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Glycosylation Changes in Cancer

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ALLIANCE of GLYCOBIOLOGISTS
For Detection of Cancer and Cancer Risk

Every Living Cell in Nature is Covered with a Dense and Complex Array of Sugar Chains (Glycans)

Varki A.: Nothing in Glycobiology Makes Sense,
Except in the Light of Evolution.
Cell 126:841-845, 2006

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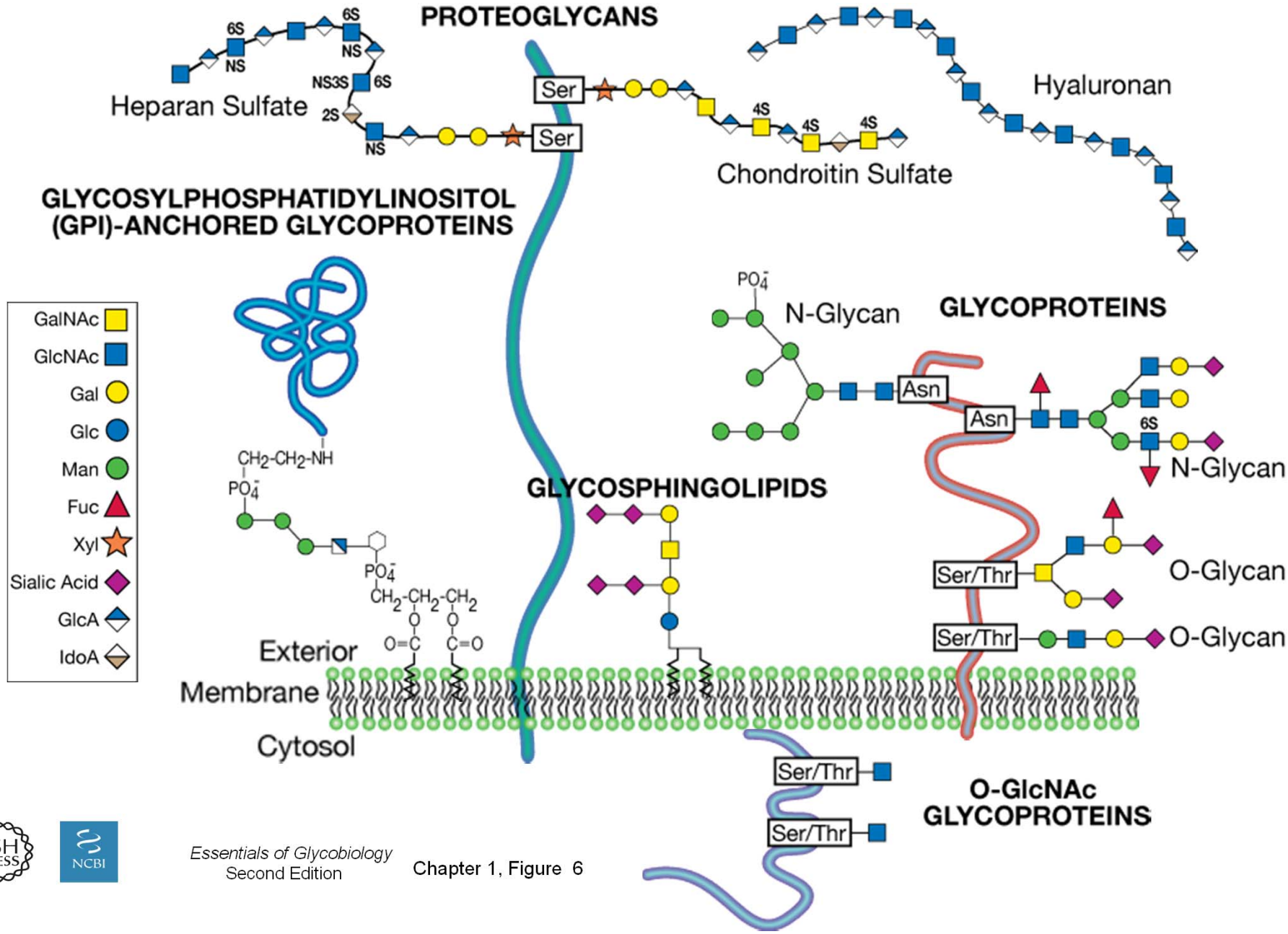
Common classes of animal glycans



FDA-Approved Cancer Biomarkers

Biomarker	Type	Source	Cancer Type	Clinical Use
α-Fetoprotein	**Glycoprotein**	Serum	Liver	Monitoring
α-Fetoprotein-L3	**Glycoprotein**	Serum	Liver	Risk
DCP	Protein	Serum	Liver	Risk
Human chorionic gonadotropin-β	**Glycoprotein**	Serum	Testicular	Staging
CA19-9	**Carbohydrate**	Serum	Pancreatic	Monitoring
CA125	**Glycoprotein**	Serum	Ovarian	Monitoring
Pap smear	Cervical smear	Cervix	Cervical	Screening
CEA	**Glycoprotein**	Serum	Colon	Monitoring
EGF receptor	**Glycoprotein**	Colon	Colon	Selection of therapy
KIT	Protein (IHC)	GI tumor	GI stromal tumors	Diagnosis & selection of therapy
Thyroglobulin	**Glycoprotein**	Serum	Thyroid	Monitoring
PSA	**Glycoprotein**	Serum	Prostate	Monitoring
CA15-3	**Glycoprotein**	Serum	Breast	Monitoring
CA27-29	**Glycoprotein**	Serum	Breast	Monitoring
Cytokeratins	Protein (IHC)	Breast tumor	Breast	Prognosis
Estrogen & progesterone receptors	Protein (IHC)	Breast tumor	Breast	Selection of therapy
HER2/NEU	**Glycoprotein (IHC)**	Breast tumor	Breast	Prognosis & selection of therapy
HER2/NEU	**Glycoprotein**	Serum	Breast	Monitoring
HER2/NEU	DNA (FISH)	Breast tumor	Breast	Prognosis & selection of therapy
Chromosomes 3, 7, 9, and 17	DNA (FISH)	Urine	Bladder	Screening & monitoring
NMP22	Protein	Urine	Bladder	Screening & monitoring
Fibrin/FDP	Protein	Urine	Bladder	Monitoring
BTA	Protein	Urine	Bladder	Monitoring
CEA and mucin	**Glycoprotein**	Urine	Bladder	Monitoring

