Why Glycobiology?

Learning Objectives

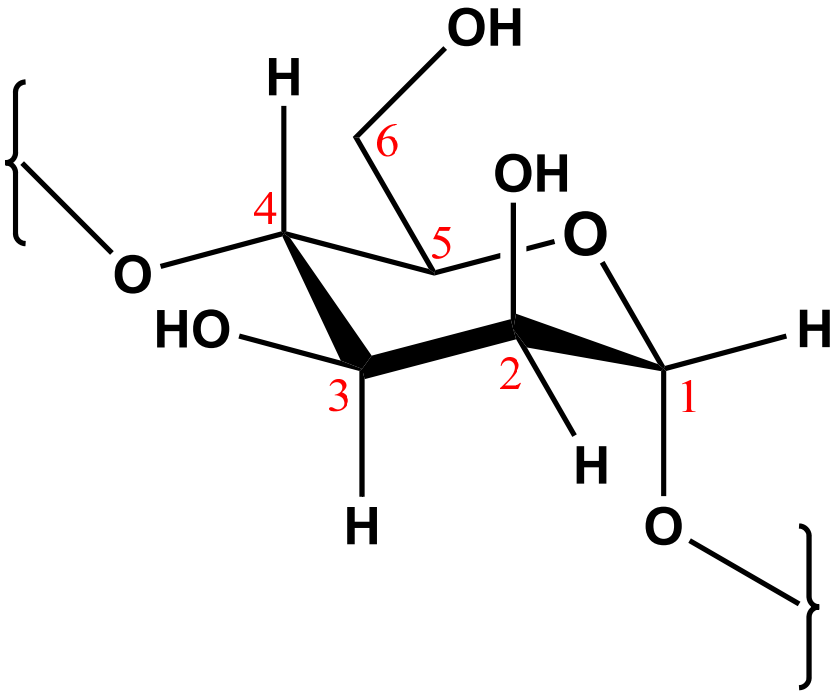
- ***This lecture provides an overview of the glycobiology***
- ***Understand what are glycans***
- ***Learn where are glycans most located in vivo***
- ***Review the types of interactions made by glycans***
- ***Evaluate the structures of glycans***
- ***Learn about glycans as drugs***
- ***Learn about proteins that bind glycans***
- ***Study the molecular mechanisms of glycan function***

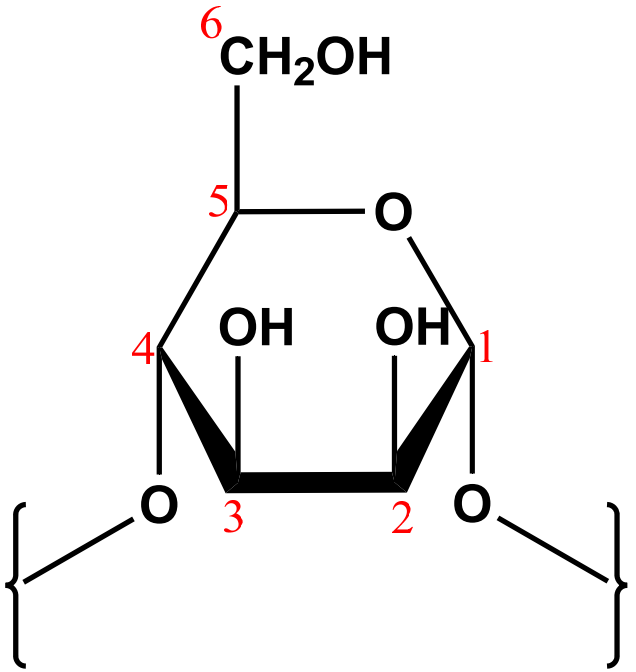
Why Glycobiology?

- ***What are Glycans"***
  - ✓ *What does 'glycans' refer to?*
  - ✓ *Broad classification*
- ***Glycans on Proteins and Cell Surfaces***
  - ✓ *Glycans dominate protein surface*
  - ✓ *Glycans dominate cell surface too*
- ***Glycan Modulation of Physiology and Pathology***
  - ✓ *Different types of interactions*
- ***Glycans and Structural Diversity***
  - ✓ *Types of scaffolds*
  - ✓ *Types of conformations and substitutions*
- ***Glycans as Drugs***
  - ✓ *Fondaparinux*
  - ✓ *Oseltamivir/Zanamivir*
  - ✓ *Other clinically approved drugs*
- ***Glycan Binding Proteins***
  - ✓ *Lectins*
  - ✓ *Glycosaminoglycan binding proteins*
- ***Unique Mechanisms of Glycan Function***

What are Glycans?

- ***What does the term 'glycan' refer to?***
  - ✓ *…. constituents possessing a saccharide residue (below)*
  - ✓ *…. single residue or a chains*
  - ✓ *…. linear or branched chain*
  - ✓ *…. free or could be linked to another molecule (small or large)*

