Carbohydrate Engineering for Generating Sialylated Glycoproteins in Insect Cells

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Biotechnology & Pharmaceuticals

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ACTIVASE
ALTEPLASE
A RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR

ACTIVASE
ALTEPLASE
A RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR

Lilly

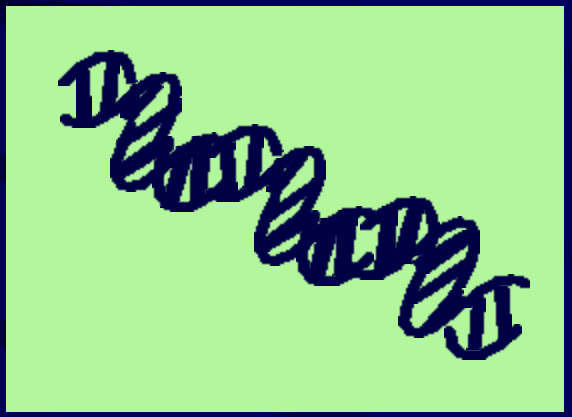
Humulin®
human insulin
(rDNA origin)

Genentech, Inc.
Medicines

Humulin®
human insulin
(rDNA origin)

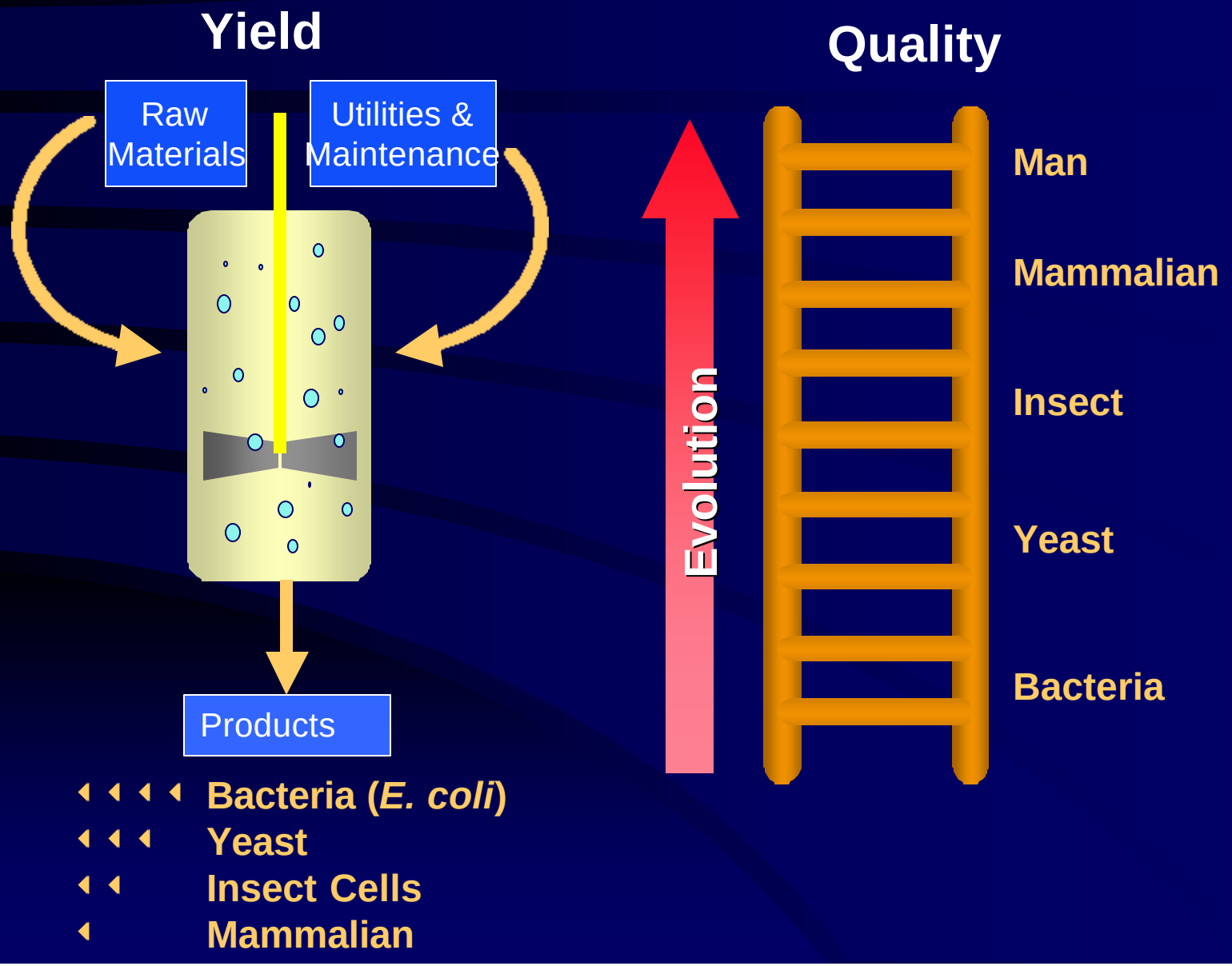
The Living Factory

Okay, cells, here's what they're tellin' us to produce!

