Кононов Леонид Олегович

ХИМИЯ УГЛЕВОДОВ И ГЛИКОБИОЛОГИЯ

https://углеводы.su

Лекция 6

Углевод-связывающие белки

6. *Essentials of glycobiology*, A. Varki, et al. (Eds.), 3d edn., 2017, Ch. 28–38.

10. *The Sugar Code. Fundamentals of glycosciences*, H.-J. Gabius (Ed.), 2009, Ch. 13, 14, 15, 16, 19.

38. M. E. Taylor, K. Drickamer. *Curr. Opin. Cell. Biol*. **2007**, *19*, 572.

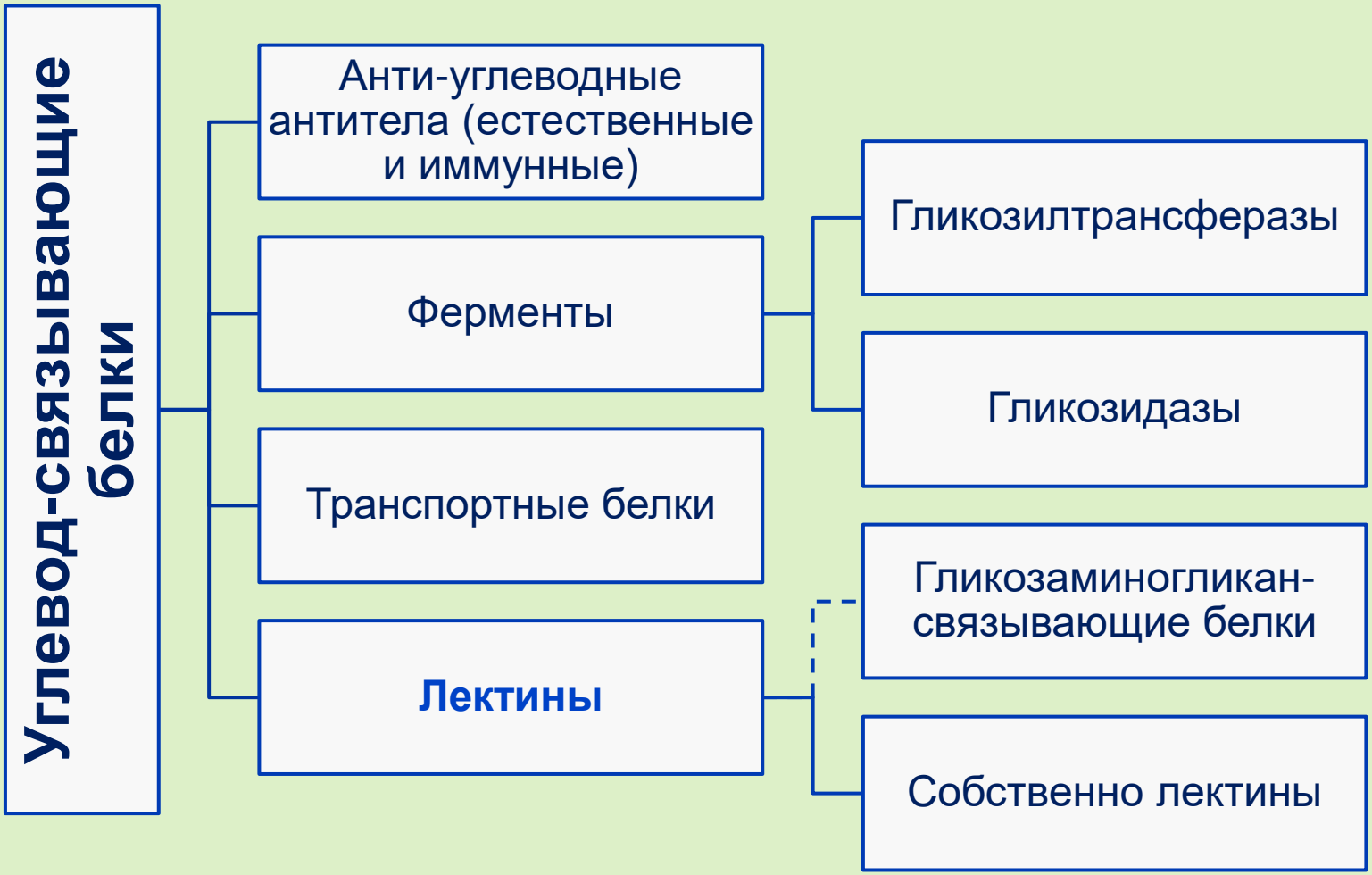
41. N. Sharon. *Biochem. Soc. Trans*. **2008**, *36*, 1457.

49. H.-J. Gabius, S. André, J. Jiménez-Barbero, A. Romero, D. Solís. *Trends Biochem. Sci.* **2011**, *36*, 298.

62. D. Solís, N. V. Bovin, A. P. Davis, J. Jiménez-Barbero, A. Romero, R. Roy, K. Smetana Jr., H.-J. Gabius. *Biochim. Biophys. Acta* **2015**, *1850*, 186.

Лектины и другие углевод-связывающие белки

Углевод-связывающие белки



Лектины — это специфические белки, обладающие свойством избирательно связывать углеводы и углеводные компоненты гликоконъюгатов различной природы. Лектинами принято считать только те углевод-связывающие белки, которые не обладают специфической гликоферментативной активностью и имеют неиммунное происхождение, т.е. не являются антителами.

Сравнение двух основных классов углевод-связывающих белков

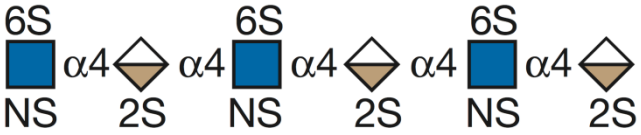
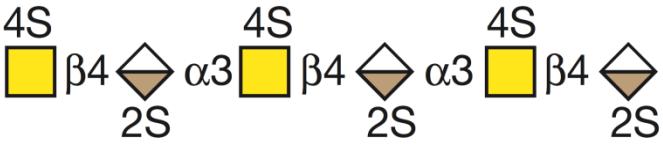
	Lectins[a]	Glycosaminoglycan-binding proteins[b]
Shared evolutionary origins	yes (within each group)	no
Shared structural features	yes (within each group)	no
Defining AA residues involved in binding	often typical for each group	patch of basic amino acid residues
Type of glycans recognized	N-glycans, O-glycans, glycosphingo-lipids (a few also recognize sulfated glycosaminoglycans)	different types of sulfated glycosaminoglycans
Location of cognate residues within glycans	typically in sequences at outer ends of glycan chains	typically in sequences internal to an extended sulfated glycosaminoglycan chain
Specificity for glycans recognized	stereospecificity high for specific glycan structures	often recognize a range of related sulfated glycosaminoglycan structures
Single-site binding affinity	often low; high avidity generated by multivalency	often moderate to high
Valency of binding sites	multivalency common (either within native structure or by clustering)	often monovalent
Subgroups	C-type lectins, galectins, P-type lectins, I-type lectins, L-type lectins, R-type lectins etc.	heparan sulfate–binding proteins, chondroitin sulfate–binding proteins, dermatan sulfate– binding proteins
Types of glycans recognized within each group	can be similar (e.g., galectins) or variable (e.g., C-type lectins)	classification itself is based on type of glycosaminoglycan chain recognized

Гликозаминогликан (ГАГ)-связывающие белки

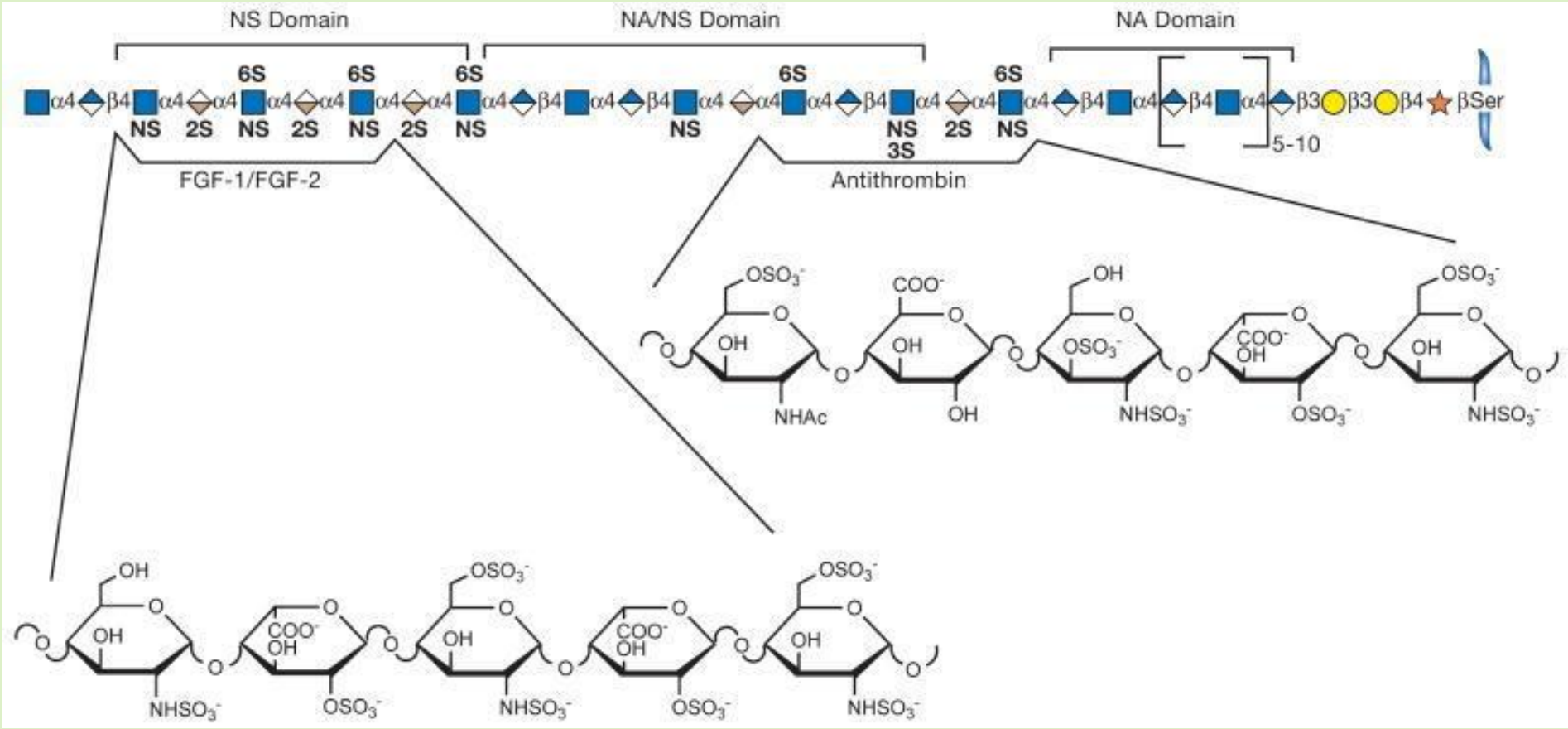
Примеры белков, связывающихся с ГАГ

Cell/matrix interactions	Coagulation/ fibrinolysis	Lipolysis	Inflammation	Growth factors and morphogens
laminin	antithrombin	lipoprotein lipase	cytokines (IL-2, IL-7, IL-8)	FGFs and FGF receptors
fibronectin	heparin cofactor II	hepatic lipase		HGF; scatter factor
vitronectin	tissue factor pathway inhibitor	apoE	chemokines (e.g., MIP-1β, SDF-1, etc.)	VEGF
thrombospondin		apoB		TGF-β
tenascin	thrombin	apoA-V		BMPs
various collagens	protein C inhibitor		TNF-α	Hedgehogs
amyloid proteins	tPA and PAI-1		L and P selectins	Wnts
			superoxide dismutase	
			microbial adhesins	

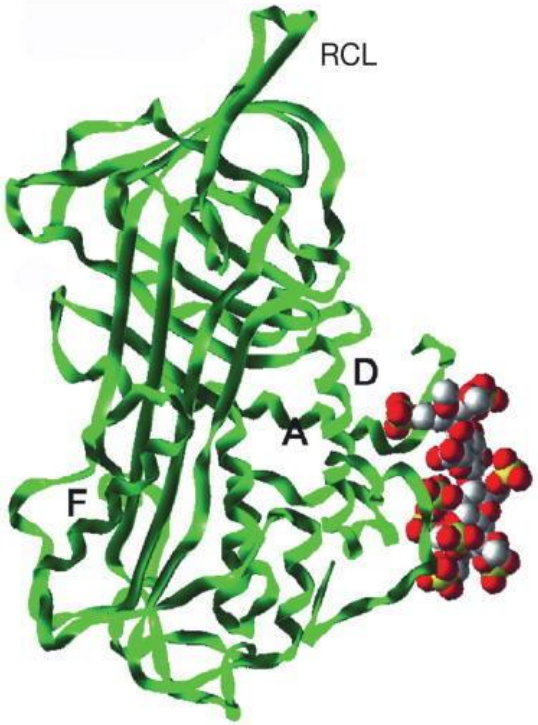
Примеры олигосахаридов, которые узнаются ГАГ-связывающими белками

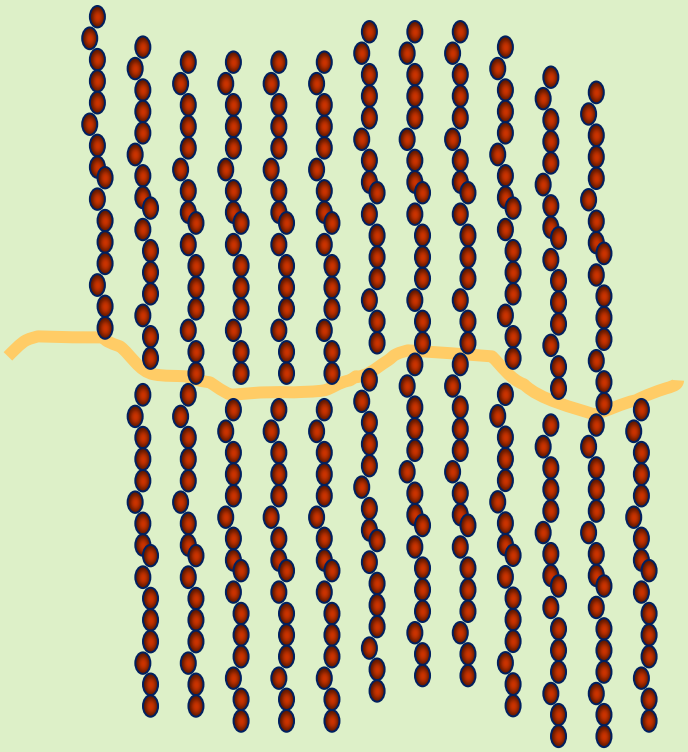
Protein	Glycosaminoglycan partner	Oligosaccharide
Antithrombin	heparin/heparan sulfate	
Fibroblast growth factor 2	heparin/heparan sulfate	
Lipoproteinlipase	heparin/heparan sulfate	
Heparin cofactor II	dermatan sulfate	
Herpes simplex virus Glycoprotein gD	heparin/heparan sulfate	

Доменная структура гепарансульфата/гепарина: сайты связывания с различными лигандами

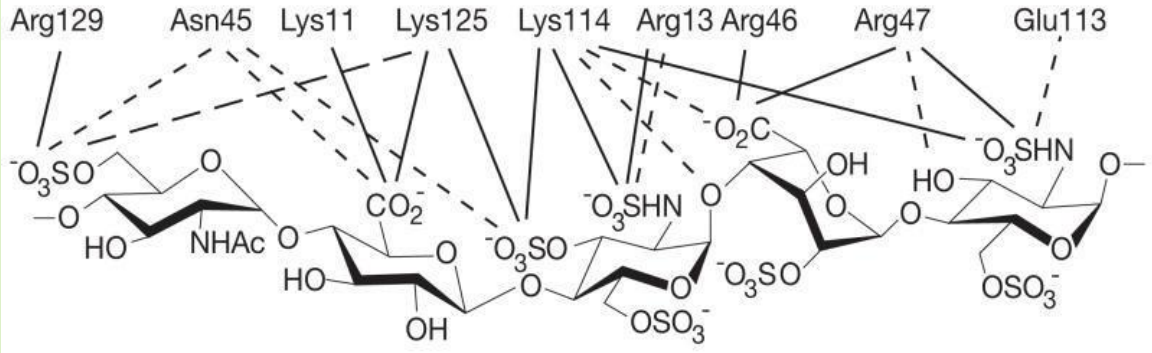


Комплекс антитромбина с пентасахаридным фрагментом гепарина





протеогликан

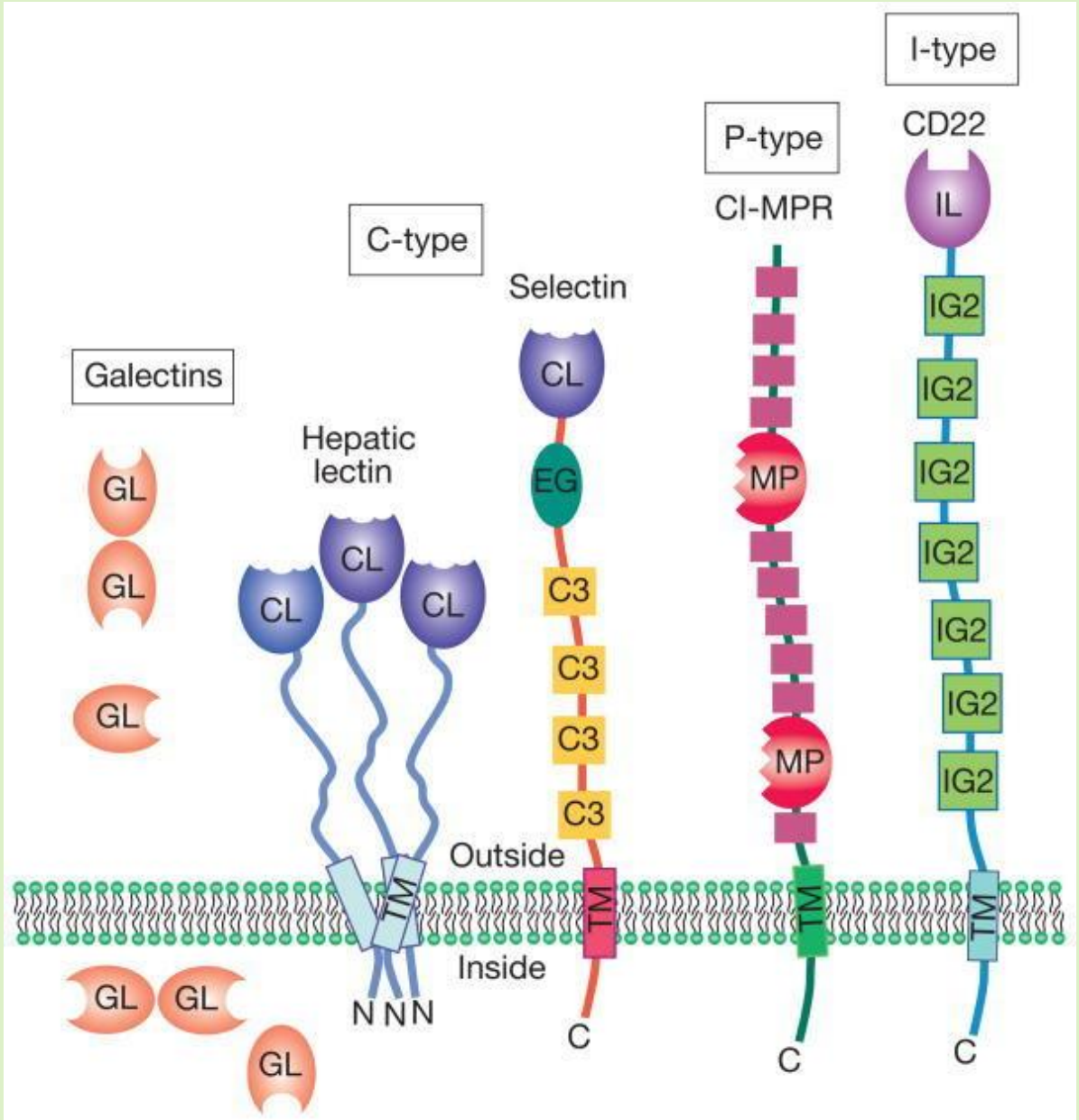


Классификация лектинов

Варианты классификации лектинов

- Классификация, основанная на природе гликановых последовательностей, с которыми лектин связывается наиболее прочно
  - Например, β-галактозид-связывающие лектины = галектины.
- Классификация, основанная на анализе гомологии аминокислотных последовательностей белковых цепей и сходства их третичных структур.
  - Углевод-связывающие домены (CRD) каждой группы (типа) лектинов содержат консервативные мотивы последовательностей аминокислот.
  - Общая доменная архитектура (мотив укладки) каждого типа лектинов сходна для представителей одного типа (имеет характерный «фолд») и различается для лектинов разных типов.

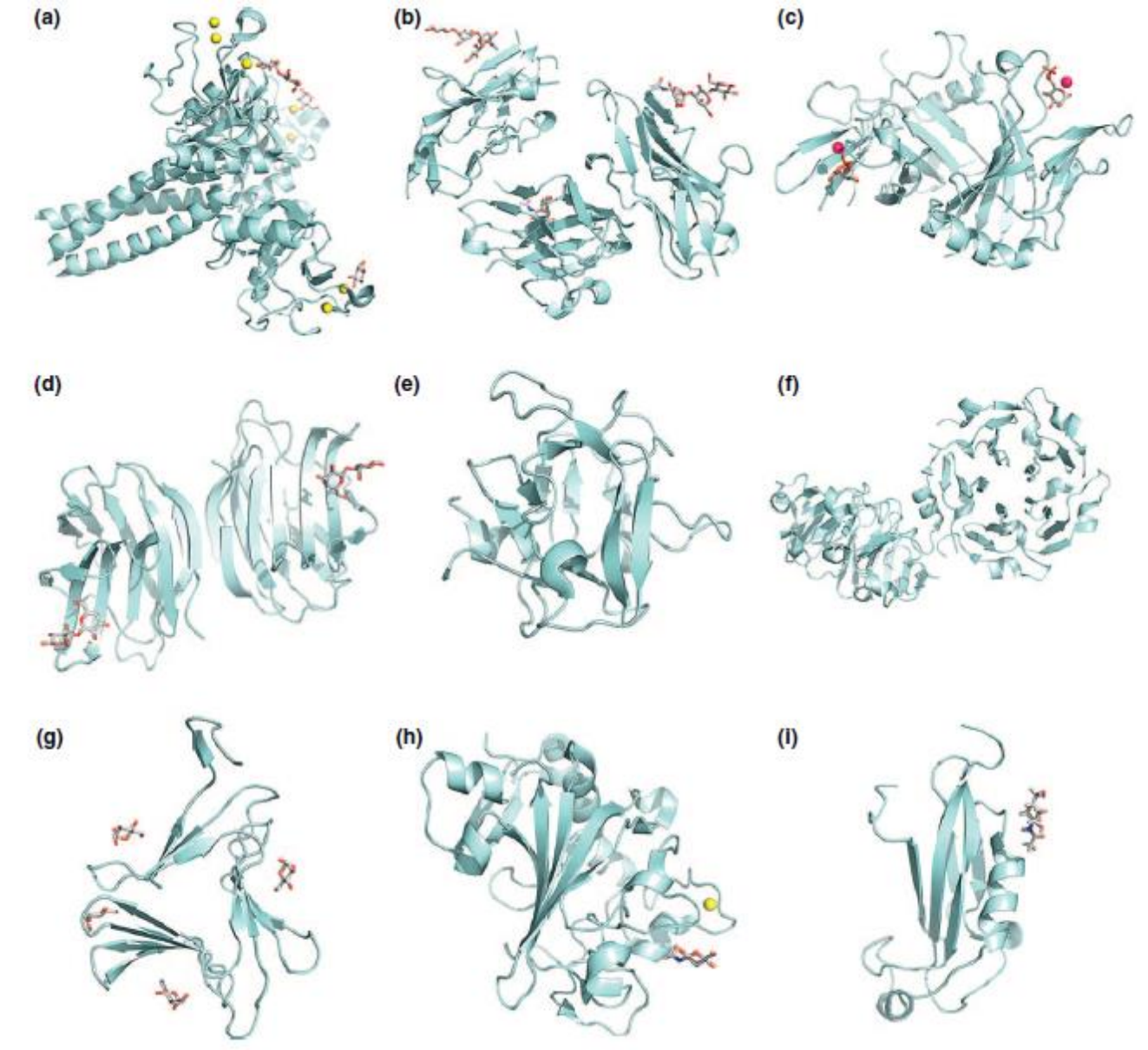
Основные типы лектинов (на основе анализа первичной/третичной структуры белка)



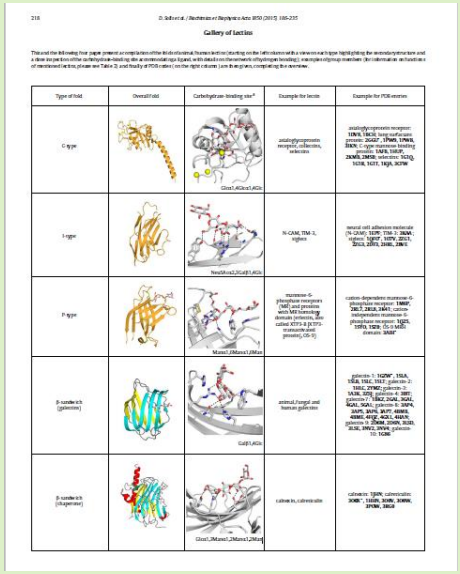
Углевод-связывающие домены (CRDs):

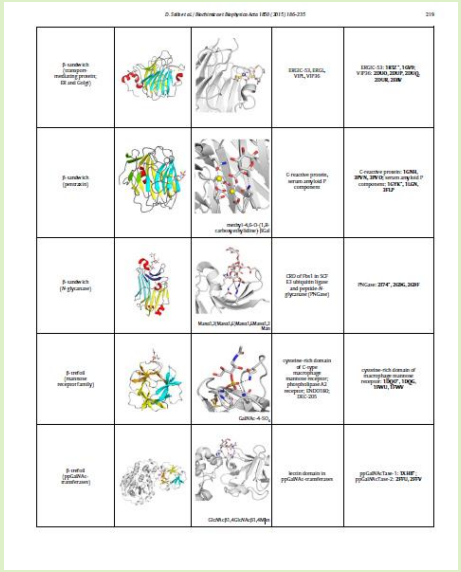
- (CL) C-type lectin CRD;
- (GL) S-type lectin CRD;
- (MP) P-type lectin CRD;
- (IL) I-type lectin CRD.
- (EG) EGF-like domain;
- (IG2) immunoglobulin C2-set domain;
- (TM) transmembrane region;
- (C3) complement regulatory repeat.

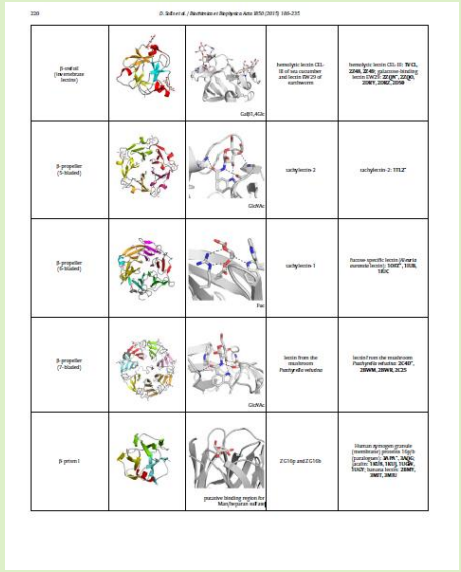
Разнообразие типов третичных структур («фолдов») лектинов: несколько примеров

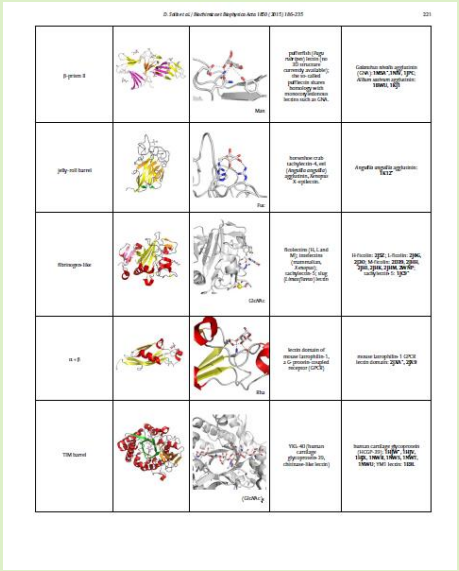


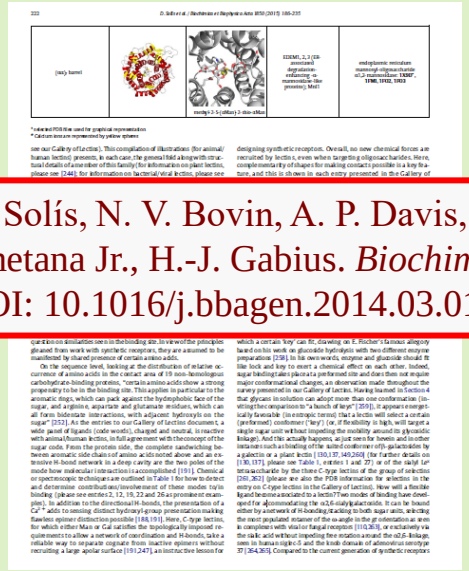
Галлерея лектинов: все известные типы третичных структур («фолдов») лектинов











62. D. Solís, N. V. Bovin, A. P. Davis, J. Jiménez-Barbero, A. Romero, R. Roy, K. Smetana Jr., H.-J. Gabius. *Biochim. Biophys. Acta* **2015**, *1850*, 186-235. DOI: 10.1016/j.bbagen.2014.03.016.

Лектины растений

Лектины L- и R-типов

Лектины растений

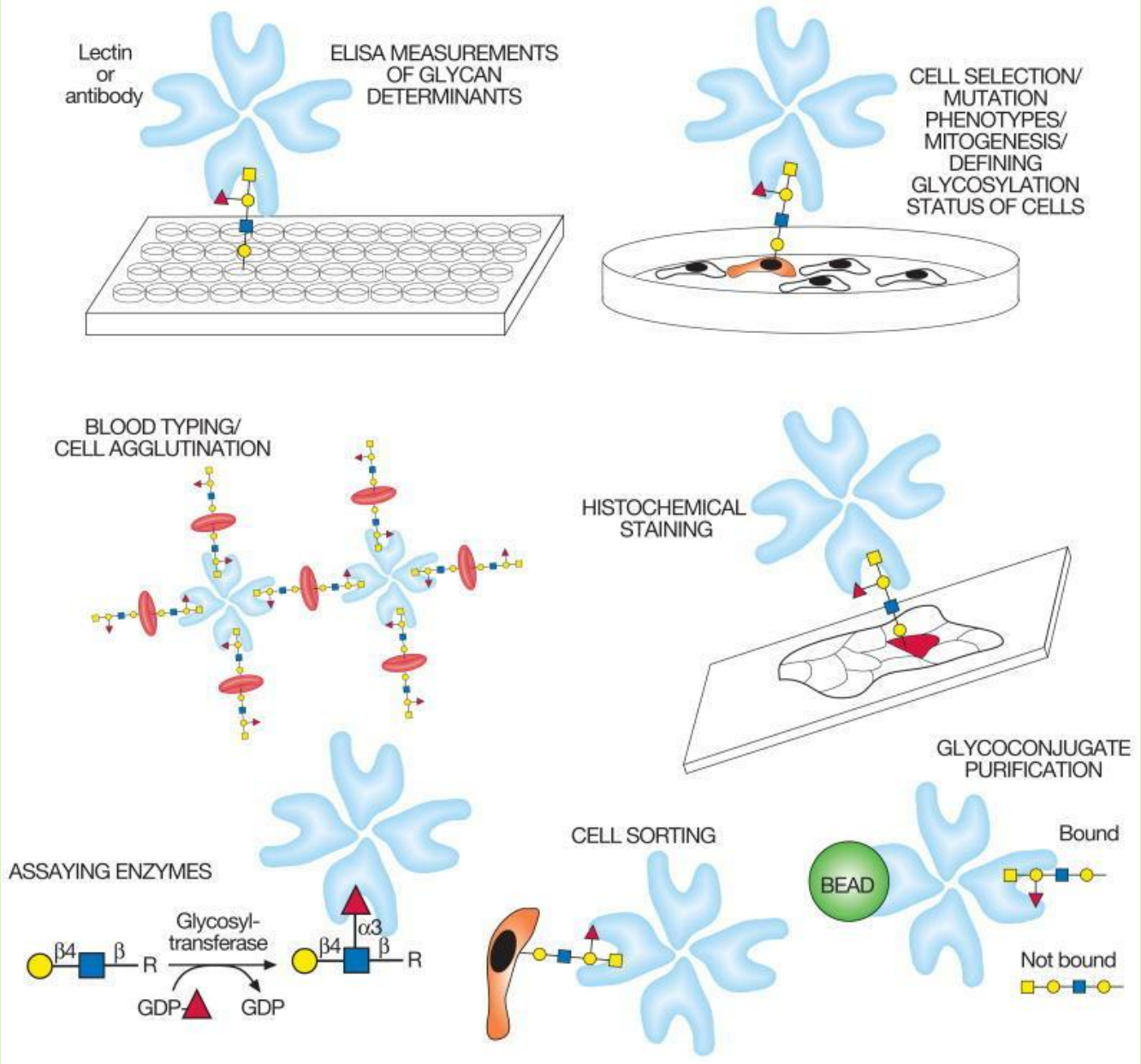
Лектины: что это такое и чем вредно?

04.04.2015

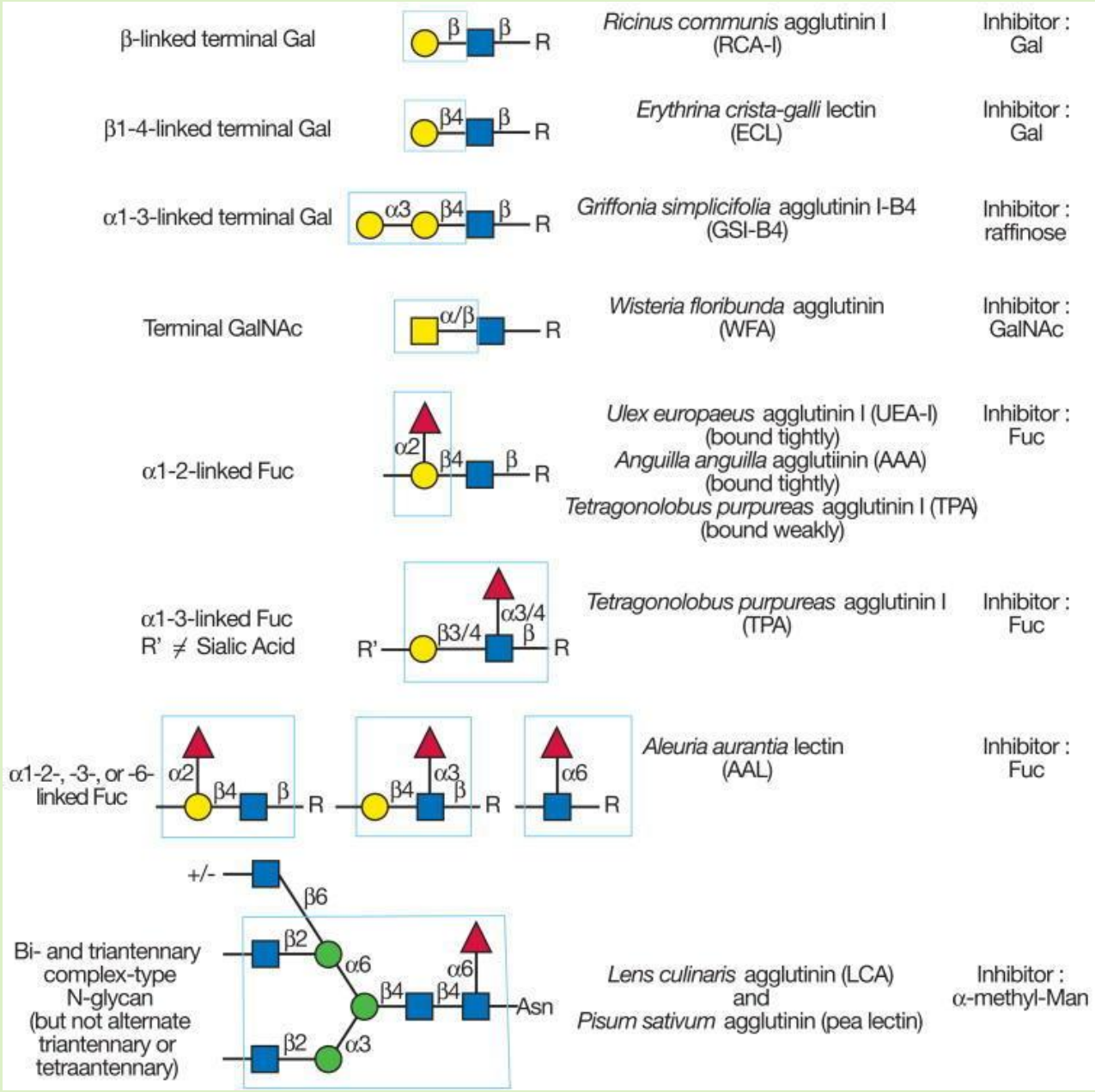
Лектинами называют очень разнообразное семейство углевод-связывающих белков. В природе настолько много лектинов, что они содержатся во всех растительных и животных организмах. Без преувеличения можно сказать, что без лектинов нет жизни.

А если так, почему тогда известно, что лектины вредны, и что с их употреблением в пищу надо быть очень острожным?

