Carbohydrate biomaterials in biomedicine. The present and the future.

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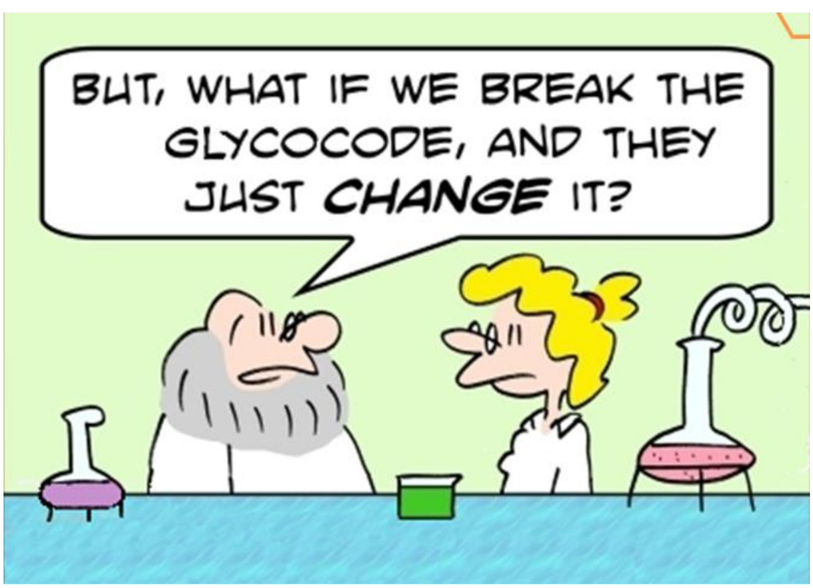


Introduction to carbohydrates

characteristic, *classification,*

structures

Outline

1. Basic characteristic of carbohydrates
2. Enzymatic synthesis of carbohydrates:
   glycosyltransferases, glycosidases
3. Glyco-nanomaterials

Recommended literature

J. McMurry: Organic Chemistry; Brooks/Cole Publishing, 1995

T. K. Lindhorst: Essentials of carbohydrate chemistry and biochemistry; Wiley, 2007 (3rd ed.)

P. Bojarová-Fialová, V. Křen: Enzymatic approaches to *O*-glycoside introduction: Glycosidases. In *Comprehensive Glycoscience* (J. P. Kamerling, Ed.); Elsevier: Oxford, 2007, Vol. 1, pp. 453-487.

P. Bojarová, V. Křen: Glycosidases: a key to tailored carbohydrates. *Trends Biotechnol*. 2009, *27*, 199.

K. Slámová, P. Bojarová, L. Petrásková et al.: β-*N*-Acetylhexosaminidase: What's in a name…? *Biotechnol. Adv*. 2010, *28*, 682.

T. Desmet, W. Soetaert, P. Bojarová, et al.: Enzymatic glycosylation of small molecules: Challenging substrates require tailored catalysts. *Chem. Eur. J*. 2012, *18*, 10786.

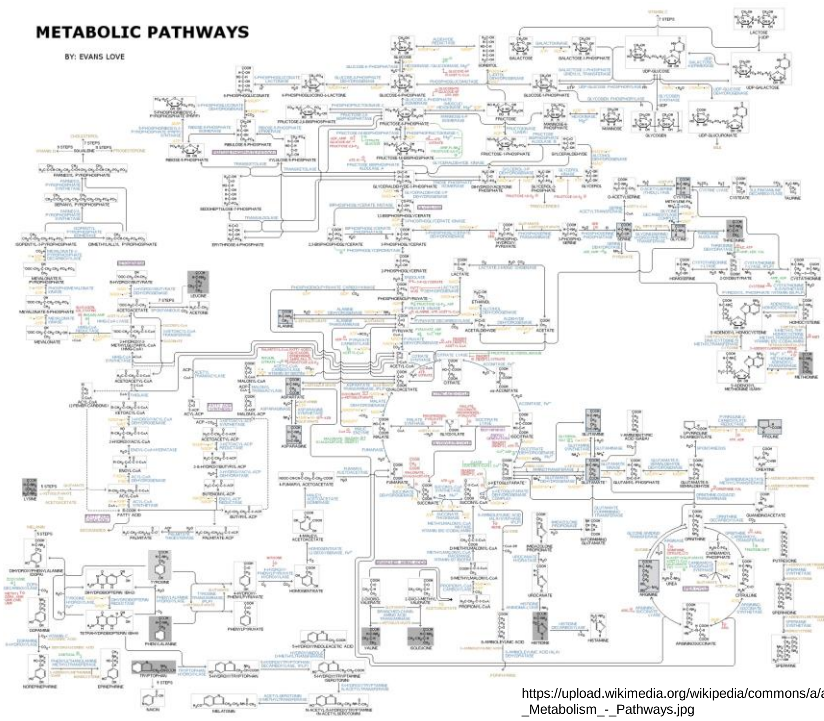
P. Bojarová, R. R. Rosencrantz, L. Elling, et al.: Enzymatic glycosylation of multivalent scaffolds. *Chem. Soc. Rev*. 2013, *42*, 4774.

P. Bojarová, V. Křen: Sugared biomaterial binding lectins: achievements and perspectives. *Biomater. Sci.*, 2016, *4*, 1142.

Carbohydrates
Introduction, definition

- Lat. *saccharum* = sugar
- empirical formula $\mathbf{C_m(H_2O)_n}$ (carbon, hydrogen, oxygen); also N and S
- **polyhydroxyaldehydes** (aldoses, CHO)

  or **polyhydroxyketones** (ketoses, C=O)
- the most prolific natural compounds
- metabolic precursors of most other molecules
- chemical characteristics:
  - (1) Presence of at least one centre of asymmetry
  - (2) Both linear and cyclic forms
  - (3) Natural polymers via glycosidic bonds
  - (4) Hydrogen bonds with water or other molecules



https://upload.wikimedia.org/wikipedia/commons/a/a8/Human_Metabolism_-_Pathways.jpg

Carbohydrates
Introduction, definition

- macromolecular polysaccharides tasteless, badly water soluble (starch, agar) or insoluble (cellulose)
- **sugars** = low-molecular soluble carbs, sweet
- relative sweetness scale: sucrose – 100 %

Saccharide	Swetness (%)
Sucrose	100
Fructose	173
Glucose	74
Sorbitol	48
Maltose	32
Rhamnose	32
Galactose	32
Lactose	16

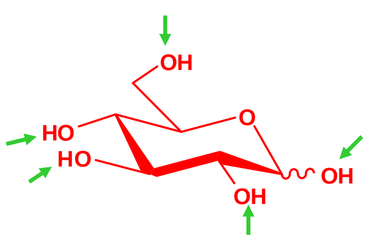
Not sweet

- **honey** – the first sweet substance known to humans
  - originally ritual and medical substance, not food (only in Ancient World)
  - work-up of blossom nectar:
    1. reduction of water content to 15-19%
    2. hydrolysis of most sucrose to Glc (31 %) and Fru (38 %) (invertase)
    3. some Glc oxidized to gluconic acid (glucose oxidase)
  - emulsion of microcrystals of hydrates of Glc and Fru in a thick syrup
  - nowadays its importance beaten by sugar cane and sugar beet

Carbohydrates
Function

- Plants and other autotrophs create them by photosynthesis X other organisms dependent on nutrition (245-499 g/d – central Europeans ca 50% excess)
- Until 1960s recognized mainly as a structural element and energy source
- **Functions in nature**:
  1. Energy source (glucose, fructose)
  2. Energy storage (starch, glycogen, inulin)
  3. Protective and building material (cellulose, chitin)
  4. Information and recognition functions (blood groups, fertilization, immunity)
  5. Components of complex compounds (nucleic acids, hormones, coenzymes)
- Industrial material (paper, textile fibers, ethanol, antibiotics, …)

Carbohydrates
Information code



Two identical hexopyranoses ⇒ **11** different disaccharides

X

Two identical aas ⇒ **1** dipeptide

Oligomer	**Compound type**	**Possible oligopeptides**	**Possible oligosaccharides**
Dimer	AA/AB	**1**/2	**11**/20
Trimer	AAA/ABC	1/6	120/720
Tetramer	AAAA/ABCD	1/24	1424/34560
Pentamer	AAAAA/ABCDE	1/12	17872/2144640

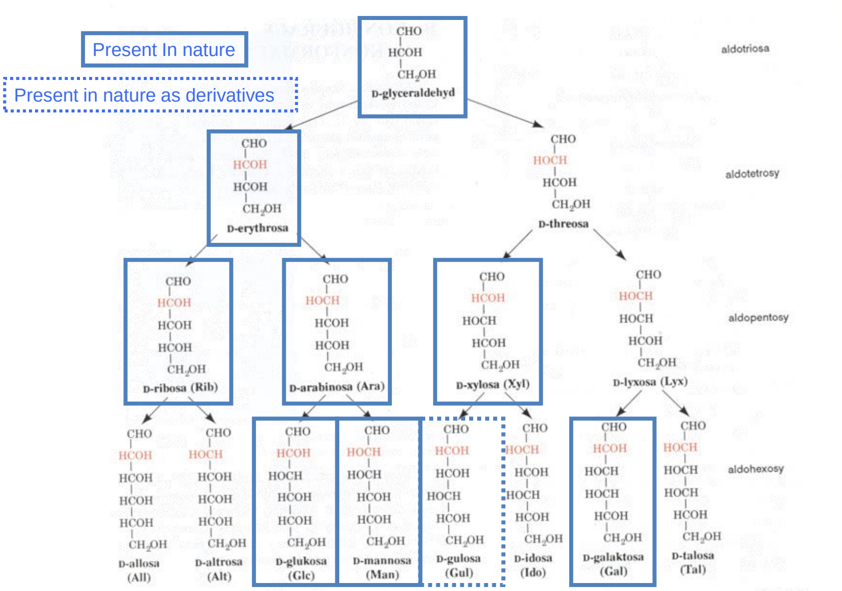
Carbohydrates
Classification

Monosaccharides: one sugar unit (aldoses, ketoses)
- contain asymmetric carbs (*n* isomeric centers ⇒ 2^n stereoisomers)

Oligosaccharides: 2-10 carbohydrate units
- disaccharides - 2 units (sucrose, maltose, lactose, trisaccharides - 3 units (raffinose), etc