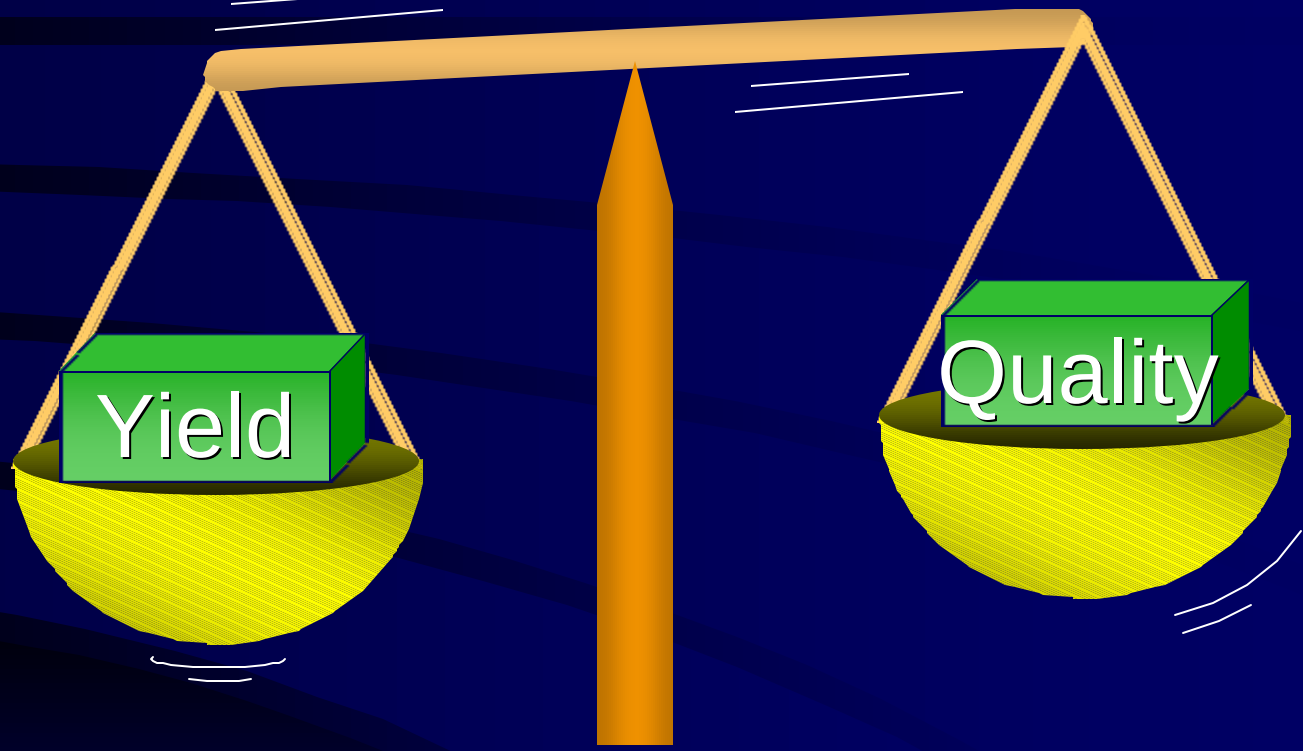


Product

Choosing a Production System



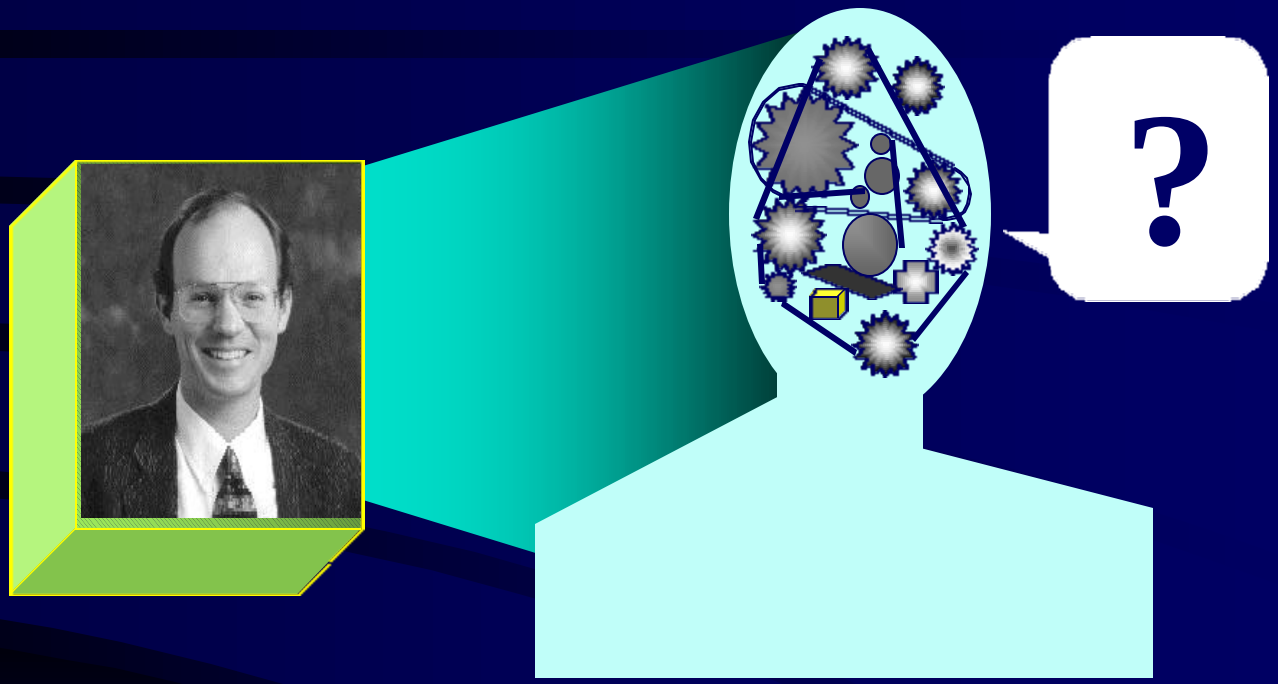
The Engineering Problem



Efficient and inexpensive production scheme

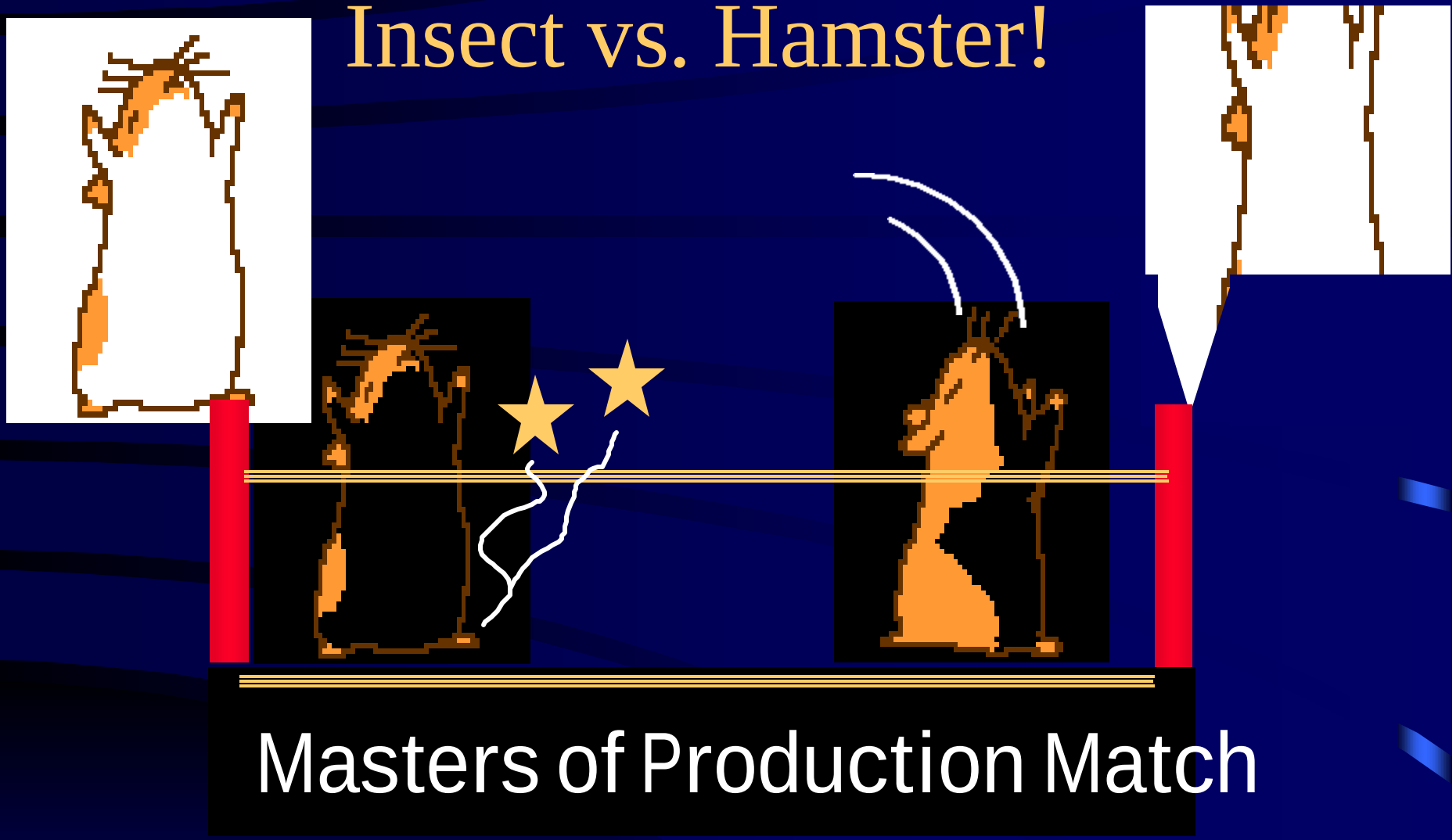
Essential pharmacological properties

Once upon a time...



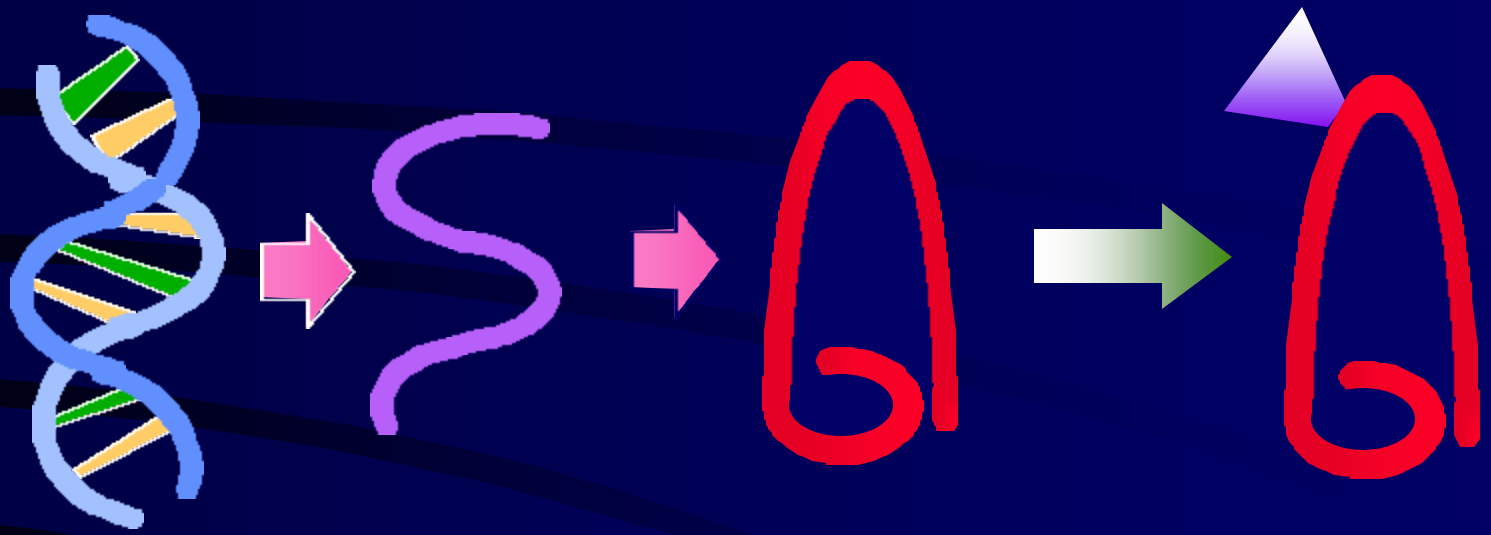
Goal: High yields and high quality

Insect vs. Hamster!