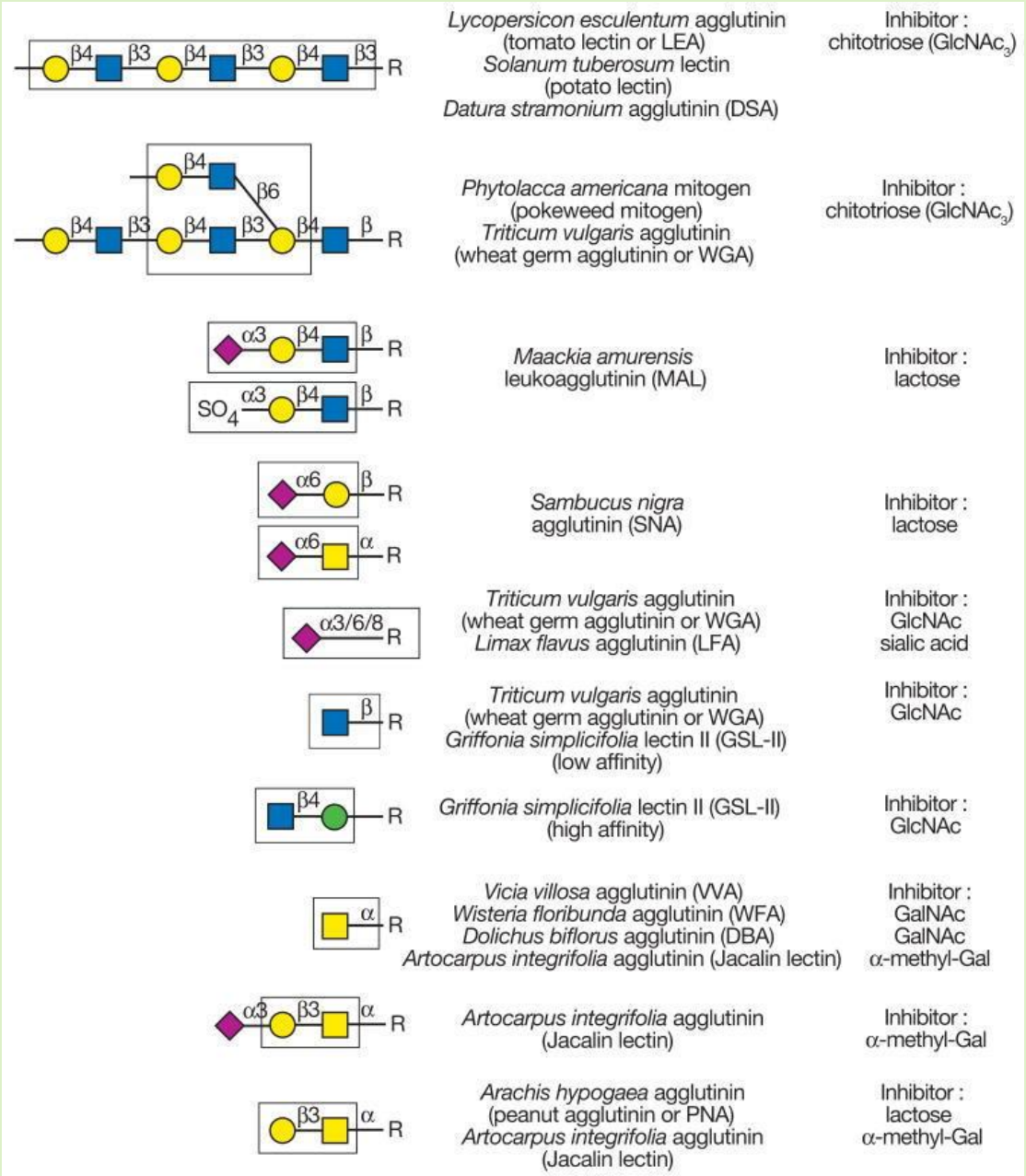
Использование лектинов в гликобиологии: лектины – инструменты исследования



Гликаны, прочно связывающиеся с лектинами

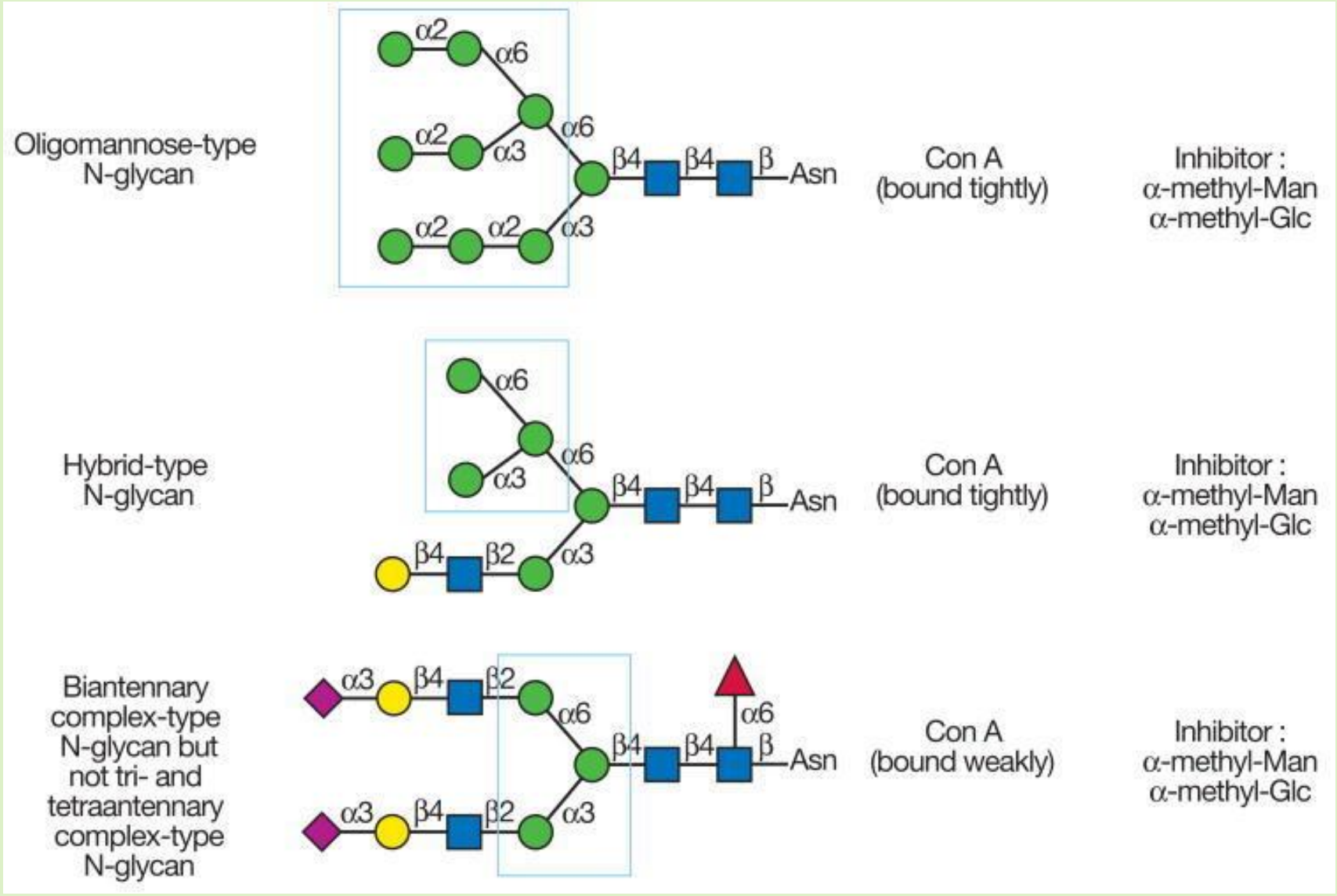


Гликаны, узнаваемые лектинами растений



Lycopersicon esculentum agglutinin (tomato lectin or LEA) *Solanum tuberosum* lectin (potato lectin) *Datura stramonium* agglutinin (DSA)	Inhibitor : chitotriose ($GlcNAc_3$)
Phytolacca americana mitogen (pokeweed mitogen) *Triticum vulgaris* agglutinin (wheat germ agglutinin or WGA)	Inhibitor : chitotriose ($GlcNAc_3$)
Maackia amurensis leukoagglutinin (MAL)	Inhibitor : lactose
Sambucus nigra agglutinin (SNA)	Inhibitor : lactose
Triticum vulgaris agglutinin (wheat germ agglutinin or WGA) *Limax flavus* agglutinin (LFA)	Inhibitor : GlcNAc sialic acid
Triticum vulgaris agglutinin (wheat germ agglutinin or WGA) *Griffonia simplicifolia* lectin II (GSL-II) (low affinity)	Inhibitor : GlcNAc
Griffonia simplicifolia lectin II (GSL-II) (high affinity)	Inhibitor : GlcNAc
Vicia villosa agglutinin (VVA) *Wisteria floribunda* agglutinin (WFA) *Dolichus biflorus* agglutinin (DBA) *Artocarpus integrifolia* agglutinin (Jacalin lectin)	Inhibitor : GalNAc GalNAc α-methyl-Gal
Artocarpus integrifolia agglutinin (Jacalin lectin)	Inhibitor : α-methyl-Gal
Arachis hypogaea agglutinin (peanut agglutinin or PNA) *Artocarpus integrifolia* agglutinin (Jacalin lectin)	Inhibitor : lactose α-methyl-Gal

N-Гликаны, узнаваемые конканавалином А (ConA) из *Canavalia ensiformis*

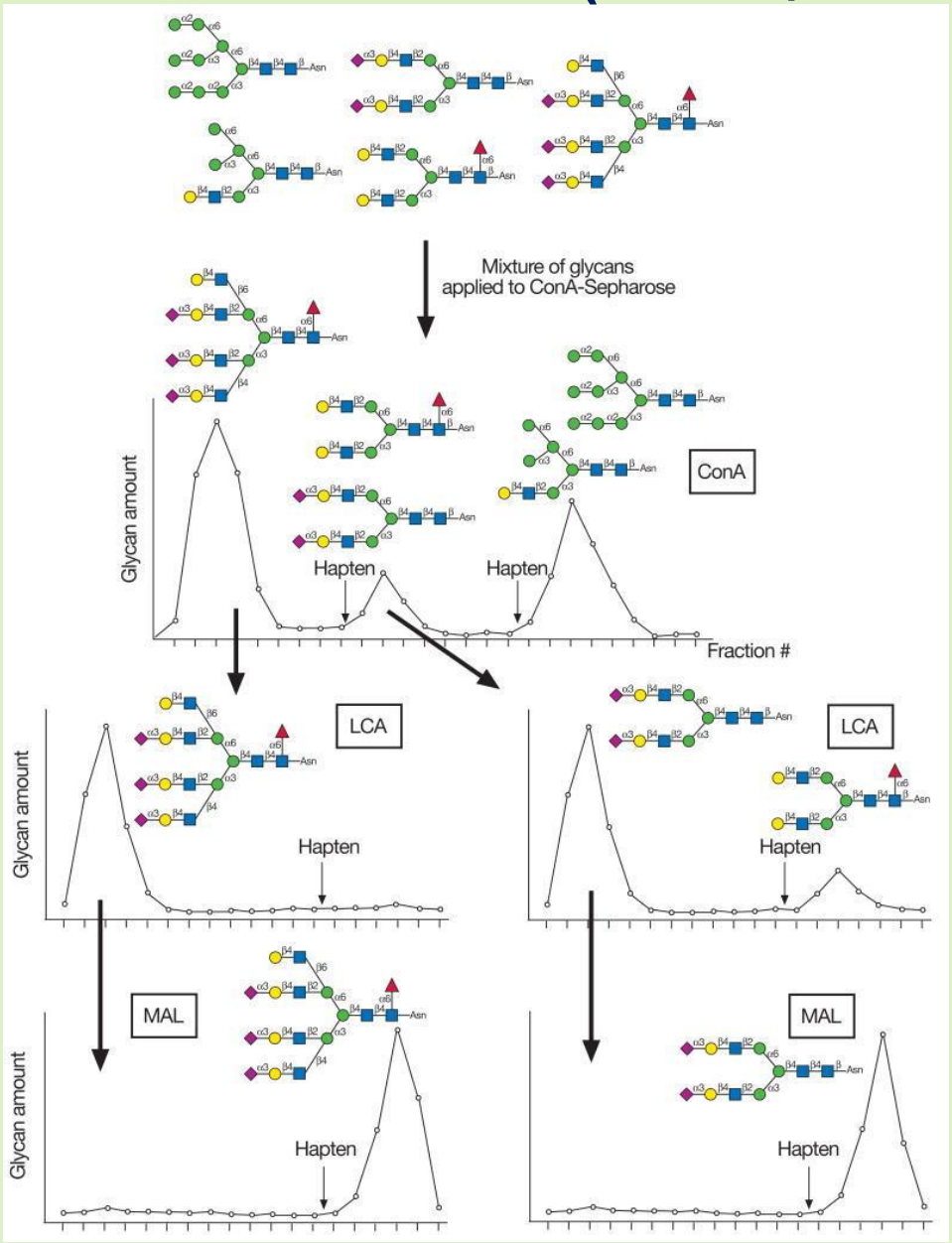


Аффинная хроматография: на колонках иммобилизованы лектины (ConA, LCA, MAL)

α-GlcOMe

α-ManOMe

Lac



α-ManOMe

α-ManOMe

Lac

Лектины клеток животных

Селектины, коллектины, фиколины, сиглеки, галектины

Функции лектинов клеток животных

Activity	Example of lectin
Recognition of stem region of *N*-glycans, a signal for ubiquitin conjugation when accessible in incorrectly folded glycoproteins	F-box proteins Fbs1 and Fbs2, which comprise the ligand-specific part of SCF[b] ubiquitin ligase complexes
Molecular chaperones with dual specificity for $Glc_2/Glc_1Man_9GlcNAc_2$ and protein part of nascent glycoproteins in the ER	Malectin/ribophorin I complex, calnexin, calreticulin
Targeting of misfolded glycoproteins with $Man_{8-5}GlcNAc_2$ as carbohydrate ligand to ER-associated degradation (ERAD)	EDEM1,2[c]/Mnl1 (Htm1) (lectins or glycosidases?), Yos9p (MRH[d] domain) in yeast, erlectin (XTP3-B[e]) and OS-9[f] in mammals
Intracellular routing of glycoproteins and vesicles and apical delivery	Comitin, ERGIC53[g] and VIP36[h] (probably also ERGL[i] and VIPL[j]), galectins-3, -4 and -9, P-type lectins
Intracellular transport and extracellular assembly	Non-integrin 67 kDa elastin/laminin-binding protein
Enamel formation and biomineralization	Amelogenin
Inducer of membrane superimposition and zippering (formation of Birbeck granules)	Langerin (CD207)
Cell type-specific endocytosis	Cysteine-rich domain (β-trefoil) of the dimeric form of mannose receptor for GalNAc-4-SO_4-bearing glycoprotein hormones in hepatic endothelial cells, dendritic cell and macrophage C-type lectins (mannose receptor family members (tandem-repeat type) and single-CRD[k] lectins such as trimeric langerin/CD207 or tetrameric DC-SIGN[l]/CD209), hepatic and macrophage asialoglycoprotein receptors, HARE[m], P-type lectins
Recognition of foreign glycans (β1,3-glucans, cell wall peptidoglycan, LOS[n] and LPS[o]), mycobacterial glycolipid or host-like epitopes	CR3[p] (CD11b/CD18, Mac-1 antigen), C-type lectins such as collectins, DC-SIGN, dectin-1, Mincle and RegIIIγ (murine)[q] or HIP/PAP (human), ficolins, galectins, immulectins, intelectins, *Limulus* coagulation factors C and G, siglecs, tachylectins
Recognition of foreign or aberrant glycosignatures on cells (including endocytosis or initiation of opsonization or complement activation) and of apoptotic/necrotic cells (glycans or peptide motifs)	Collectins, C-type macrophage and dendritic cell lectins, CR3 (CD11b/CD18, Mac-1 antigen), α/Θ-defensins, ficolins, galectins, pentraxins (CRP, limulin), RegIIIγ (HIP/PAP), siglecs, tachylectins
Targeting of enzymatic activity in multimodular proteins	Acrosin, *Limulus* coagulation factor C, laforin, β-trefoil fold ($(QxW)_3$ domain) of GalNAc-Ts[r] involved in mucin-type *O*-glycosylation, frequent in microbial glycosylhydrolases for plant cell wall polysaccharides, termed carbohydrate-binding modules
Bridging of molecules	Cerebellar soluble lectin, cytokines (e.g. IL-2[s]-IL-2R and CD3 of TCR), galectins
Induction or suppression of effector release (H_2O_2, cytokines etc.)	Chitinase-like YKL-40, galectins, I-type lectins (e.g. CD33 (siglec-3), siglecs-7 and -9), selectins and other C-type lectins such as CD23, BDCA2 and dectin-1, Toll-like receptor 4
Alteration of enzymatic activities in modular proteins/receptor endocytosis via lattice formation	Mannan-binding lectin (acting on meprins); galectins
Cell growth control, induction of apoptosis/anoikis and axonal regeneration	Amphoterin and other heparin-binding proteins, cerebellar soluble lectin, chitinase-like lectins, C-type lectins, galectins, hyaluronic acid-binding proteins, siglecs (e.g. CD22 and CD33)
Cell migration and routing	Galectins, hyaluronic acid-binding proteins (CD44, hyalectans/lecticans, RHAMM[t]), I-type lectins, selectins and other C-type lectins
Cell–cell interactions	Galectins, gliolectin, I-type lectins (e.g. siglecs, N-CAM[u], P_0 or L1), selectins and other C-type lectins such as DC-SIGN or macrophage mannose receptor
Cell–matrix interactions	Calreticulin, discoidin I, galectins, heparin- and hyaluronic acid-binding lectins including hyalectans/lecticans
Matrix network assembly	Galectins (e.g. galectin-3/hensin), non-integrin 67 kDa elastin/laminin-binding protein, proteoglycan core proteins (C-type CRD and G1 domain of hyalectans/lecticans)

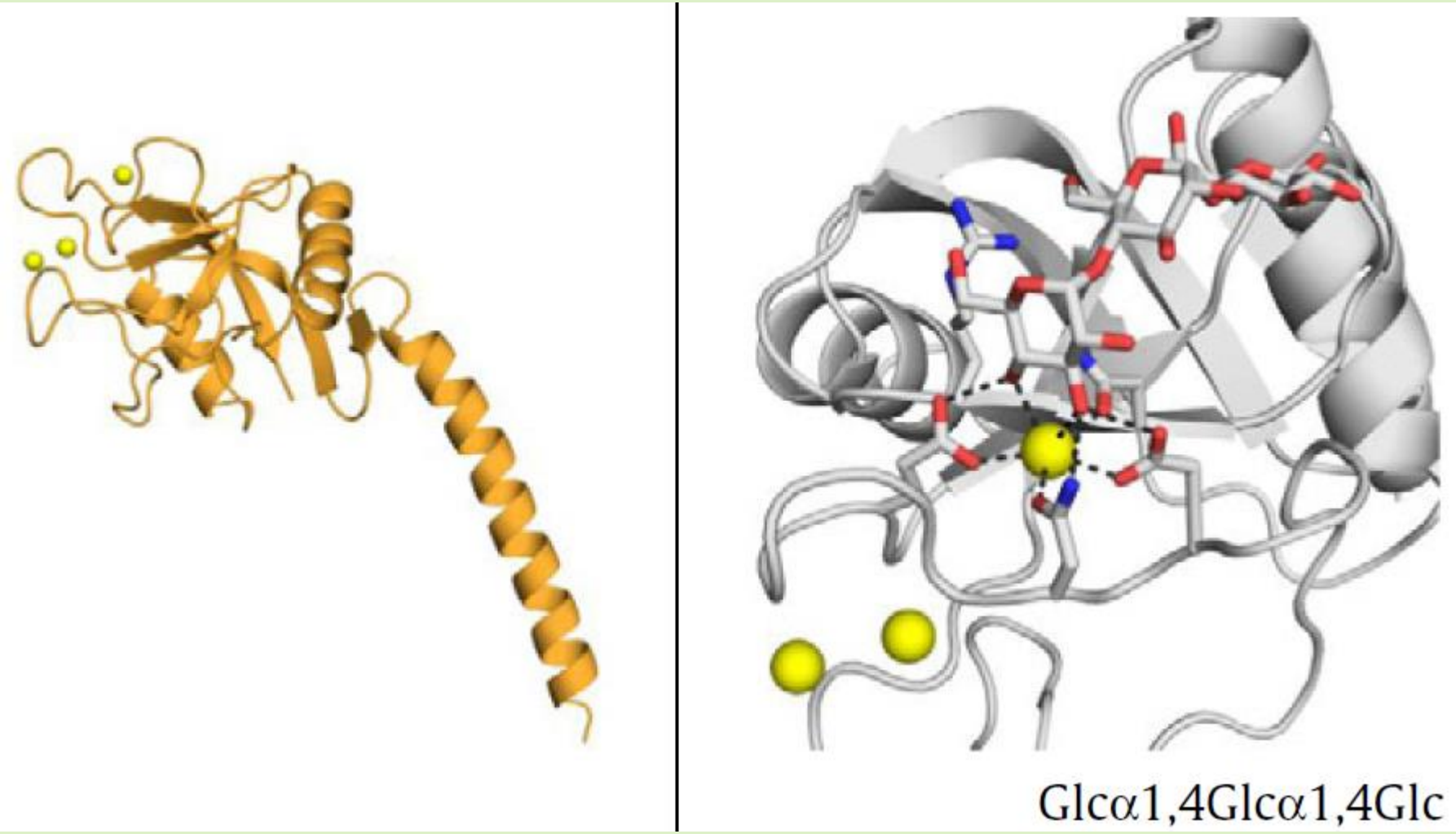
Что узнают лектины млекопитающих?

- ASGPR (свое)
- галектины (свое и чужое-?)
- сиглеки (свое и чужое)
- цитокины (свое)
- коллектины (чужое)
- фиколины (чужое)
- Man-связывающие белки С-типа (чужое)
- DC-SIGN (чужое)

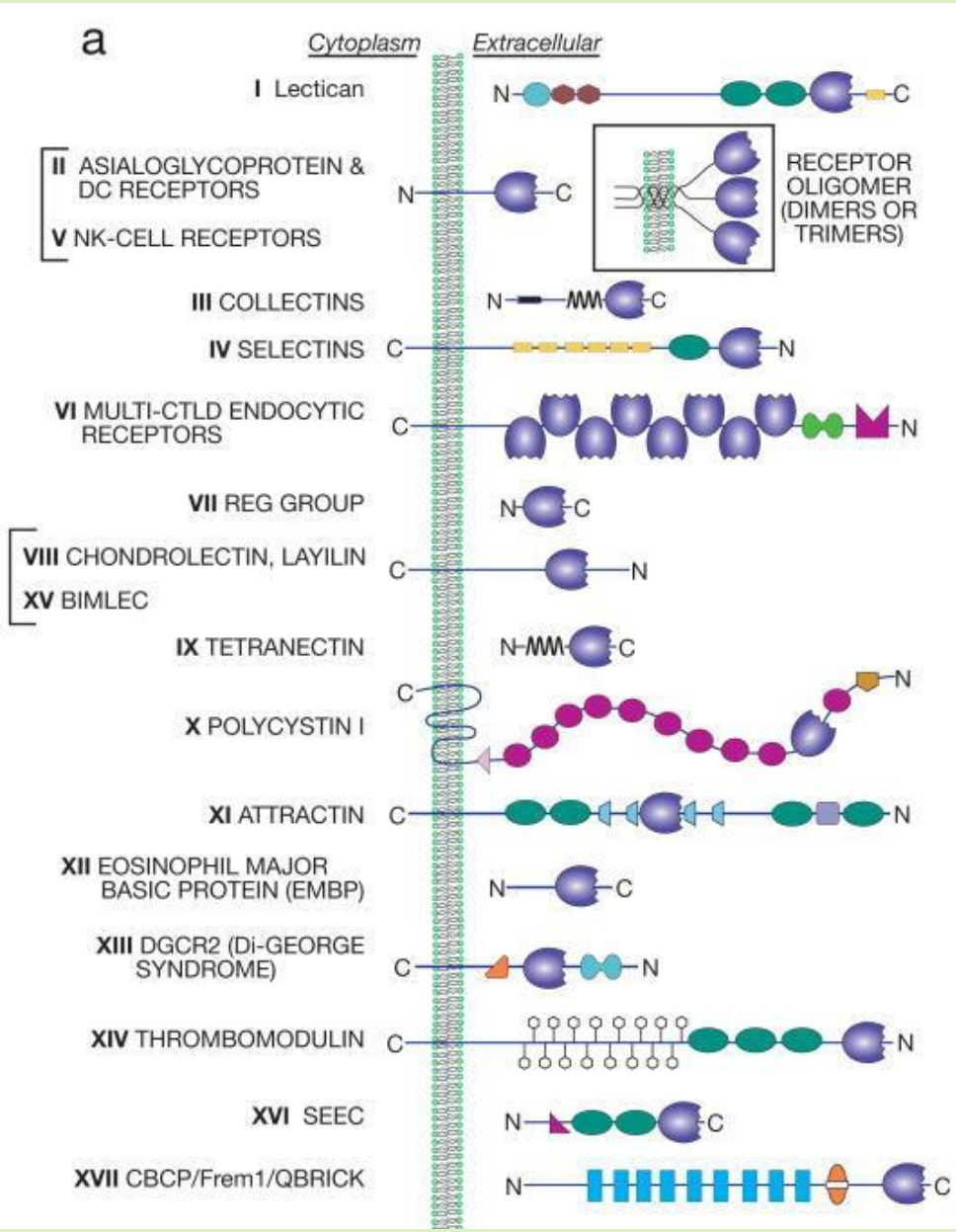
Лектины С-типа

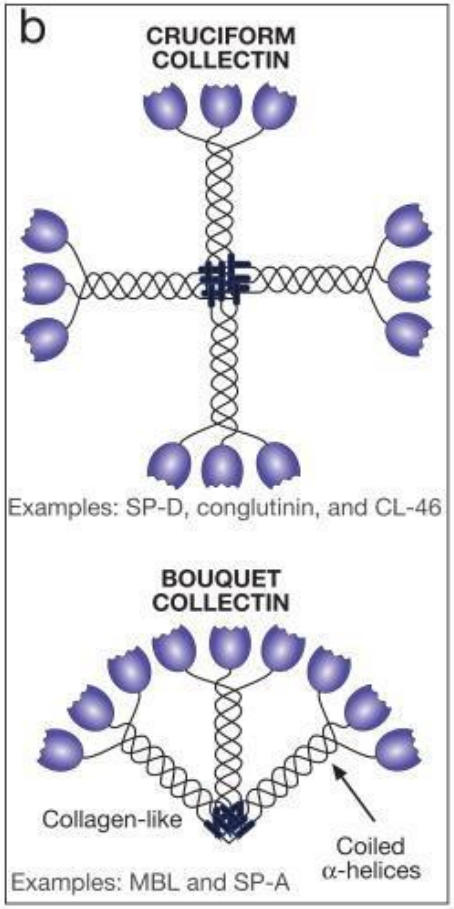
**Селектины, коллектины,
асиалогликопротеиновый рецептор,
рецепторы дендритных клеток,
рецепторы эндоцитоза**

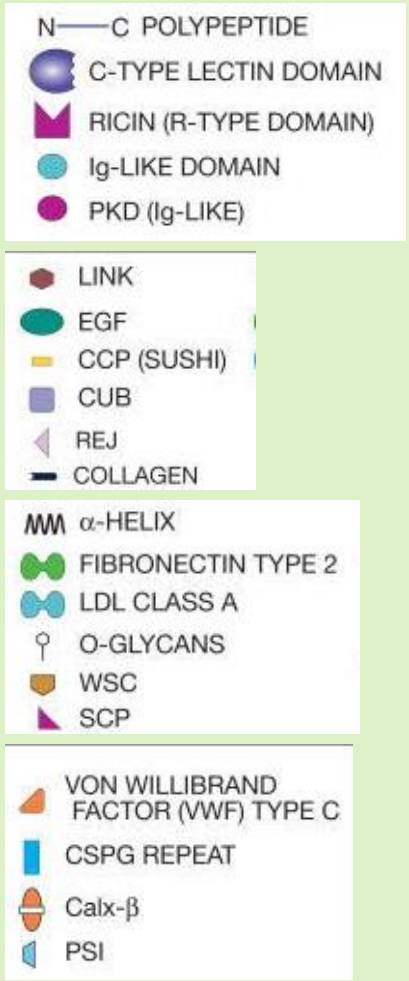
Типичная третичная структура лектинов С-типа: β-сэндвич



17 Групп лектинов С-типа: доменное строение

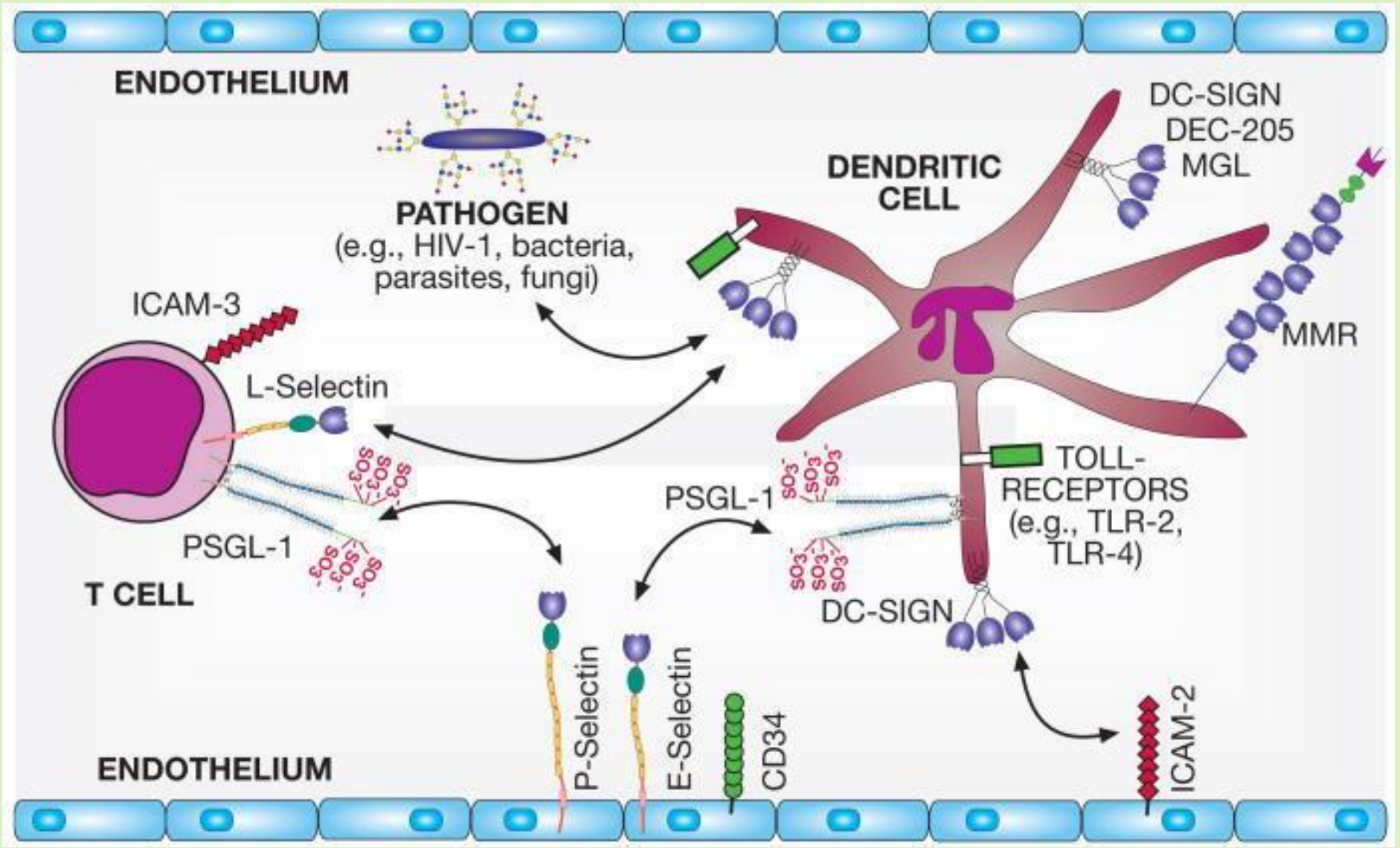




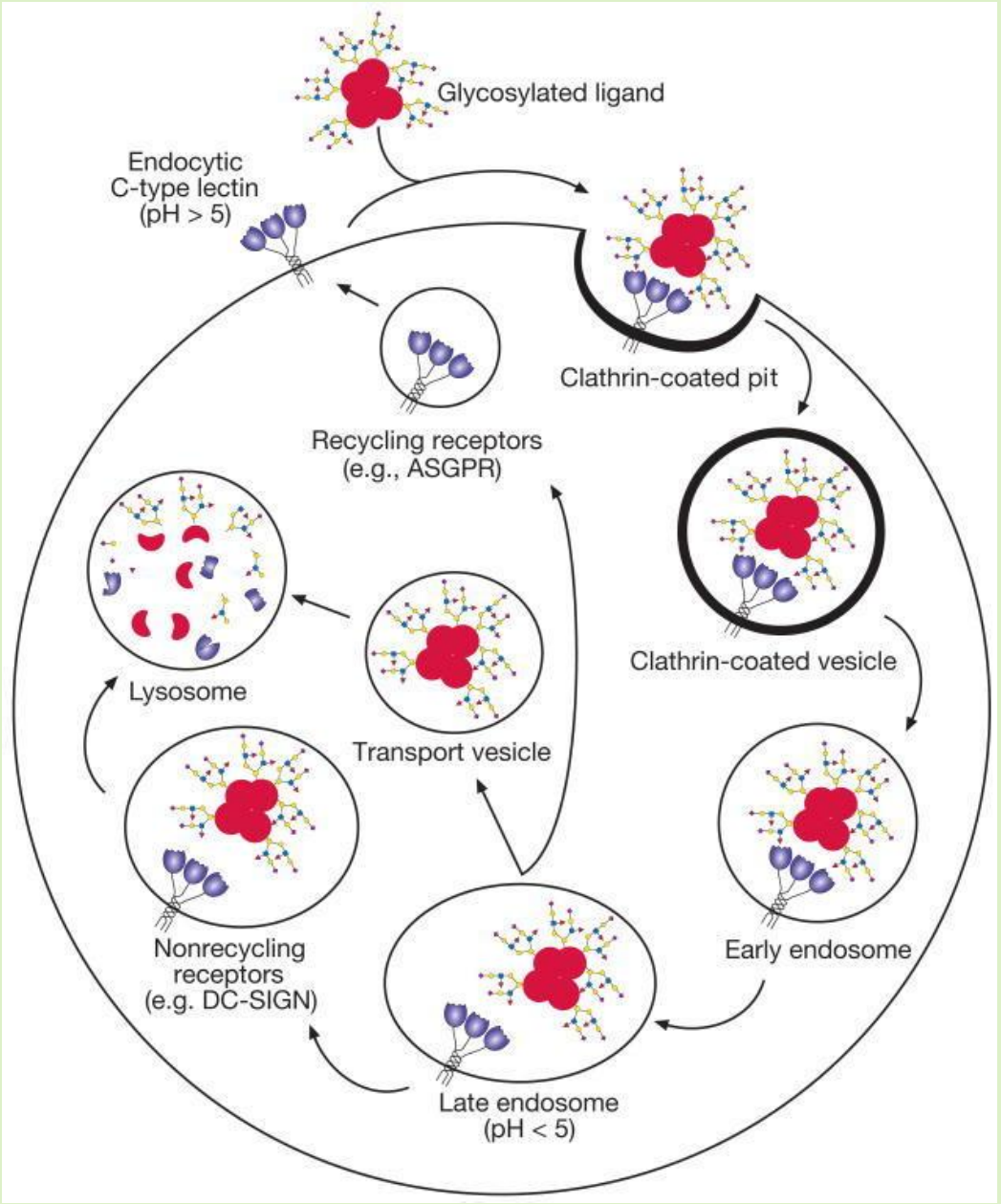


- (DC) Dendritic cell;
- (NK cell) natural killer cell;
- (MBP) mannose-binding protein;
- (SP-A and SP-D) surfactants;
- (CCP) complement control protein;
- (CTLD) C-type lectin domain.

Лектины С-типа: система врожденного иммунитета, распознавание патогенов и клеточная адгезия



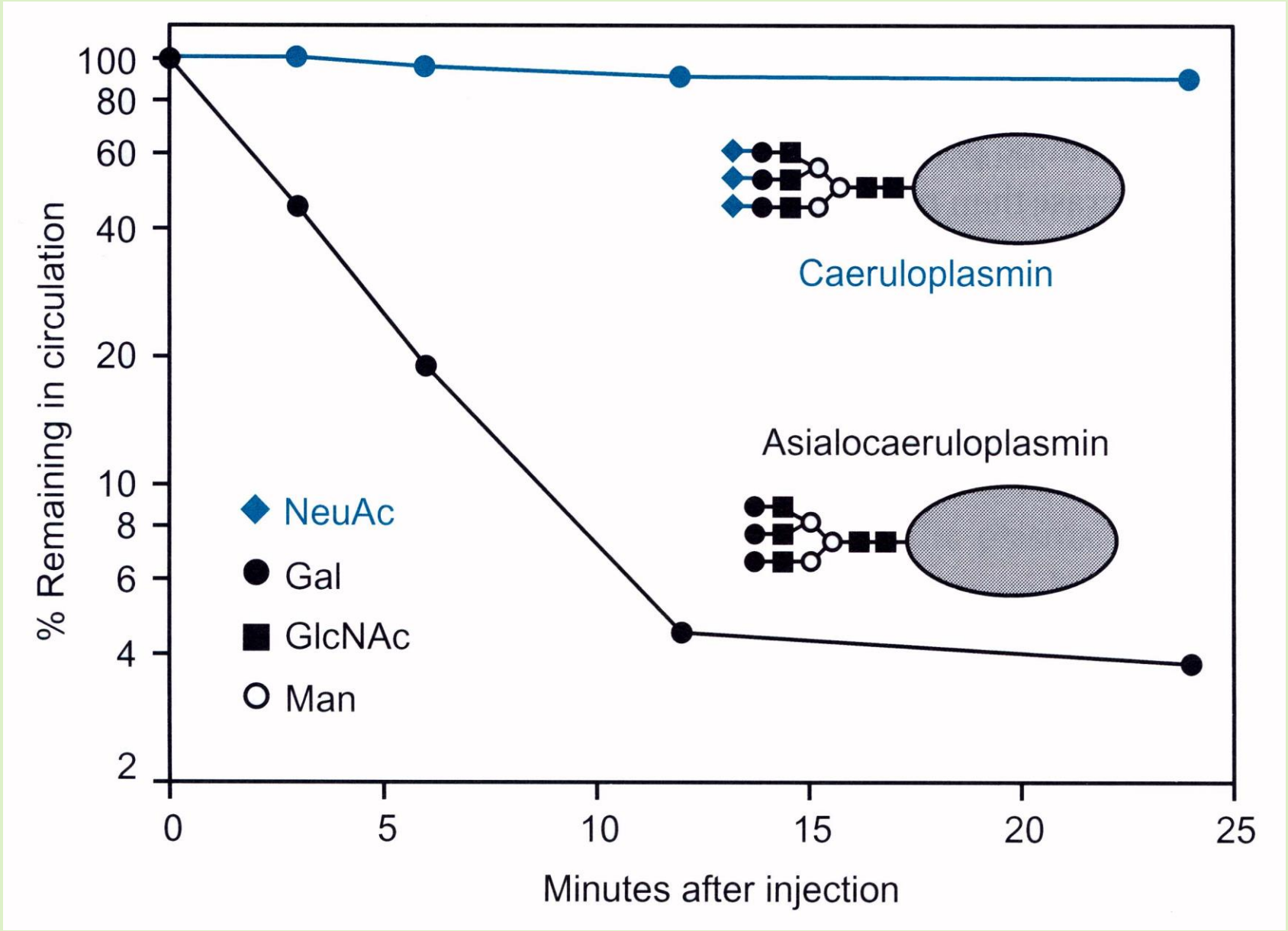
Лектины С-типа как рецепторы эндоцитоза



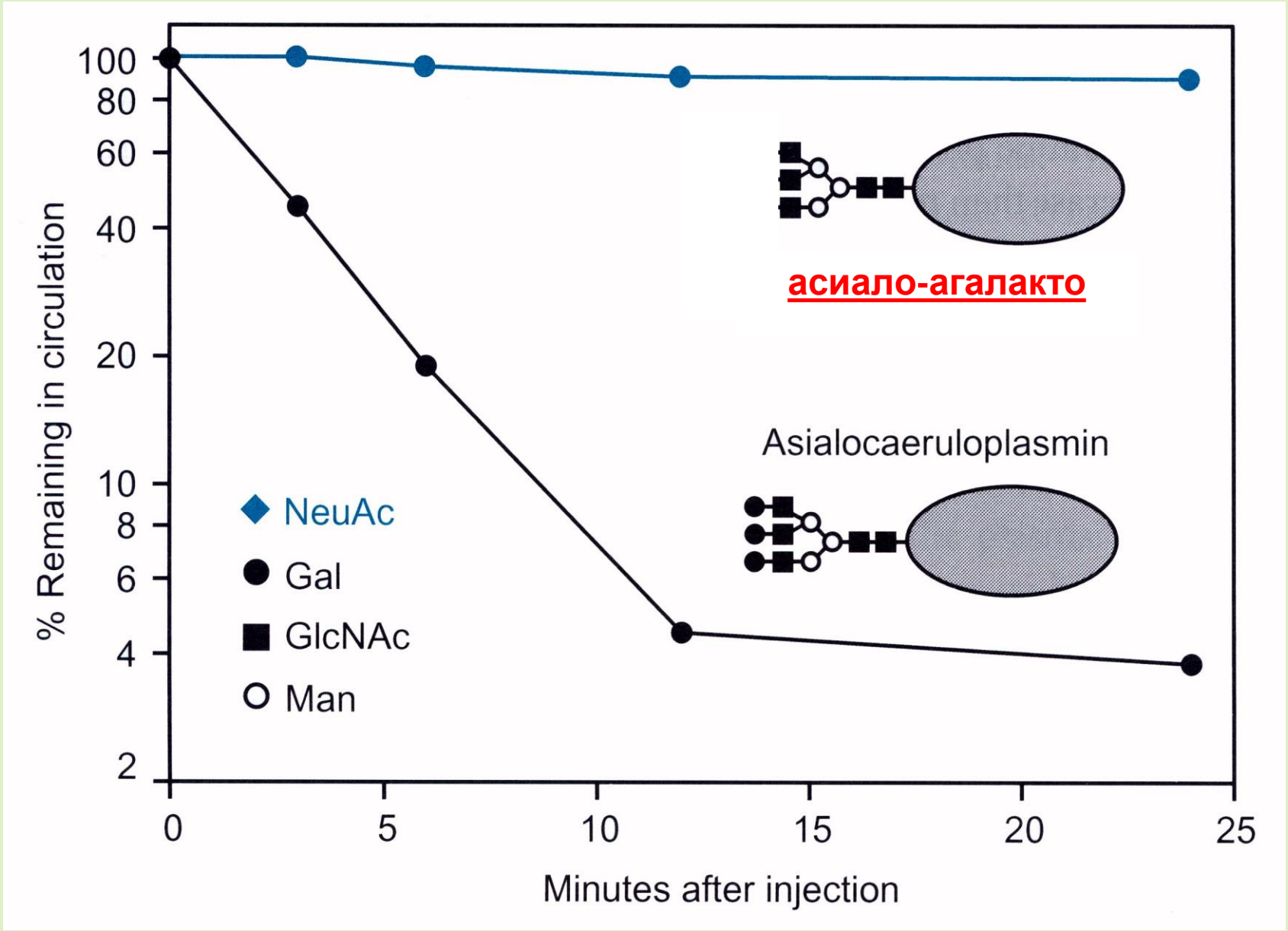
Асиалогликопротеиновый рецептор

Лектин С-типа

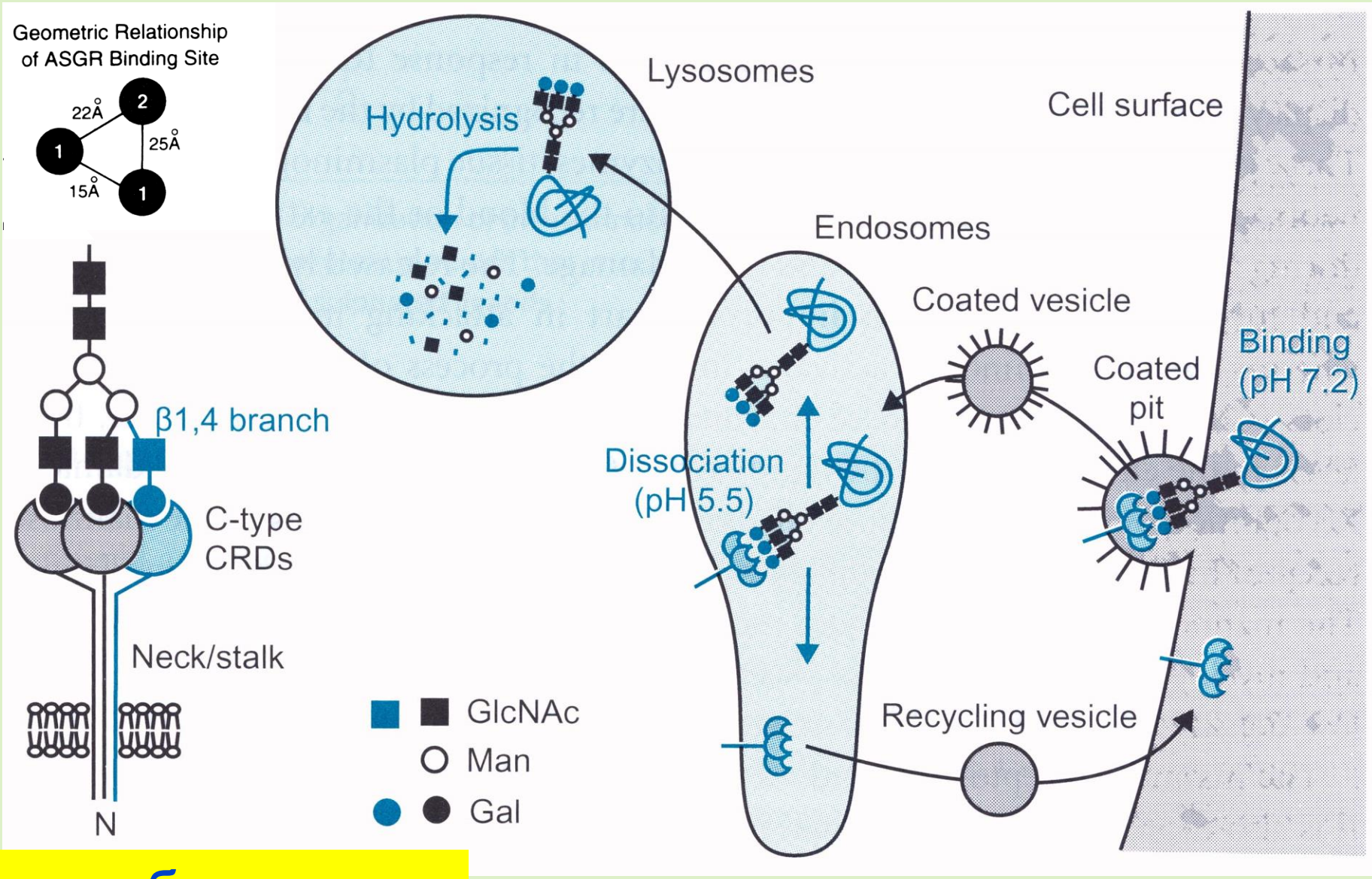
ASGPR – асиалогликопротеиновый рецептор: как открыли первый лектин млекопитающих



