Lecture 14 Carbohydrate recognition in cell adhesion and signalling

Learning objects

- The classification of sugar-binding proteins based on structures of carbohydrate-recognition domains
- The roles of mannose-binding protein and other C-type lectins in innate immunity
- How the selectins function as cell-adhesion molecules
- The adhesion and signalling functions of siglecs in the immune system
- How galectins modulate adhesion and signalling in lymphocytes (淋巴细胞) and other cells

- Lectins serve as receptors for specific glycans
- recognize foreign cell surfaces
- mediate or modulate immune responses to pathogens
- bind to endogenous carbohydrates
- mediate adhesion or signalling events at the cell surface

1 Animal lectins can be classified according to their structures

Carbohydrate-recognition domains (CRD)

- CRDs are responsible for the recognition functions of the lectins
- Other domains in the lectins mediate subsequent responses
- Different types of CRD have distinct ancestry (家谱) and different polypeptide folds
- The overall folds of CRDs are determined by conserved residues that are characteristic of each family of CRDs
- These residues form the hydrophobic core and in some cases disulphide bonds and binding sites for divalent cations

Type	Structure	Typical ligands	Examples of function
calnexin	β-Sandwich	Glc_1Man_9	Protein sorting in the ER (内质网)
M-type	α-Helical barrel	Man_8	ER-associated protein degradation
L-type	β-Sandwich	Man_{5-9}	Protein sorting in the ER
P-type	Unique β-rich structure	Man6-P	Protein sorting post-Golgi
C-type	Unique mixed α/β structure	Various	Cell adhesion (selectins); glycoprotein clearance; innate immunity (collectins)
Galectins	β-Sandwich	β-Galactosides	Glycan cross-linking in the extracellular matrix
I-type	Immunoglobulin superfamily	Sialic acid	Cell adhesion (siglecs)
R-type	β-Trefoil	Various	Enzyme targeting; glycoprotein hormone turnover

2 Mannose-binding protein (MBP) is a host defence molecule that initiates the lectin pathway of complement activation (补体激活)

innate immune system (先天性免疫系统)

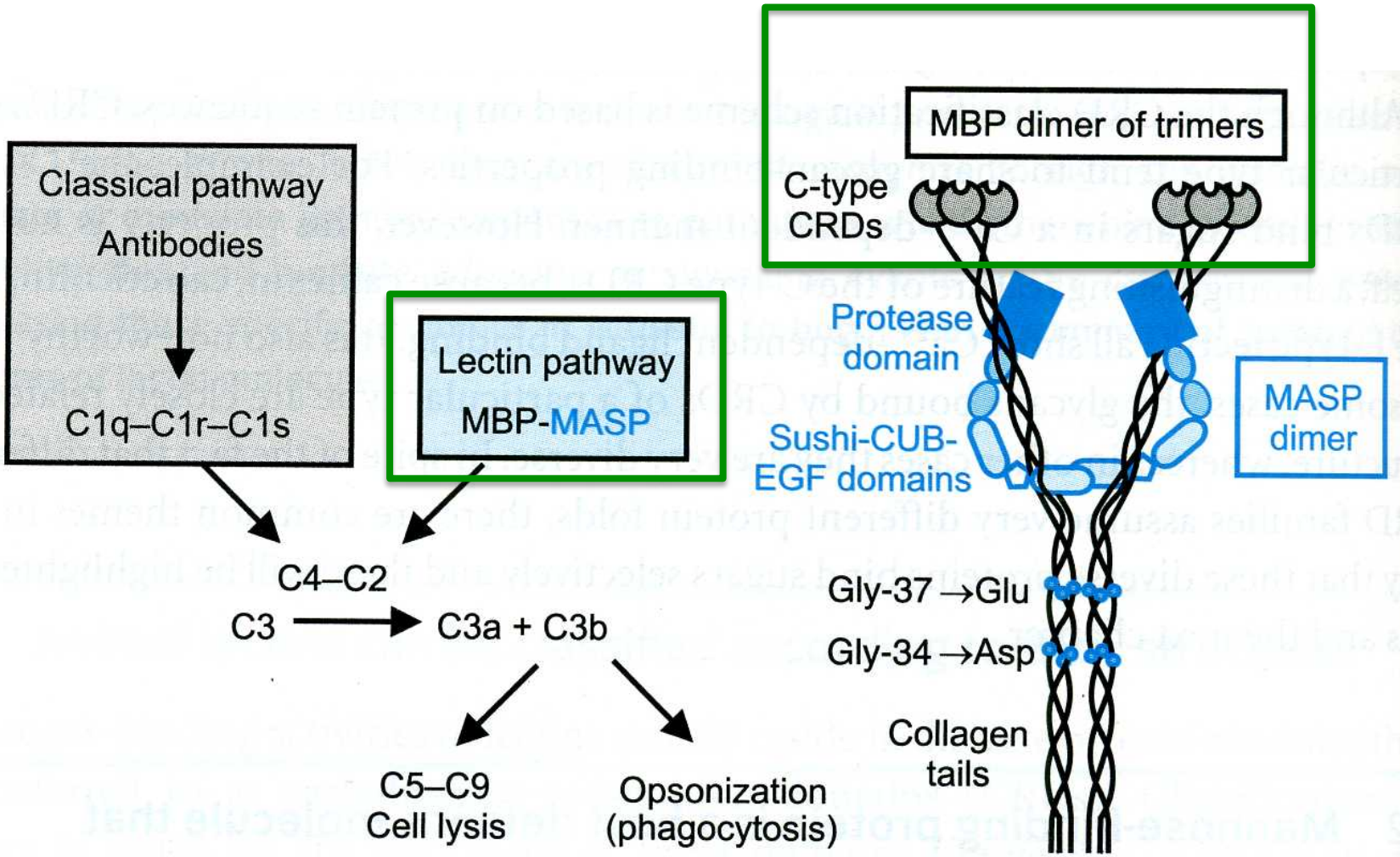
- Distinguished from adaptive immune system (获得性免疫系统)
- cells of the innate system recognize and respond to pathogens in a generic way
- provide immediate defense against infection, and are found in all classes of plant and animal life

The complement system(补体系统) helps or “complements” the ability of antibodies and phagocytic cells (噬菌细胞) to clear pathogens from an organism.

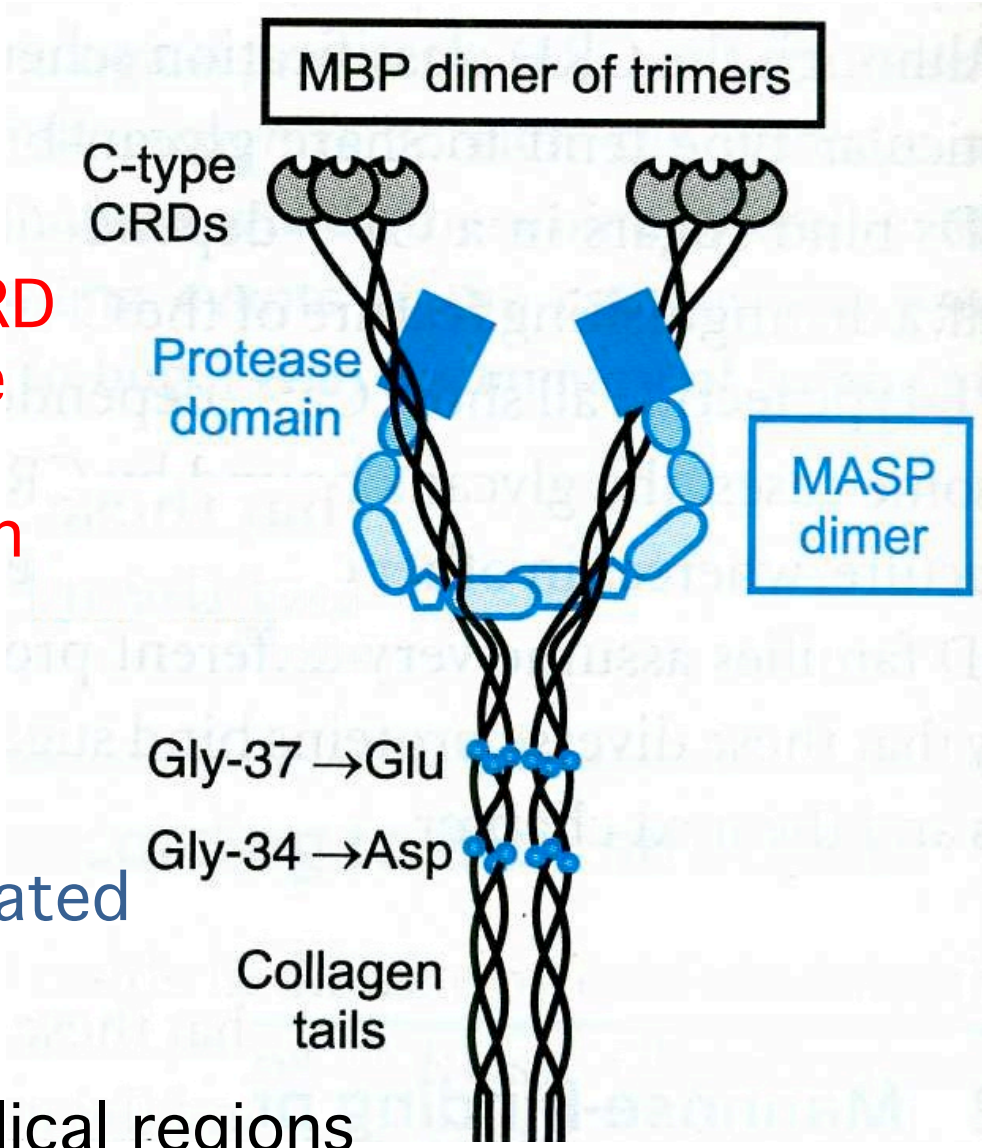
It is part of the innate immune system and does not change over the course of an individual's lifetime.

MBP

- Binds to carbohydrate structure on the surface of microorganisms
- Using the ability to distinguish exogenous structures from mammalian glycans
- To provide a mechanism for identification and neutralization of pathogen
- Circulates constitutively in the blood stream provides a type of innate immune response


Classical pathway
Antibodies
C1q–C1r–C1s
Lectin pathway
MBP-MASP
C4–C2
C3
C3a + C3b
C5–C9
Cell lysis
Opsonization
(phagocytosis)
MBP dimer of trimers
C-type
CRDs
Protease
domain
Sushi-CUB-
EGF domains
MASP
dimer
Gly-37 →Glu
Gly-34 →Asp
Collagen
tails

1.Fixed distance between CRD
2.Orientation: face the same direction
Suitable to the role of MBP in interacting with surfaces of microorganisms



Association with MBP-associated serine protease (MASP)

Trimer stabilized by two helical regions

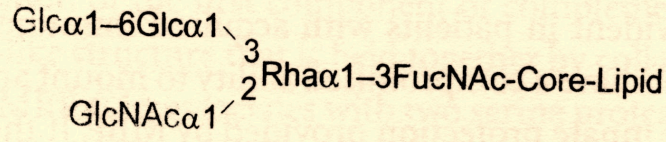
A neck of coiled coil of α-helices
An extended tail forms a collagen-like helix

3 Pathogen recognition by MBP results from both monosaccharide-binding specificity and oligomer geometry

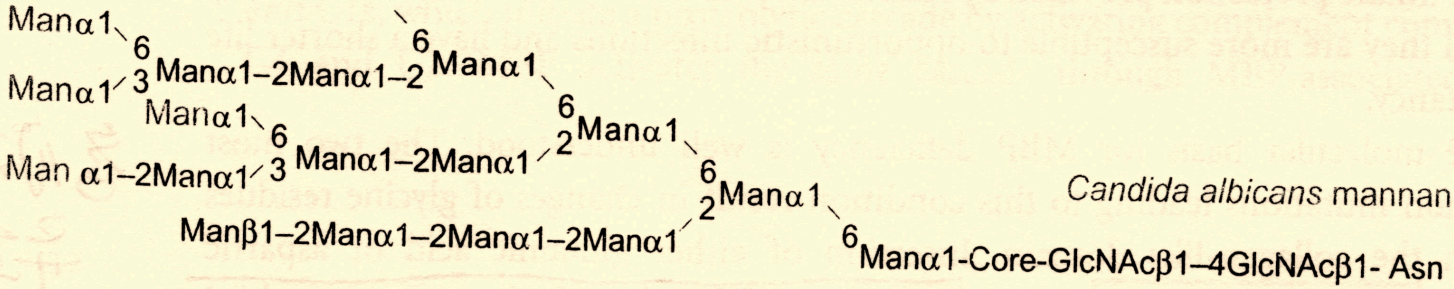
- The ability of MBP to distinguish between self and non-self is based on the different compositions and structures of mammalian glycoproteins and glycolipids compared with those on the surfaces of most microorganisms.
- Glycans on bacteria, yeast, and fungi differ widely, but many share a few key characteristics
- They usually serve structural roles
- Repetitive structures with terminal Glc, GlcNAc and Man

- Recognition by MBP takes advantages of both of these features
- MBP shows equal affinity for Glc, Man and GlcNAc in terminal positions