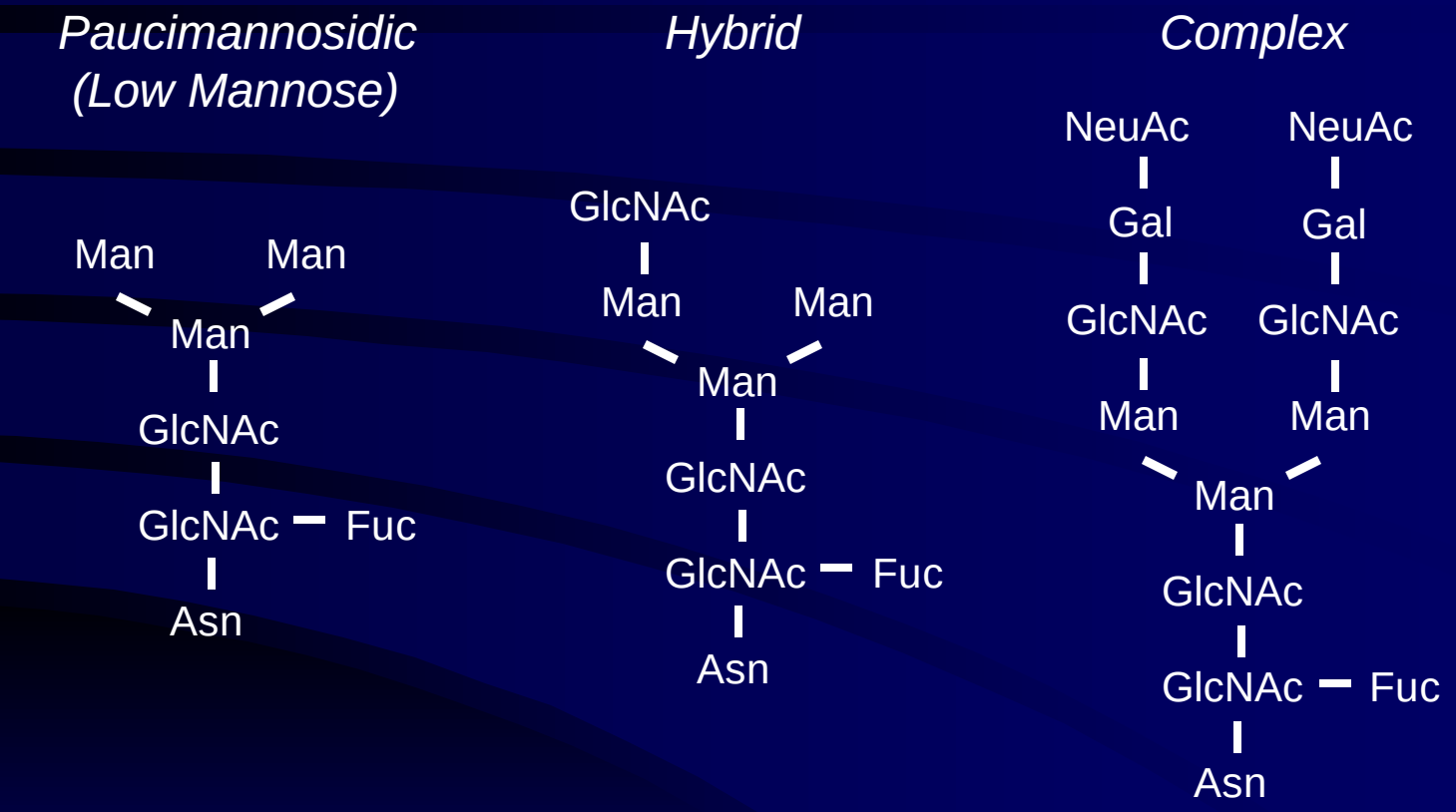
Masters of Production Match

Where do insect cells fall short?



DNA → RNA → Folded Protein → Active Sugar-modified Protein

N-glycan Processing: Insect versus Mammalian

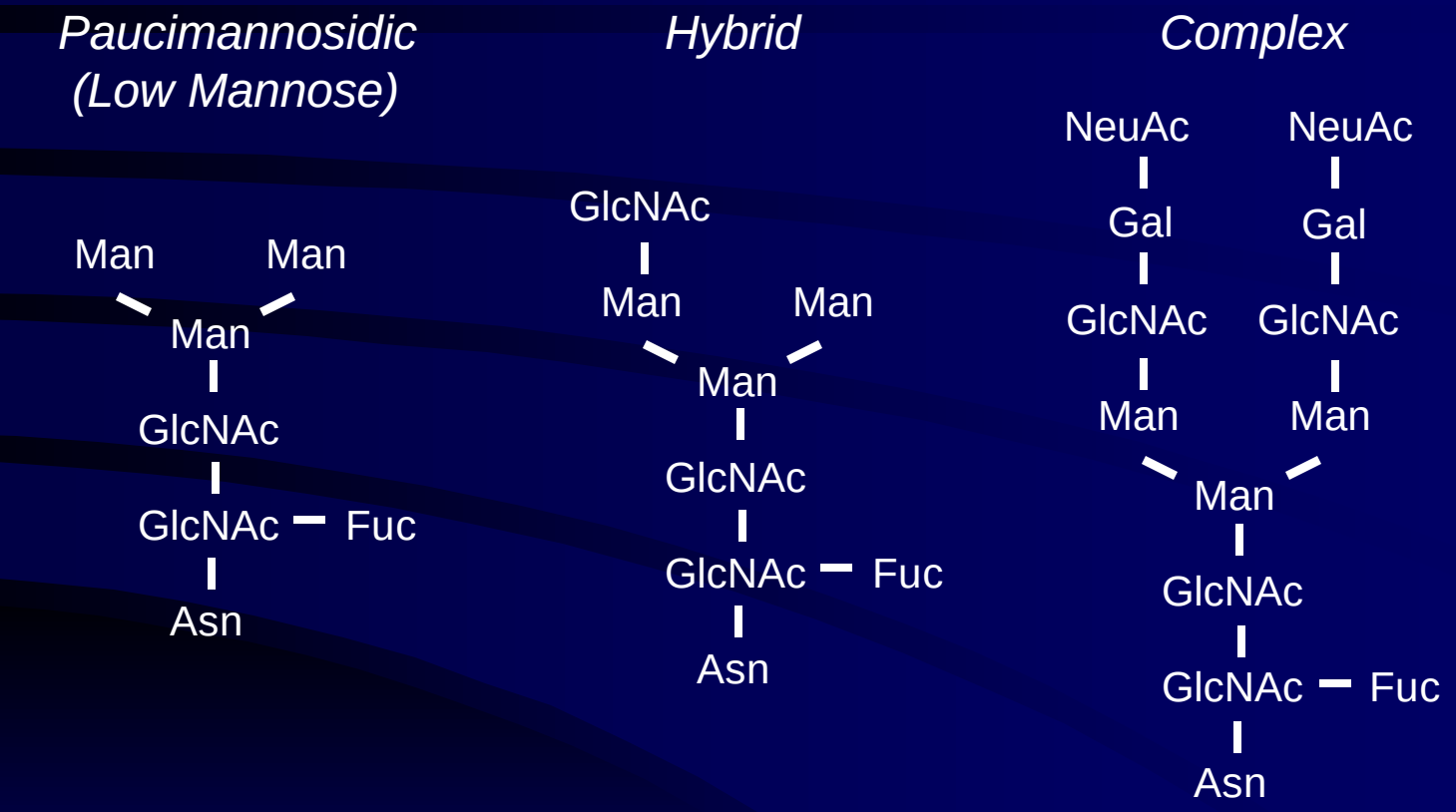


Insect Cells **Mammalian Cells**

Importance of Sialylation (NeuAc)

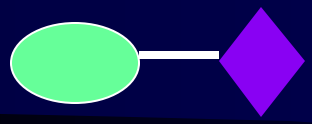
- Protein Structure
- Protein Stability
- Biological Activity
- *In Vivo* Circulatory Half-Life

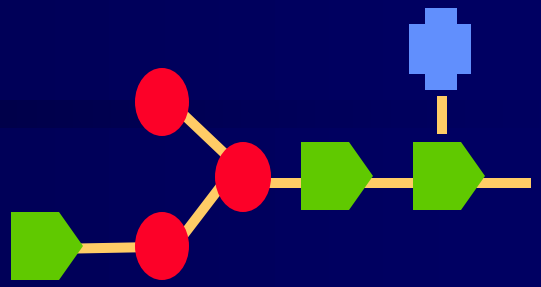
N-glycan Processing: Insect versus Mammalian