ASGPR – асиалогликопротеиновый рецептор: как открыли первый лектин млекопитающих

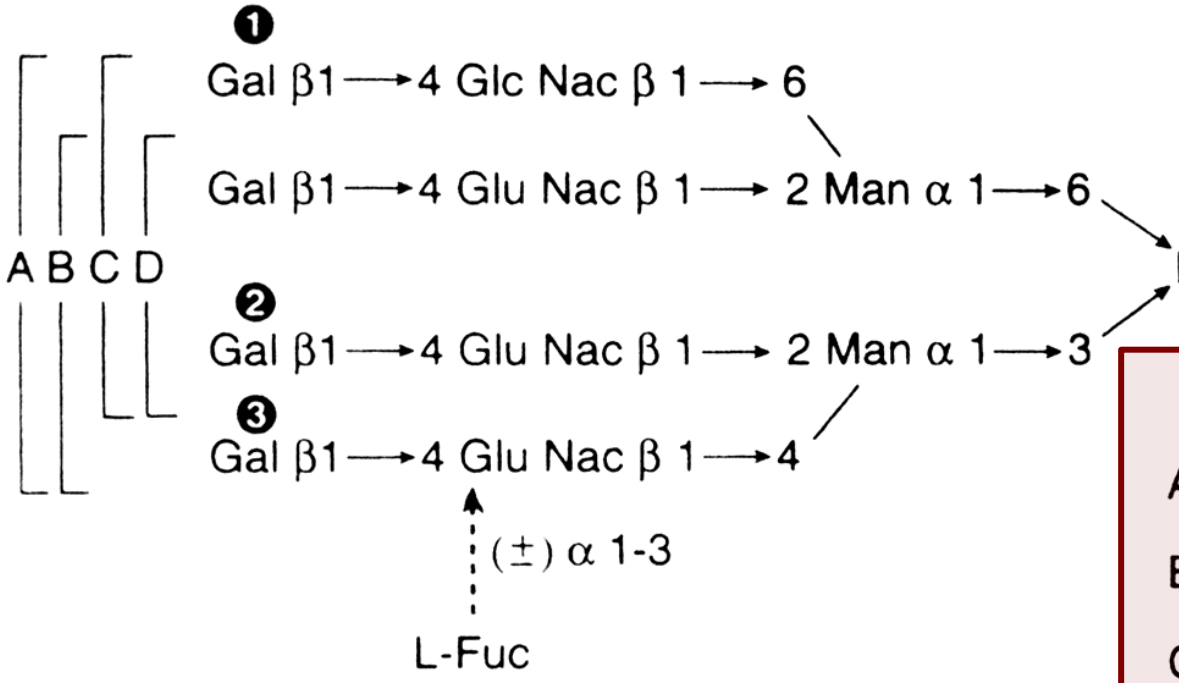


Связывание с ASGPR – первый этап рецептор-опосредованного эндоцитоза



три субъединицы

Требования к точной структуре гликана

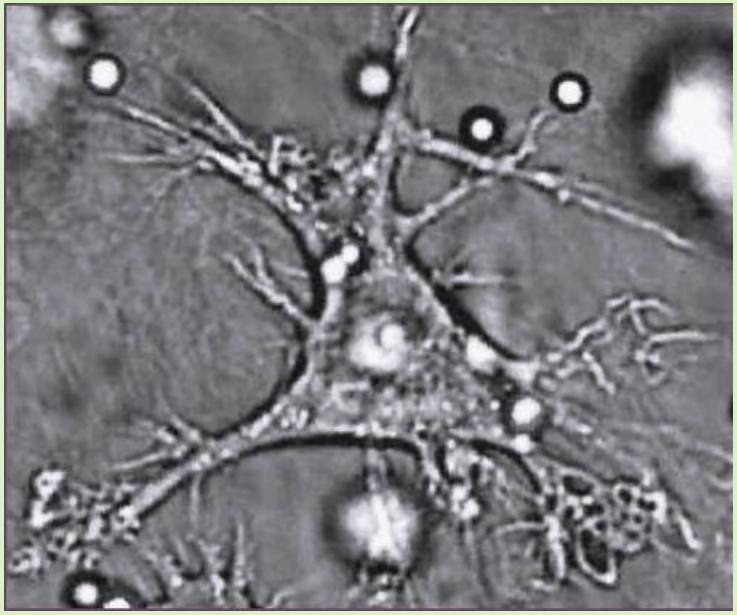


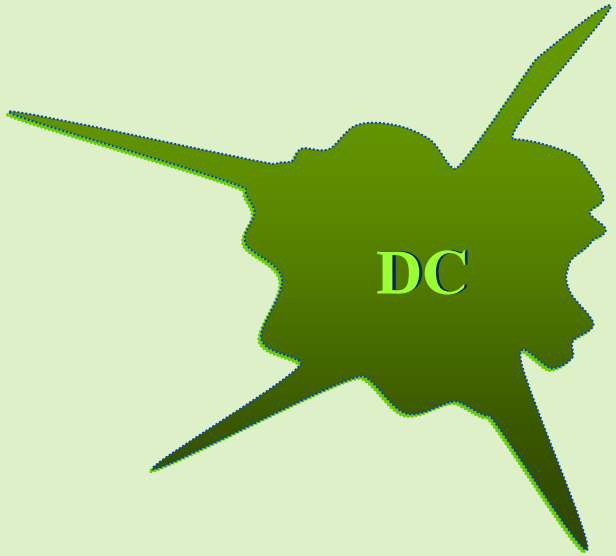
Structure	Affinity (M)
A (ASOR)	2×10^{-9}
B	5.8×10^{-9}
C	150×10^{-9}
D (AsTf)	$49{,}000 \times 10^{-9}$

Лектины на дендритных клетках

Лектины С-типа, галектины, сиглеки

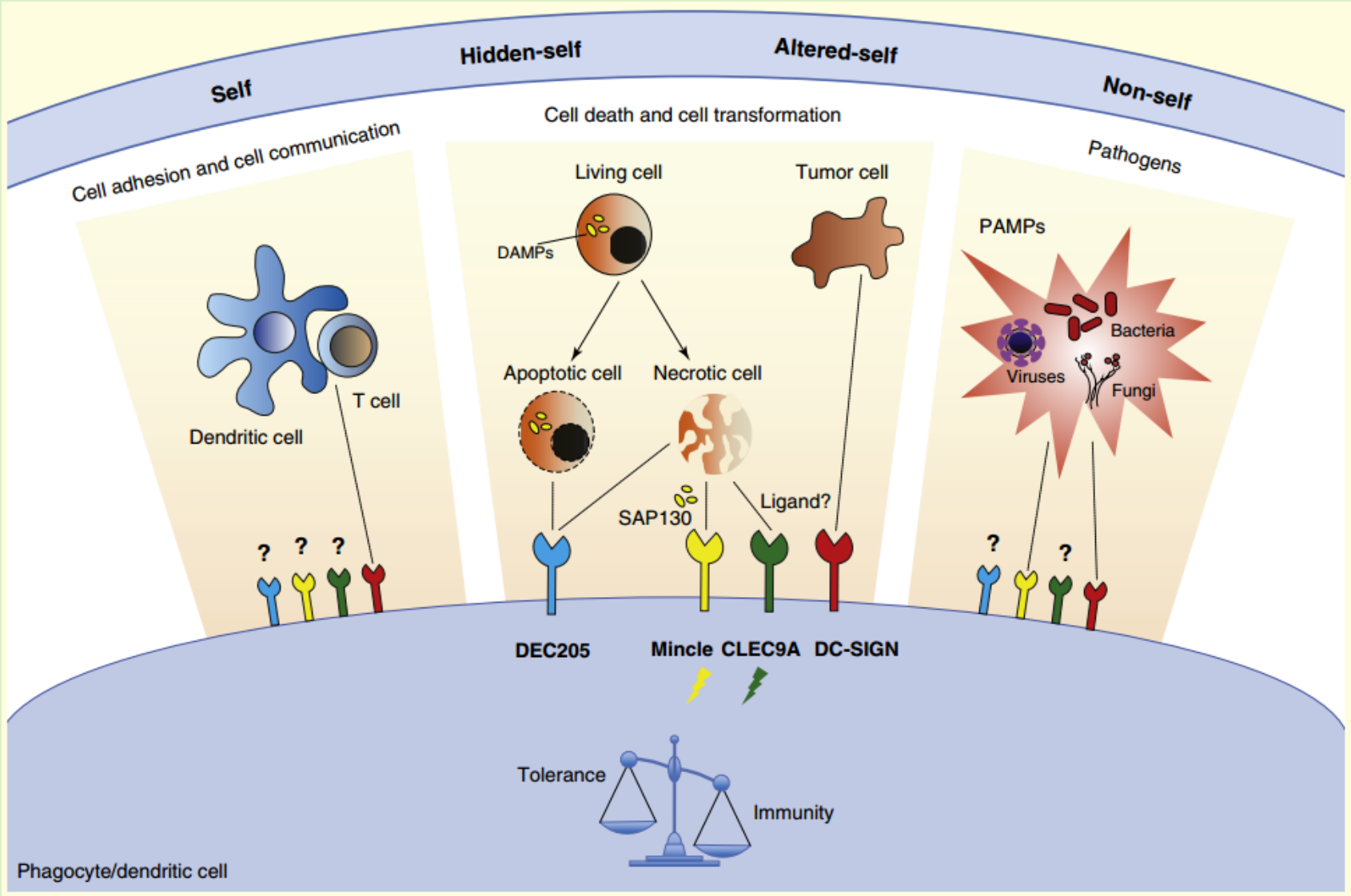
Лектины на дендритных клетках (DC)





- Лектины С-типа:
  - DC-SIGN
  - DEC-205
  - Mf Gal-рецептор
  - Man-рецептор
  - Дектин
  - CLec
  - L-Селектин
- Галектины: -1, -3, -9
- Сиглеки: -3, -7, -9, -10, -15

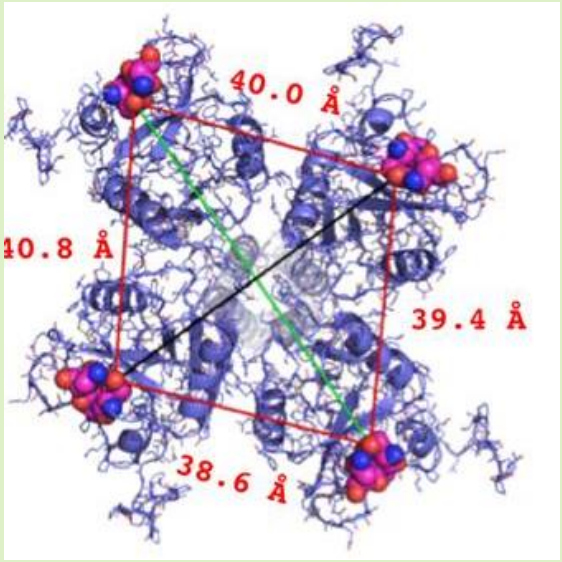
Функции лектинов С-типа на антиген-презентирующих клетках

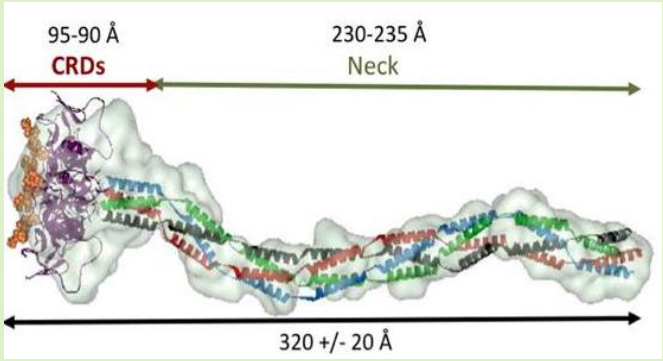


DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin

Лектин С-типа

Интернализация вириона HIV-1 (связывание gp120)

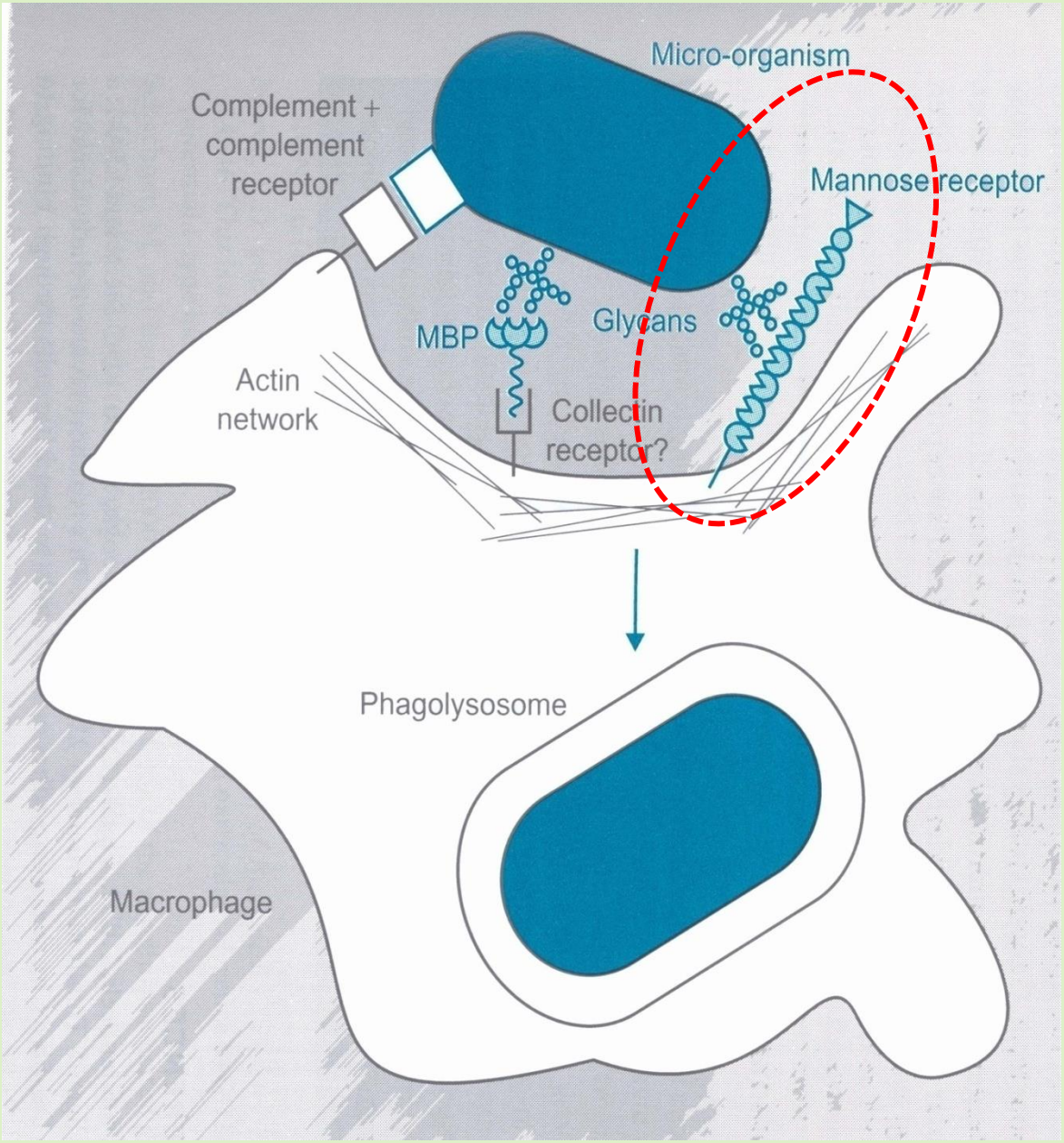




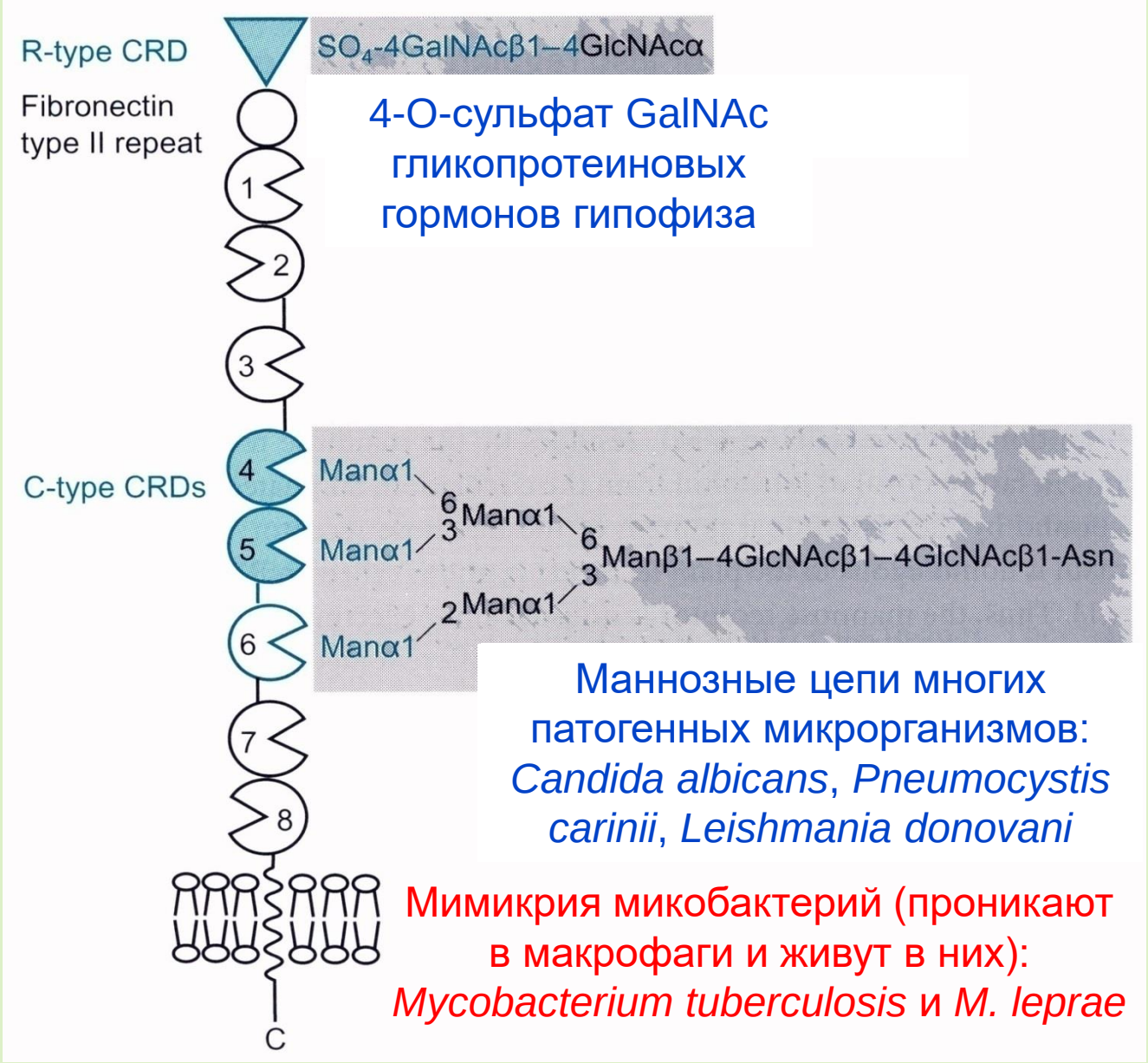
Лектин DC	Специфичность
DC-SIGN	$[GalNAc\beta1\text{-}4(Fuc\alpha1\text{-}3)GlcNAc]_2$ >Le^x-Le^x ≈ Man_9-$GlcNAc_2$ > Man_8-$GlcNAc_2$ > Le^b ≈ Le^y > Le^a ≈ Man_7-$GlcNAc_2$ ≈ $GalNAc\beta1\text{-}4(Fuc\alpha1\text{-}3)GlcNAc$ ≈ Man_6-$GlcNAc_2$ ≈ Le^x ≈ $SuLe^x$
DEC-205	$[GlcNAc\beta1\text{-}4]_3$ > Le^Y-Le^X-Le^X ≈ $KDN\alpha2\text{-}3Gal\beta1\text{-}4GlcNAc\beta$ ≈ $Gal\alpha1\text{-}3Gal\beta1\text{-}4Glc$ ≈ $GlcA\beta1\text{-}6Gal\beta$ ≈ $GlcNAc\beta$
Macrophage Gal-binding lectin (MGL)	$GalNAc\alpha1\text{-}3Gal$; LacdiNAc; GD2; A (type 2); $GalNAc\beta1\text{-}4(Fuc\alpha1\text{-}3)GlcNAc$; GM2; $GlcNAc\beta1\text{-}6GalNAc\alpha$; $GalNAc\alpha1\text{-}3GalNAc\alpha$; $GalNAc\alpha$ (Tn); 6SuGalNAcα; SiaTn
MBP	Mannans, branched Man_{14}
DCAL	Man-terminated glycans, LacdiNAc, LNnT
Dectin-1	$\beta1\text{-}3Glc_n$ (n>6); Chito-OS, su-OS, $GlcNAc\beta1\text{-}3GalNAc\alpha$, Sia_3
CLec-1	3',6',6 Su_3LacNAc > $Gal\beta1\text{-}3GalNAc\beta1\text{-}3GalNAc\alpha1\text{-}4Lac$; $LacNAc2Man\alpha3(Man\alpha6)Man\beta4GlcNAc4GlcNAc$; $Glc\beta$
CLec-2	su-OS, Sia_3, $Glc\beta$
CLec-6	Globo-H ~ 3',6',6Su_3LacNAc > Man_3
CLec-15	$Fuc\alpha1\text{-}4GlcNAc$
CLec-18	$Glc\beta$, $Neu5Ac\alpha2\text{-}6$, $Fuc\alpha1\text{-}2Gal$
galectins	$(Gal\beta1\text{-}4GlcNAc)_n$

Маннозный рецептор макрофагов (MMR, CD206)

Лектин С-типа



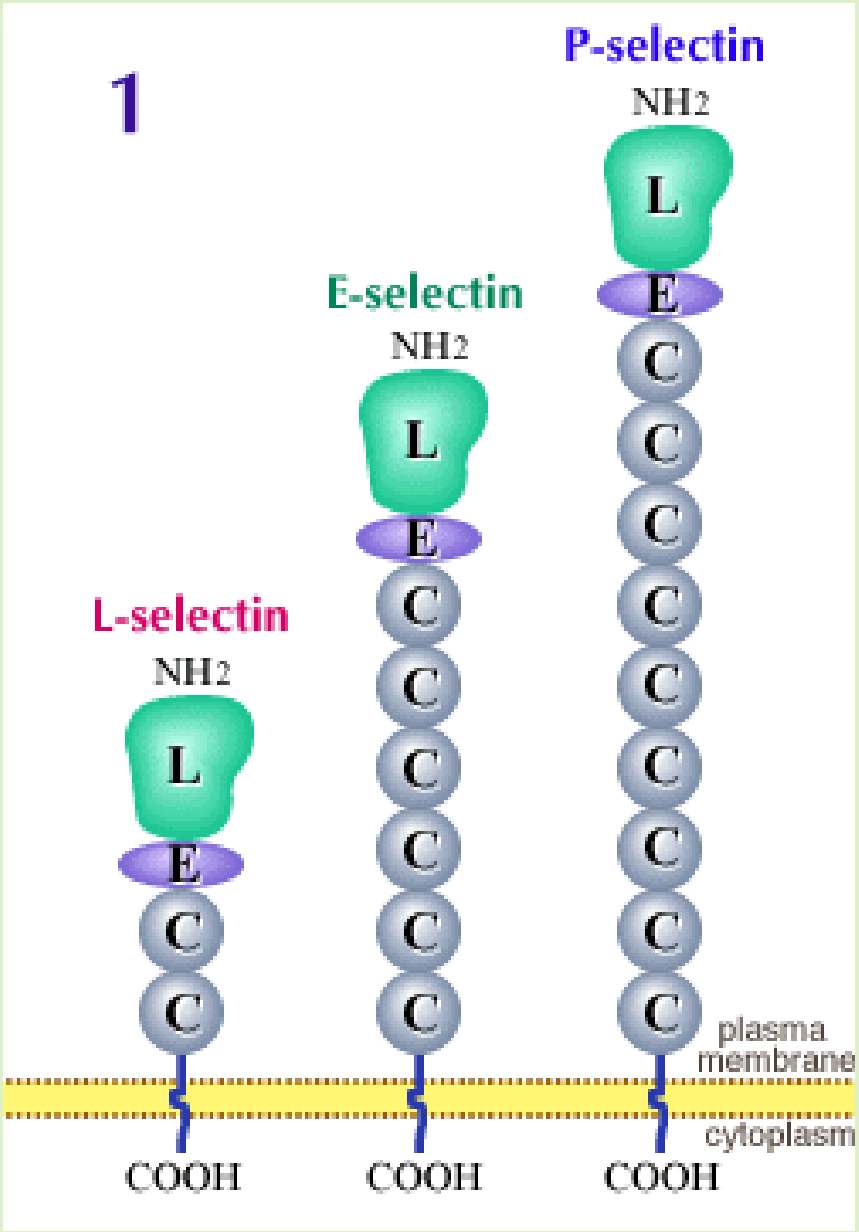
Маннозный рецептор макрофагов (MMR), DC и лимфоцитов: два разных сайта связывания



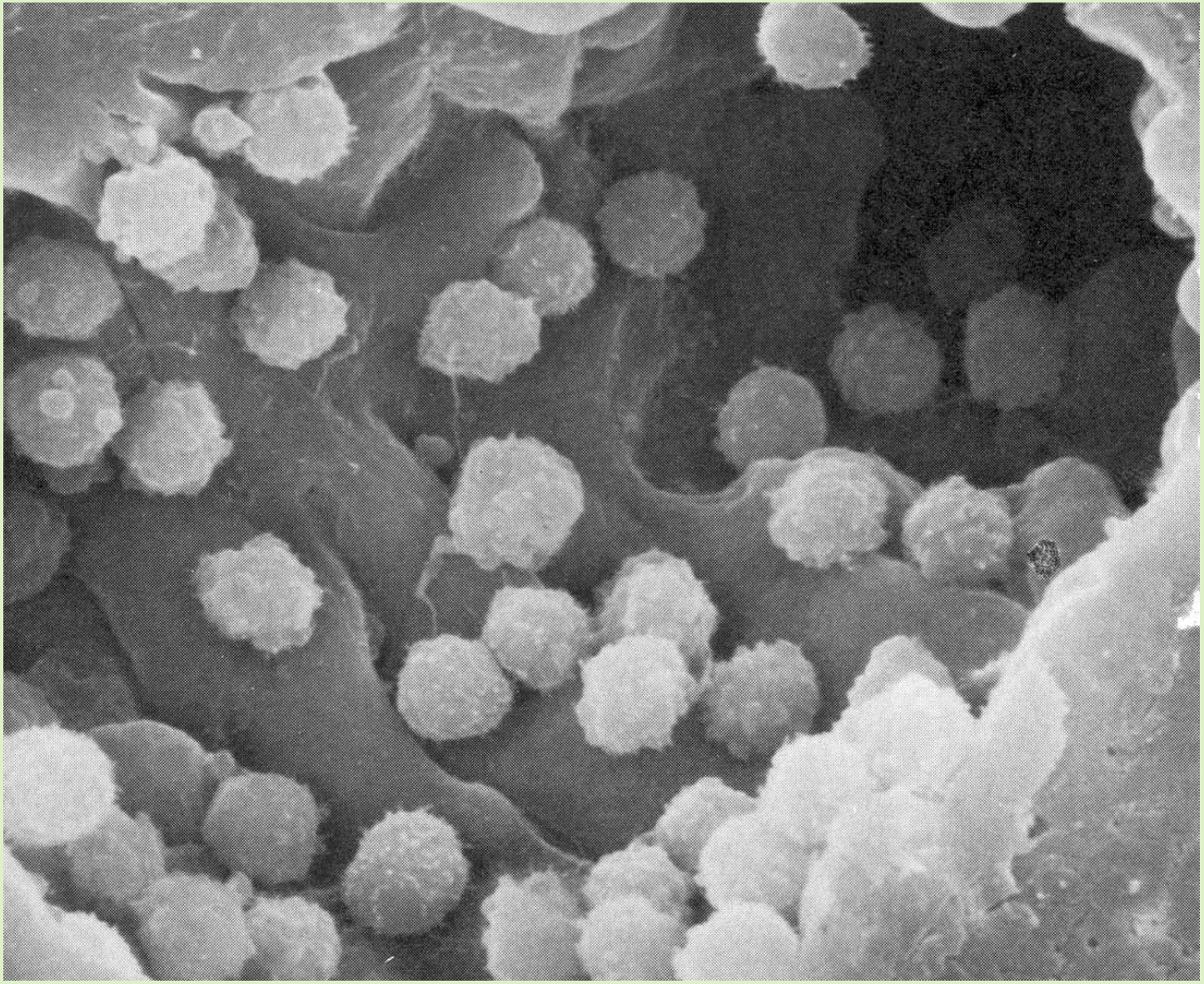
Селектины

Лектины С-типа

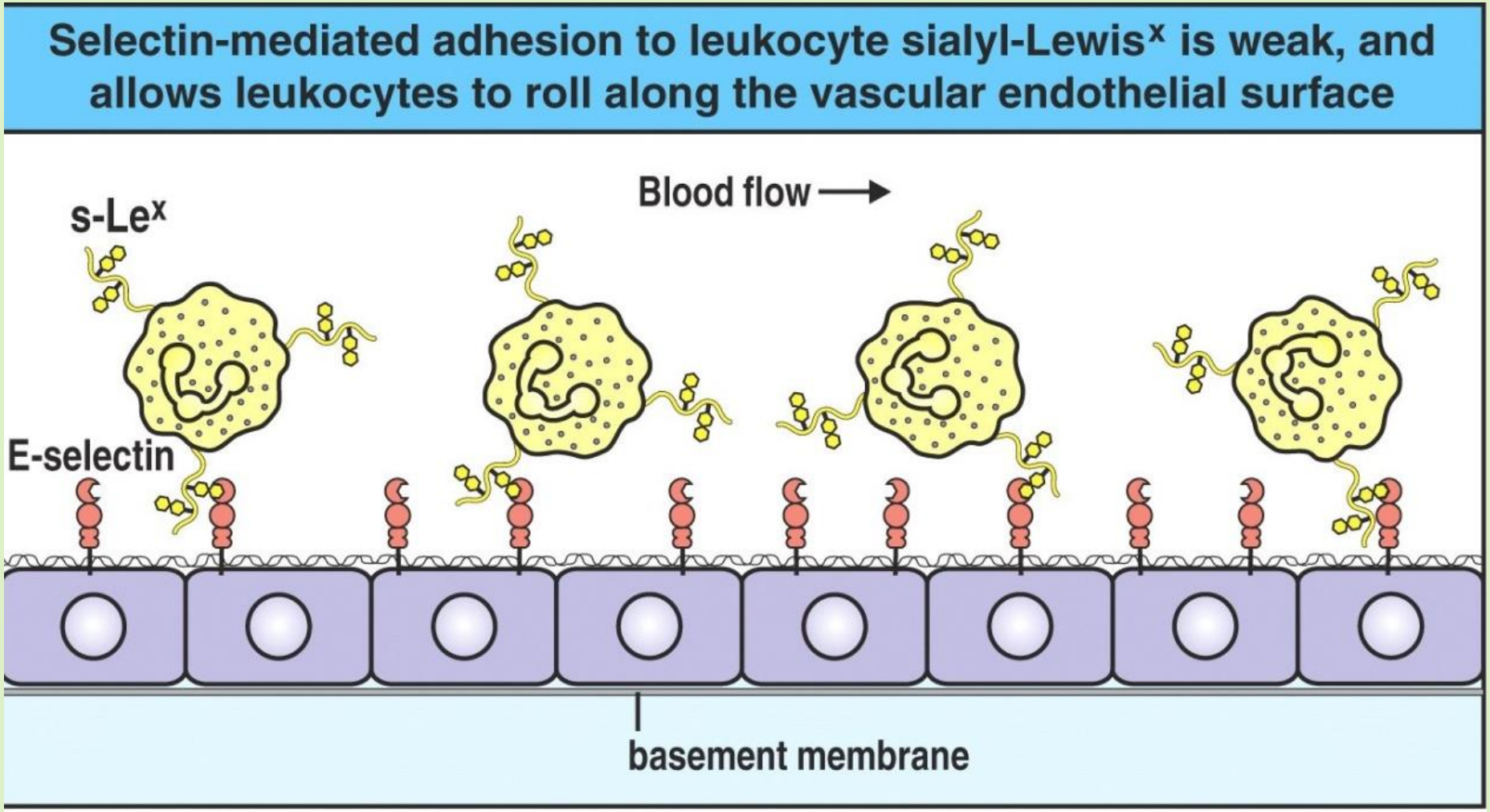
P-, E- и L-Селектины



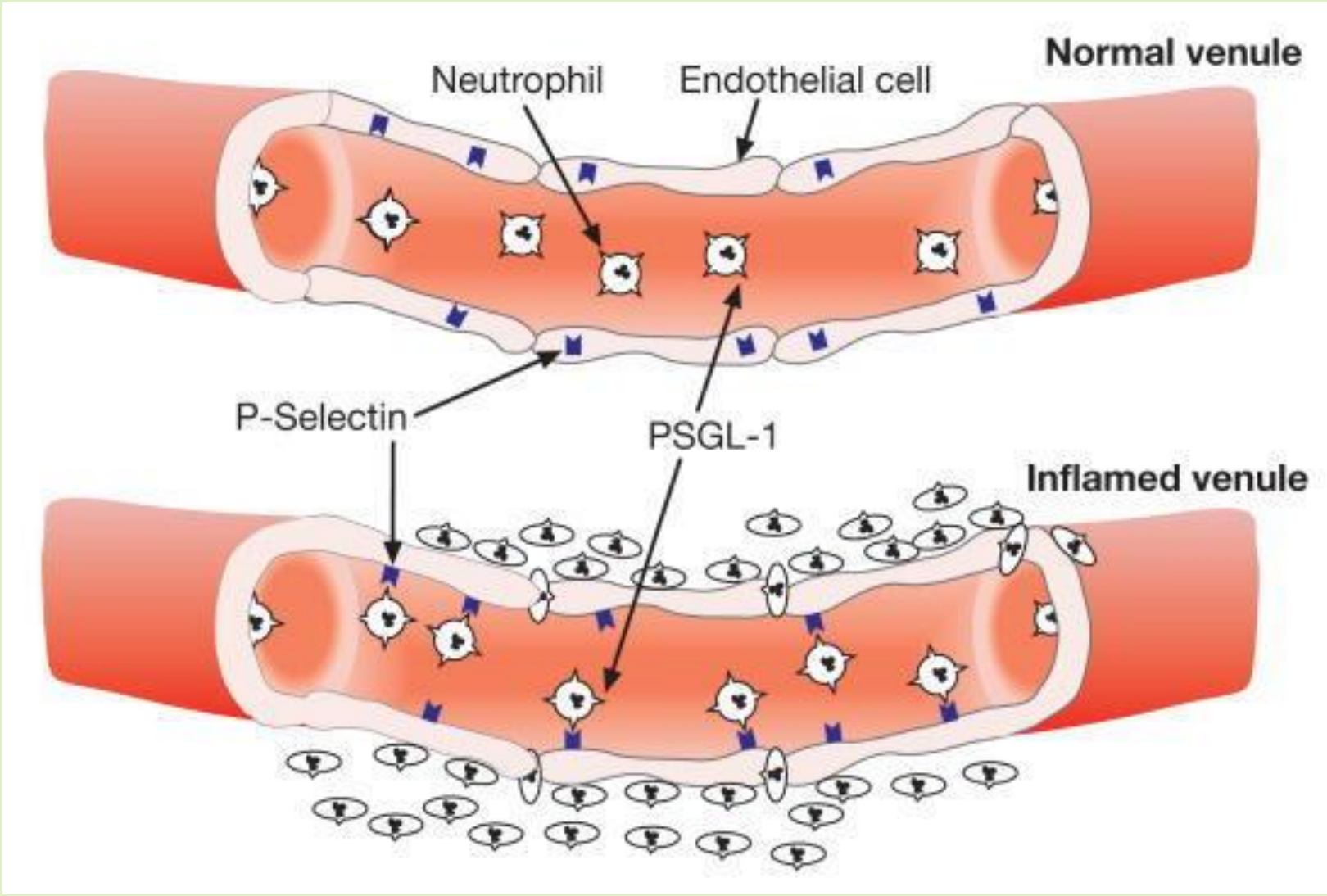
Роллинг лейкоцитов по кровеносным сосудам



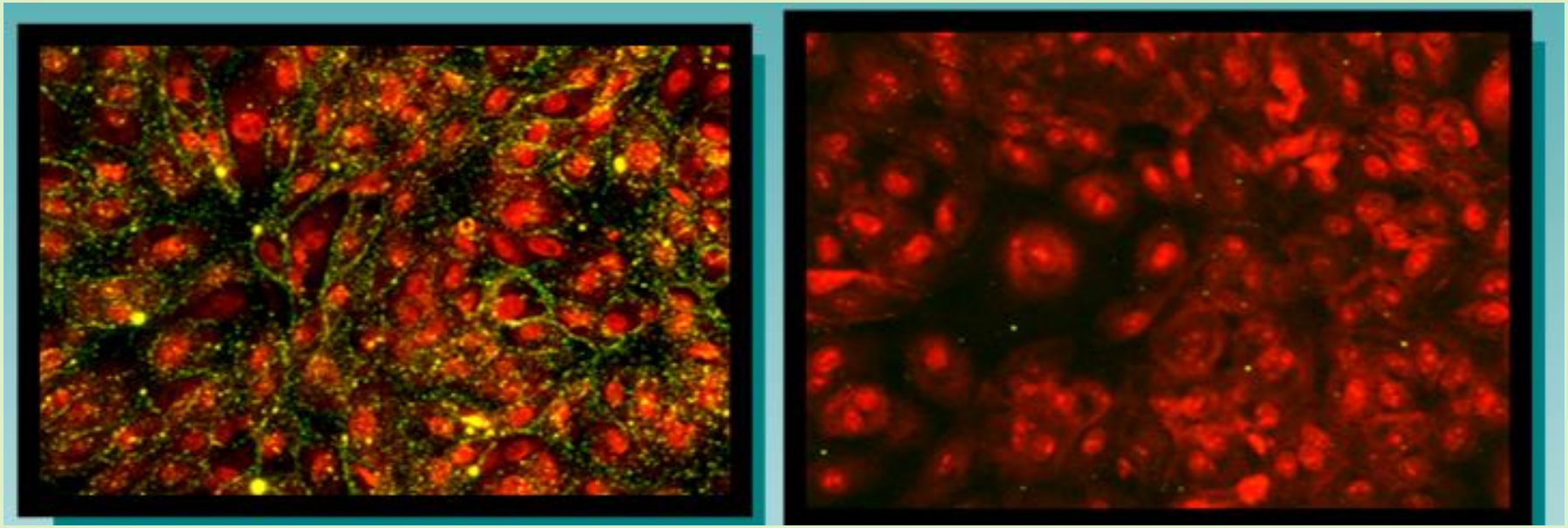
Роллинг лейкоцитов, опосредованный E- и P-селектинами



Мобилизация лейкоцитов к сайту воспаления



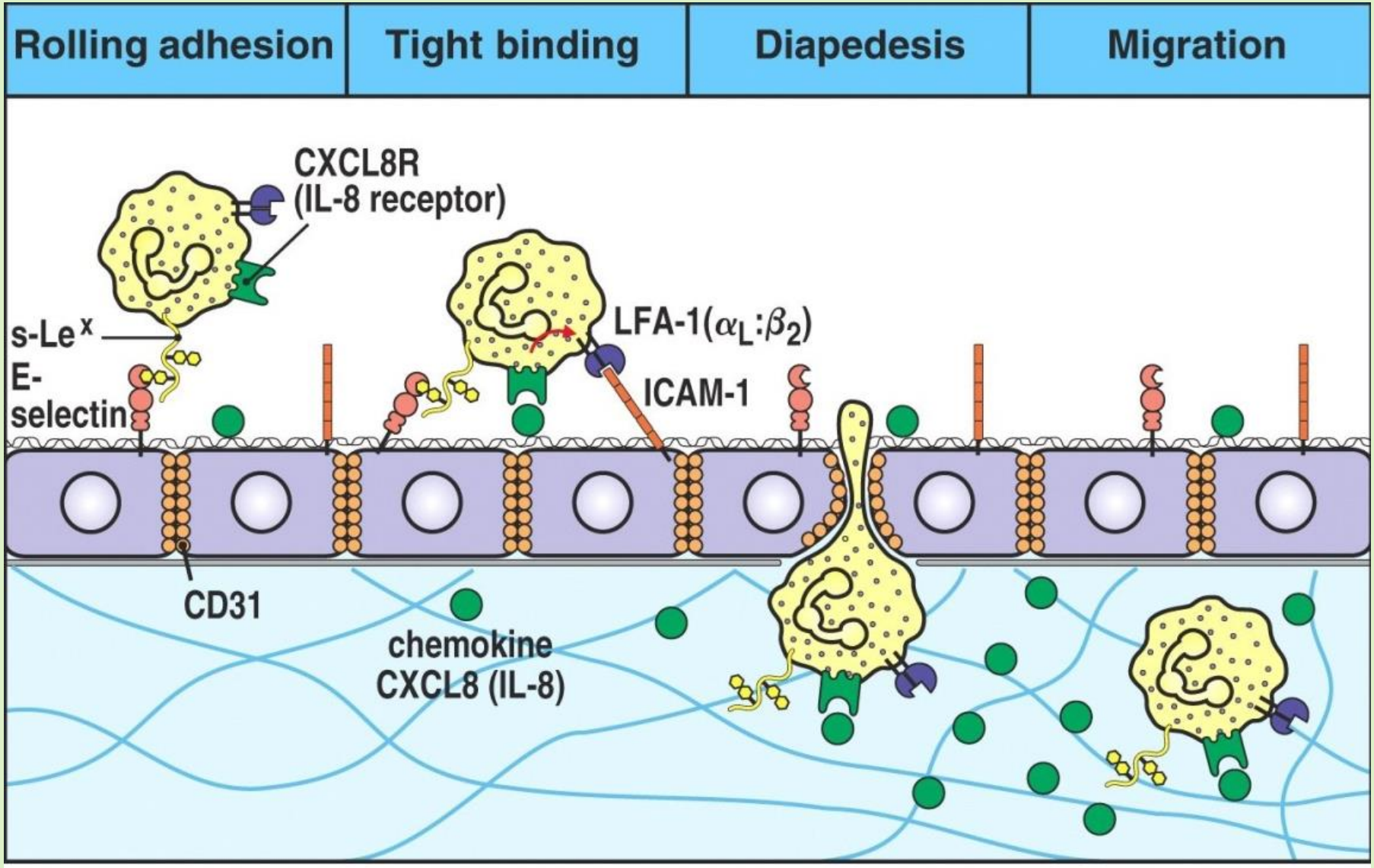
E-Селектин экспрессируется на *активированных* эндотелиальных клетках



