Carbohydrate Engineering for Generating Sialylated Glycoproteins in Insect Cells

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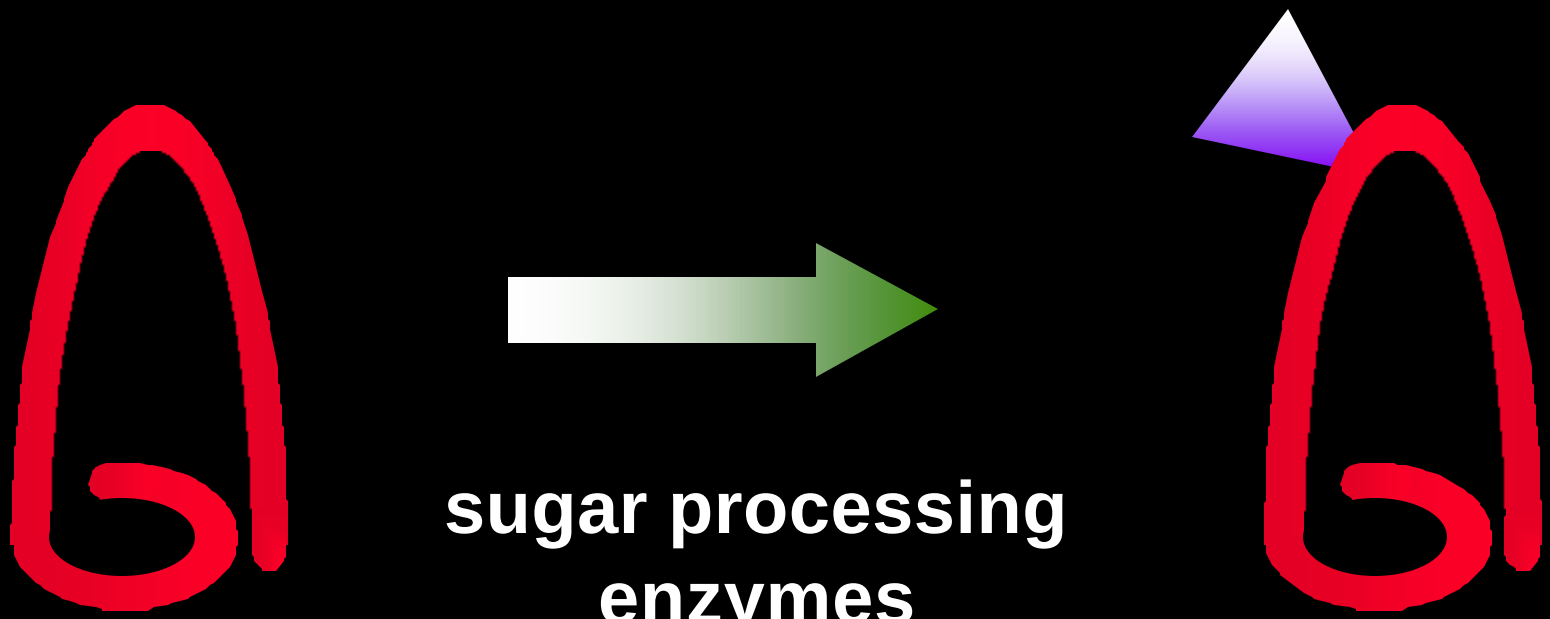
Department of Chemical Engineering
Johns Hopkins University
Baltimore, MD 21218

Oligosaccharide Processing



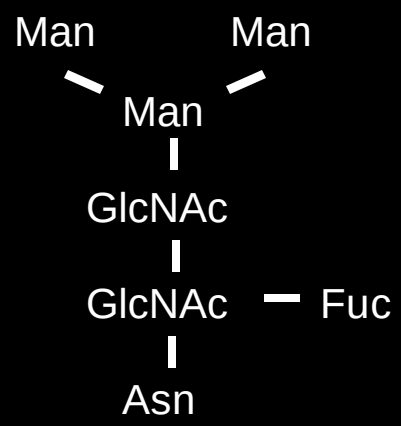
sugar processing enzymes

folded polypeptide

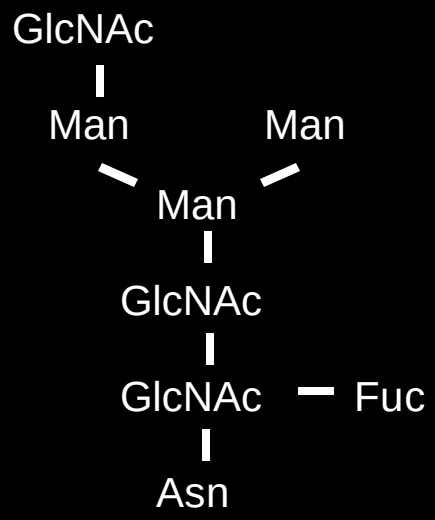
active sugar-modified protein

N-glycan Processing: Insect versus Mammalian

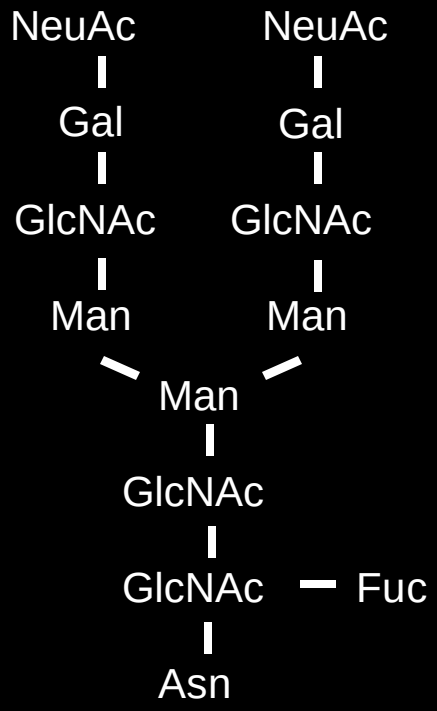
Paucimannosidic (Low Mannose)



Hybrid



Complex



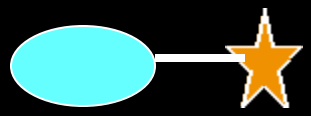
Insect Cells

Mammalian Cells

Importance of Sialylation: (N-acetylneuraminic acid, NeuAc)

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

Sialylation Reaction



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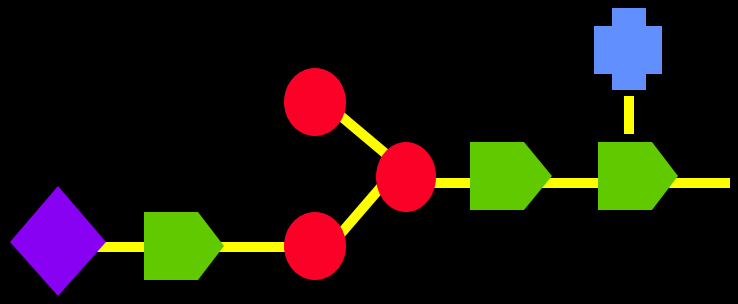
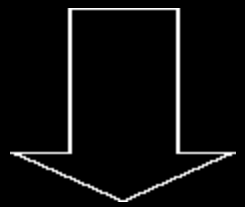
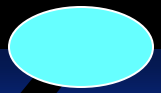