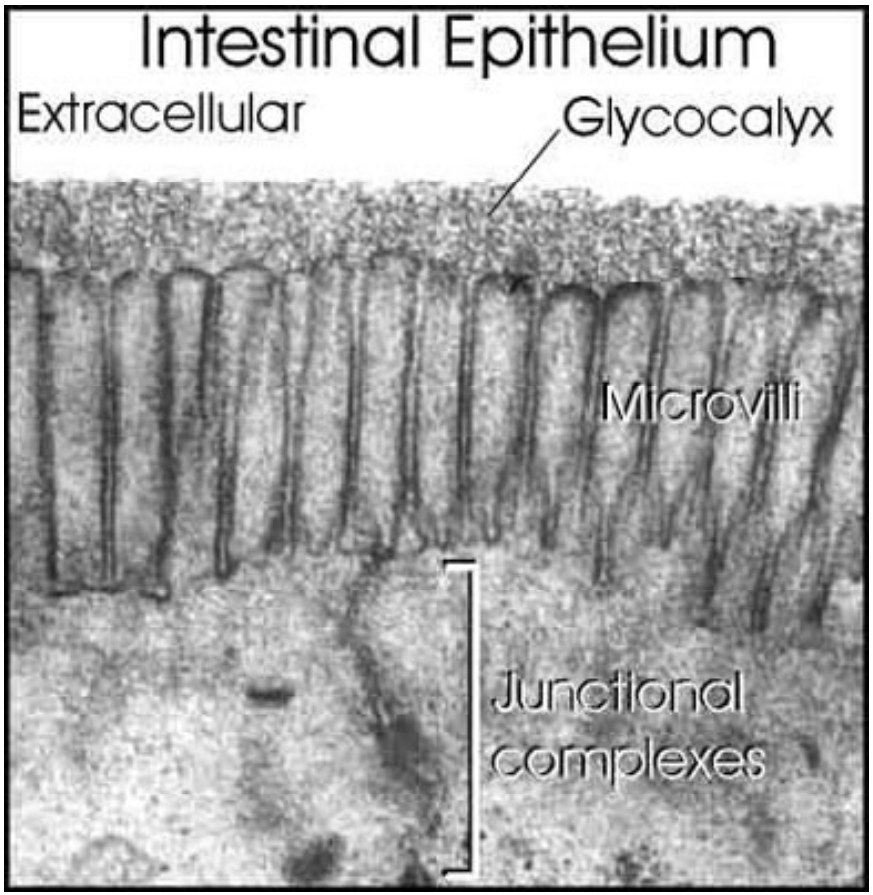


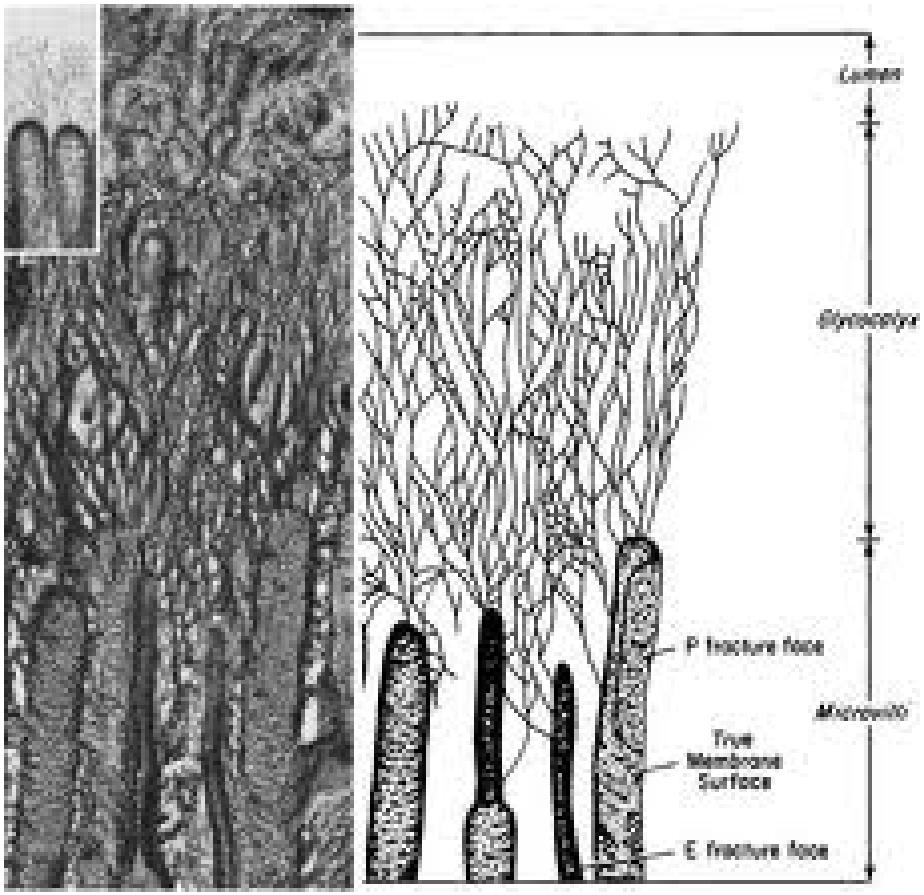


Polyhydroxyaldehyde or polyhydroxyketones and their simple derivatives, or oligomeric or polymeric compounds that can be hydrolyzed into such simple units

Glycans Dominate on Cell Surfaces

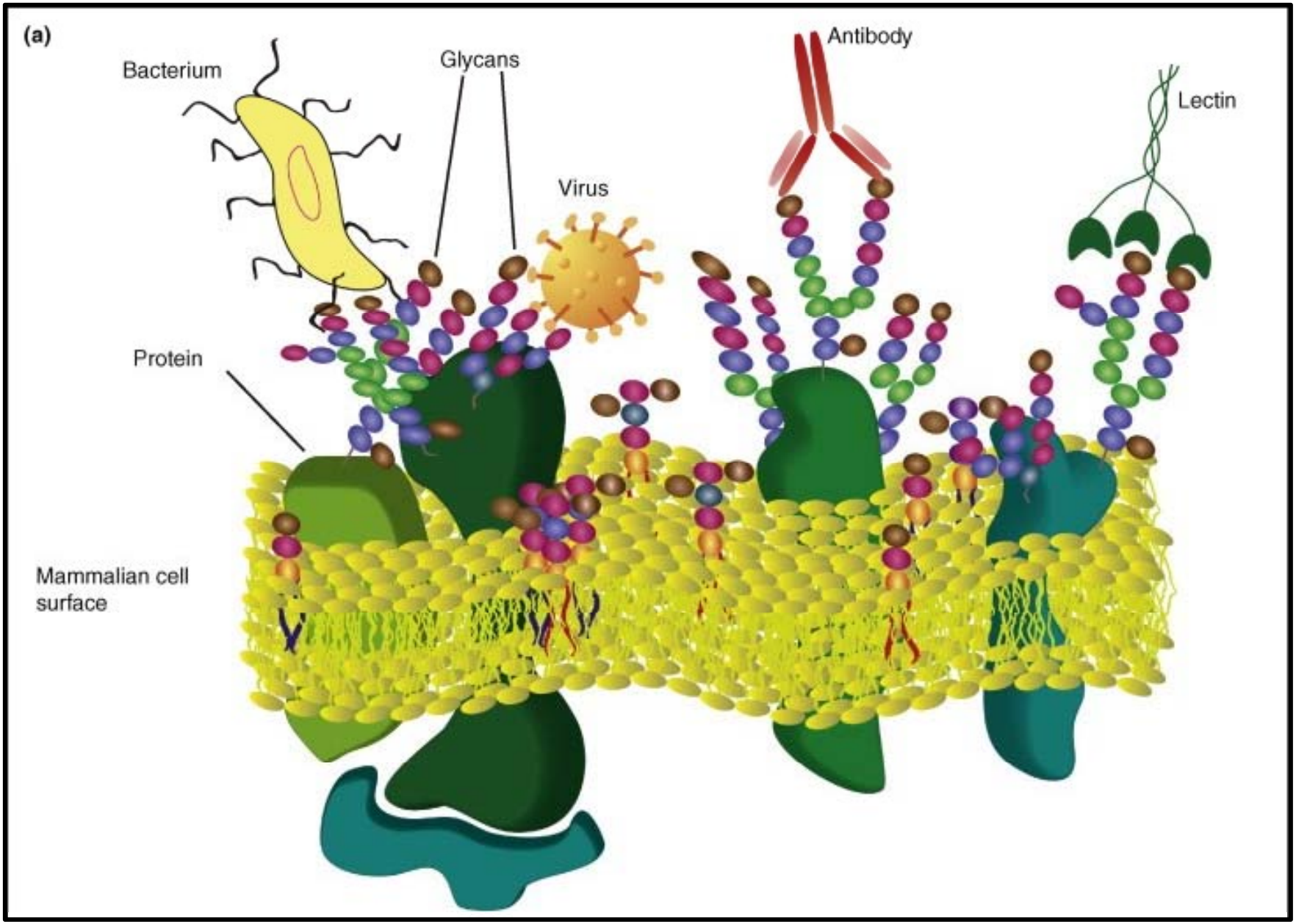
- ***Glycocalyx***
  - ✓ Is a dense, fuzzy coating on animal cell surface … external to the plasma membrane … mostly consists of carbohydrate moieties including glycolipids, glycoproteins, and proteoglycans.
  - ✓ Presents substantial physical barrier and maintains tissue structure & porosity
  - ✓ Everybody's glycocalyx is unique. Our body uses glycocalyx to distinguish self from non-self