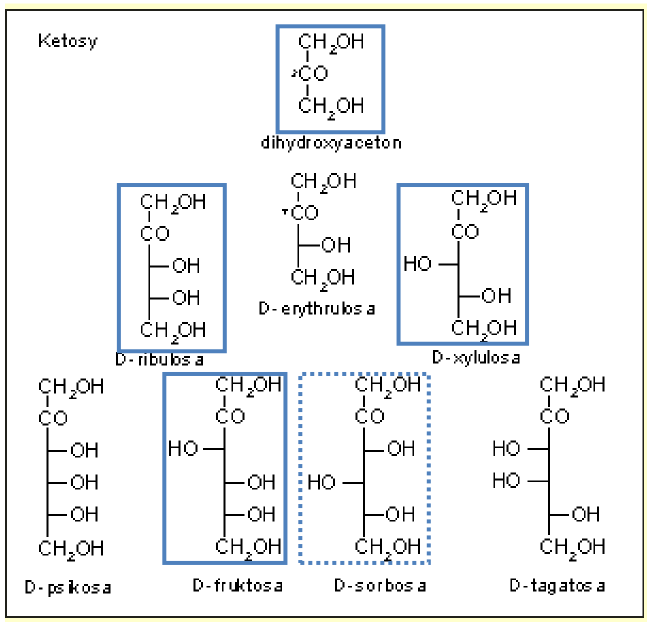
Polysaccharides (glycans): > 10 carbohydrate units
- LMW: soluble starch
- HMW (polymers): starch, glycogen, celullulose, chitin), M = až 10^6
- linear (starch) or branched (amylopectin) – the only branched biopolymers!
- homo- or heteropolysaccharides (chondroitin, hyaluronan)

Aldoses



Obr. 10-1
Stereochemické vztahy mezi D-aldosami s třemi až šesti uhlíkovými atomy. Konfigurace na druhém uhlíkovém atomu (*červené*) rozlišuje párové sacharidy.

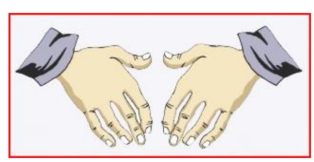
Ketoses



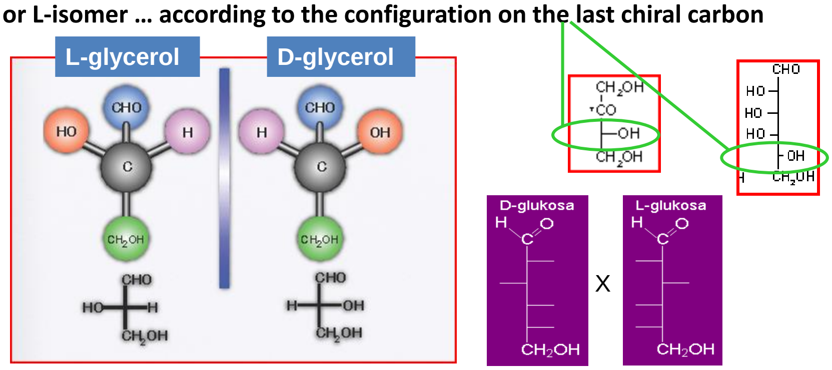
Carbohydrates

Basic terms: ENANTIOMERS

- *optical isomers (enantiomers)* – mirror image, optically active (rotate polarized light)
- dextrorotatory (+) – D+; levorotatory (-) L-
- racemic mixture indicated as DL- or (±)

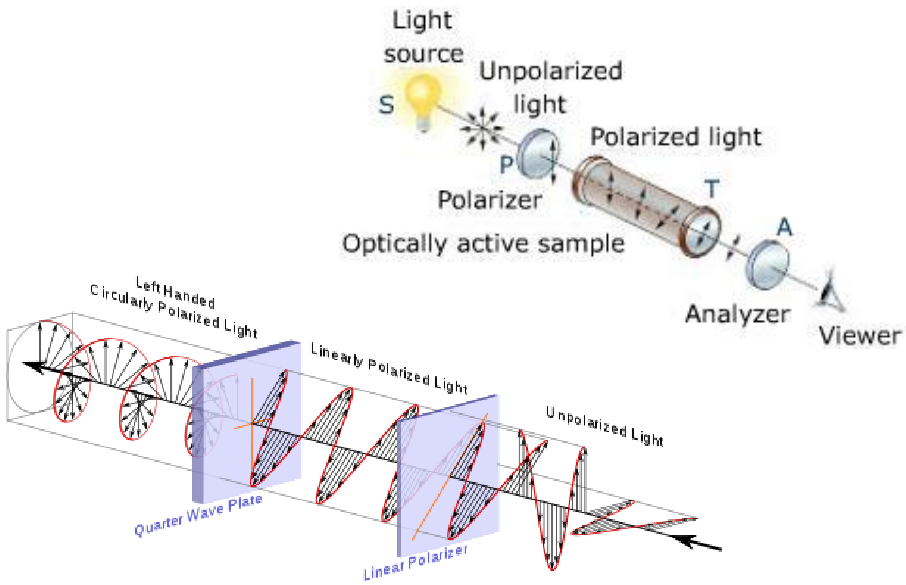


-D- or L-isomer … according to the configuration on the last chiral carbon



Carbohydrates

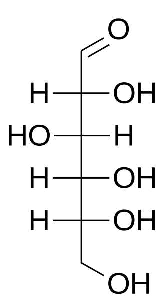
Basic terms: ENANTIOMERS



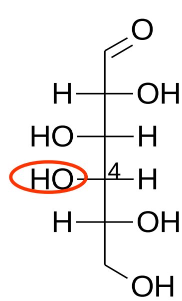
Carbohydrates

Basic terms: EPIMERS

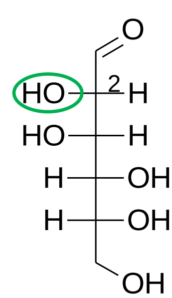
Epimer = differs by configuration at one chiral carbon



D-glucose



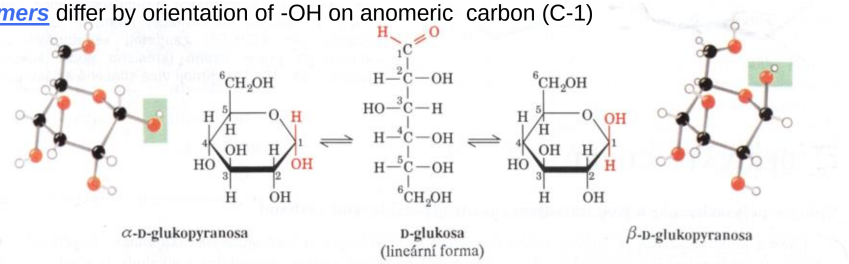
D-galactose
4-epimer of D-glucose



D-mannose
2-epimer of D-glucose

Carbohydrates
Basic terms: ANOMERS

- Reaction of –CHO with -OH at C-4 (furanoses) or C-5 (pyranoses) affords cyclic hemiacetals
- new asymmetric centre: **alpha and beta anomer**
- ***anomers*** differ by orientation of -OH on anomeric carbon (C-1)

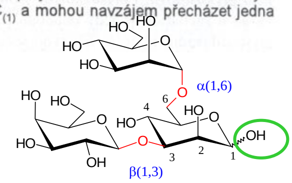


Obr. 10-5
Anomerní monosacharidy α-D-glukopyranosa a β-D-glukopyranosa v Haworthově projekci a znázorněné kuličkovým modelem. Tyto pyranosy se liší pouze konfigurací na anomerním uhlíkovém atomu $C_{(1)}$ a mohou navzájem přecházet jedna v druhou přes lineární formu.

- Glycosidic bonds are **α-** or **β-**
- anomeric -OH blocked: **non-reducing sugar**

X

- anomeric -OH free: **reducing sugar**



Carbohydrates
Basic terms: (NON)REDUCING SUGAR

- reducing properties

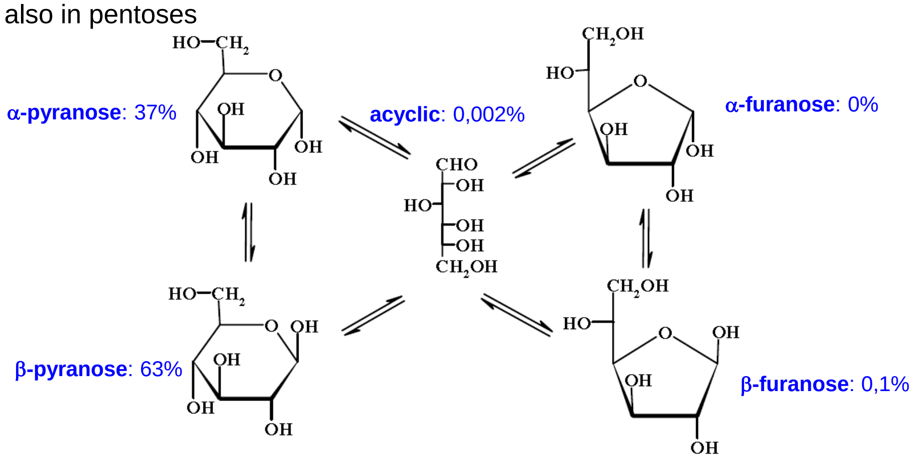
Fehling reagent ($Cu^{2+} \rightarrow Cu^{+}$)

Tollens reagent ($Ag^{+} \rightarrow Ag^{0}$)

Carbohydrates

Basic terms: MUTAROTATION

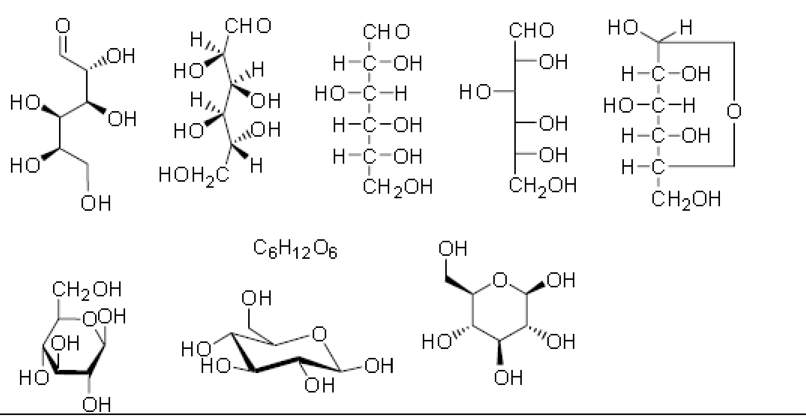
- lineár form in a low cocentration in solution
- ***mutarotation:*** balance between linear form and **cyclic structures** (α,β-pyranoses, α,β-furanoses)
- also in pentoses



Carbohydrates

FORMULAS

How to depict D-glucose?