CMP-NeuAc
(Cytidine Monophospho-
N-acetylneuraminic acid/Sialic Acid)

Galactose-terminated Structure

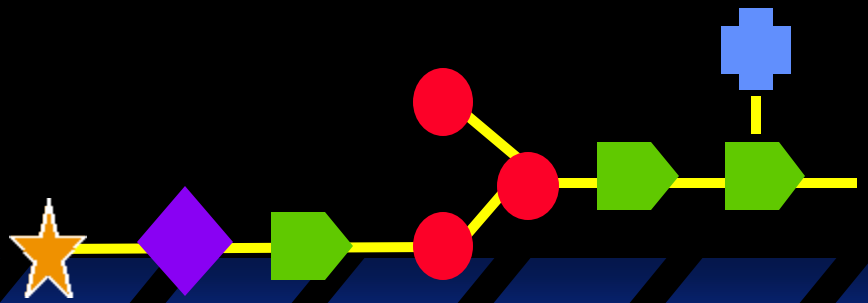


Sialyltransferase



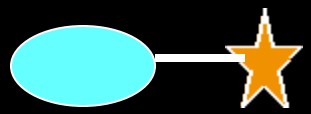
CMP

+



Sialylated Structure
(terminal NeuAc)

Sialylation Reaction



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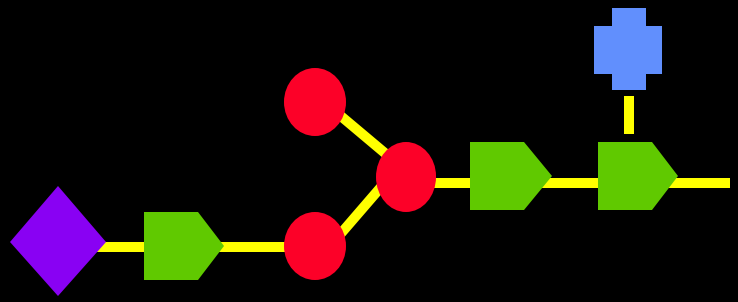


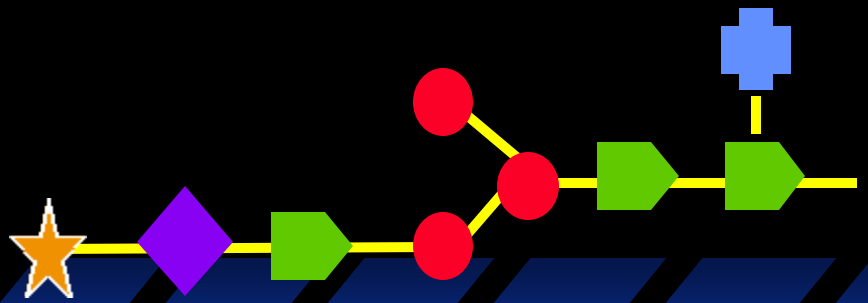
CMP-NeuAc
(Cytidine Monophospho-
N-acetylneuraminic acid/Sialic Acid)

Galactose-terminated Structure

-DJ/MI

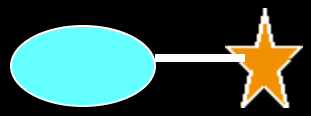
Sialyltransferase

CMP +



Sialylated Structure
(terminal NeuAc)

Sialylation Reaction



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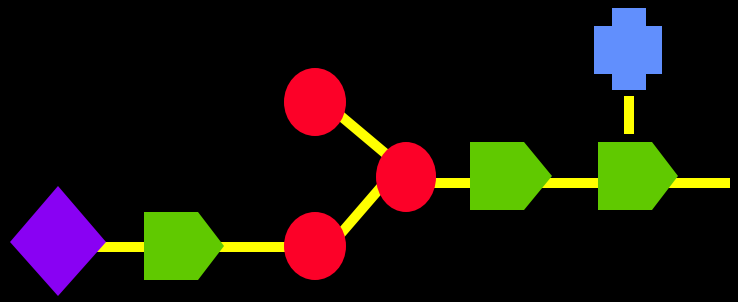


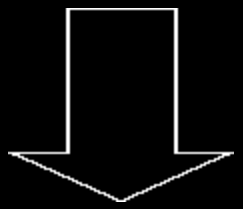
CMP-NeuAc
(Cytidine Monophospho-
N-acetylneuraminic acid/Sialic Acid)

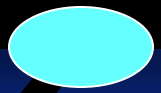
Galactose-terminated Structure

-DJ/MI

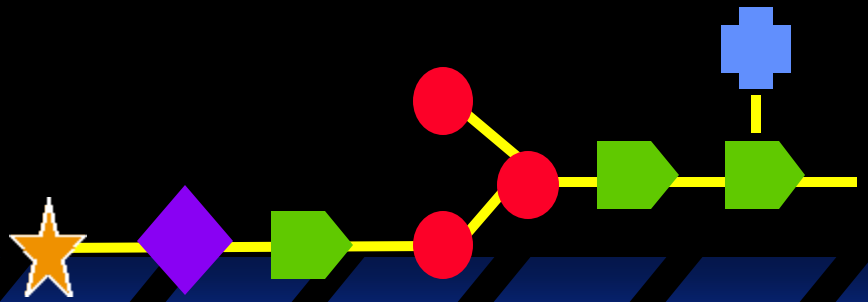
Sialyltransferase

-DJ



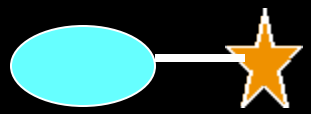


CMP +



Sialylated Structure
(terminal NeuAc)

Sialylation Reaction



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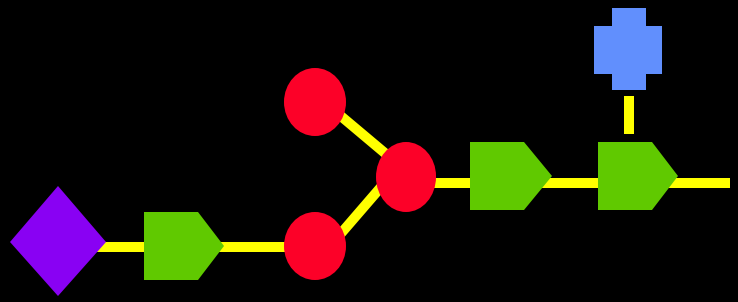
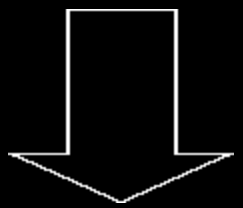


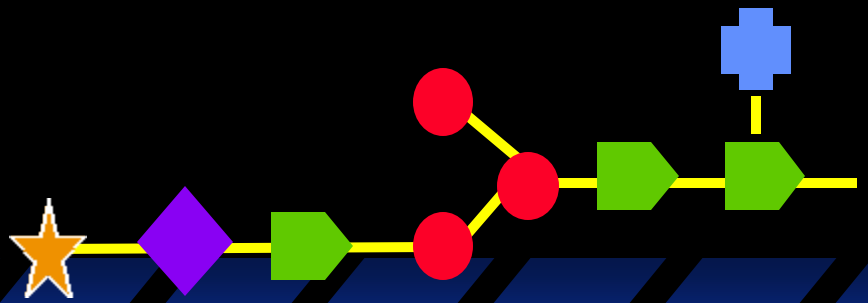
CMP-NeuAc ?

