Mammalian and Insect Cell Metabolic Engineering for Biotechnology Applications

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NSF Metabolic Engineering Working Group
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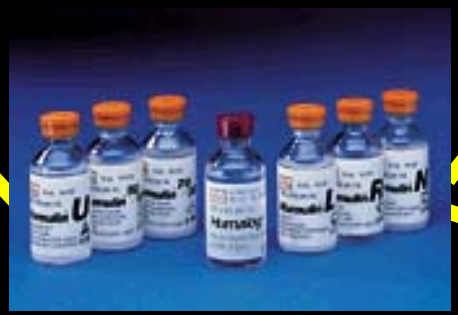
Biotechnology & Pharmaceuticals

ACTIVASE
ALTEPLASE
A RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR

REMICADE®
INFLIXIMAB
recombinant

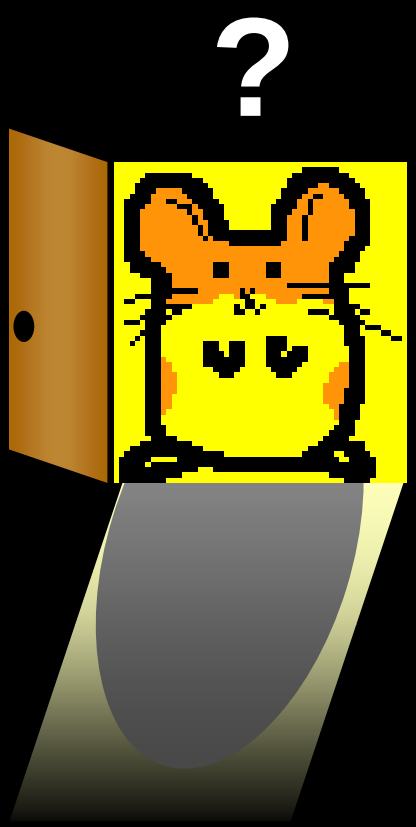


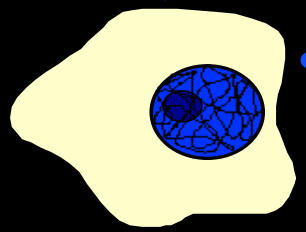
Genentech, Inc.

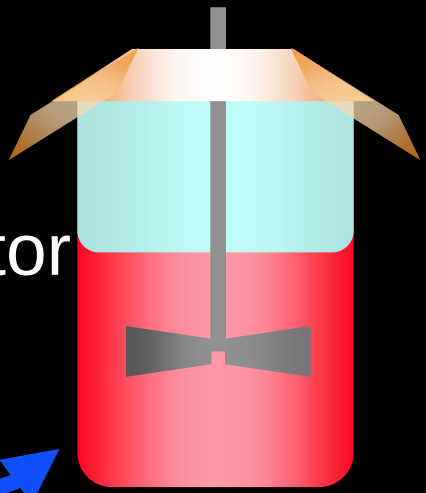
Humulin®
human insulin
(rDNA origin)

Lilly

Mammalian cell culture





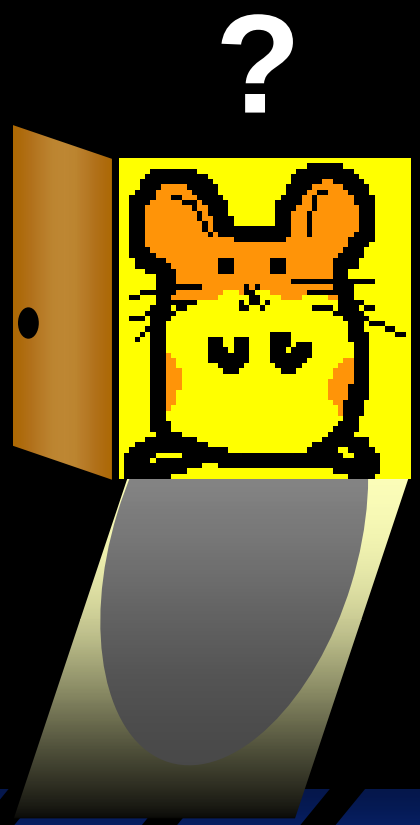


Immortal Cell Lines:
Chinese Hamster Ovary (CHO)
NS0 Mouse Myeloma
Human Embryonic Kidney: HEK 293
Baby Hamster Kidney (BHK)
SP2/0 Mouse Myeloma
Hybridomas

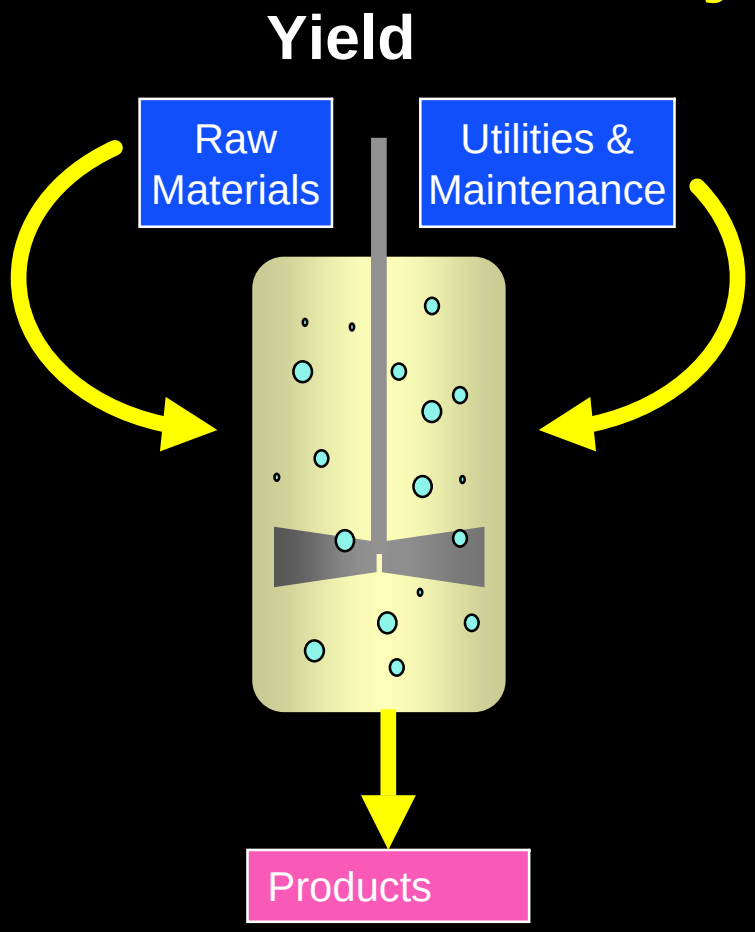
Therapeutic Proteins from Mammalian Cell Biotechnology

- 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

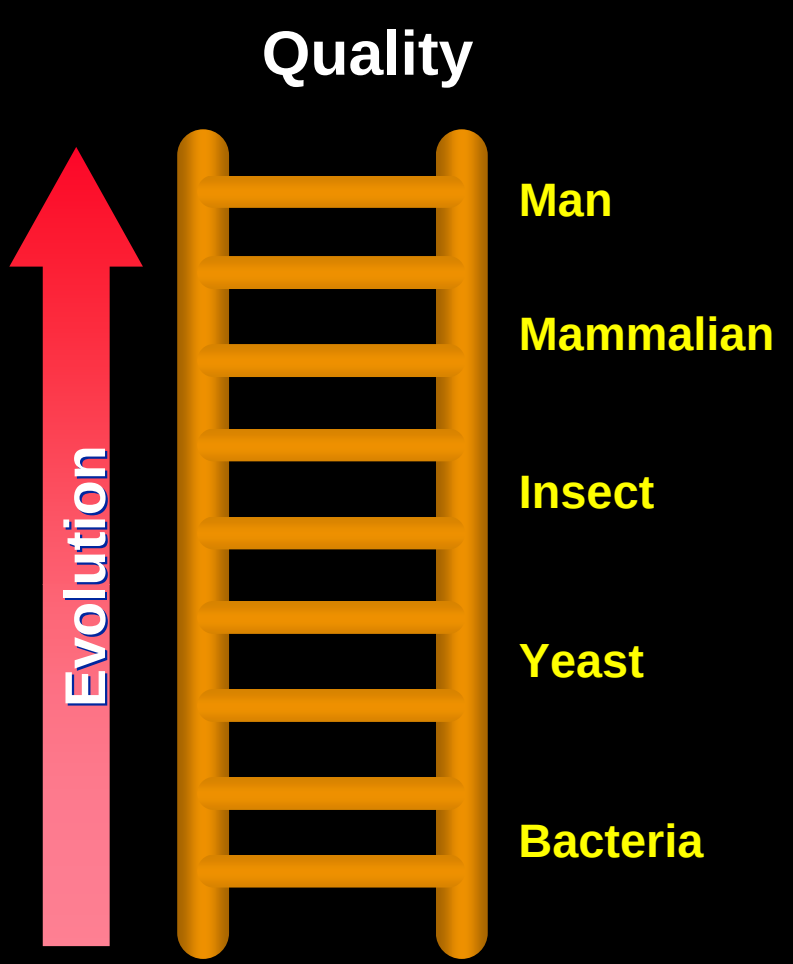
Why are mammalian cells the biotherapeutic champions?