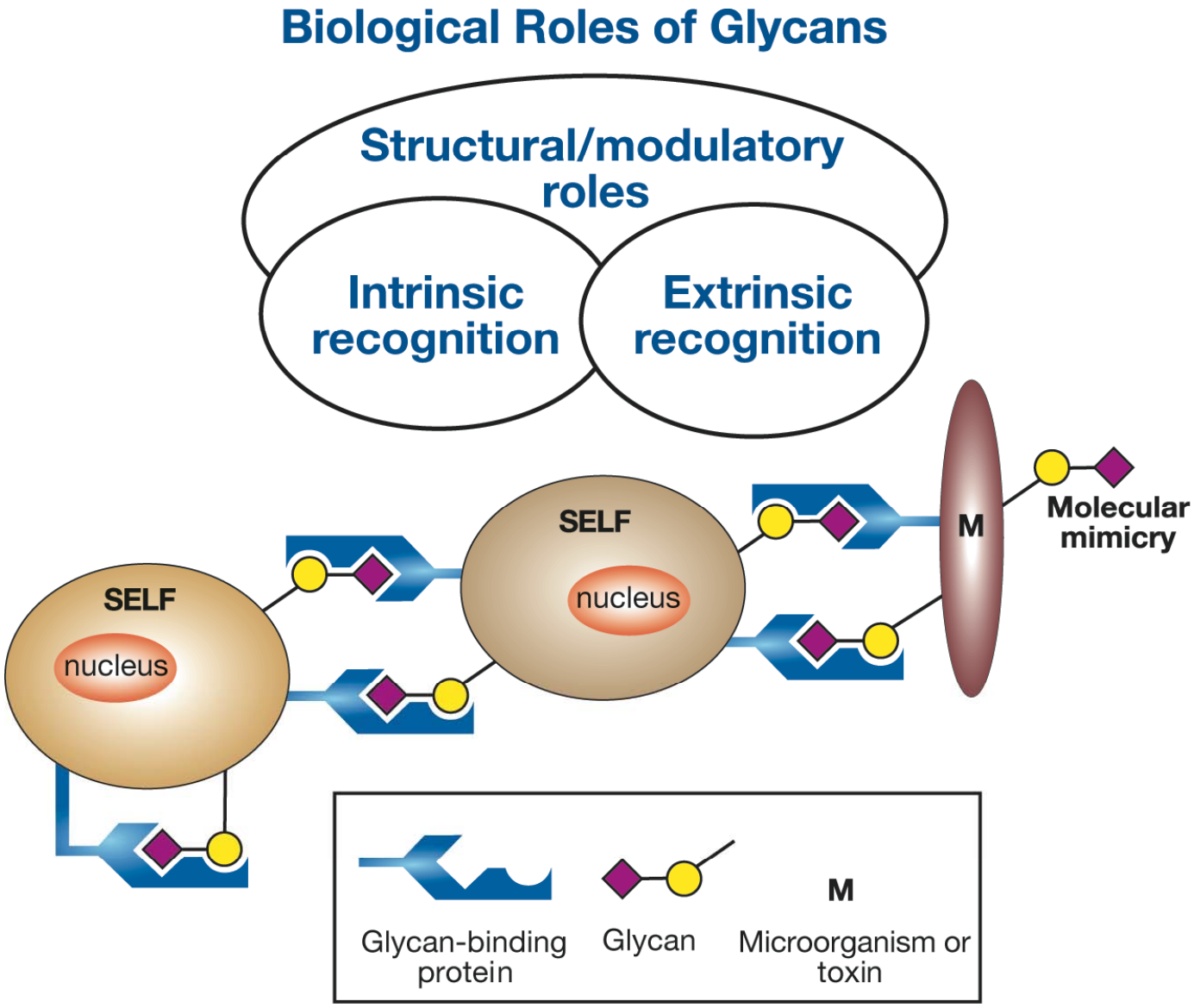


Images downloaded from 1) http://www.erin.utoronto.ca/~w3bio315/lecture2.htm; 2) http://www.comprehensivephysiology.com

Glycans On the Cell Surface Form Site of Recognition



Overview of the Biological Roles of Glycans



'Essentials of Glycobiology' 2nd Edition (Varki, A.; et al. Editors), Cold Spring Harbor, New York, (2009) pg. 76

Glycan Interactions Modulate Physiology and Pathology

Physiology/Pathology	Glycan-bearing & Glycan-interacting Entities
Microbial Infection	Host cells & Bacteria, Viruses
Immune Response	Phagocytes, Macrophages, & Microbes
Fertilization	Zona pellucida & Sperm
Leukocyte Recruitment	Leukocytes & Endothelial cells
Metastasis	Malignant cells & Host cells
Plant Flowering/Defense	Free Glycan & cell surface receptor
Blood Group Determinants	Glycans on cell surface Gp