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

Galactose-terminated Structure ◂ □-DJ/MI

Sialyltransferase ◂ -DJ



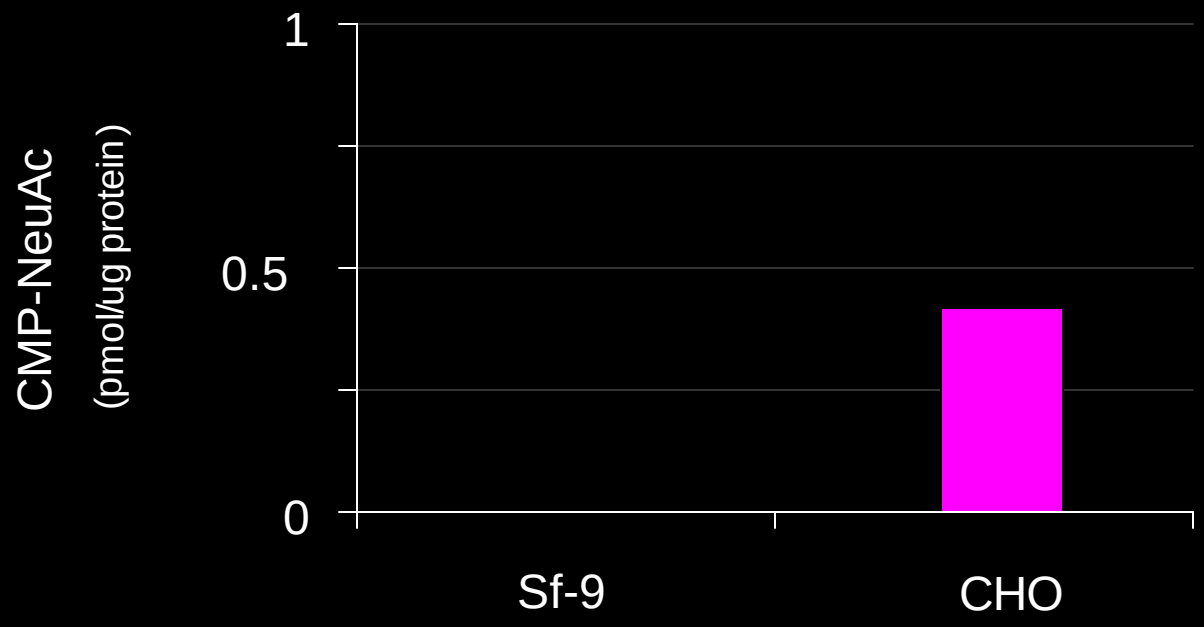


?

CMP +

Sialylated Structure
(terminal NeuAc)

CMP-NeuAc Levels



Engineer Sialic Acid Pathway

Insects

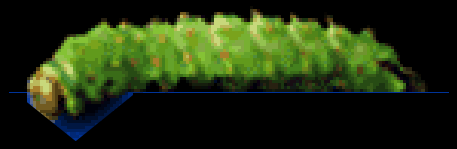
Humans

DNA

sialic acid production

CMP-NeuAc

Sialic Acid Pathway Reconstruction



UDP-N-acetylglucoseamine (UDP-GlcNAc)

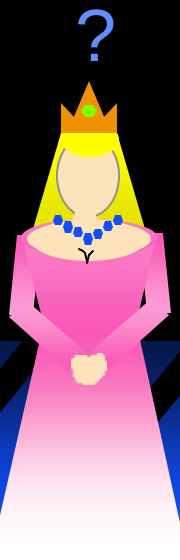
UDP-GlcNAc Epimerase

N-acetylmannoseamine (ManNAc)

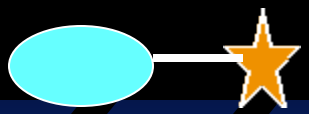
Sialic Acid Phosphate Synthase

N-acetylneuraminic acid (NeuAc)

CMP-Sialic Acid Synthase

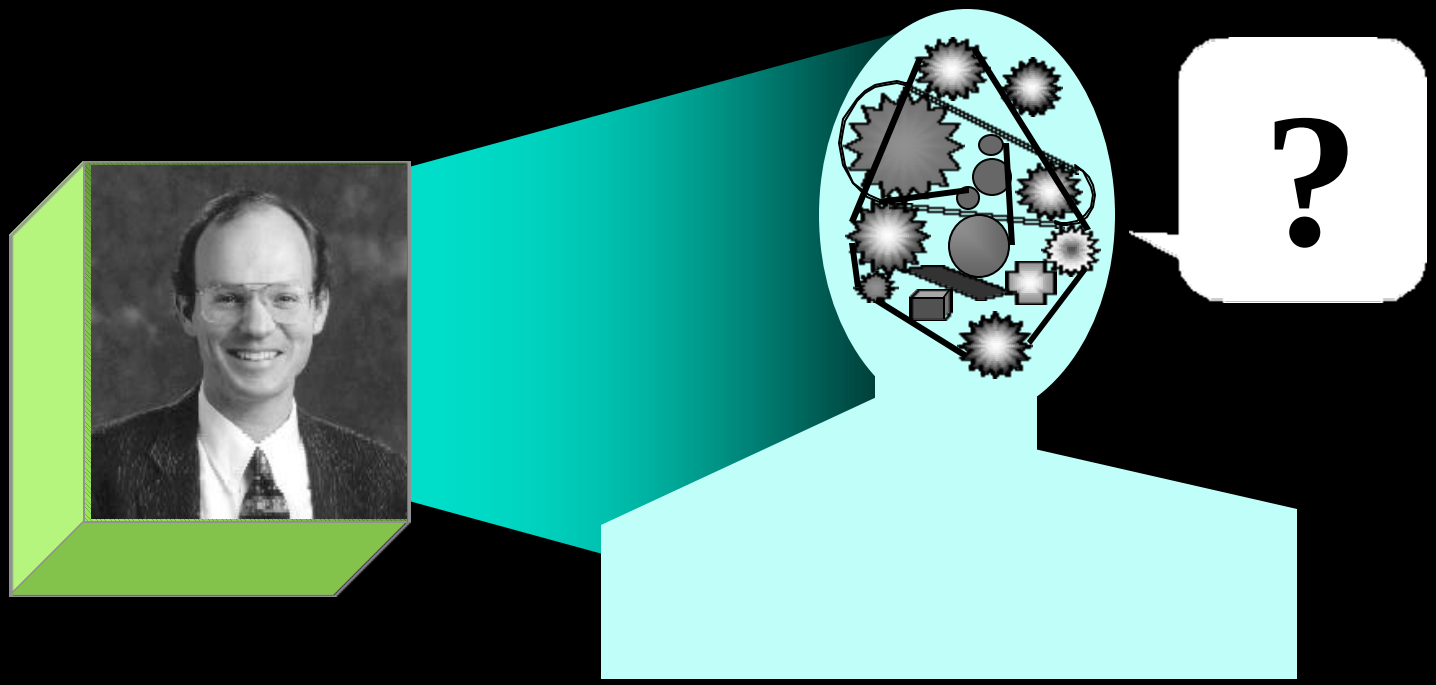


CMP-NeuAc



CMP N-acetylneuraminic acid (CMP-NeuAc)

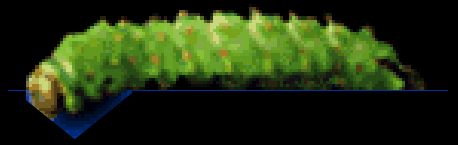
One problem with this strategy….