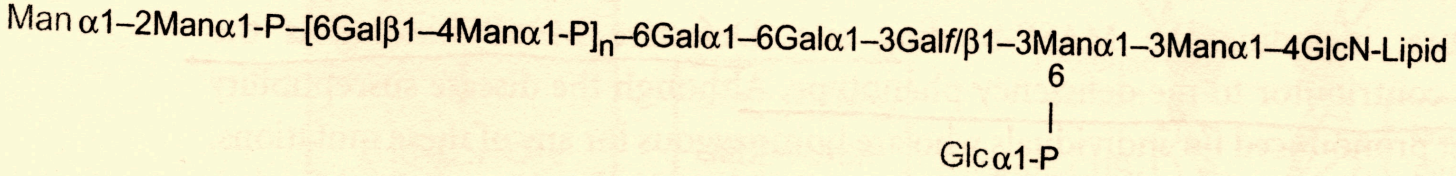
Escherichia coli lipopolysaccharide



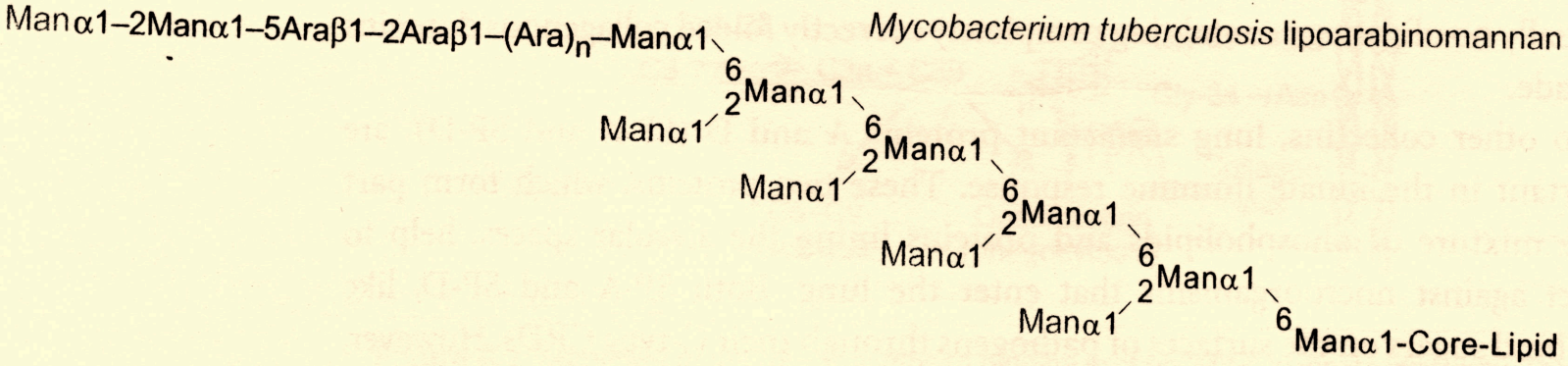
Candida albicans mannan

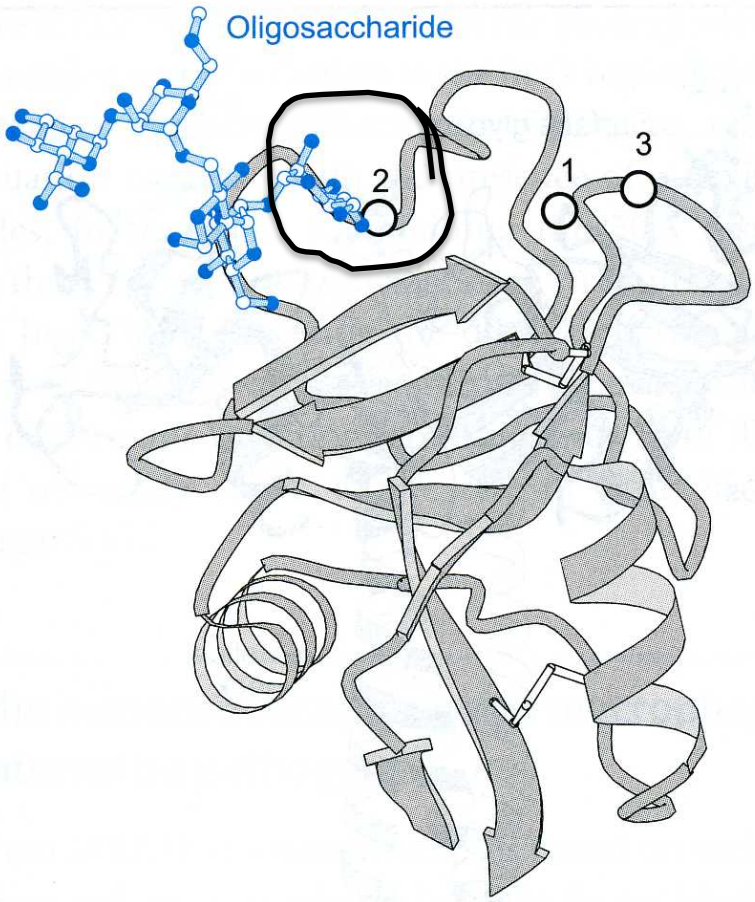


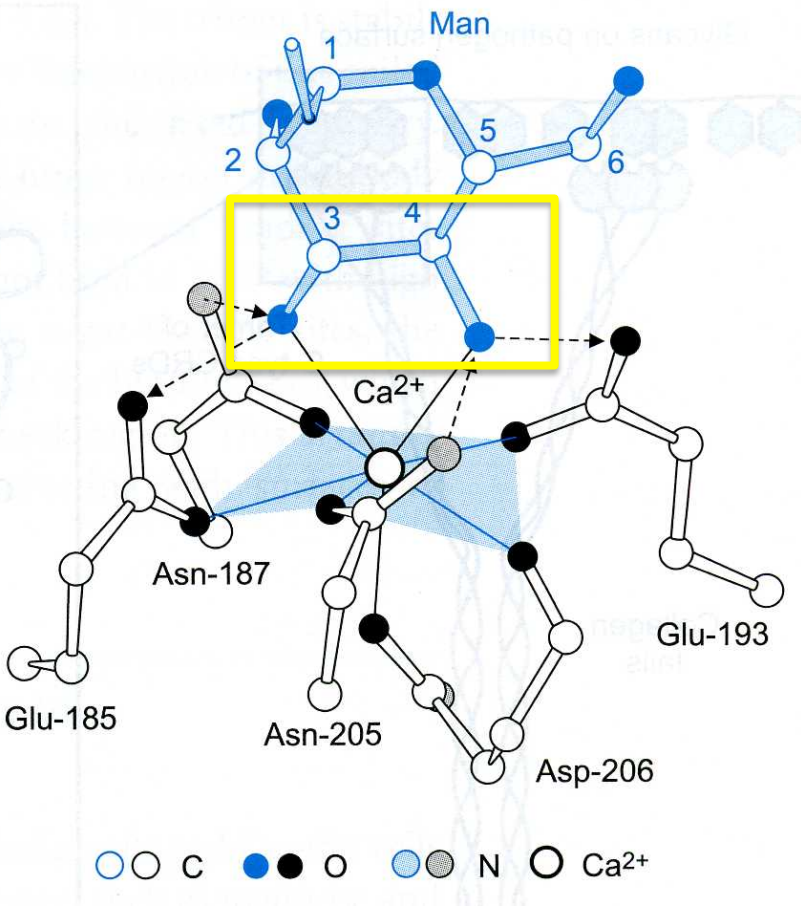
Leishmania donovani lipophosphoglycan



Mycobacterium tuberculosis lipoarabinomannan







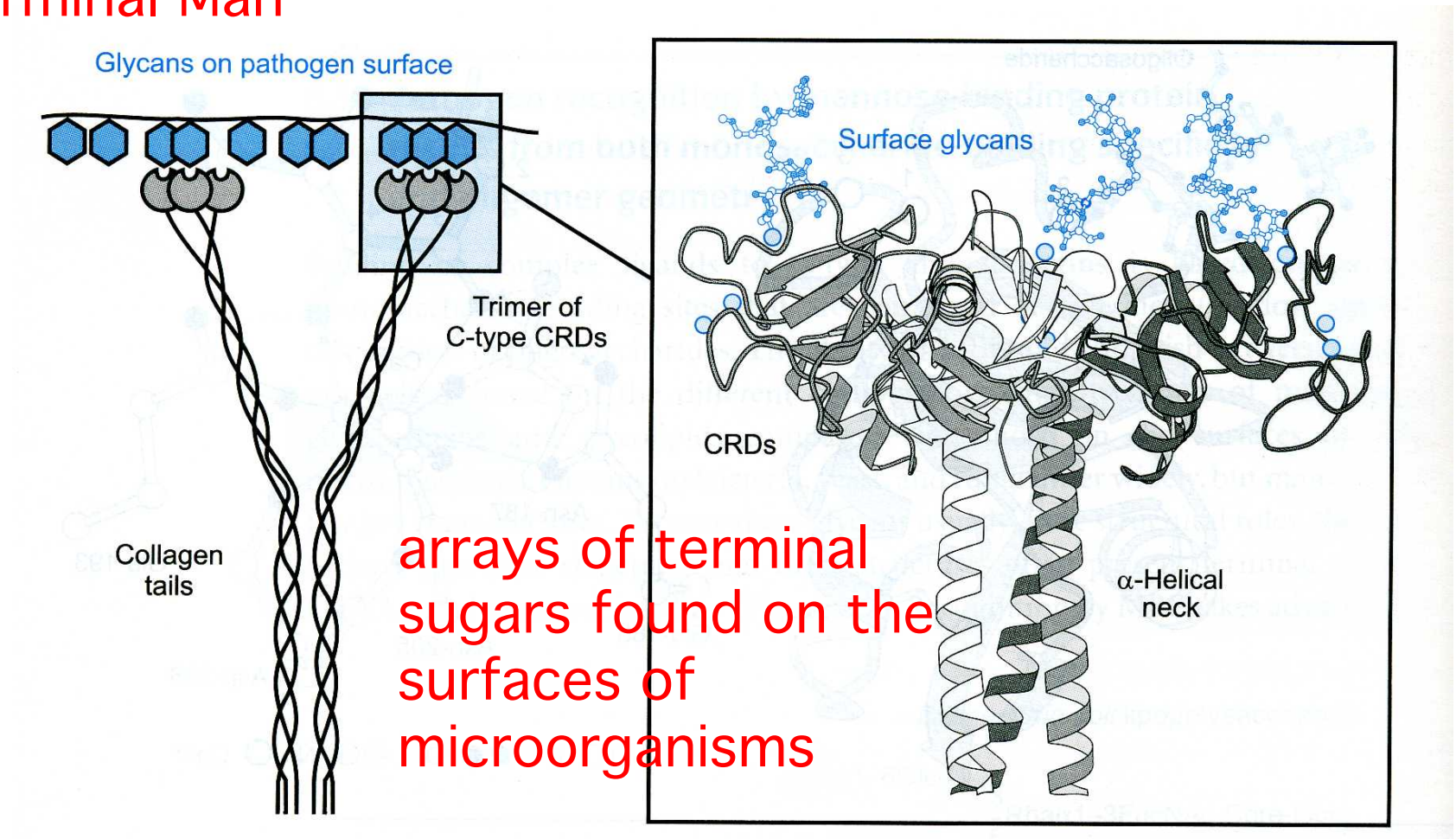
Man; Gal; innate immune response

Four hydrogen bonds

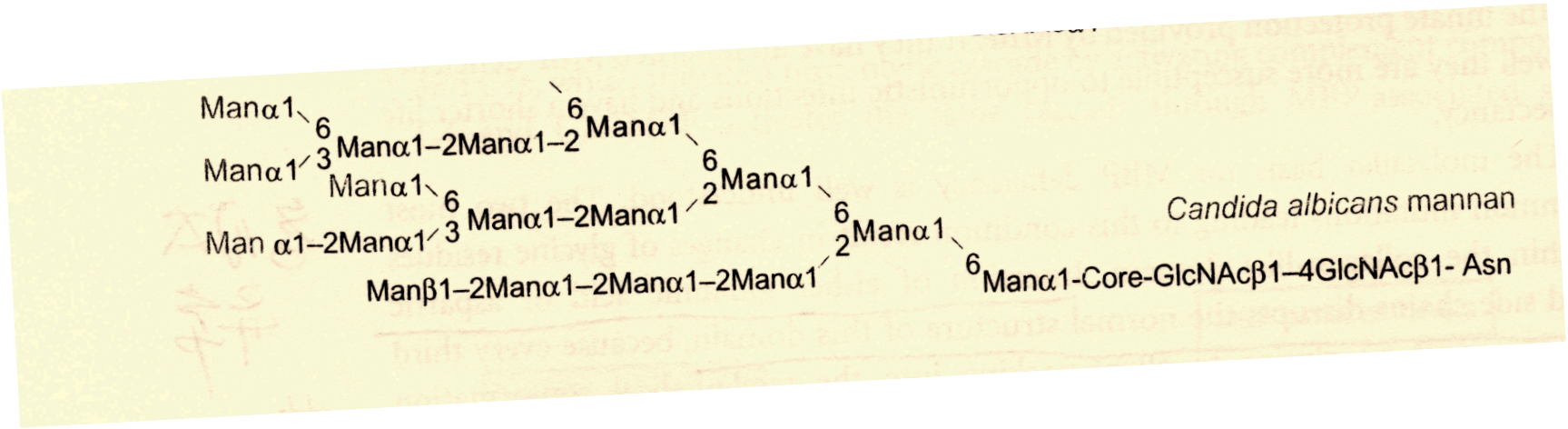
Man and GlcNAc

MBP interacts only with the terminal sugars, dissociation constant 1 mM; same as a monosaccharide

High-affinity binding requires multiple CRD and multiple terminal Man



arrays of terminal sugars found on the surfaces of microorganisms



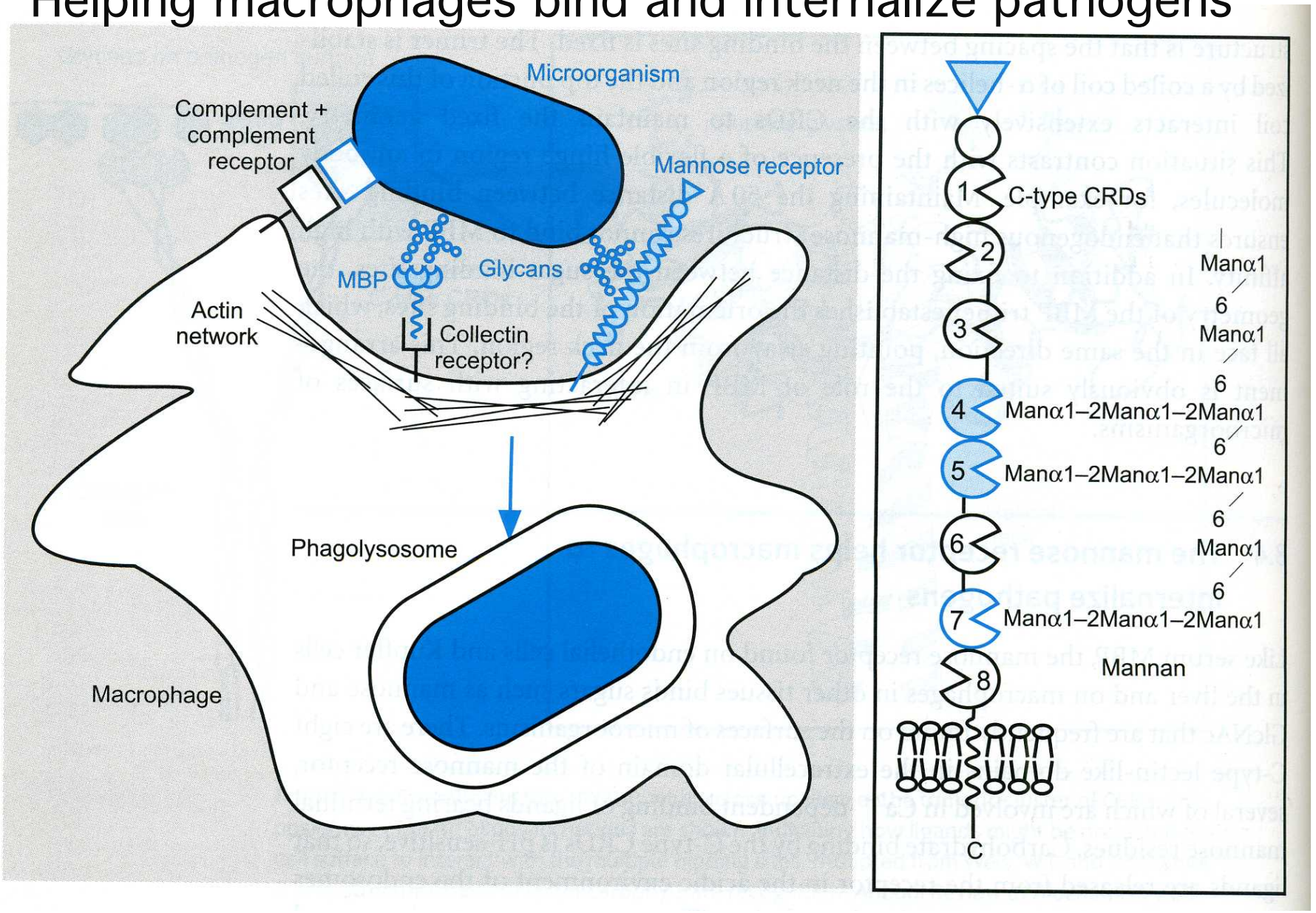
Does this high-Mannose structure bind to MBP with high affinity of not?