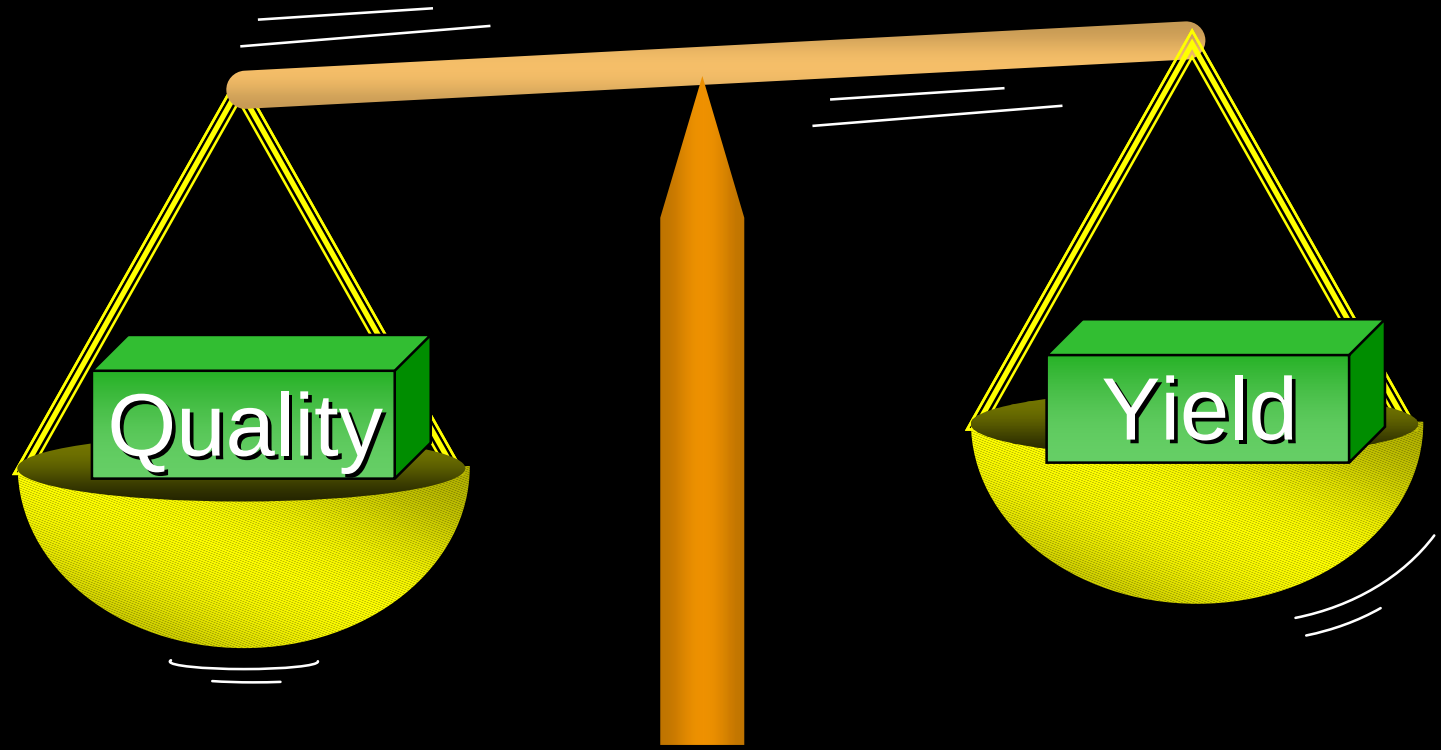
Factors in Choosing a Production System



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



The Engineering Problem: Quality versus Yield

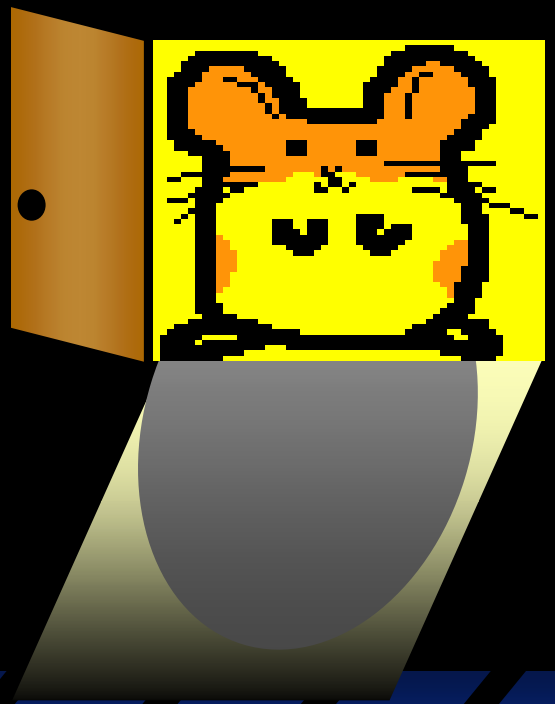


Essential pharmacological properties

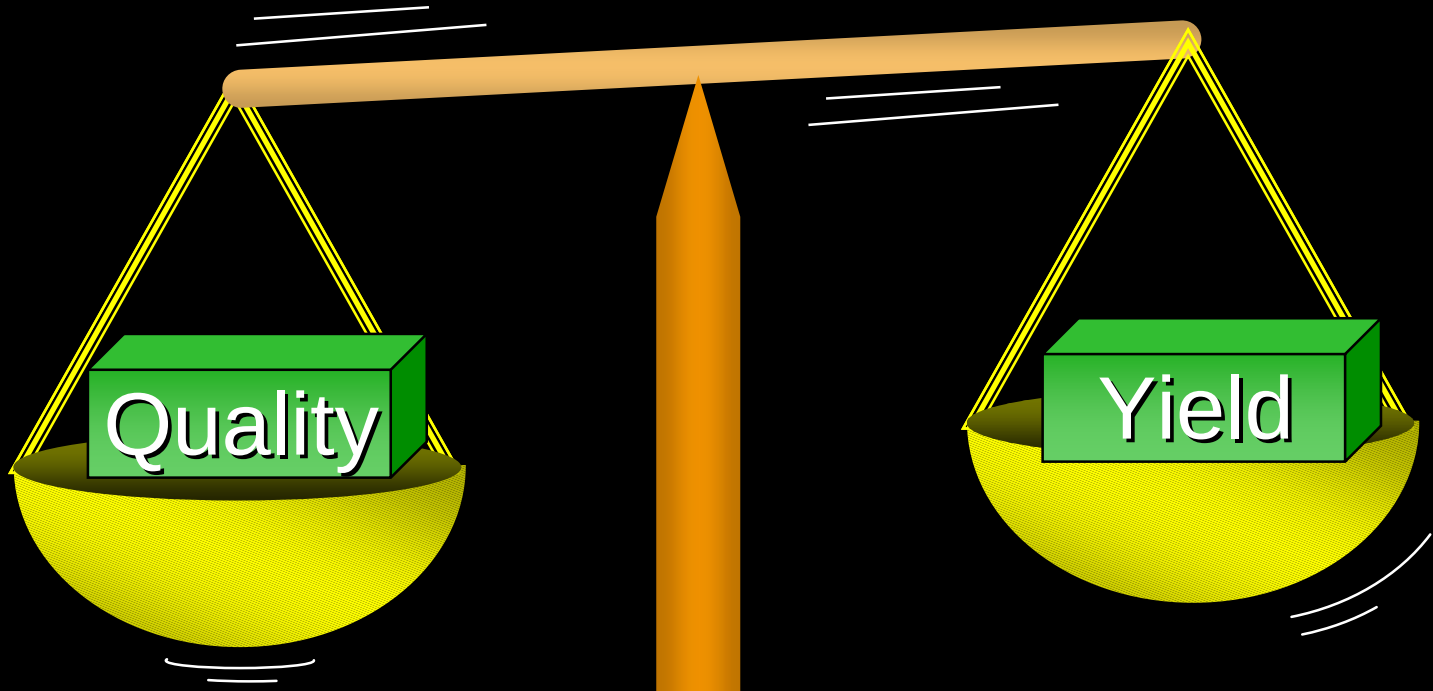
Efficient and inexpensive production scheme

Why are mammalian cells the biotherapeutic champions?

High Quality in spite of Lower Yields



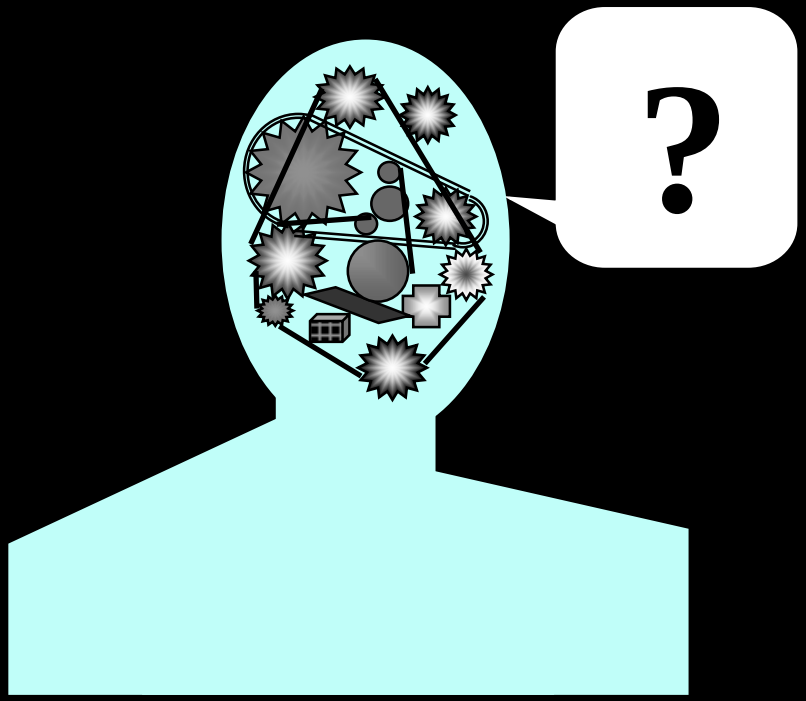
Current Approach : Quality trumps Yield



Essential pharmacological properties

Efficient and inexpensive production scheme

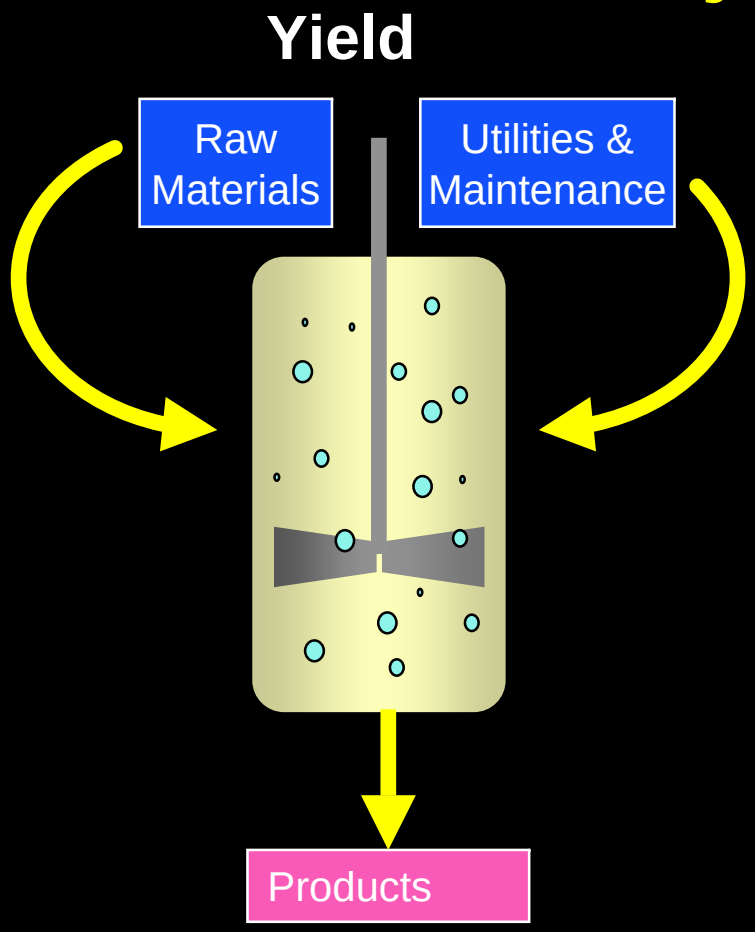
Question?...



Can we solve the engineering problem?

Can metabolic engineering provide high yields and high quality?

Factors in Choosing a Production System



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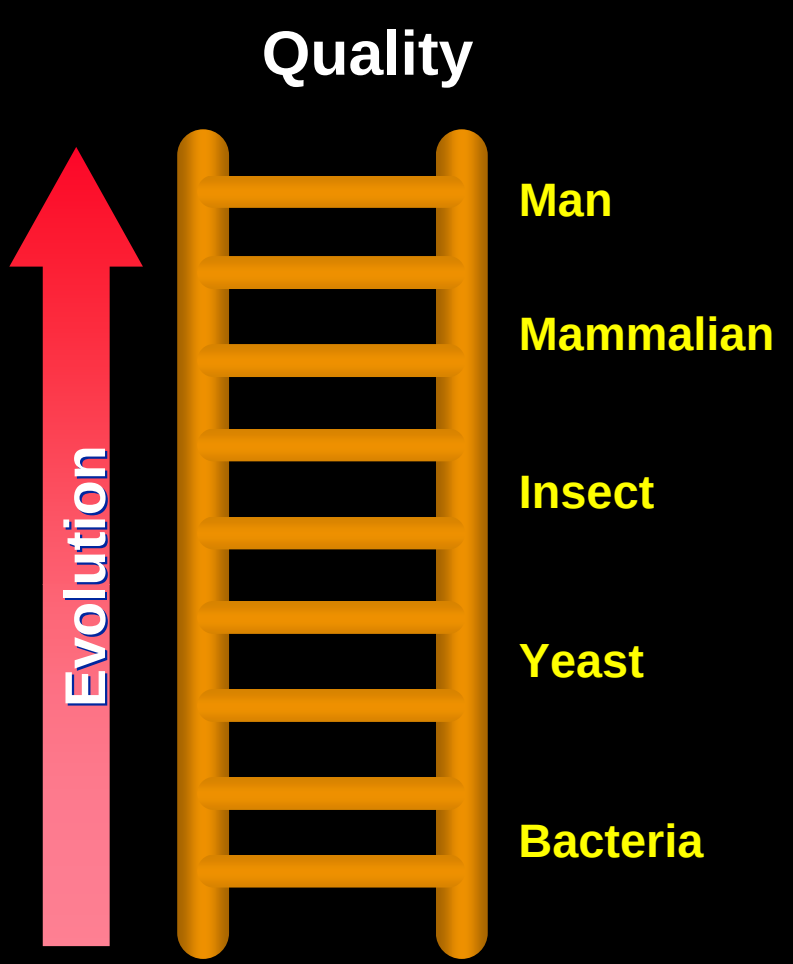
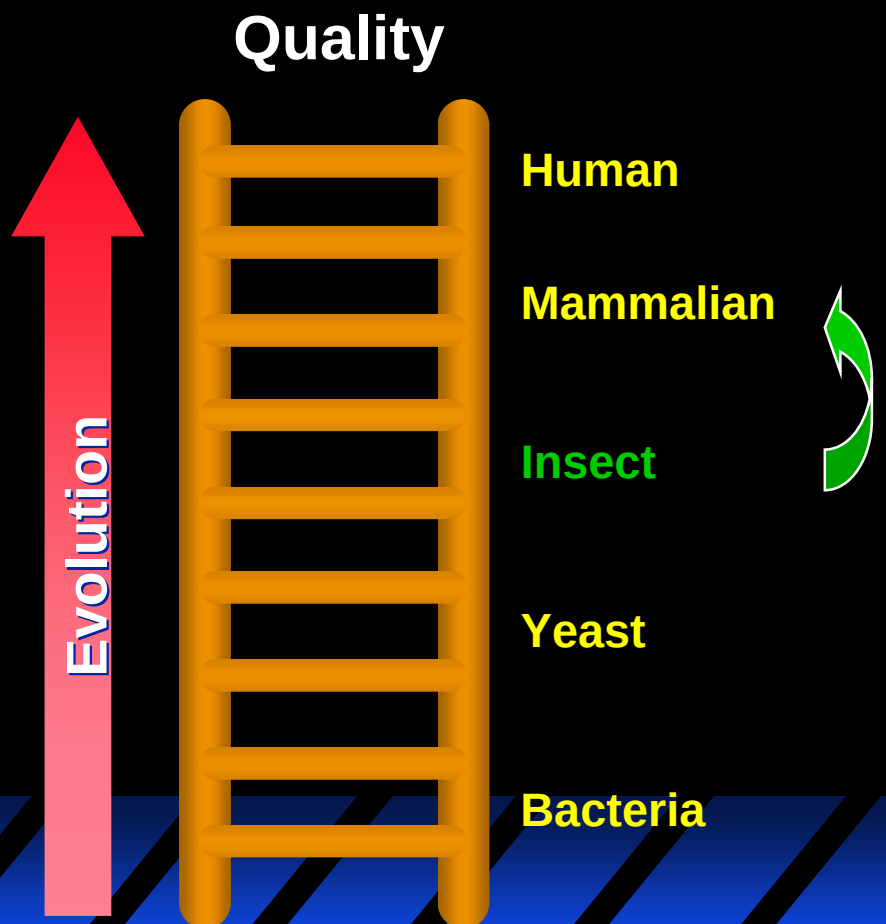
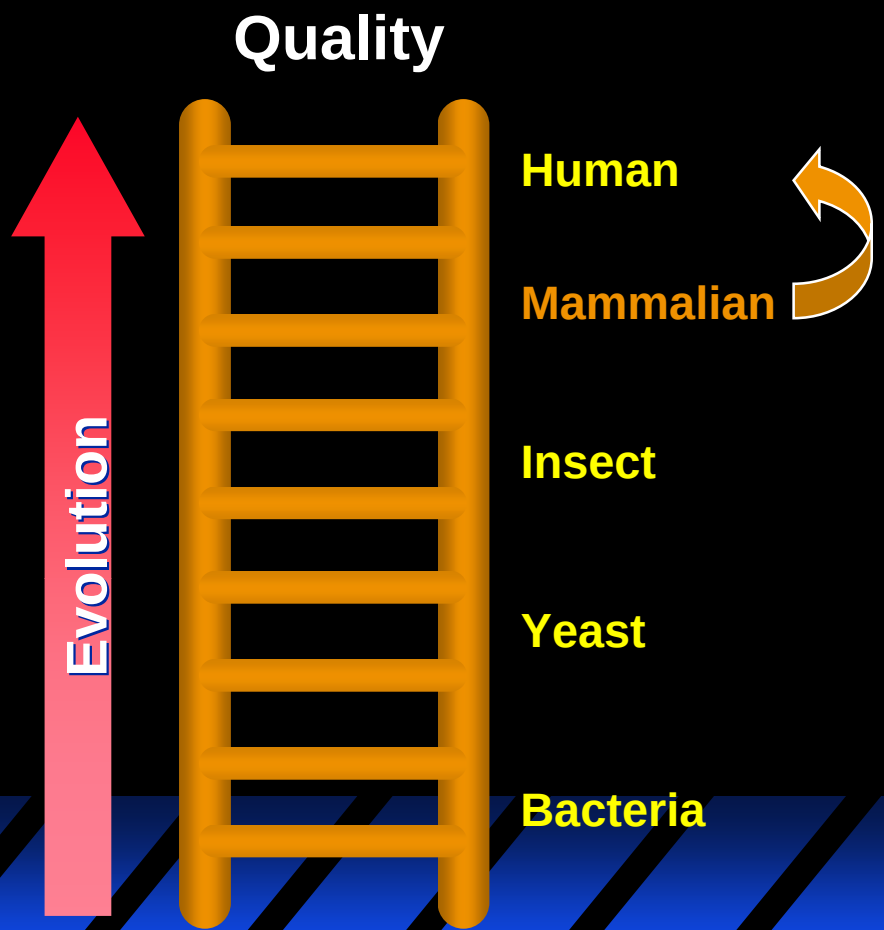