The human genes are UNKNOWN…

Sialic Acid Pathway Reconstruction

UDP-GlcNAc



UDP-GlcNAc Epimerase

ManNAc

?

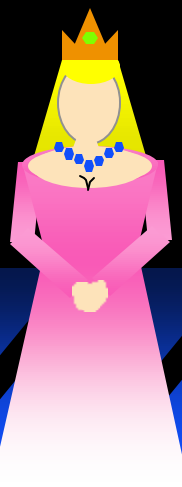
Sialic Acid Phosphate Synthase

NeuAc

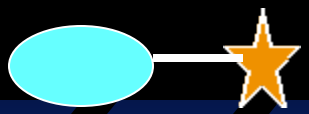
?

CMP-Sialic Acid Synthase

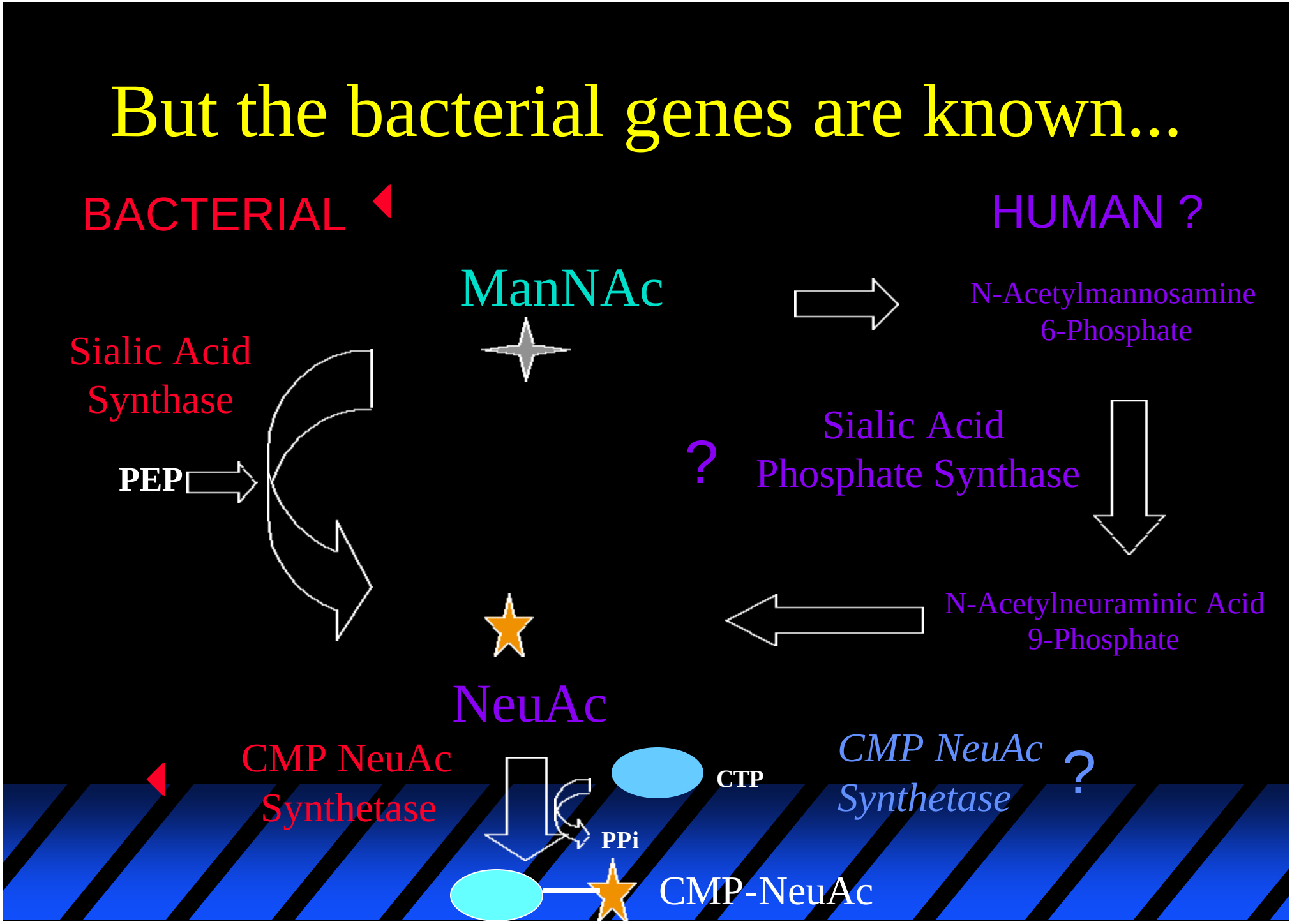
?