4 The mannose receptor helps macrophages to internalize pathogens

- Man receptors found on endothelial cells and Kupffer cells in the liver and on macrophages in other tissue
- Binds sugars such as Man and GlcNAc like serum MBP
- 8 C-type lectin-like domains
- pH-sensitive, ligands are released from the receptor in the acidic environment of the endosomes and directed to the lysosomes for degradation

Multiple different C-type CRD in a single polypeptide chain

Helping macrophages bind and internalize pathogens

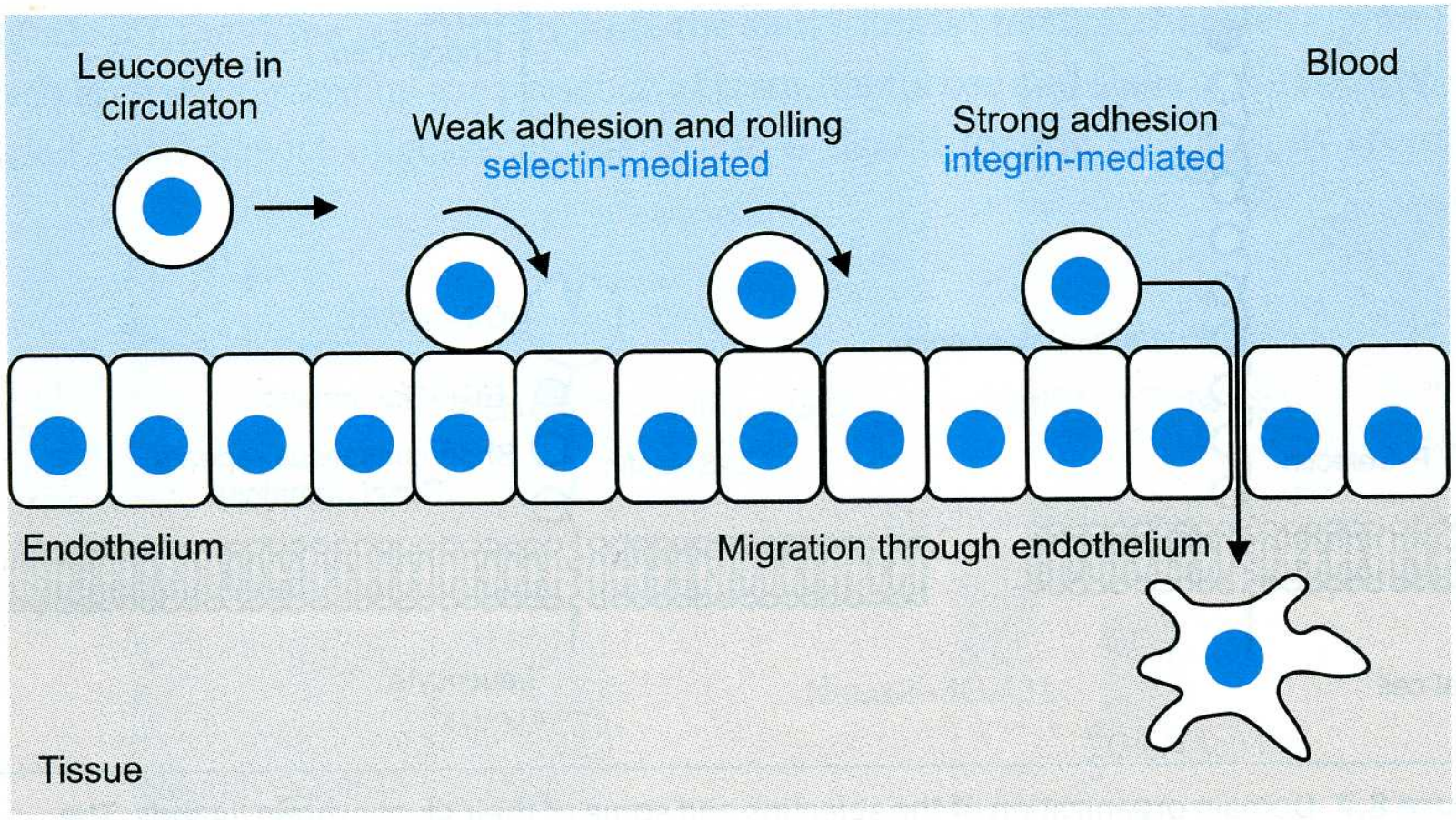


5 The selectins are cell-adhesion molecules for white blood cells

Circulating leucocytes (白细胞) must interact with endothelial cells(内皮细胞) lining blood vessels to reach the underlying tissues

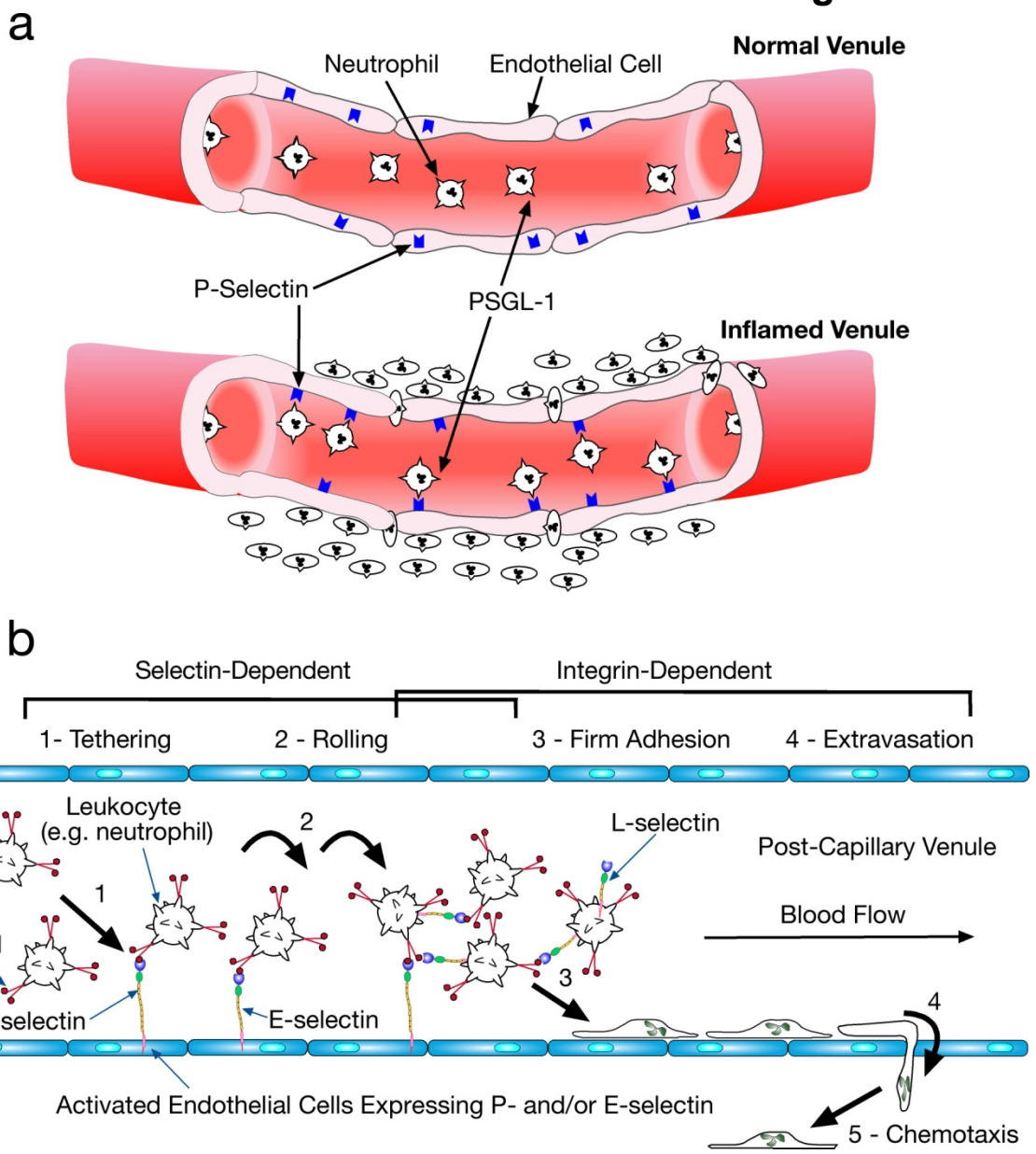
- T- and B-cell homing (归巢) to peripheral lymph nodes (淋巴结) and neutrophil (嗜中性粒细胞) migration to sites of inflammation both involved such interactions
- The first step of of leucocyte-endothlium interaction is carried out by the selectin cell-adhesion molecules
- Some of the best-understood examples of C-type lectins

The selectins mediate the initial, weak binding that leads to rolling of the lymphocytes on the endothelial surface



Steps in leucocyte interactions with endothelial cells

Tethering of circulating leukocytes to activated endothelium via interactions between selectins and their ligands

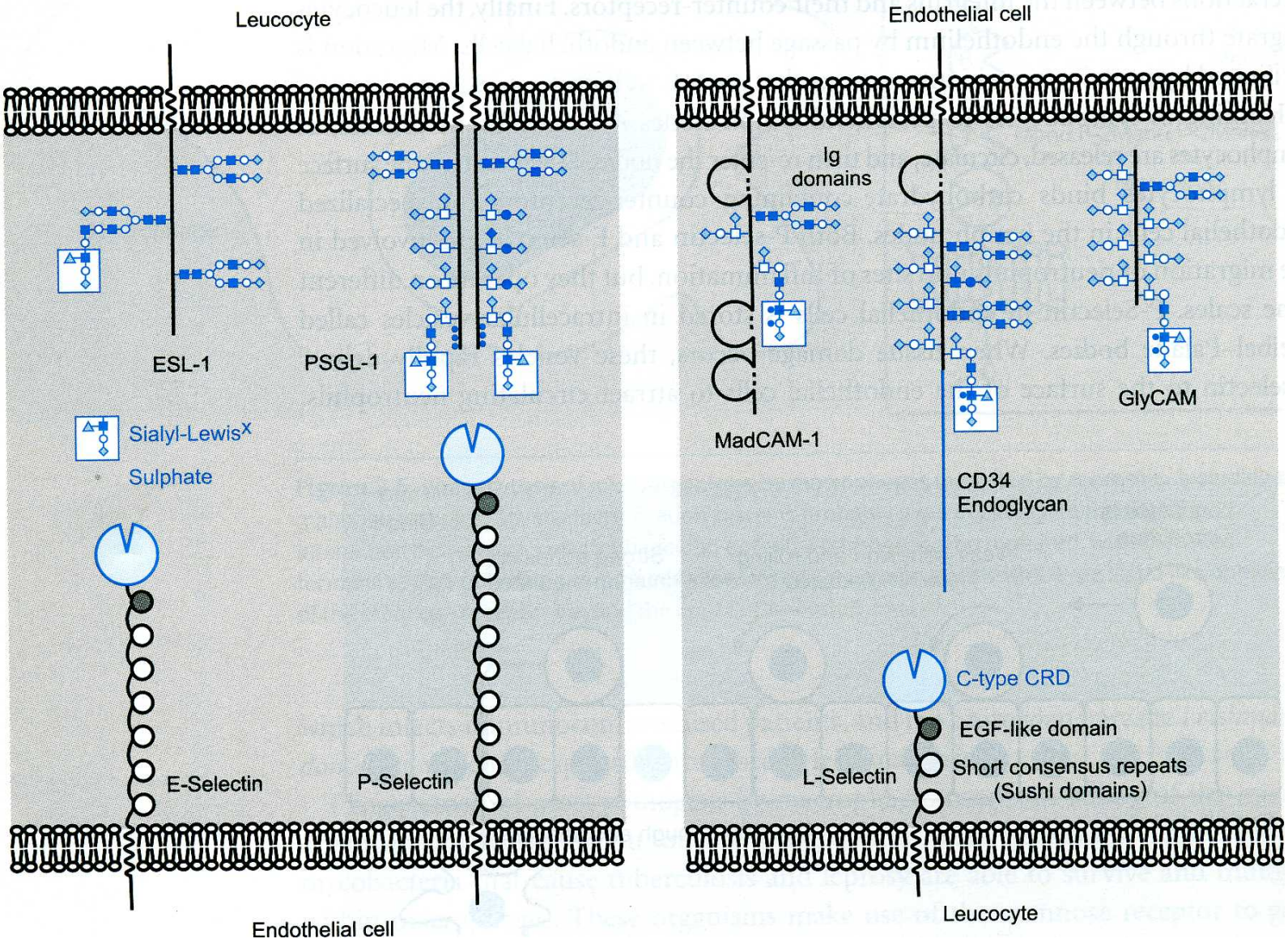


Homing of lymphocytes to peripheral lymph nodes

- Is a continuous process, as lymphocytes are released circulate and then re-enter the node
- L-Selectin on the surface of lymphocytes binds carbohydrate-containing counter-receptors on specialized endothelial cells in the lymph nodes

P-selectin and E-selectins

- They are involved in the migration of neutrophils into sites of inflammation
- They operate on different time scales
- P-selectin in endothelial cells is stored in intracellular vesicles
- When tissue damage occurs, these vesicles rapidly deliver P-selectin to the surface of the endothelial cells to attract circulating neutrophiles


Leucocyte
ESL-1
PSGL-1
Sialyl-Lewis^x
Sulphate
E-Selectin
P-Selectin
Endothelial cell
Endothelial cell
Ig
domains
MadCAM-1
CD34
Endoglycan
GlyCAM
C-type CRD
EGF-like domain
Short consensus repeats
(Sushi domains)
L-Selectin
Leucocyte

The phenotypes of knockout mice lacking on or more selectin reflect the roles of these proteins in leucocyte trafficking

- The rolling process requires a precise balance between the making and breaking of contacts between the leucocyte and endothelium
- The rate constants for ligand binding (k_{on}) and release (k_{off}) are rapid
- the extended organization of the selectin molecules also allows them to act as mechanical level arms
- The density and clustering of selectins and their glycan ligands on microvilli that project from the cell surface
- Intercations of the selectins with the cytoskeleton