(1) Can metabolic engineering improve quality of biotherapeutics from insect cells?

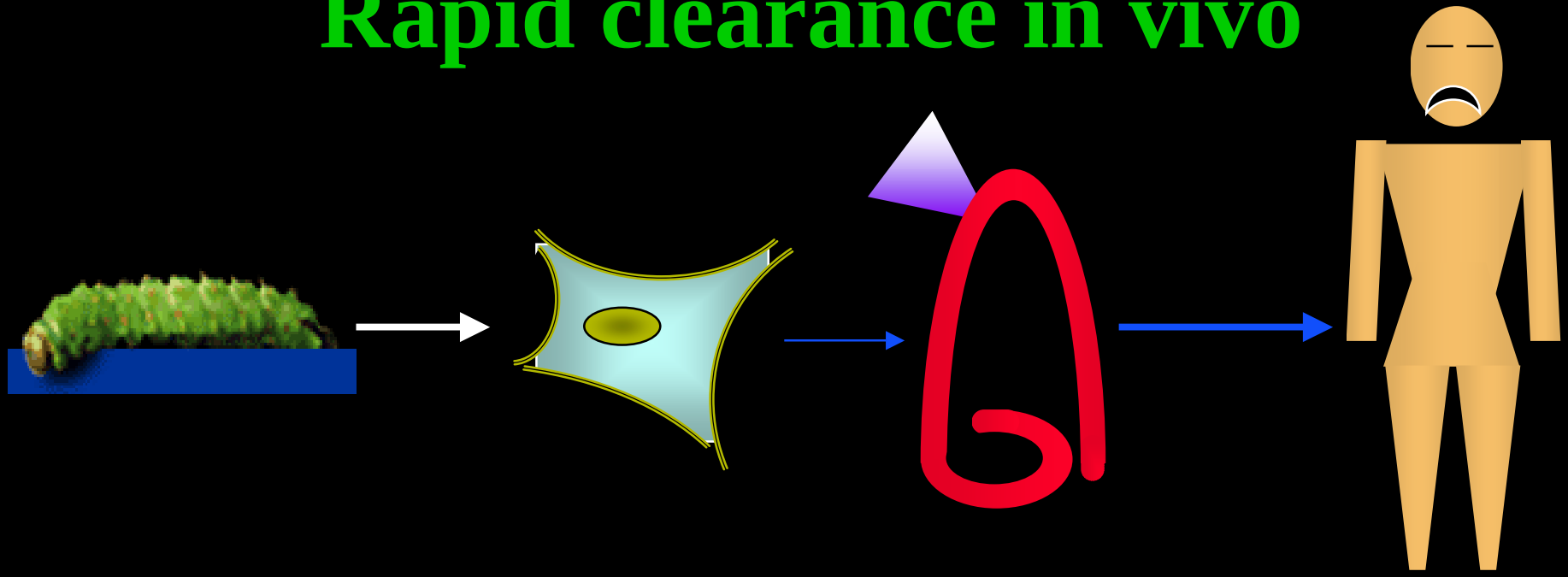


(2) Can metabolic engineering "humanize" biotherapeutic quality from mammalian cells?



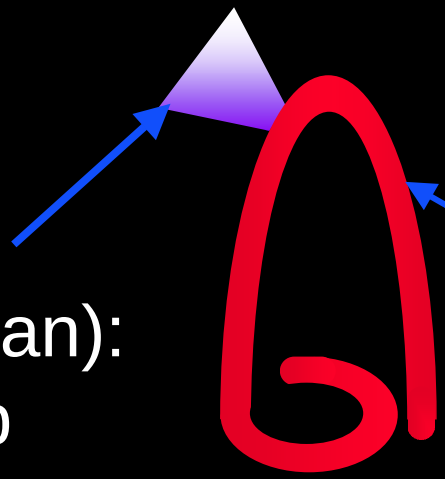
What is “quality” and why are mammalian cells champs over insects?

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?



Polypeptide Structure

Glycosylation (N-glycan):
Attached sugar group

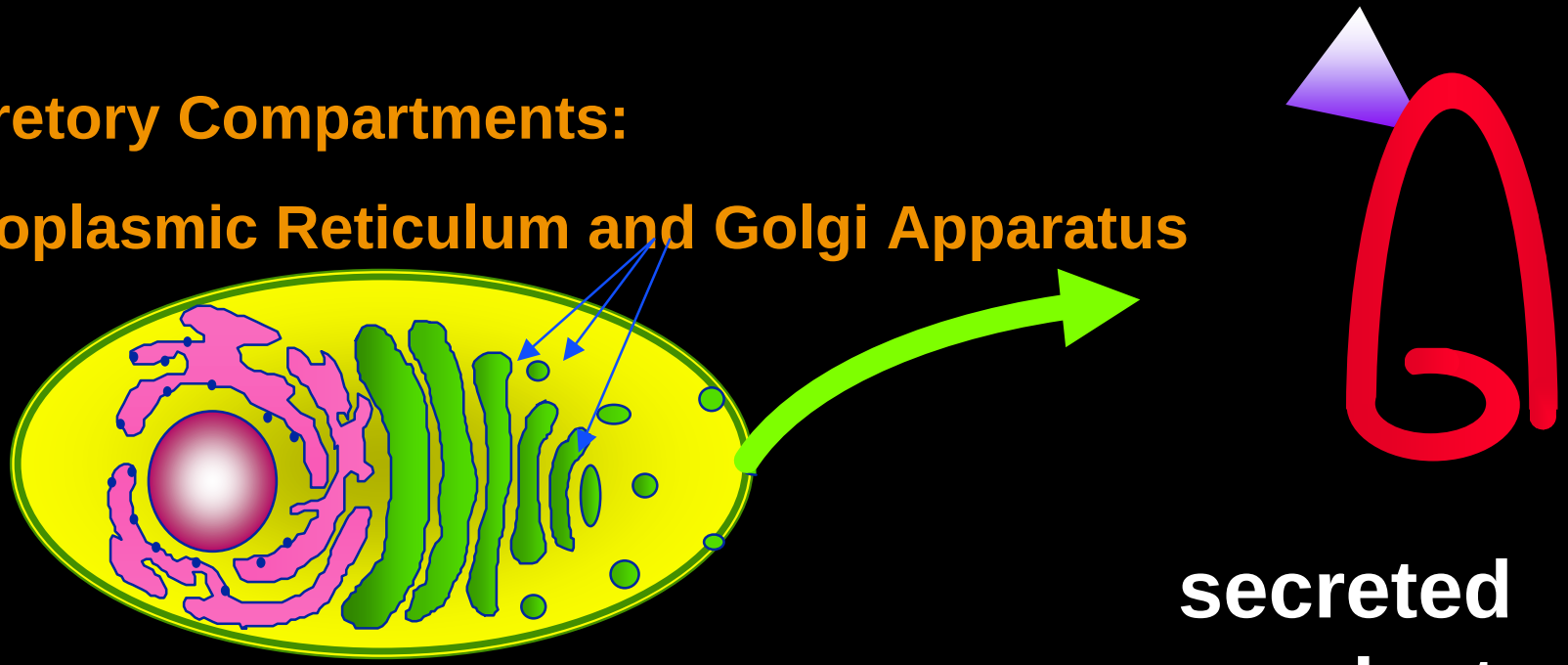
- Identical Polypetide Structures-Insect vs. Mammals
- Different Glycosylation Patterns: Insects vs. Mammals

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

Glycosylation Processing in the cell

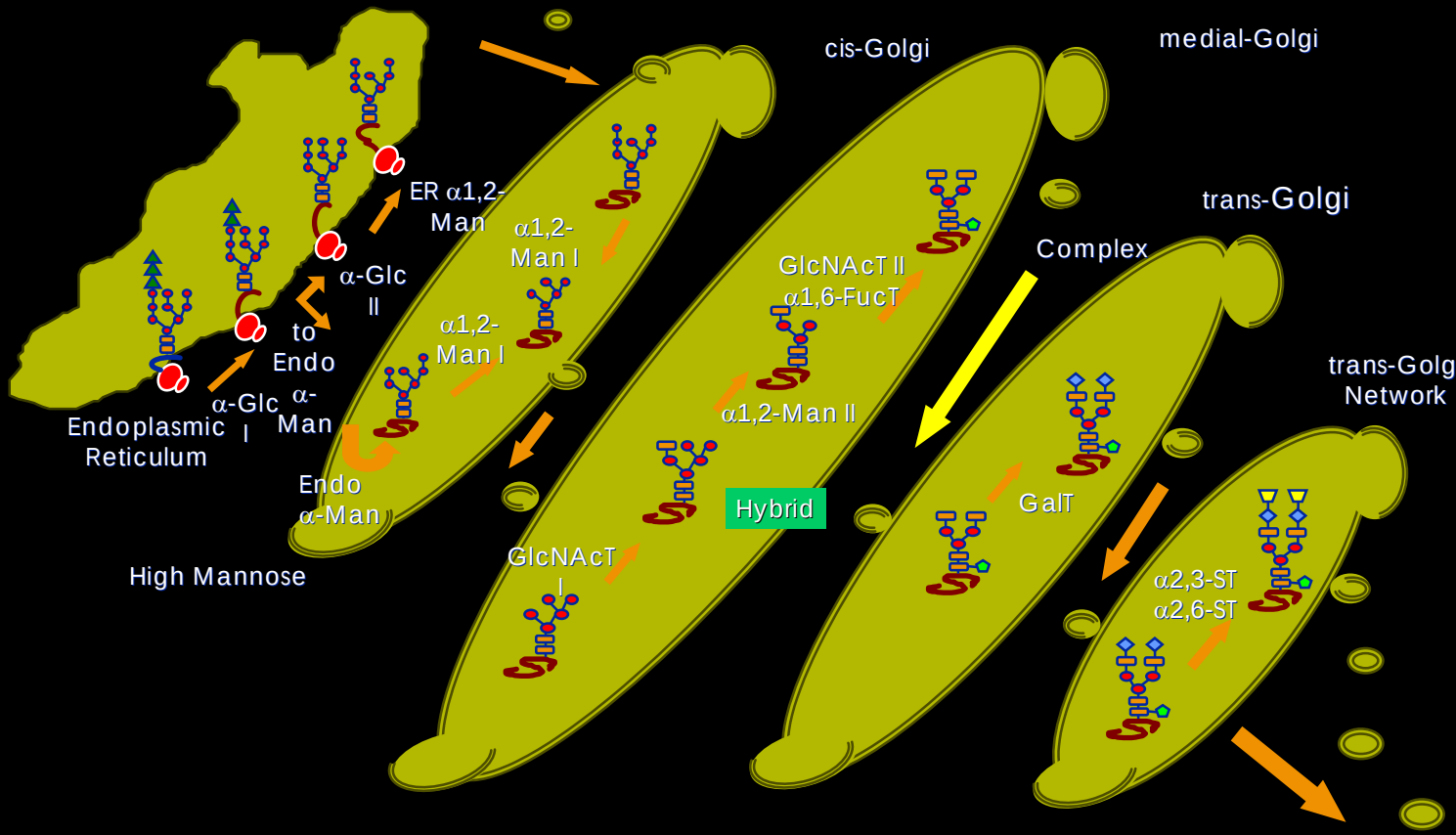
Secretory Compartments:

