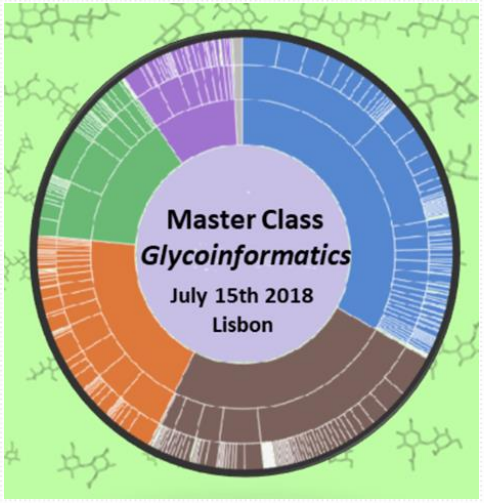


Integrated use of Databases in Structural Investigation of Complex Carbohydrates

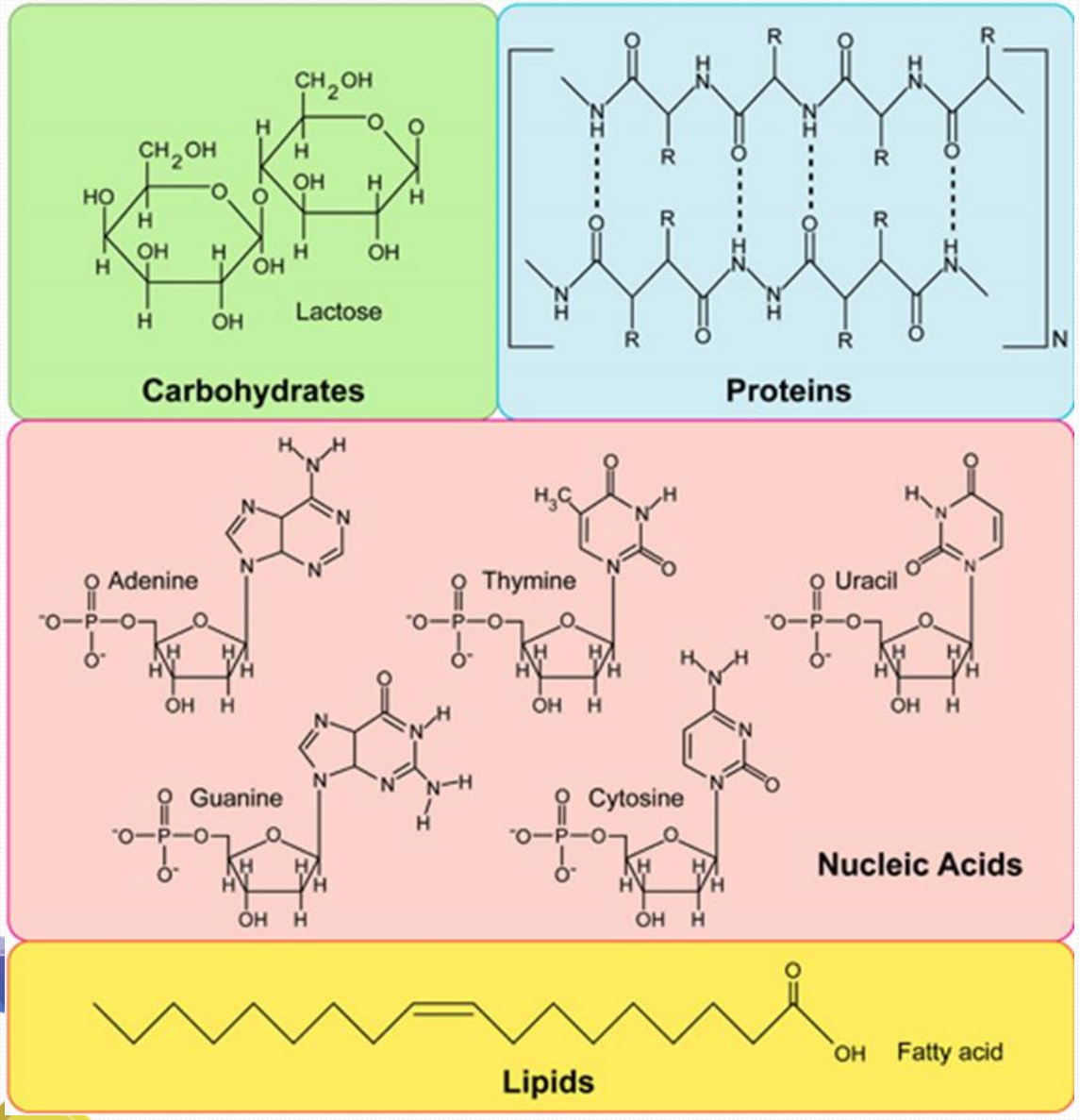
Roberta Marchetti, Ph.D.