6 Specific carbohydrate ligands for the selectins interact through extended binding sites on the C-type CRDs

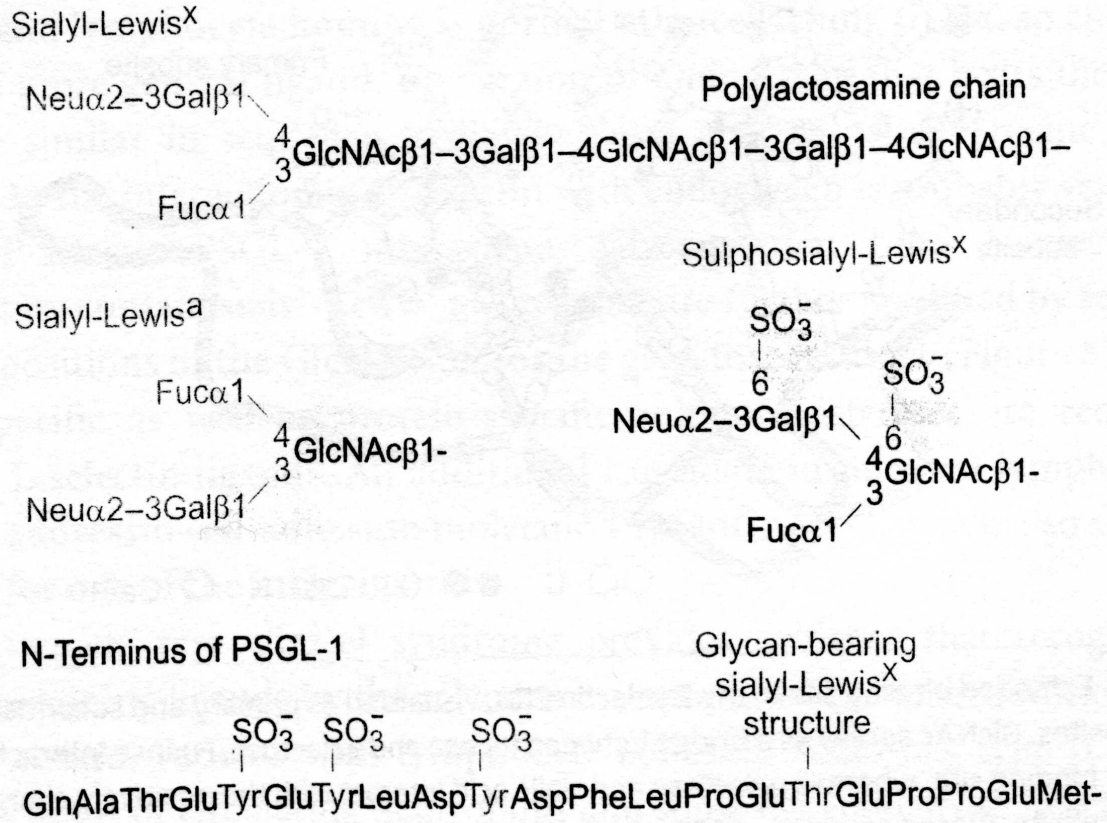
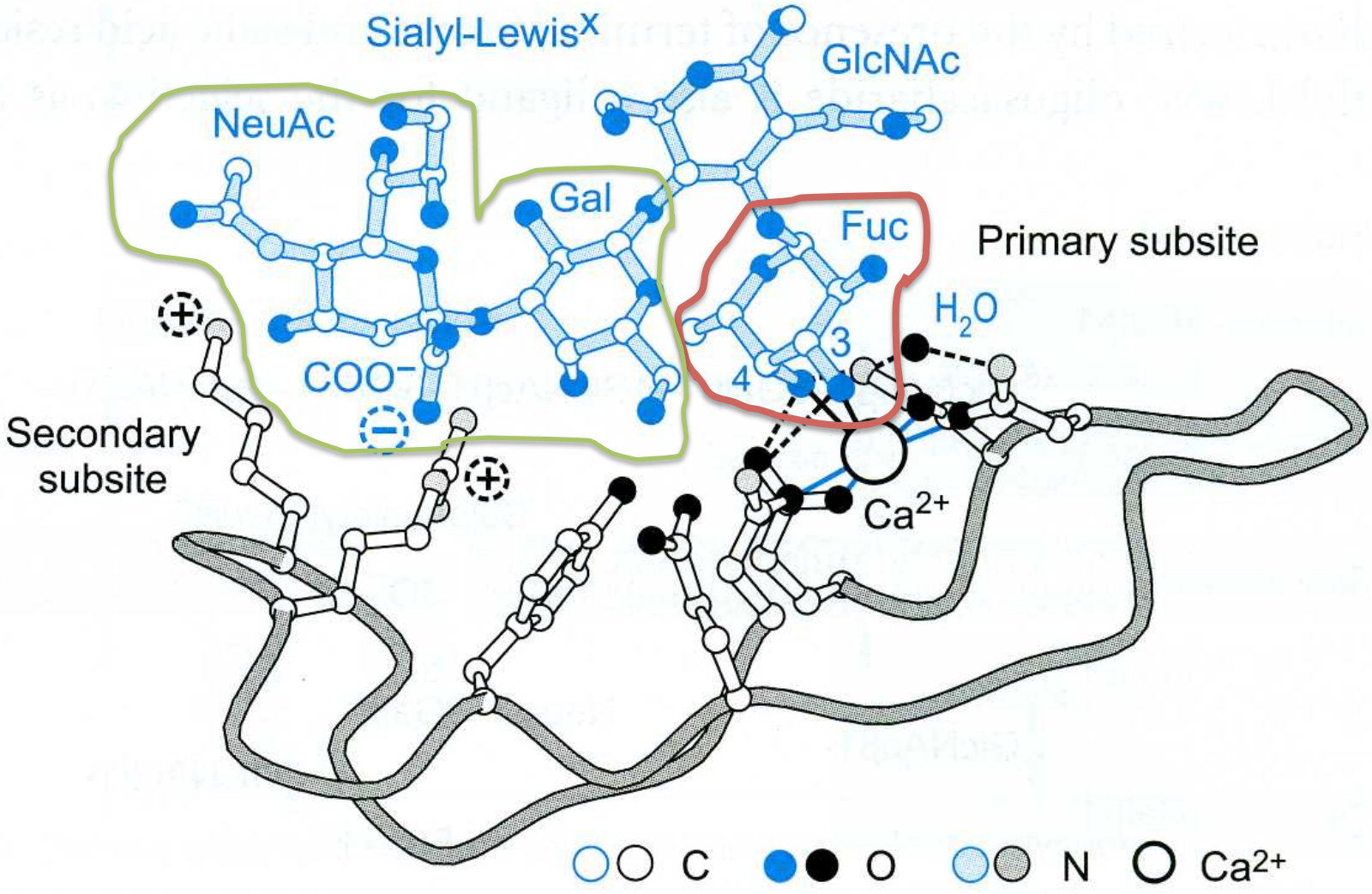
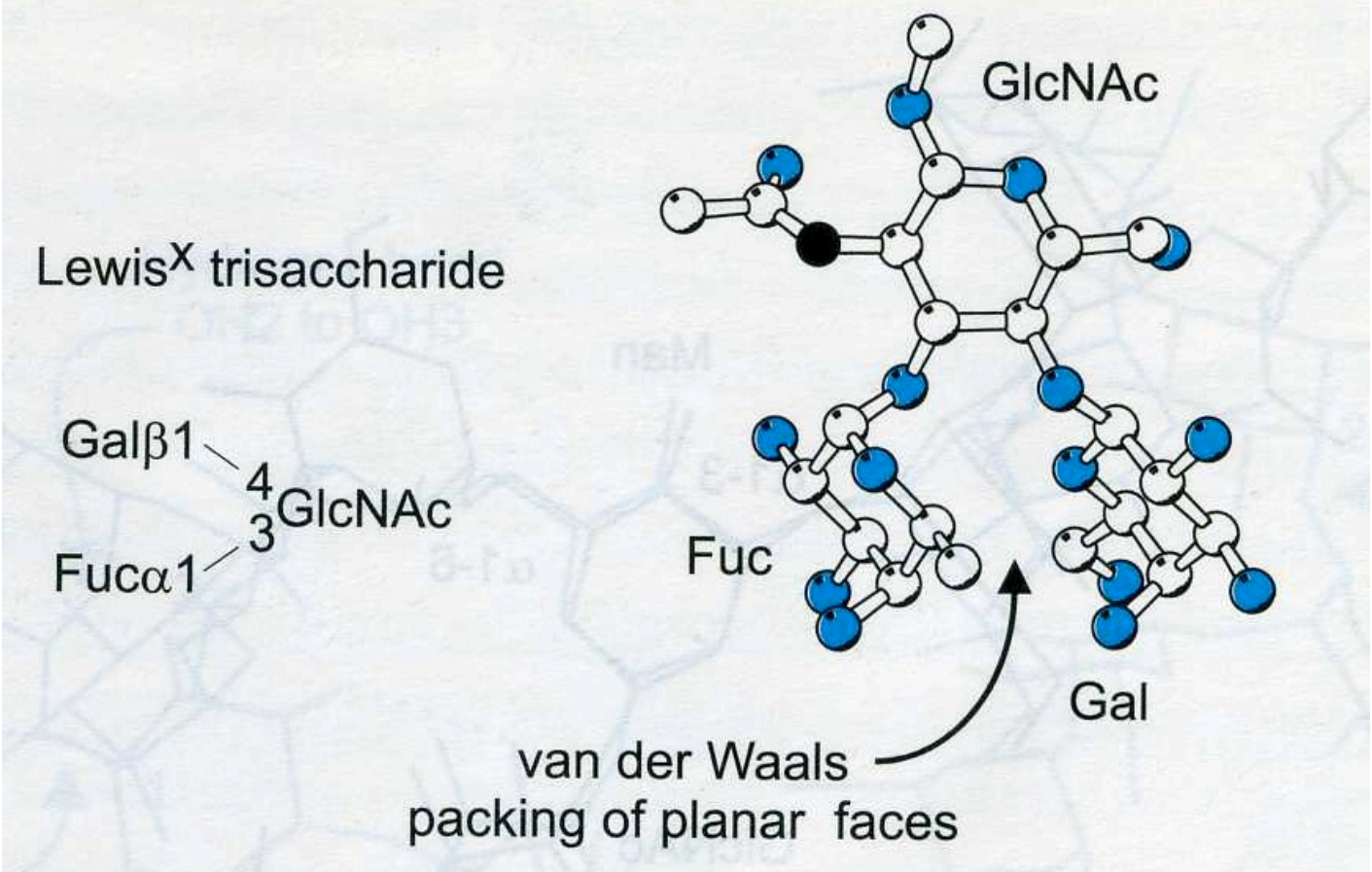


Figure 8.8 Structures of oligosaccharide and glycopeptide ligands for the selectins. Key terminal structures are highlighted in blue.

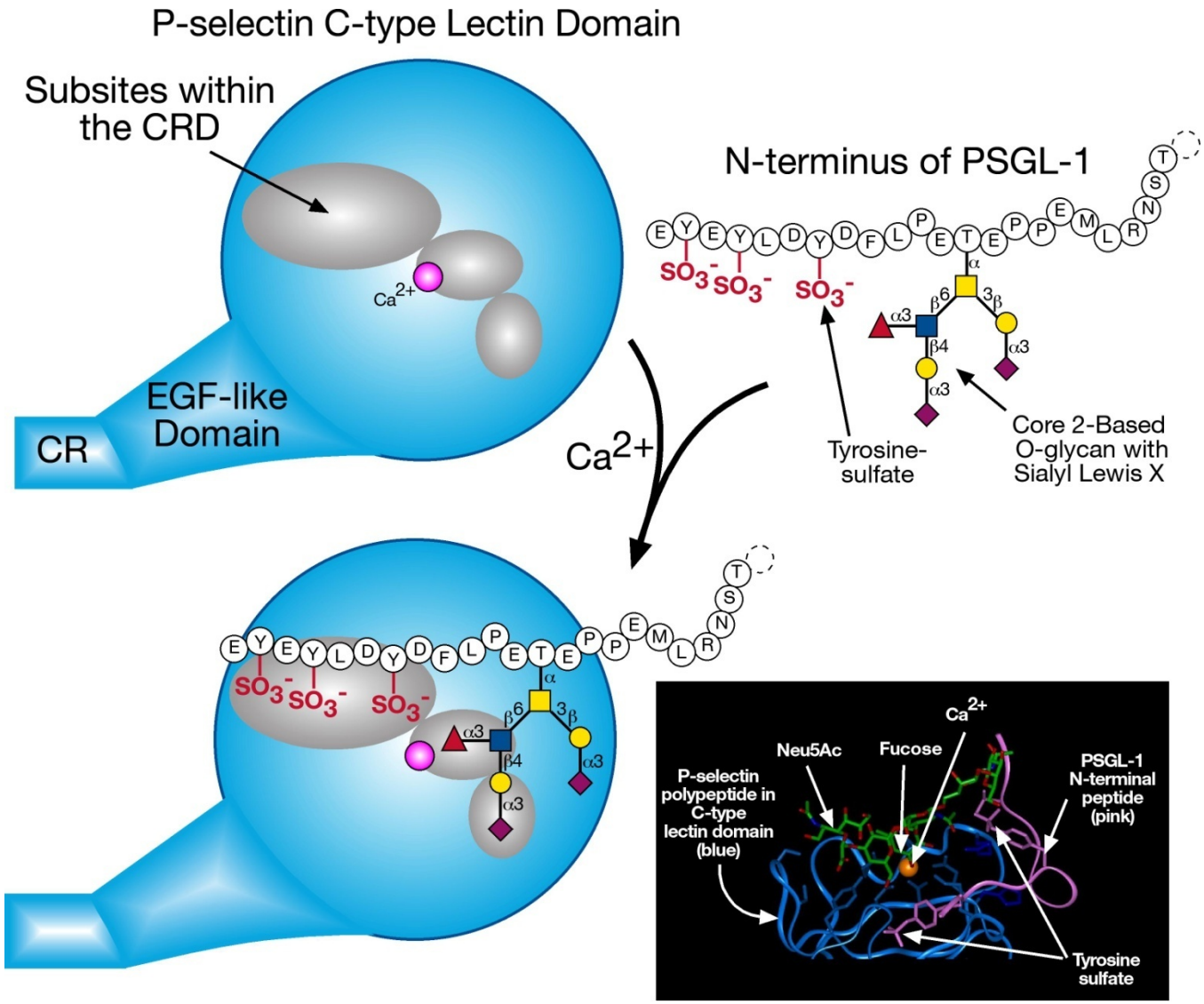

Sialyl-Lewis^X
NeuAc
Gal
GlcNAc
Fuc
Primary subsite
H_2O
3
4
COO^-
Secondary subsite
Ca^{2+}
C
O
N
Ca^{2+}

The binding of lowest- or nearly lowest-energy conformations of oligosaccharides is a general feature of lectin binding to complex ligand



This conformation remains the same when the ligands are bound to E- or P-selectin

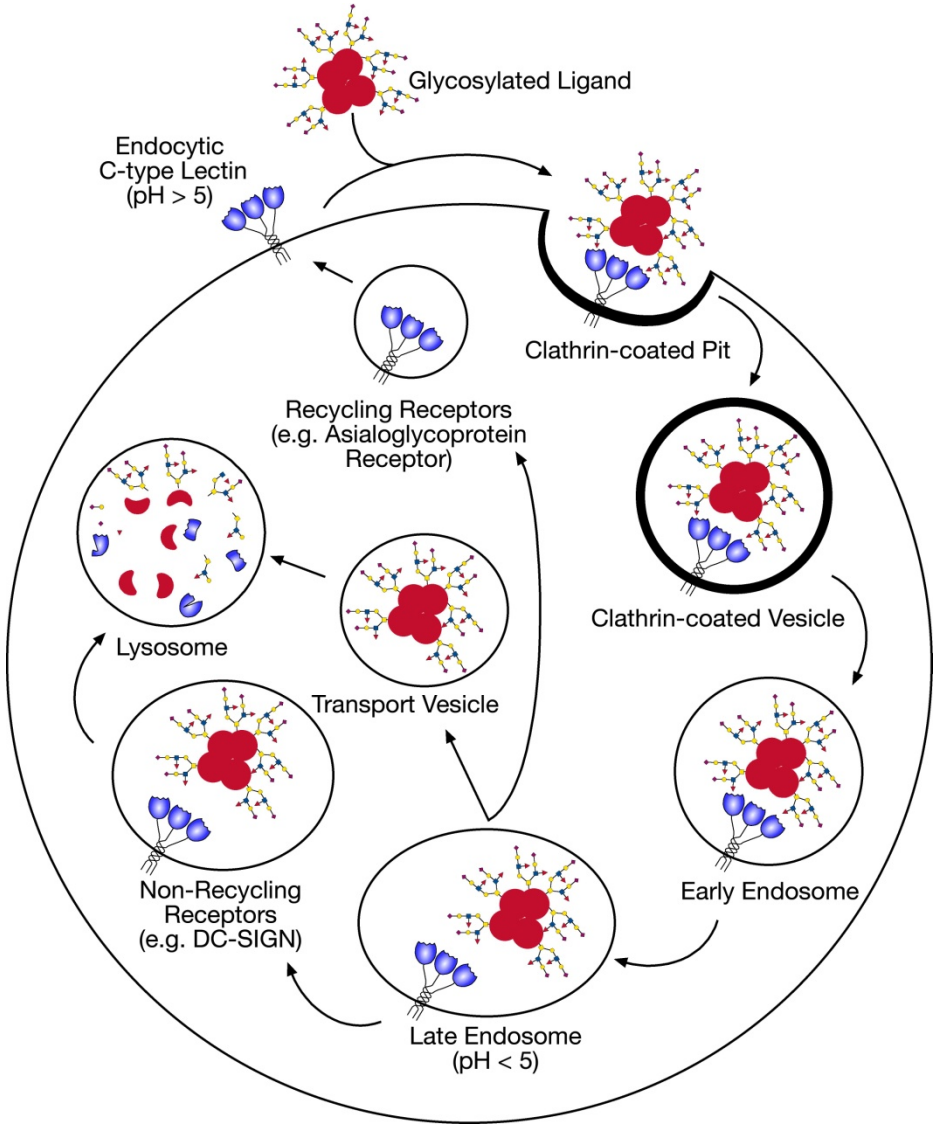
The molecular interactions between P-selectin and the amino terminus of PSGL-1



7 The selectins are also signal transduction molecules

8 C-type lectins participate in the process of antigen presentation (抗原递呈)

C-type lectins that function as endocytic receptors

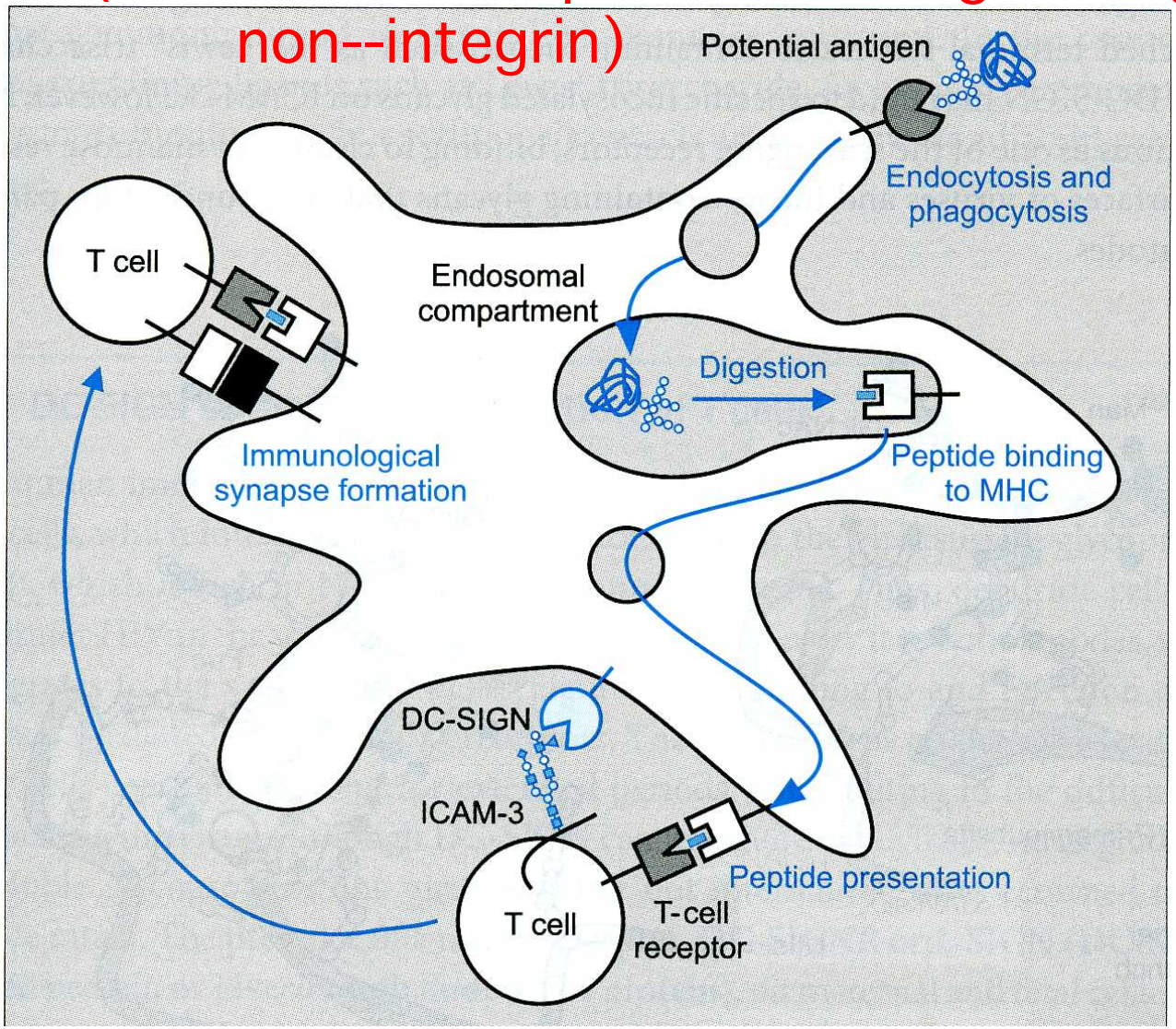


Two different roles of C-type lectin in the presentation of antigens to T cells

- The first step in the development of an antibody-mediated or cytotoxic T-cell-mediated adaptive immune response
- Several of the receptors that scavenge (清除，打扫) for potential foreign substances are members of the c-type lectin family
- Dendritic cells are resident in peripheral tissues, such as skin and the lining of the intestine, capture protein and glycoprotein antigens