Adapted from Ludwig, JA & Weinstein, JN Nature Rev. 2005

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Glycosylation Changes in Cancer

- **Altered glycosylation is a universal feature of cancer cells.**
- **This is not a random consequence of disordered biology in cancer**
- **Of all possible changes, only a very limited subset are frequently correlated with malignant transformation and tumor progression.**
- **As cancer is a "microevolutionary" process in which only fittest cells in a genetically heterogeneous population survive, specific glycan changes are likely selected for during tumor progression.**
- **Certain glycan structures are indeed well-known markers for tumor progression and/or biomarkers.**

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ALTERED GLYCOSYLATION IN CANCER

- Increased β1-6GlcNAc branching of N-glycans
- Changes in the amount, linkage, and acetylation of sialic acids
- O-glycan truncation, generating Tn & sialyl Tn antigens
- Failure of O-glycosylation, with mucin polypeptide exposure
- Expression of immature N-glycans
- Expression of nonhuman sialic acid Neu5Gc, from dietary sources
- Expression of sialylated Lewis structures and selectin ligands
- Altered expression and enhanced shedding of glycosphingolipids
- Increased expression of galectins and poly-N-acetyllactosamines
- Altered expression of ABH(O) blood-group-related structures
- Alterations in sulfation of glycosaminoglycans
- Increased expression of hyaluronan
- Loss of expression of GPI anchors.

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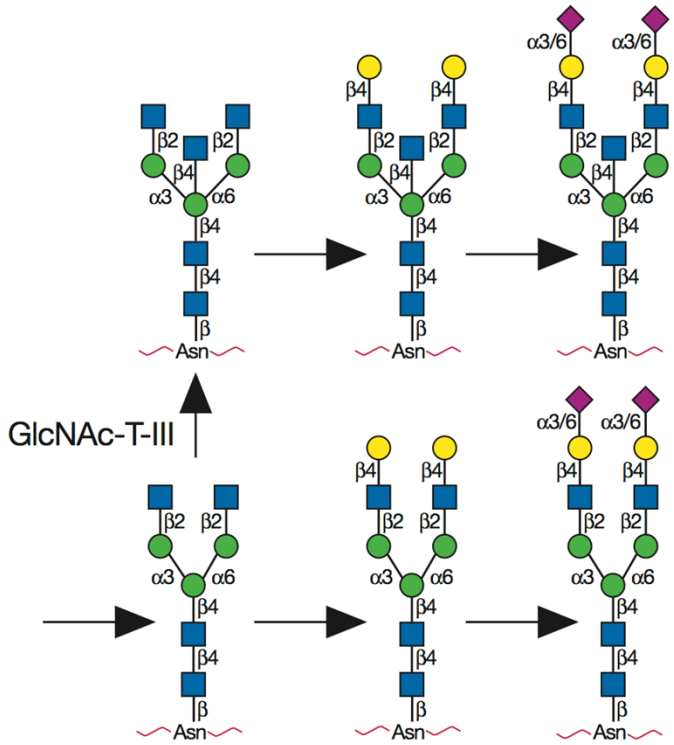
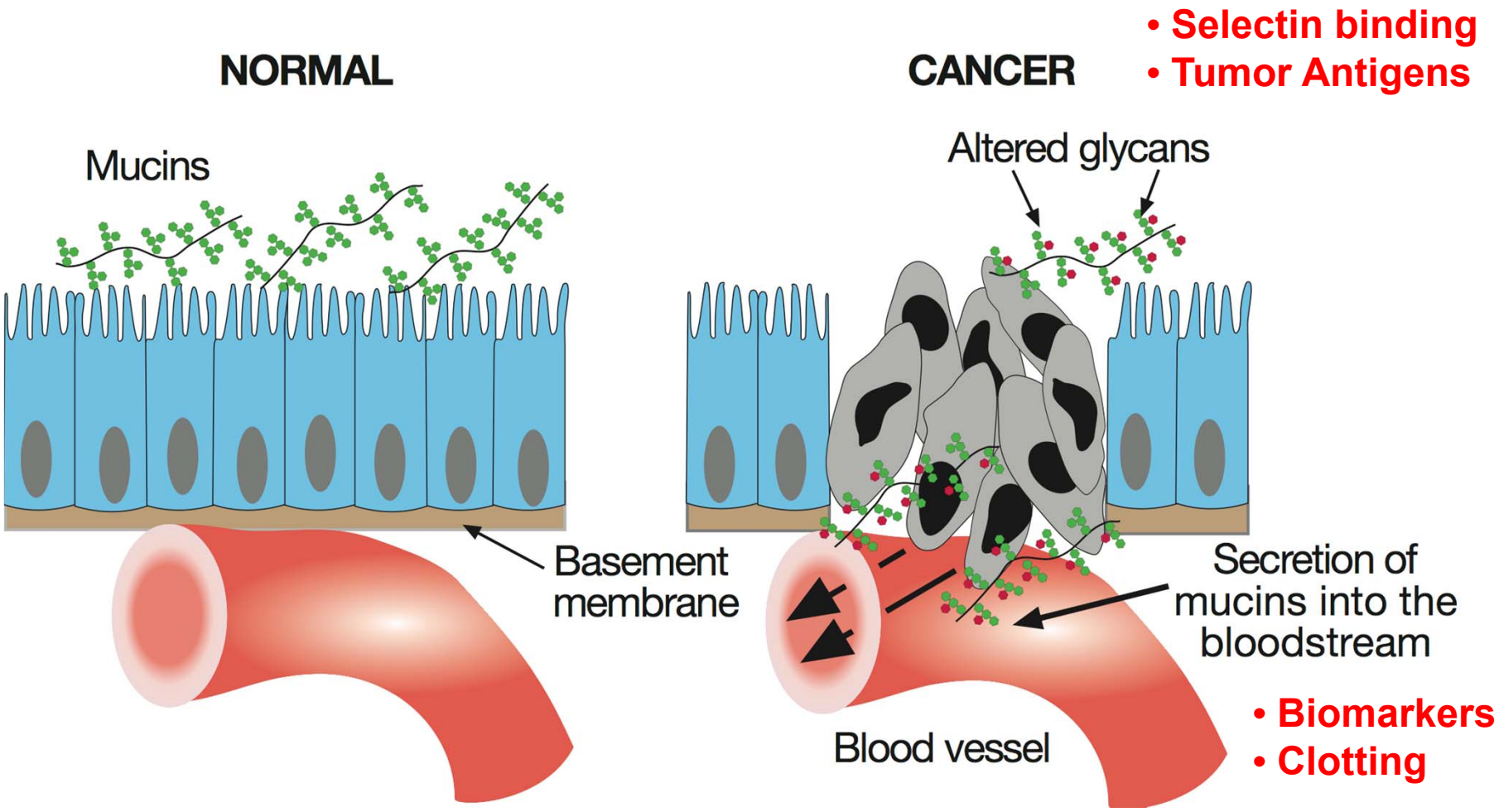
The increased size of N-glycans that occurs upon transformation can be explained by an elevation in GlcNAc transferase-V (GNT-V) activity