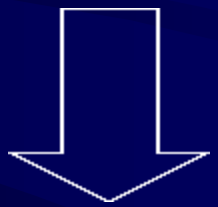
Insect Cells **Mammalian Cells**

Mammalian Galactosylation Reaction

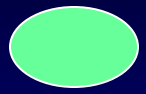


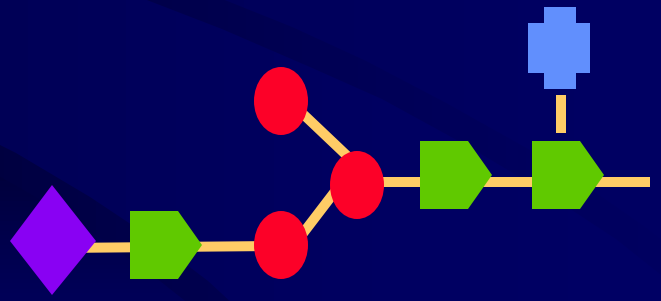


UDP-Galactose + N-acetylglucosamine Structure



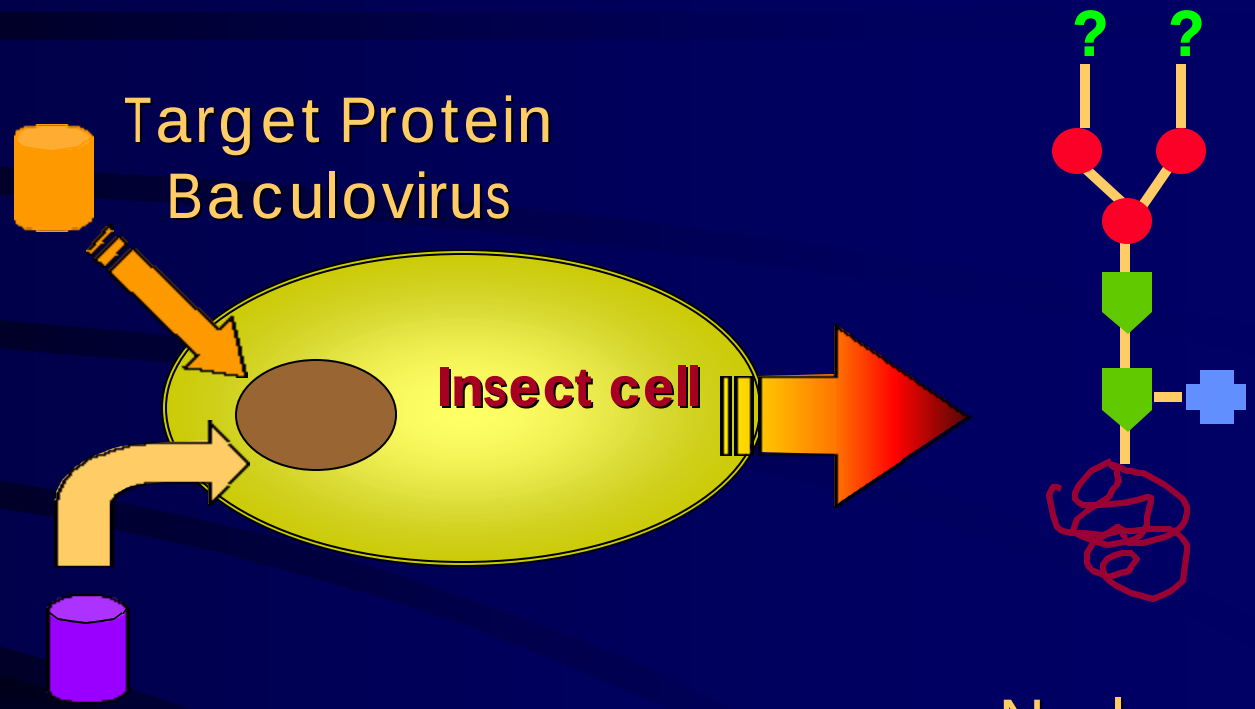
Mammalian Galactose transferase



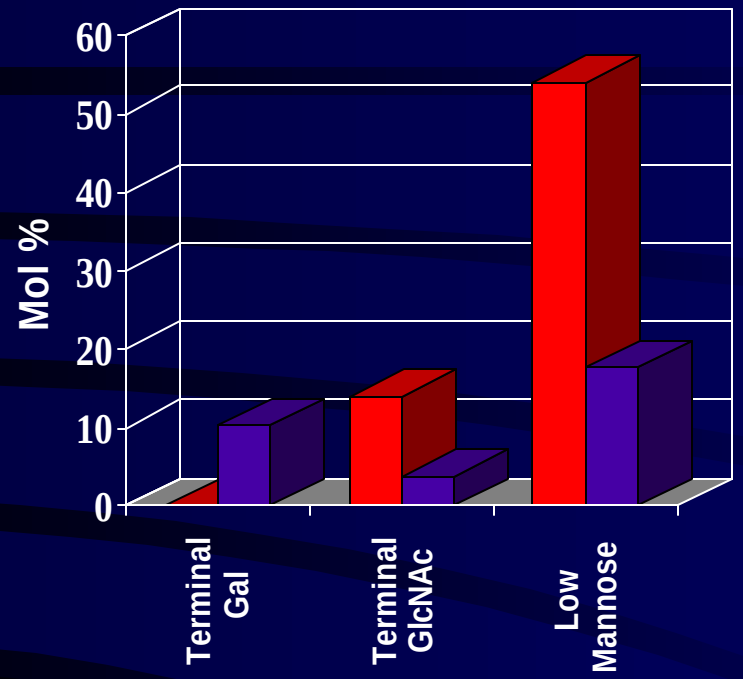


UMP + Galactose-terminated Structure ?

Engineering Galactosylation in Insect Cells

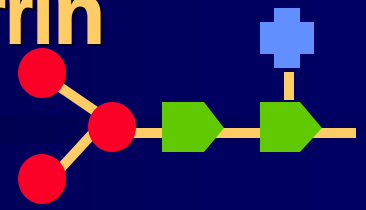


N-Glycan patterns from secreted transferrin

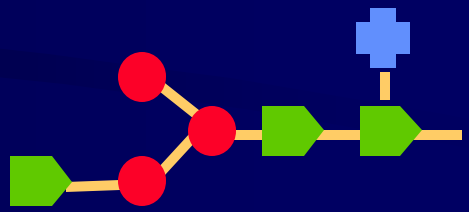


Transferrin from *T. ni*

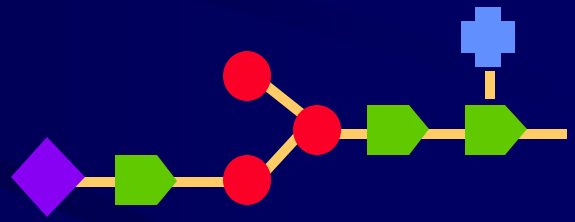
Transferrin (+ GalT) from *T. ni*



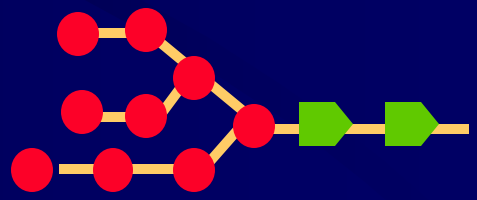
Low Mannose (Paucimannosidic



Terminal GlcNAc

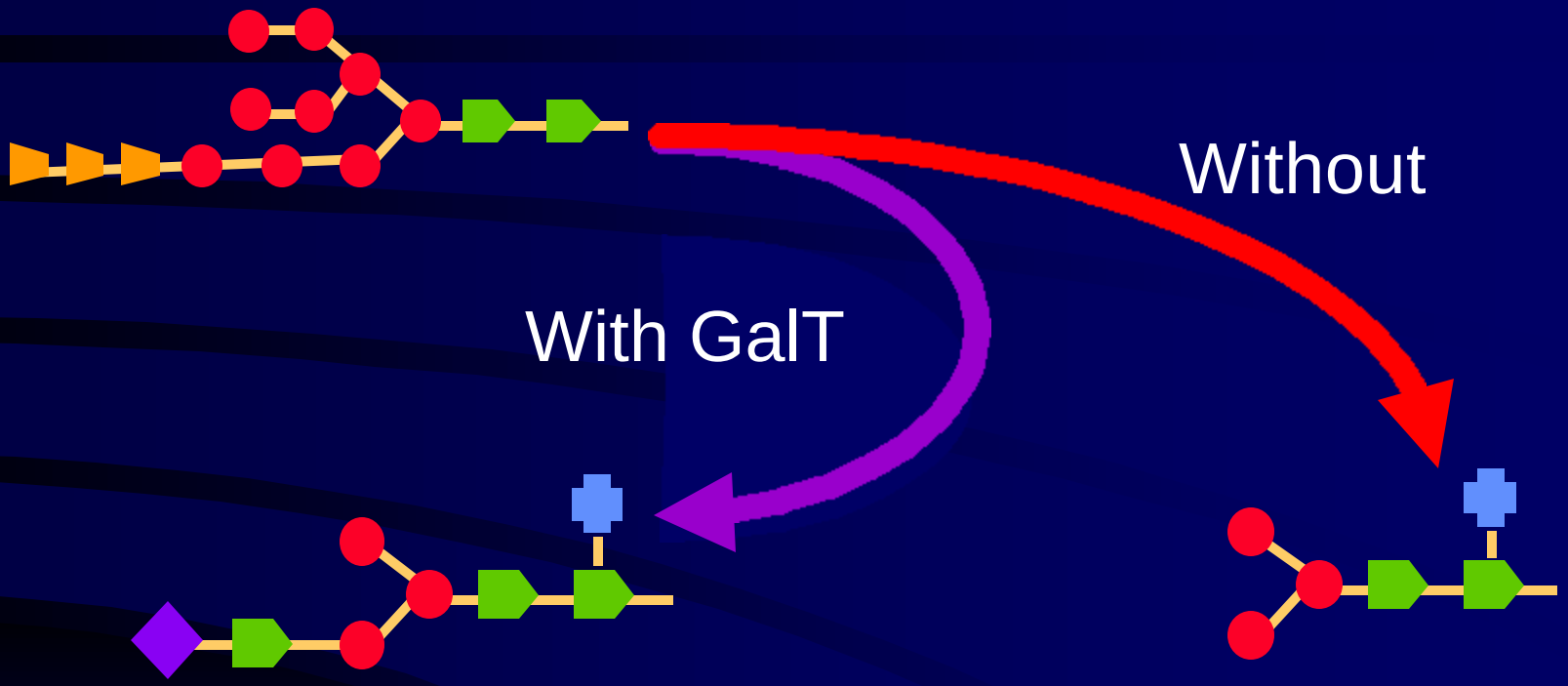


Terminal Gal



High Mannose

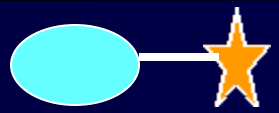
Gal Transferase make N-glycans more “mammalian-like”

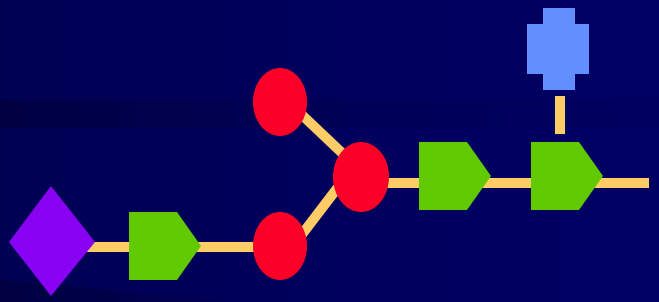


1. Adds Terminal Galactose
2. Blocks N-acetylglucosamine Removal

= Fucose; = Glucose; = Galactose

Sialylation Reaction

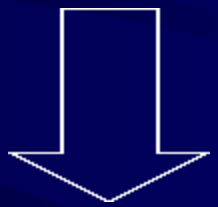




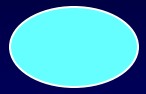
CMP-NeuAc +

(Cytidine Monophospho-
N-acetylneuraminic acid/Sialic Acid)