University of Naples "Federico II"

Four Classes of Biological Molecules

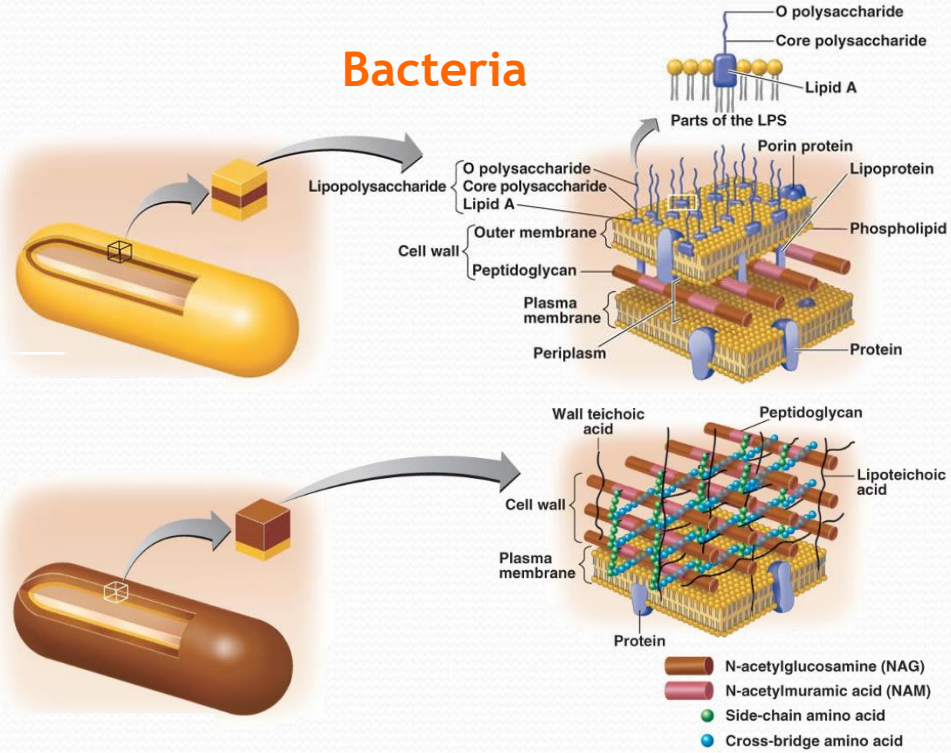
- Carbohydrates
- Proteins
- Nucleic Acids
- Lipids

Glycans are made up of individual **sugar units** linked to one another in a multitude of ways