Endoplasmic Reticulum and Golgi Apparatus

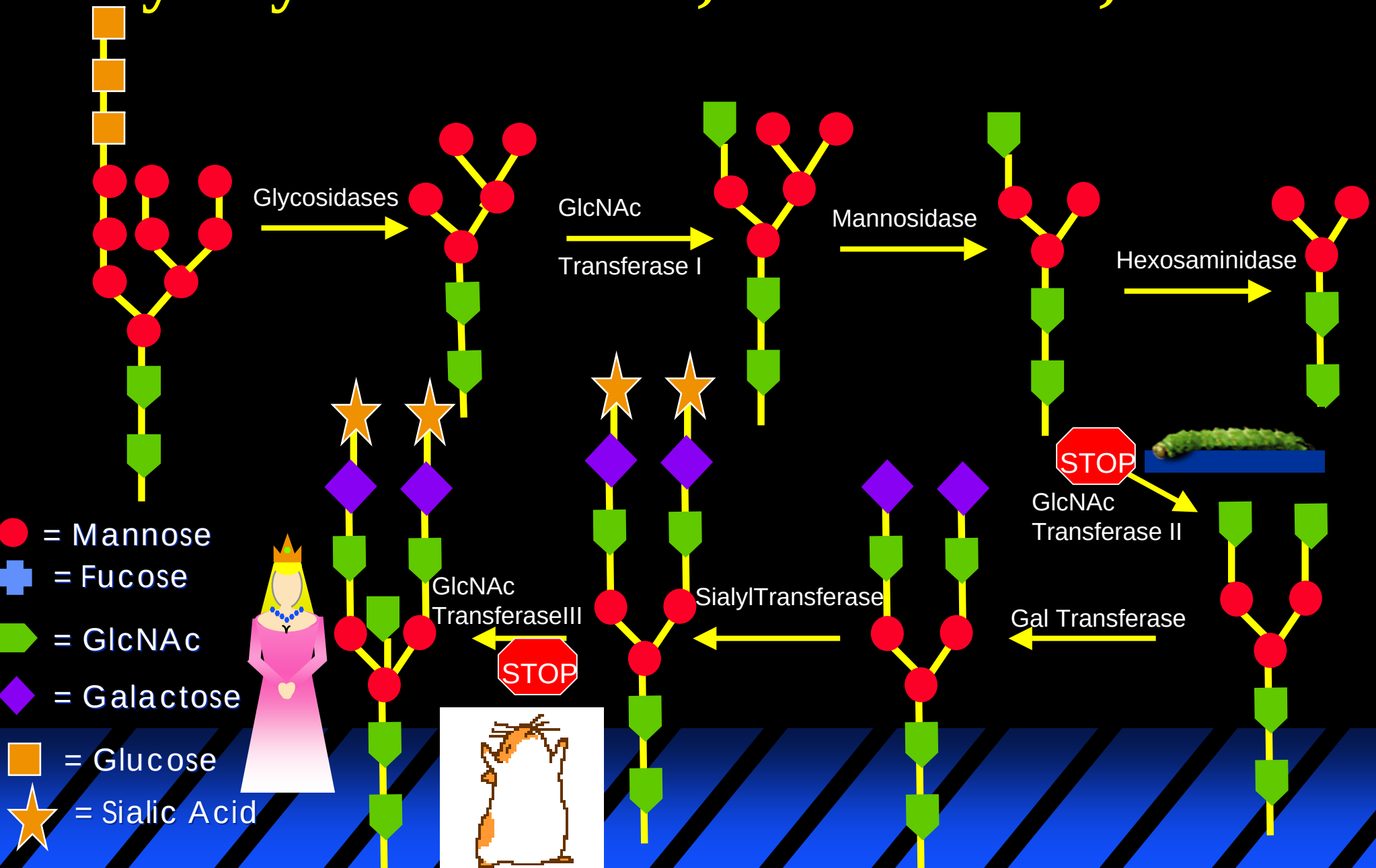


secreted product

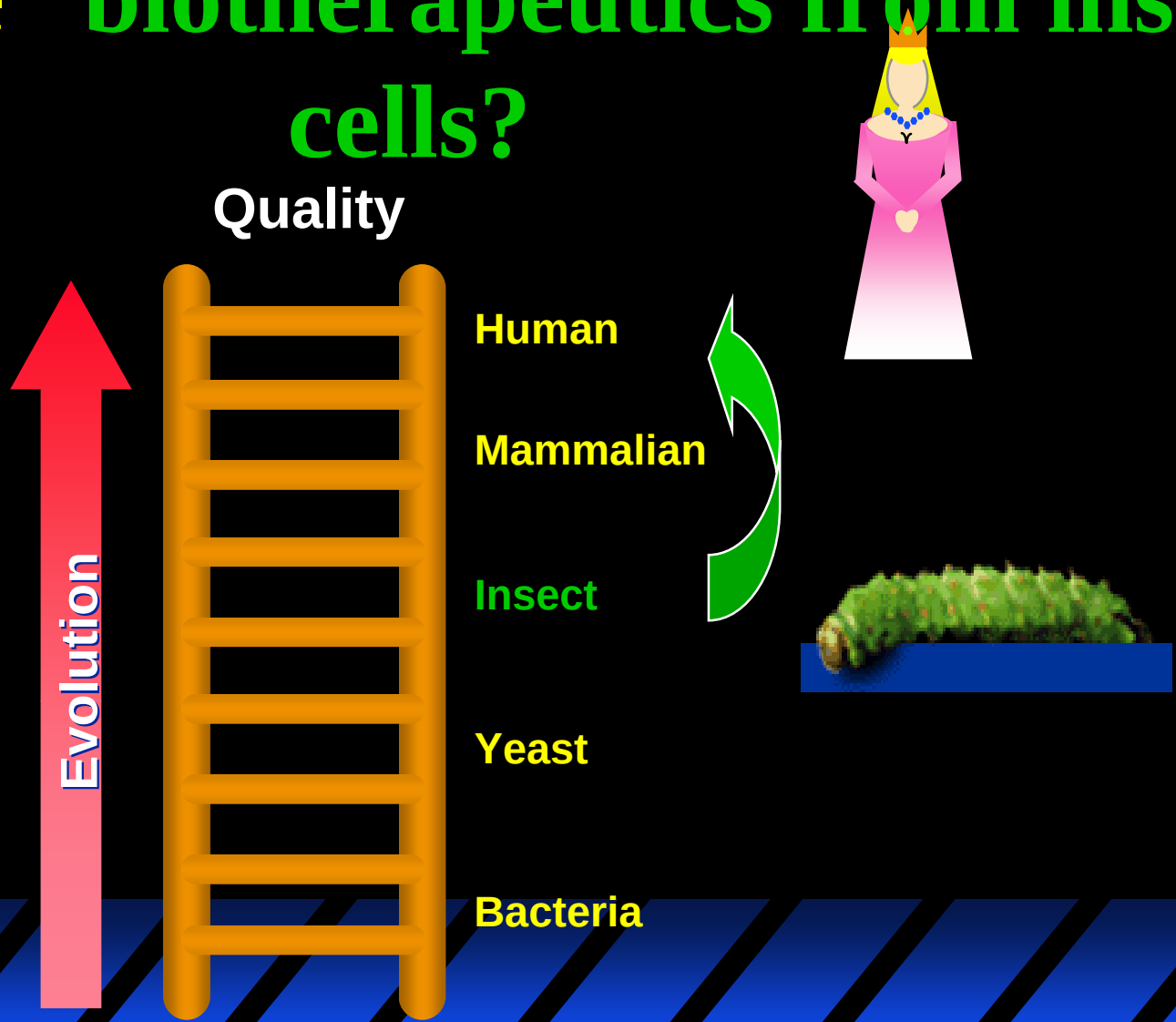
Glycosylation Processing in the Cell



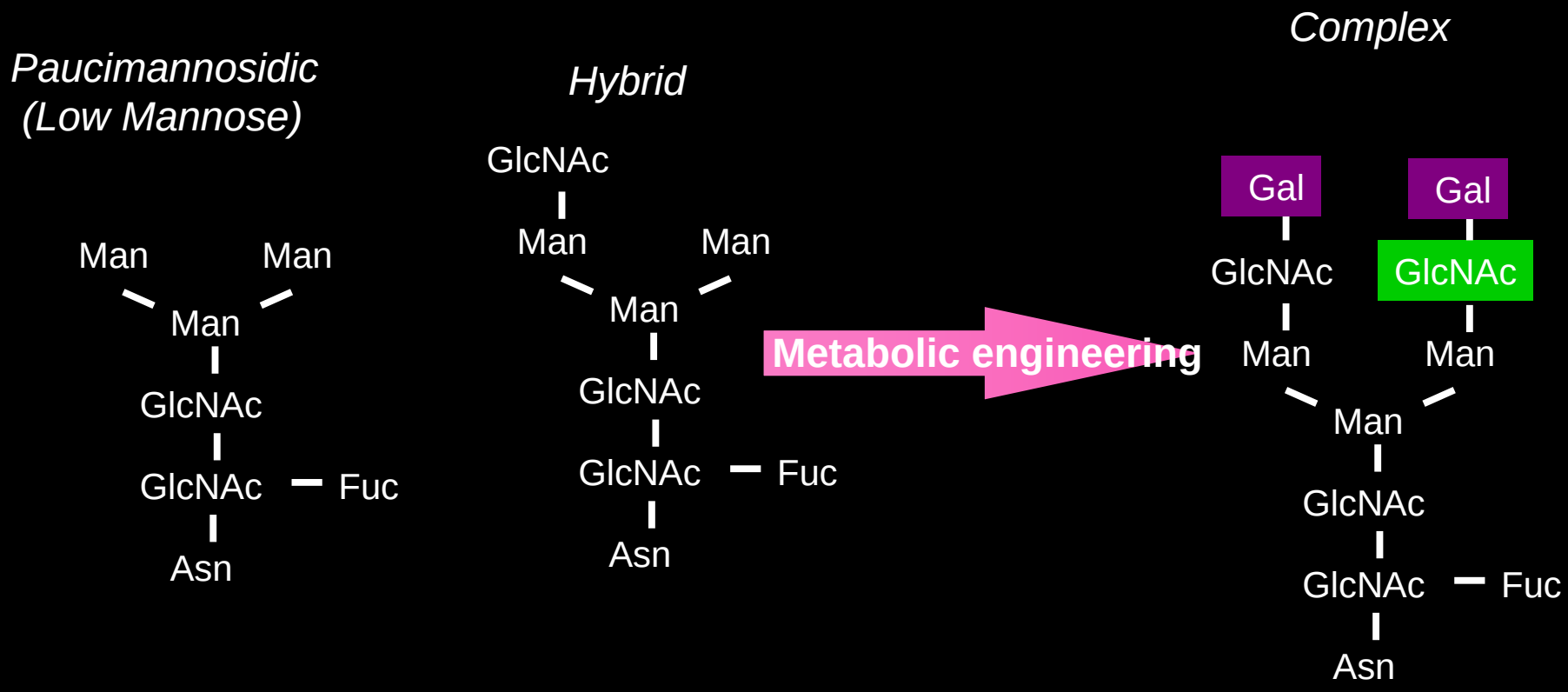
Glycosylation: Insect, Mammalian, Human



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



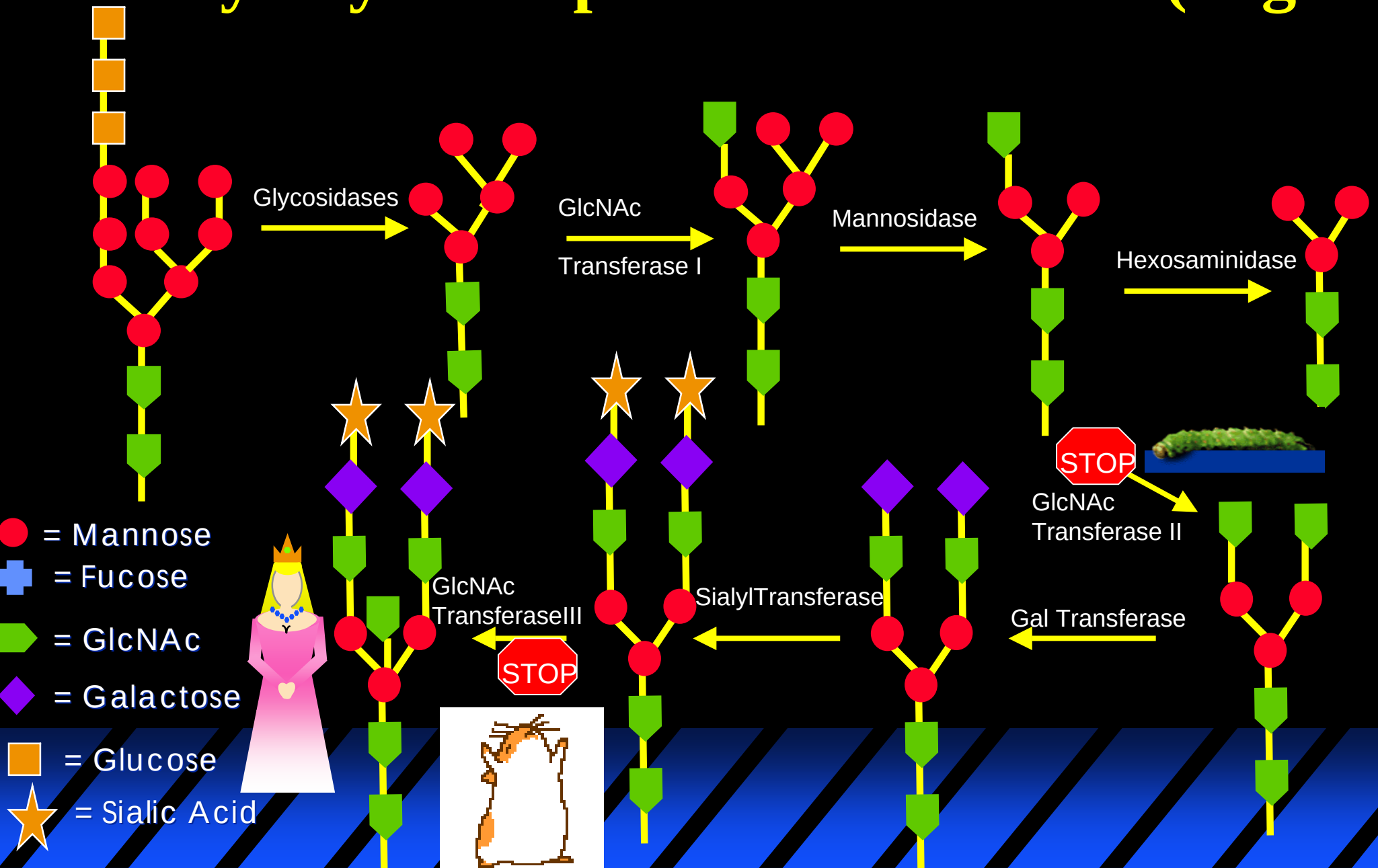