Glycans May Dominate on the Protein Surface too

- ***Glycans Extend Way Out From the Protein Surface***

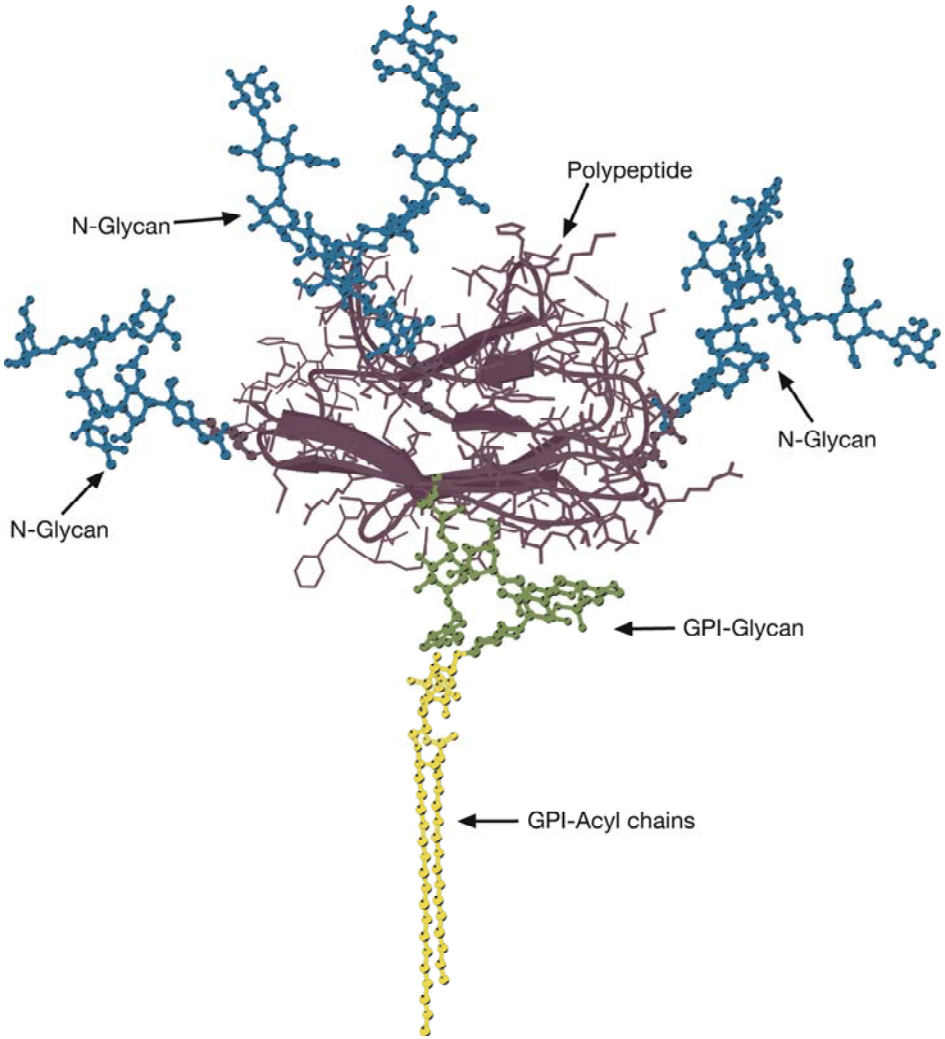


FIGURE 1.3. Schematic representation of the Thy-1 glycoprotein including the three *N*-glycans (*blue*) and a glycosylphosphatidylinositol (GPI-glycan, *green*) lipid anchor, whose acyl chains (*yellow*) would normally be embedded in the membrane bilayer. Note that the polypeptide (*purple*) represents only a relatively small portion of the total mass of the protein.

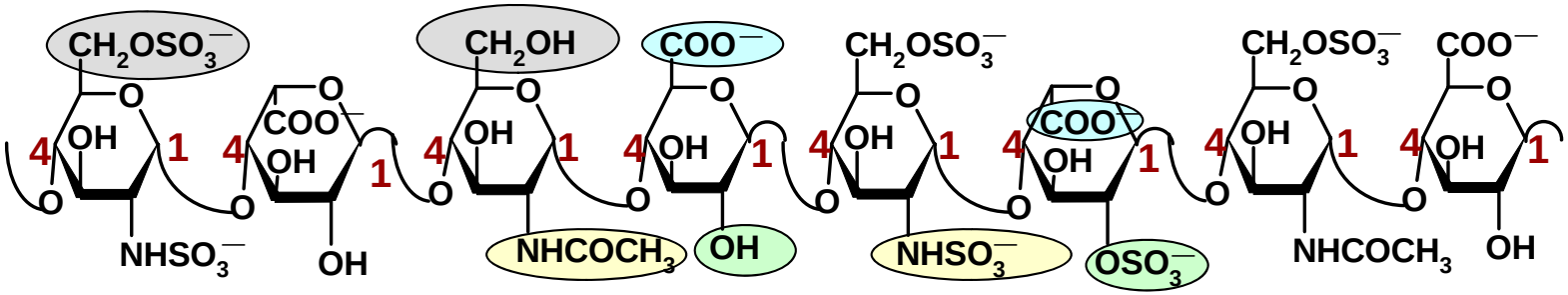
'Essentials of Glycobiology' 2nd Edition (Varki, A.; et al. Editors), Cold Spring Harbor, New York, (2009) pg. 10

Glycans Present Phenomenal Structural Diversity … 1

- ***The Fundamental Carbohydrate Scaffold and Its Implications***
  - ✓ *Many monosaccharide types*
    - Glc, Gal, Man, Xyl, Fuc, NeuAc, ……
    - 16 hexoaldoses, 8 hexoketoses, 8 pentoaldoses, 4 pentoketoses, ….
    - 2 common sialic acids (NeuAc and Kdn) + numerous others
    - Compare these with 20 amino acid residues and 8 nucleosides (oxy and deoxy)
  - ✓ *Many points of attachment*
    - A 1→4 attachment is different from 1→6 attachment
    - Possibilities 1→2, 1→3, 1→4, 1→6
    - Two additional possibilities arising from anomeric center
    - Compare with one attachment type for proteins (amide bond) and nucleic acids (phosphodiester bond)
  - ✓ *Many different substituents possible*
    - Amine ($-NH_2$)
    - Amide ($-NHCOCH_3$)
    - Ester ($-OSO_4^{-2}$, $-OPO_3^{-2}$)
    - Deoxy (-OH → -H)

Glycans Present Phenomenal Structural Diversity … 2

- ***An Example from the Glycosaminoglycan (GAG) Class of Glycans***
  - ✓ A specific GAG – heparin/heparan sulfate (H/HS) – is orders of magnitude more complex than any known biopolymer!
  - ✓ Each H/HS sequence can be a potential modulator of protein function