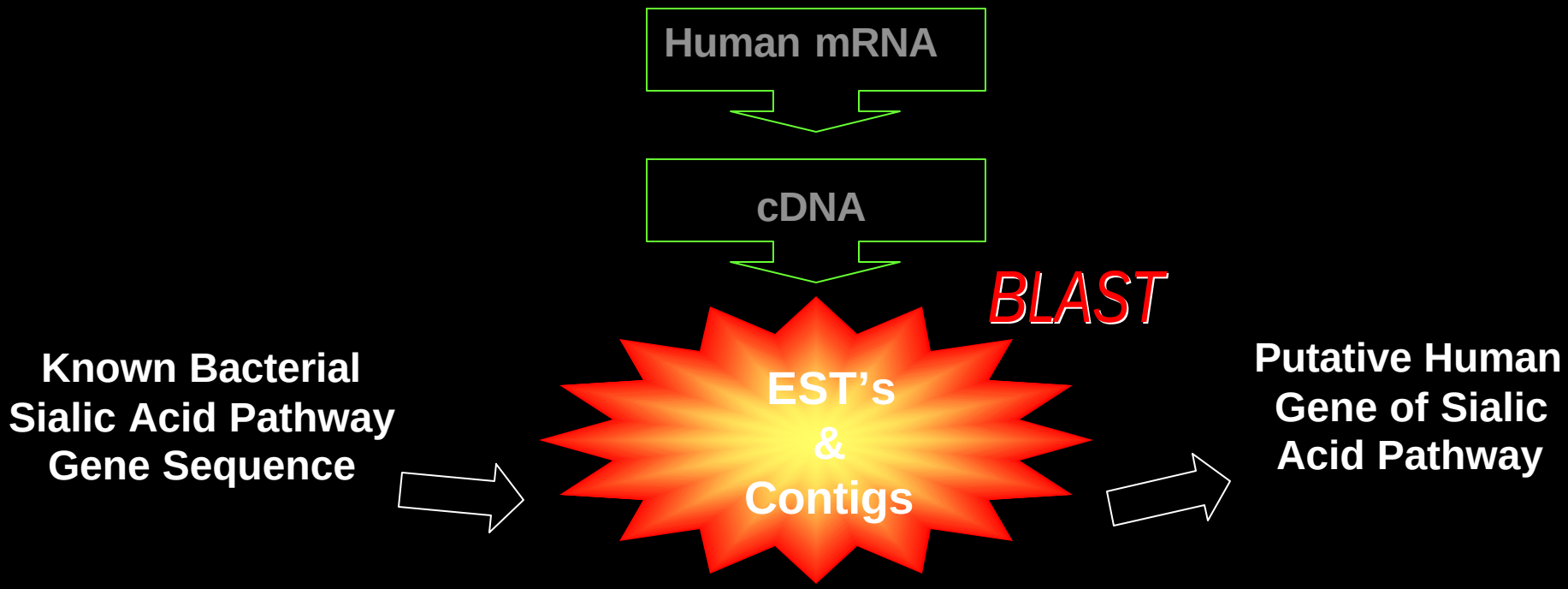
CMP-NeuAc



But the bacterial genes are known...



Identify putative human genes from bacterial homologs?



Human Genome Sciences, Inc., Databases

Sialic Acid Pathway Reconstruction

UDP-GlcNAc

X *UDP-GlcNAc Epimerase* X

ManNAc
(from medium)

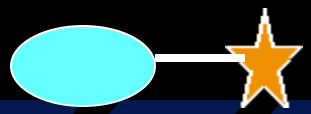
ManNAc

Sialic Acid Phosphate Synthase ?

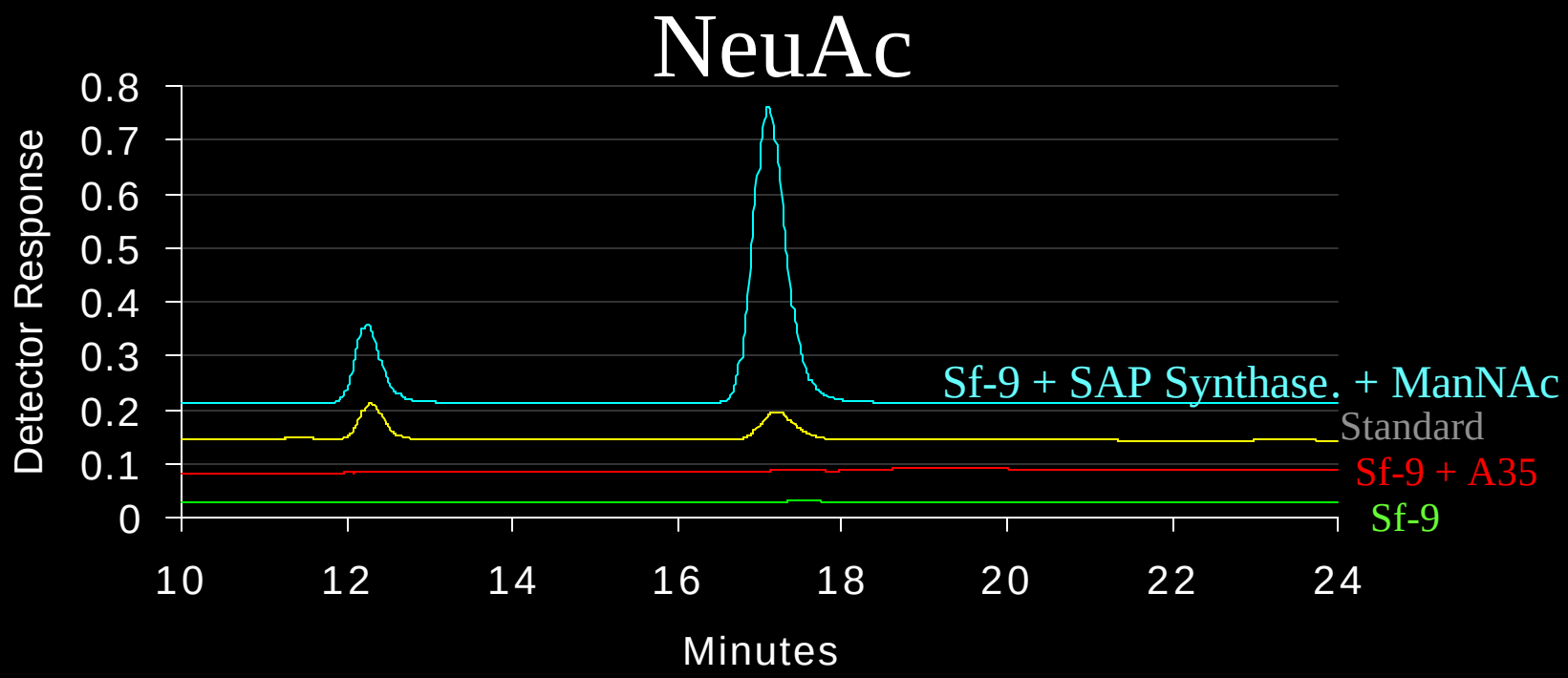
? NeuAc

? *CMP-Sialic Acid Synthase* ?

CMP-NeuAc



Sialic Acid Phosphate Synthase Infection with ManNAc Feeding



Sialic Acid Pathway Reconstruction

UDP-GlcNAc

X *UDP-GlcNAc Epimerase* X

ManNAc
(from medium)