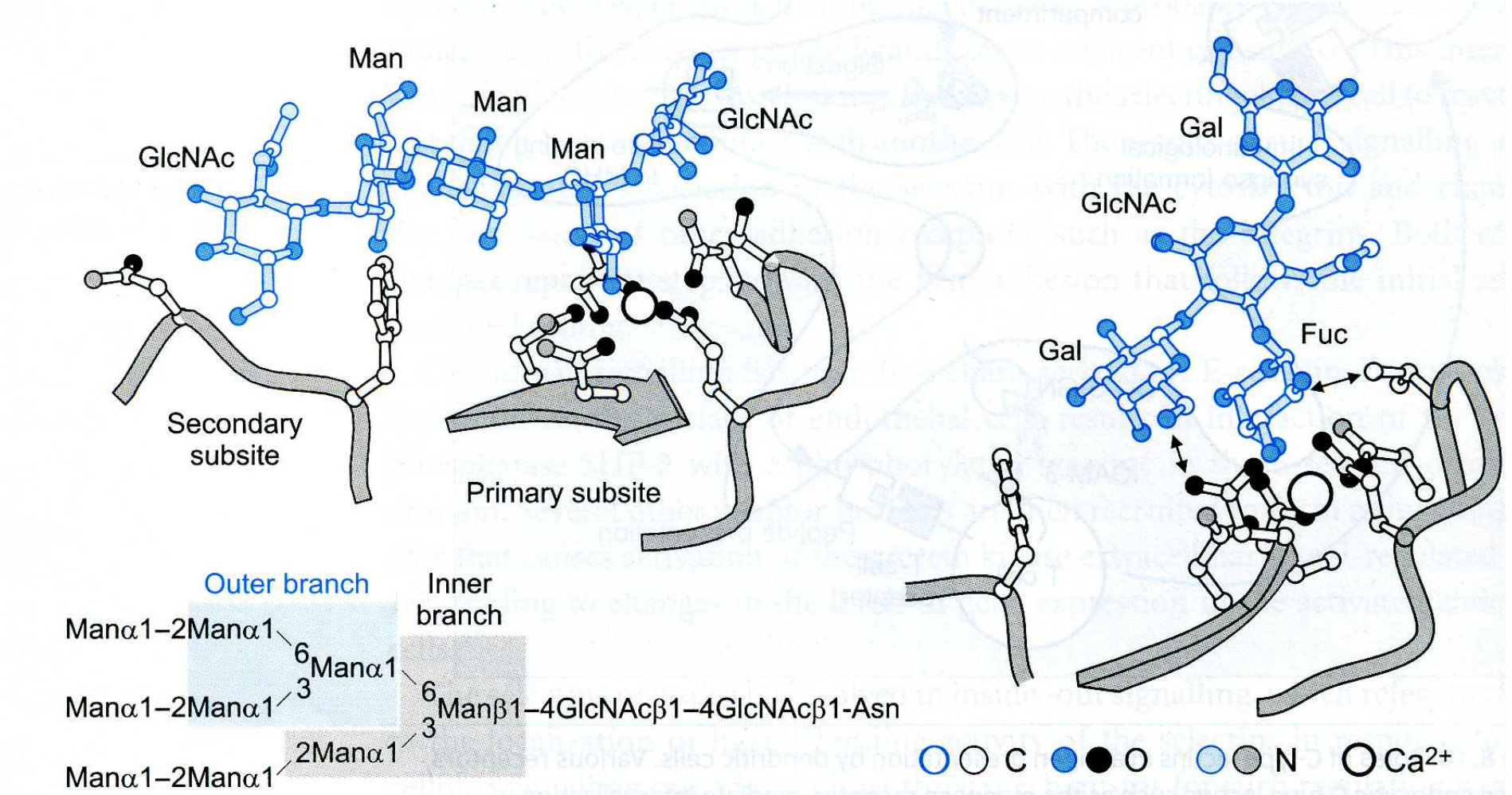
- The internalized antigens are digested to produce peptide fragments and after the dendritic cells migrate to lymph nodes
- Fragments of the antigens return to the cell surface in complex with molecules of the major histocompatibility complex (MHC)
- The sampling process is facilitated by receptors that bring the surfaces of dendritic cells and T cells near to each other

Carbohydrates on intercellular adhesion molecule 3 (ICAM-3) on lymphocytes are bound by the C-type lectin DC-SIGN (dendritic cell-sepcific ICAM-3-grabbing non--integrin)



DC-SIGN

- A tetrameric transmembrane protein containing C-terminal C-type CRDs that are projected from the cell surface by an a-helix neck region
- The CRDs bind high-Mannose, N-linked oligosaccharides and also a variety of glycans that bear branched terminal structures containing fucose, e.g. the Lewis^x trisaccharide
- It also functions as one of the scavenging receptors, binding to clusters of mannose residues on surface of viruses and fucose-containing glycans on parasitic nematodes (线虫)

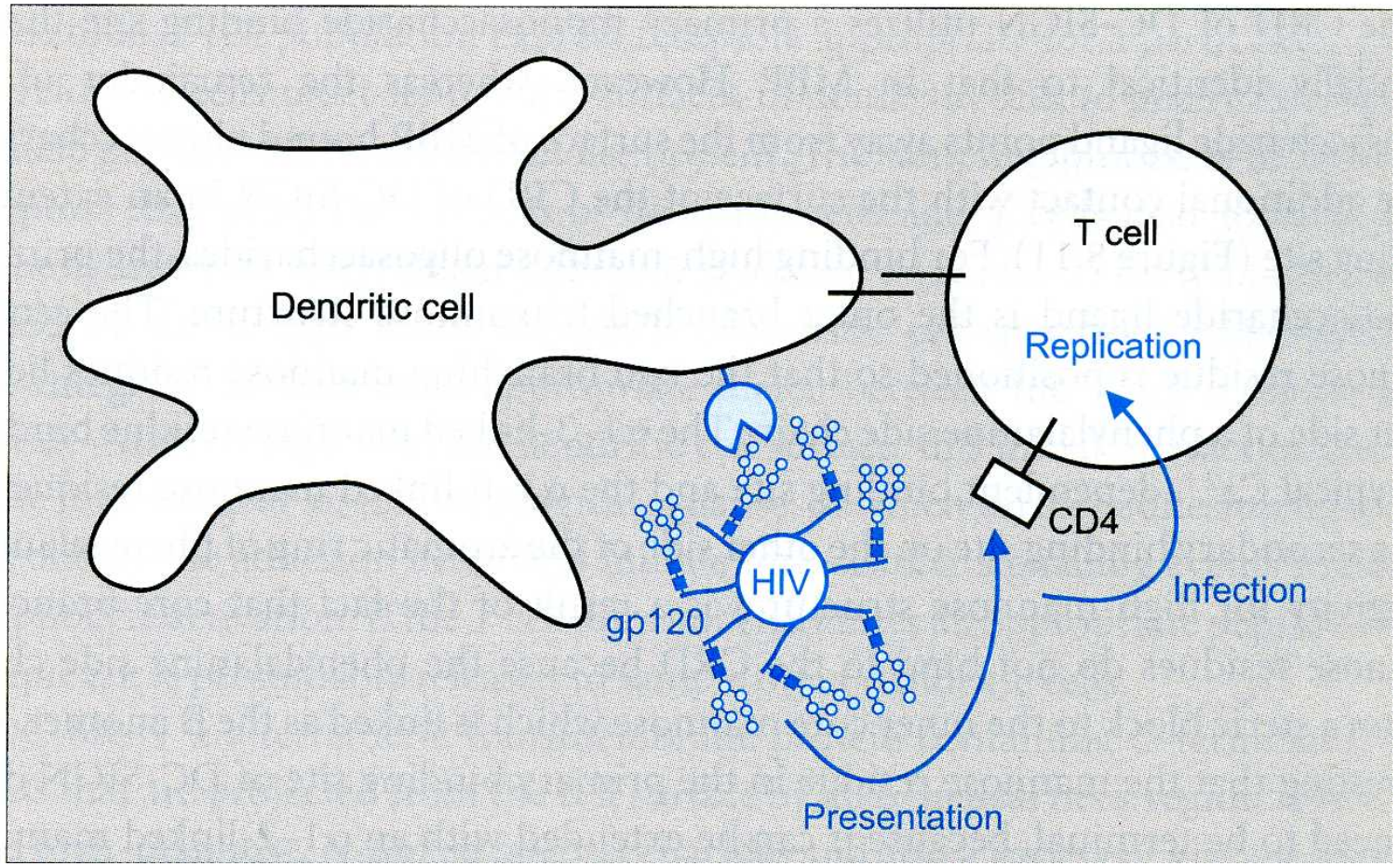

Man
Man
Man
GlcNAc
GlcNAc
Secondary subsite
Primary subsite
Outer branch
Inner branch
Manα1–2Manα1
6
Manα1
Manα1–2Manα1
3
6
Manβ1–4GlcNAcβ1–4GlcNAcβ1-Asn
3
2Manα1
Manα1–2Manα1
Gal
GlcNAc
Gal
Fuc
C
O
N
Ca2+

9 DC-SIGN enhances HIV infection of T cells

- The human immunodeficiency virus (HIV) utilizes DC-SIGN to facilitate infection of T cells, which initiates AIDS
- DC-SIGN binds to gp120, the viral surface glycoprotein rich in N-linked high-mannose glycans
- Dendritic cells that encounter HIV can carry it to lymph nodes
- Then HIV migrates to the surface of T cells
- HIV on the surface of dendritic cells can remain latent for extended periods

Compare DC-SIGN and selectin

- Both of them participate in reversible adhesion events between two cells or between a cell and a virus
- These interactions talk place in rather different contexts, in the presence of shear flow or under static conditions
- The selectins bind to complex glycan ligands expressed on a limited number of glycoproteins
- DC-SIGN binds to relatively common types of glycan


Dendritic cell
T cell
Replication
CD4
HIV
gp120
Infection
Presentation

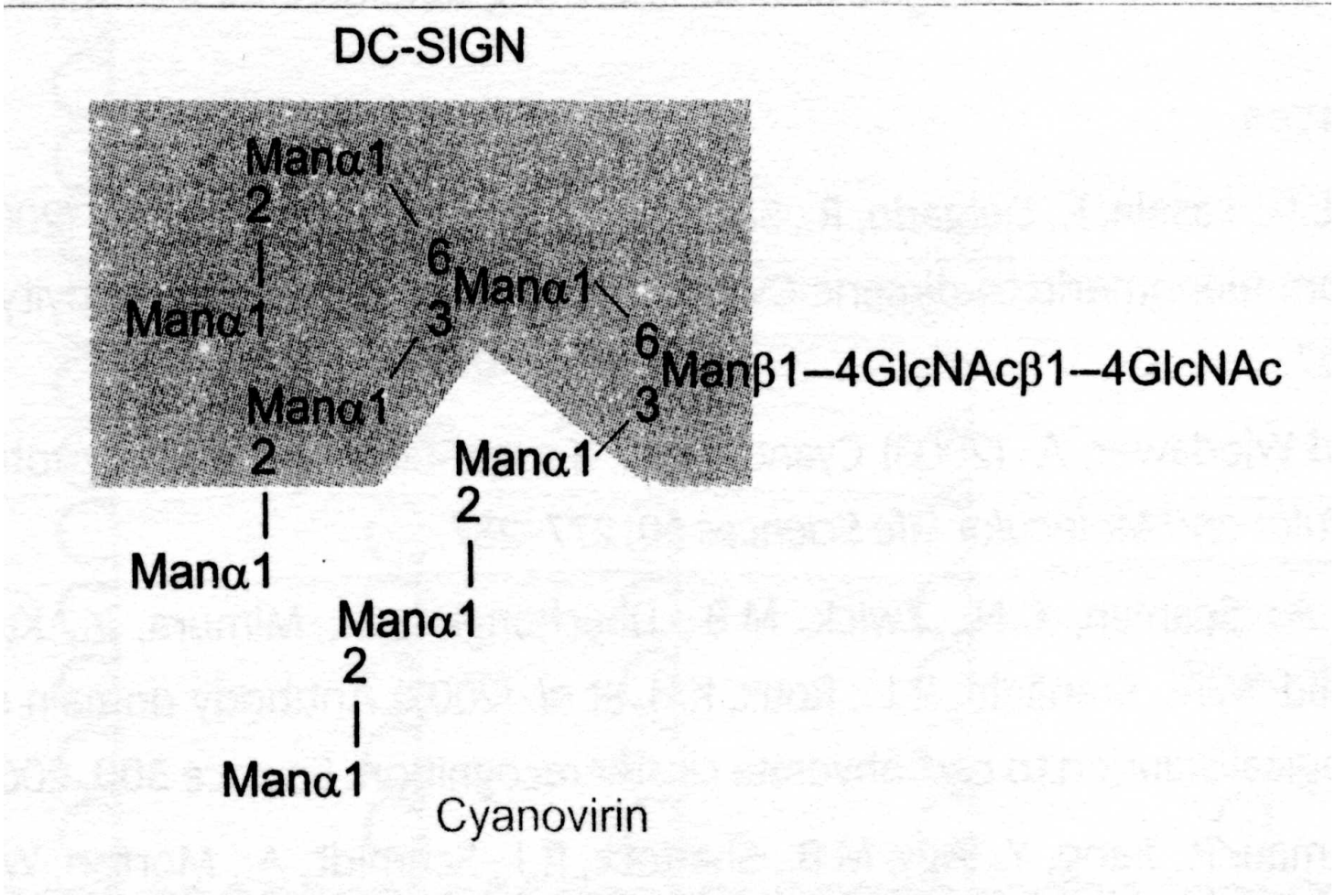
Glycotherapeutics: drugs and antibodies that prevent HIV infection mimic recognition by DC-SIGN