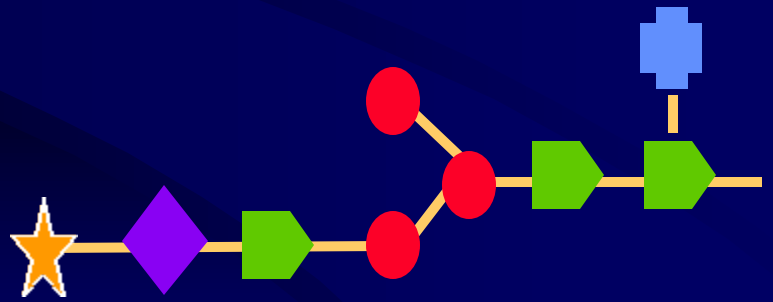
Galactose-terminated Structure



Sialyltransferase

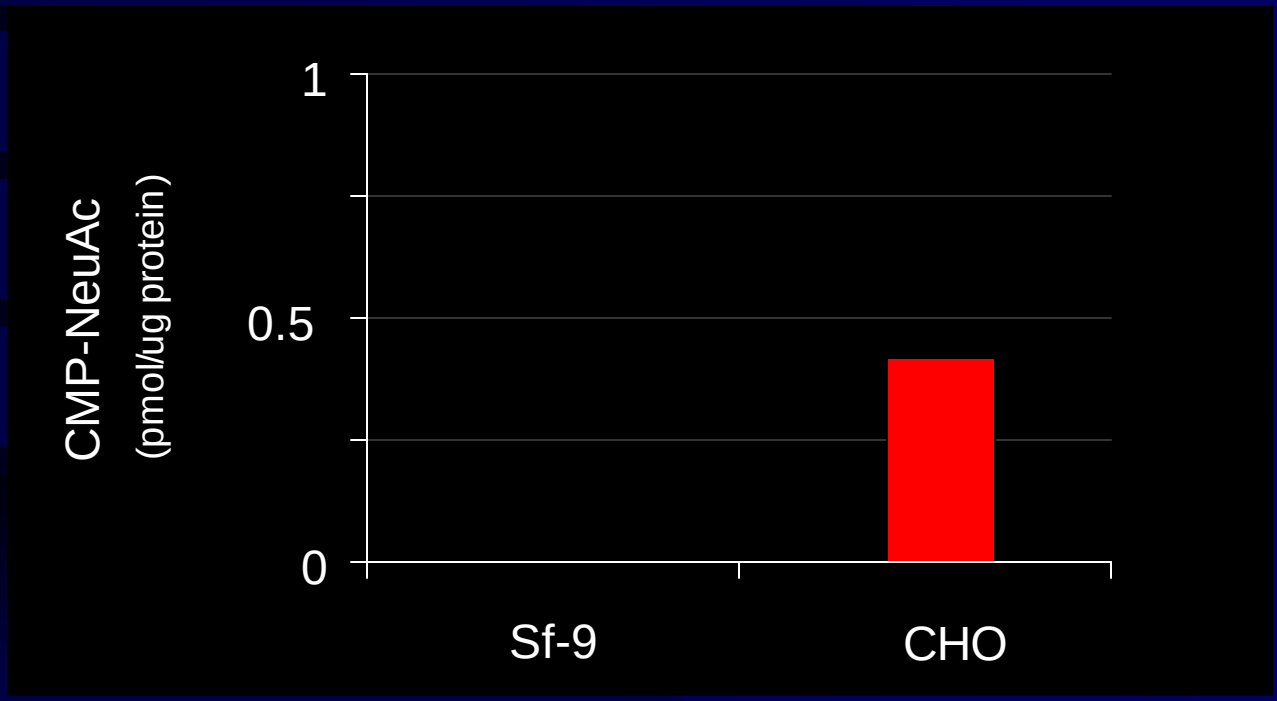


CMP +

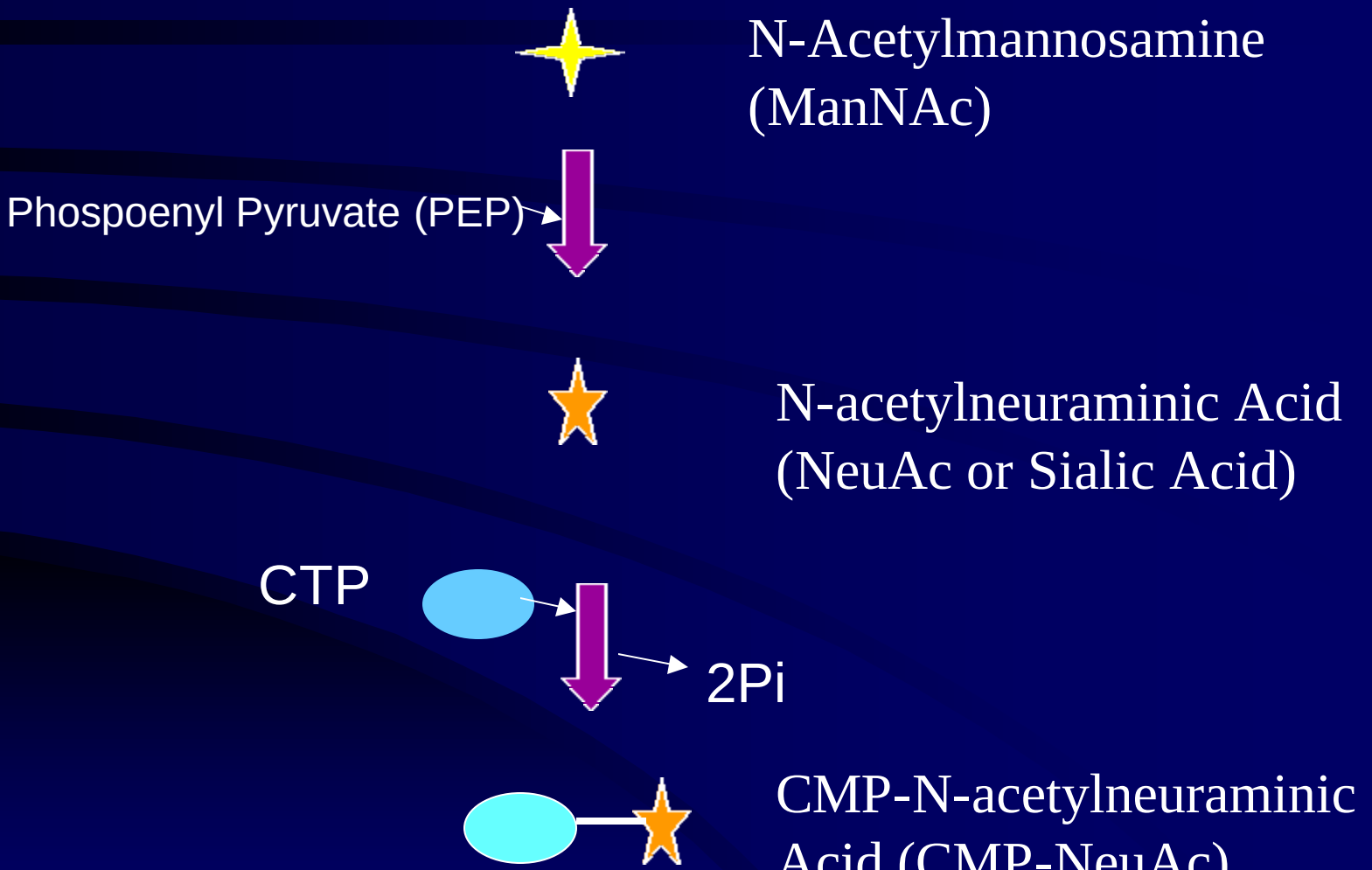


Sialylated Structure
(terminal NeuAc)