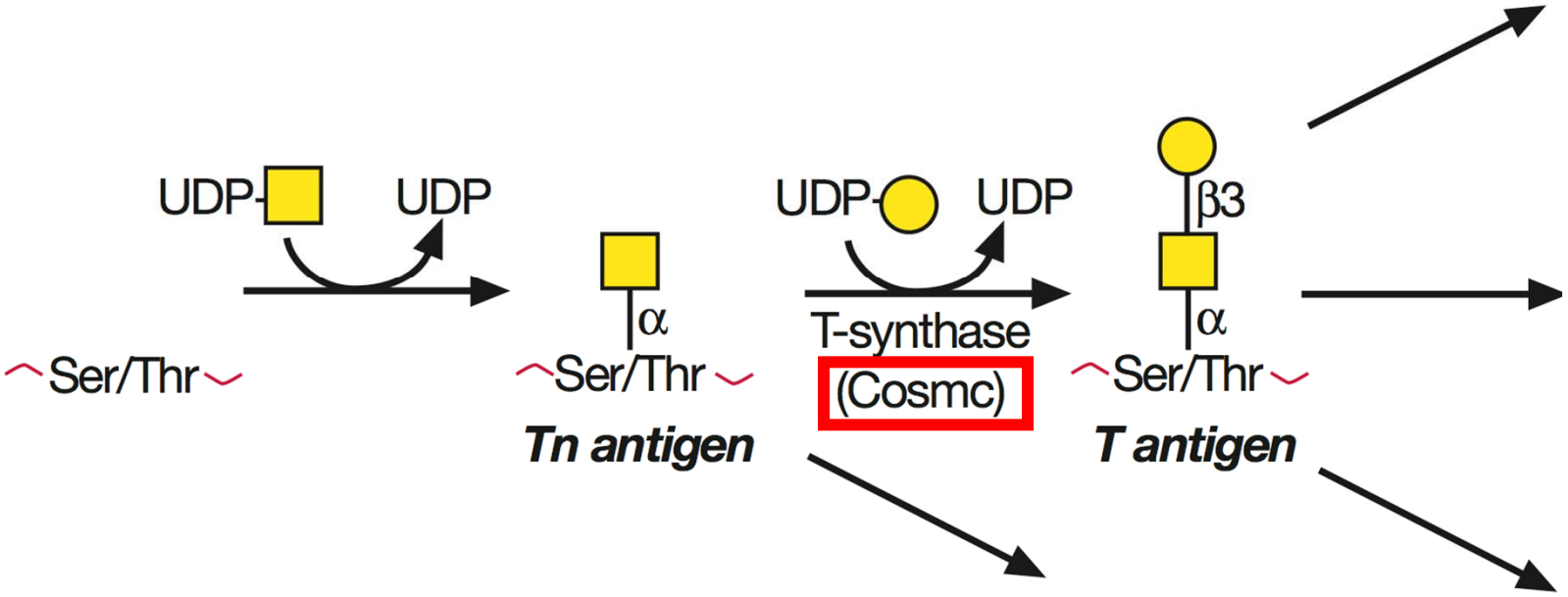
Upregulated In Cancer

Loss of normal topology and polarization of epithelial cells in cancer results in secretion of mucins into the bloodstream