Humanizing Insect Cell Glycosylation: Add GlcNAc and Galactose (Gal)



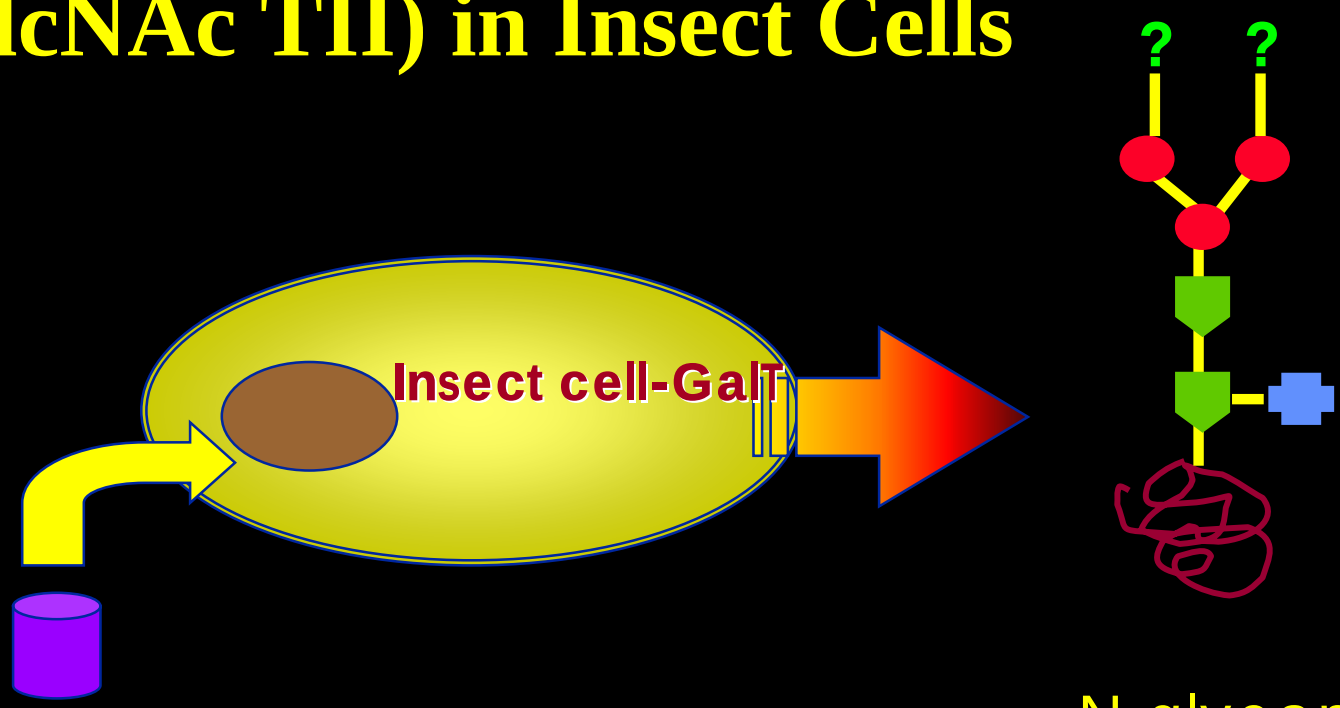
Insect Cells

Engineered Insect Cells?

Glycosylation patterns from T. ni (High 5)



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

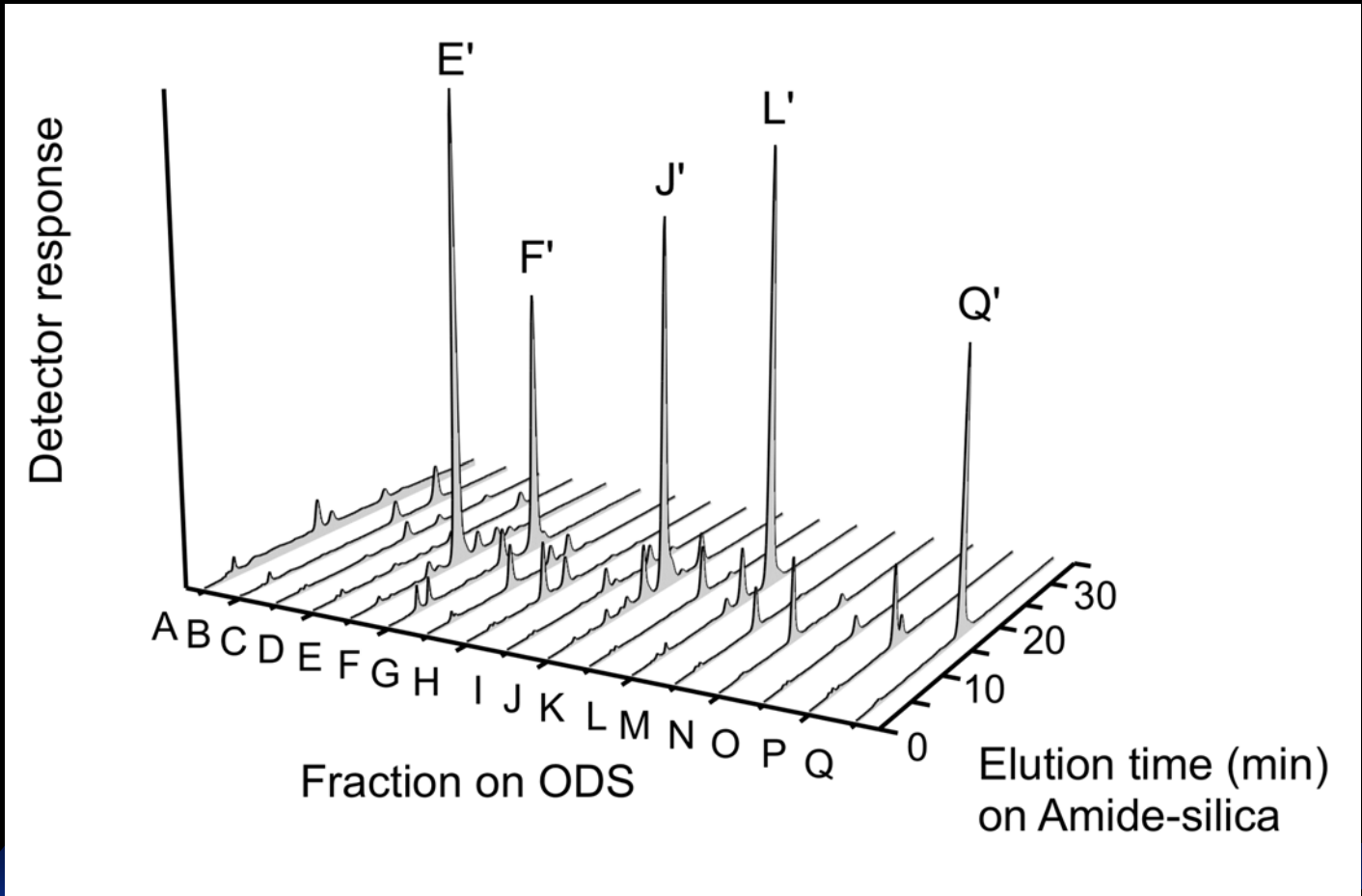


Baculovirus with Mammalian GlcNAc Transferase TII (GlcNAc TII)/ target protein

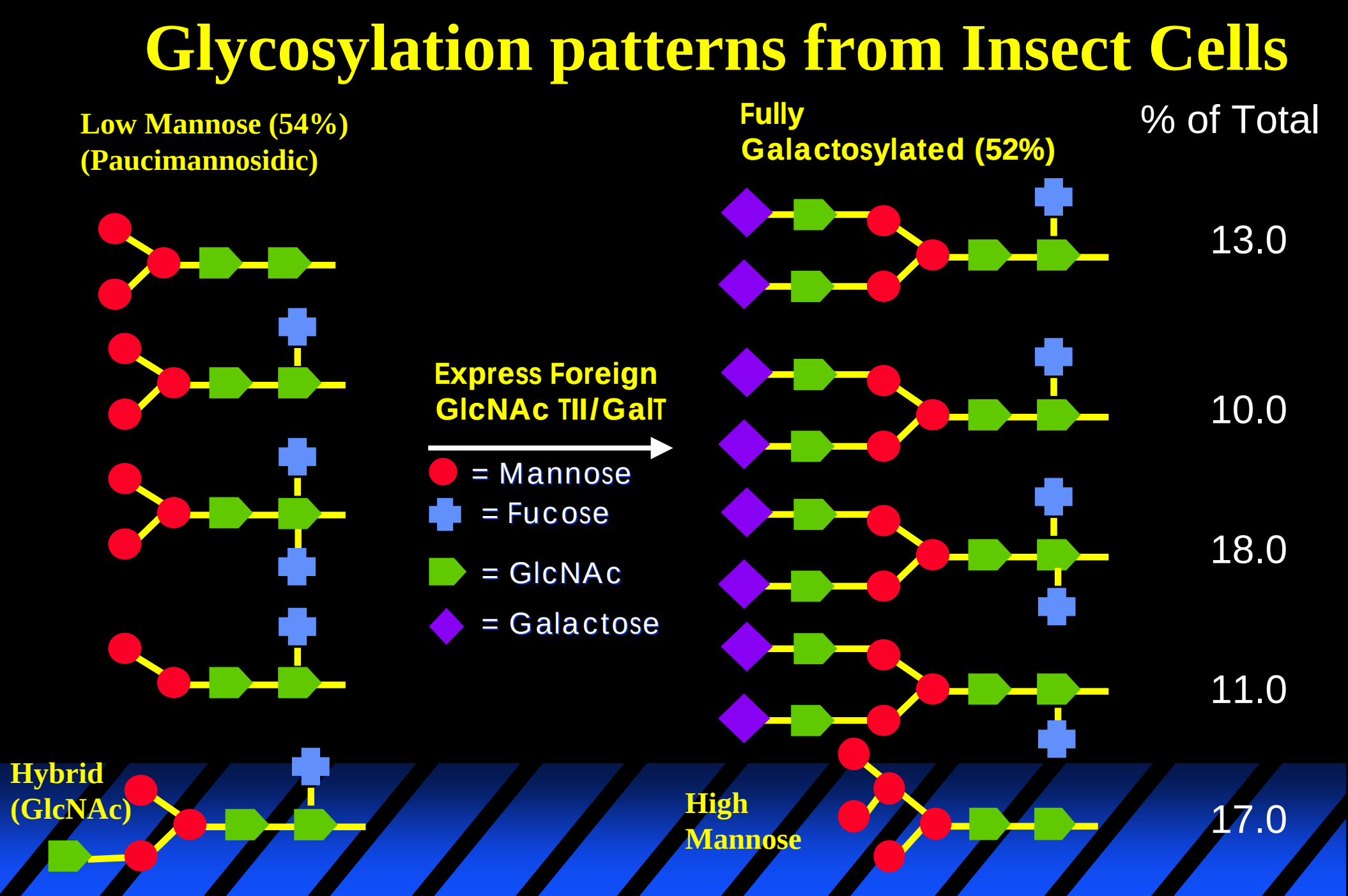
N-glycan patterns?