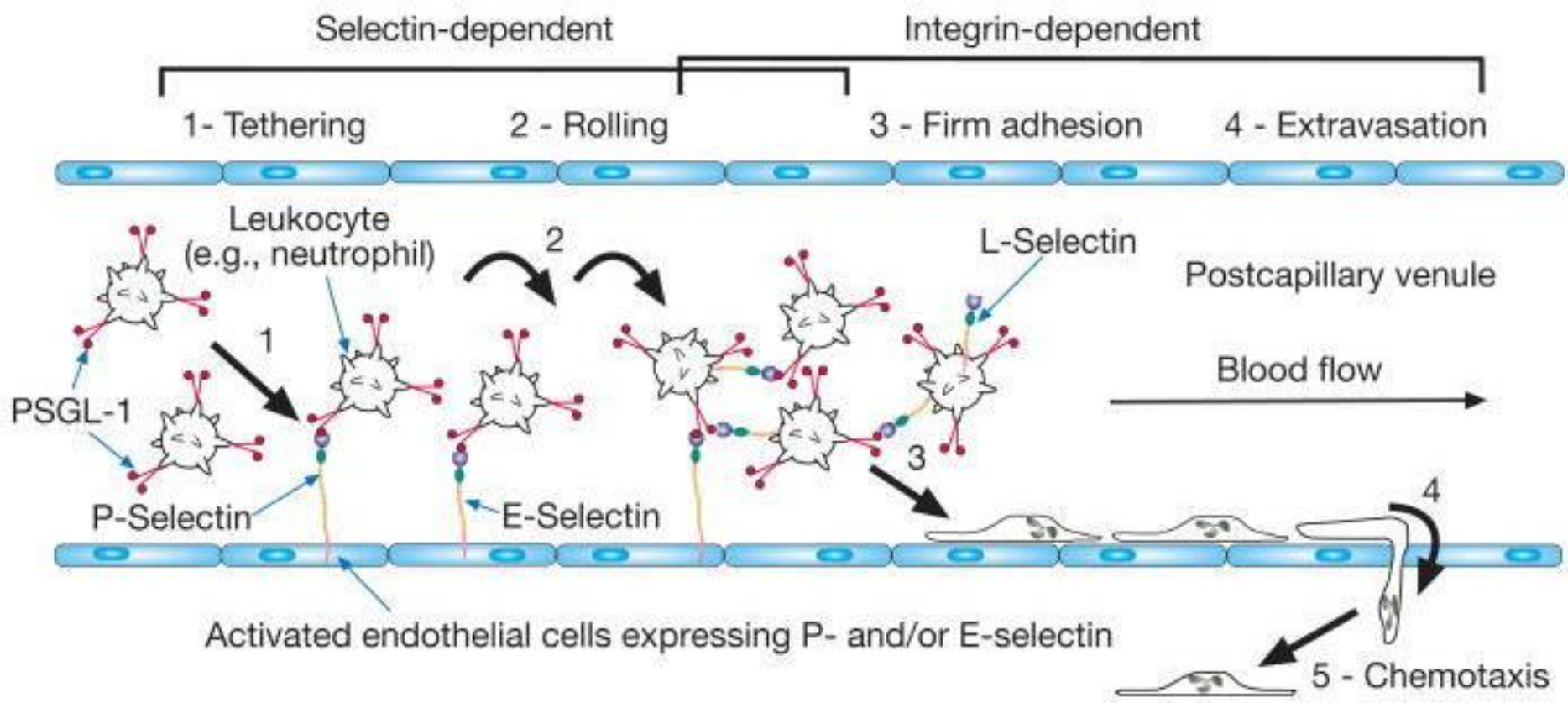
Активированные клетки
+ SiaLe^x-флуоресцентная частица

То же с неактивированными клетками

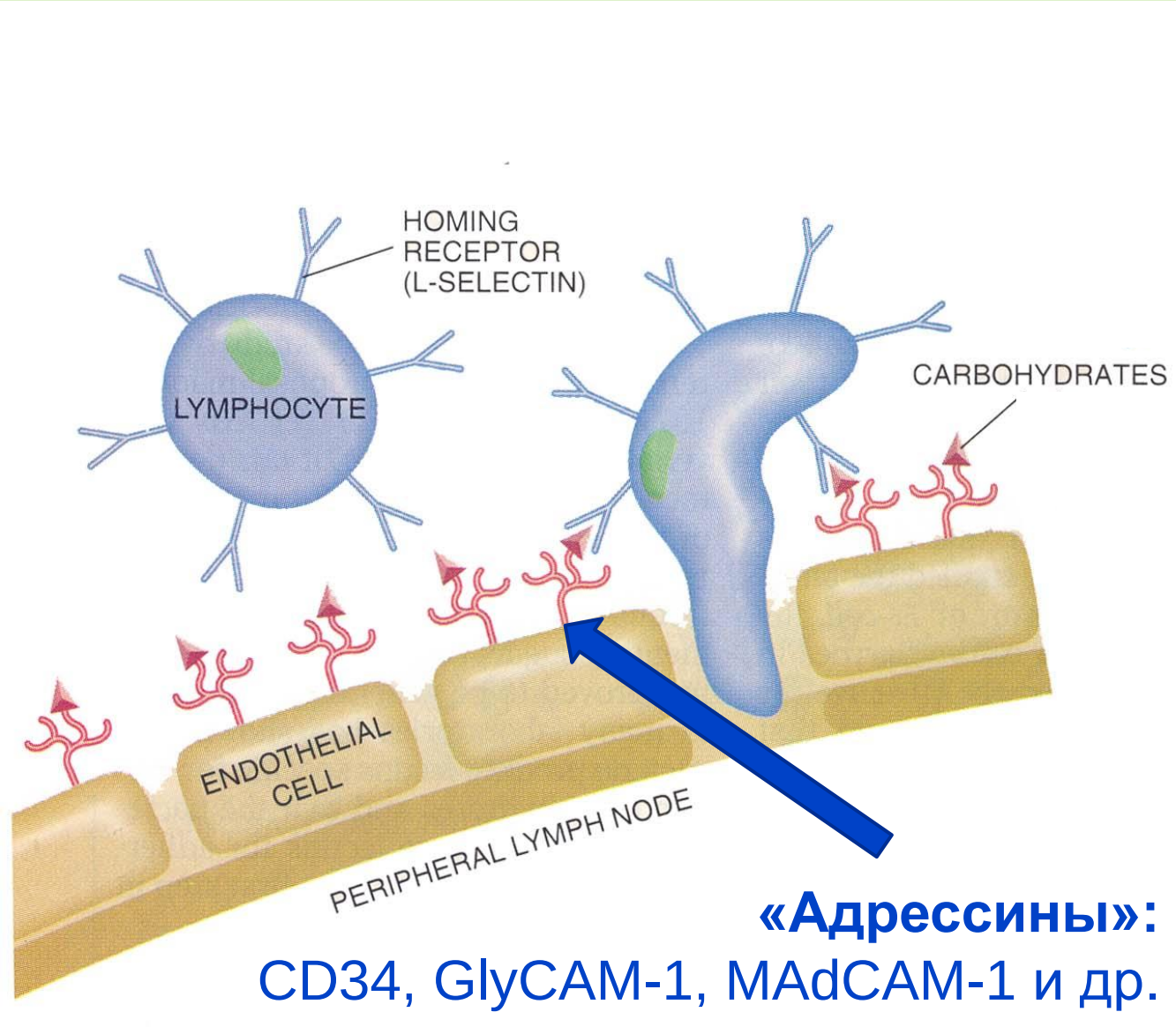
Прикрепление лейкоцитов к эндотелиальным клеткам и экстравазация из сосуда в ткань



Мобилизация лейкоцитов к сайту воспаления: этапы



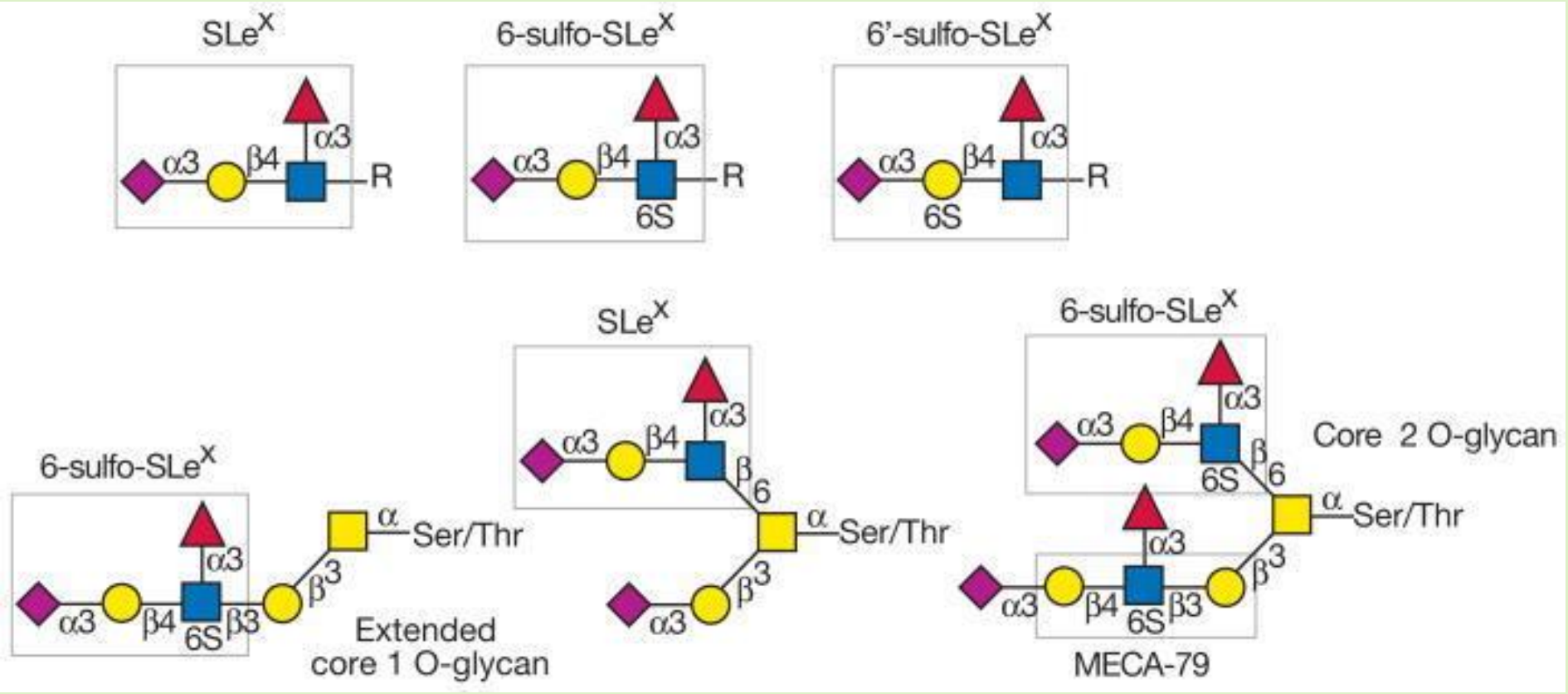
L-Селектин-опосредованный роллинг: возвращение в лимфоидную ткань («хоминг»)



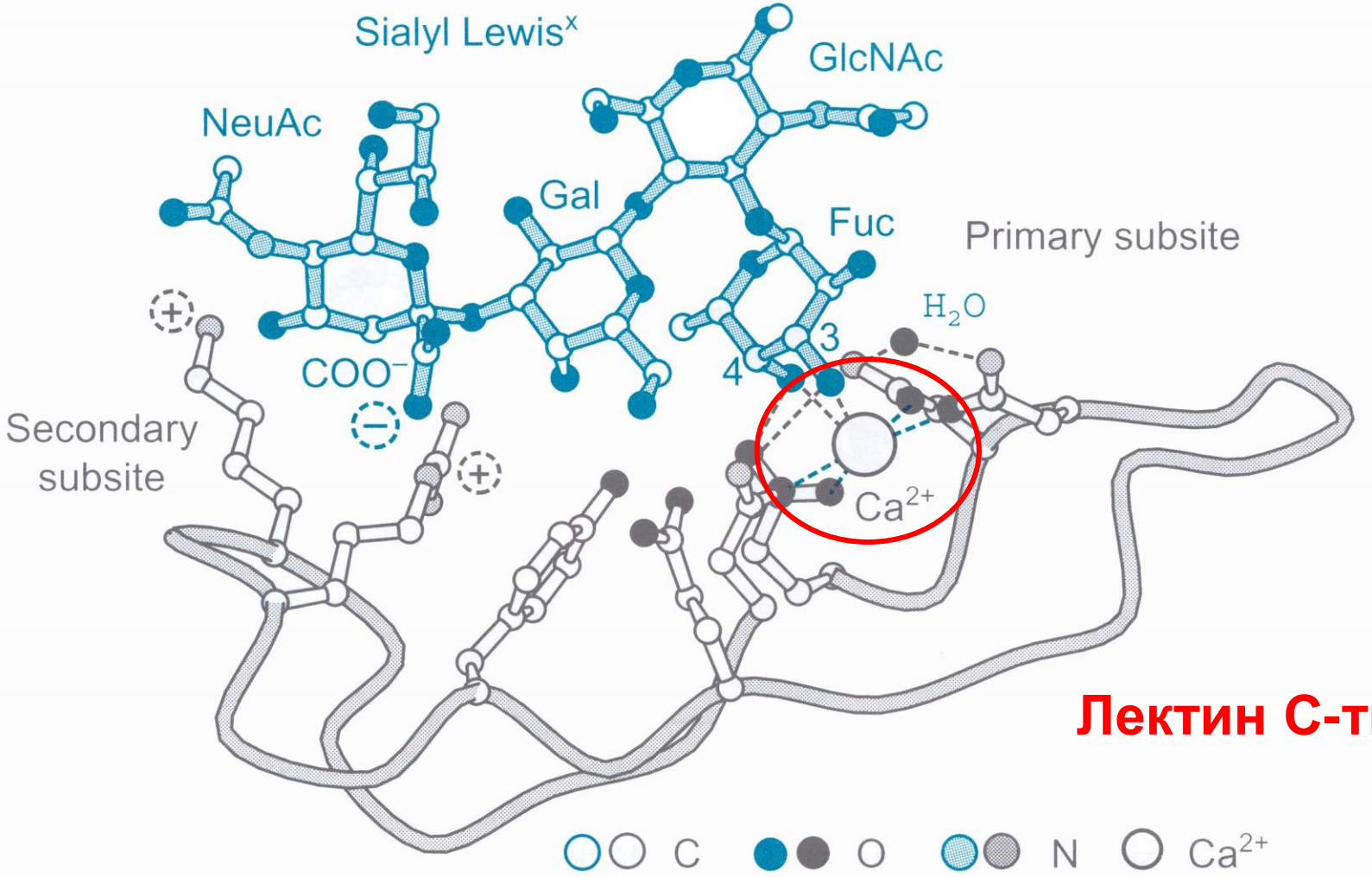
«Адрессины»:
CD34, GlyCAM-1, MAdCAM-1 и др.

Гликаны – медиаторы селектин-опосредованной адгезии клеток

SiaLe^x – общий лиганд всех селектинов

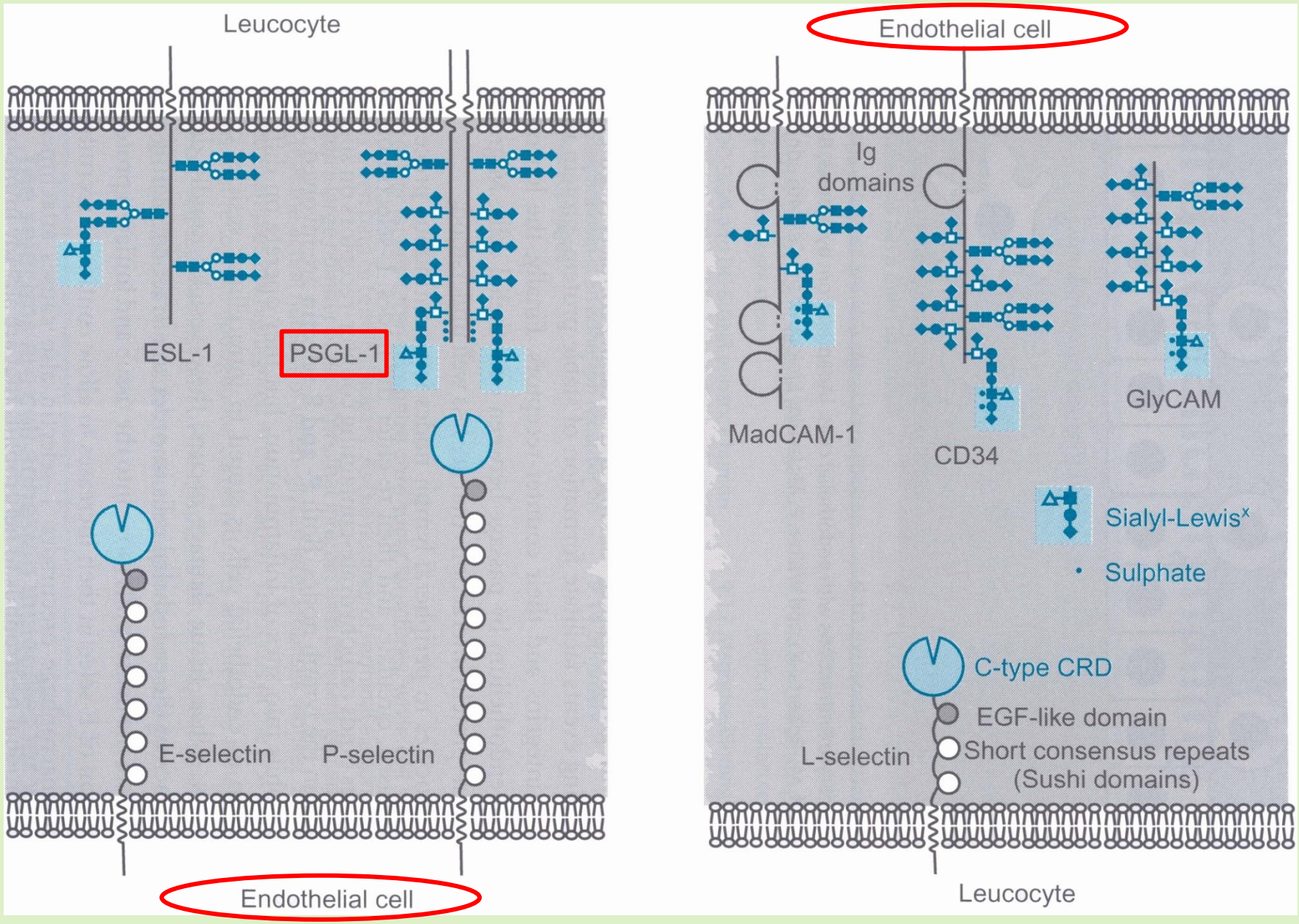


Комплекс E-селектина с SiaLe^x

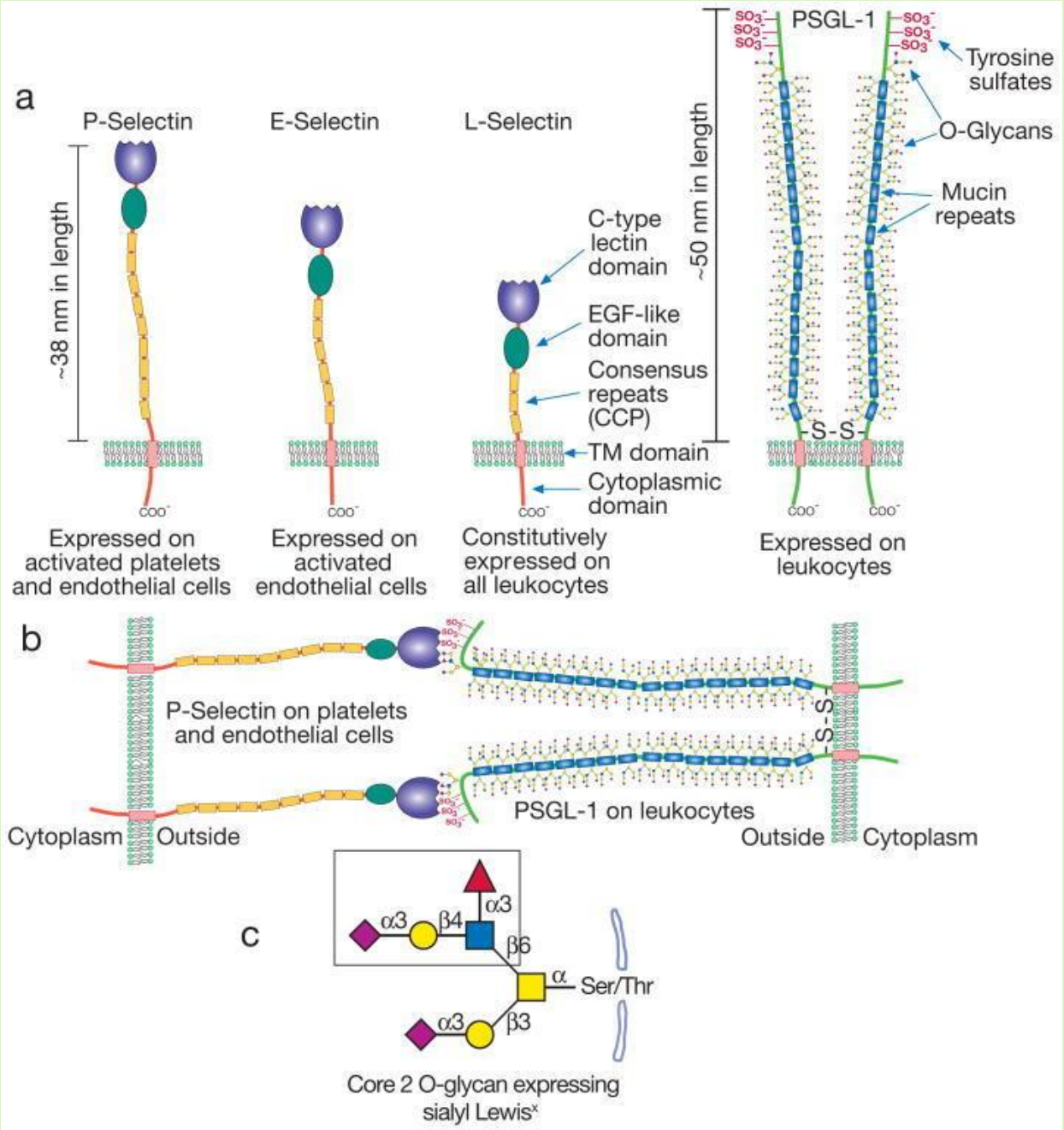


Лектин C-типа

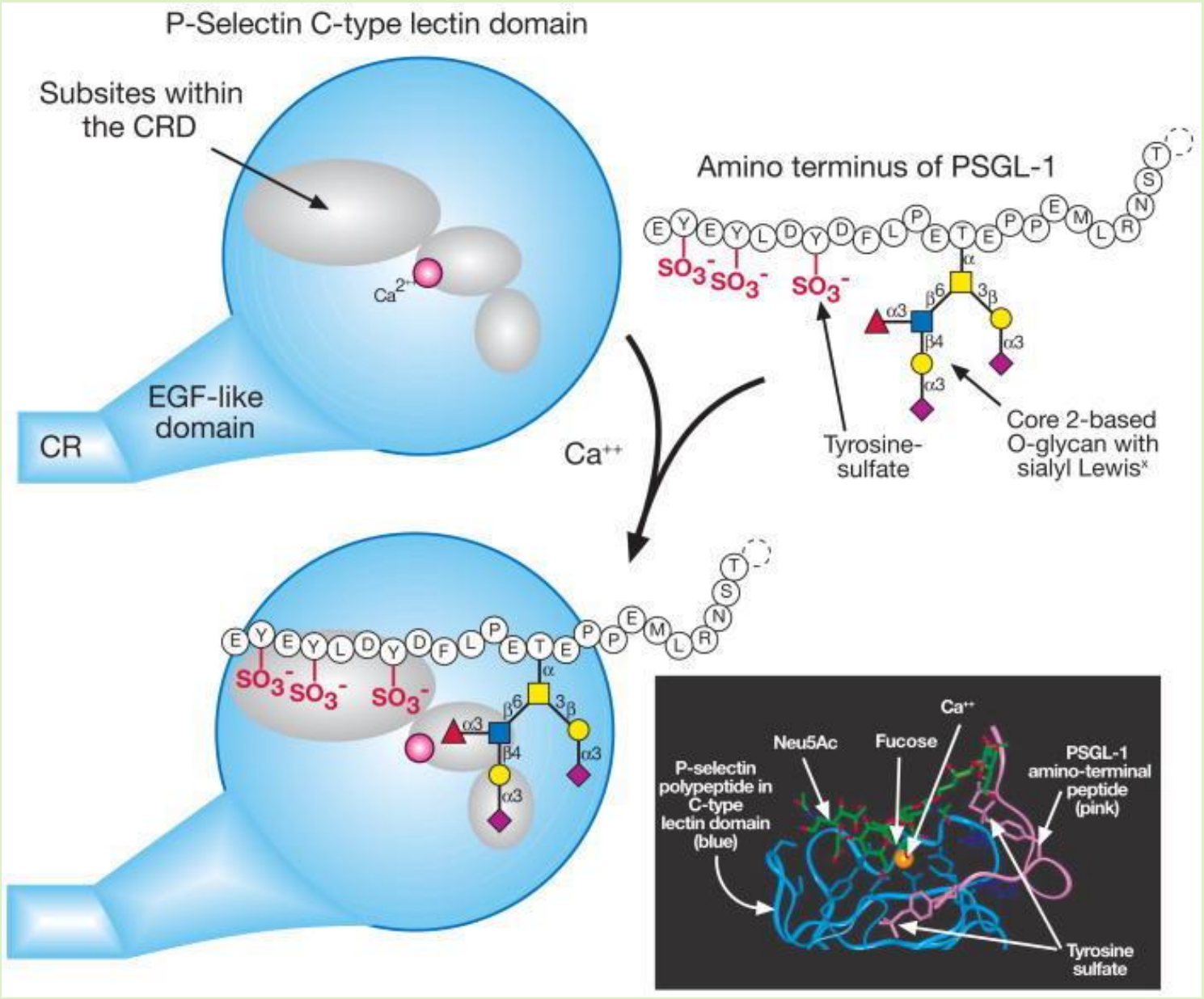
Селектины и их рецепторы



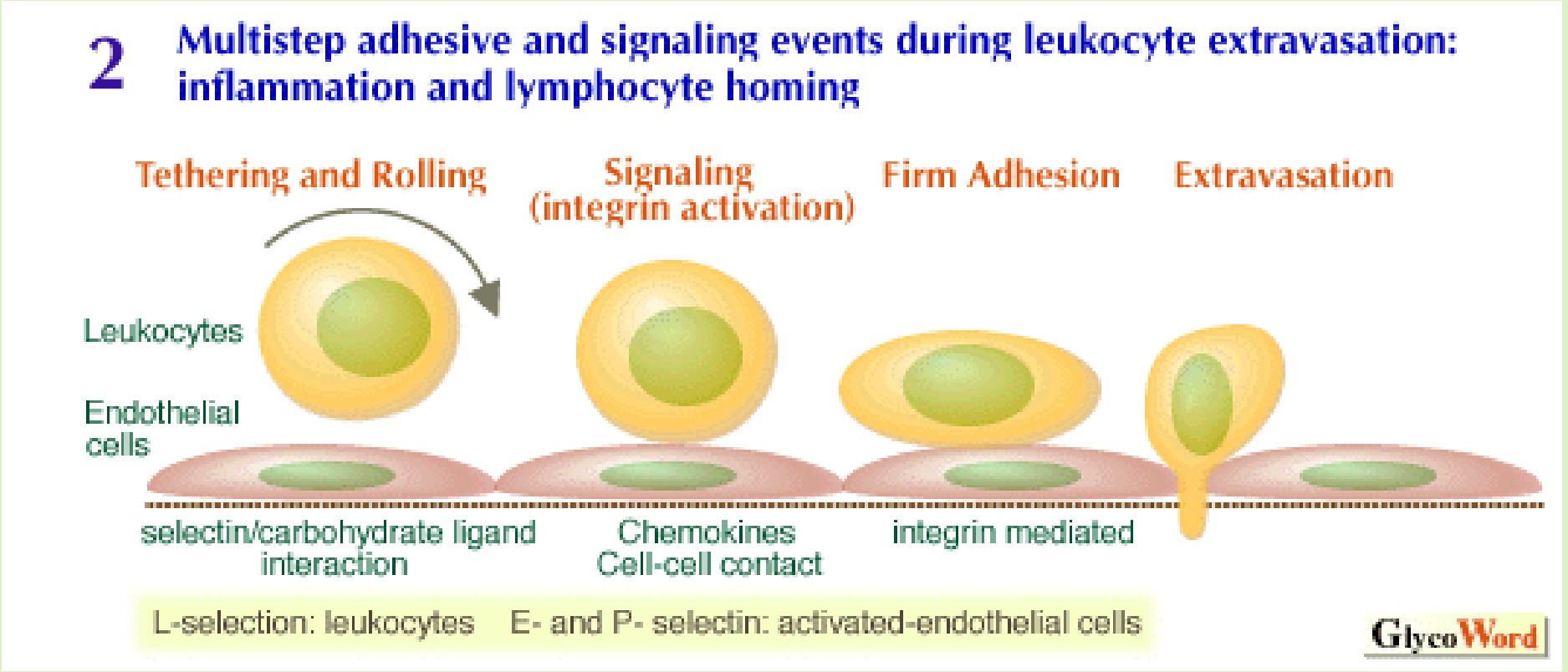
Доменная структура P-, E-, L-селектинов и PSGL-1



Взаимодействие между P-селектином и амино-концом его лиганда – PSGL-1



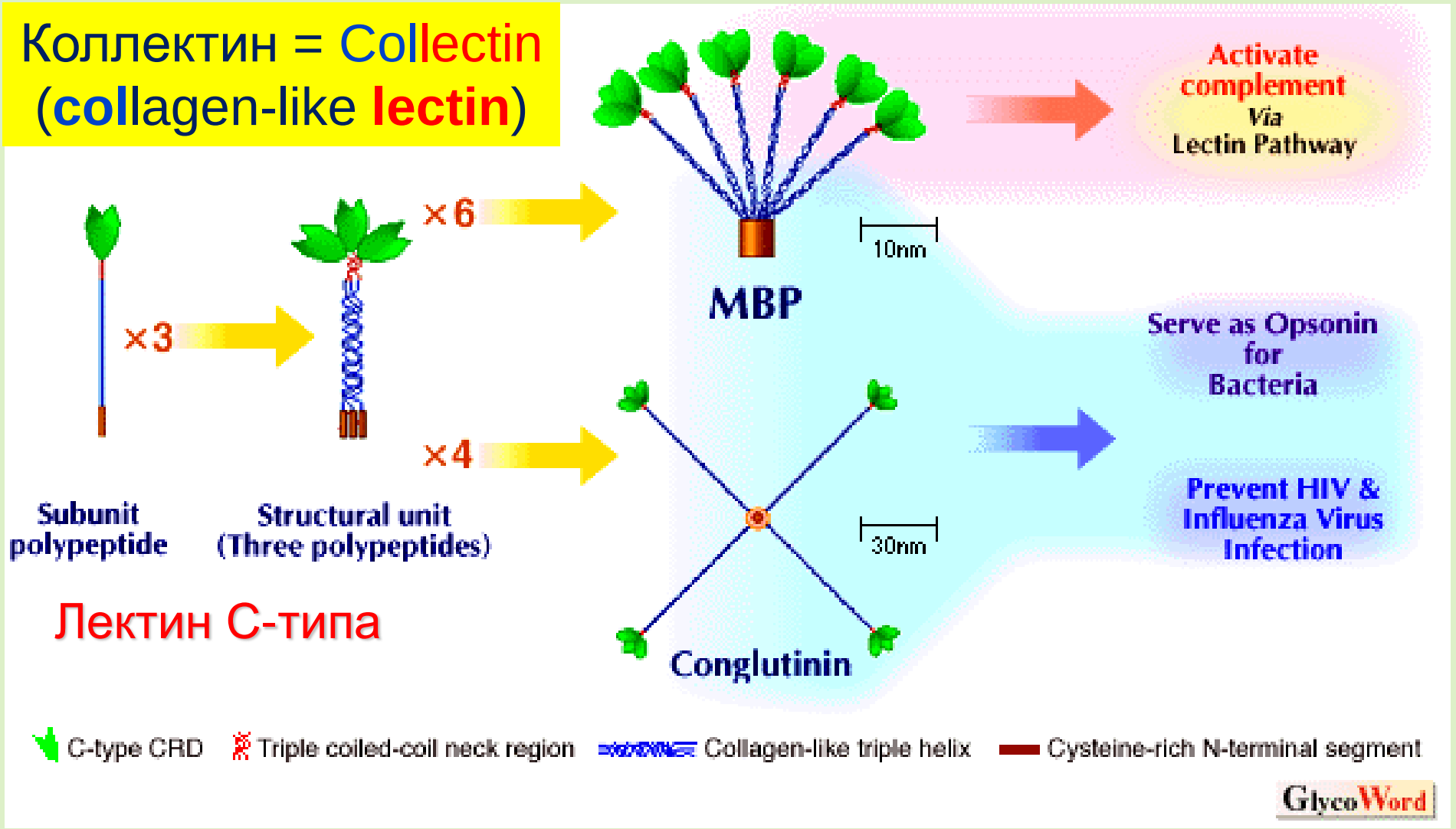
Роллинг лейкоцитов по кровеносным сосудам



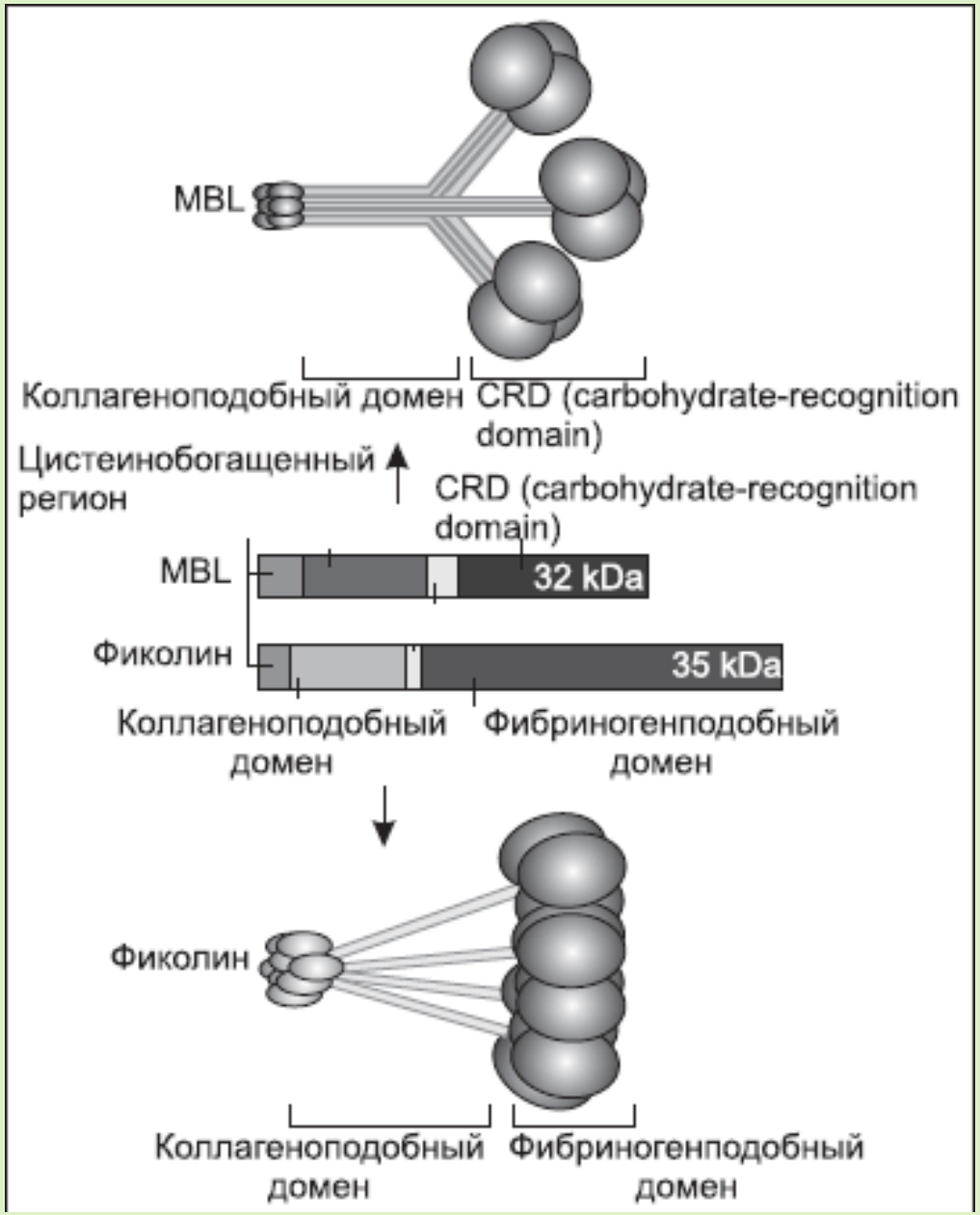
Коллектины и фиколины

Коллектины и родственные немембранные белки: MBP (MBL) и конглютинин

Сходство с белками системы комплемента



Строение коллектинов и фиколинов



Лектиновый путь активации комплемента: действие коллектинов и фиколинов

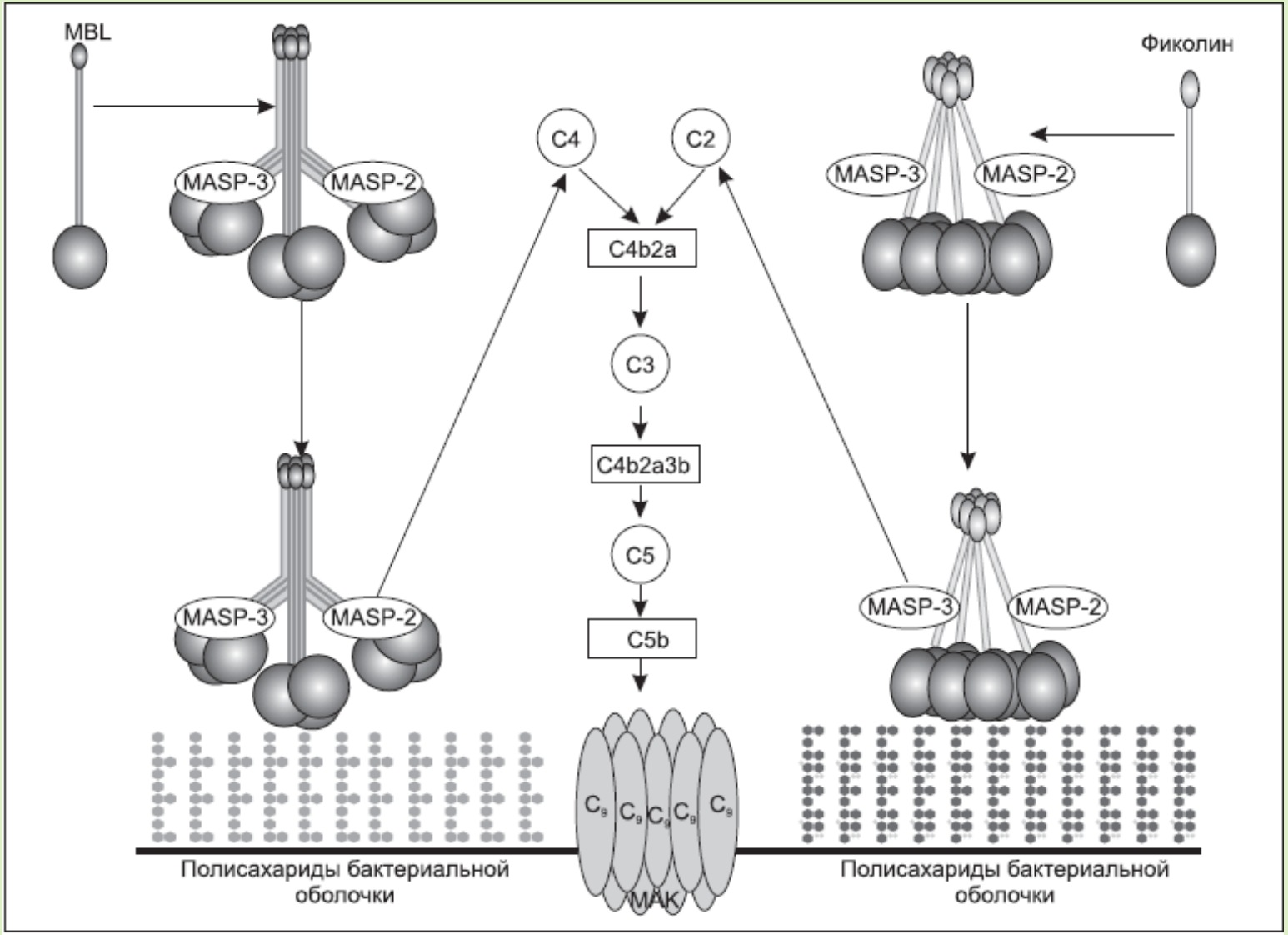
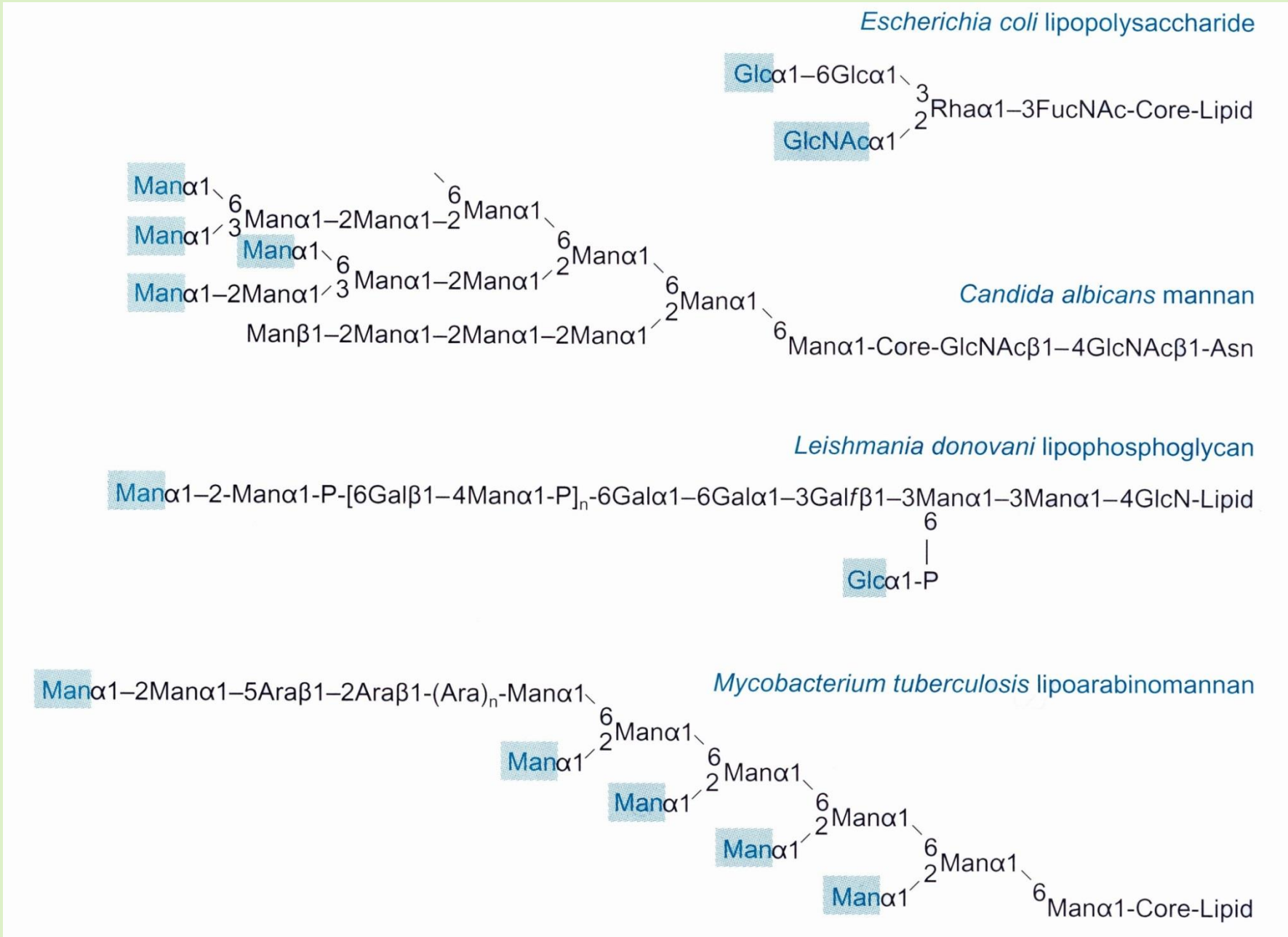


Рисунок 5. Механизм действия MBL и фиколинов

Примечание: С — компоненты комплемента, МАК — мембраноатакующий комплекс, MASP — MBL-ассоциированные сериновые протеазы.