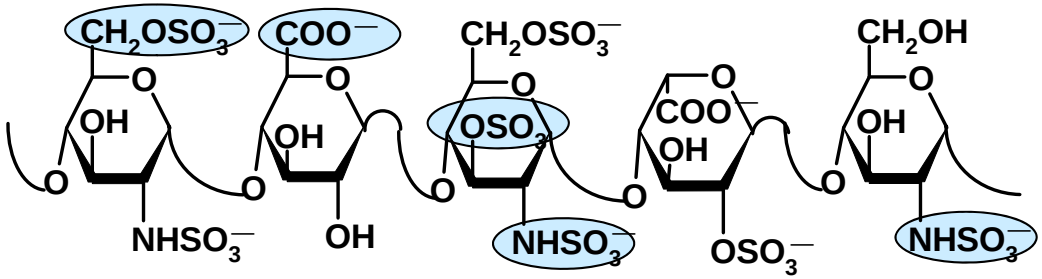
Heparin / Heparan Sulfate

Theoretical Number of Hexameric Units

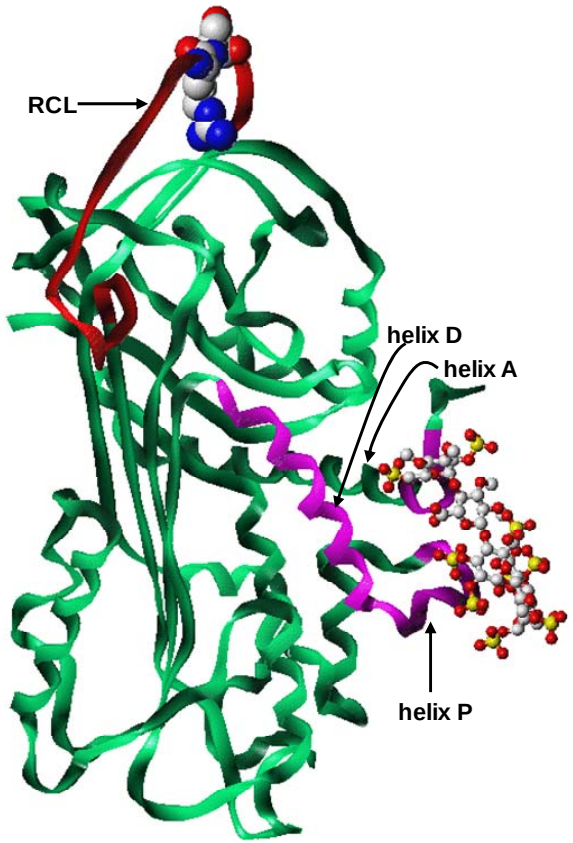
Nucleic Acids	$(4 \text{ nucleotides})^6$	$\approx 4 \times 10^3$ sequences
Peptides	$(20 \text{ residues})^6$	$\approx 64 \times 10^6$
GAGs	$(96 \text{ disacc. units})^6$	$\approx 0.8 \times 10^{12}$
GAGs (w/ $^1C_4$; $^4C_1$; ^2S_O; $^OS_2$)	$48^6 + (48 \times 4)^6$	$\approx 50 \times 10^{12}$

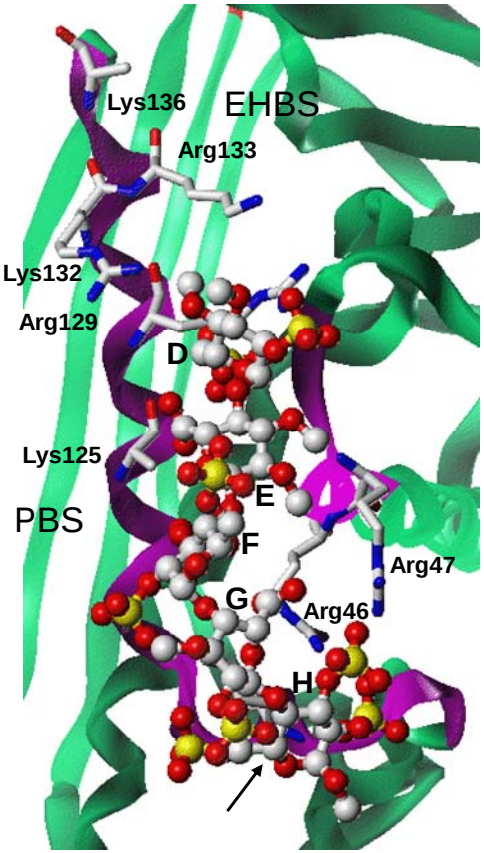
A Major Class of Anticoagulants is Polysaccharide-based

- ***Fondaparinux***



High Specificity Pentasaccharide Sequence

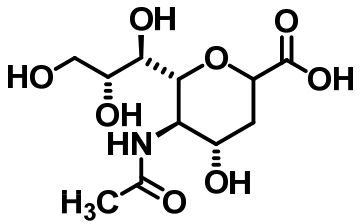




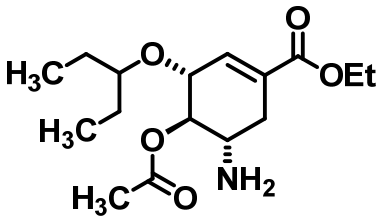
Proc. Natl. Acad. Sci. USA 94 (1997) 14683-8; *Nat. Struct. Mol. Biol.* 11 (2004) 857-62; *Nat. Struct. Mol. Biol.* 11 (2004) 863-7; *EMBO J.* 25 (2006) 2029-37; *Proc. Natl. Acad. Sci. USA* 107 (2010) 645-50

A Major Class of Anti-Virals is Polysaccharide-based

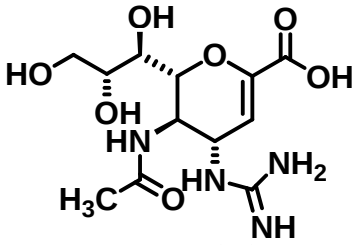
- ***Oseltamivir and Zanamivir***
  - ✓ *Influenza virus utilizes hemagglutinin (HA) and neuraminidase (NA) to infect and propagate. HA recognizes host cell surface sialic acids and NA helps spread following cleavage of these sialic acid groups*
  - ✓ *Oseltamivir and zanamivir competitively inhibit NA and attenuate replication*



5-Acetyl-neuraminic acid (Sialic Acid)