Collaborator: Don Jarvis, Univ. of Wyoming

HPLC Analysis of Glycosylation

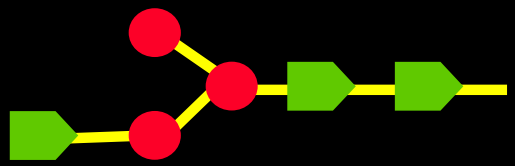


Glycosylation patterns from Insect Cells



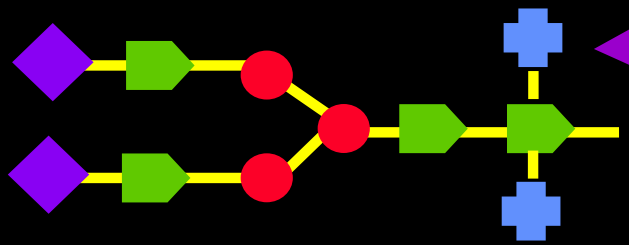
GlcNAc TII/Gal T makes N-glycans more “mammalian” –fully galactosylated

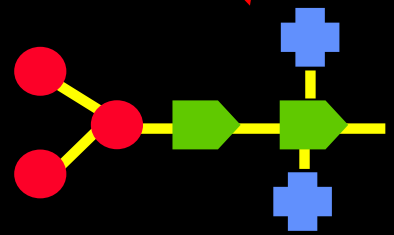
Insect N-glycan



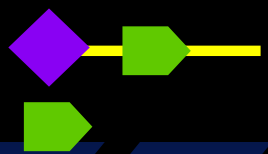
Without GlcNAc TII/Gal T

With GlcNAc TII/Gal T



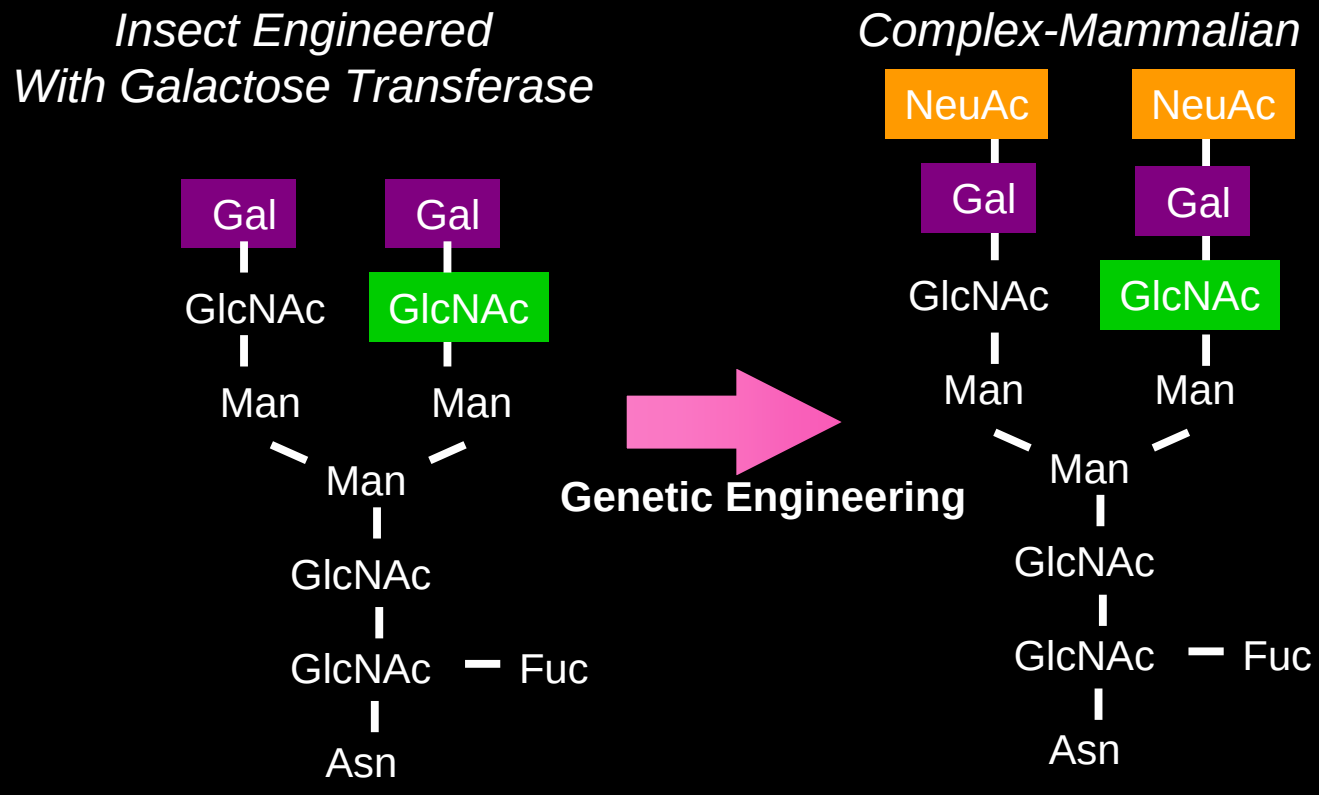


1. Adds GlcNAc and Terminal Galactose
2. Blocks Removal of GlcNAc



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

Next, we must add sialic acid to insect cell glycosylation

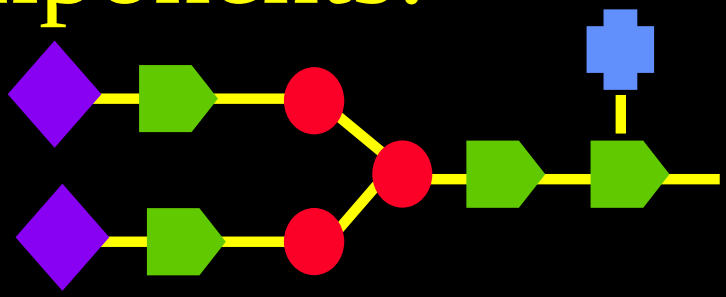


Engineered Insect Cells

Re-Engineered Insect Cells?

Sialylation reaction requires three components:

1. Galactose-terminated Structure



✓

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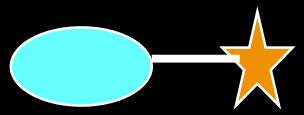
2) CMP-Sialic acid-CMP-Neu5Ac
(nucleotide sugar-Cytidine Monophospho-N-acetylneuraminic acid)



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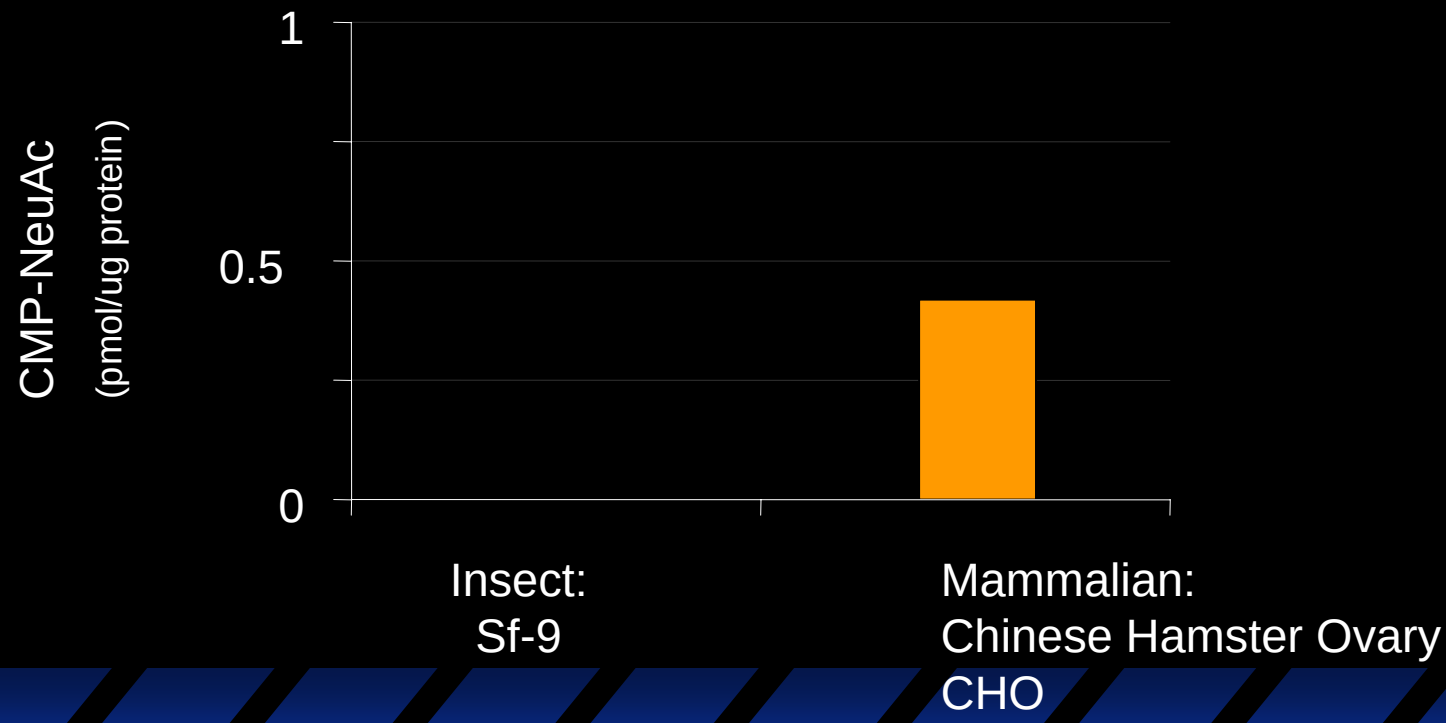
3) Sialyltransferase

?

But CMP-Sialic Acid is not present in Insect Cells



Can we make CMP-Sialic Acid?

UDP-GlcNAc

X *UDP-GlcNAc Epimerase/ ManNAc Kinase*

N-Acetylmannosamine (ManNAc)

Phosphoenyl Pyruvate (PEP)

X *Sialic Acid Synthase*

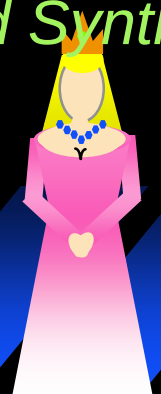
N-acetylneuraminic Acid (NeuAc or Sialic Acid)

X *CMP-Sialic Acid Synthase*

CTP

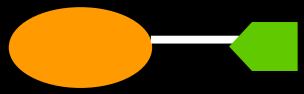
2Pi

?