Лиганды коллектинов



Спектр микроорганизмов, распознающихся коллектином MBL

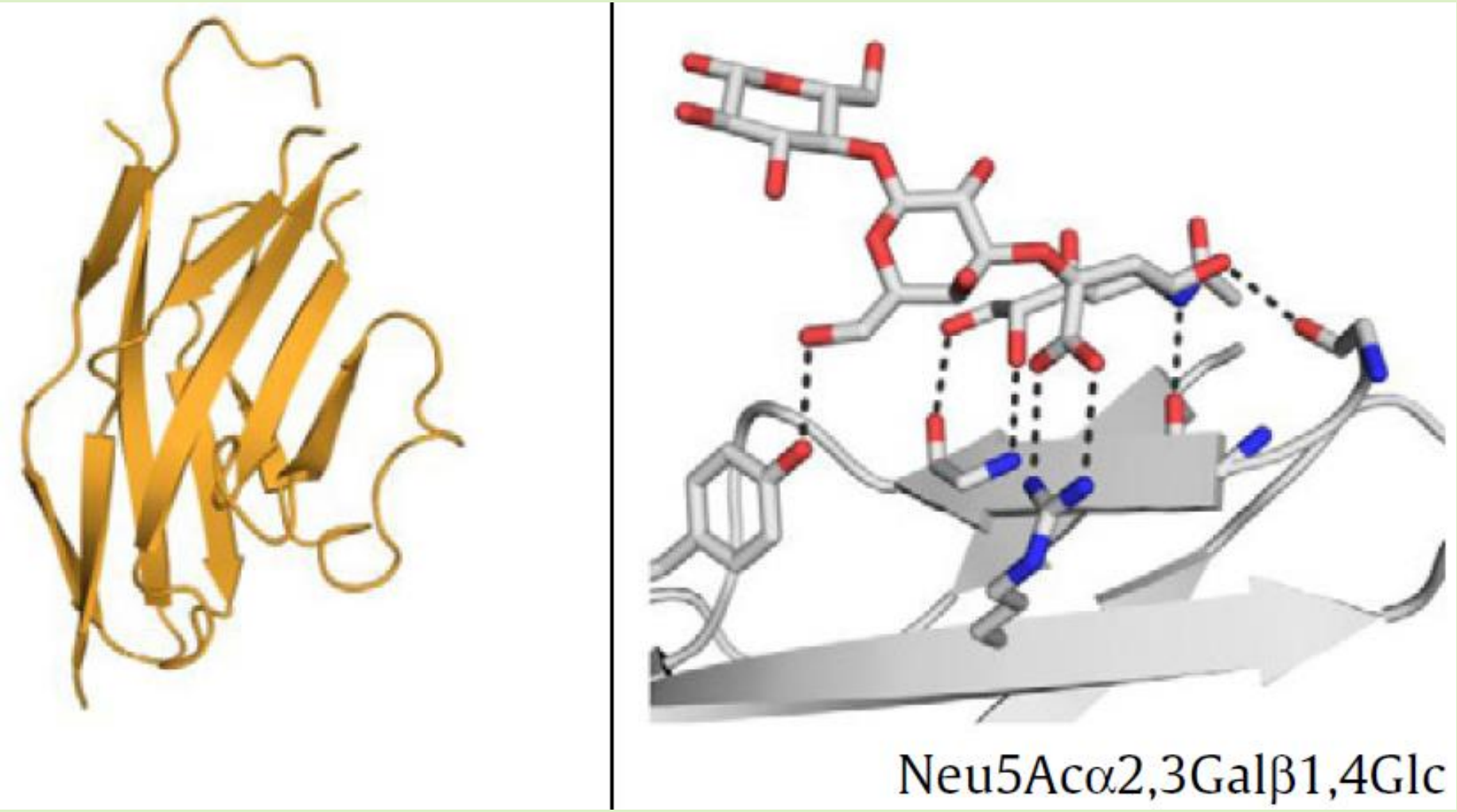
Группа микроорганизмов	Микроорганизмы
Бактерии	*Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Enterococcus* spp., *Listeria monocytogenes, Haemophilus influenzae, Neisseria meningitidis, Neisseria gonorrhoeae, Escherichia coli, Klebsiella* spp., *Pseudomonas aeruginosa, Salmonella montevideo, Salmonella typhimurium, H.pylori, Chlamydia trachomatis, Chlamydia pneumonia, Proprionibacterium acnes, Mycobacterium avium, Mycobacterium tuberculosis, Mycobacterium leprae, Leishmania chagasi*
Вирусы	ВИЧ-1, -2, вирус простого герпеса, вирус гриппа А, вирус гепатита В, вирус гепатита С
Грибы	*Aspergillus fumigatus, Candida albicans, Cryptococcus neoformans, Saccharomyces cerevisiae*
Простейшие	*Plasmodium falciparum, Cryptosporidium parvum, Trypanosoma cruzi*

Сиглеки

Лектины I-типа

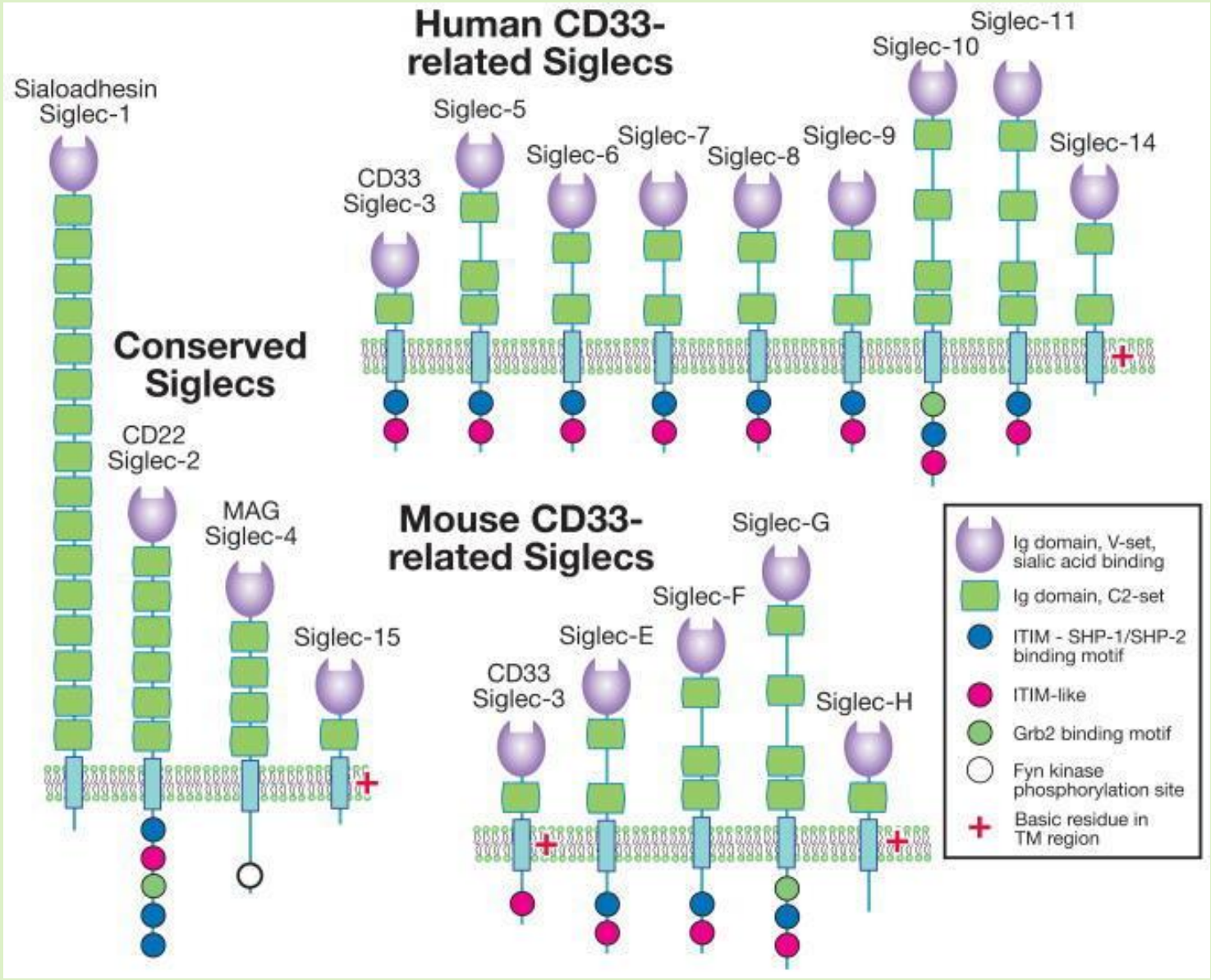
Siglec (sialic acid–binding, immunoglobulin-like lectin)

Типичная третичная структура лектинов I-типа: Ig-подобный фолд

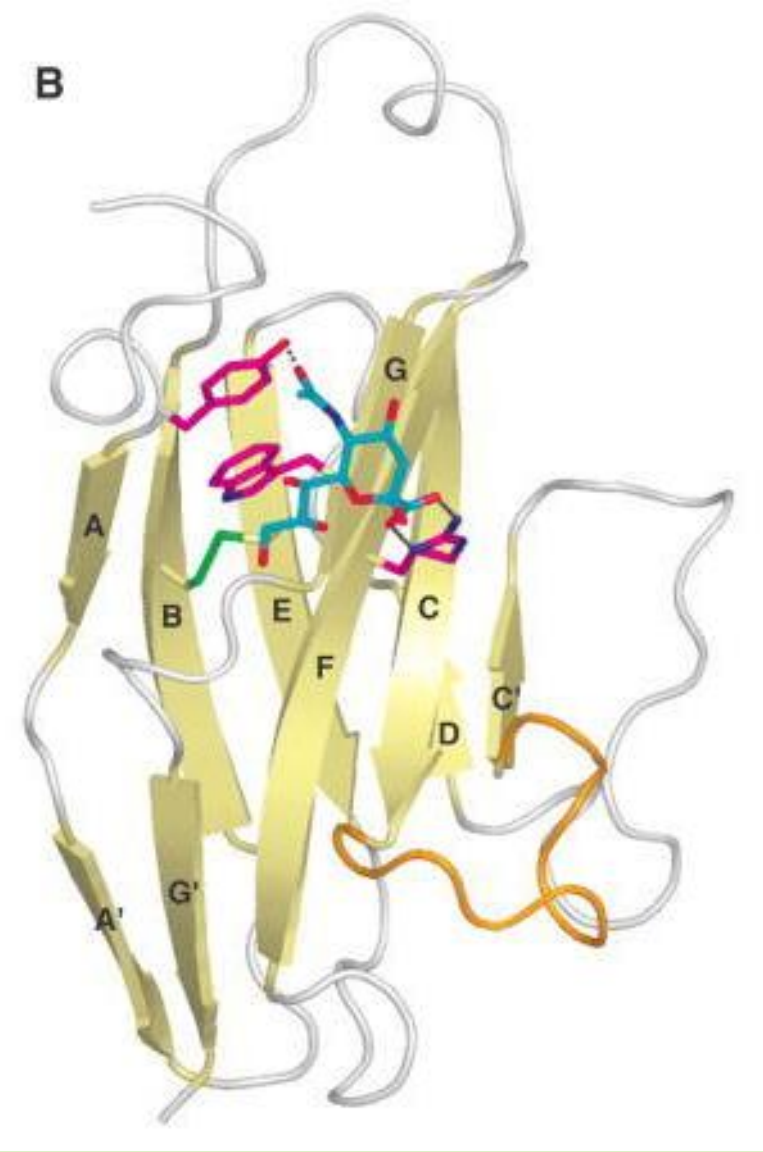


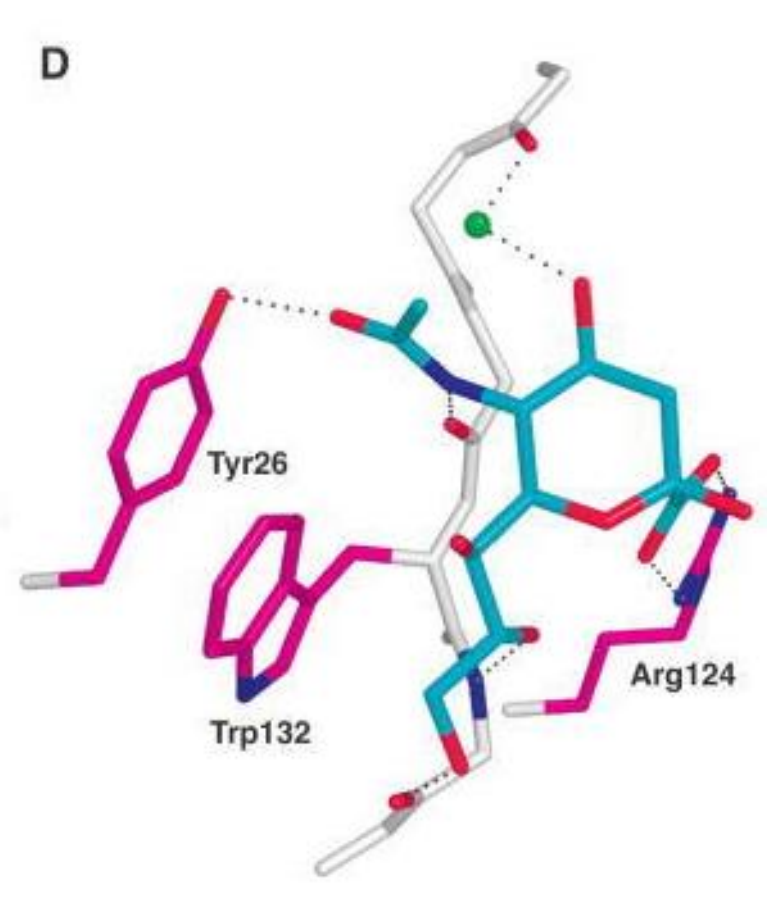
Сиглеки

Siglec (sialic acid–binding, immunoglobulin-like lectin)

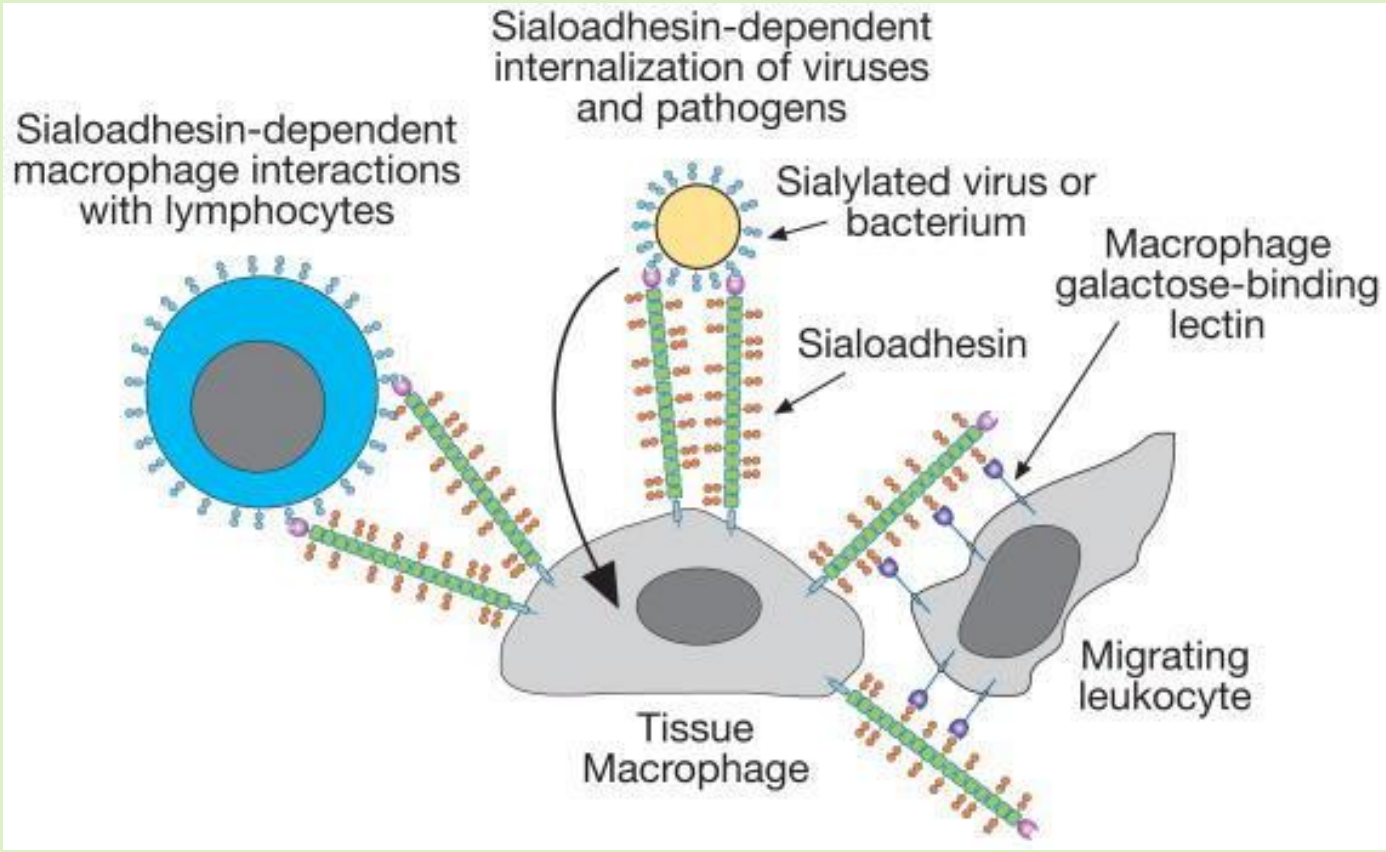


Комплекс сиглека-7 с сиаловой кислотой

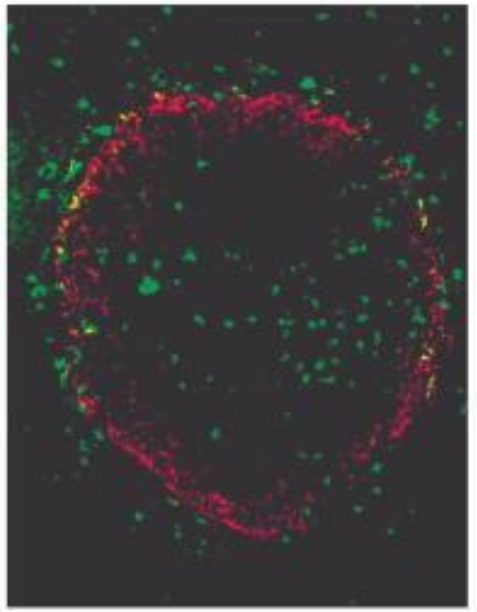




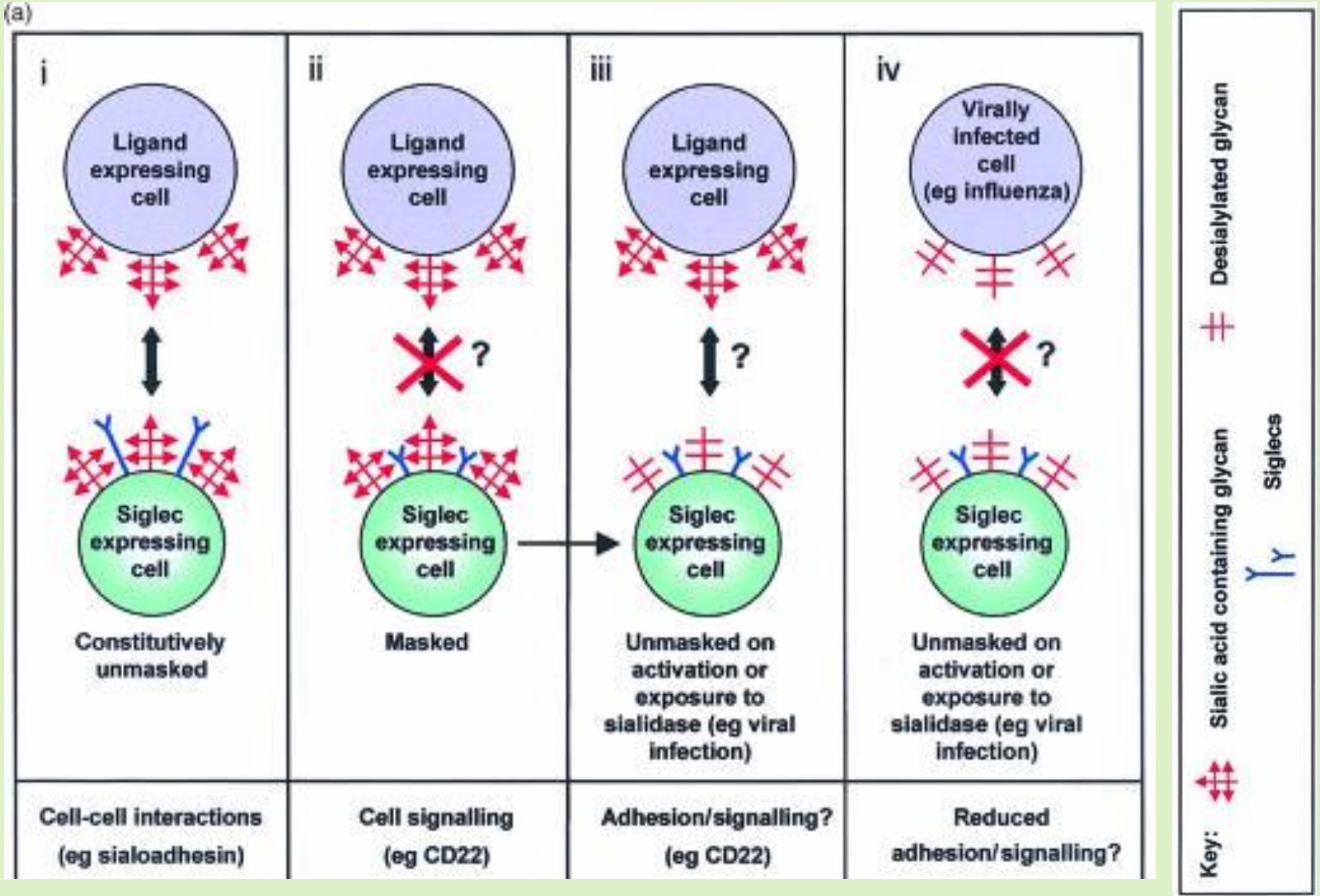
Биологические фунции, медиируемые сиглеком-1 (сиалоадгезин, Sn, CD169)



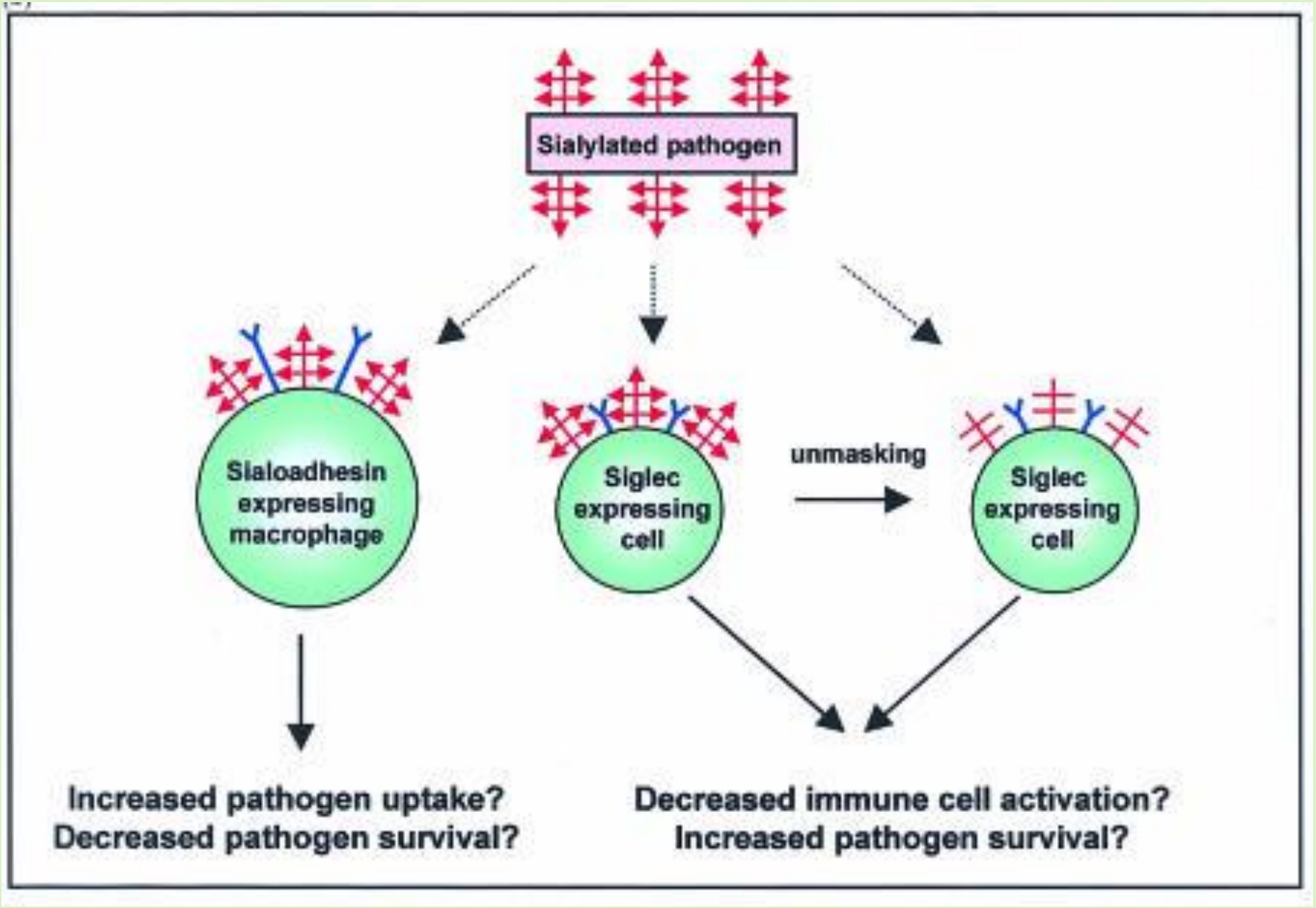
Сиглек-1 на макрофагах



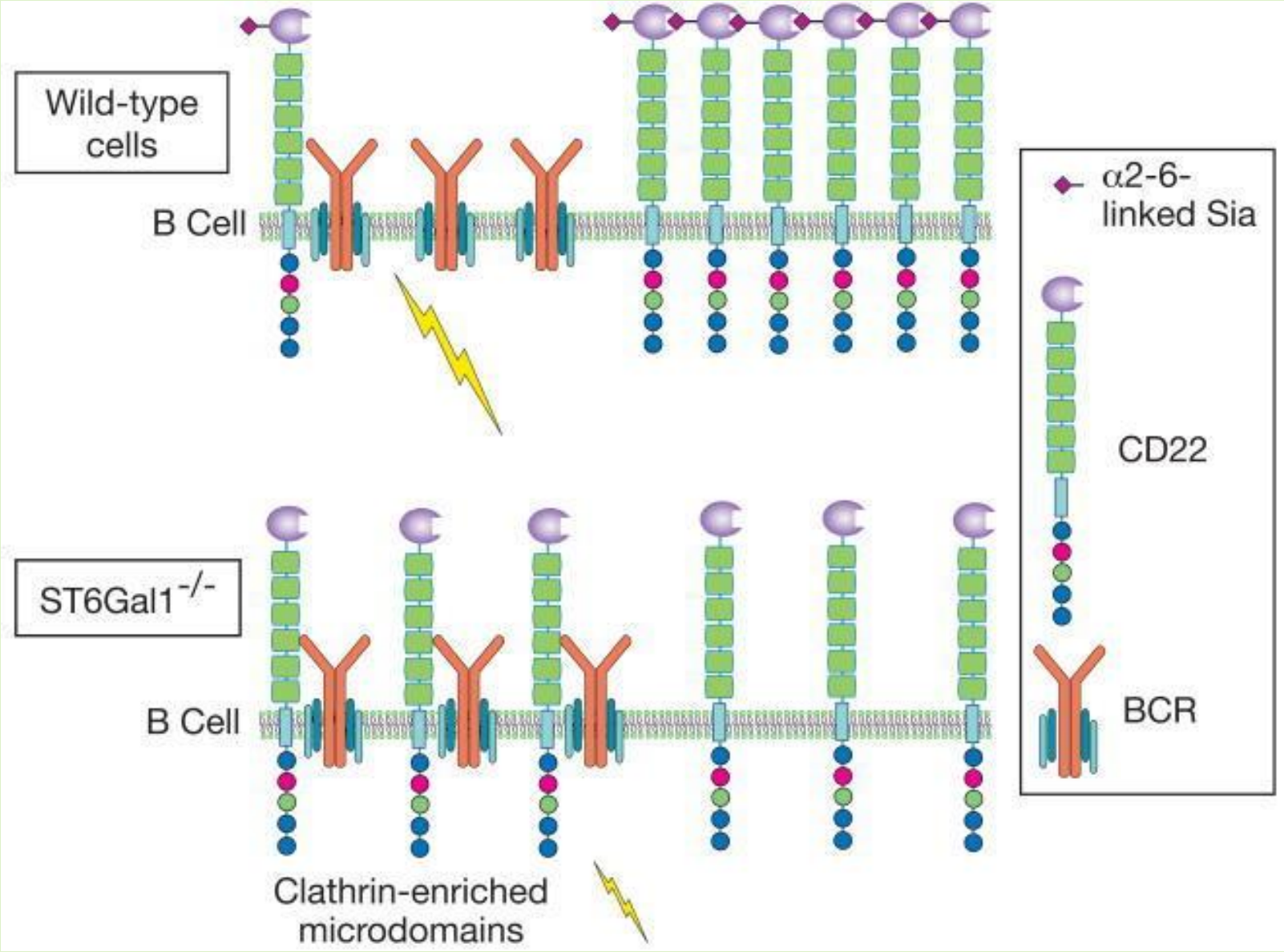
«Маскировка» сиглека вследствие *цис*-взаимодействий



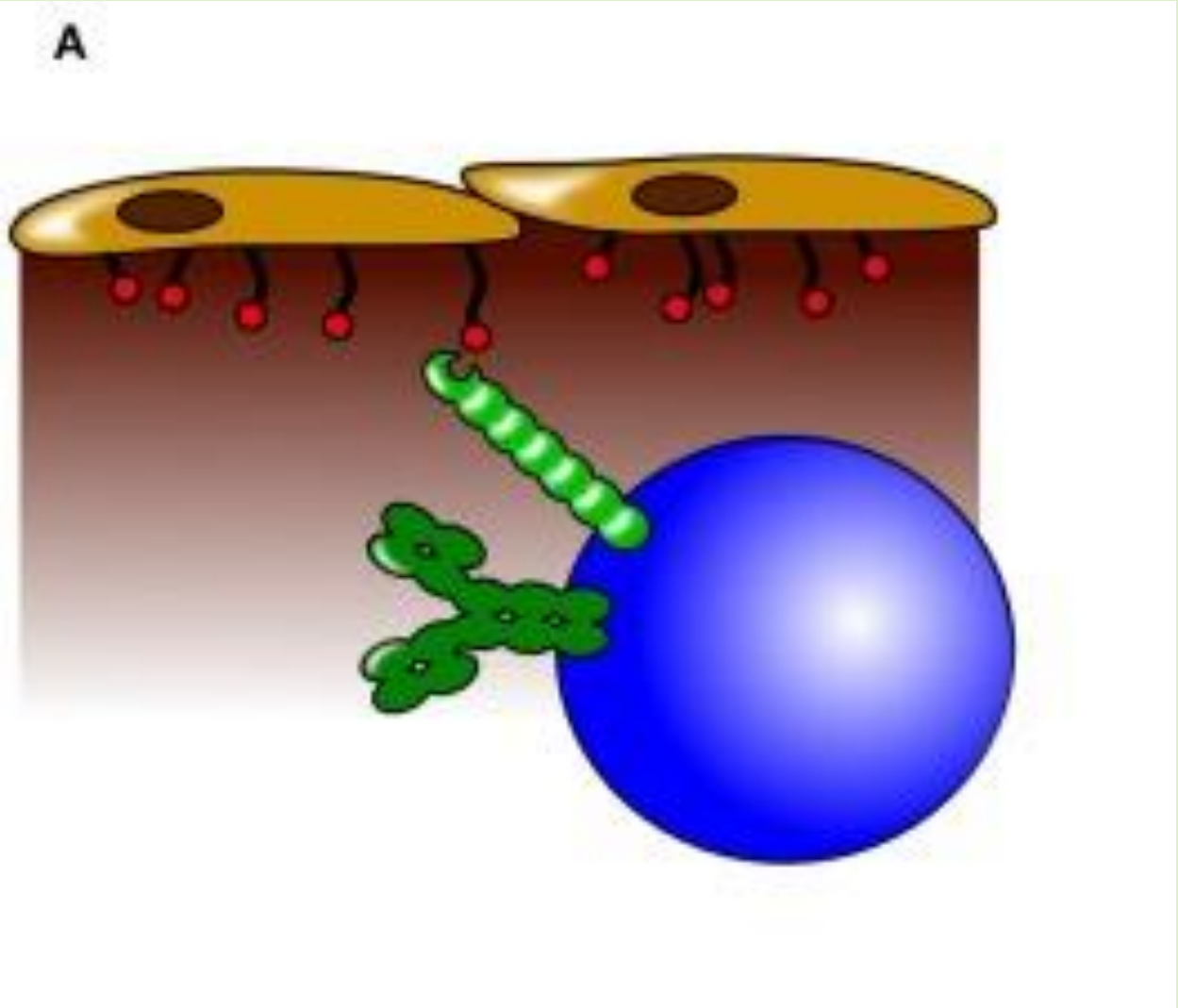
«Демаскирование» сиглека: десиалилирование



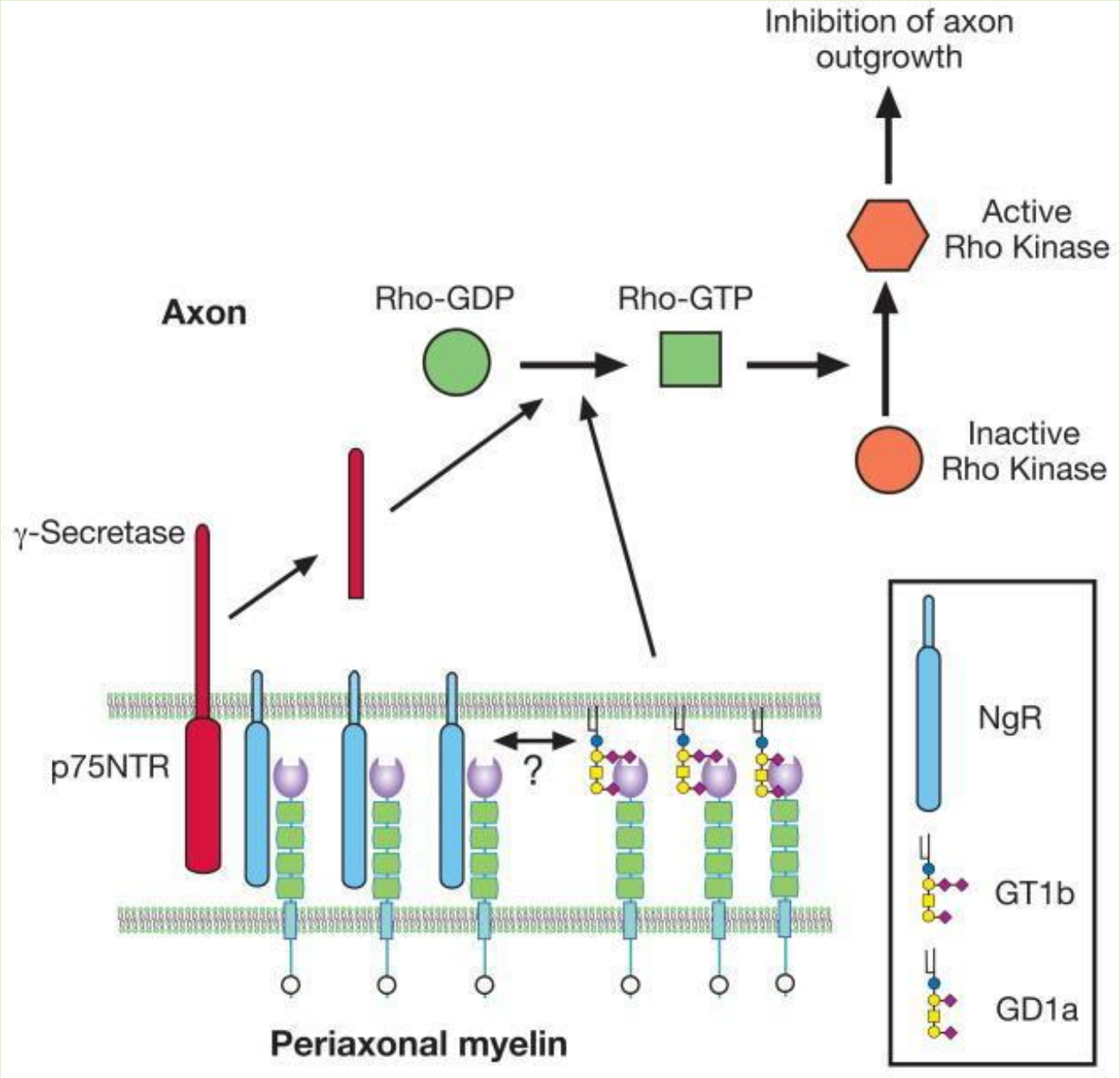
Сиглек-2 (CD22) на В-лимфоцитах: *цис*-взаимодействие модулирует сигнал BCR