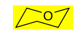


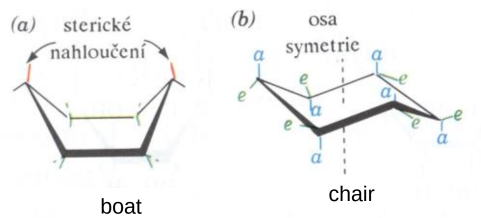
configuration prefix „*gluco*“ expresses stereochemistry
(2S,3R,4S,5S,6R)-6-(hydroxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetr(a)ol

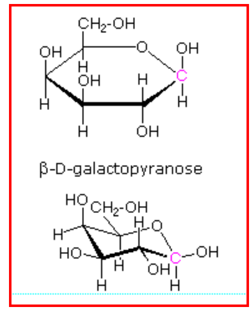
Carbohydrates

FORMULAS

Conformation formulas

- the most stable is pyranose form „chair“: $^4C_1$  and $^1C_4$ 





- the most stable of hexoses: β-D-glucopyranose

Enzymatic synthesis of glycosidic bond: glycosyltransferases

Enzymatic synthesis in carbohydrate chemistry

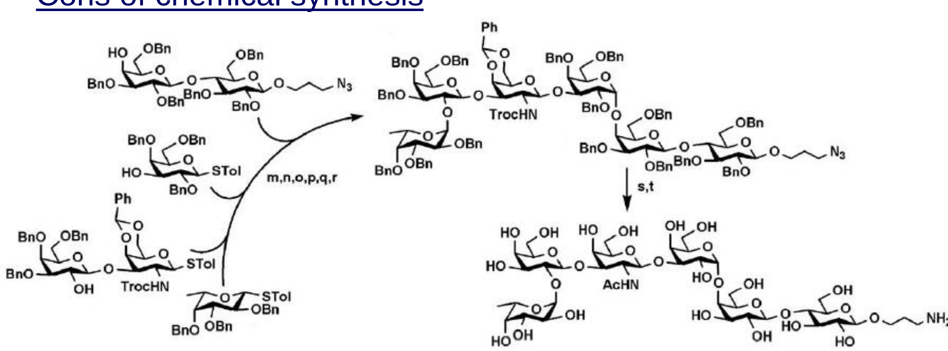
Cons of chemical synthesis

- Many (de)protection steps – groups of the same reactivity
- Organic solvents, silikagel, toxic catalysts (Hg, Cd, Ag; strong acids/ bases, cancerogens).
- Low overall yield despite almost-quantitative individual steps – many steps needing purifications

1	step 90 %	overall yield 90 %
5	steps 90 %	overall yield 60 %
10	steps 90 %	overall yield 35 %
20	steps 90 %	overall yield 12 %

Enzymatic synthesis in carbohydrate chemistry

Cons of chemical synthesis



Hexasaccharide Globo H

(m) *p*-TolSCl, AgOTf, TMSOTf, 78 °C; (n) TTBP, 78 to 20 °C; (o) p-TolSCl, AgOTf, 78 °C; (p) TTBP, 78-20 °C; (q) p-TolSCl, AgOTf, 78 °C; (r) TTBP, 78-20 °C; (s) NaOH, THF; Ac_2O, py, DMAP; (t) trimethylphosphine (PMe_3), THF, NaOH; H_2, Pd(OH).

Enzymatic synthesis in carbohydrate chemistry

Why enzymatic synthesis?

- High selectivity, low number of steps (1 – 2)
- Sensitive substrates (natural compounds…)
- Deprotected reagents and product
- Water medium
- Recyclable separation materials (GPC)
- Libraries of products (glycorandomisation)
- Green chemistry, SuSy, cell factories

Enzymová syntéza v cukerné chemii

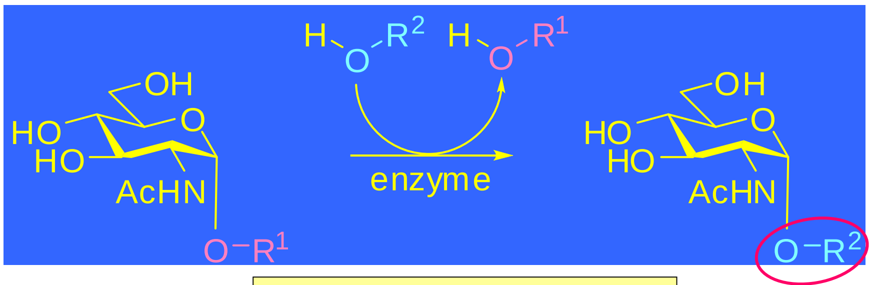
What are the bottlenecks of enzymatic synthesis?

- Availability, price and stability of required enzymes
- Multidisciplinary approach (microbiology, biochemistry, chemistry, molecular biology)
- Difficult separation of regioisomers
- Risk of low yields

Important terms of enzymatic synthesis

stereoselectivity

regioselectivity



O-glycosidic bond

substrate specificity (D,A)

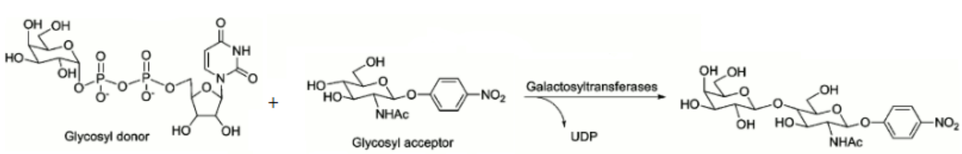
retaining

(retains anomeric configuration) x inverting

Main Carbohydrate Active enZYmes

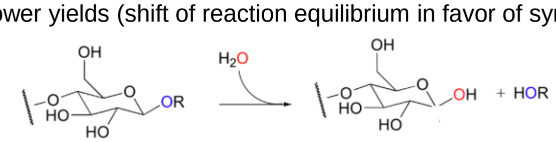
Glycosyltransferases (EC 2.4)

- carbohydrate synthesis under physiological conditions
- strict stereoselectivity and regioselectivity; lower stability
- donors – sugar nucleotides (*in situ* generation by multienzymatic systems)



Glycosidases (glycoside hydrolases; EC 3.2.1)

- carbohydrate hydrolysis in vivo, necessary modification of reaction conditions
- stereoselectivity, low regioselectivity and substrate specificity, robustness
- lower yields (shift of reaction equilibrium in favor of synthesis)



Glycosyltransferases

Leloir glycosyltransferases

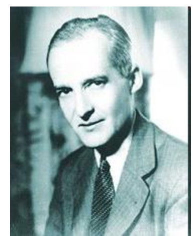
– use sugar nucleotide donors

Non-Leloir glycosyltransferases

– use other substrates than nucleotides (phosphates)

Luis Federico Leloir (1906 – 1987)

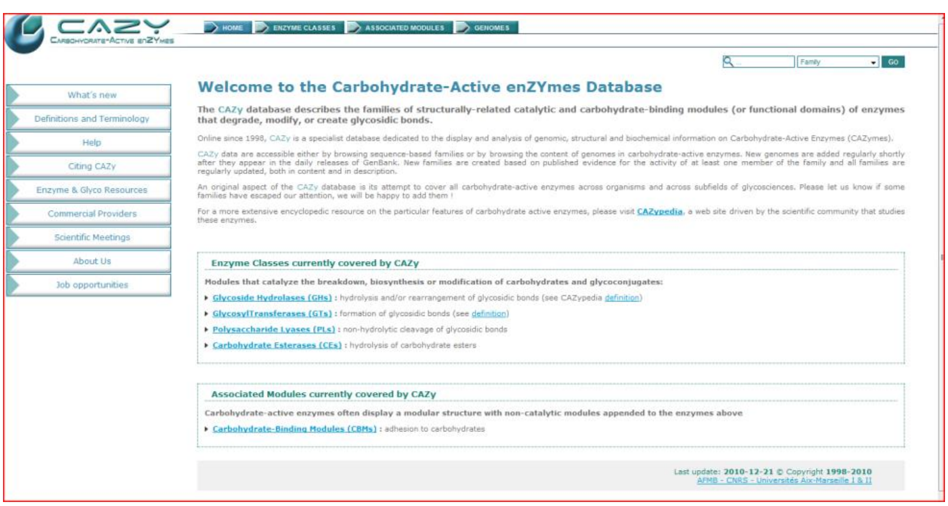
- Argentinian medical doctor and biochemist
- 1970 Nobel prize for chemistry
- discovered sugar nucleotides (1948)



CAZy database; http://www.cazy.org/

= *Carbohydrate-Active enZYmes Database,* gathers enzymes processing glycosidic bond and proteins binding sugars

- http://www.cazypedia.org/ … public encyklopaedia of CAZymes



Classification and characteristic of CAZy

= Henrissat, B.: A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem. J.* (1991) 280, 309-316.