ManNAc

Sialic Acid Phosphate Synthase

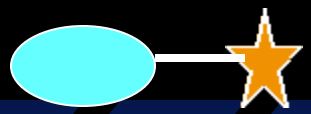
NeuAc

CMP-Sialic Acid Synthase

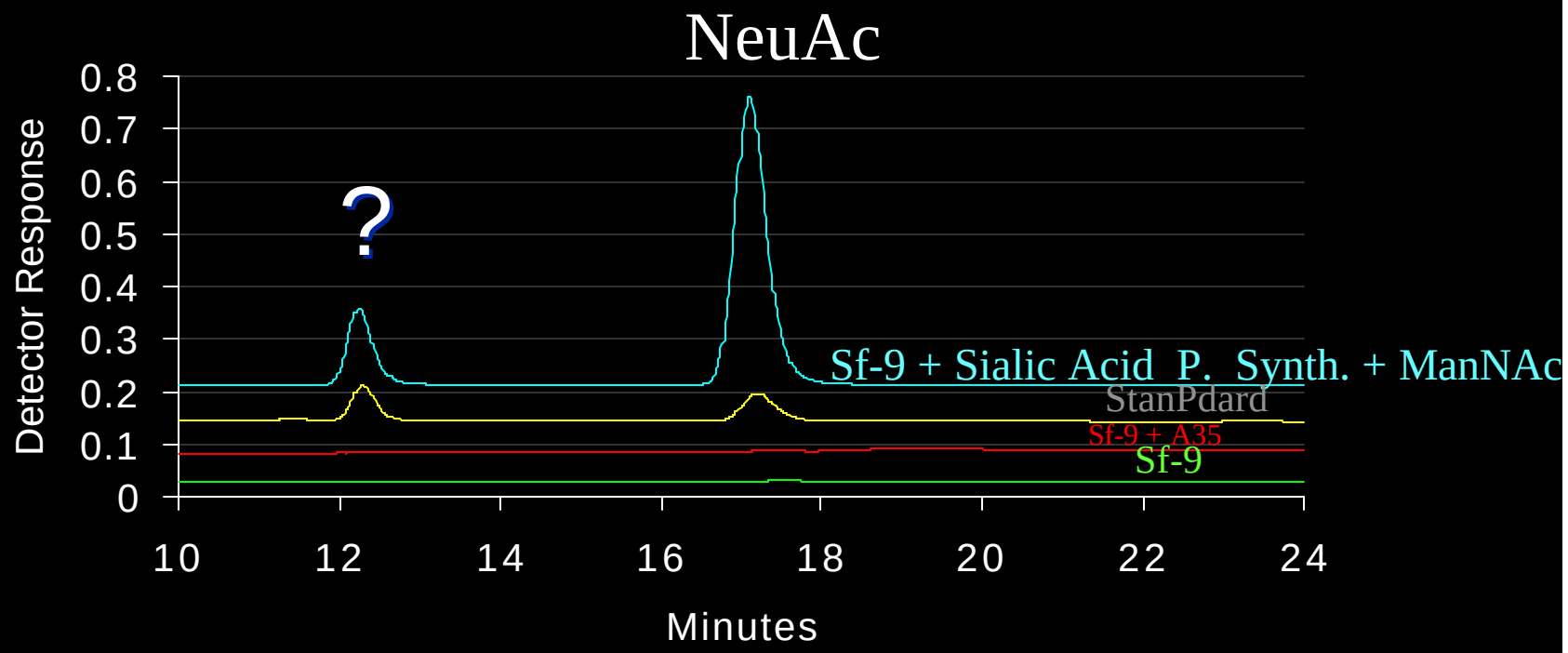
?

?

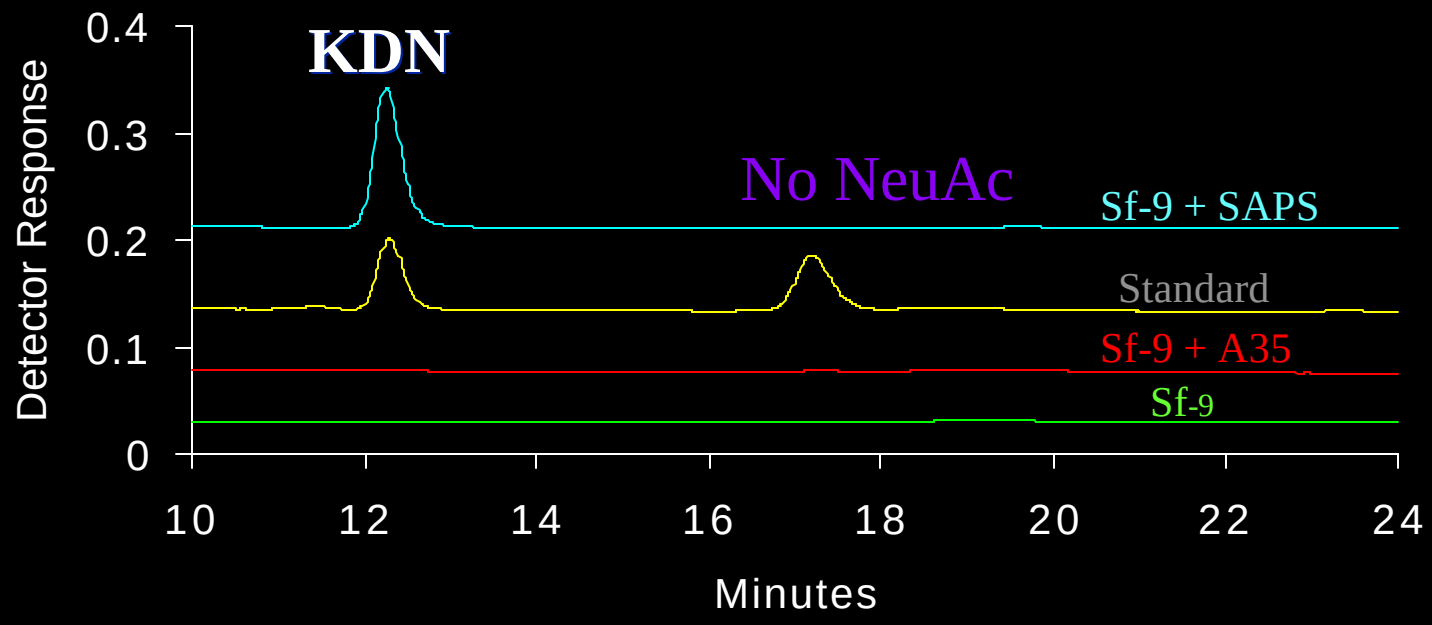
CMP-NeuAc



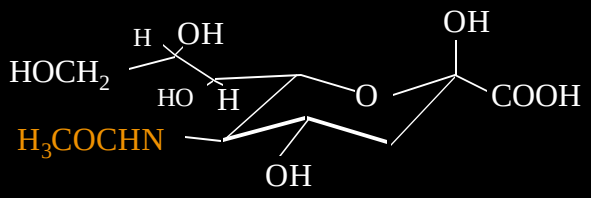
Sialic Acid Phosphate Synthase Infection with ManNAc Feeding



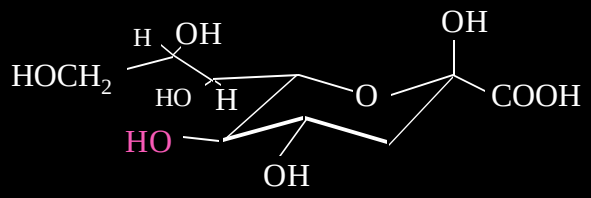
Sialic Acid Phosphate Synthase Infection without ManNAc Feeding



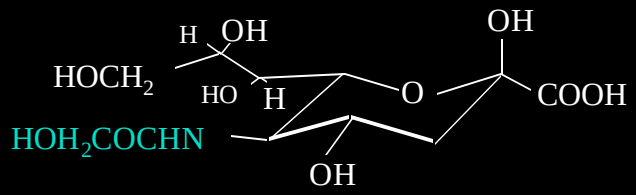
The Sialic Acids



N-Acetylneuraminic Acid
(NeuAc)



3-Deoxy-D-*glycero*-D-*galacto*-
Nonulosonic Acid
(KDN)



N-Glycolylneuraminic Acid
(Neu5Gc)