Сиглек-2 («демаскированный»): хоминг В-лимфоцитов в костный мозг

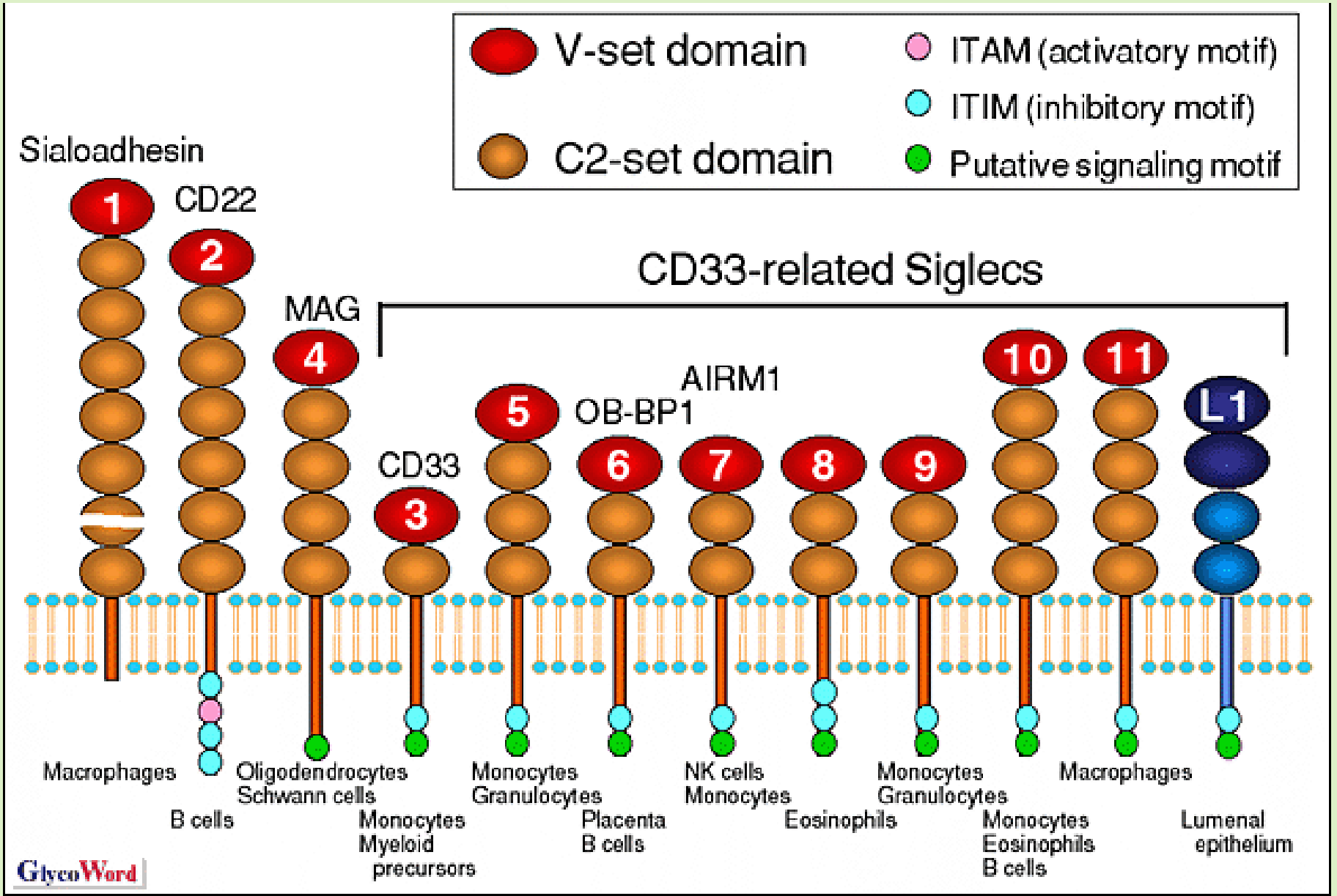


Ингибирование роста аксонов сиглеком-4 (MAG)

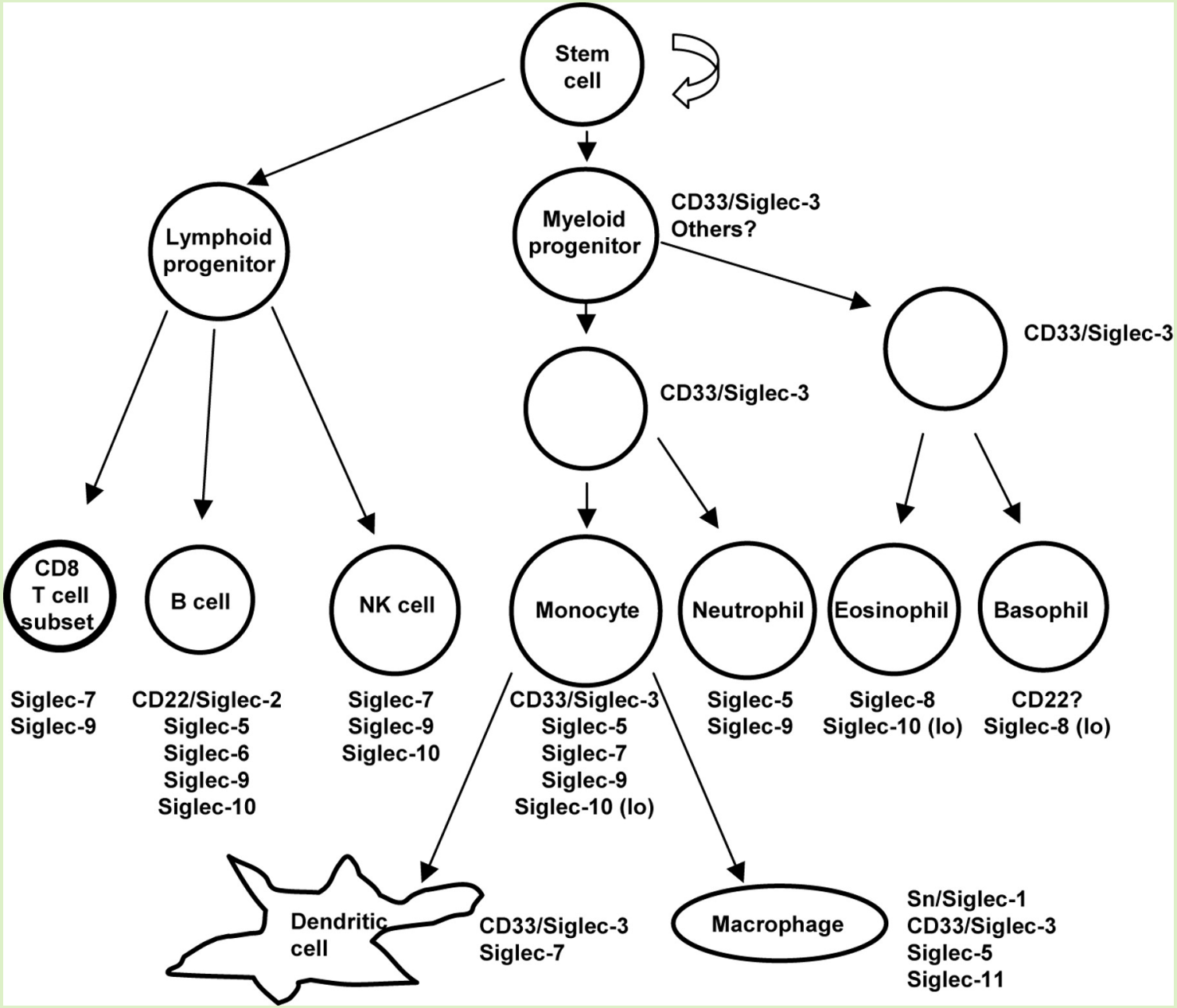


Сиглеки человека: экспрессия

Siglec (sialic acid–binding, immunoglobulin-like lectin)



Сиглеки человека: экспрессия на типах клеток



Углеводная специфичность сиглеков

Oligosaccharide	R1	R2	R3
N-Glycan	+		
O-Glycan	+		+
Glycolipid	+	+	

◆ = Sia ● = Gal ■ = GlcNAc ■ = GalNAc ▲ = Fuc

	Relative Recognition by Human Siglec										
	1	2	3	4	5	6	7	8	9	10	11
◆α6●β4■β-R1	-	++	++	-	-	-	-	-	+	+	-
◆α3●β4■β-R1	++	-	+	+	++	-	-	+	++	++	+
◆α8◆α3●β4■β-R1	+	-	+	-	-	-	++	+	+	-	++
◆α3●β3■β-R1	++	-	+	+	-	-	-	+	+	+	-
◆α3●β4■β-R1 (■ α3 ▲)	+	-	+	-	-	-	-	+	+	-	-
◆α3●β3□β-R2/α-R3	+	-	+	+	-	-	-	-	+	-	-
◆α3●β3□β-R2/α-R3 (□ α6 ◆)	?	?	?	++	-	-	+	-	+	?	?
□β-R2/α-R3 (□ α6 ◆)	+	+	+	-	-	+	+	+	+	+	-

Известные и предполагаемые функции сиглеков

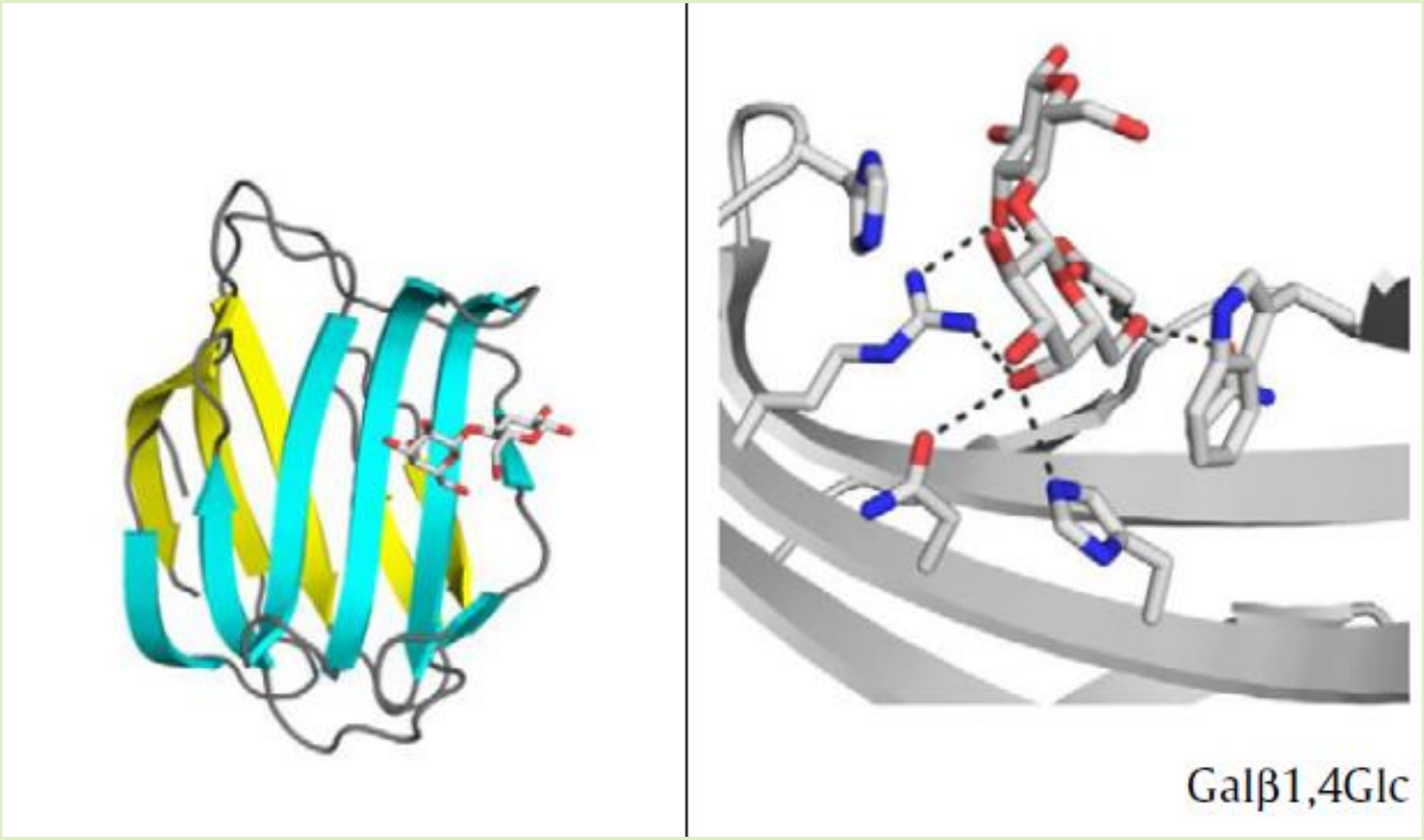
With the exception of MAG and CD22, wherein biological roles in glial cells (maintenance of myelin organization and inhibition of neurite outgrowth) and B cells (dampening the response to BCR ligation), respectively, are well documented, we do not yet know the physiological functions of any other Siglec. The fact that Sn and CD33rSiglecs are found predominantly on cells of the innate immune system suggest that this is where their primary functions lie. Given the highly conserved preference of Sn for α2-3-linked Neu5Ac and to a lesser extent for α2-8-linked Neu5Ac, its presence on tissue macrophages, and the lack of cytosolic signaling motifs, it is reasonable to speculate that Sn's primary role may actually lie in recognition and phagocytosis of bacterial pathogens that express Sias. In keeping with this, α2-3-and α2-8-linked Neu5Ac (and not Neu5Gc) are the dominant sialylated motifs on bacterial pathogens studied to date. Of course, one cannot rule out an additional intrinsic role of Sn in interactions with other cell types within the hematopoietic or lymphoid systems. The CD33rSiglecs stand in striking contrast to Sn, having rapidly evolving Sia-binding preferences and expression patterns, and inhibitory cytosolic motifs. The best explanation for the findings to date is that these molecules serve to detect the host "sialome" as "self," thereby down-regulating innate immune cell reactivity via their ITIM motifs. In keeping with this notion, a very recent study using siRNA and other techniques has demonstrated a "constitutive repressor activity" of CD33 on human monocytes that involves phosphoinositide 3-kinase–mediated intracellular signaling and requires Sia recognition to function optimally. However, it was also reported that one CD33-related Siglec could be involved in bacterial uptake.

In further exploring Siglec functions, we must remember that potential ligands could be formed not only by Sias on the same cell surface (or on the Siglec itself), but also on other cell surfaces or on soluble glycoproteins. Direct cell–cell interactions could thus potentially occur among Siglec-positive cells or between Siglec-positive cells and another other cell type. Soluble sialylated glycoprotein ligands could also interact directly with Siglec-positive cells, bridge between two such cells, or serve to inhibit cell–cell interactions involving Siglecs.

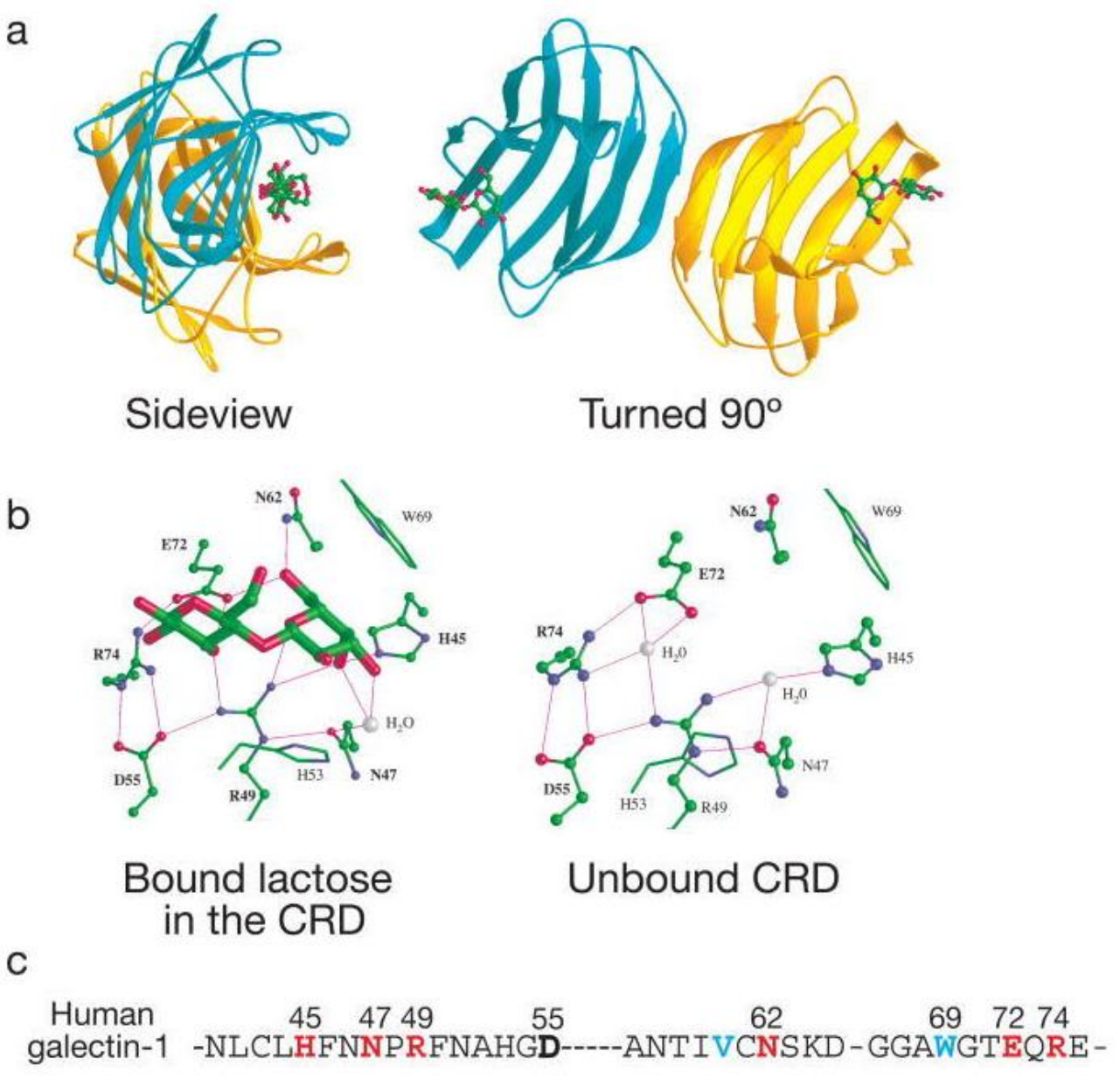
Галектины

Лектины S-типа

Типичная третичная структура галектинов: β-сэндвич



Третичная структура галектинов: галектин-1



Галектины человека

a Galectins in humans

Galectin-1
Galectin-2
Galectin-7
Galectin-10
Galectin-13
Galectin-14

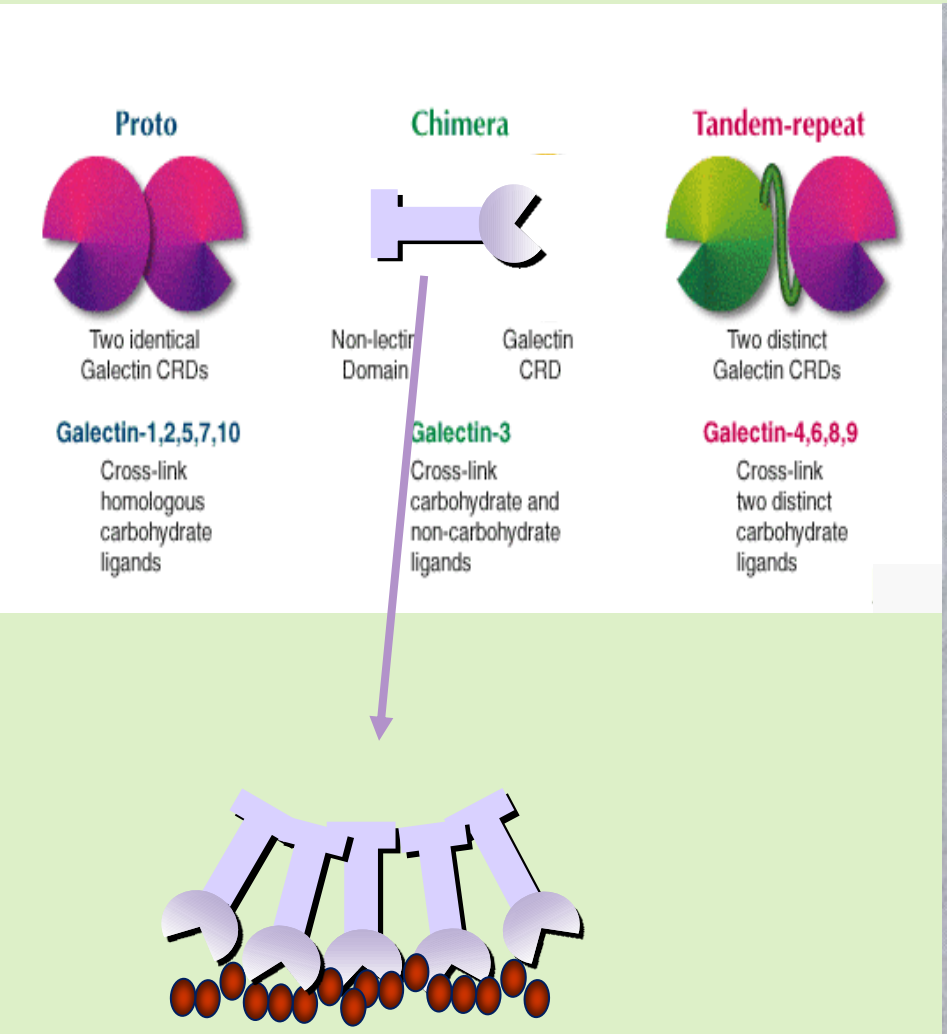
Galectin-3

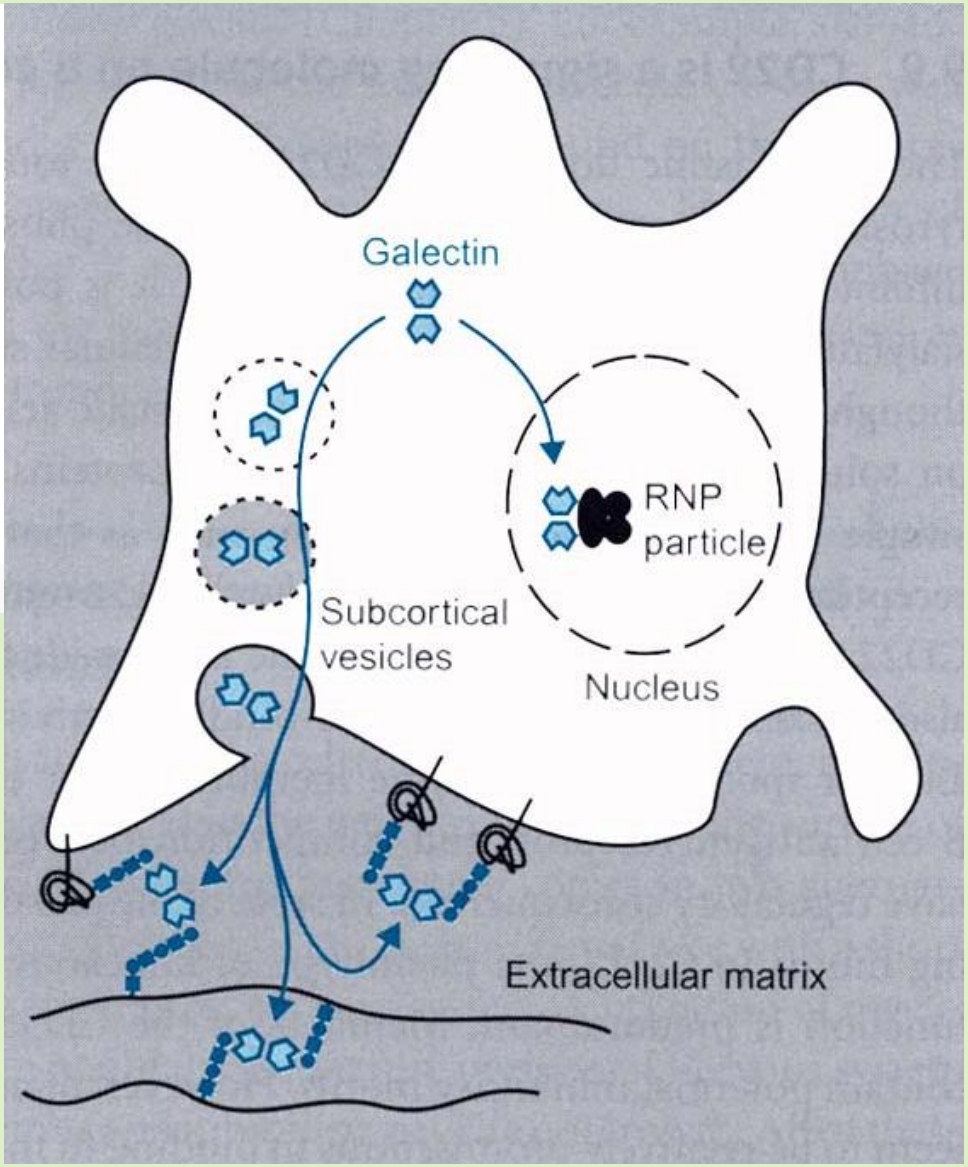
Galectin-4
Galectin-8
Galectin-9
Galectin-12

b Sequence alignments of some human galectins

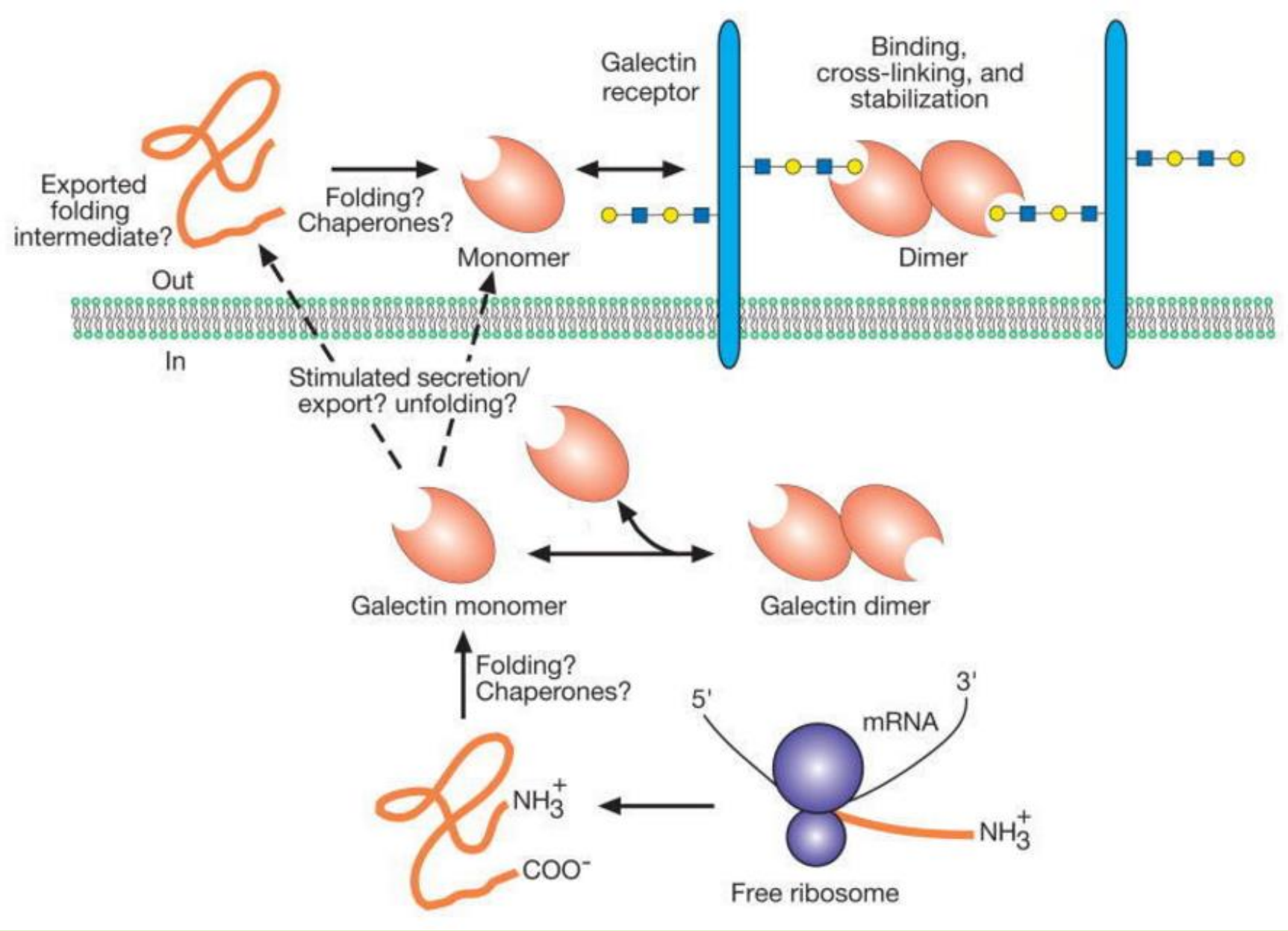
```
                41  *  *  *                  56      *      66   *   71 *  *
Galectin-1  -NLCLHFNPRFNAHGD------ANTIVCNSKD-GGAWG-TEQRE-
Galectin-2  -KLNLHFNPRFS----------ESTIVCNSLD-GSNWG-QEQRE-
Galectin-3  -DVAFHFNPRFNENN-------RRVIVCNTKL-DNNWG-REERQ-
Galectin-4  -DVAFHFNPRFDG---------WDKVVFNTLQ-GGKWG-SEERK- (N-term)
            -DIALHINPRMG----------NGTVVRNSLL-NGSWG-SEEKK- (C-term)
Galectin-7  -DAALHFNPRLD----------TSEVVFNSKE-QGSWG-REERG
```

Галектины

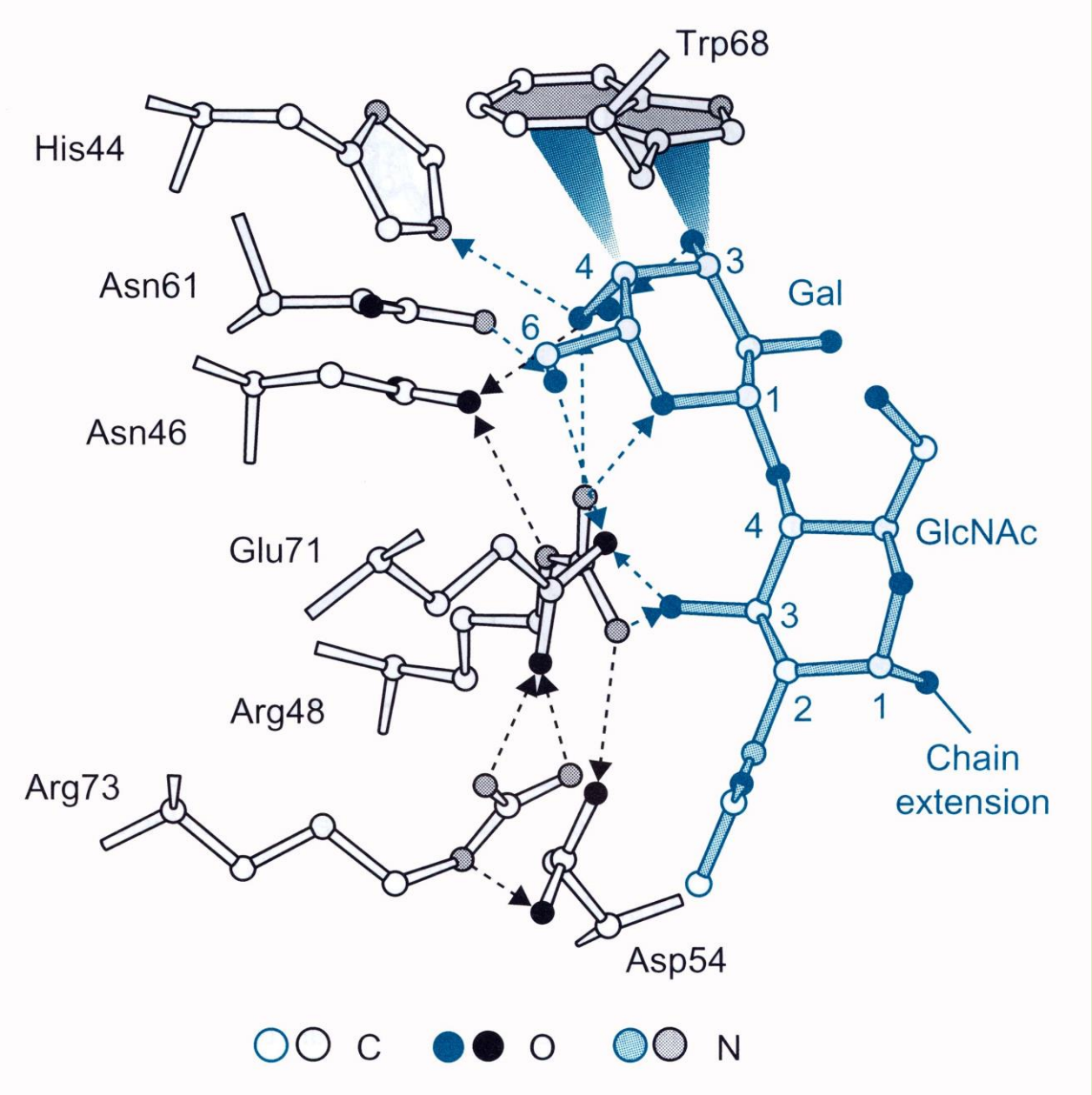

Proto
Chimera
Tandem-repeat
Two identical Galectin CRDs
Non-lectin Domain
Galectin CRD
Two distinct Galectin CRDs
Galectin-1,2,5,7,10
Cross-link homologous carbohydrate ligands
Galectin-3
Cross-link carbohydrate and non-carbohydrate ligands
Galectin-4,6,8,9
Cross-link two distinct carbohydrate ligands


Galectin
RNP particle
Subcortical vesicles
Nucleus
Extracellular matrix

Биосинтез галектинов: галектин-1



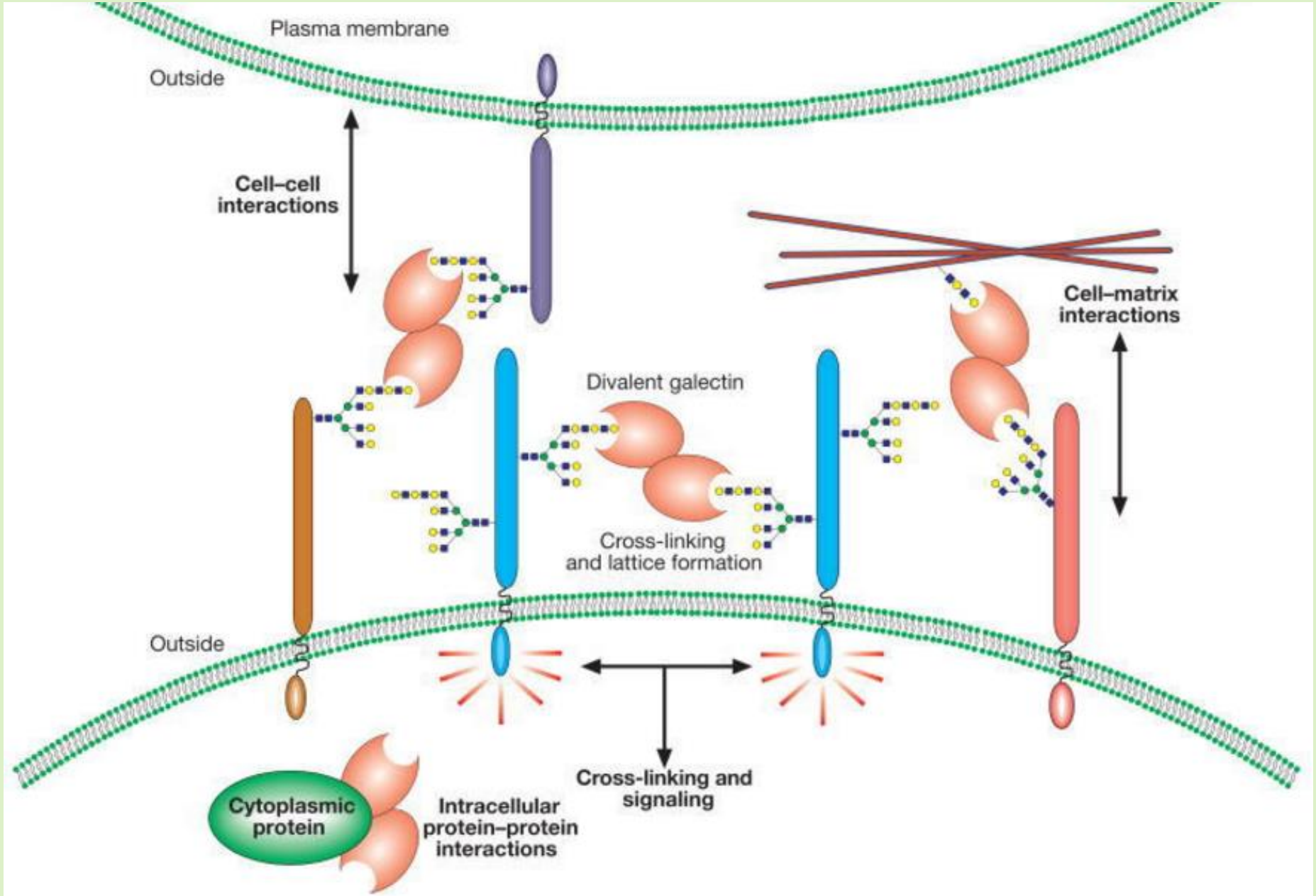
Комплекс галектина-1 с Galβ1-4GlcNAc



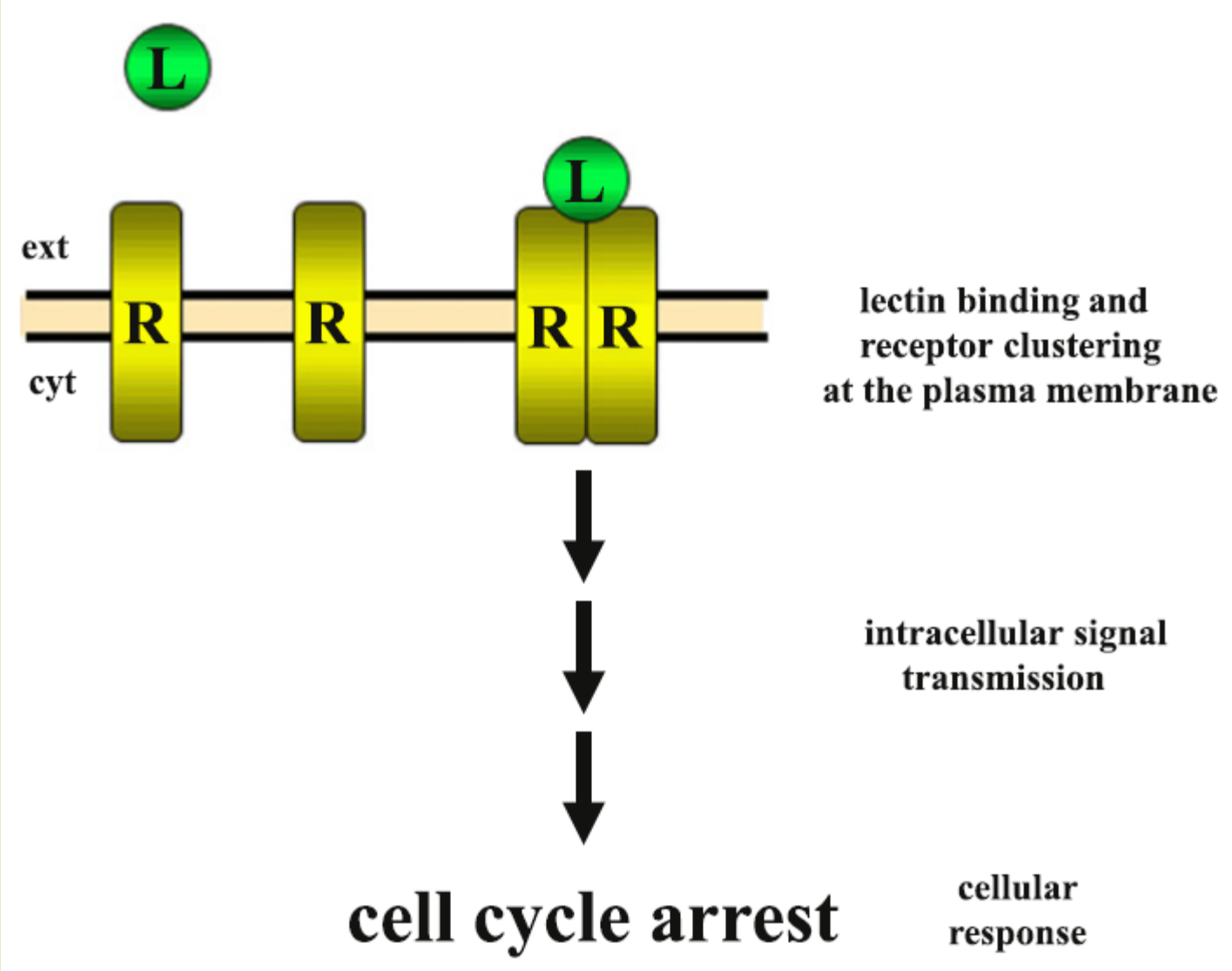
Галектины могут узнавать больше, чем терминальный остаток Galβ1-4GlcNAc