- 1991: 35 GH families → 2017: 145 GH families
- 2017: 490,000 GHs, 360,000 GTs
- classification based on primary sequence – evolutionary relations
- reflects on 3D-architecture of the enzyme molecule
- information on mechanism; substrate specificity is rather not defined

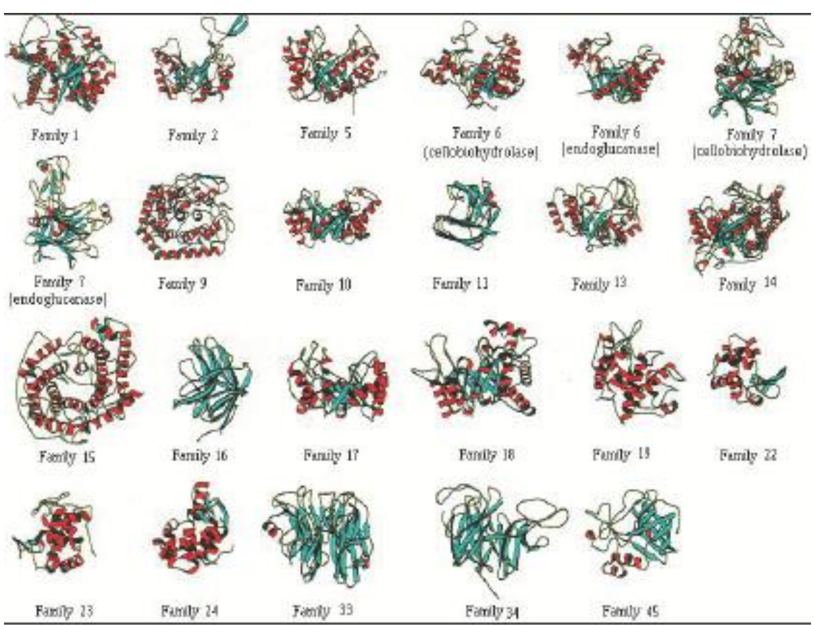
Present state in glycosidases

- GH1 – GH145
- crystal structure - 75% families

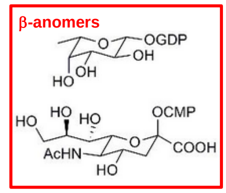
Present state in glycosyltransferases

- 2017: 105 GT families
- ca 100 X-ray structures in total
- high chemical diversity

CAZy: 3D models of enzymes of GH families

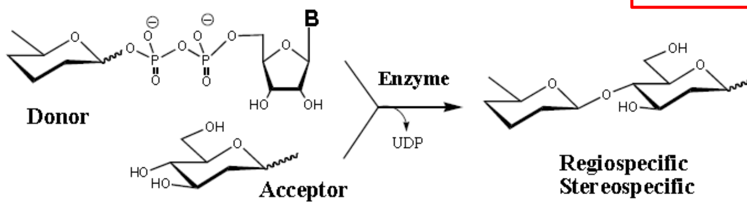


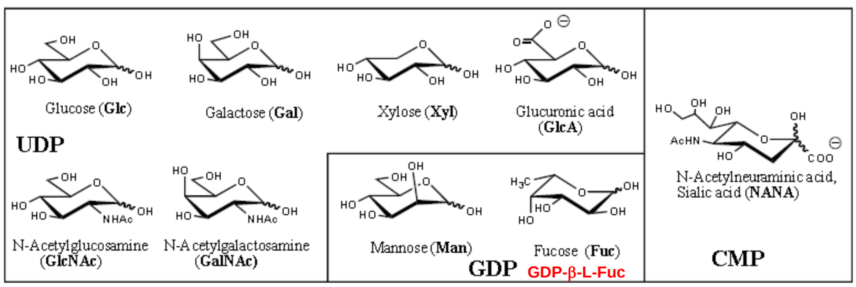
Glycosyltransferases (EC 2.4)



Mammalian glycosyltransferases

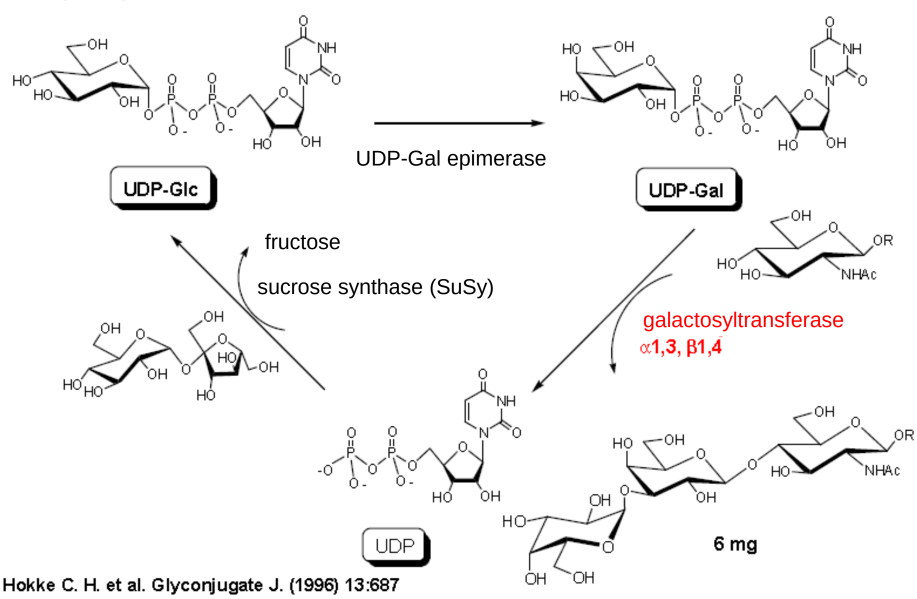
- over 200 GTs use only 9 sugar nucleotides





Glycosyltransferases (EC 2.4)

Recycling of nucleotide donors

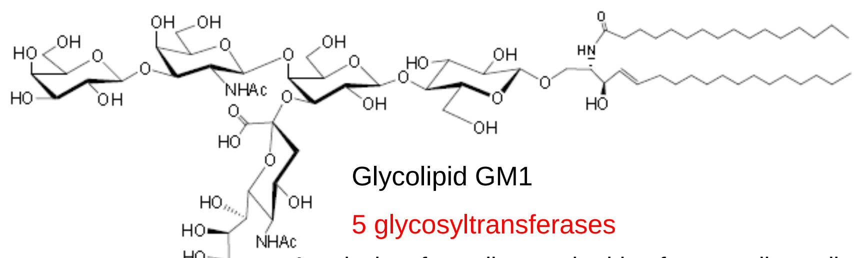


Hokke C. H. et al. Glyconjugate J. (1996) 13:687

Glycosyltransferases (EC 2.4)

Acceptors

- Glycosides, oligo- and polysaccharides
- Protein residues Tyr, Ser, Thr → O-gp / Asn → N-gps
- Lipids → glycolipids
- DNA, natural and non-natural compounds



Glycolipid GM1

5 glycosyltransferases

A typical surface oligosaccharide of mammalian cells

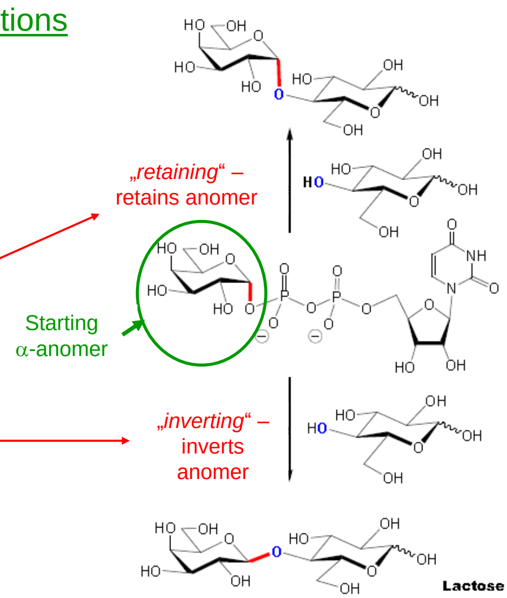
Glycosyltransferases (EC 2.4)

Glycosyltransferase reactions

- regiospecific
- stereospecific
- one enzyme – one bond

α1,4-galactosyltransferase

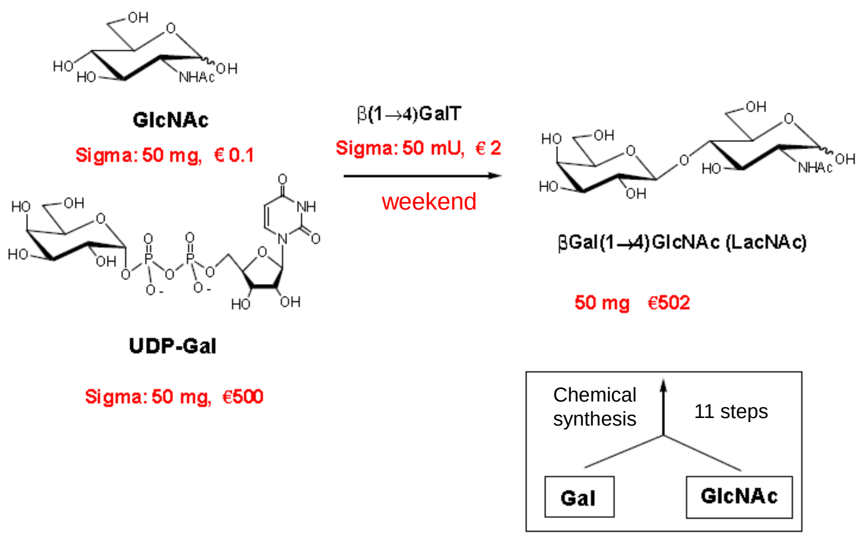
β1,4-galactosyltransferase (lactose synthase)



Glycosyltransferases (EC 2.4)

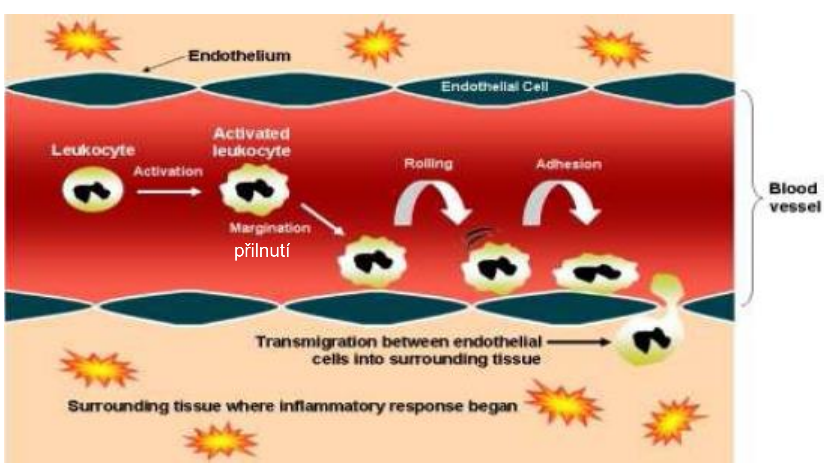
Enzymatic synthesis of βGal(1→4)GlcNAc (LacNAc)

Commercial bovine β1,4-galactosyltransferase (lactose-synthase)



Glycosyltransferases (EC 2.4)