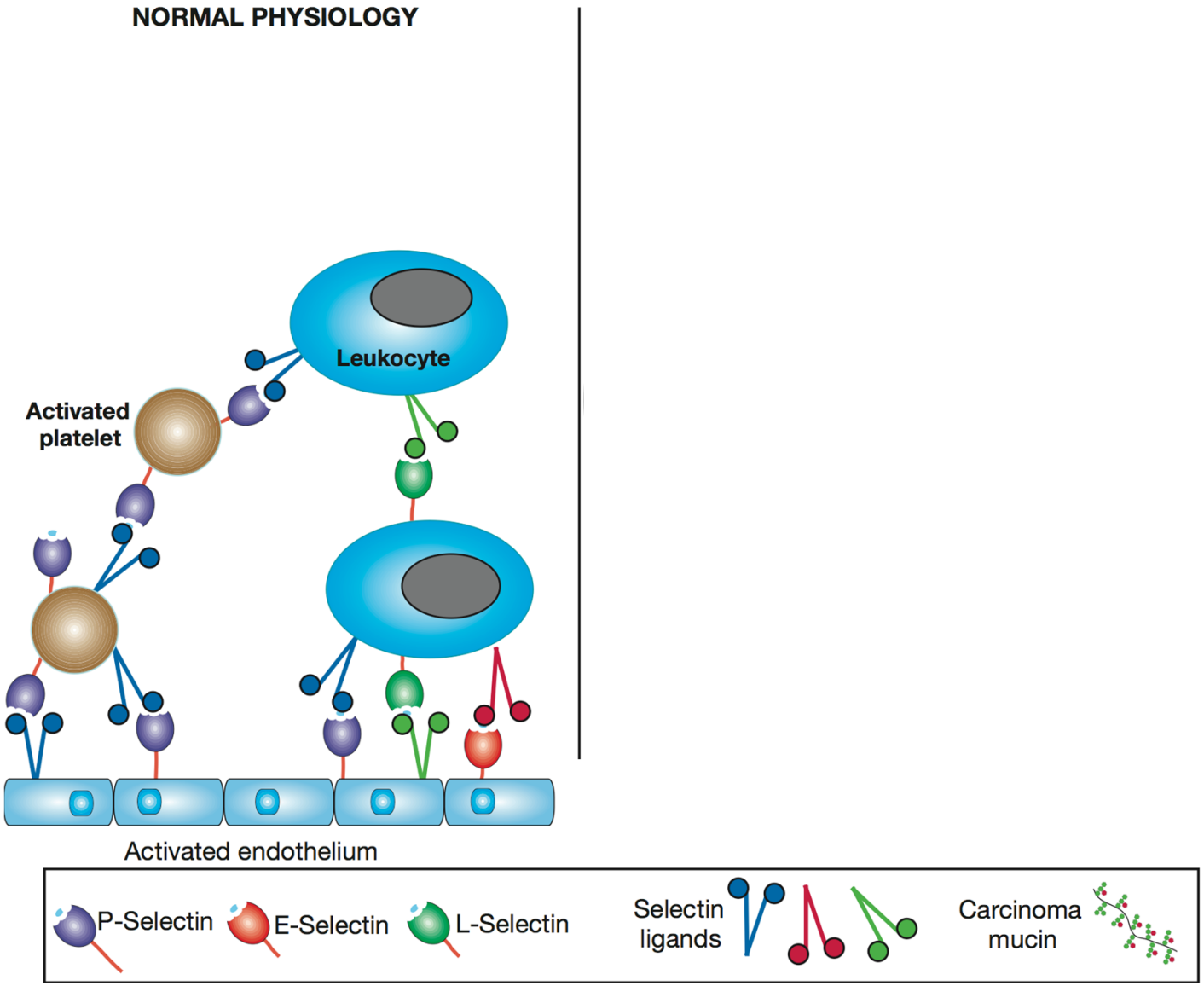


Incomplete glycosylation in the O-linked pathway results in expression of the Tn antigen, the sialylated Tn antigen

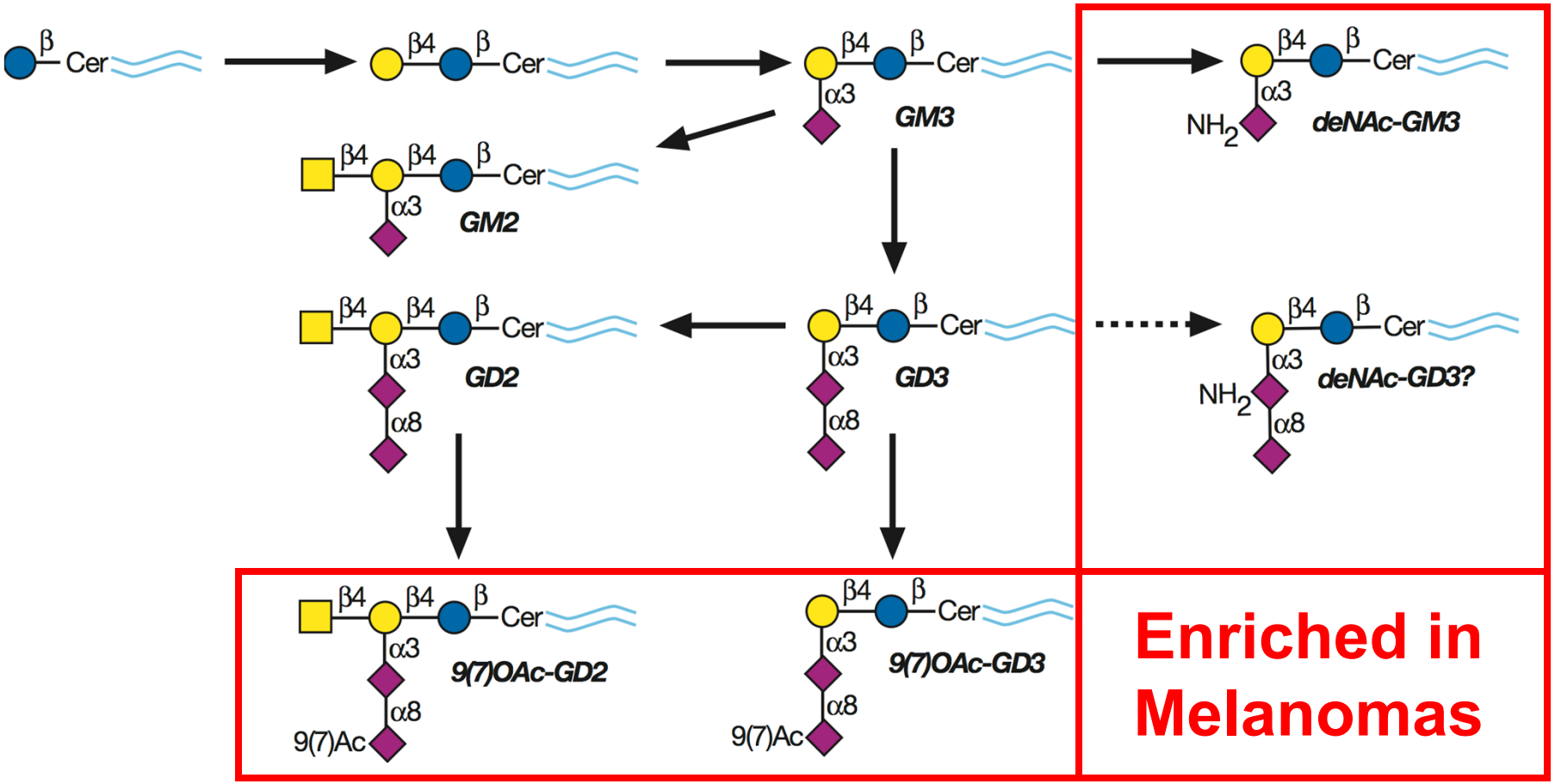
Mutated in many Carcinomas. Single hit on X Chromosome