Interaction of DC-SIGN with gp120

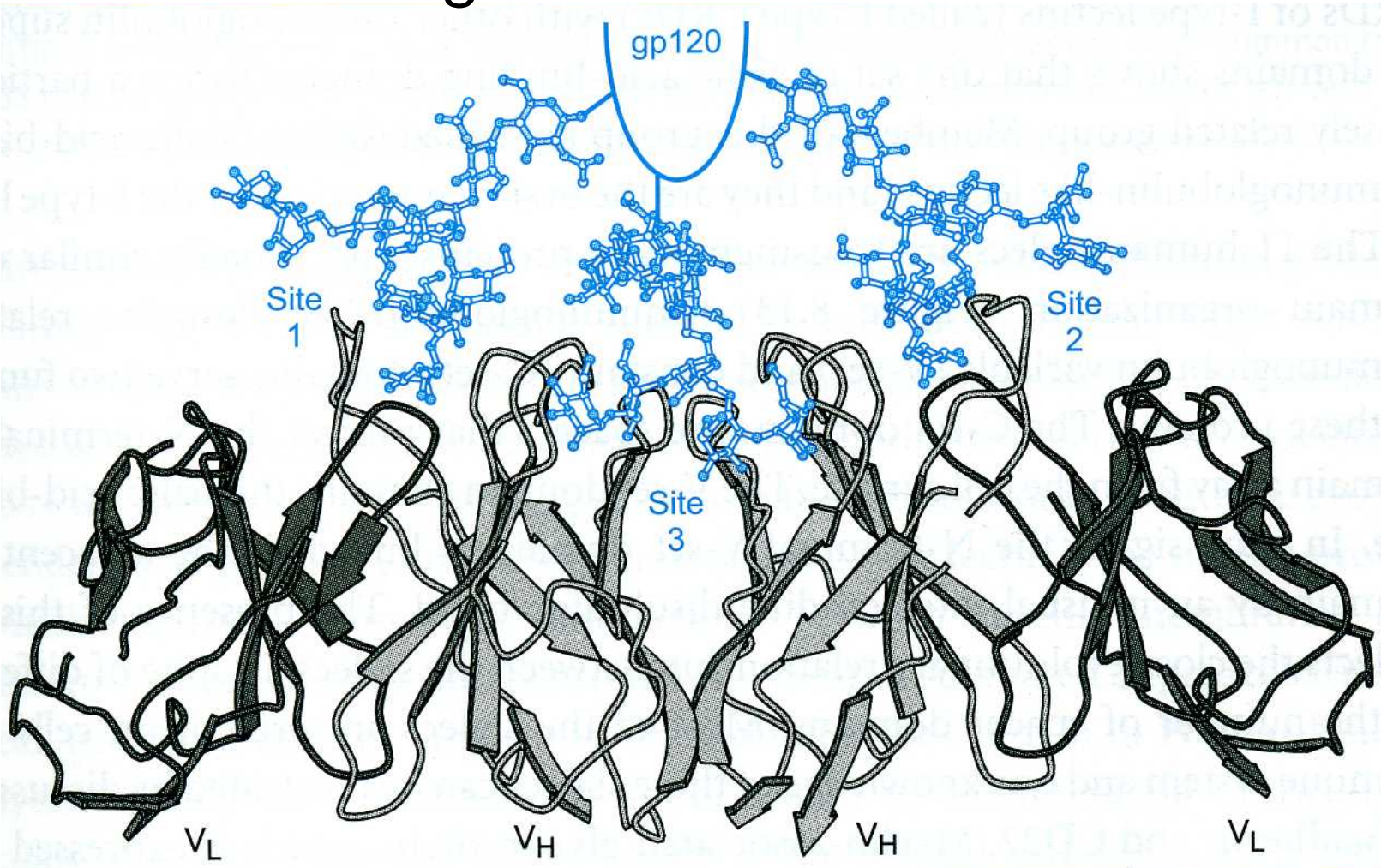
- Two unusual properties of the viral glycoprotein
- The close spacing of multiple high-mannose oligosacchrides on the protein
- The relatively large size of the glycans, mostly Man_7, Man_8 and Man_9.
- Inaccessibility due to close packing; shield conserved portions from immune system
- Allows the virus to use DC-SIGN as a means of trafficking to lymph nodes

Cyanovirin-N

- A small protein that binds with high affinity to Man8- and Man9-type glycans
- It blocks HIV infection of T cells by preventing gp120 from binding to the CD4 receptor
- The recognized parts in gp120 is different:

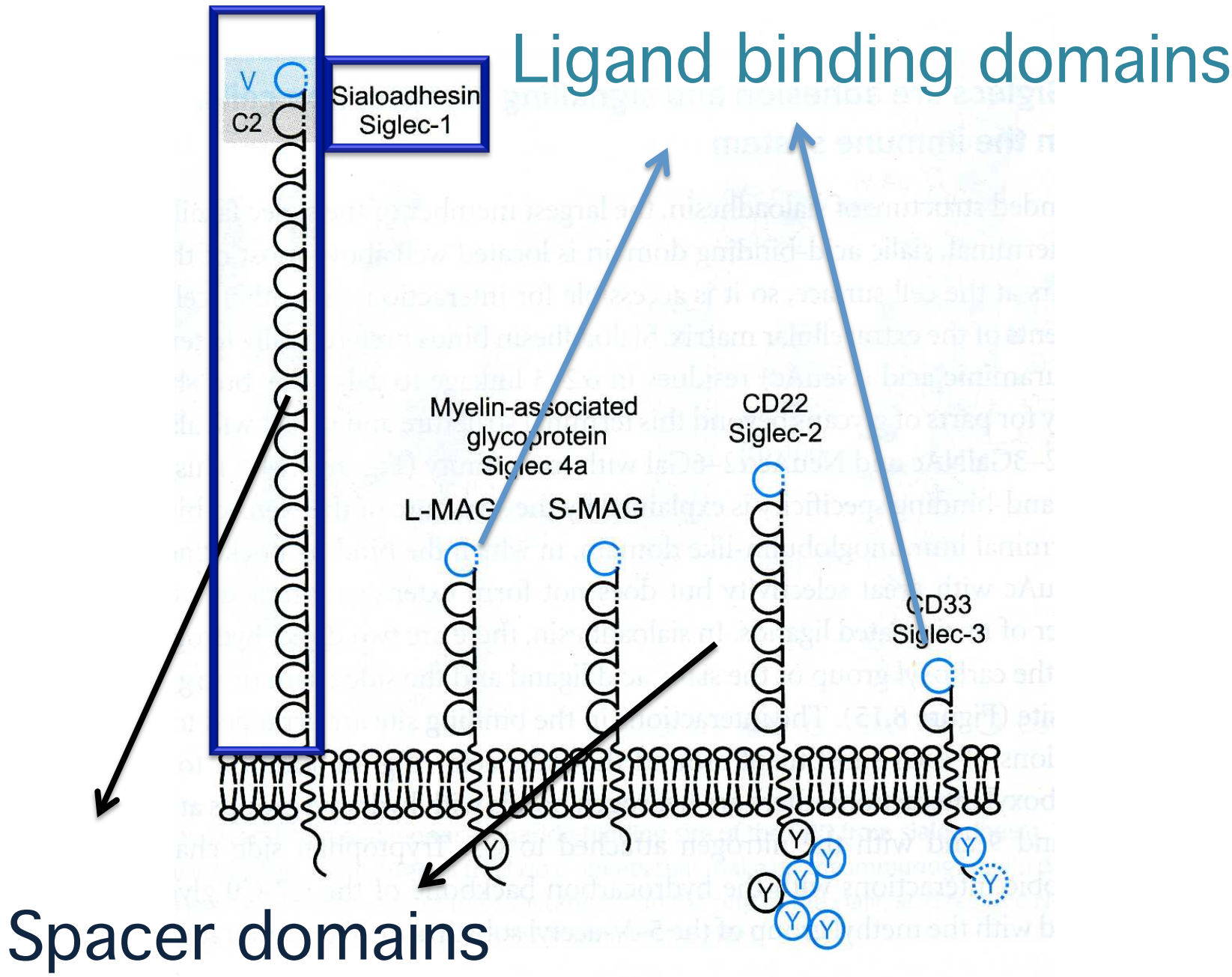

DC-SIGN
Manα1
2
Manα1
6
3
Manα1
6
3
Manβ1–4GlcNAcβ1–4GlcNAc
Manα1
2
Manα1
Manα1
2
Manα1
Manα1
2
Manα1
Cyanovirin

Neutralizing antibody, 2G12, binds to high-mannose oligosacchrides: closely spaced and rigid conformation



10 I-type lectins are composed of immunoglobulin-like domains

- Receptors that bind carbohydrate ligands through immunoglobulin superfamily domains are referred to as I-type lectins
- One set of sialic acid-binding domains forms a particularly closely related group
- Siglecs (sialic acid-binding immunoglobulin-like lectins)


Ligand binding domains
V
C2
Sialoadhesin
Siglec-1
Myelin-associated
glycoprotein
Siglec 4a
L-MAG
S-MAG
CD22
Siglec-2
CD33
Siglec-3
Y
Spacer domains

11 Siglecs are adhesion and signalling receptors on cells in the immune system

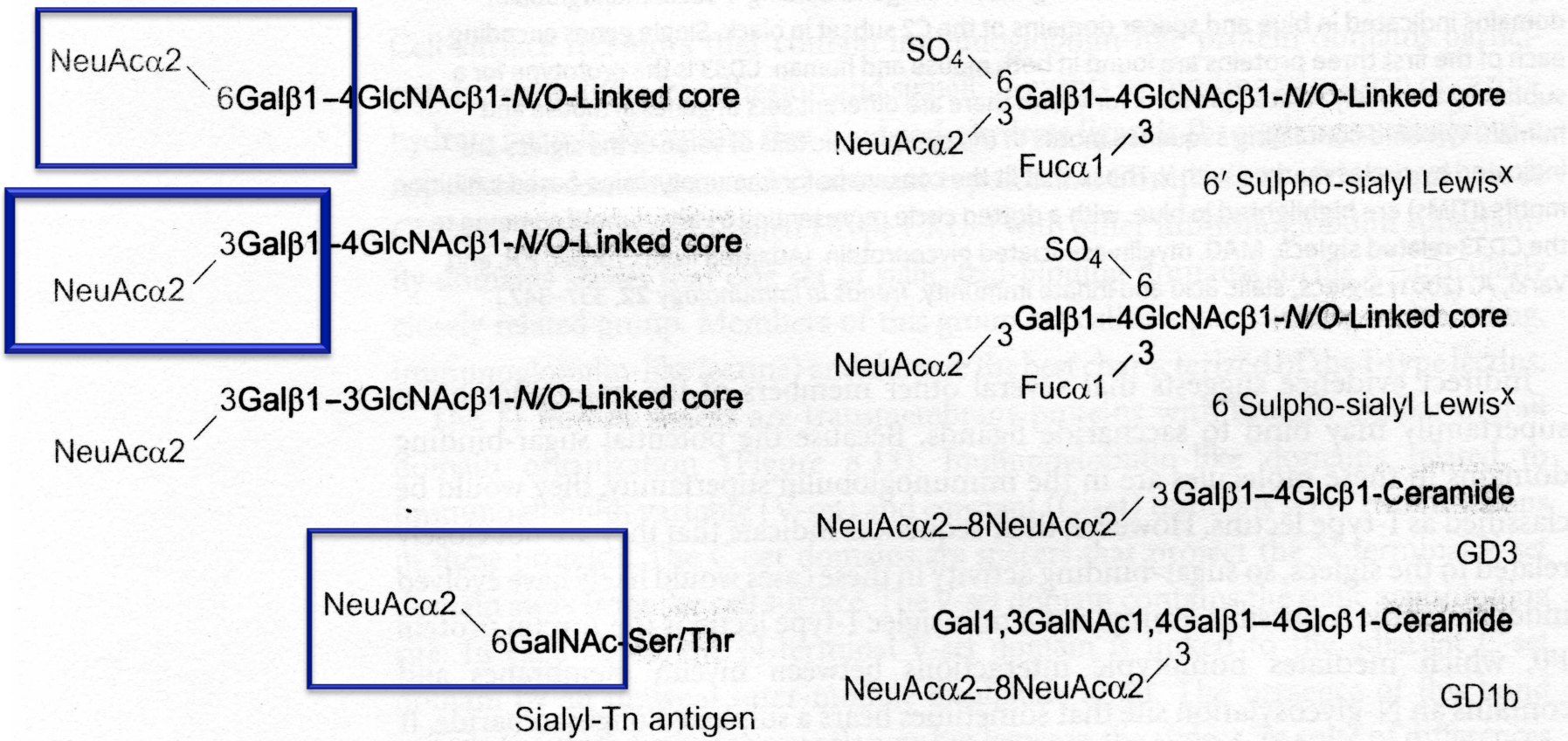
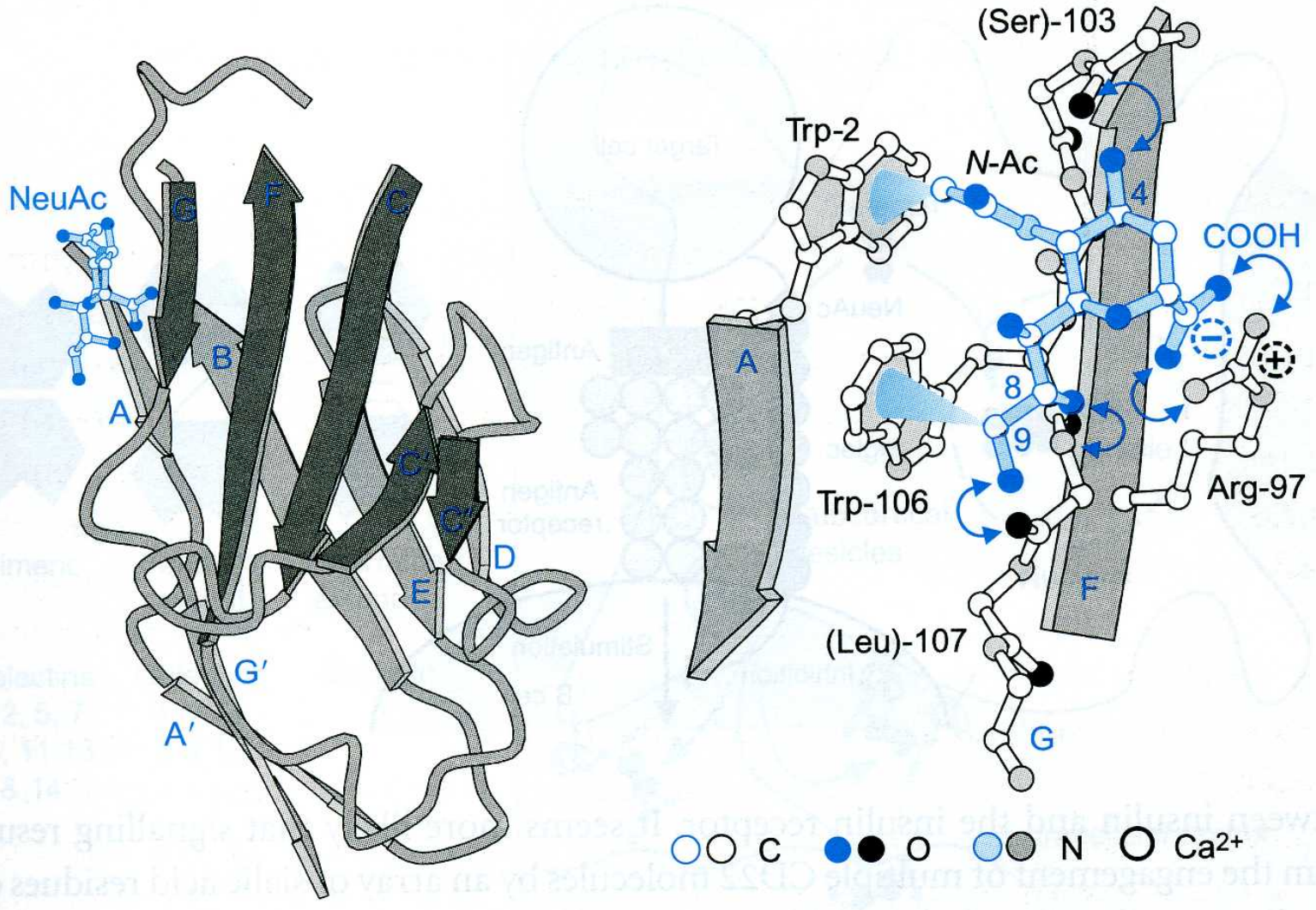
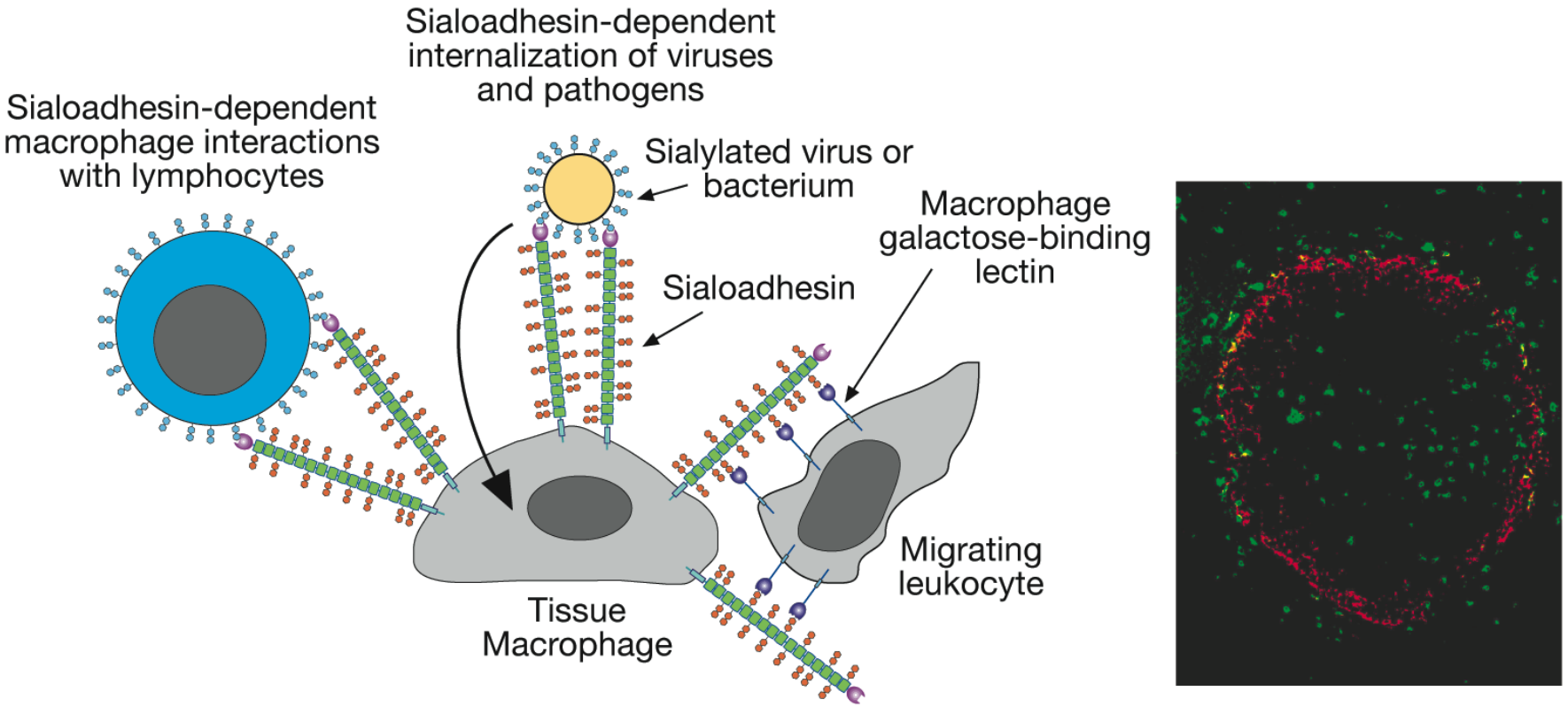


