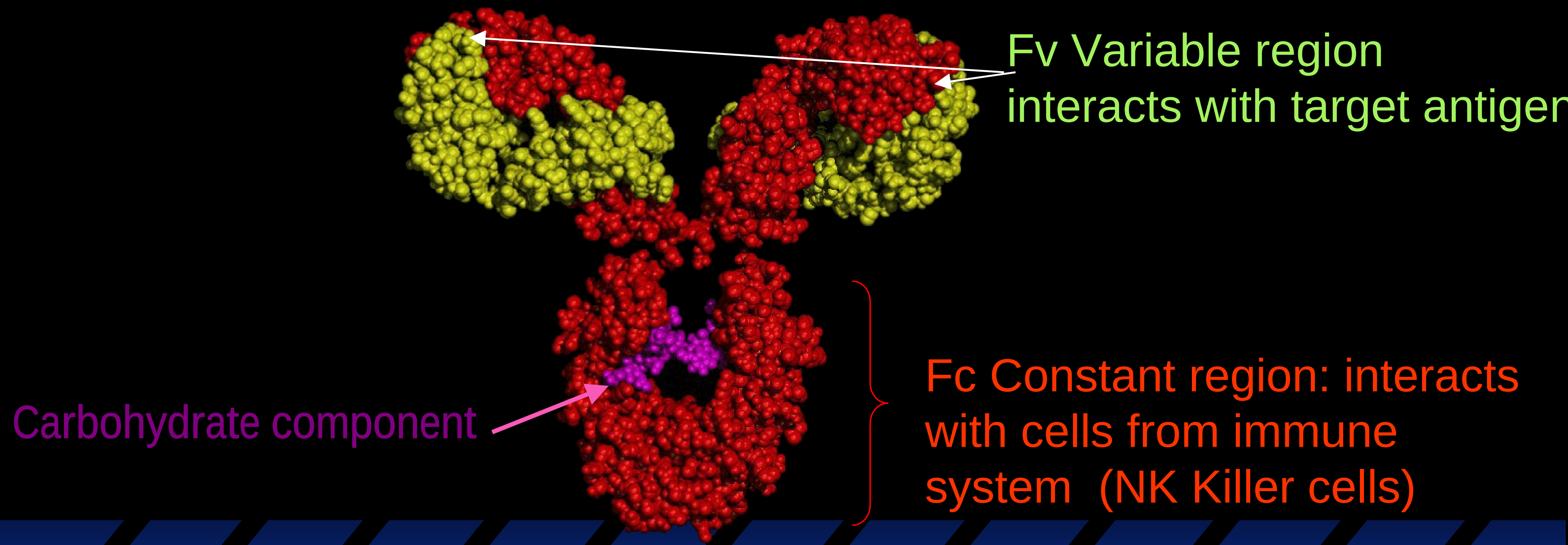
Immune Killer Cell Binding to Fc Region of Antibody



(* Proportional to mean fluorescence by FACS analysis)

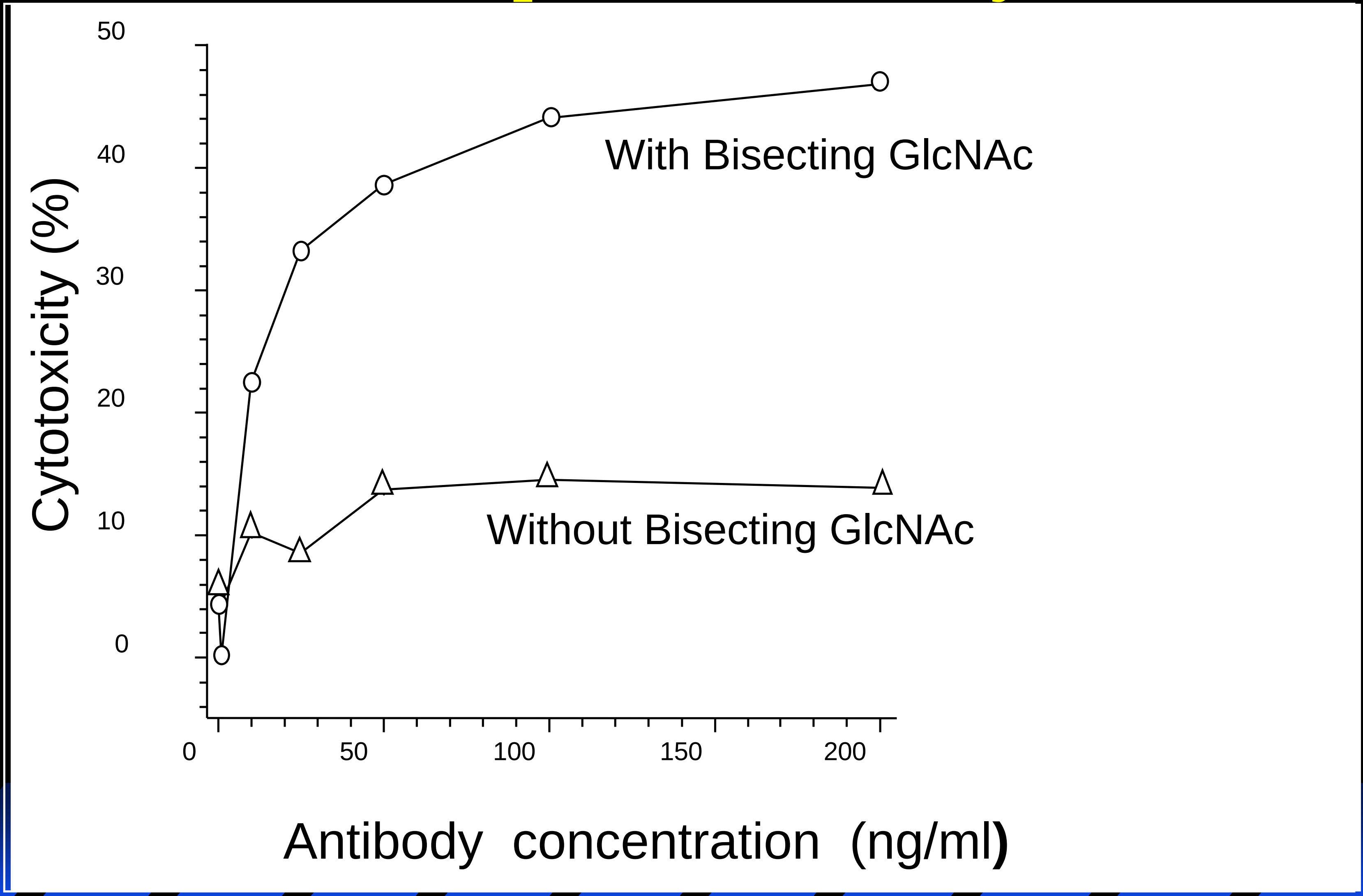
Courtesy of P. Umana, Glycart, Inc.