(Gal, GalNAc, Fuc, Sia, Su)-Galβ1-4GlcNAc-X-X-X

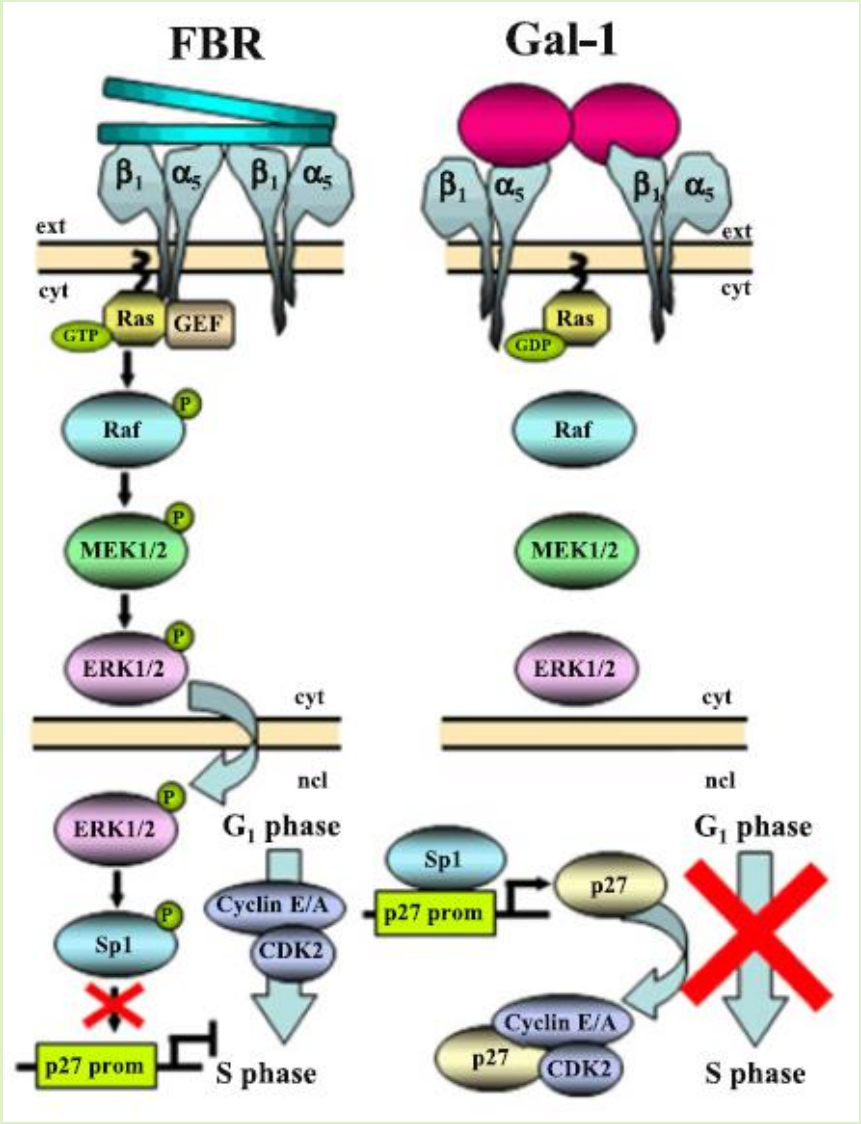
Функциональные взаимодействия галектинов



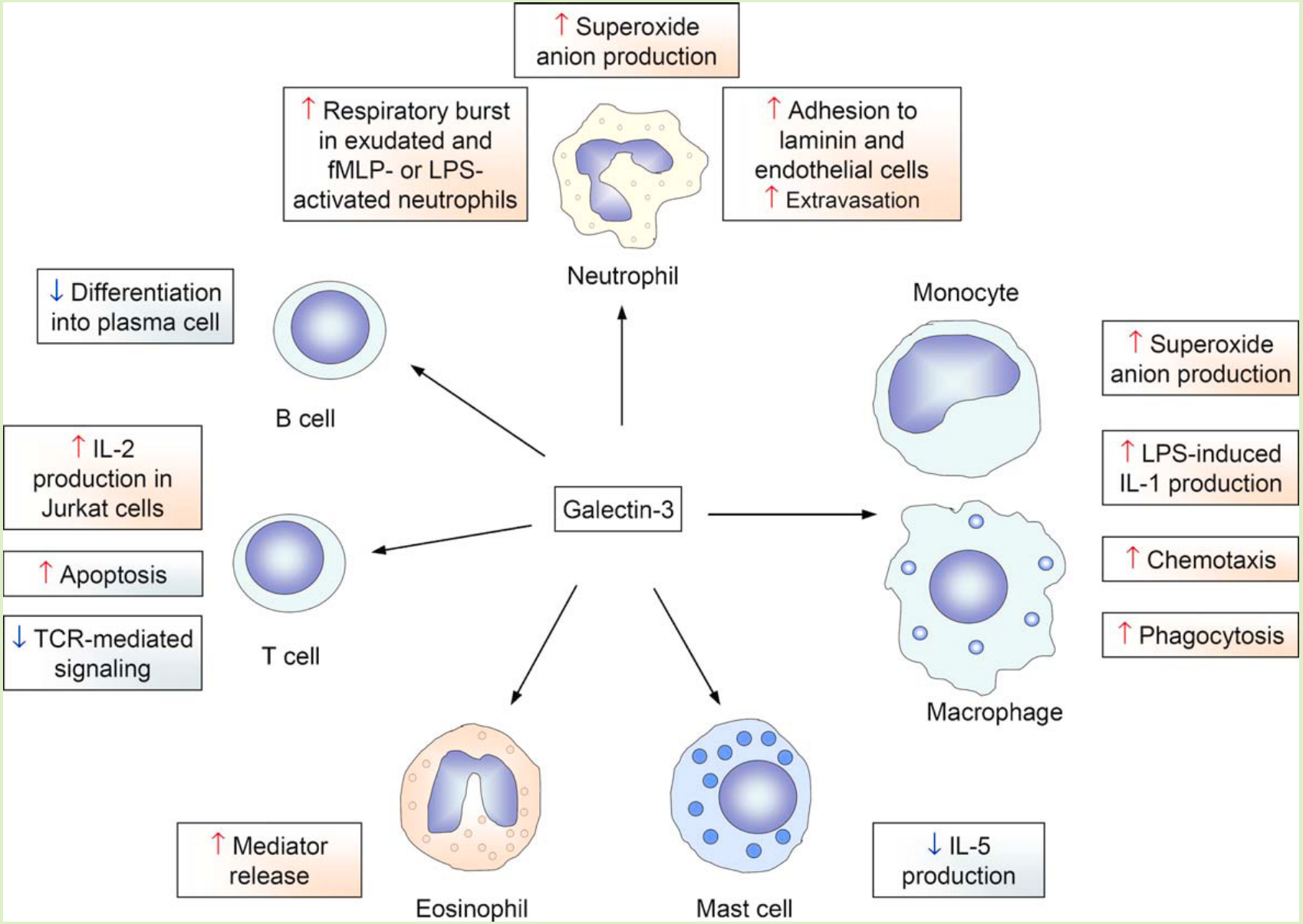
Транслирование «углеводной информации» в эффекты: связывание лектина – сигнал



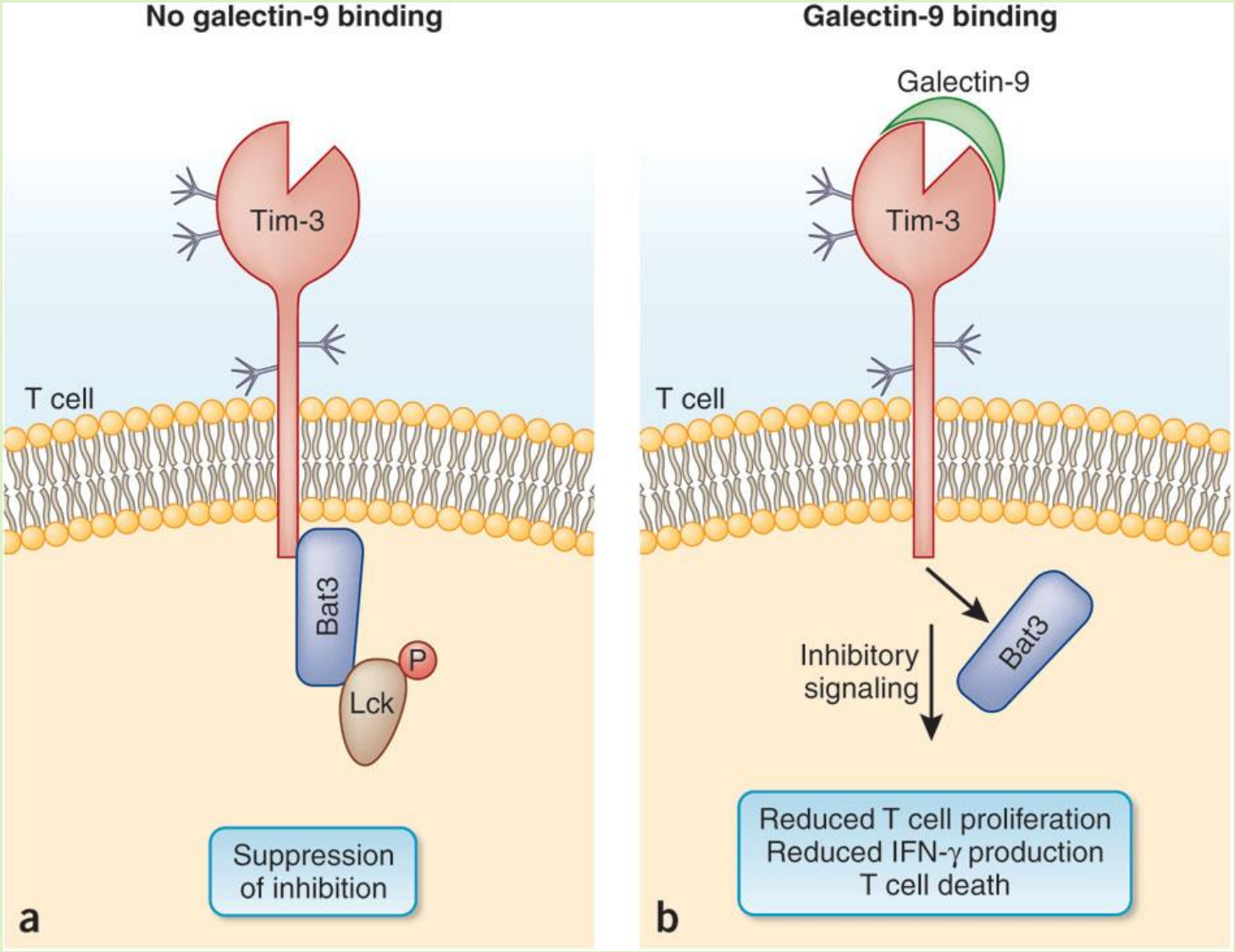
Связывание галектина-1 с фибронектиновым рецептором – сигнал к остановке клеточного цикла



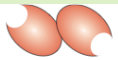
Галектин-3


↑ Superoxide anion production
↑ Respiratory burst in exudated and fMLP- or LPS-activated neutrophils
↑ Adhesion to laminin and endothelial cells
↑ Extravasation
Neutrophil
↓ Differentiation into plasma cell
B cell
Monocyte
↑ Superoxide anion production
↑ LPS-induced IL-1 production
↑ Chemotaxis
↑ Phagocytosis
Macrophage
↑ IL-2 production in Jurkat cells
↑ Apoptosis
↓ TCR-mediated signaling
T cell
Galectin-3
↑ Mediator release
Eosinophil
Mast cell
↓ IL-5 production

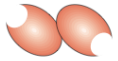
Апоптоз Т-лимфоцита, вызванный связыванием с галектином-9



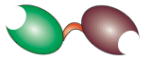
Биологические роли галектинов: апоптоз, рак, иммунный ответ и воспалительные процессы



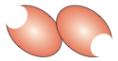
Galectin-1
- involved in Treg cell function and enhances Treg formation
- conflicting results on effects on T-cell viability
- mediates adhesion of thymocytes to thymic epithelium
- induces apoptosis in $CD4^+CD8^+$ double positive thymocytes
- induces shift in Th1 response to Th2 (decreases IFNγ; increases IL-5)
- reduces TNFα, IL-1β, IL-12, IL-2 and IFNγ
- increases IL-10 production in both naive and activated T cells
- inhibits mast cell degranulation
- reduces pathology-associated graft-versus-host disease, Con A-induced hepatitis, experimental allergic encephalomyelitis, myasthenia gravis and rheumatoid arthritis
- reduces acute inflammatory responses
- expression in endothelial cells up-regulated by activation
- induces apoptosis-independent phosphatidylserine (PS) exposure (Ca^{++}-dependent) in neutrophils
- inhibits chemotaxis of neutrophils
- inhibits extravasation of neutrophils
- activates NADPH-dependent respiratory burst in neutrophils
- induces maturation of dendritic cells



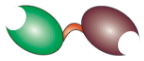
Galectin-2
- induces T-cell apoptosis under some conditions
- decreases IFNγ and TNFα while increasing IL-10 and IL-5
- involved in the pathogenesis of atheroma formation
- induces apoptosis-independent PS exposure (Ca^{++}-dependent) of neutrophils



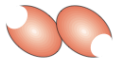
Galectin-4
- induces IL-6 production in T cells
- induces apoptosis-independent PS exposure (Ca^{++}-independent) of neutrophils



Galectin-7
- intracellular expression induces apoptosis of tumor cells
- extracellularly can inhibit growth of cells

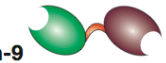


Galectin-8
- activates Rac-1 in T cells
- activates NADPH-dependent respiratory burst of neutrophils
- modulates integrin-mediated neutrophil adhesion of neutrophils

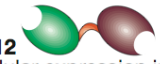


Galectin-10
- highly expressed in eosinophils (Charcot-Leyden crystal protein)
- involved in Treg function

Galectin-3
- blocks apoptosis of T cells when overexpressed intracellularly
- endogenously involved in T-cell viability
- extracellularly induces apoptosis of T cells
- promotes adhesion of thymocytes to thymic epithelium
- enhances Th2 immune responses
- enhances adhesion of naive T cells to DCs
- binds TCR, reducing TCR mediated T cell activation
- inhibits IL-5 production in eosinophils
- induces mast cell degranulation independent of antigen-mediated IgE stimulation
- exacerbates Th2 immune responses (asthma)
- expressed on surface of macrophages (also called Mac-2 antigen)
- enhances phagocytosis of macrophages
- enhances respiratory burst of macrophages
- enhances LPS-induced IL-1β secretion of macrophages
- inhibits apoptosis (intracellularly)
- blocks IL-4-induced survival of activated B cells
- favors plasma cell differentiation
- exhibits an anti-apoptotic role in B-cell lymphomas
- expression induced in dendritic cells by *T. cruzi* infection
- enhances pro-inflammatory cytokine release in endothelial cells
- expression up-regulated in tumor endothelial cells
- induces chemotaxis of neutrophils
- enhances extravasation of neutrophils
- activates NADPH-dependent respiratory burst of neutrophils
- induces activation of neutrophils
- induces release of IL-8 of neutrophils
- mediates interaction of neutrophils with laminin and fibronectin (both directly and indirectly)
- enhances leukocyte adhesion to endothelium

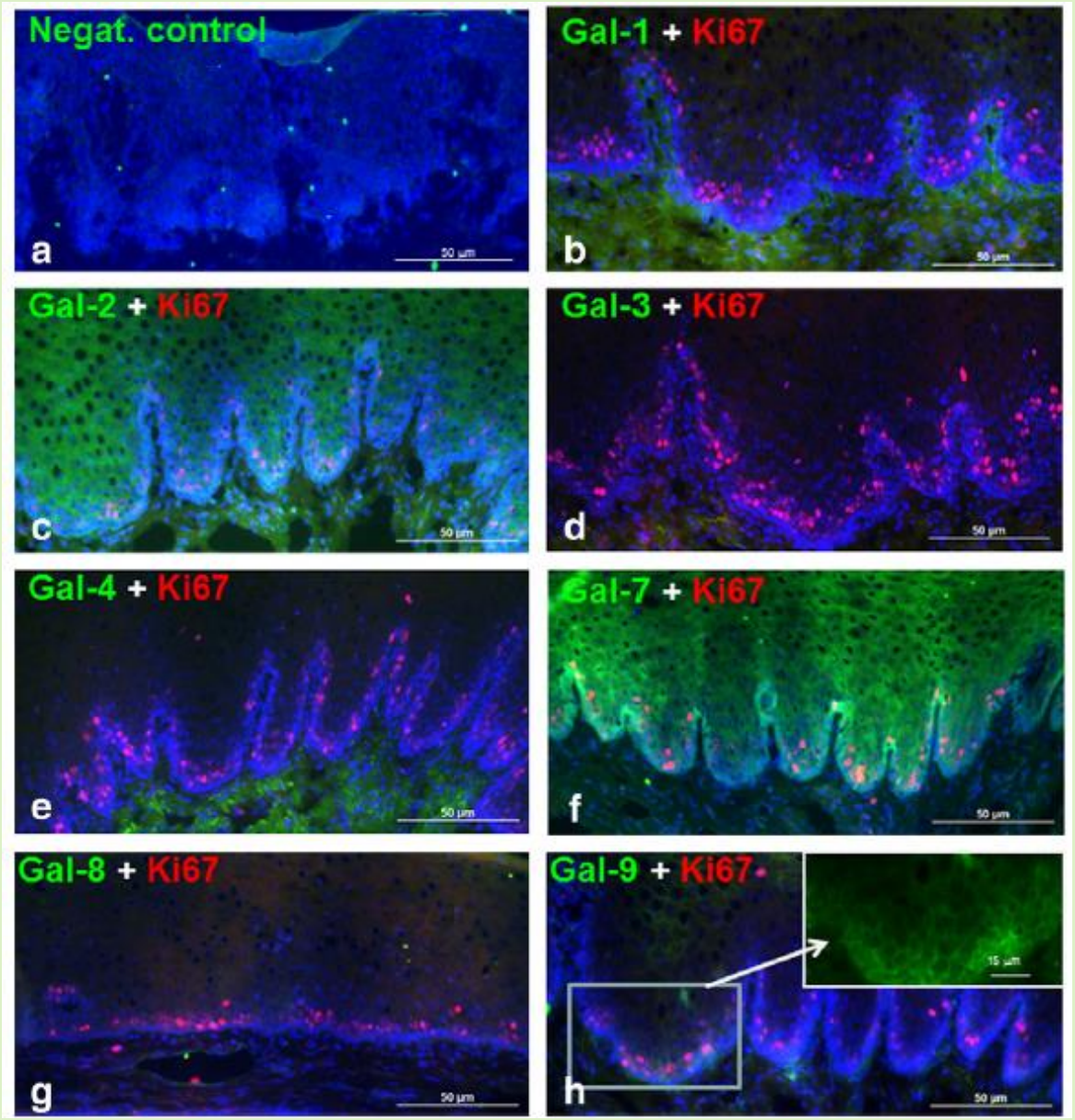


Galectin-9
- induces apoptosis in thymocytes and T cells
- induces selective loss of $CD4^+$ Th1 cells
- induces selective loss of $CD8^+$ T cells
- induces eosinophil chemotaxis, activation, superoxide generation
- induces moderate degranulation of eosinophil
- expression in endothelial cells induced by virus infection
- induces maturation of dendritic cells

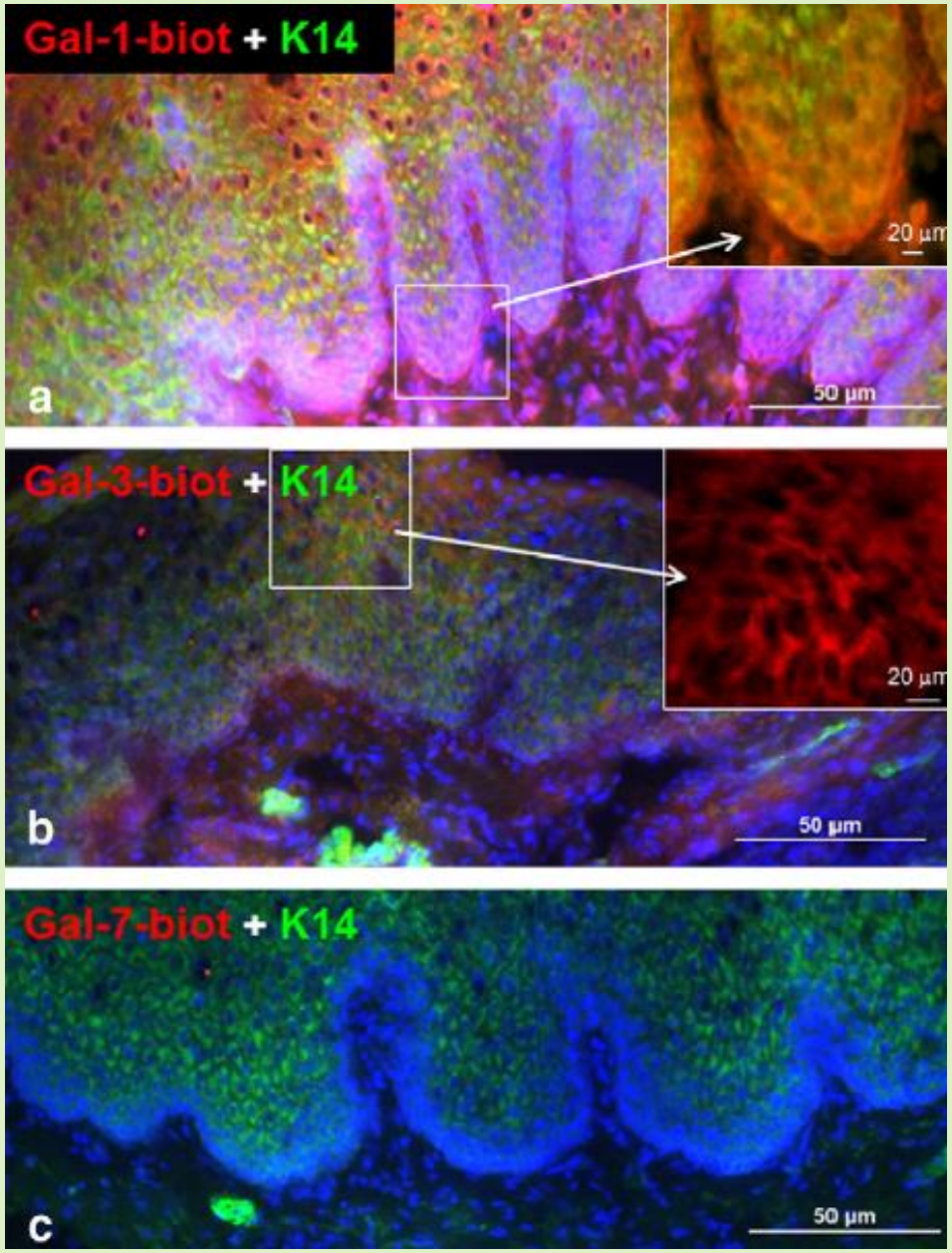


Galectin-12
- intracellular expression induces apoptosis of tumor cells
- can cause cell cycle arrest and growth suppression

Галектины в эпителиальных клетках



Сайты связывания галектинов в эпителии

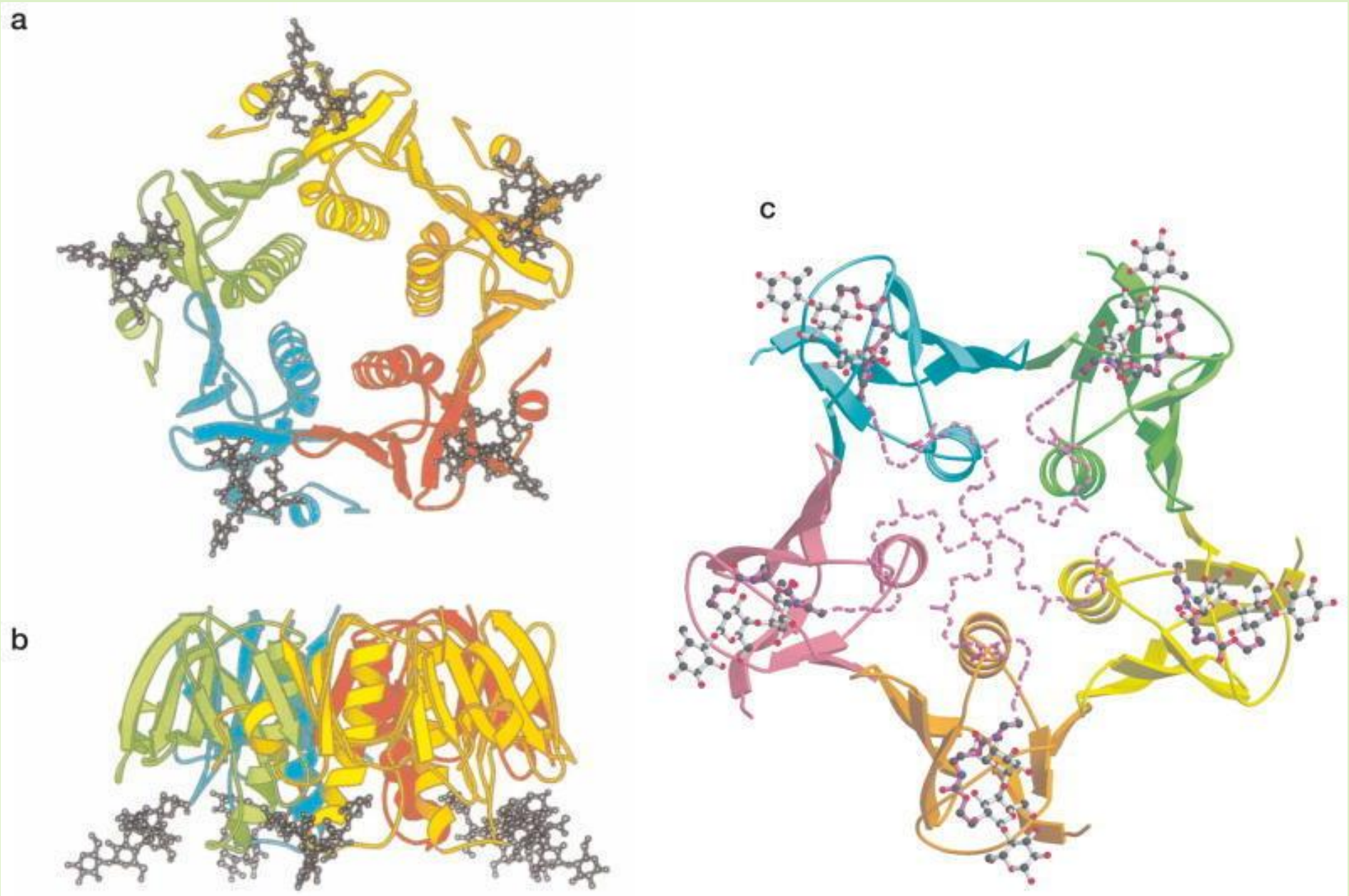


Бактериальные токсины

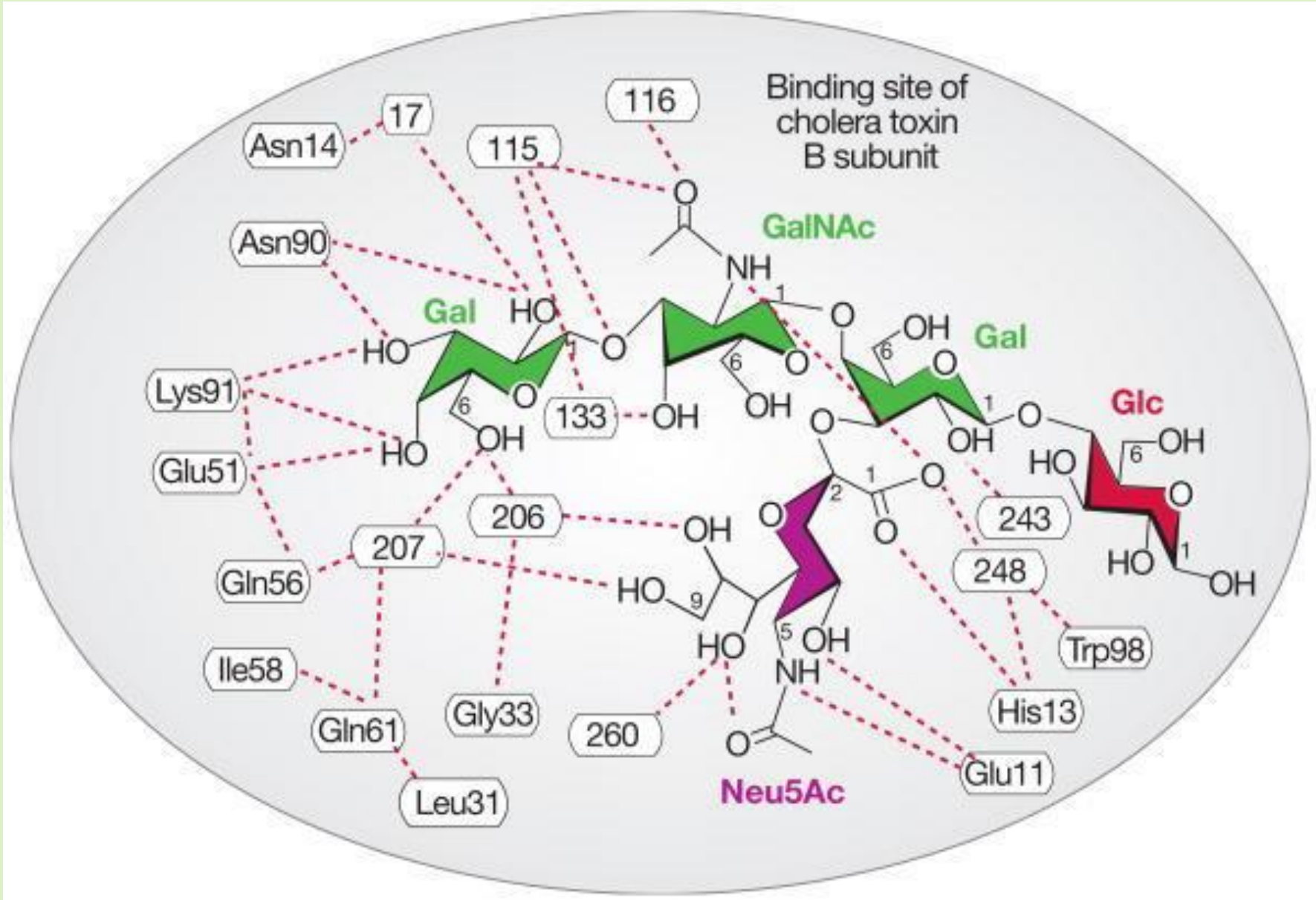
Бактериальные токсины

Clostridium tetani (tetanus toxin)	GD1b, GT1b
Clostridium botulinum (neurotoxin, type A-F)	GQ1b, GT1b, GD1a
Clostridium perfringens (δδ toxin)	GM2
Clostridium perfringens (ε toxin)	Neu5Ac
Vibrio cholera (cholera toxin)	GM1
Vibrio mimicus (hemolysin) ,	GD1a, GT1b
Vibrio parahemolyticus (thermostable direct hemolysin)	GT1
E. coli (heat-labile enterotoxin)	GM1
E. coli (heat-stable enterotoxin *b*)	sulfatide
Bordetella pertussis (pertussis toxin)	GD1a; Neu5Acα2-6Galβl-4GlcNAc
Staphylococcus aureus (*a* toxin)	Neu5AcGalGlcNAcGalGlc
Staphylococcus aureus (*y* hemolysin, leucocidin)	GM1

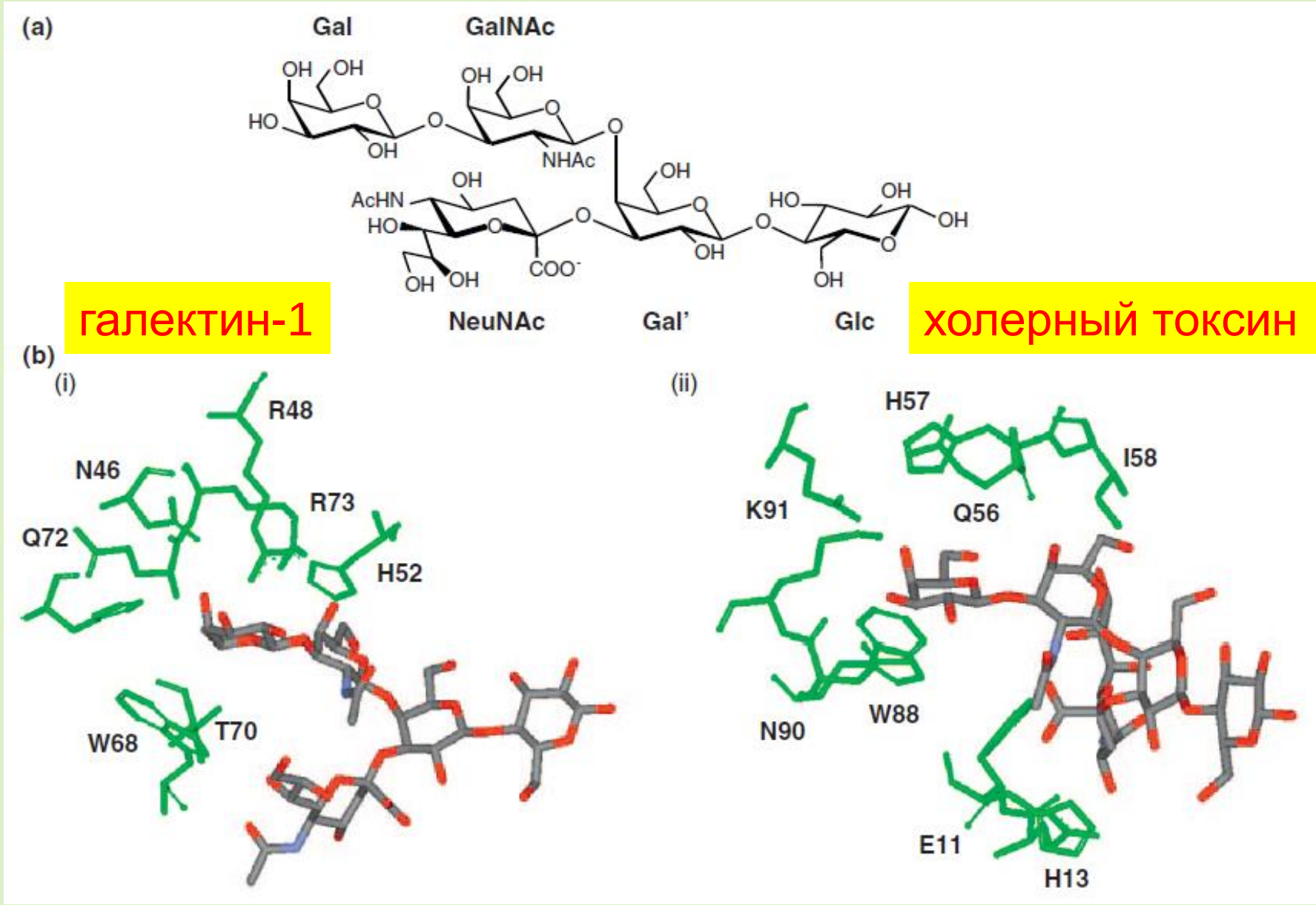
Пентасахарид GM1 и холерный токсин (пентамер)



Пентасахарид GM1 и холерный токсин



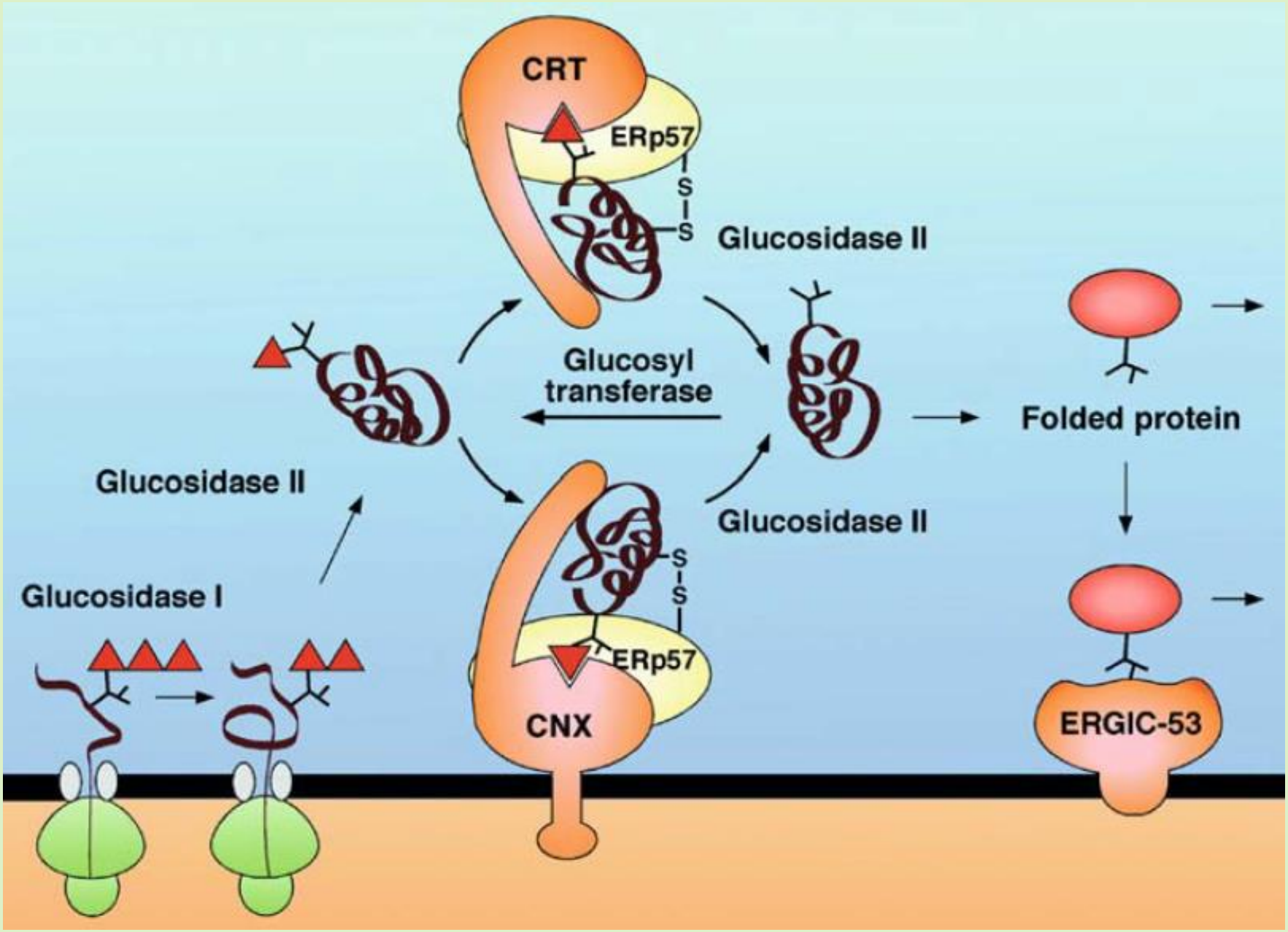
Селекция конформеров пентасахарида GM1 галектином-1 и холерным токсином



Лектины-шапероны

Лектины L-типа

Сворачивание белков в ЭПР: лектины-шапероны



Другие лектины

Цитокины связываются с углеводами

примеры	углеводная специфичность
IL-1α	$(Neu5Ac\text{-}Gal\text{-}GlcNAc\text{-}Man)_2Man\text{-}GlcNAc_2$
IL-1β	GPI якорь, GM4
IL-2	Man_5/Man_6
IL-4	Neu5Ac-1,7-лактон
IL-7	Neu5Acα2-6GalNAcα
TNFα	$Man_3/GlcNAc\beta 1\text{-}4GlcNAc$

Дефензины тоже связываются с углеводами...

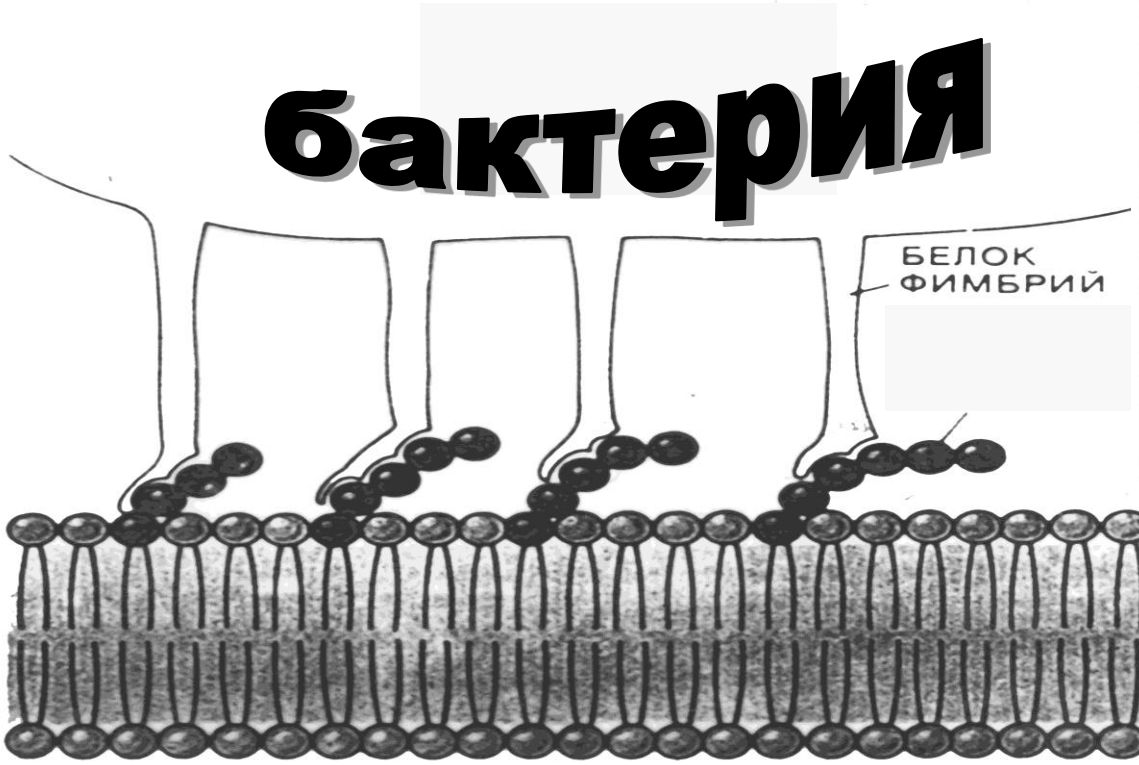
Межклеточная адгезия, опосредованная углеводами

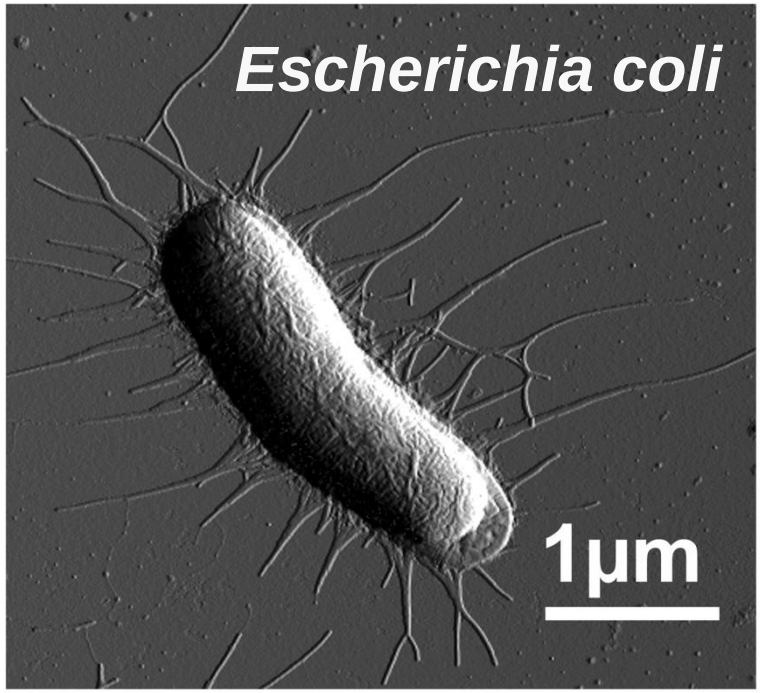
Число гликан-связывающих рецепторов с потенциальной ролью в адгезии клеток

Receptor family	Number of human receptors	Receptors with proposed roles in cell-cell adhesion
C-type lectin	24	L, E and P-selectin DC-SIGN Scavenger receptor C-type lectin?
Galectins	9	Several possible
Siglecs	13	Myelin-associated glycoprotein (MAG)

Узнавание углеводов клетки-хозяина лектином бактерии – первый этап адгезии

P-Фимбрии – PapG: галабиоза
Фимбрии типа 1 – FimH: манноза





7. *Comprehensive Glycoscience. From Chemistry to System Biology*, 2007, Ch. 3.28.3.1, p. 636 (2346).