Figure 8.14 Sialic acid-containing ligands for some of the siglecs. Sialoadhesin and CD22 probably bind common N- and O-linked glycans attached to glycoproteins. Some of the CD33-related siglecs bind to specific ligands such as 6′ sulpho sialyl-Lewis^x (human siglec 8), 6 sulpho sialyl-Lewis^x (human siglec 9), and disialyl motifs found in GD3 and GD1b (human siglec 7).


NeuAc
G
F
C
B
A
C′
C″
D
E
G′
A′
(Ser)-103
Trp-2
N-Ac
4
COOH
A
8
9
Trp-106
Arg-97
F
(Leu)-107
G
C
O
N
Ca2+

Biological functions mediated by sialoadhesin



12 Extracellular galectins have roles in cell adhsion and cell signalling

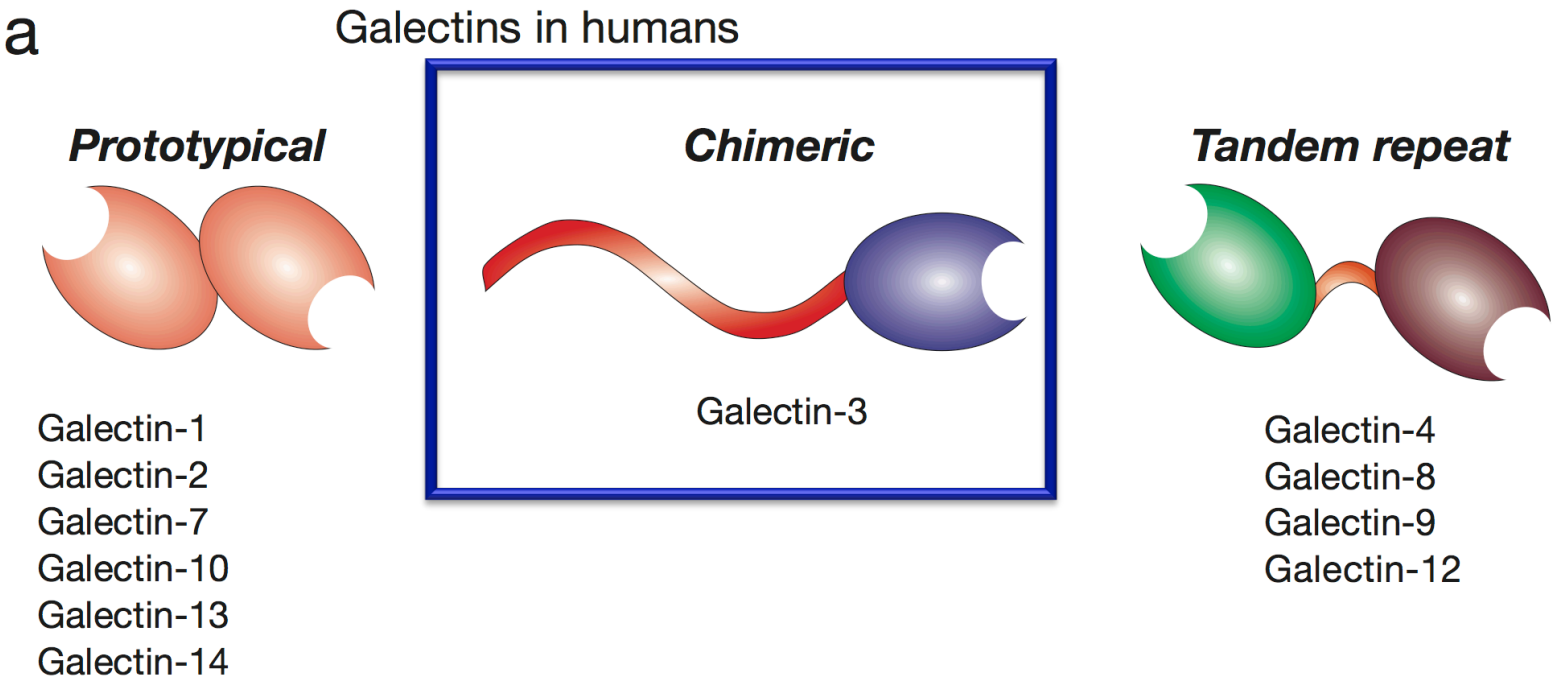
Galectins

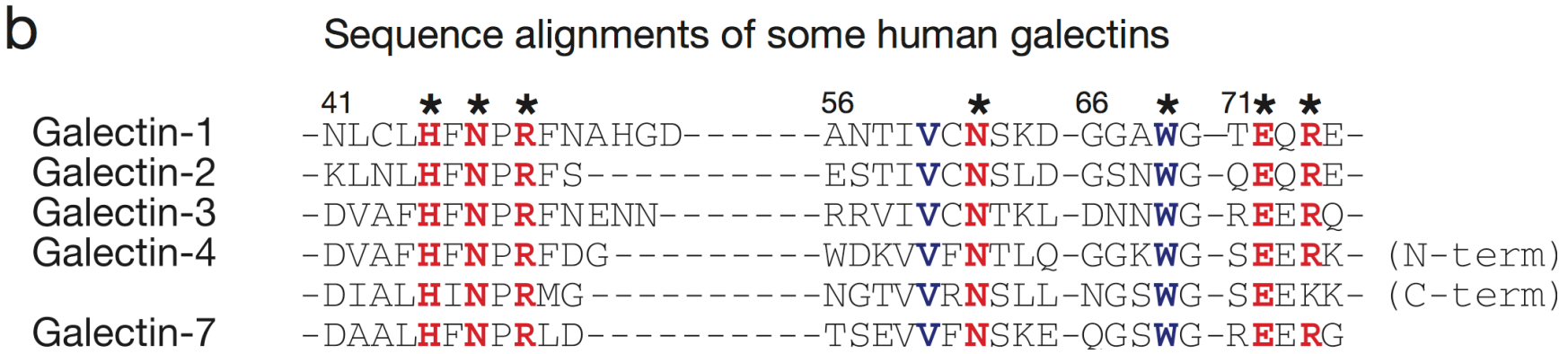
- A family of soluble proteins that bind β-galactosides
- Have a wide cell and tissue distirbution and proposed roles in the nucleus
- Reach on cell surface with a process they become enclosed in membrane vesicles
- Mediate or modulate cell-cell interactions, cell-matrix adhesion, and transmembrane signalling
- Phenotype of knockout mice: function in specific developmental process

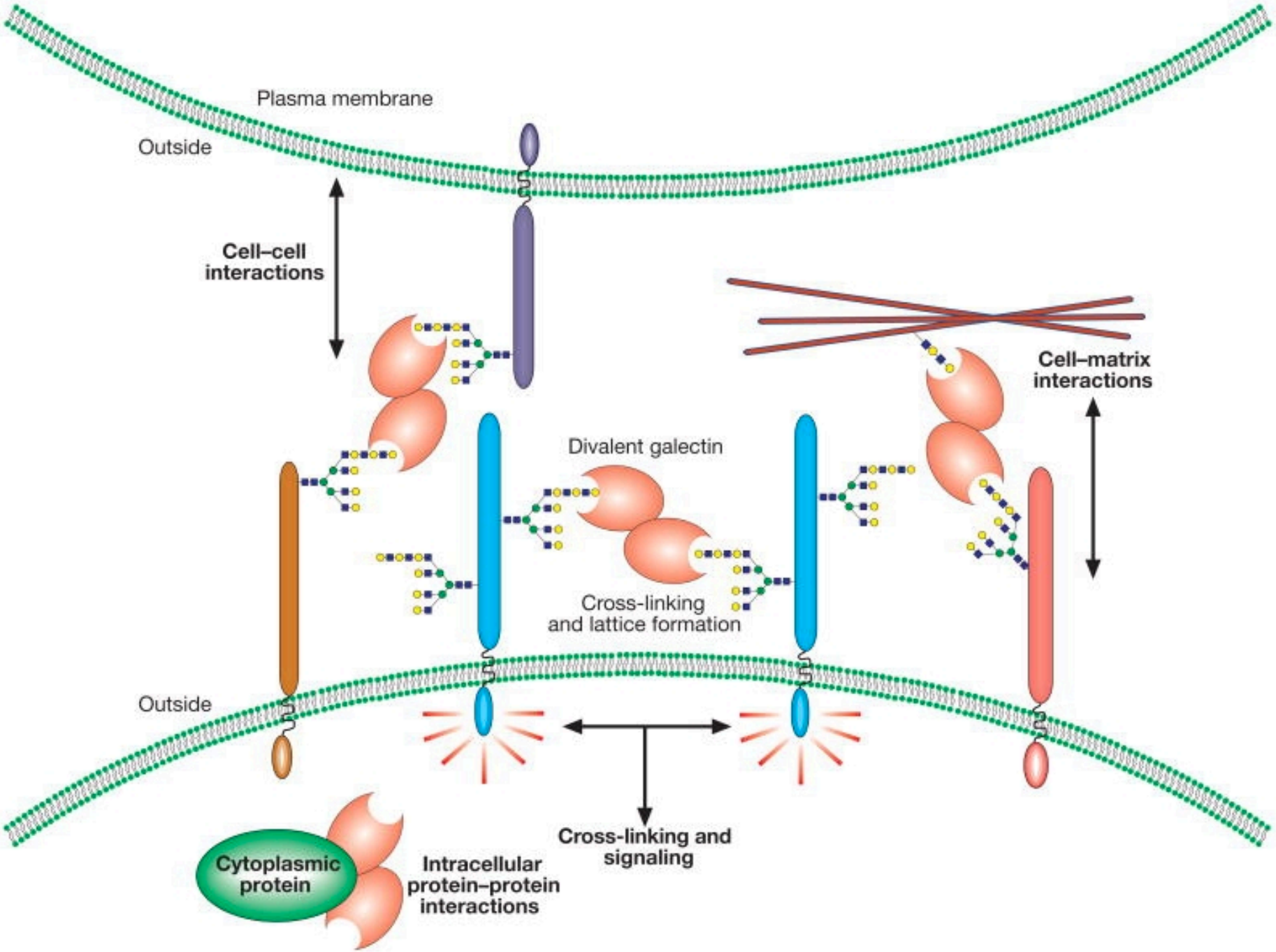
Numerous potential ligands for galectins are found outside cells

- They bind tightly to the polylactosamine chains
- Cell-surface glycoproteins and matrix include integrin (整联蛋白), fibronectin (纤连蛋白), laminin (层粘连蛋白) and tenascin (生腱蛋白)
- They don’t have hydrophobic membrane anchors, so they mediate adhesive events by crosslinking two glycans