Metabolic Engineering of Glycosylation Alters Antibody Structure/Function



Umana et al., Nature Biotechnol.

Effects of Bisecting GlcNAc on Therapeutic Efficacy



Courtesy of P. Umana, Glycart, Inc.

Blood, 2002, Vol. 99, No. 3, pp. 754-758

Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgRIIIa

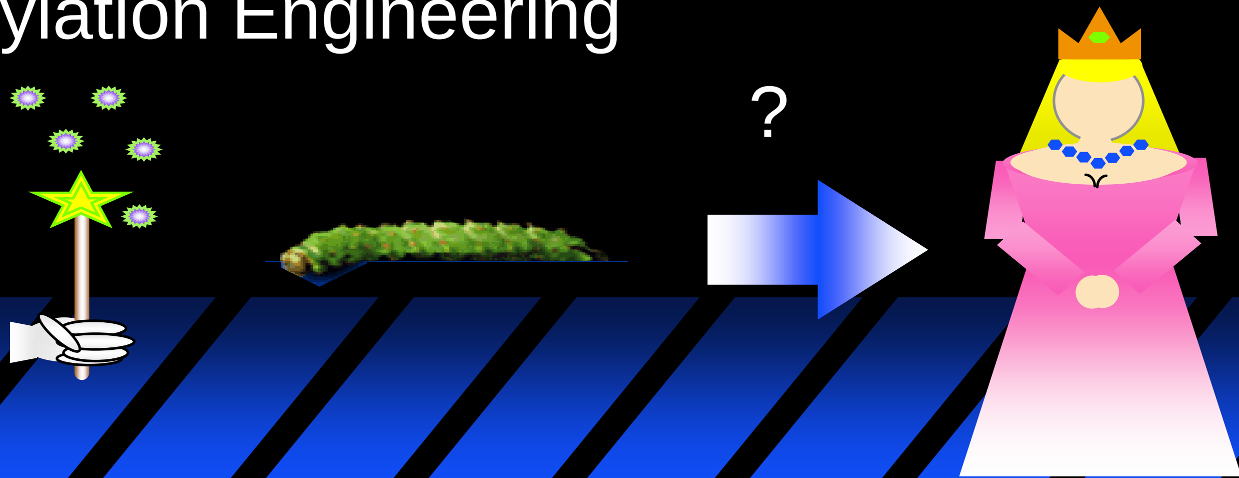
Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, Watier H.

Clinical Outcome after 12 months Rituxan® therapy

	Objective response rate (% patients)	Molecular response rate (% patients)
Homozygous high affinity	90	83
Low affinity carrier (homozygous+heterozygous)	51	29

Conclusions

- Engineering Tradeoff: High Yields versus High Quality
  - Mammalian: Higher Quality and Lower Yields
  - Insect Cells: Lower Quality and Higher Yields
- Can Metabolic Eng. Solve the Engineering Problem?
  - High Quality & High Yield?
- Insect Cells: Higher Quality through Glycosylation Engineering
- Mammalian Cells: Improved Antibody Quality through Glycosylation Engineering



Acknowledgements

- Johns Hopkins University
  - Yuan C. Lee
  - Noboru Tomiya
  - Lee Pitts
  - Shawn Lawrence
  - Badarulhisam Abdul Rahma
  - Eric Ailor
- FDA
  - Willie F. Vann
- University of Wyoming
  - Donald Jarvis
- Temple University
  - Karen Palter
- Human Genome Sciences
  - Timothy Coleman
  - Kathy Huddleston
  - Nam Nguyen
  - Joelle Porter
  - Dave Parmelee
- NSF and NIH Metabolic Engineering Grants