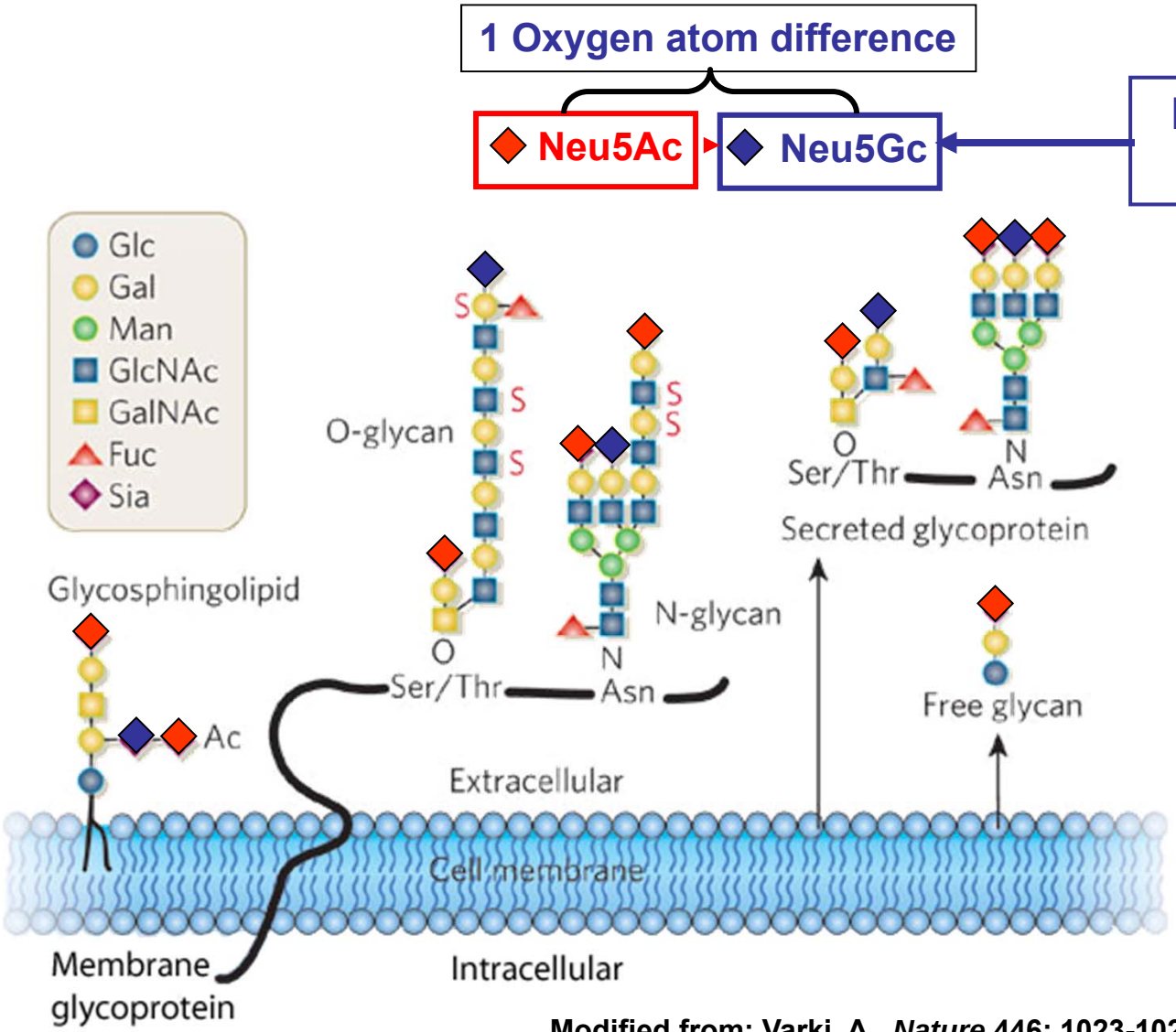
Potential interactions that could occur between tumor cells and selectins
(All shown in vitro. Most shown in vivo)



Some pathways for expression of gangliosides in human neuroectodermal tumors



Two Major Kinds of Sialic Acids on Mammalian Cells



Missing/Immunogenic in Humans?