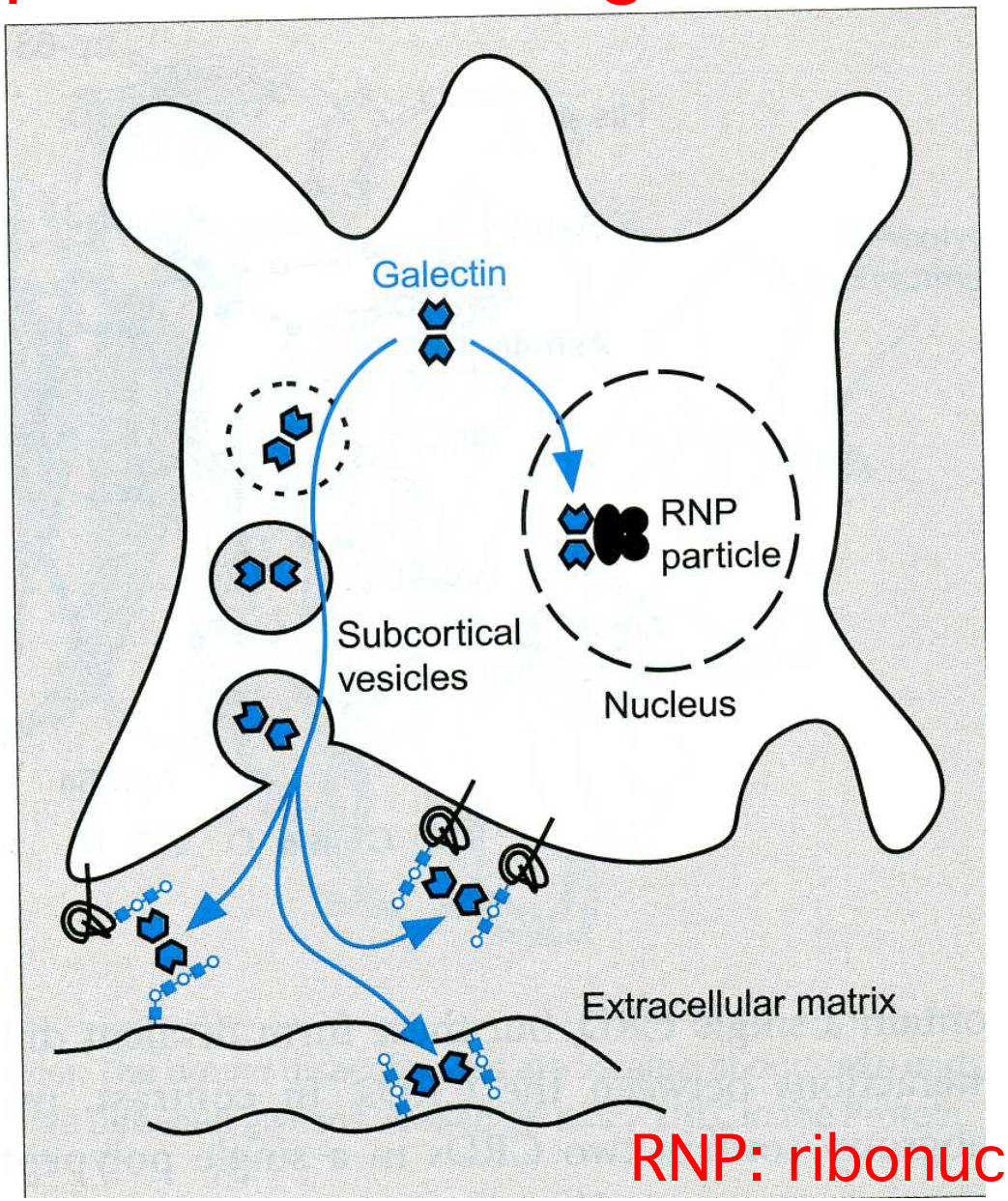
Different types of galectins in humans





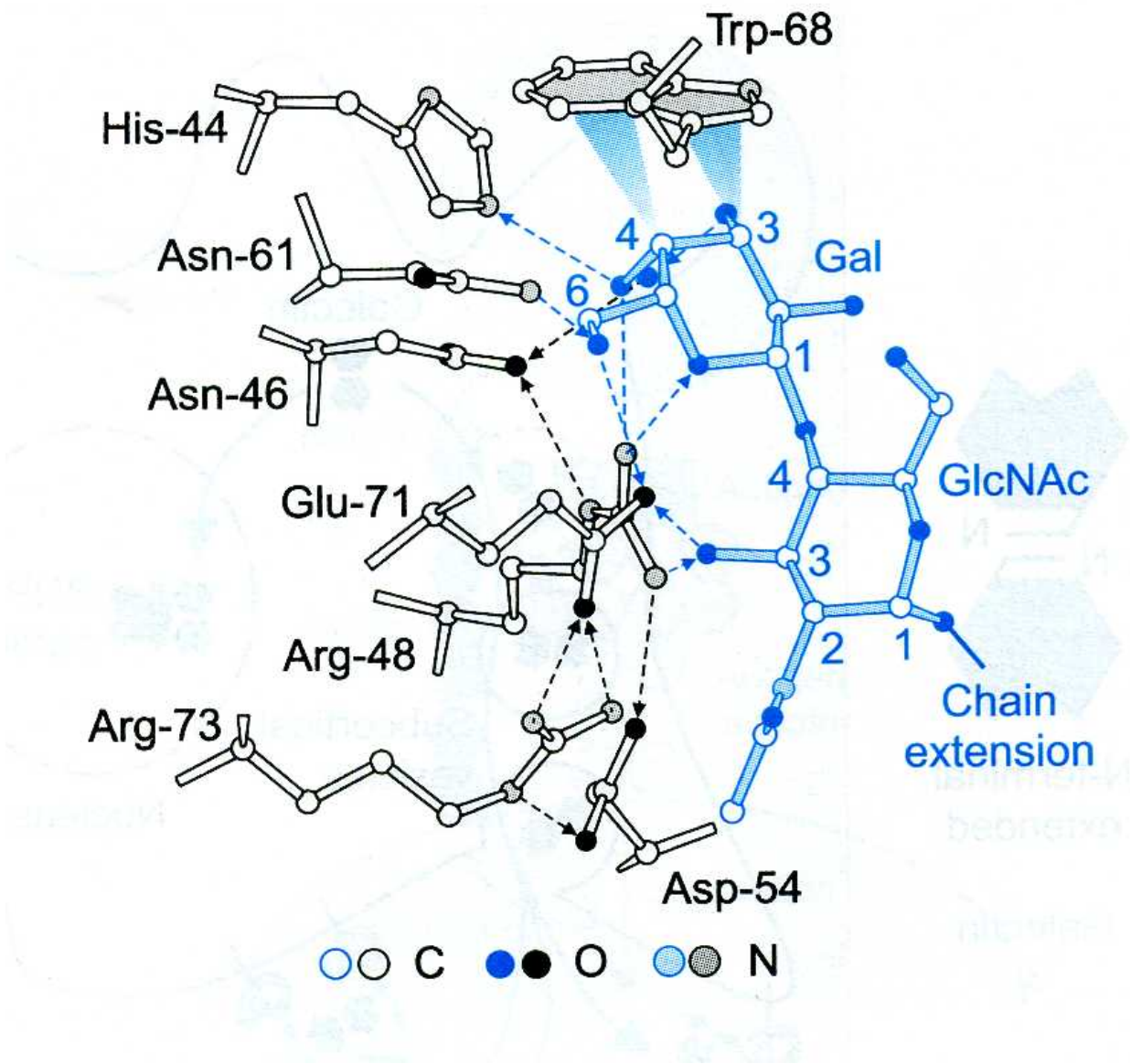

Plasma membrane
Outside
Cell–cell interactions
Cell–matrix interactions
Divalent galectin
Cross-linking and lattice formation
Outside
Cytoplasmic protein
Intracellular protein–protein interactions
Cross-linking and signaling

Two proposed roles of galectins

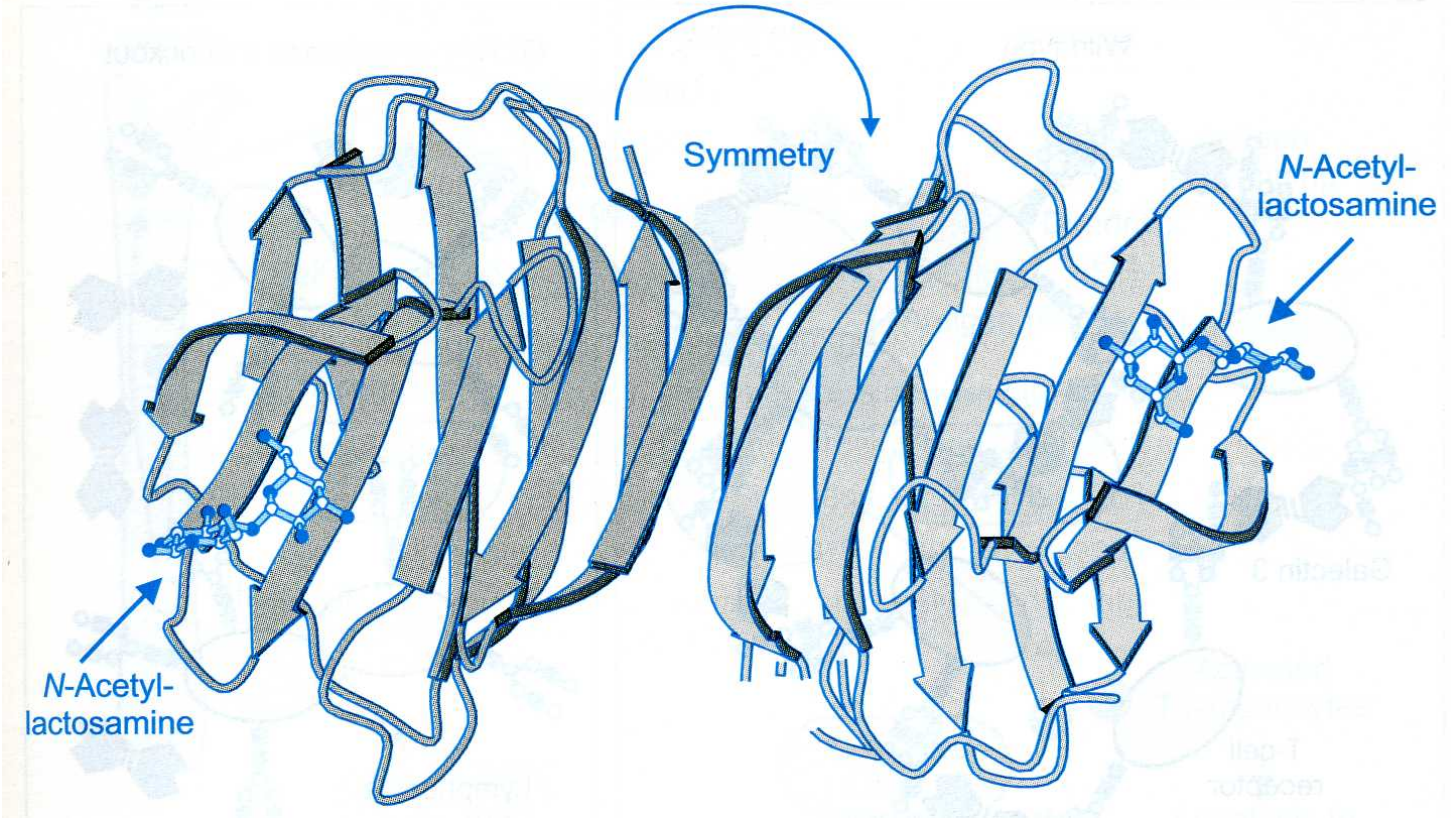


RNP: ribonucleoprotein

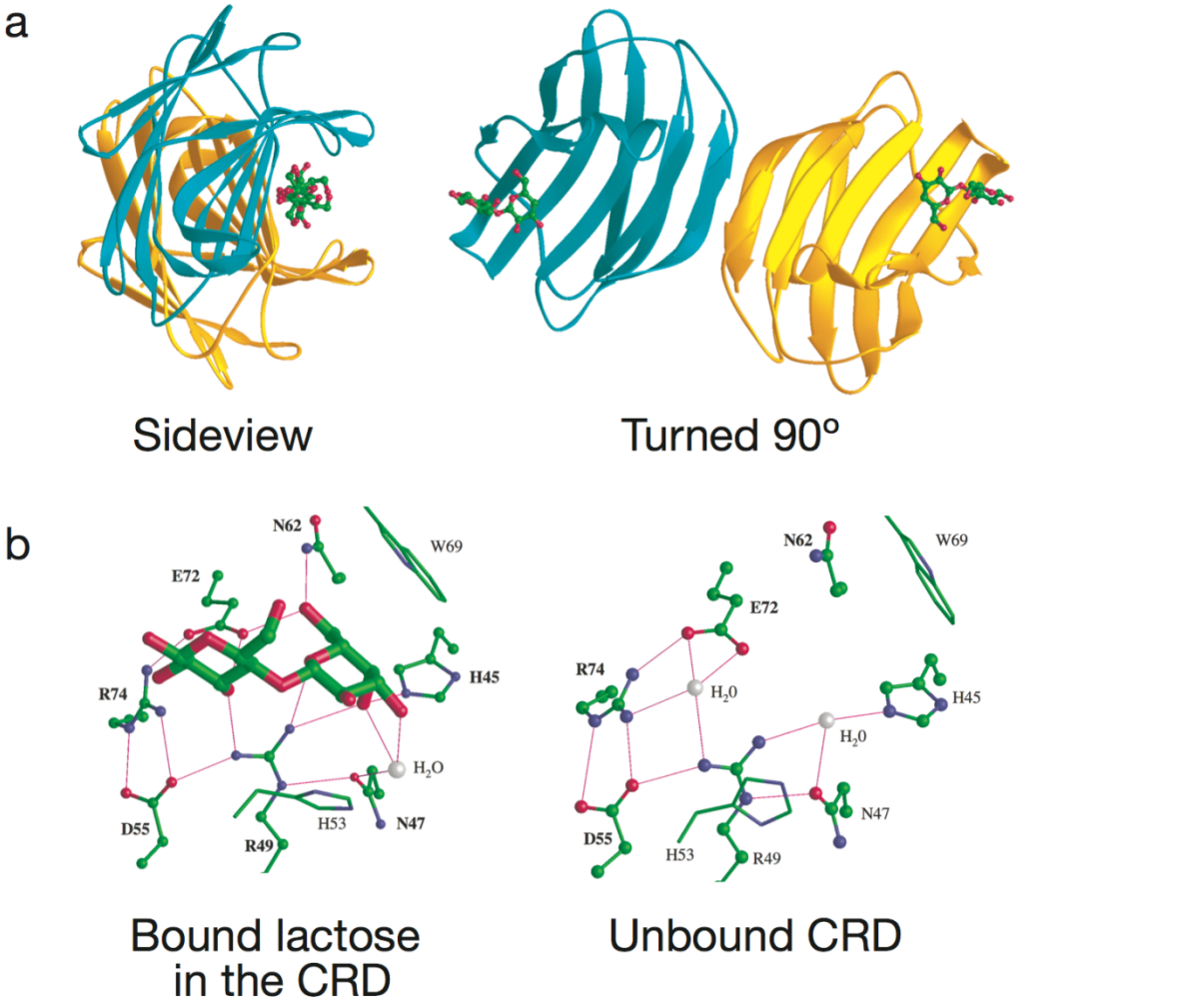
Binding to lactose/Galβ1-4GlcNAc



Two binding sites extend from opposite ends of the dimer

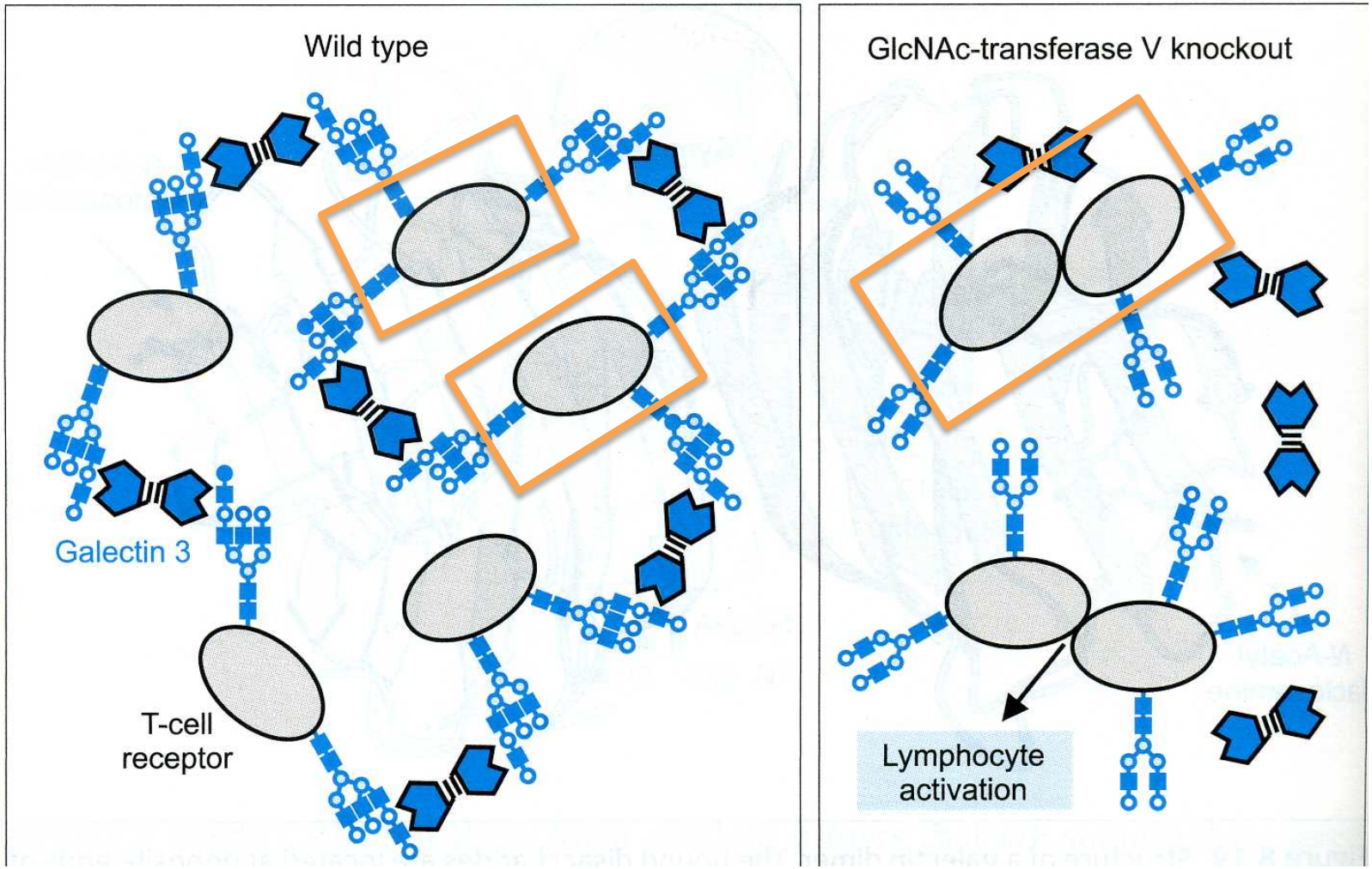


Structures of galectins



13 Galectins modulate activation of T cells and control cell survival by triggering or inhibiting apoptosis (凋亡)

Suppression of T-cell receptor activation by galectin 3



- Mice lack polylactosamine chain show increased susceptibility to autoimmune diseases and have hypersensitive T cells
- Due to enhanced clustering of T-cell receptors
- A similar phenotype can be induced in wild type cells by pretreatment with lactose, but not other sugars

Function of galectins in apoptosis (凋亡) of T cells