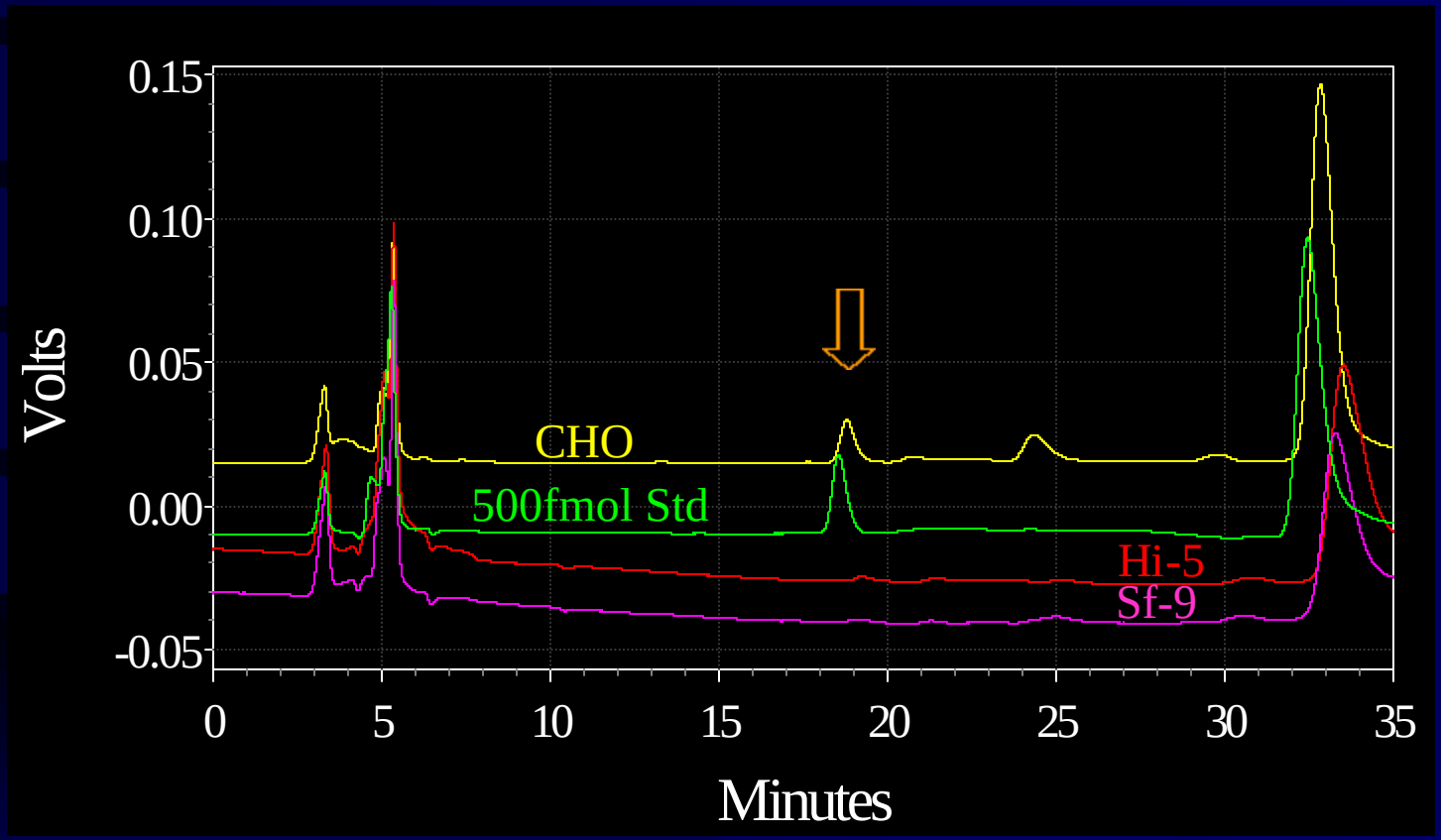
CMP-NeuAc Levels



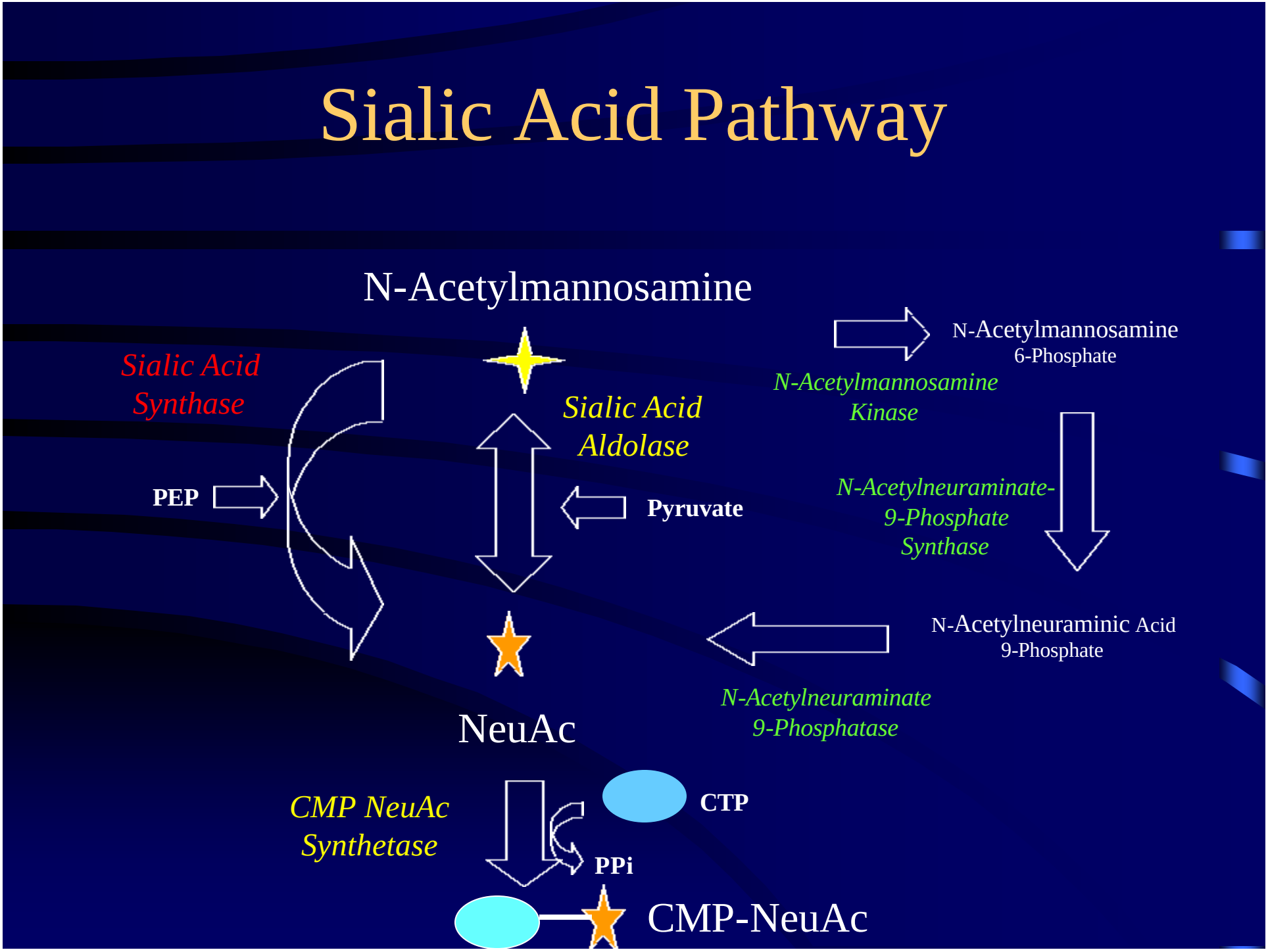
Sialic Acid Pathway



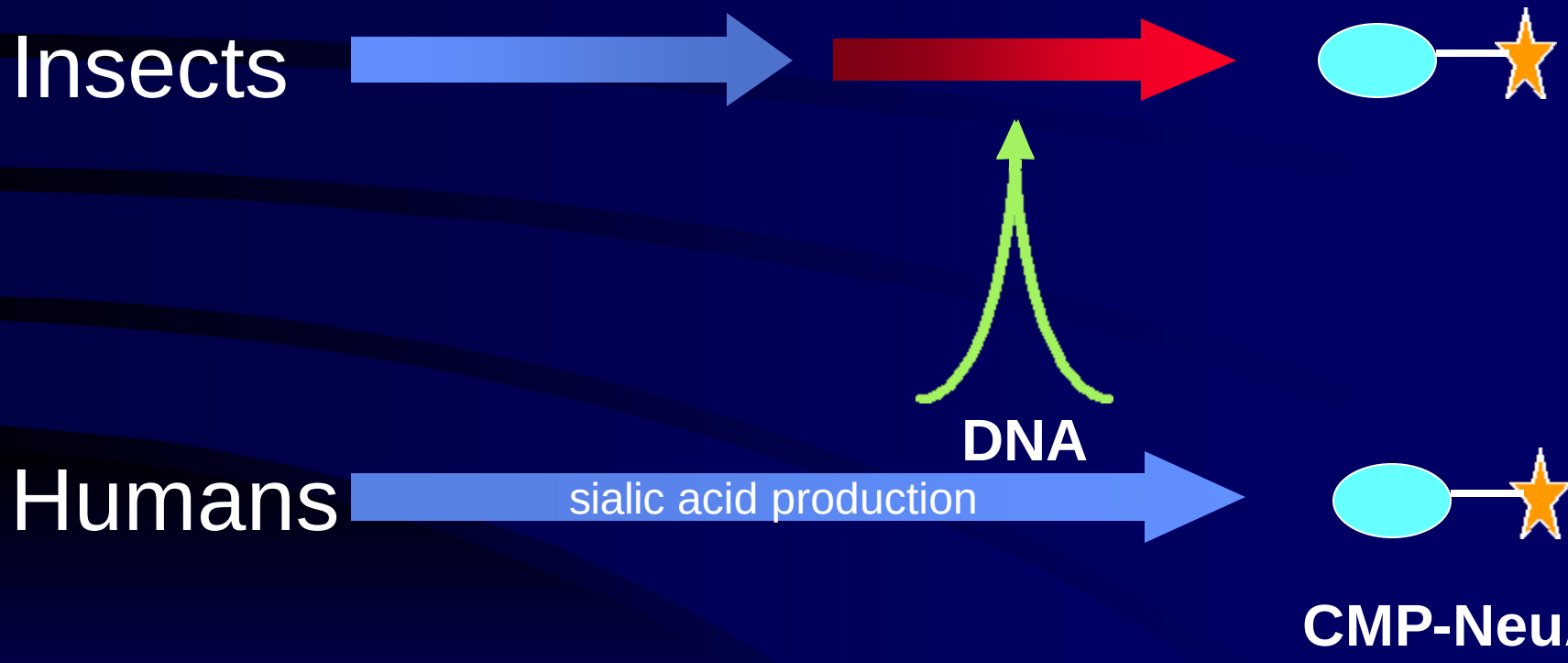
Free NeuAc (Sialic Acid) Levels



Sialic Acid Pathway



Engineer Sialic Acid Pathway



Bioinformatics Strategy

- Human Gene Identification: Human Genome Sciences, Inc.
  - mRNA $\rightarrow$ cDNA $\rightarrow$ EST
  - EST's collected into database
  - EST's assembled into contigs based on homology with themselves
  - Perform homology search using bacterial gene
  - Probe contigs for putative ORF of human gene