Present In Human Cancers and Fetuses?!

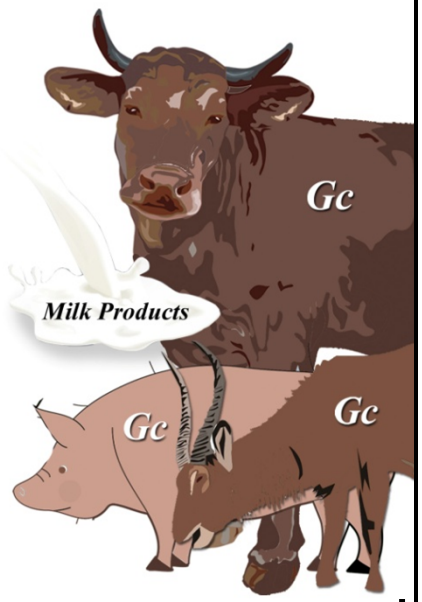
Anti-Neu5Gc Antibodies In Human Cancer?!

Modified from: Varki, A. *Nature* 446: 1023-1029, 2007

Contamination of Humans and Biotherapeutic Products by Incorporation of "Gc" (a.k.a., NGNA/Neu5Gc) Despite Anti-Gc Antibody Responses : Implications and Mechanisms

Human Genetic Mutation
Loss of Gc

Gc = Neu5Gc
(*N*-Glycolylneuraminic acid)



Gc in Food

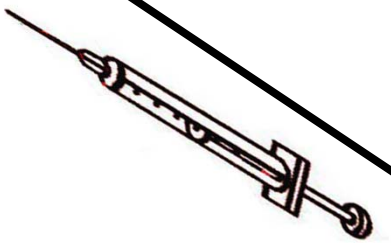
Hedlund et.al. ***Proc.Nat'l.Acad.Sci.U.S.A.*** 105:18936-18941, 2008

Pham et al. *Blood* 114:5225-35. 2009

Gc Incorporation

Increased risk of Cancer Heart Attacks?

Chronic Inflammation

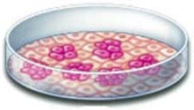


Ghaderi et al. *Nat Biotechnol* (in press)

Serum Substitutes

Serum

Animal Cell Lines



Stabilizers

?

Induced Anti-Gc Antibodies

Human Body
No Intrinsic Gc

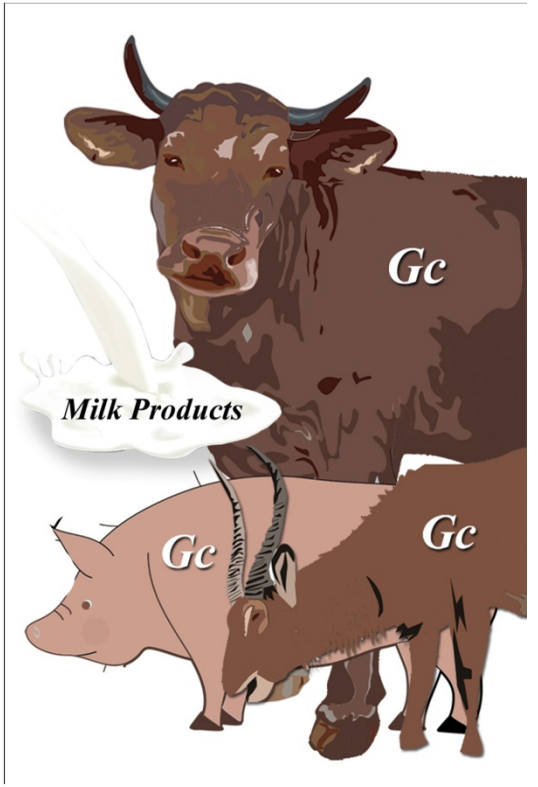
Drug Reactions?
Transplant Rejection?

Gc incorporated into Biotherapeutic Products & Cells: Antibodies, Cytokines, Enzymes, Hormones, Stem Cells, etc.

Contamination of Humans by Dietary Gc* in the Face of Anti-Gc Antibody Responses: Opportunities for Early Detection, Diagnosis, Prognosis and Therapy of Cancer

*Gc = Neu5Gc
(*N*-Glycolylneuraminic acid)

Genetic Mutation
Loss of Gc



Gc in Food

Gc Incorporation

Epithelium of Prostate, Colon, Breast, etc.

Chronic Inflammation

Increased risk of carcinomas of these organs?