Oseltamivir



Zanamivir

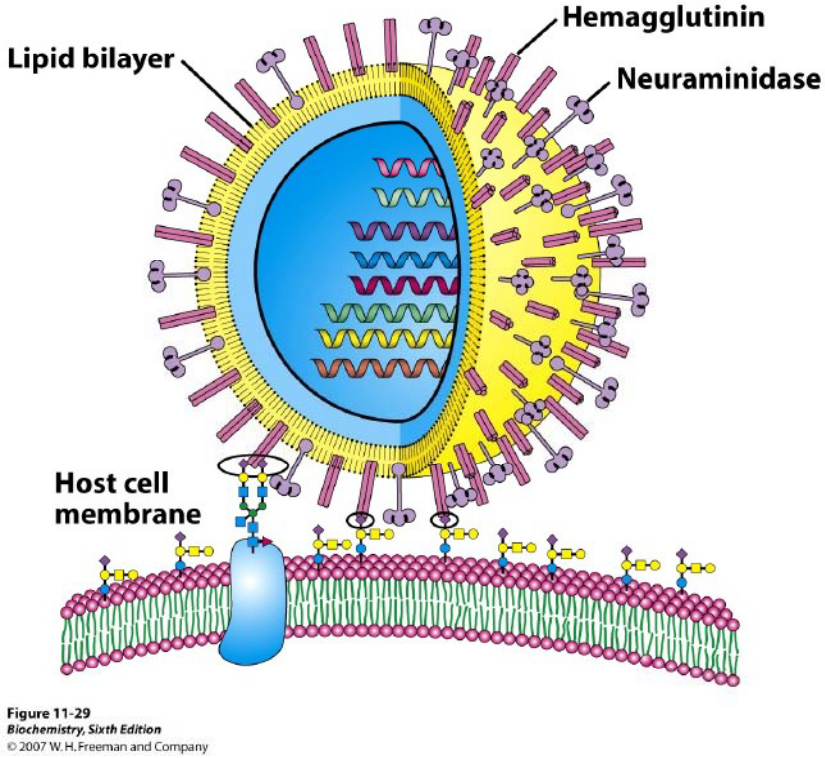


Figure 11-29
Biochemistry, Sixth Edition

Nature Presents a Large Number of Glycan-Binding Proteins … 1

- ***Classes of Carbohydrate-Binding Proteins***
  - ✓ *Lectins*
    - *L-type lectins* (leguminous plants derived lectins)
    - *C-type lectins* (Ca^{2+} dependent lectins)
    - *R-type lectins* (ricin-like lectins)
    - *I-type lectins* (immunoglobulin-like lectins, siglecs = sialic acid-recognizing immunoglobulin family of lectins)
    - *P-type lectins* (man-6-phosphate recognizing lectins)
    - *Galectins* (β-gal recognizing lectins)
    - …..

    (NOTE: no universal classification of lectins)

Nature Presents a Large Number of Glycan-Binding Proteins … 2

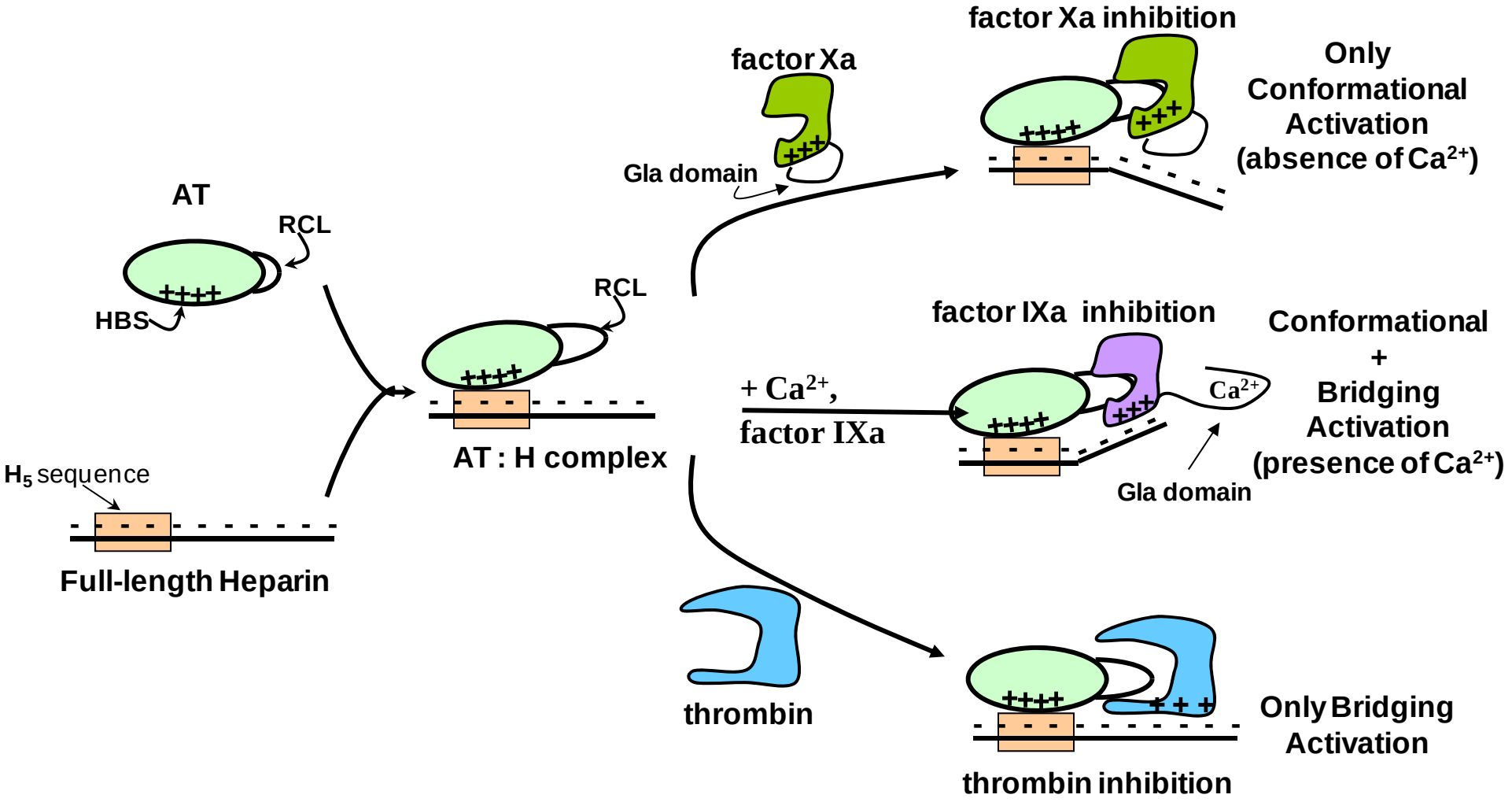
- ***Classes of GAG-Binding Proteins***
  - ✓ *Serpins*
    - Antithrombin, heparin cofactor II, protease nexin 1, ZPI, ….
  - ✓ *Enzymes*
    - Coagulation proteases, GAG biosynthetic enzymes, ….
  - ✓ *Chemokines*
    - Platelet factor 4, γ-interferon, MIP-1β (CCL4), RANTES (CCL5), MCP-3 (CCL7), IL8 (CXCL8), …
  - ✓ *Growth factors and their receptors*
    - FGF and its receptor, VEGF and its receptor, …
  - ✓ *Viral envelope proteins*
    - Herpes simplex glycoproteins gD, gB, gC and others, HIV glycoproteins gP120, dengue virus proteins
  - ✓ *Lipid-binding proteins*
    - Apolipoprotein E, apolipoprotein B, lipoprotein lipase, …
  - ✓ *Numerous other proteins*
    - 435 Proteins apparently constitute the heparin interactome (Ori A., et al. *J. Biol. Chem.* **2011**, *286*:19892-19904)

Yet, Glycan-Based Drugs in the Clinic are Only a Handful!