CMP-N-acetylneuraminic Acid (CMP-NeuAc)

Mammalian Enzymes Engineered into Insect Cells



UDP-GlcNAc

- *UDP-GlcNAc Epimerase/ ManNAc Kinase*

N-Acetylmannosamine (ManNAc)

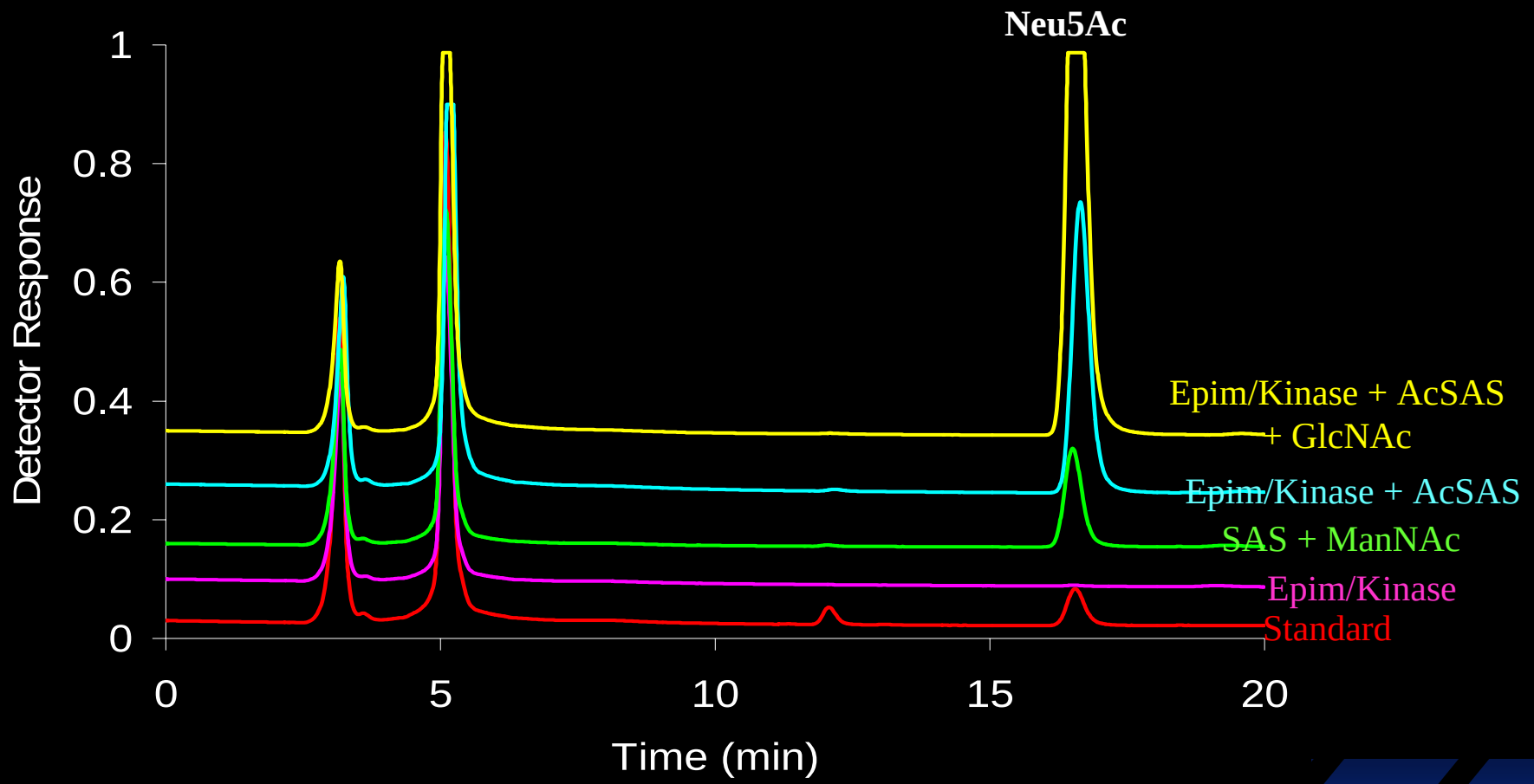
Phosphoenyl Pyruvate (PEP)

- *Sialic Acid Synthase (SAS)*

?

N-acetylneuraminic Acid (NeuAc or Sialic Acid)

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



Can we make CMP-Sialic Acid?

UDP-GlcNAc

UDP-GlcNAc Epimerase/ ManNAc Kinase

N-Acetylmannosamine (ManNAc)

Phosphoenyl Pyruvate (PEP)

Sialic Acid Synthase

N-acetylneuraminic Acid (NeuAc or Sialic Acid)

CMP-Sialic Acid Synthase

CTP

2Pi

?

CMP-N-acetylneuraminic Acid (CMP-NeuAc)

Can we make CMP-Sialic Acid?

Add ManNAc in Medium

N-Acetylmannosamine (ManNAc)

Phosphoenyl Pyruvate (PEP)

✔ *Sialic Acid Synthase*

N-acetylneuraminic Acid (NeuAc or Sialic Acid)

✔ *CMP-Sialic Acid Synthase*

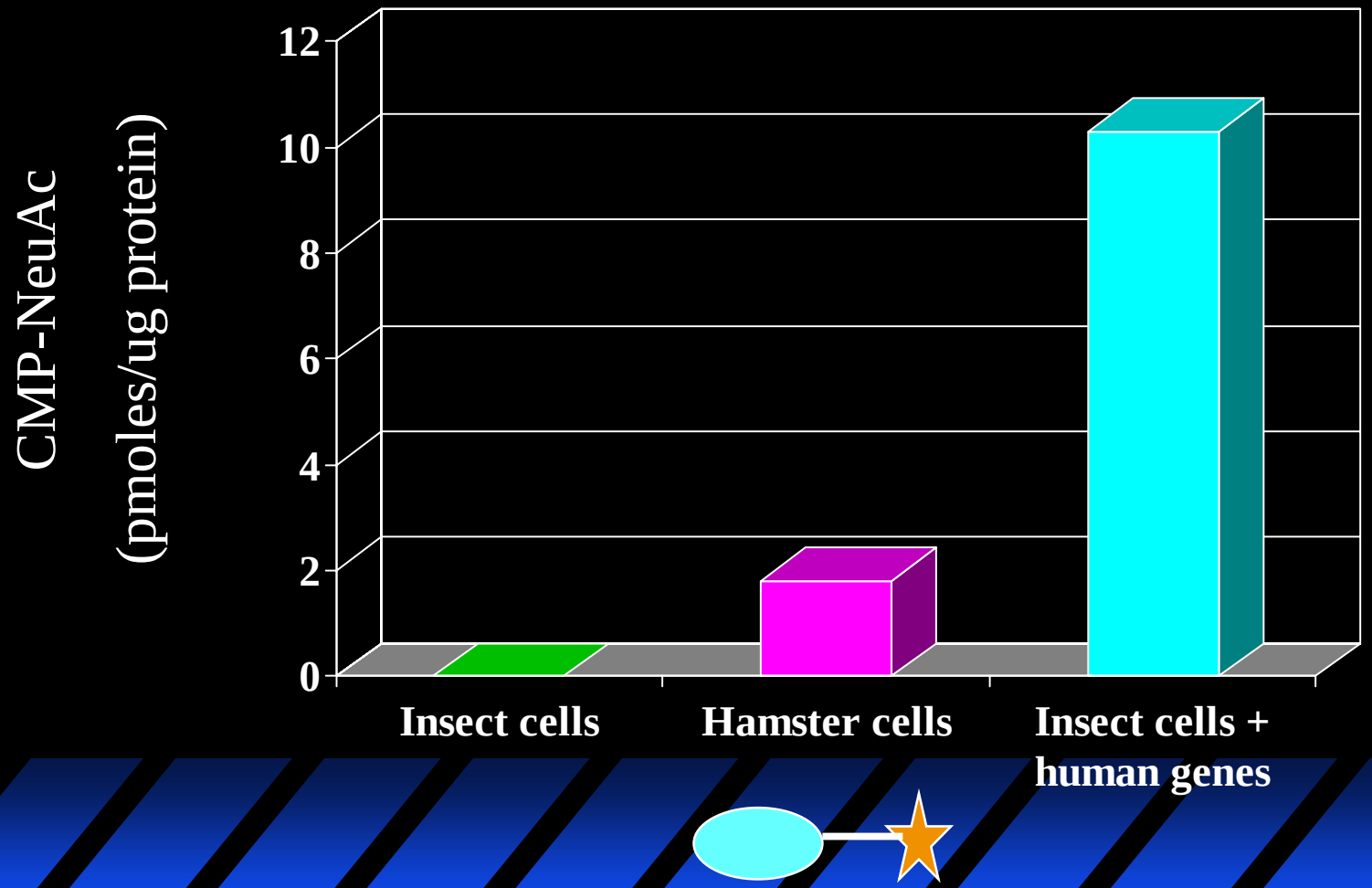
CTP

2Pi

?

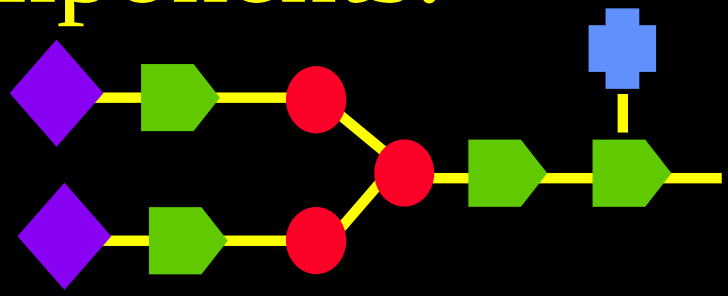
CMP-N-acetylneuraminic Acid (CMP-NeuAc)

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



Sialylation reaction requires three components:

1. Galactose-terminated Acceptor



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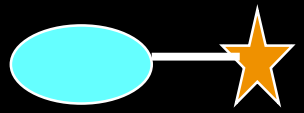
2) CMP-Neu5Ac (nucleotide sugar)
(Cytidine Monophospho-
N-acetylneuraminic acid/Sialic Acid)



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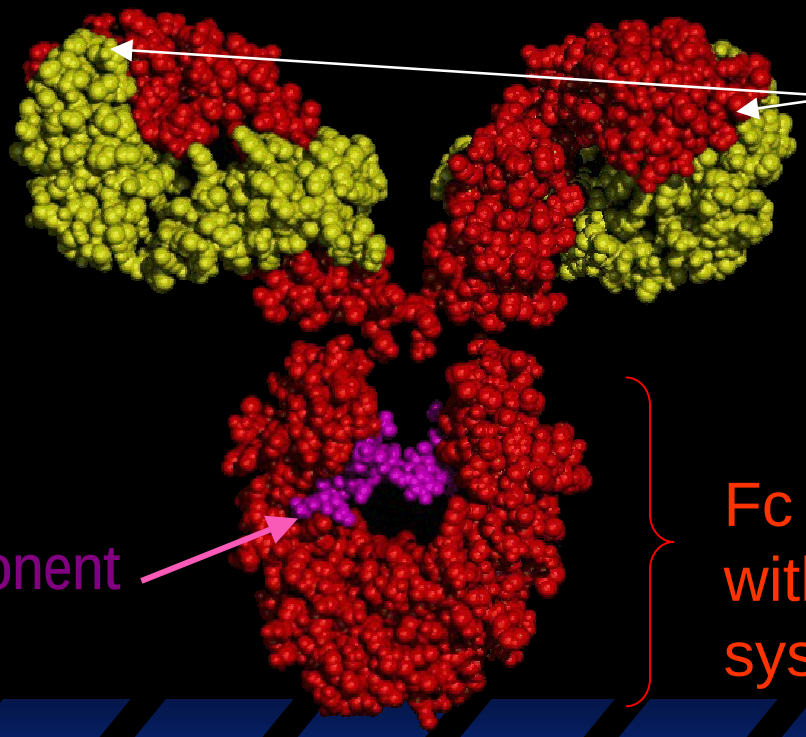
3) Sialyltransferase

Don Jarvis-Wyoming

But you have to put them all together too….

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



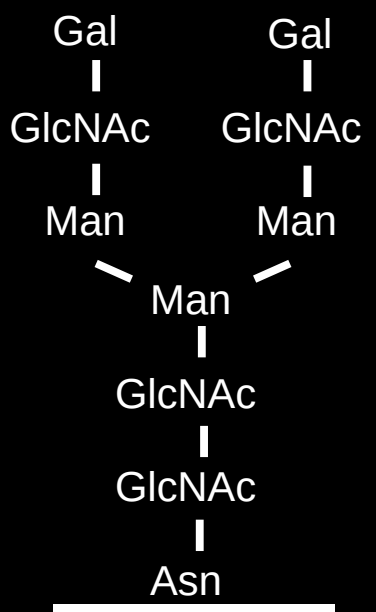
Fv Variable region
interacts with target antigen

Carbohydrate component

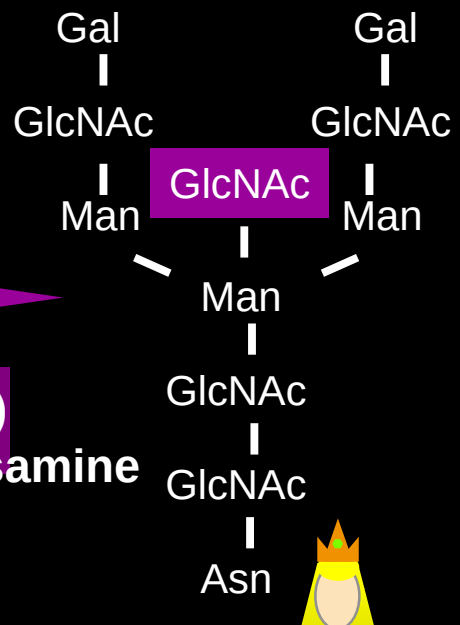
Fc Constant region: interacts with cells from immune system (NK Killer cells)

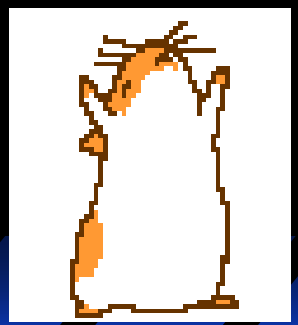
Umana et al., Nature Biotechnol.