Sialic Acid Pathway Reconstruction

UDP-GlcNAc

UDP-GlcNAc Epimerase

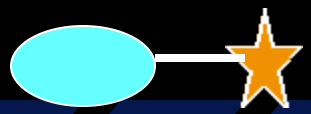
? ManNAc

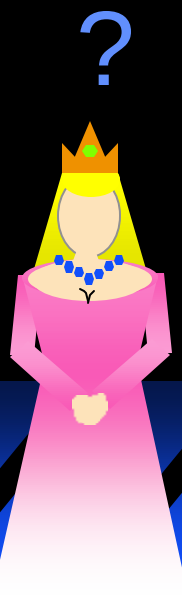
Sialic Acid Phosphate Synthase

? NeuAc

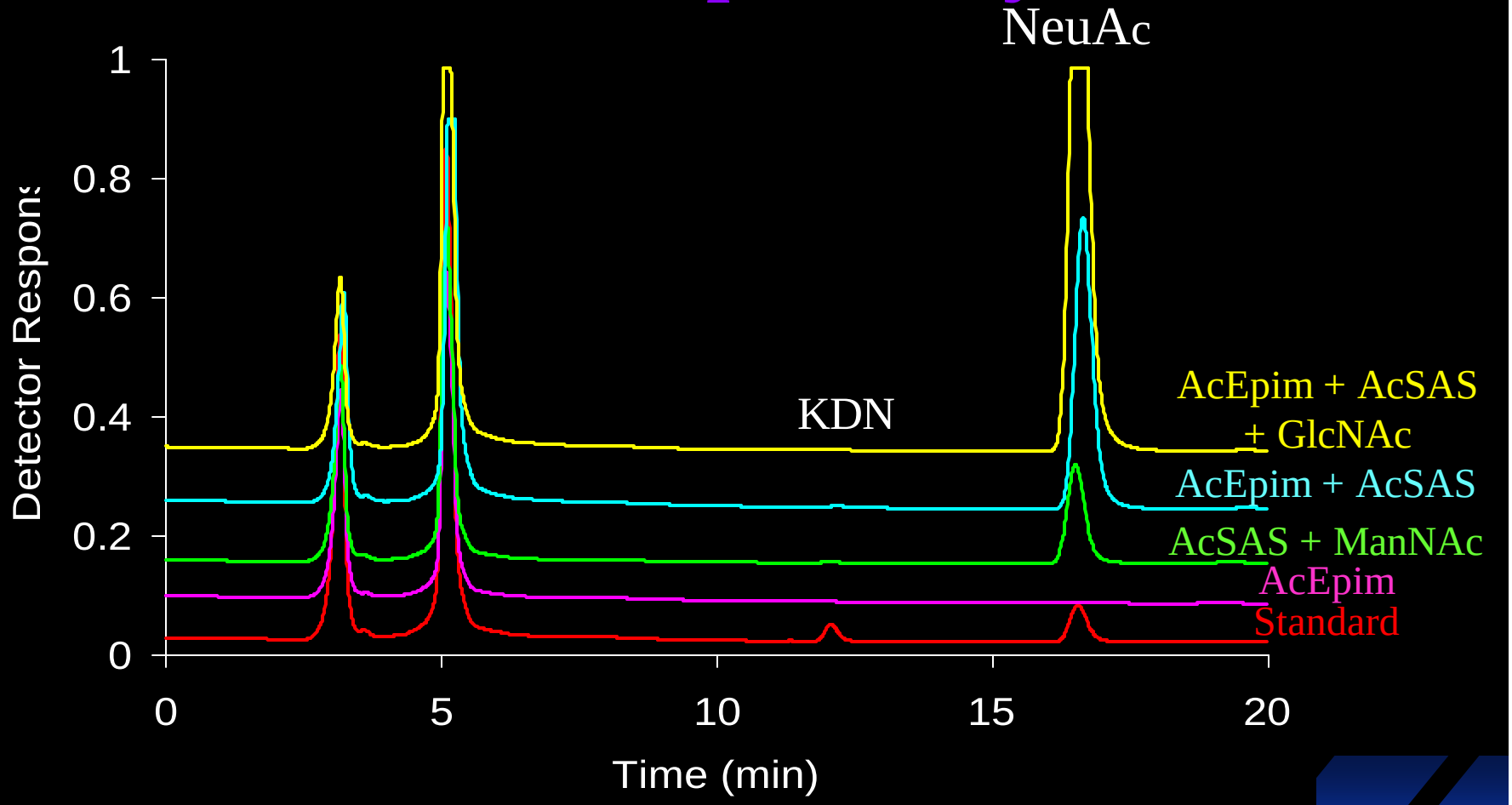
CMP-Sialic Acid Synthase

CMP-NeuAc



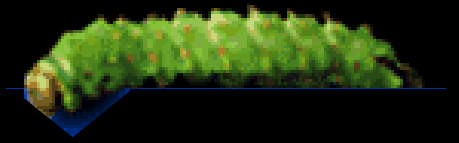


UDP-GlcNAc Epimerase + Sialic Acid Phosphate Synthase



Sialic Acid Pathway Reconstruction

Native Insect Metabolism



UDP-GlcNAc

UDP-GlcNAc Epimerase

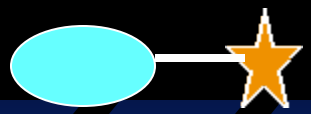
ManNAc

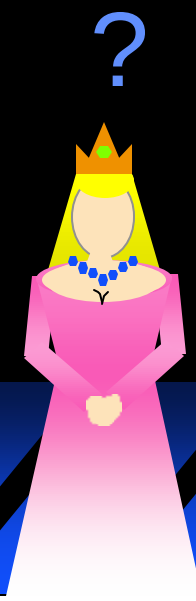
Sialic Acid Phosphate Synthase

NeuAc

CMP-Sialic Acid Synthase

CMP-NeuAc





Sialic Acid Pathway Reconstruction

Insect Metabolism

GlcNAc

UDP-GlcNAc

UDP-GlcNAc Epimerase

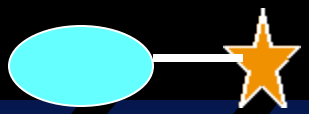
ManNAc

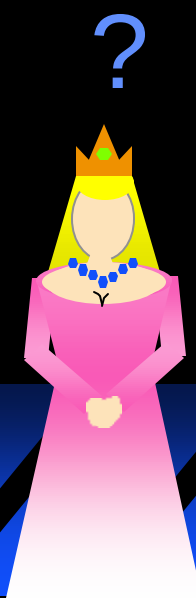
Sialic Acid Phosphate Synthase

NeuAc

CMP-Sialic Acid Synthase

CMP-NeuAc





Sialic Acid Pathway Reconstruction

Insect Metabolism

UDP-GlcNAc

UDP-GlcNAc Epimerase

ManNAc

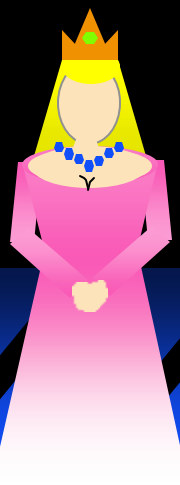
Sialic Acid Phosphate Synthase

NeuAc

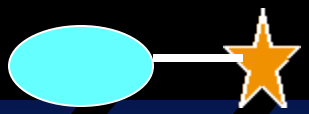
CMP-Sialic Acid Synthase

?

?