22. *Glycoscience and Microbial Adhesion*. K. Lindhorst, S. Oscarson (Eds.), 2009, 186 pp.

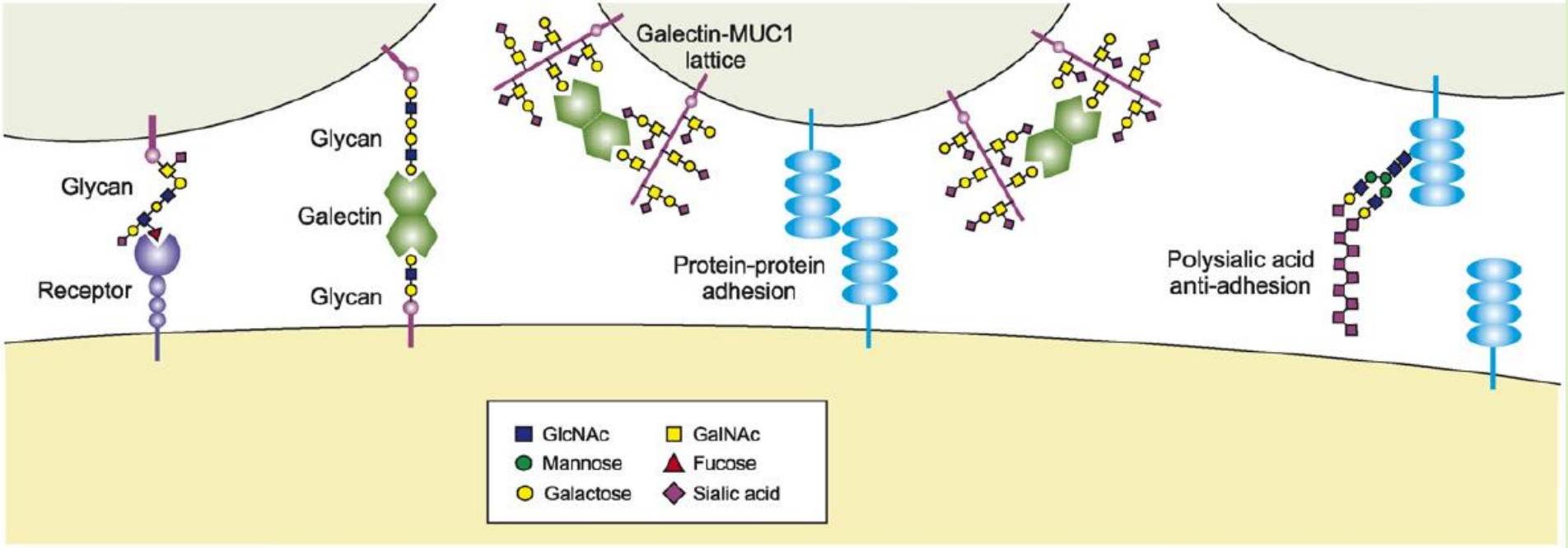
61. A. Bernardi, *et al*. Multivalent glycoconjugates as anti-pathogenic agents. *Chem. Soc. Rev.* **2013**, *42*, 4709.

Углеводы поверхности клеток – сайты присоединения бактериальных патогенов

Organism	Target tissue	Carbohydrate	Structure
E. coli Type 1	Urinary	Manα3Manα6Man	GP
E. coli P	Urinary	Galα4Gal	GL
E. coli S	Neural	NeuAc (α2-3)Galβ3GalNAc	GL
E. coli CFA/1	Intestinal	NeuAc (α2-8)	GP
E. coli F1C	Urinary	GalNAcβ4Galβ	GL
E. coli F17	Urinary	GlcNAc	GP
E. coli K1	Endothelial	GlcNAcβ4GlcNAc	GP
E. coli K99	Intestinal	NeuAc(α2-3)Galβ4Glc	GL
C. jejuni	Intestinal	Fucα2GalβGlcNAc	GP
H. pylori	Stomach	NeuAc(α2-3)Galβ4GlcNAc	GP
		Fucα2Galβ3(Fucα4)Gal	GP
K. pneumoniae	Respiratory	Man	GP
N. gonorrhoea	Genital	Galβ4Glc(NAc)	GL
N. meningitidis	Respiratory	[NeuAc(α2–3)] Galβ4GlcNAcβ3Galβ4GlcNAc	GL
P. aeruginosa	Respiratory	L-Fuc	GP
	Respiratory	Galβ3Glc(NAc)β3Galβ4Glc	GL
S. typhimurium	Intestinal	Man	GP
S. pneumoniae	Respiratory	NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc	GL
S. suis	Respiratory	Galα4Galβ4Glc	GL

GP = glycoprotein, GL = glycolipids

Адгезия клеток: модели взаимодействия гликан–рецептор

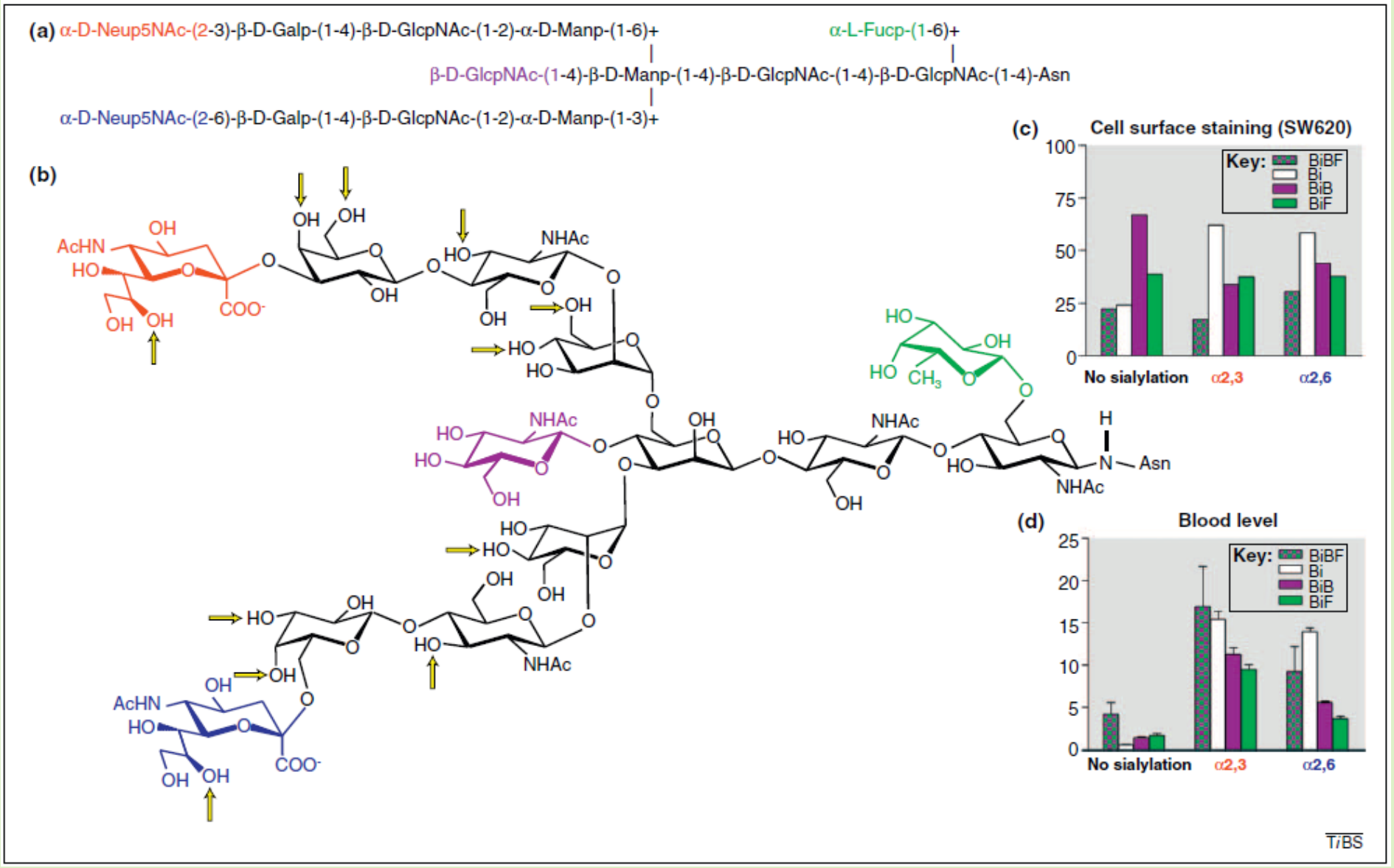


Механизм углевод-белкового связывания

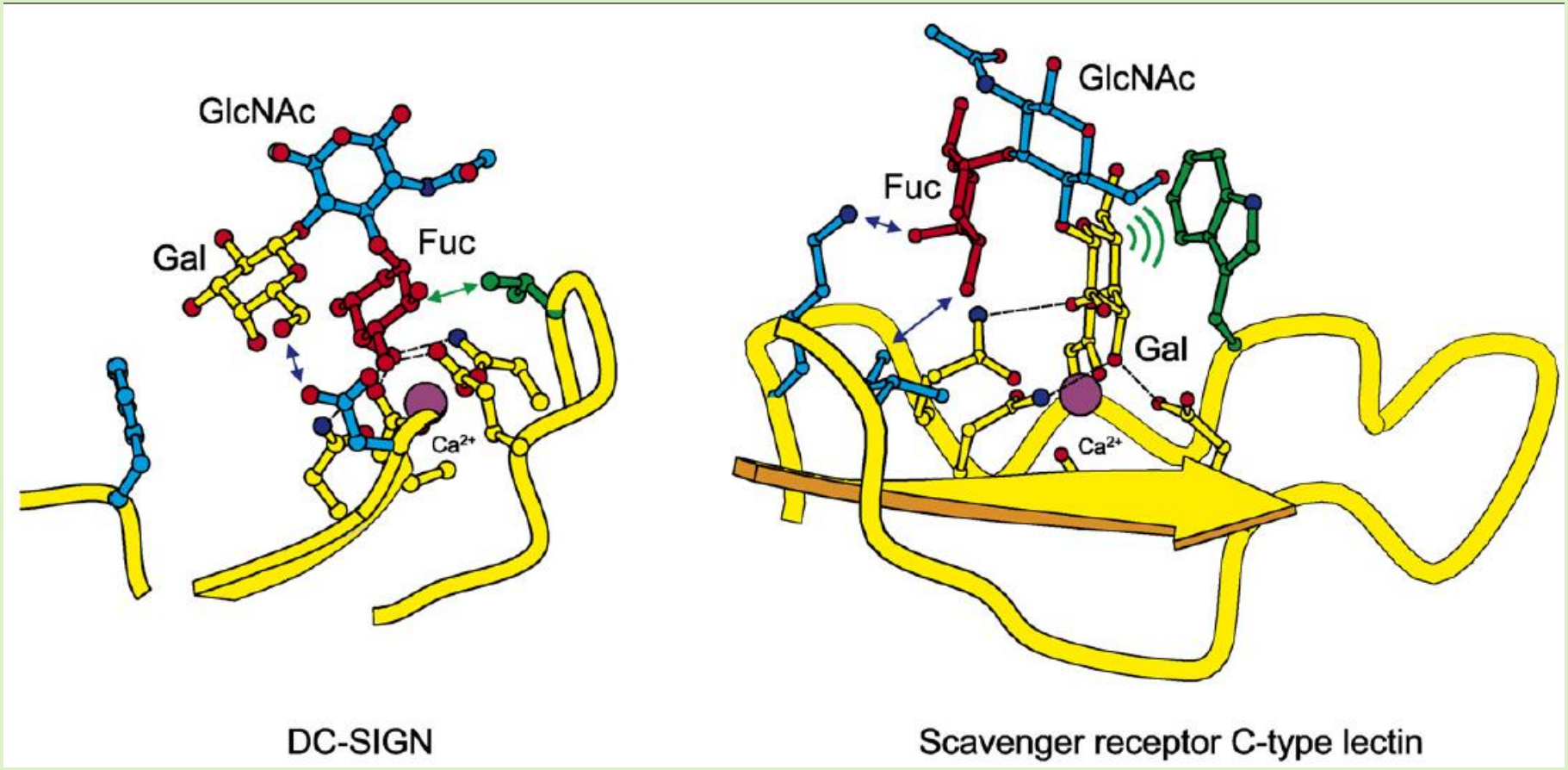
Шесть уровней регуляции аффинности связывания гликана с лектином

- 1. Mono- and disaccharides (including anomeric position and substitutions)
- 2. Oligosaccharides (including branching and substitutions)
- 3. Spatial parameters of oligosaccharides
  - a. Shape of oligosaccharide (differential conformer selection)
  - b. Conformational flexibility differences between isomers
- 4. Spatial parameters of glycans in natural glycoconjugates
  - a. Shape of glycan chain (examples: modulation of conformation by substitutions not acting as lectin ligand, such as core fucosylation or introduction of bisecting GlcNAc in N-glycans, influence of protein part)
  - b. Cluster effect with bi- to pentaantennary N-glycans or branched O-glycans (including modulation by substitutions, please see a.)
- 5. Cluster effect with different, but neighboring, glycan chains on the same glycoprotein (e.g. in mucins) or a glycoprotein–glycolipid complex (e.g. integrin–ganglioside complexes)
- 6. Cluster effect with different glycoconjugates on the cell surface in spatial vicinity forming microdomains

Влияние строения на связывание с лектинами



Два различных механизма связывания трисахарида Le^x с рецептором



Стратегическая роль Ca^{2+} в активности лектинов

Function	Lectin type
Structural role in stabilizing the lectin domain or organizing the site for ligand binding (no direct contact to ligand), oligomerization of subunits	Leguminous lectins homologous to concanavalin A, lectin chaperones involved in quality control (calnexin, calreticulin), animal lectin-type cargo receptors (i.e. ERGIC53b and VIP36c), *Anguilla anguilla* agglutinin, discoidin I
Structural role and direct contact to anionic group(s) of ligand or neutralization of repulsive forces between anionic charges in ligand and lectin	Pentraxins, laminin G-like module, annexin A2, cation-dependent mannose-6-phosphate receptor
Direct contact to neutral group(s) of ligand with/without structural role	Most C-type lectins, *Cucumaria echinata* lectin III (β-trefoil fold), *Pseudomonas aeruginosa* lectin I (two coordination bonds) and lectin II (four coordination bonds)

Как установить факт связывания белка с гликаном?

Установление структуры лектинов, факта и деталей комплексообразования

Box 1. Experimental approaches to determine lectin structure and complex formation[a]

Atomic force microscopy: measurement of binding strength under force for surface-presented lectin/ligand pairs indicates lectin potential to engage in transient or firm contacts.

Biodistribution: determination of clearance from serum and organ uptake of radioiodinated lectin after i.v. injection.

Chemical mapping: analysis of inhibitory potency of ligand derivatives (mostly deoxy, fluoro or *O*-methyl) in a binding assay yields information on hydrogen bonding contribution and steric aspects at each position.

Circular dichroism: monitoring yields insights into secondary structure elements; ellipticity changes at distinct wavelengths reflect involvement of aromatic amino acid residues in ligand binding.

Fluorescence-activated cell scanning (cytofluorometry): binding of labeled lectin to cell surfaces (measuring mean fluorescence intensity and percentage of positive cells) characterizes the glycophenotype; when performed with a panel of lectins (Table 3), this is termed glycophenotyping.

Fluorescence correlation spectroscopy: monitoring of translational diffusion of a fluorescent protein in solution indicates shape properties and detects ligand-induced changes.

Fluorescence titration: quenching of intrinsic protein fluorescence (from Tyr and especially Trp residues) by ligand presence indicates alterations in their microenvironment/surface presentation and/or direct ligand contact.

Gel filtration: elution profile yields information on hydrodynamic behavior as an indication of quaternary structure and respective influence of the ligand.

Hemagglutination: erythrocyte agglutination to determine inhibitory potential by saccharides (classical assay for lectin activity). Note: the glycomic profile on erythrocytes varies.

Isothermal titration calorimetry: measurement of heat released or absorbed by ligand binding enables determination of association constant (K_a), stoichiometry (n) and enthalpy of binding (ΔH°), thereby facilitating calculation of entropy (ΔS°).

Lectin cyto- and histochemistry: localization of lectin-reactive sites in cell preparations and frozen/fixed tissue sections.

Saturation transfer difference NMR spectroscopy: magnetization transfer (spin diffusion) from, in this example, saturated protein to ligand protons maps spatial vicinity (below 5 Å).

Small angle neutron/X-ray scattering: spectrum yields information on quaternary structure and shape and detects ligand-induced changes.

Surface plasmon resonance: measurement of changes in refractive index near a planar surface presenting lectin/ligand in resonance units (RUs; 1000 RUs equal 1 ng of mass per mm^2) leads to equilibrium and kinetic constants.

Transferred nuclear Overhauser effect spectroscopy: signal generation by through-space dipolar interaction between two ligand protons, preferably separated by a glycosidic bond, helps to define bound-state topology.

Ultracentrifugation: relative protein sedimentation reflects average molecular mass (equilibrium analysis) and hydrodynamic parameters (velocity analysis).

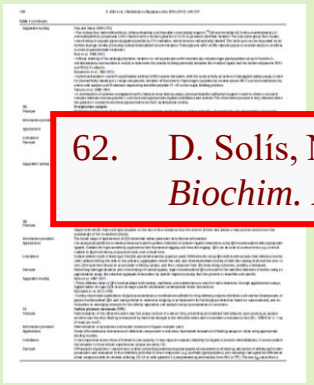
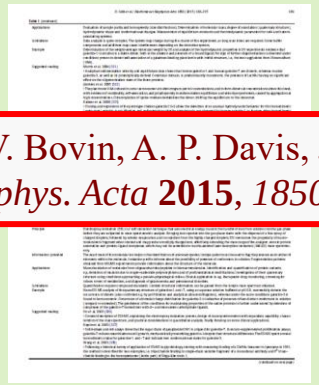
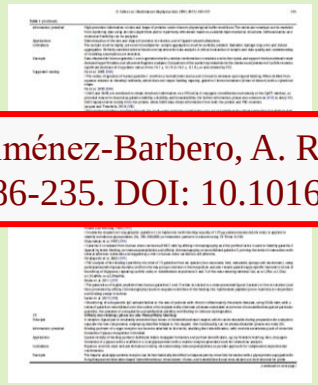
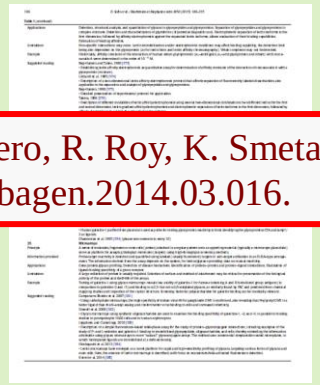
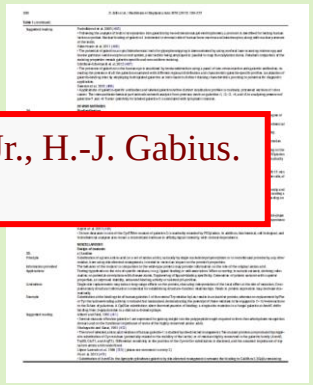
X-ray crystallography: diffraction pattern yields electron density map; crystallization might require unphysiological conditions, packing can cause artifacts and will preclude the separation of static from dynamic disorder.

[a]See also Figure 4, where experimental data for these techniques are presented.

Экспериментальные методы изучения углевод-белковых взаимодействий



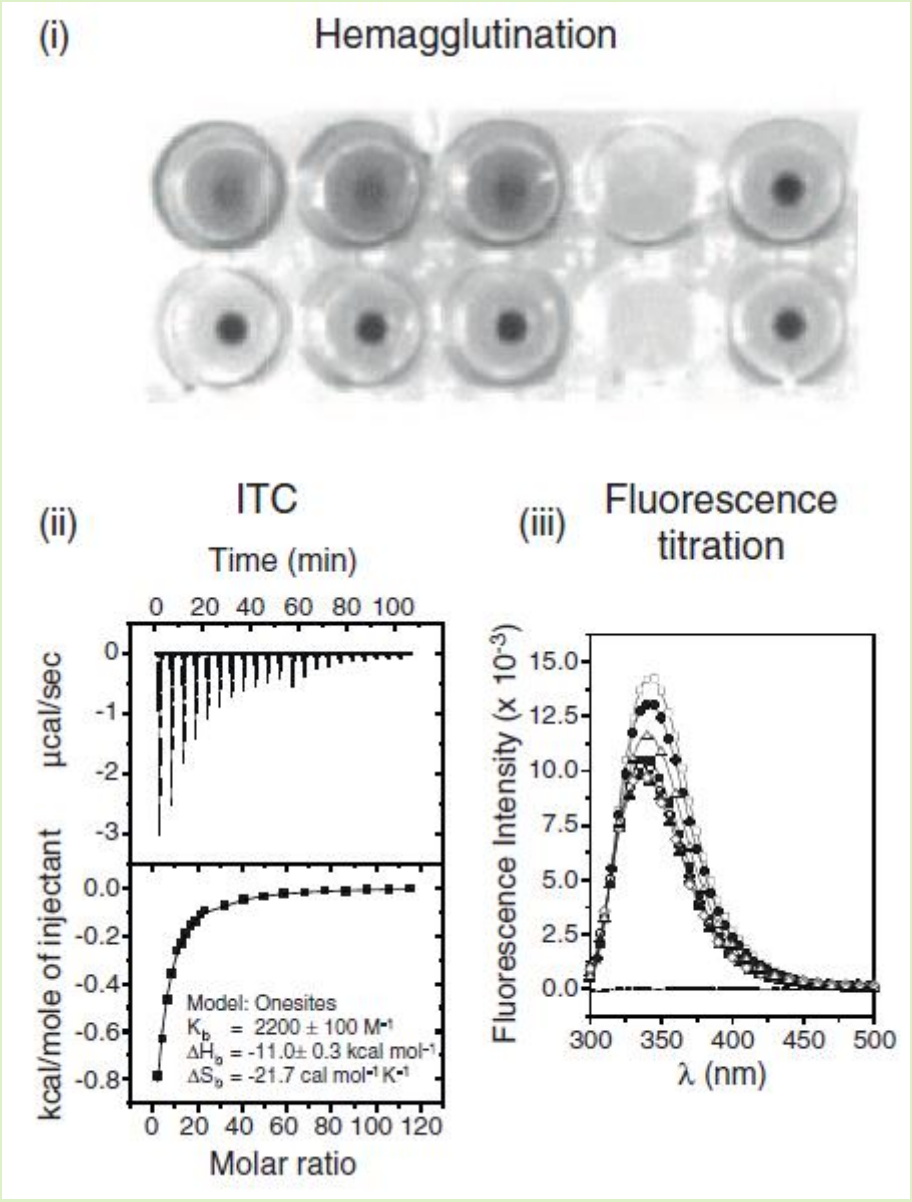


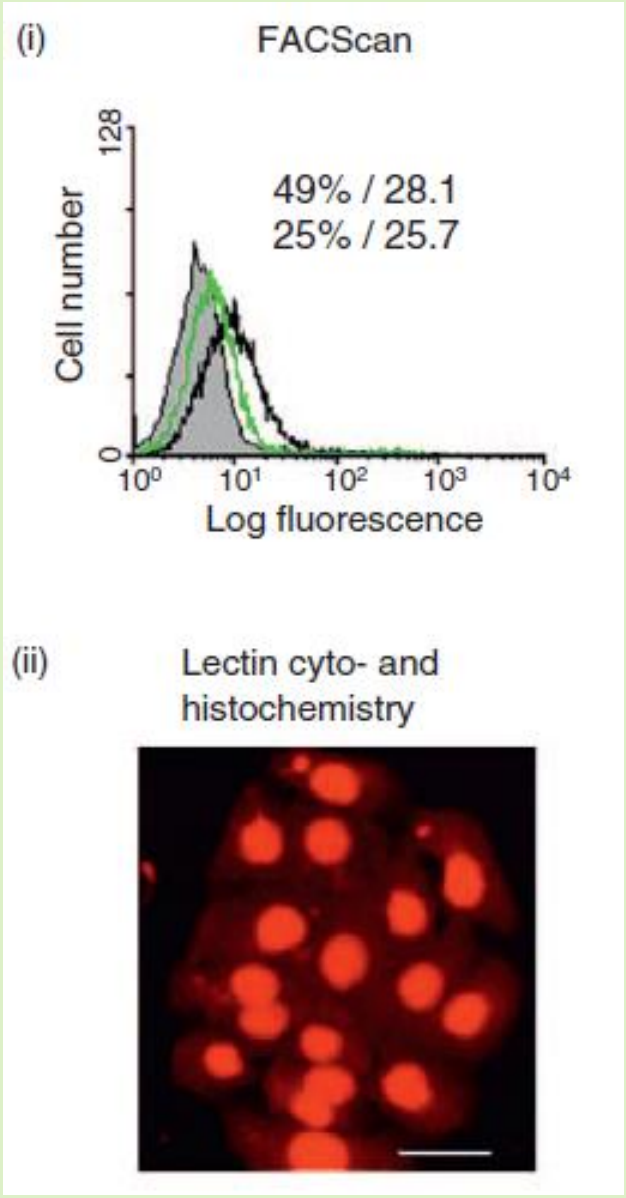









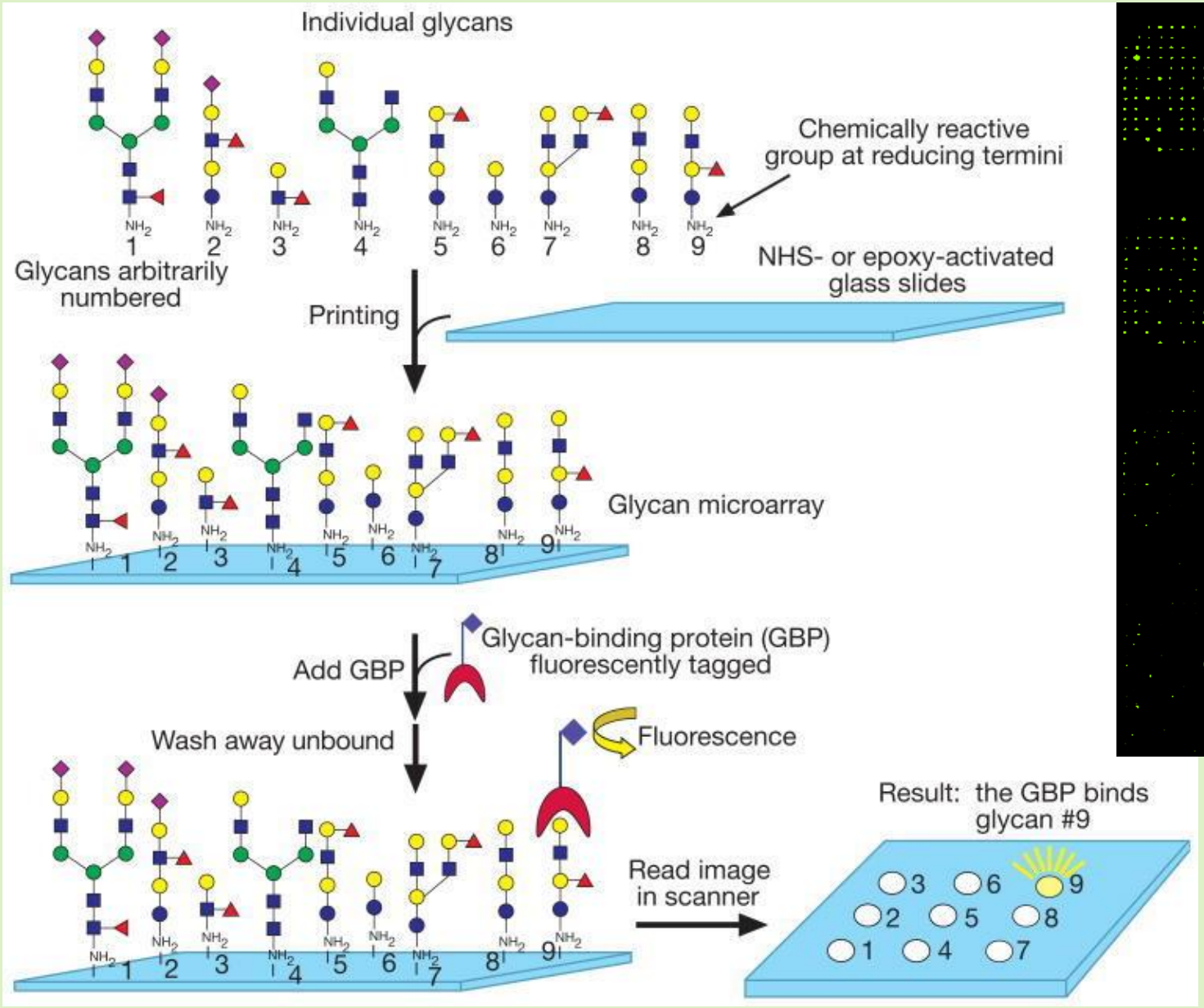

62. D. Solís, N. V. Bovin, A. P. Davis, J. Jiménez-Barbero, A. Romero, R. Roy, K. Smetana Jr., H.-J. Gabius. *Biochim. Biophys. Acta* **2015**, *1850*, 186-235. DOI: 10.1016/j.bbagen.2014.03.016.

Выявление связывания белков с гликанами

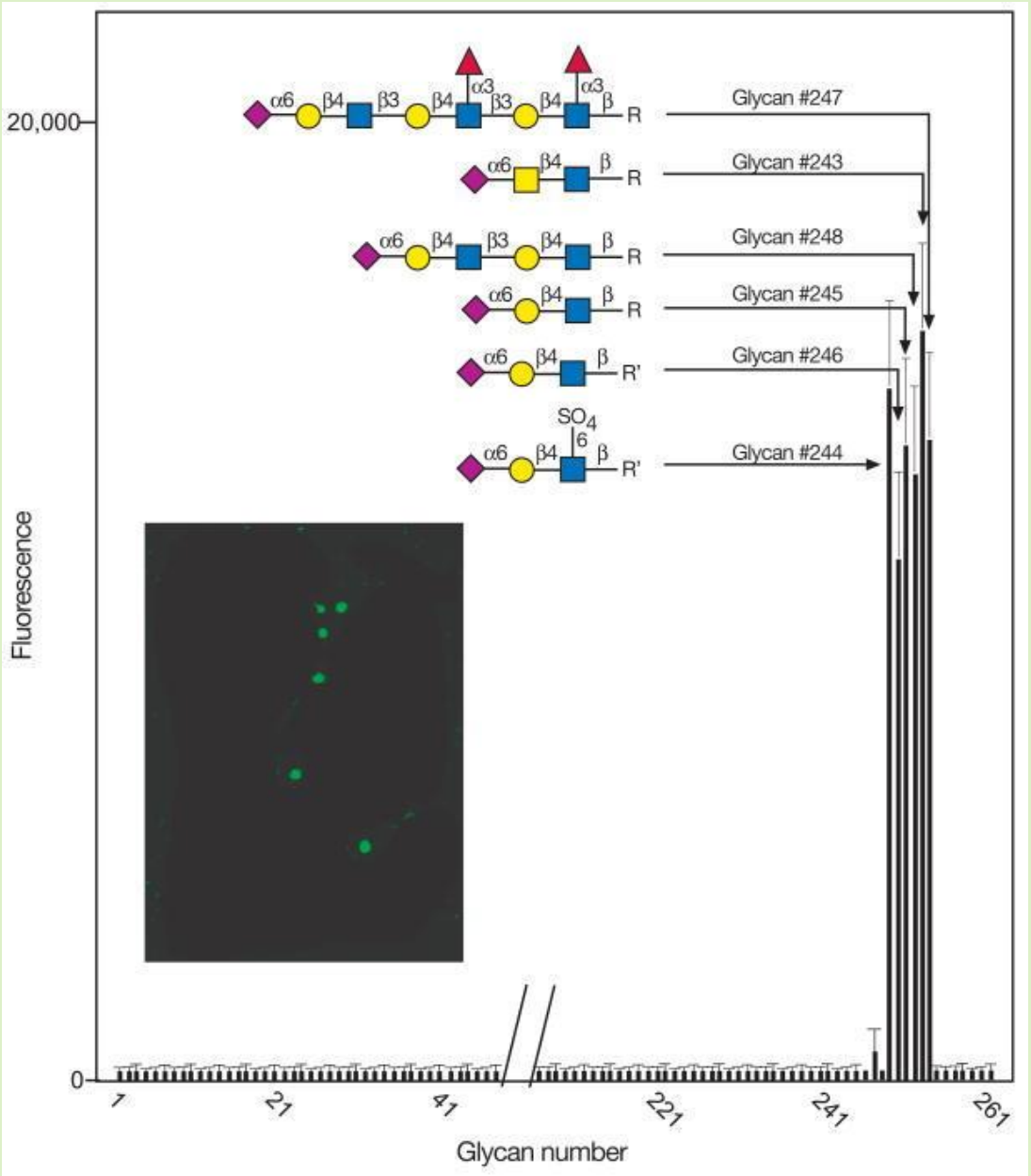




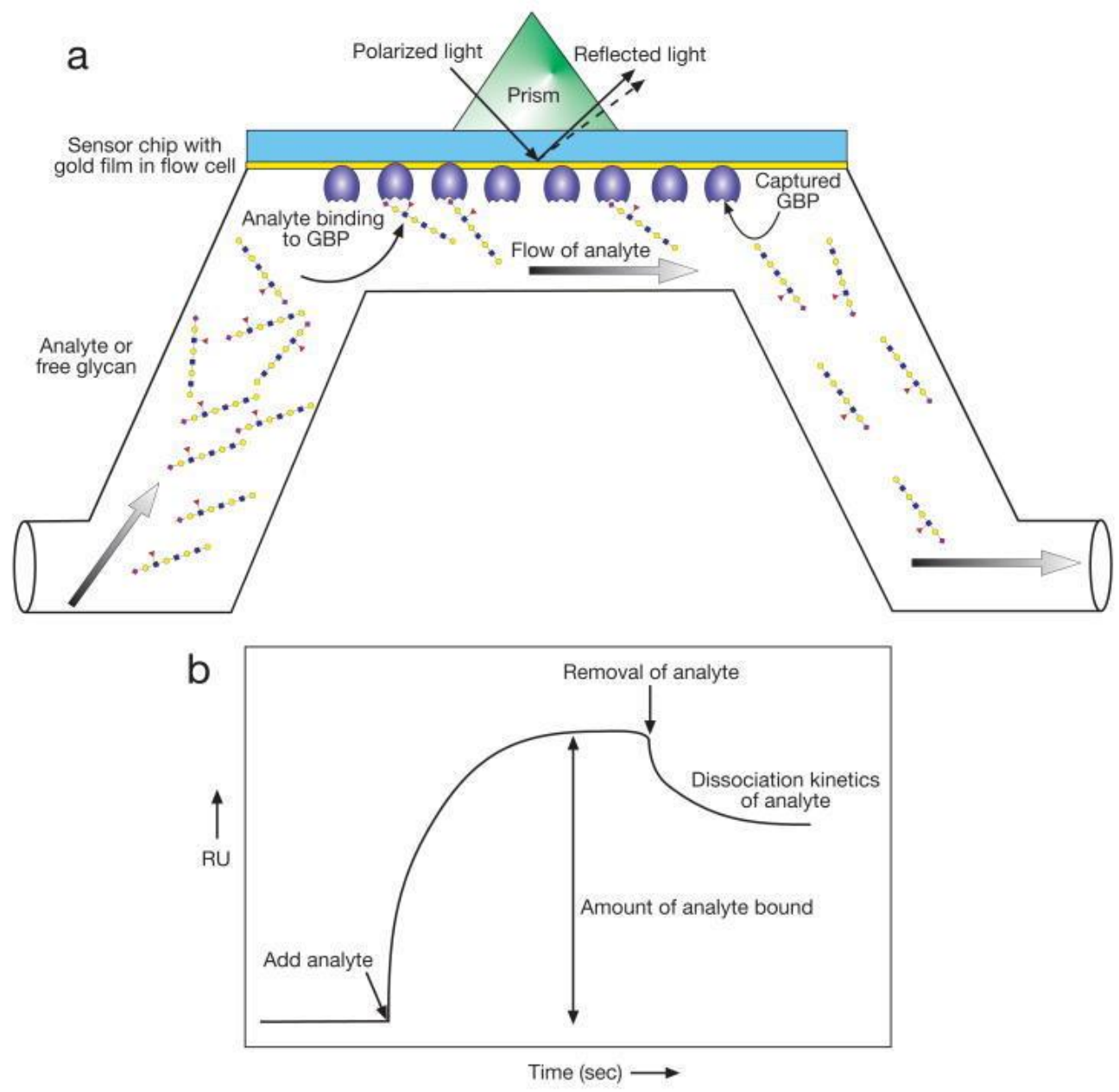
«Гликочип» – основной инструмент



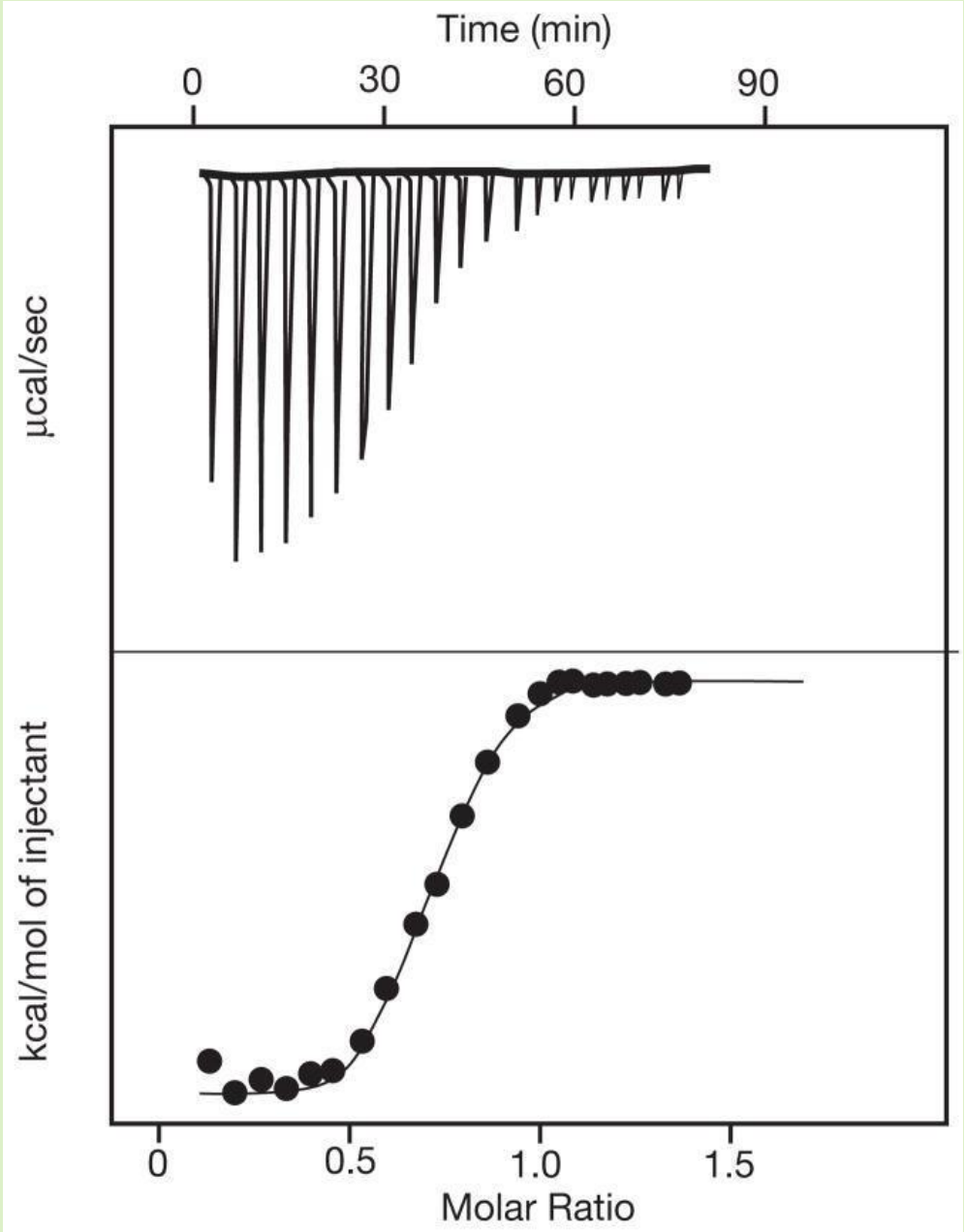
Связывание вируса гриппа А с гликанами, иммобилизованными на «гликочипе»



Поверхностный плазмонный резонанс (SPR)



Микрокалориметрия



$$\mathrm{Lectin\,(L)} + \mathrm{Glycan\,(G)} \underset{K_2}{\overset{K_1}{\rightleftarrows}} \mathrm{LG}$$

$$K_\mathrm{a} = [\mathrm{LG}]/[\mathrm{L}][\mathrm{G}] = k_1/k_2$$

$$K_\mathrm{d} = [\mathrm{L}][\mathrm{G}]/[\mathrm{LG}] = 1/K_\mathrm{a} = k_2 k_1$$

$$\Delta G_\mathrm{o} = RT \ln K = \Delta H - \mathrm{T}\Delta S$$

Конец лекции 6

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