Induced Anti-Gc Antibodies

QUANTITATE Gc in URINE

QUANTITATE ANTI-Gc ANTIBODIES IN BLOOD

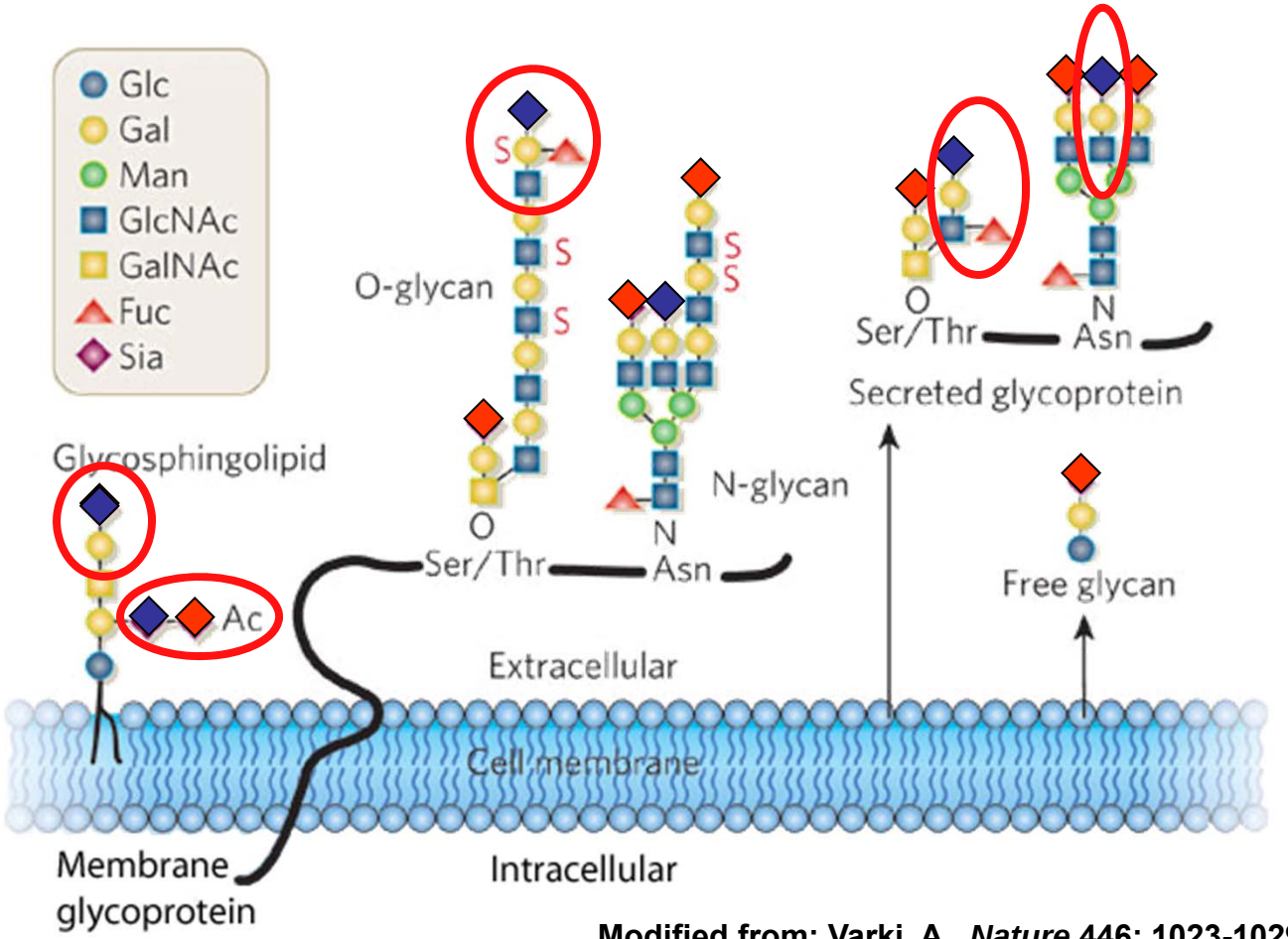
PREDICT CANCER RISK

EARLY DETECTION

PROGNOSIS AND MONITORING

ELIMINATE Gc FROM THE BODY

Each Neu5Gc-Containing Glycan Represents a Distinct Immune Epitope



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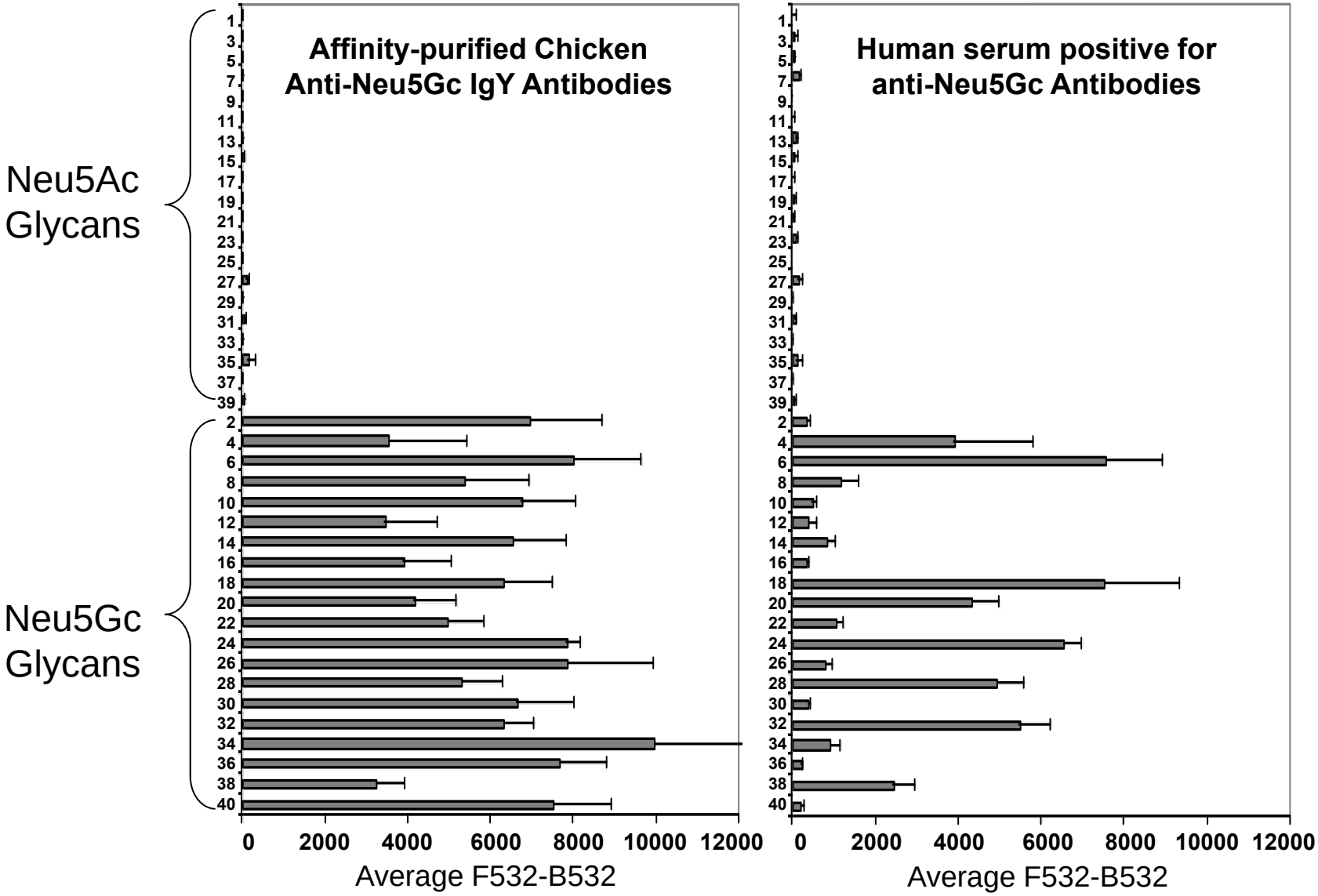
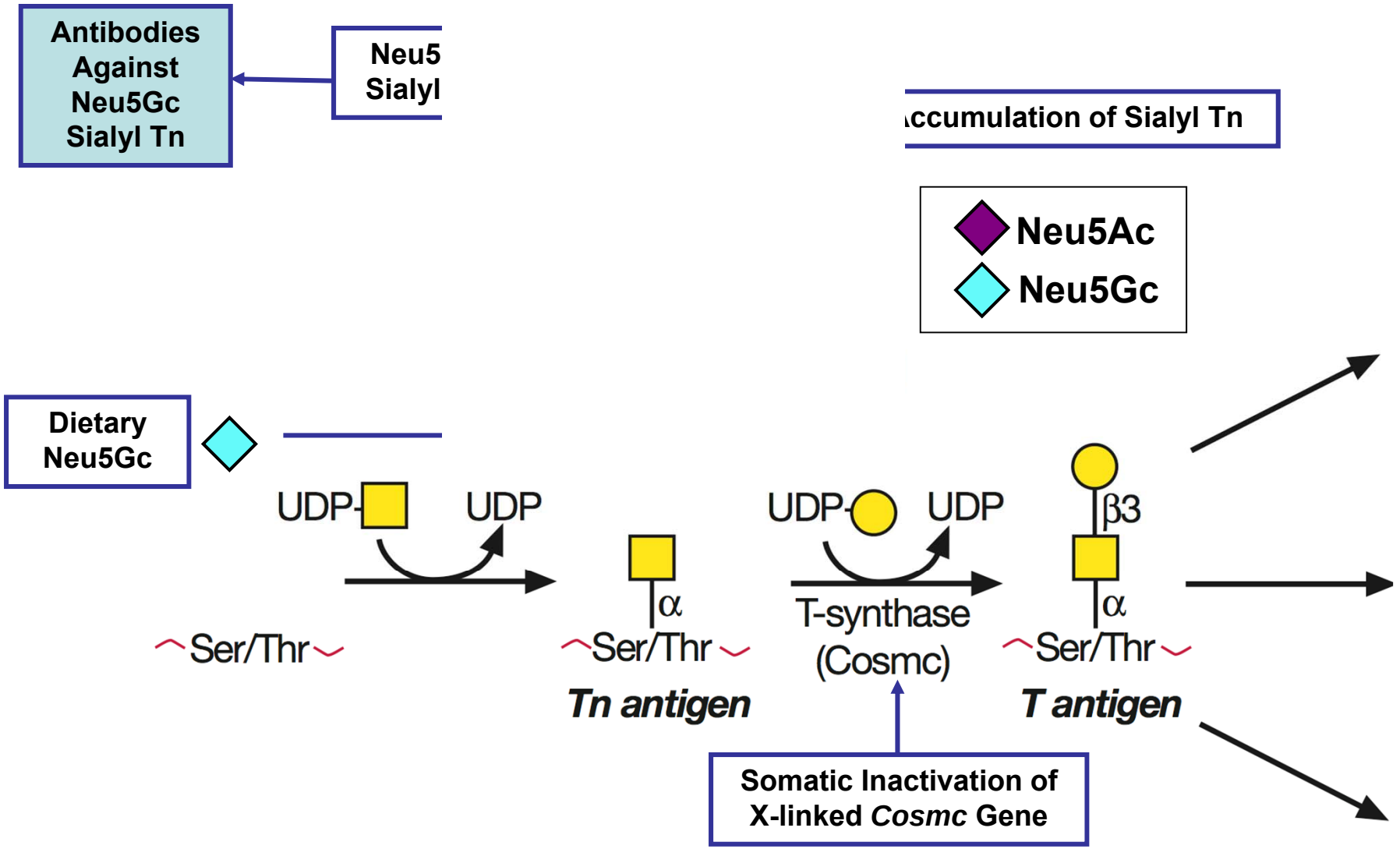
A Novel Sialoglycan Array that Allows Detection of Neu5Gc-Specific Antibodies



Incomplete O-linked glycosylation results in expression of sialylated Tn antigen in cancer

Incorporation of Dietary Neu5Gc generates Neu5Gc-Sialyl Tn and antibodies against it



Slide Modified from Varki et al. *Essentials of Glycobiology*, Chapter 44, Figure 3

ALTERED GLYCOSYLATION IN CANCER: POTENTIAL FOR BIOMARKER DISCOVERY

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