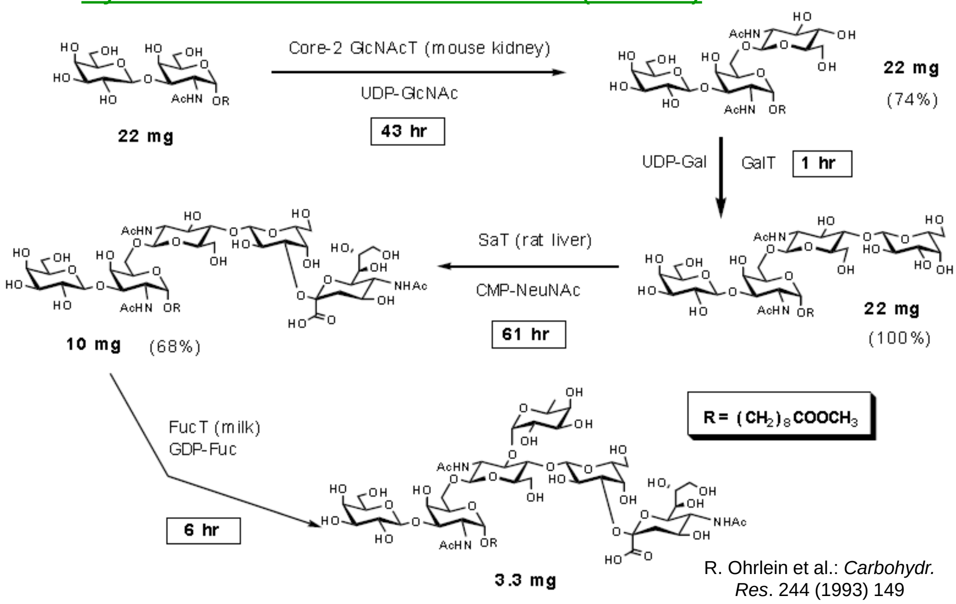
Anti-inflammatory tetrasaccharide Sialyl-Le^x



- inflammation accompanied by enhanced selectins on endothel
- selectins bind glycoproteins on white blood cells
- Sialyl Le^X hinders binding of white blood cells to endothel – suppresses inflammation

Glycosyltransferases (EC 2.4)

Synthesis of SLex hexasaccharide (1 week)



R. Ohrlein et al.: *Carbohydr. Res*. 244 (1993) 149

Glycosyltransferases (EC 2.4)

Pros and cons - summary

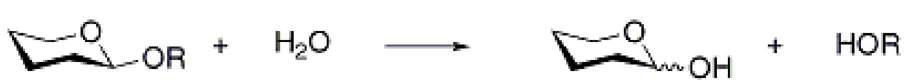
- high yields
- 100% regio- and stereoselectivity
- reaction in water medium ⇒ ecology

- limited enzyme availability (commercial expensive)
- one enzyme – one reaction
- sugar nucleotides expensive and not-so-stable
- narrow substrate specificity

Enzymatic synthesis of glycosidic bond:
glycosidases, glycosynthases

Glycoside hydrolases (EC 3.2.1)

- *in vivo* cleavage of glycosidic bonds (*exo*-, *endo*-)

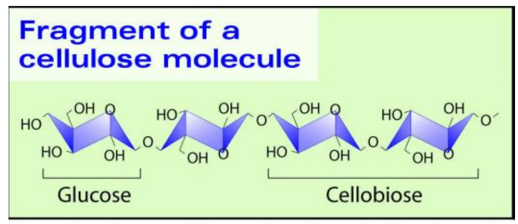


- Common enzymes in nature
  - – degradation of biomass (cellulose)
  - – antibacterial defence strategies (lysozyme)
  - – pathogenic mechanisms (viral neuraminidases)
  - – common cellular functions (biosynthesis of N-gps)
  - – digestion of carbohydrates …

Functions of glycosidases

Biomass

- Biological material from living organisms
- Plant and animal debris, municipal waste
- Production of energy (burning, electricity), biofuels



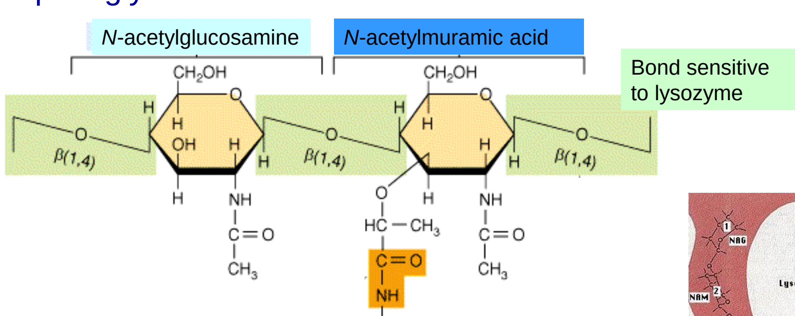
Alternating glucose residues are in an inverted orientation so the cellobiose (a disaccharide) is the repeating structural unit.

Cellulose

- the most stable comformation – alternate β(1-4)Glc, hydrogen bonds
- hydrolysis difficult – microbial cellulases (+ some ruminants)

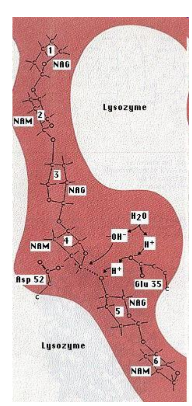
Functions of glycosidases

Peptidoglycan – cell wall of bacteria



Lysozyme

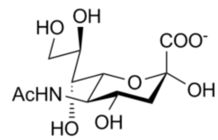
- tears, spit, mother milk, mucus, egg white
- deficient in babies on formula
- first X-ray structure – 1965 (D. C. Phillips)



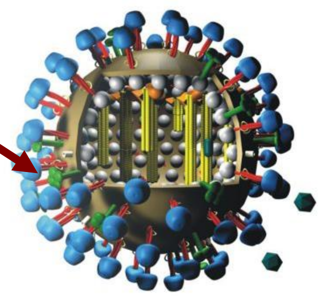
Functions of glycosidases

Viral neuraminidase

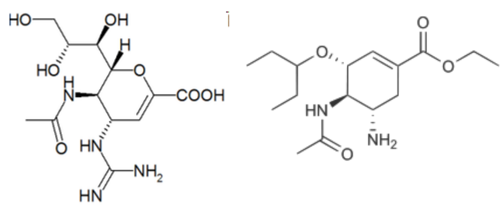
- neuraminidase (sialidase; EC 3.2.1.18)
  – cleaves terminal sialic acid



- membrane enzyme on surface of influenza virus, antigenic determinant

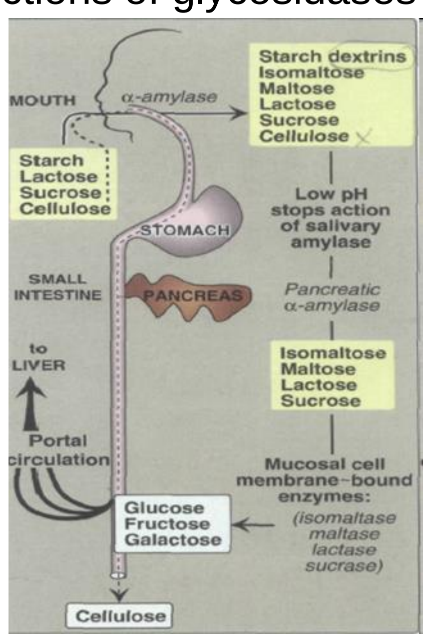


- neuraminidase inhibitors
  – target of anti-influenza medicines:
- zanamivir (Relenza) a oseltamivir (Tamiflu)



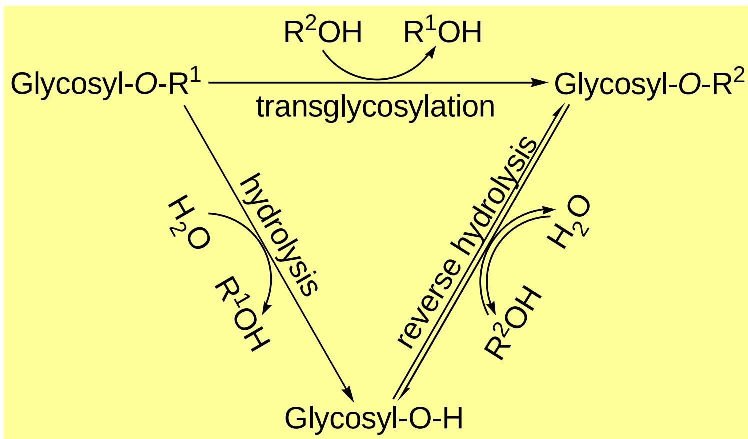
Functions of glycosidases

Digestion of carbs



Glycosidases (EC 3.2.1)

- Synthesis of glycosidic bonds by changing of reaction conditions: reduction of water activity, activated D
  - reverse hydrolysis (condensation)
  - transglycosylation (glycosyl transfer onto acceptor)

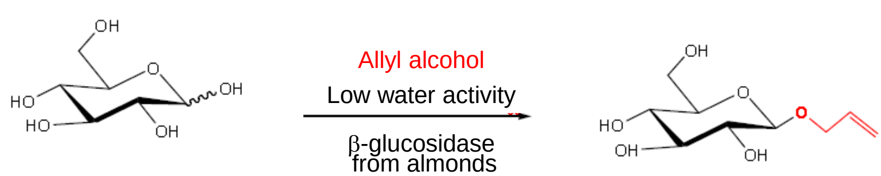


Glycosidases (EC 3.2.1)

Reverse hydrolysis – thermodynamically directed

- free monosaccharide + nucleophile → introduction of aglycon

- runs until equilibrium



Donor

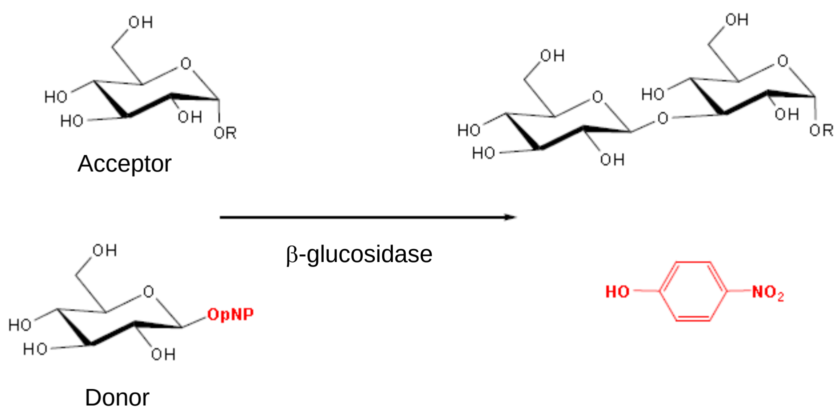
$$\text{Glycosyl-OH} + \text{R'OH} \rightleftharpoons \text{Glycosyl-OR'} + H_2O$$

Ducret, A., et al. (2006) J. Mol. Cat. B. Enzym. 38:91.
van Rantwijk, F., et al. (1999) J. Mol. Cat. B. Enzym. 6:511.