Humanizing Hamster&Mouse Glycosylation: Bisecting GlcNAc

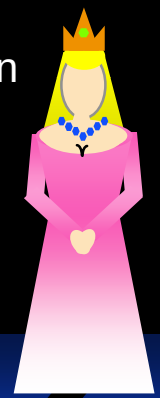


Metabolic engineering:
Add Bisecting N-acetylglucosamine (GlcNAc)
By expressing N-acetylglucosamine Transferase III (GlcNAc TIII)



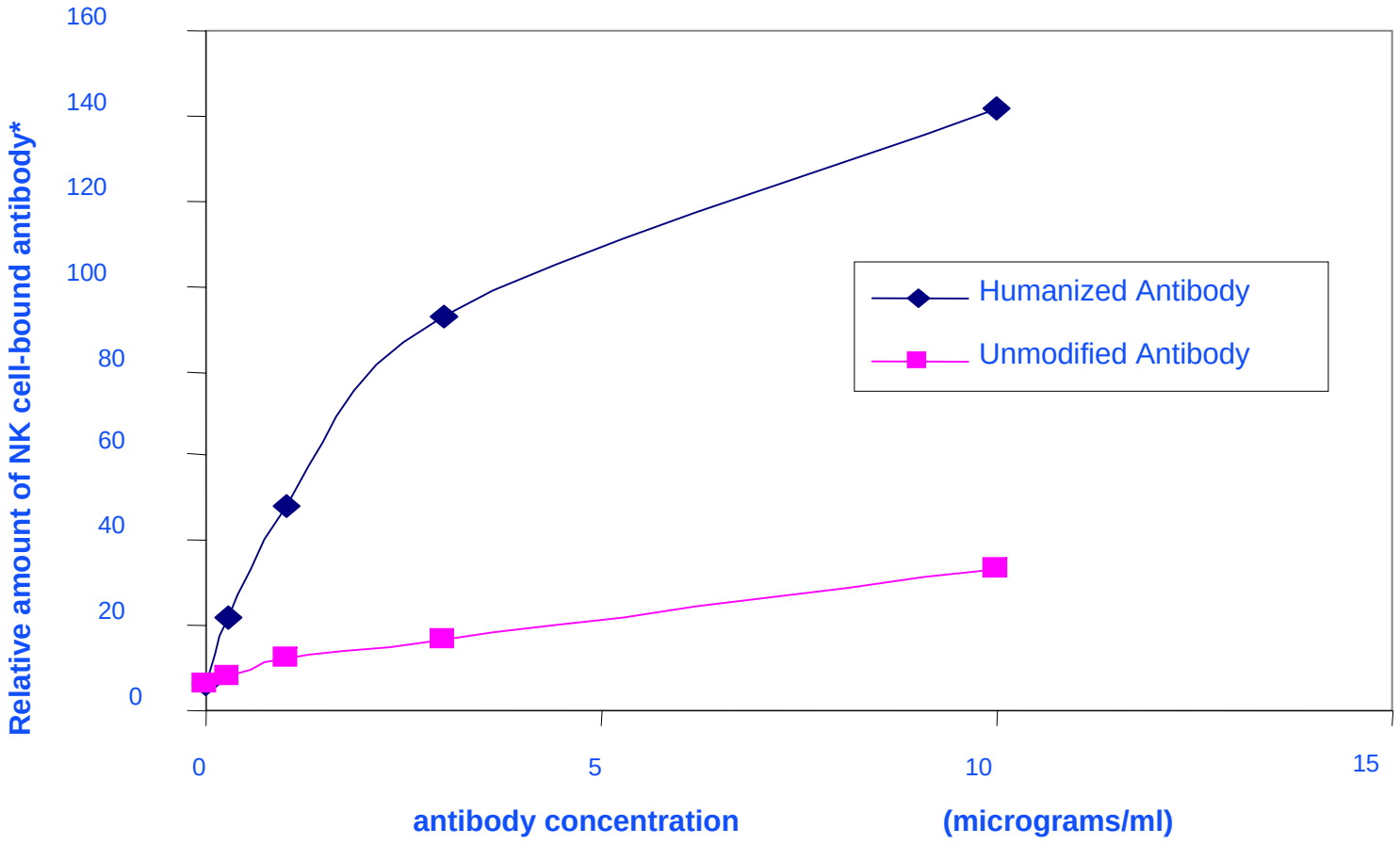


Chinese Hamster Ovary (CHO) and NSO Mouse Cells



Human cells and Humanized CHO&NSO

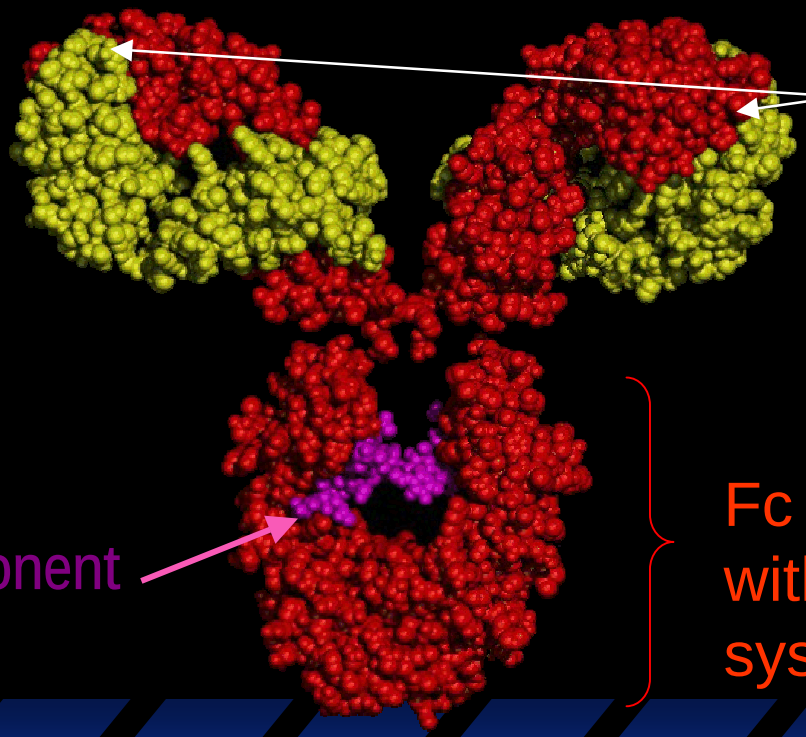
Immune Killer Cell Binding to Fc Region of Antibody



(* Proportional to mean fluorescence by FACS analysis)

Courtesy of P. Umana, Glycart, Inc.

Metabolic Engineering of Glycosylation Alters Antibody Structure/Function



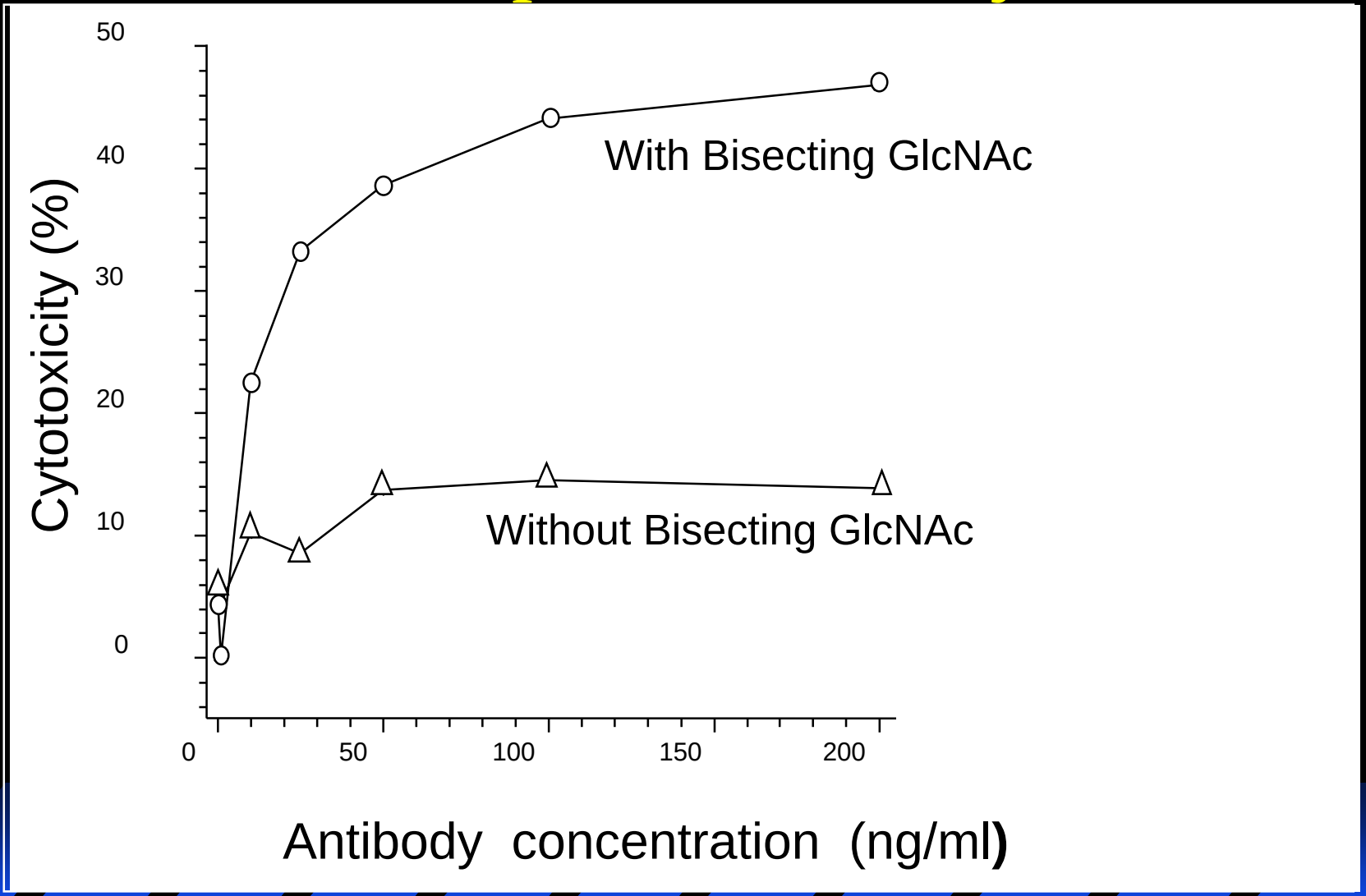
Fv Variable region
interacts with target antigen

Carbohydrate component

Fc Constant region: interacts with cells from immune system (NK Killer cells)

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

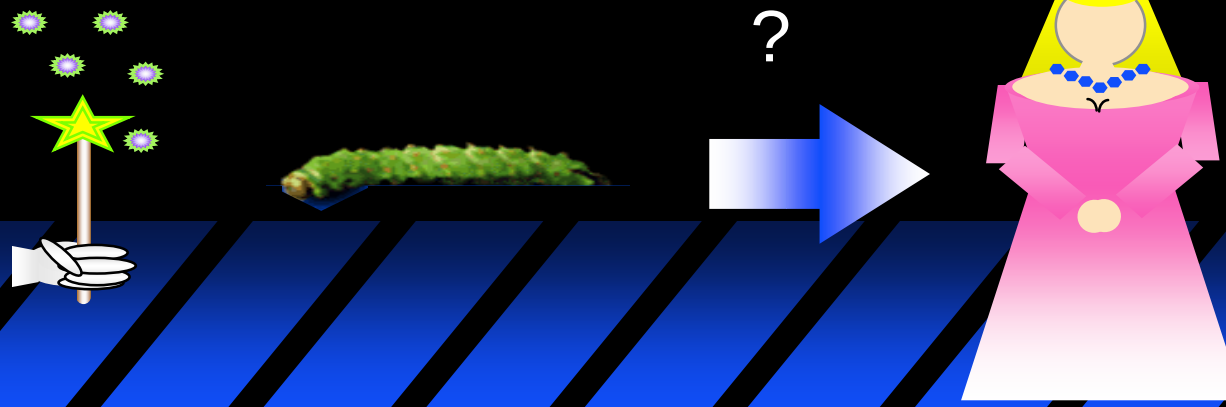
Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, Watier H.

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



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