Human-Bacterial Homology

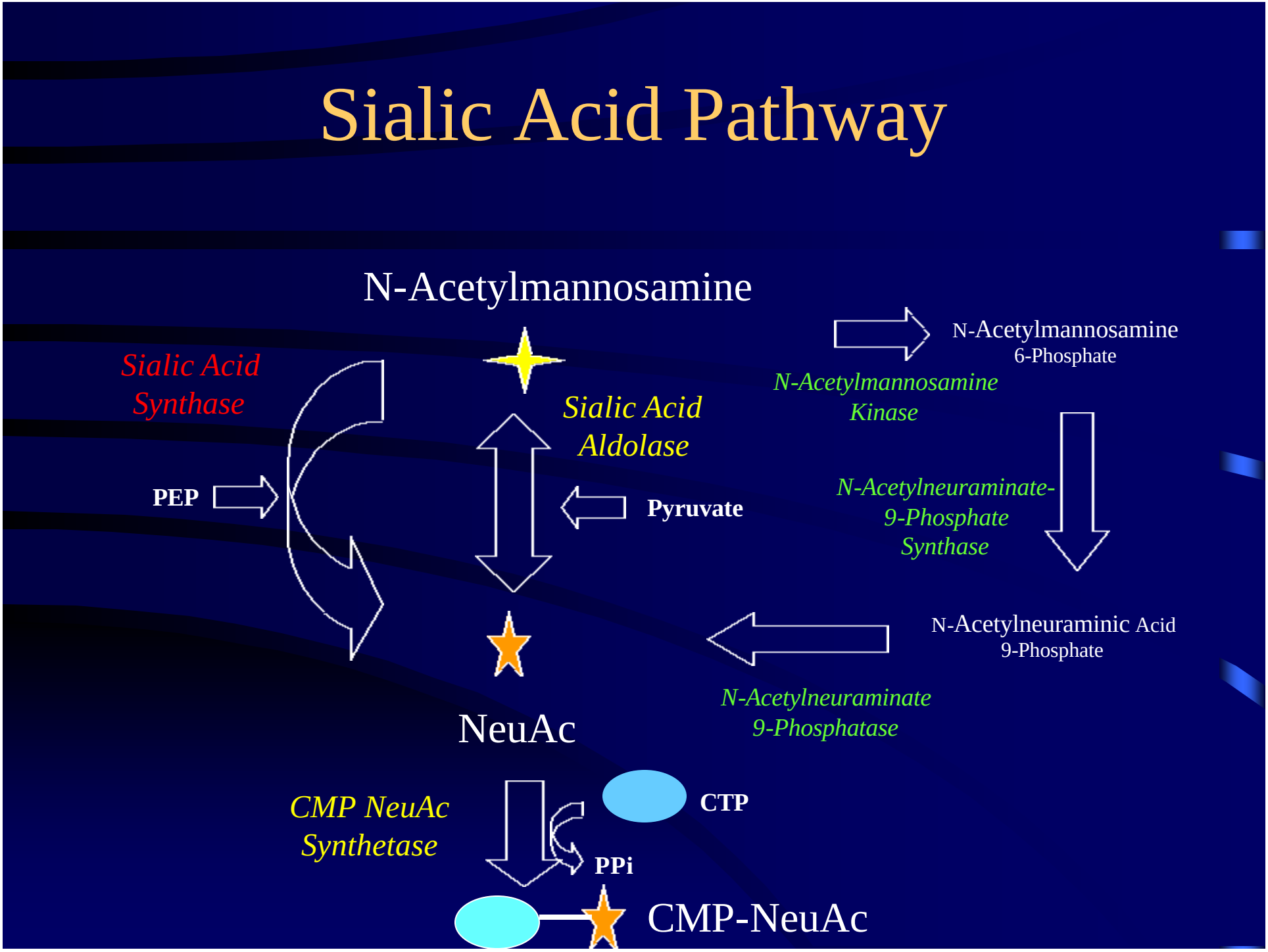
CMP Sialic Acid Synthetase

Human

E. coli

Identitical Proteins

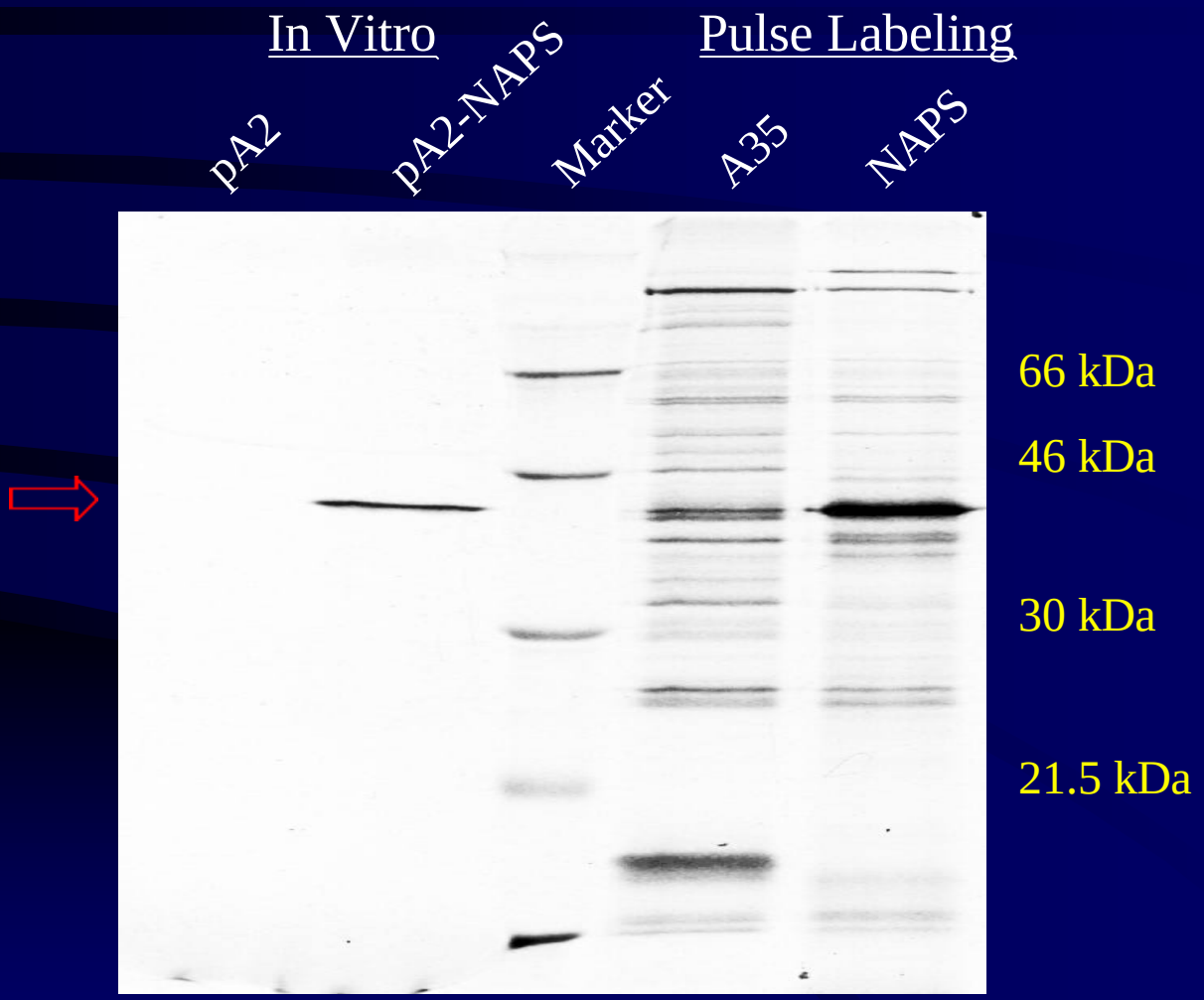
Sialic Acid Pathway



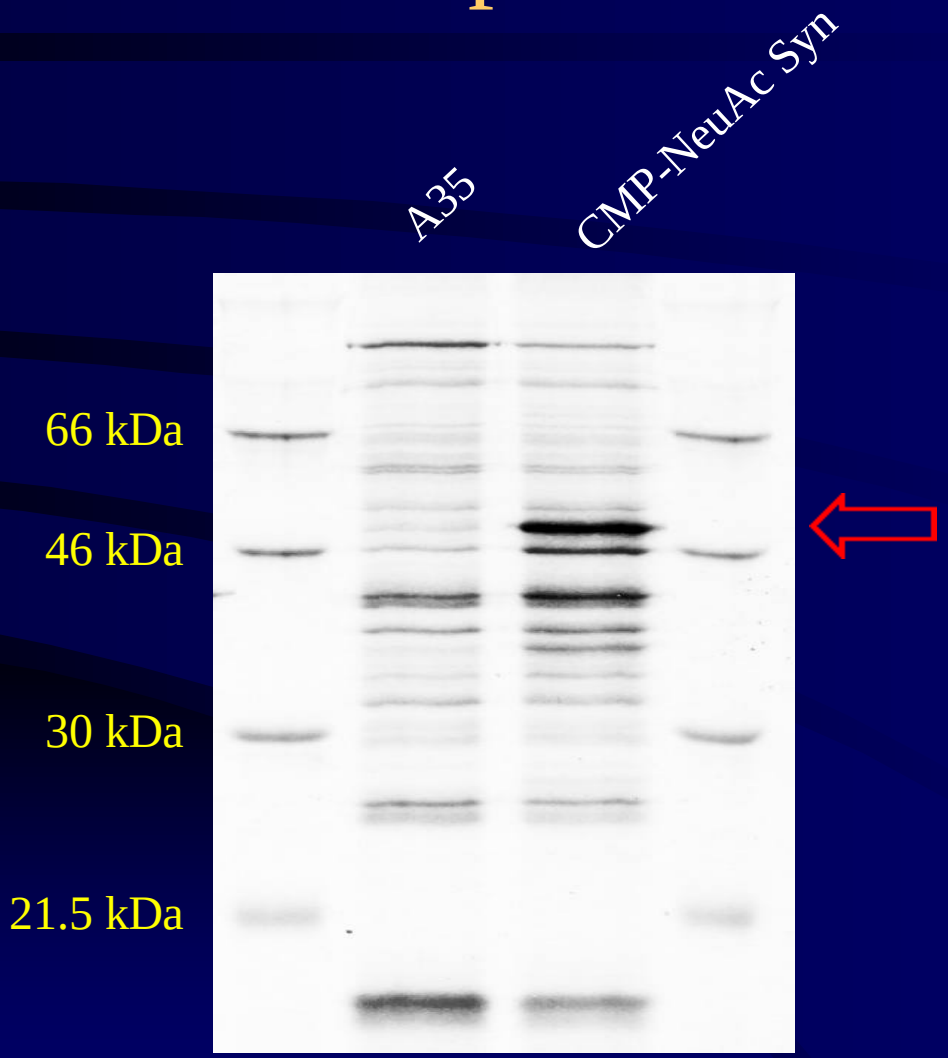
Putative Human Gene Homology

	Similarity	Identity
CMP NeuAc Synthase		
E. coli	25%	19%
M. musculus	91.2%	90.7%
NeuAc Phosphate Synthase		
E. coli	56%	36%

Putative NeuAc Phosphate Synthase (NAPS) Expression in Insect Cells



Putative CMP-NeuAc Synthase Expression



N-Terminal Protein Sequencing

NeuAc Phosphate Synthase

MPLELELCP

1 . . . 5 . . . .