- ***Clinically Approved Drugs***
  - ✓ ***Anticoagulants*** (Heparin, Low Molecular Weight Heparins (enoxaparin, tinzaparin, dalteparin), and Fondaparinux)
  - ✓ ***Antivirals*** (Zanamivir)
  - ✓ ***Antibiotics*** (Aminoglycosides (neomycin, kanamycin, streptomycin, kanamycin, …))
  - ✓ ***Congestive Heart Failure drugs*** (Cardiac glycosides (digoxin, ….))
  - ✓ ***Antiacid*** (Sucralfate)
  - ✓ ***Diuretic*** (Mannitol)
  - ✓ ***Anti-Arthritic*** (Glucosamine sulfate)
  - ✓ ***Anti-Diabetic*** (Acarbose (tetrasaccharide), miglitol (aza-sugar))
  - ✓ ***Anti-Cancer*** (Carbohydrate vaccines in development)
  - ✓ ***Other Indications*** (Hyaluronic acid (eye disorders, osteoarthritis, wound healing)

Mechanisms of Glycan Action Are Unique

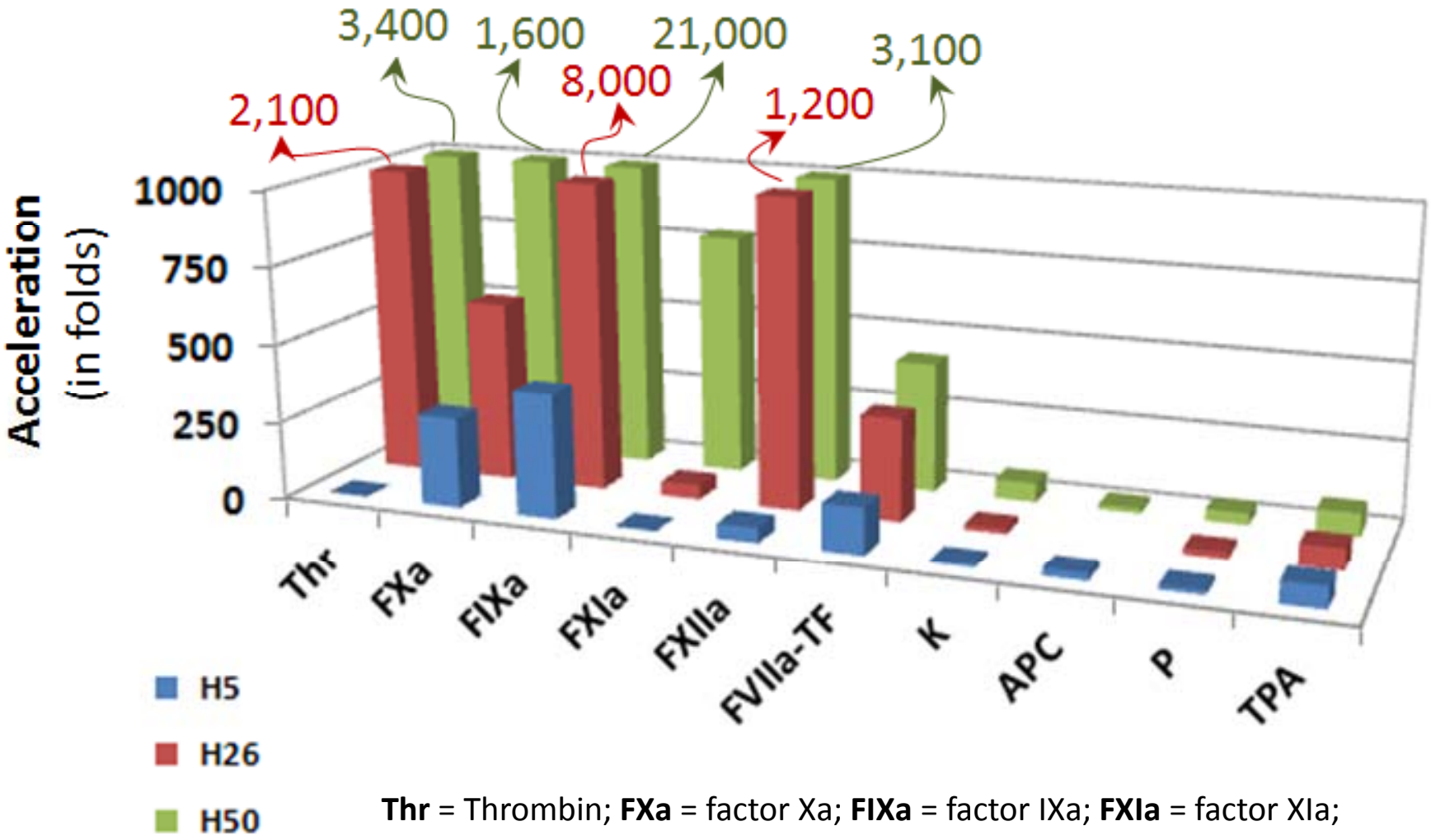
- ***Unique Use of Polymeric Chain of Heparin***
  - ✓ *Targeted elimination of coagulation enzymes through distinct mechanisms*



Med. Res. Rev. (2004) *24*:151-181

Modulation is Different for Different Proteins

- ***Importance of Saccharide-Dependent Unique Binding Geometries***
  - ✓ *Exact saccharide structures remain unknown to date*



Thr = Thrombin; **FXa** = factor Xa; **FIXa** = factor IXa; **FXIa** = factor XIa;

FXIIa = factor XIIa; **FVIIa-TF** = factor VIIa – tissue factor; **K** = kallikrein;

APC = activated protein C; **P** = plasmin; **TPA** = tissue-type plasminogen activator

Thromb. Hemost. (2004) *92*:929-939

Mechanisms of Glycan Action Are Unique

- ***GAG Modulation of Angiogenesis***
  - ✓ *Dimerization of receptor as a means of intracellular signaling*

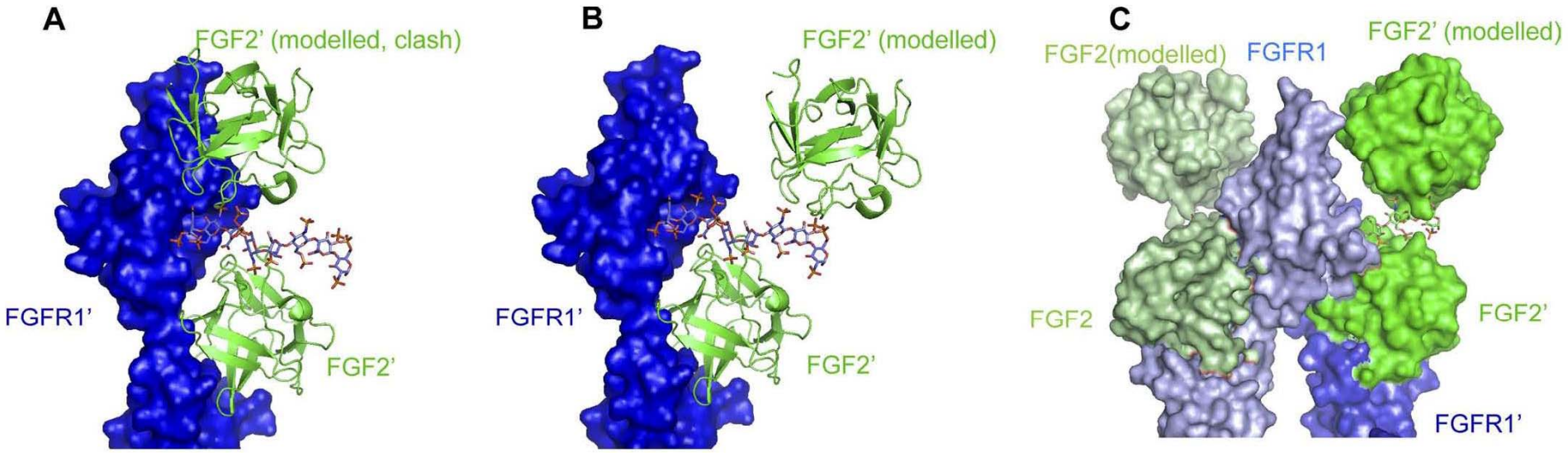
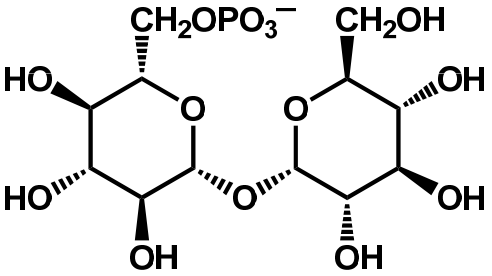