Glycosidases (EC 3.2.1)

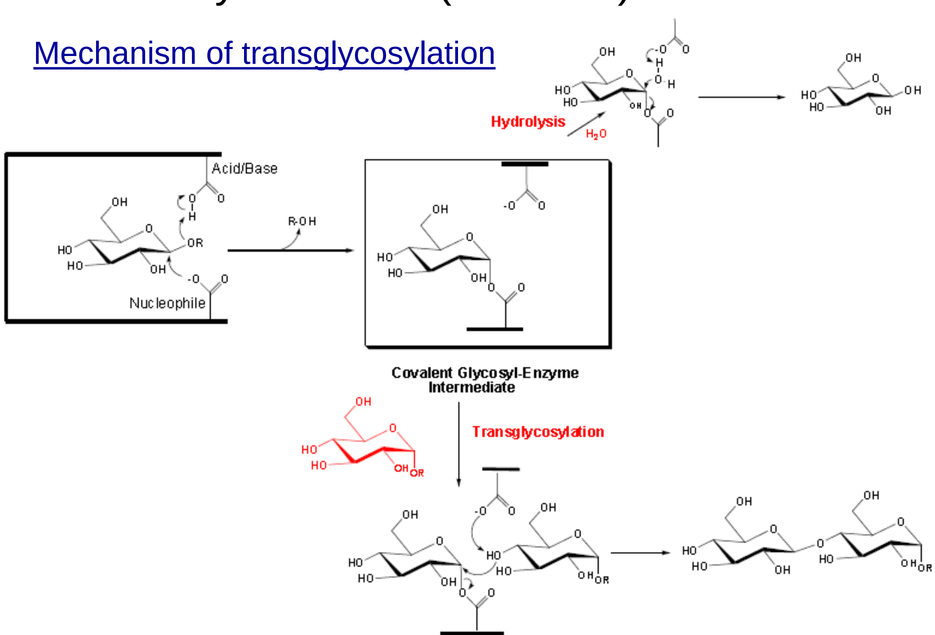
Transglycosylation – kinetically directed

- **activated donor, acceptor in excess**
- **runs fast, product is also substrate (reversible)**



Glycosidases (EC 3.2.1)

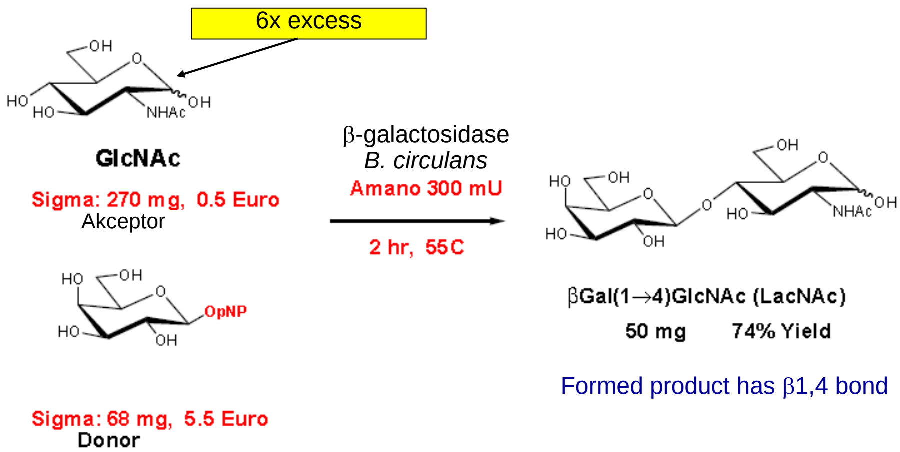
Mechanism of transglycosylation



Glycosidases (EC 3.2.1)

Enzymatic synthesis of βGal(1→4)GlcNAc (LacNAc)

Commercial β-galactosidase from *B. circulans*

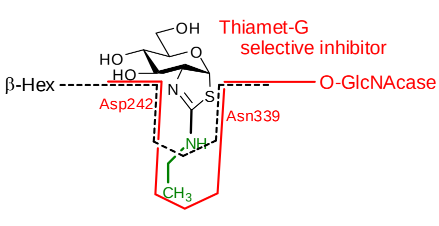


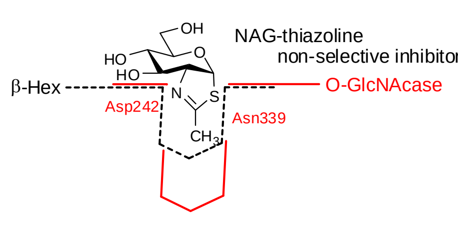
Vetere et al., J. Carb. Chem. (1999) 18:515

Glycosidases (EC 3.2.1)

Glycosidase inhibitors as therapeutics

- **human *O*-GlcNAc-ase (OGA)**
- **protein tau is physiologically strongly glycosylated (OGT), in Alzheimer disease it is deglycosylated (OGA) and hyperphosphorylated**
- **selective inhibition of OGA ⇒ slower progress of Alzheimer disease**
- **thiamet G**

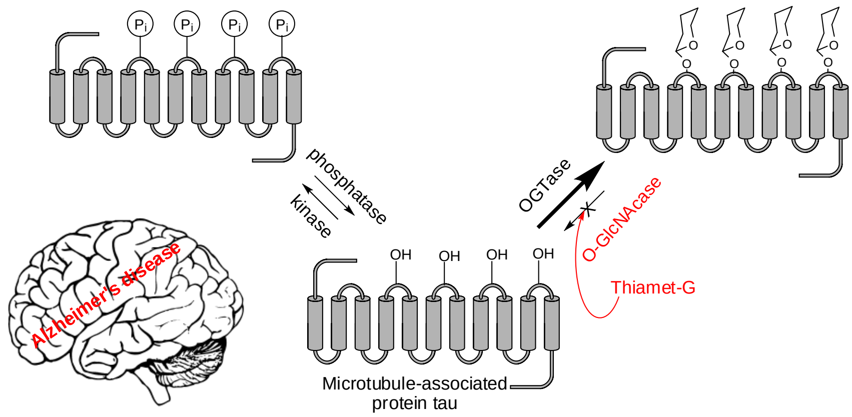




Glycosidases (EC 3.2.1)

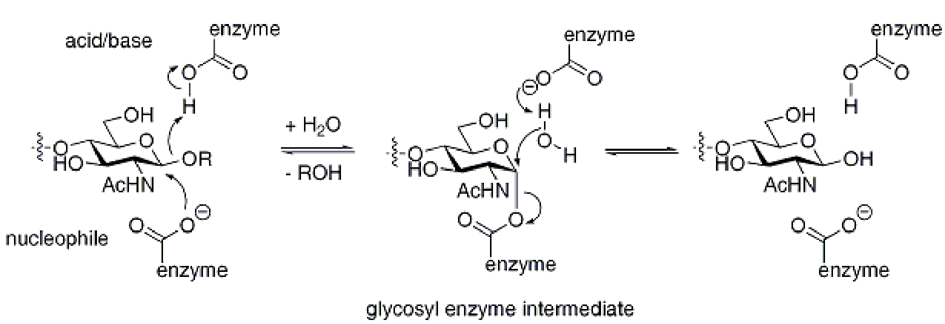
Glycosidase inhibitors as therapeutics

- Regulation of hyperphosphorylation of protein tau



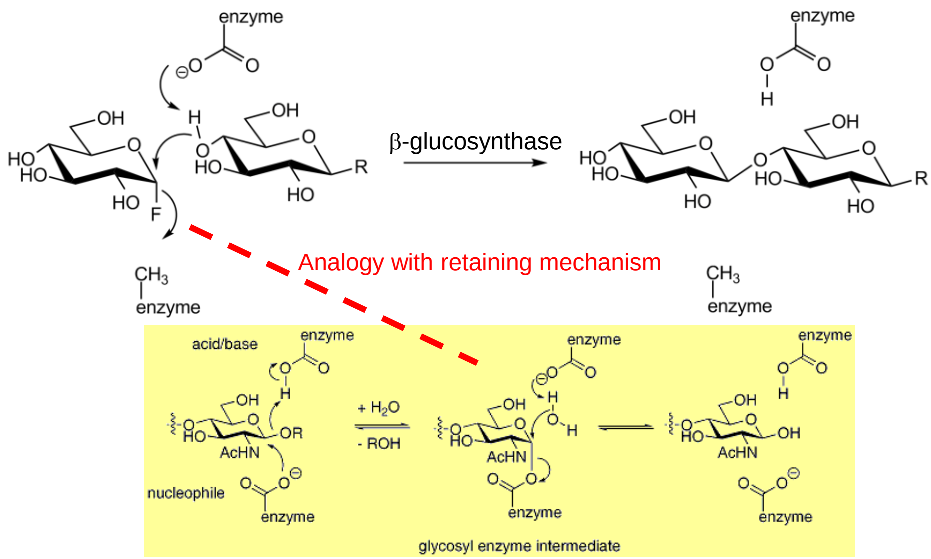
Mutant glycosidases - glycosynthases

- 1998, S. G. Withers and A. Planas
- mutation of catalytic nucleophile (Asn or Gln) into non-nucleophile (Ala, Gly, Ser)
- mutant enzyme is hydrolytically inactive (use for synthesis)
- substrate of opposite anomeric configuration (glycosyl fluorides)
- revolution in enzymatic synthesis of carbohydrates



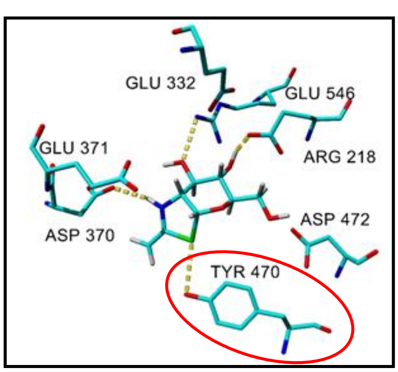
Mutant glycosidases - glycosynthases

- covalent intermediate enzyme-substrate not formed



Mutant glycosidases - transglycosidases

- glycosynthase concept does not work – different mechanism
- mutant transglycosidase from β-*N*-acetylhexosaminidase
    - water stabilizing tyrosine residue exchanged
    - chitooligomers of GlcNAc