CMP-NeuAc



Sialic Acid Pathway Reconstruction

Insect Metabolism

UDP-GlcNAc

X *UDP-GlcNAc Epimerase* X

ManNAc (from medium)

ManNAc

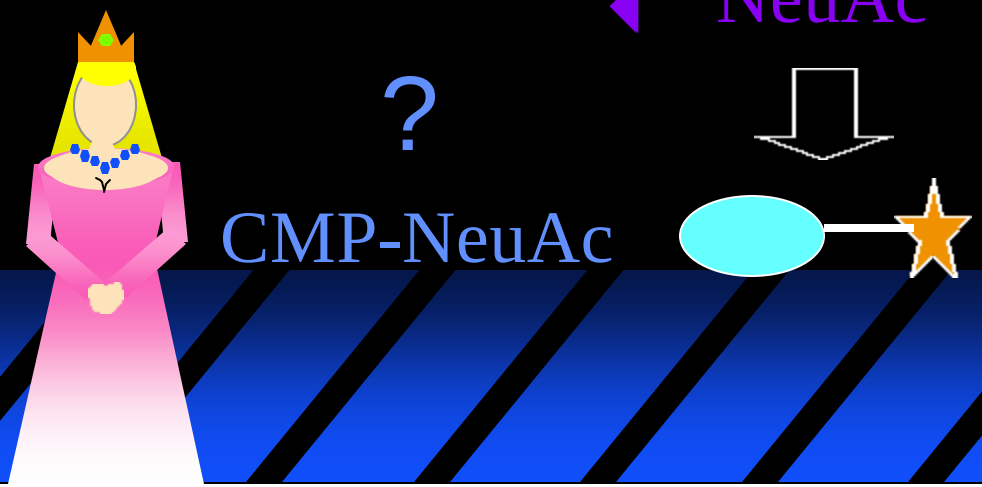
Sialic Acid Phosphate Synthase

NeuAc

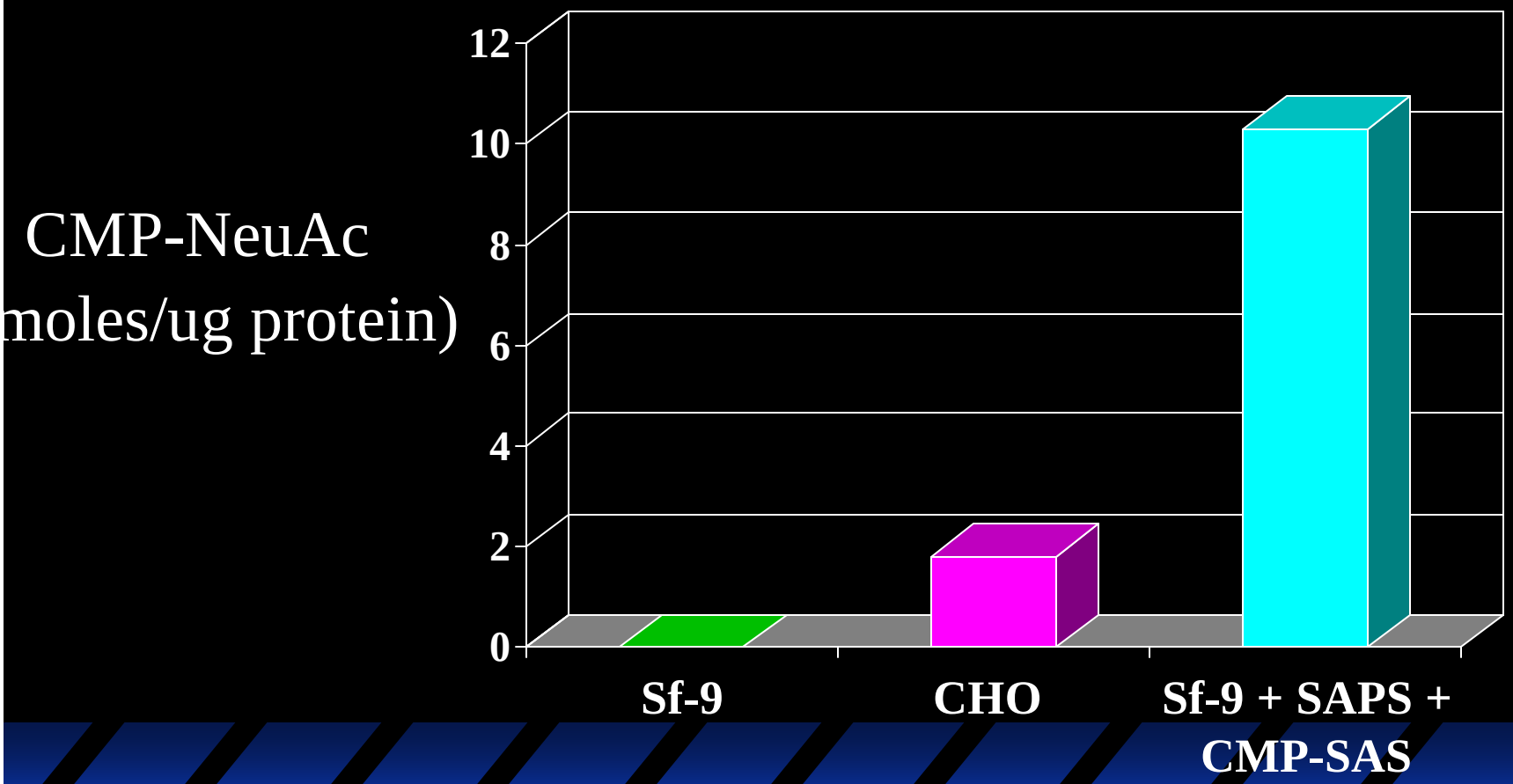
?

CMP-Sialic Acid Synthase

CMP-NeuAc



CMP-NeuAc Levels: SA Phosphate Synthase + CMP-SA Synthase + ManNAc



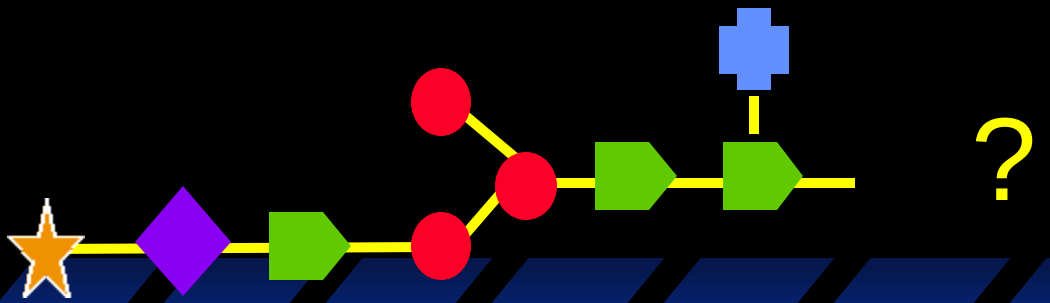
Sialylation Reaction

MP-NeuAc

ytidine Monophospho-
acetylneuraminic acid/Sialic Acid)

Galactose-terminated Structure

Sialyltransferase



?

CMP +

Sialylated Structure
(terminal NeuAc)

Acknowledgements

- Johns Hopkins University
  - Yuan C. Lee
  - Noboru Tomiya
  - Lee Pitts
  - Shawn Lawrence
  - Badarulhisam Abdul Rahman
- FDA
  - Willie F. Vann
- University of Wyoming
  - Donald Jarvis and students
- Human Genome Sciences
  - Timothy Coleman
  - Kathy Huddleston
  - Nam Nguyen
  - Joelle Porter
  - Dave Parmelee
  - Irina Knyazev
- USDA
  - Jim Slavicek
- NIH
  - Yossi Shiloach