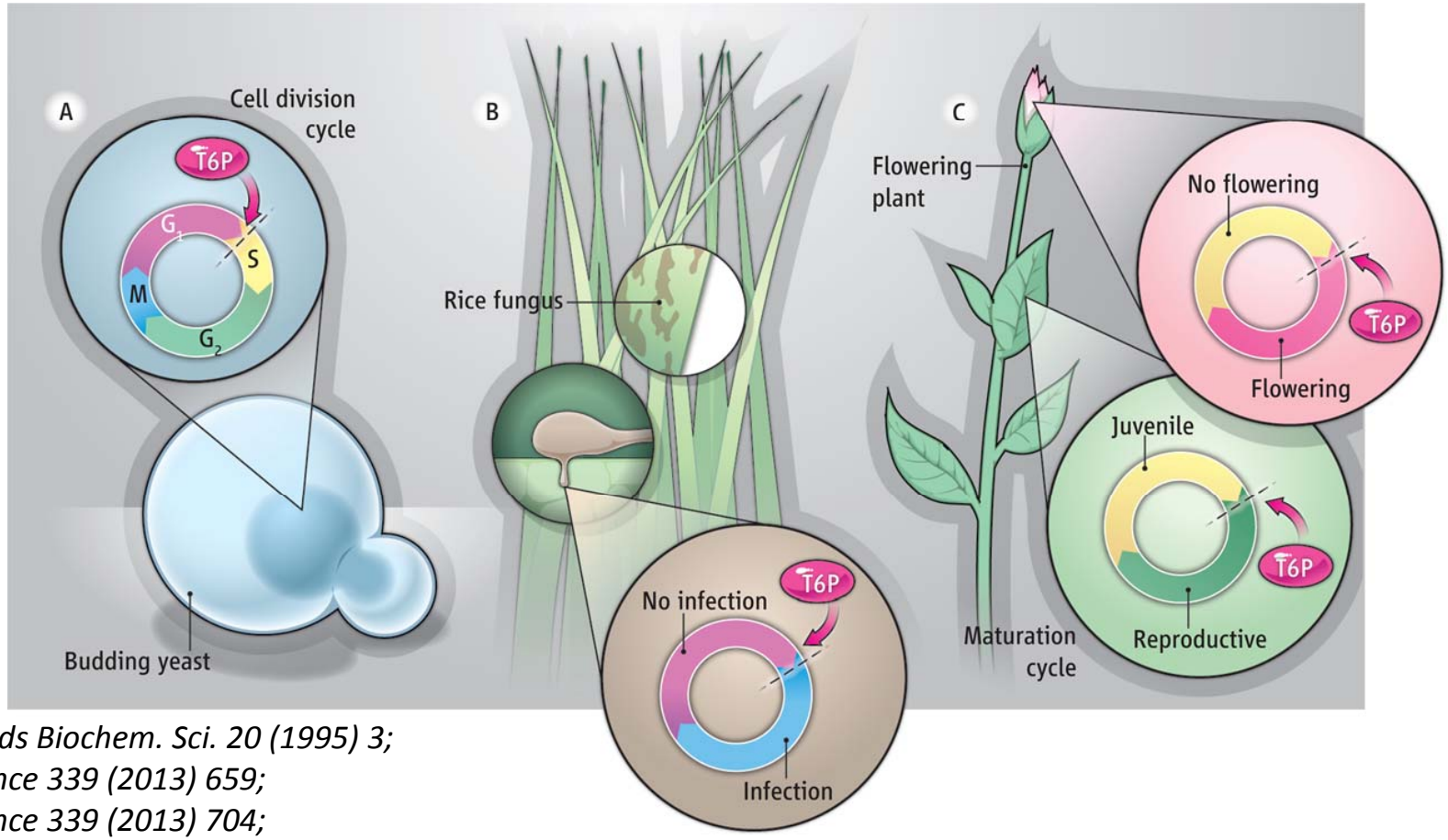
FIGURE 6. **Modeled structures of FGF2·FGFR·HM complexes based on the symmetric ternary complex (1FQ9).** *A* and *B*, FGF2 modeled to the 1:1:1 FGF2·FGFR1 D2D3·HM_8 heteromeric half-complex of the crystal structure. FGF2 is depicted as *green schematics*, HM in *sticks*. FGFR1 D2D3 is symbolized by a *blue surface*. *A*, modeled structure according to the HM_6 like $(FGF2)_2$·HM complex (Fig. 5, *A* and *B*, *IVa*). FGFR1 D2 and FGF2 overlap sterically. This complex cannot be formed. *B*, modeled structure with the second possible $(FGF2)_2$·HM_8 binding mode (Fig. 5*B*, *IVb*). No sterical clashes indicate that this complex is a possible intermediate of ternary complex formation. *C*, modeled ternary complex of a 4:2:2 FGF·FGFR·HM stoichiometry according to the heteromeric half-complexes depicted in *B*. This proposed complex is sterically possible as an intermediate assembly state for FGF-induced FGFR signaling.

J. Biol. Chem. 2010, 285:26628-26640

Same Glycan in Flowering, Yeast Budding and Fungal Growth!

- ***Trehalose 6-phophate***
  - ✓ *Unique role of this sugar in decision-making*
    - a) In the budding yeast *S. cerevisiae* …. Decision to proceed through cell division is controlled by T6P
    - b) In the rice blast fungus *M. oryzae* … T6P senses environment and initiates infectious growth
    - c) T6P regulates flowering both in the leaf and in the shoot apical meristem





Trends Biochem. Sci. 20 (1995) 3;
Science 339 (2013) 659;
Science 339 (2013) 704;