Tyr470Phe

Tyr470Asn

Tyr470His

Glycosidases (EC 3.2.1)

Pros and cons - summary

- average to high yields
- 100% stereoselectivity, good regioselectivity
- reaction in water medium ⇒ ecology
- broad tolerance to substrate functionalization

- possible formation of product mixtures
- necessary screening and optimisation
- lower yields than GTs
- not all enzymes well available

Glyconanomaterials

in biomedicine research

Pavla Bojarová

Akademie věd České republiky

FAKULTA BIOMEDICÍNSKÉHO INŽENÝRSTVÍ ČVUT V PRAZE

Institute of Microbiology AV ČR

FBMI ČVUT

MBÚ macro PRAGUE RWTH AACHEN UNIVERSITY

Lectins

William C. Boyd, 1954

- "lectin" from lat. *legere* (read, pick)
- originally plant proteins able to agglutinate cells by specifically binding sugar units
- first application – determination of blood groups (agglutination of erythrocytes)

wheat germs

Ricinus communis

Canavalia ensiformis

Present definition:

- proteins that specifically recognize and bind free or bound carbohydrates
- no enzymatic activity

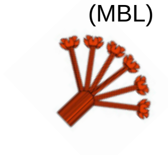
Lectins

- in all types of organisms (bacteria, viruses, plants, animals, humans)
- easy detection, simple isolation, good commercial availability and recombinant expression

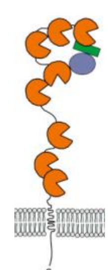
Galectin-3

Galectin-1

Mannan binding lectin (MBL)

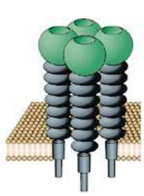


Dectin-1

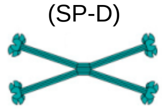


Mannose receptor

DC-SIGN

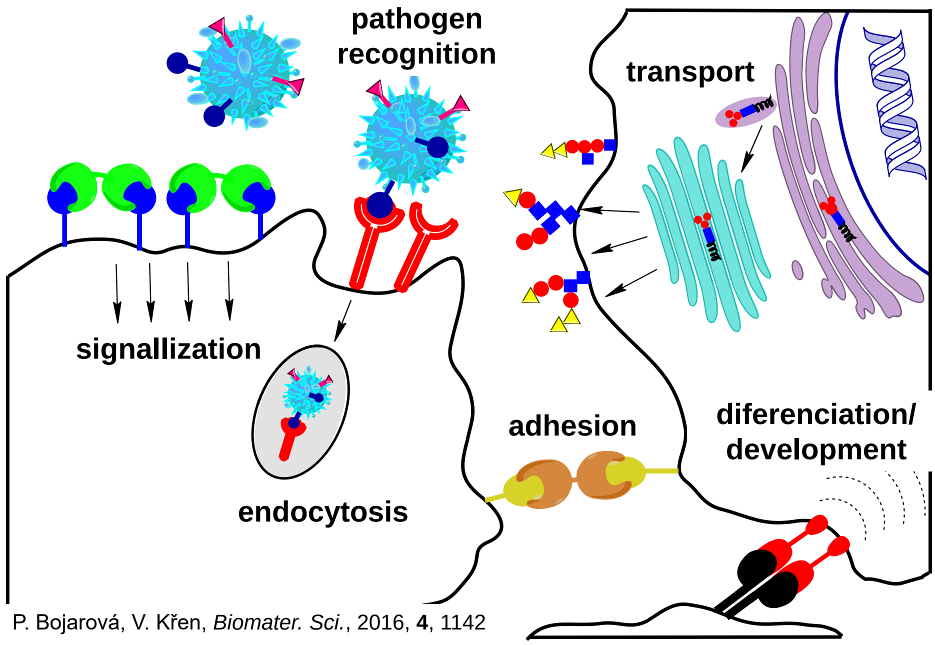


Surfactant protein D (SP-D)



Lectins *in vivo*

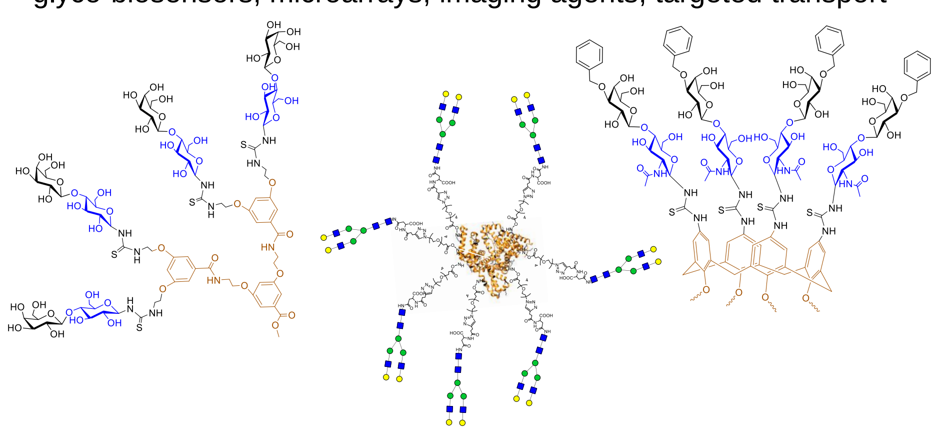
deciphering of sugar code ⇨ SIGNAL



P. Bojarová, V. Křen, *Biomater. Sci.*, 2016, **4**, 1142

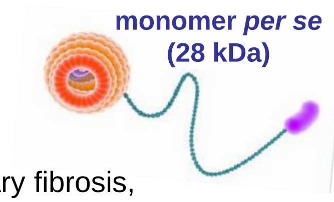
Multivalency

- **weak monovalent** interaction lectin-glycan (K_a ≈ mM-µM)
- **MULTIVALENCY** – **amplification** of biological response *in vivo*
- multivalent **glycomimetics** – mimic *in vivo* design
- glyco-biosensors, microarrays, imaging agents, targeted transport

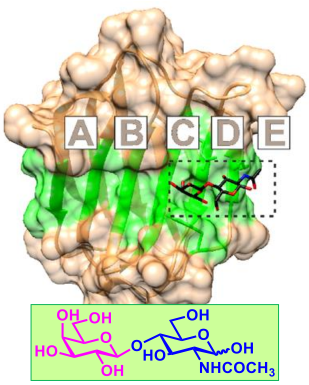


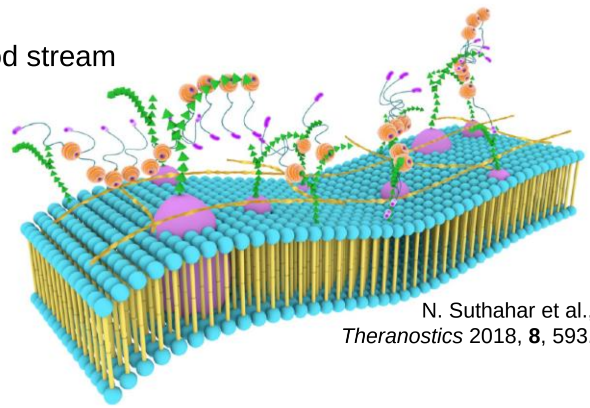
D. Laaf, P. Bojarová et al., *Trends Biotechnol*. 2019, **37**, 402.

Galectin-3



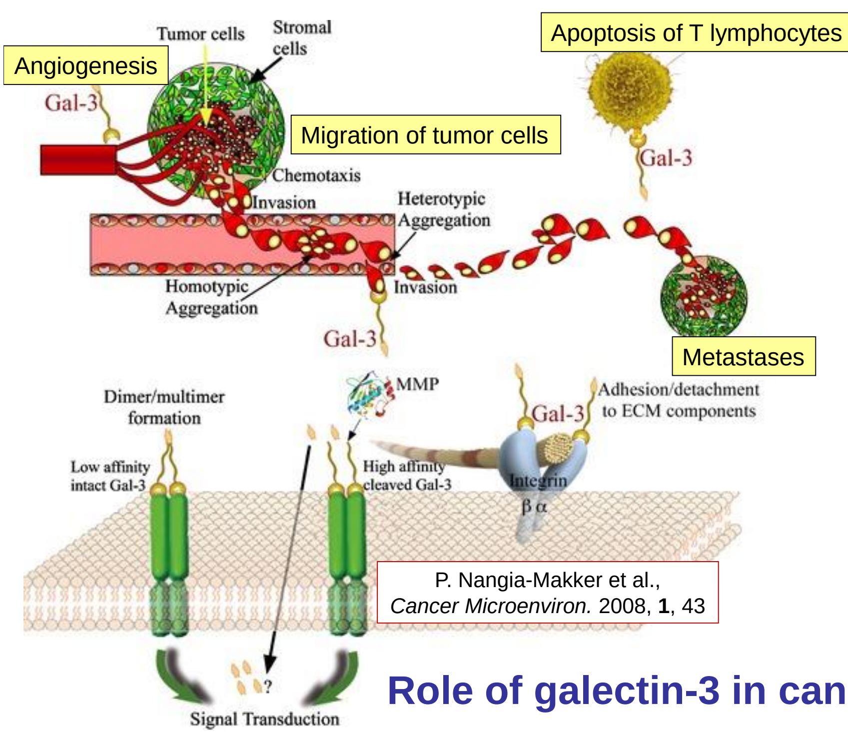
- galectins: animal (human) lectins
- cancer, kardiopathologies, idiopatic pulmonary fibrosis, metabolic disorders
- exogenous Gal-3 in blood stream