CMP-NeuAc Synthase

MDS . . . LQRNSRGGQG

1 . . 27 . . 30 . . . . 35 .

Sialic Acid Pathway

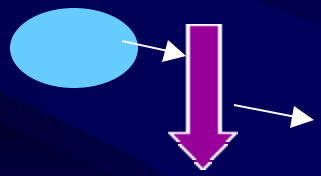
N-Acetylmannosamine (ManNAc)

Phospoenyl Pyruvate (PEP)

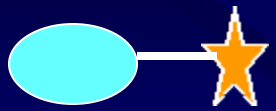
NeuAc Phosphate Synthase

N-acetylneuraminic Acid (NeuAc or Sialic Acid)

CTP

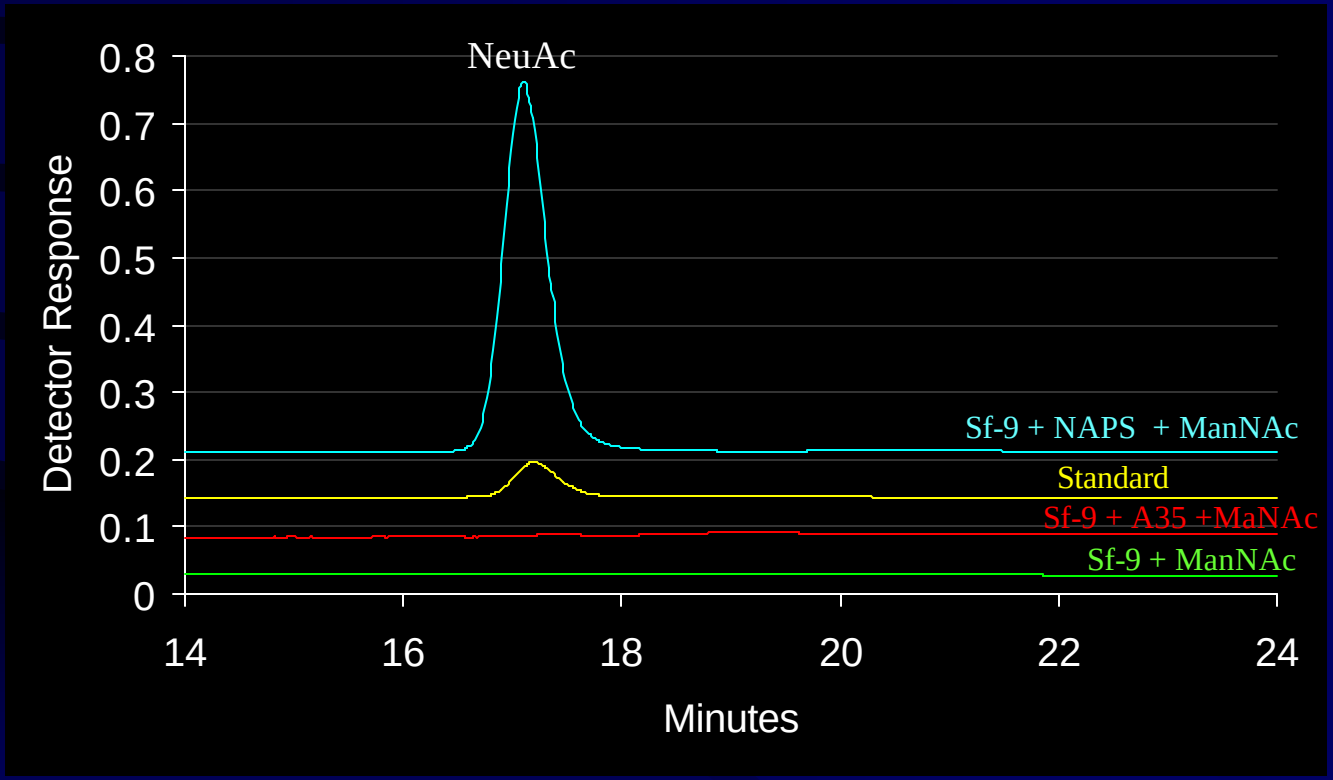


2Pi



CMP-N-acetylneuraminic Acid (CMP-NeuAc)

NeuAc Phosphate Synthase Infection with ManNAc Feeding



Sialic Acid Pathway

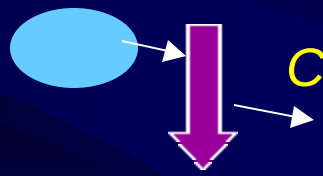
N-Acetylmannosamine (ManNAc)

Phospoenyl Pyruvate (PEP)

NeuAc Phosphate Synthase

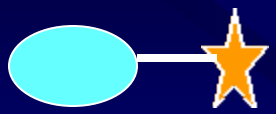
N-acetylneuraminic Acid (NeuAc or Sialic Acid)

CTP



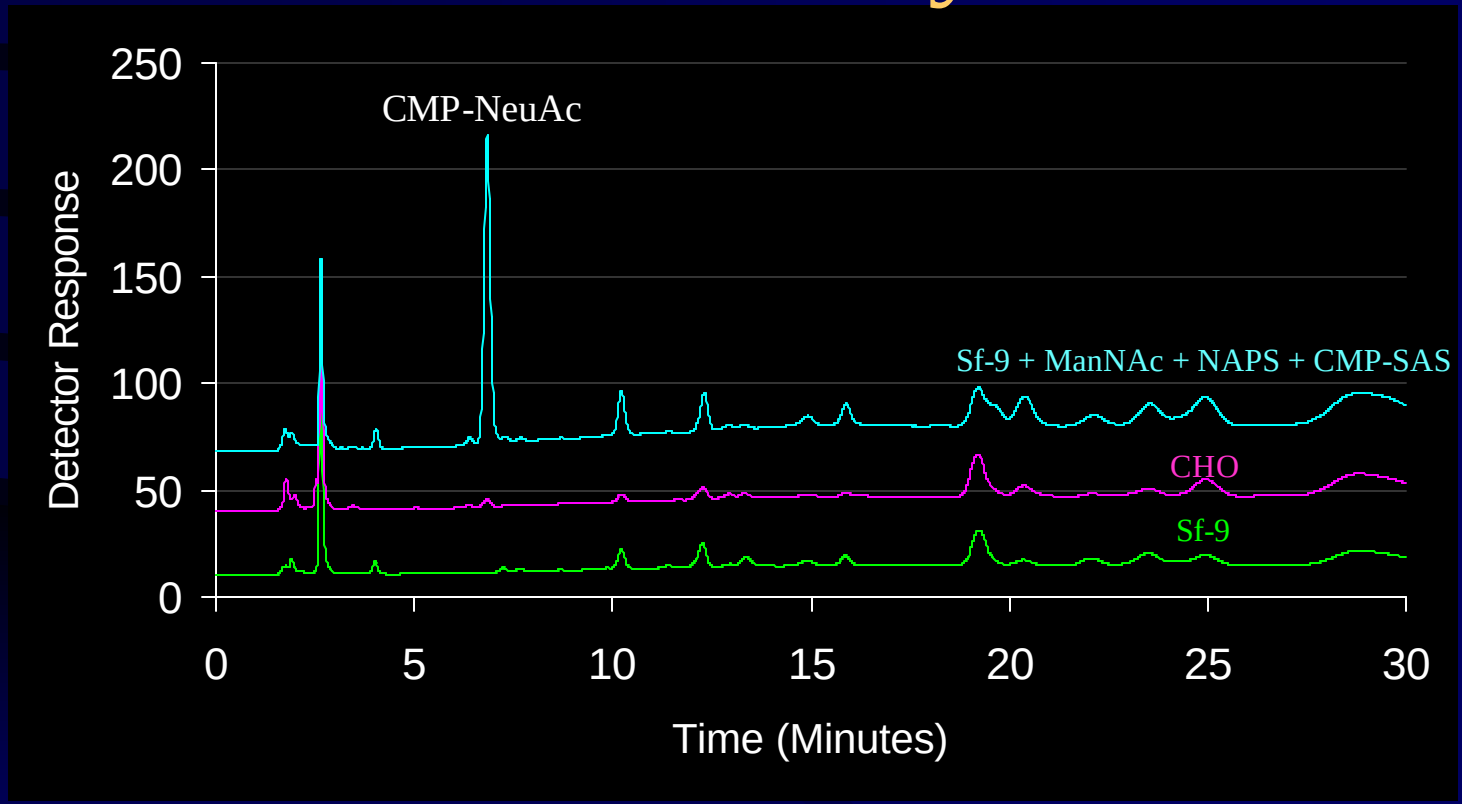
CMP-NeuAc Synthase

2Pi



CMP-N-acetylneuraminic Acid (CMP-NeuAc)

Coinfection: NeuAc-P-Synthase
+
CMP-NeuAc Synthase



CMP-Neu5Ac Levels

Cell Line	Culture Conditions	CMP-Neu5Ac (pmol/μg protein)
Sf-9	Uninfected	0
	Uninfected + ManNAc	0
	+ A35	0
	+ NAPS + CMP-NeuAc S.	0
	+ NAPS + CMP-NeuAc S. + ManNAc	10.3
CHO	Unsupplemented	0.3
	+ ManNAc	1.8

From Pest to Princess