N. Suthahar et al., *Theranostics* 2018, **8**, 593.

Heterogenous crosslinked complexes/ lattices with multivalent ligands

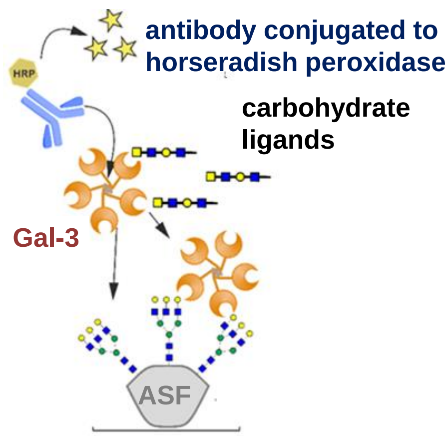


P. Nangia-Makker et al., *Cancer Microenviron.* 2008, **1**, 43

Role of galectin-3 in cancer

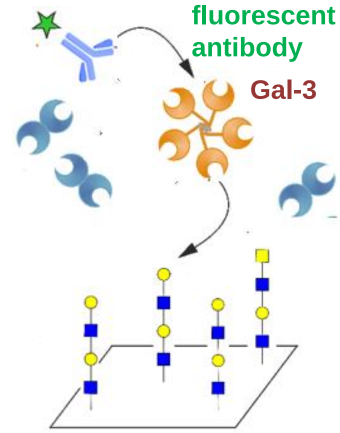
Interaction between galectins and carbohydrates

Enzyme Linked Immunosorbent Assay (ELISA)



Competitive ELISA

- imobilized asialofetuin

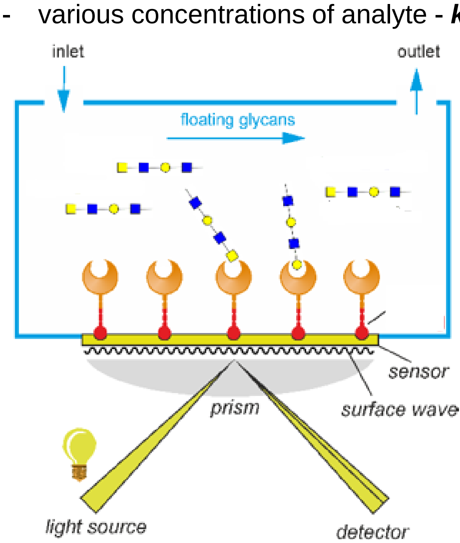


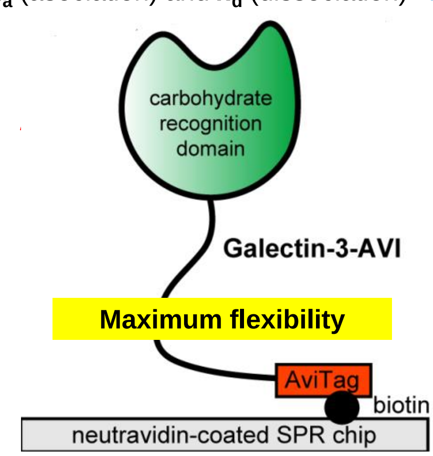
Direct ELISA

- capturing galectin from blood serum

Surface plasmon resonance

- asociation/dissociation of molekules on the biosensor surface – changes of mass result in changed refractive index
- various concentrations of analyte - k_a (asociation) and k_d (dissociation) - K_d

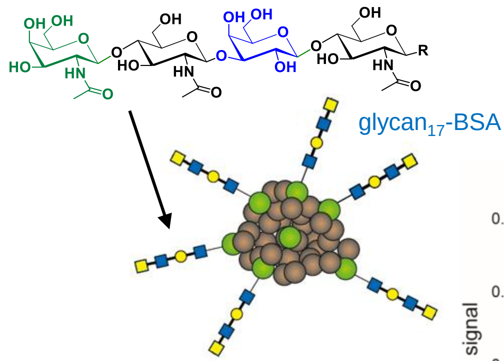


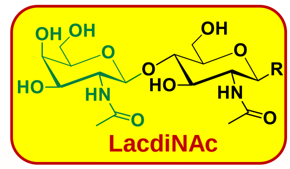


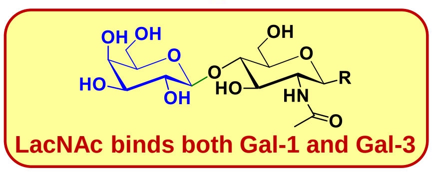
L. Bumba, P. Bojarová et al., *Int. J. Mol. Catal.* 2018, **19**, 372.

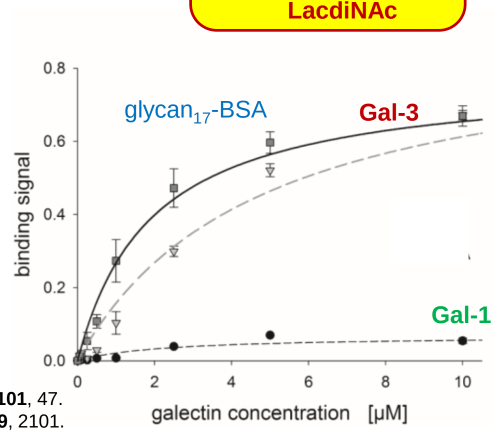
Enzymatic synthesis of tailored glycans

- terminal **LacdiNAc epitope is selective** for Gal-3 in contrast to Gal-1





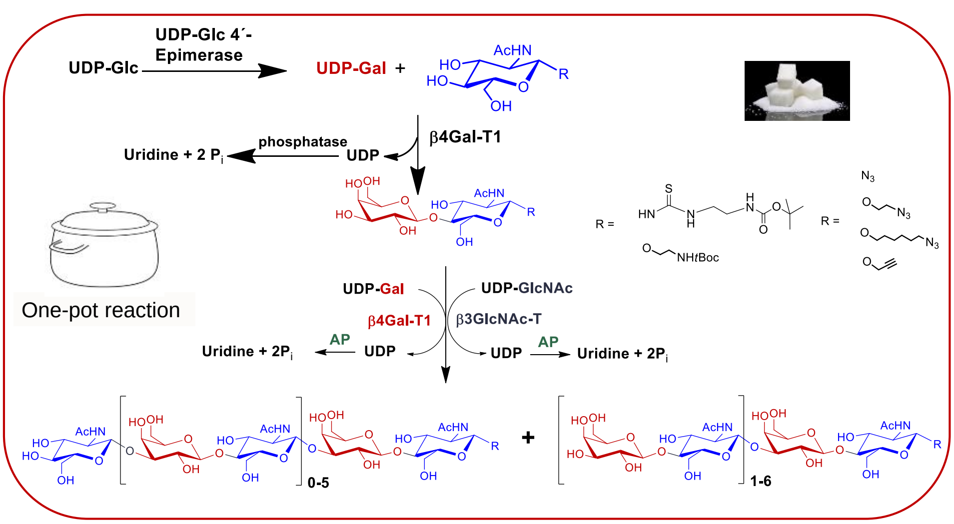




A.Šimonová, P. Bojarová et al. *J. Mol. Catal. B* 2014, **101**, 47.
D. Laaf, P. Bojarová et al. *Adv. Synth. Catal.* 2017, **359**, 2101.

Enzymatic synthesis of tailored glycans

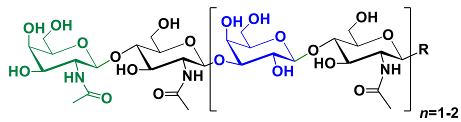
Library of selective recombinant glycosyltransferases

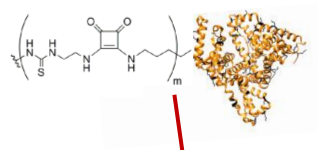


C. Rech, P. Bojarová et al. *Adv. Synth. Catal*. 2011, **353**, 2492. P. Bojarová et al. *Chem. Soc. Rev.* 2013, **42**, 4774.

Tailored glycans as ligands of Gal-3

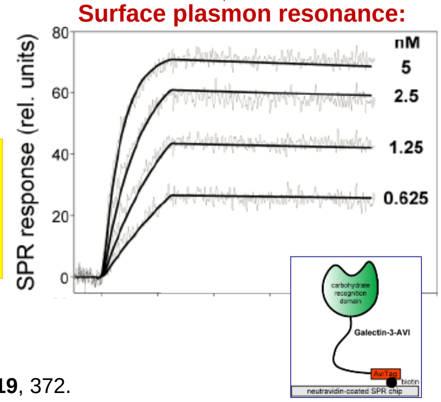
LacdiNAc-(LacNAc)$_n$R



R = 

ELISA: IC_{50} = 11 nM

Surface plasmon resonance:



Very fast association and slow dissociation

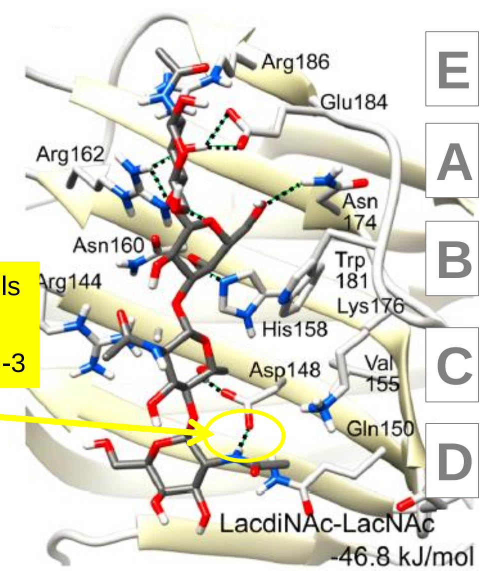
$(LacdiNAc\text{-}LacNAc)_{21}$-BSA: K_d = 14 pM

L. Bumba, P. Bojarová et al., *Int. J. Mol. Catal.* 2018, **19**, 372.

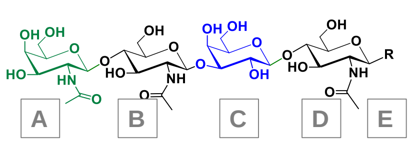
Tailored glycans as ligands of Gal-3

Binding groove of Gal-3:

five binding subsites (A-E)



Tetrasaccharide LacdiNAc-LacNAc fills the whole binding groove of Gal-3
Forms extra hydrogen bonds with Gal-3



L. Bumba, P. Bojarová et al., *Int. J. Mol. Catal.* 2018, **19**, 372

Summary - glyconanomaterials

- New protein construct **Gal-3-AVI** for surface plasmon resonance
- **Enzymatic synthesis of** tailored carbohydrate epitopes
- Multivalent presentation of LacdiNAc-LacNAc tetrasaccharide – **sub-nanomolar affinity**

Acknowledgement

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Faculty of Biomedical Engineering, Kladno