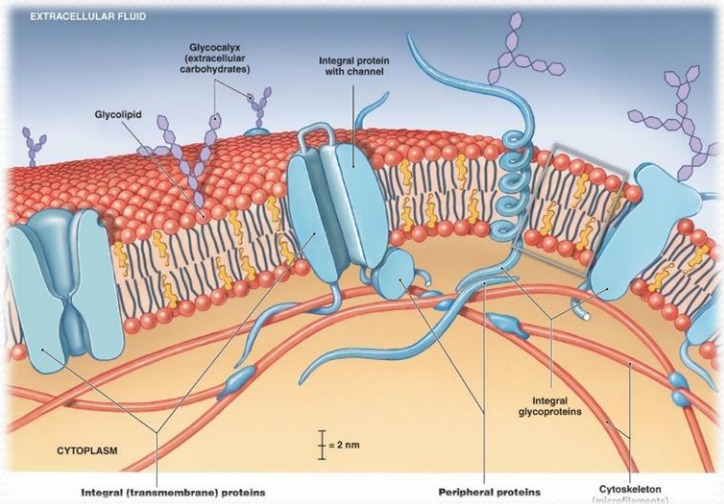


GLYCOCONJUGATES AS KEY MEDIATORS IN CELLULAR SOCIAL LIFE

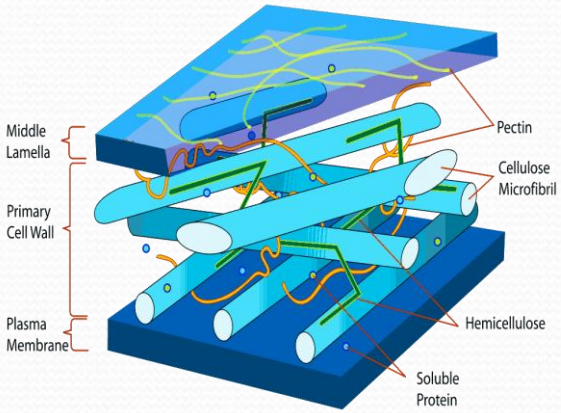
Bacteria



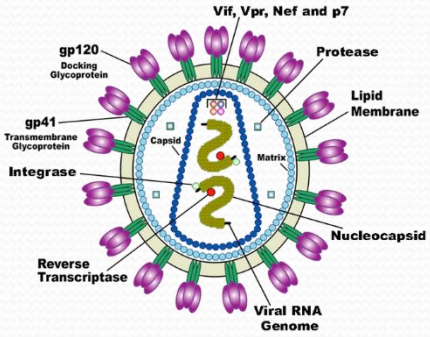
Humans/Animals



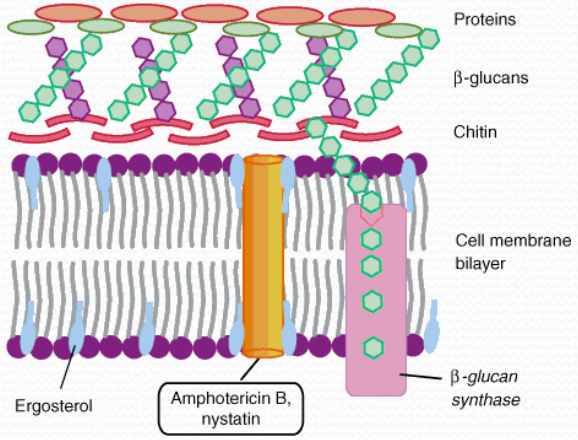
Plants



Viruses



Fungi

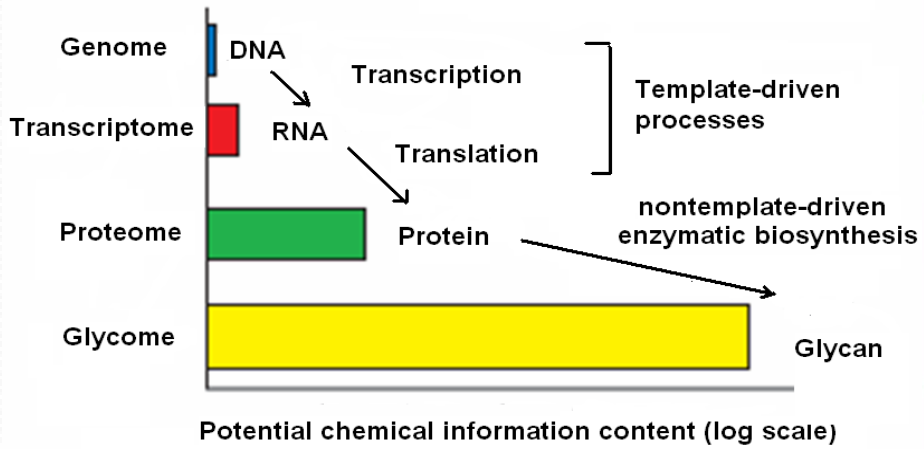


Glycans play critical functions in the areas of cell signaling, molecular recognition, immunity, and inflammation

Complexity of Glycans

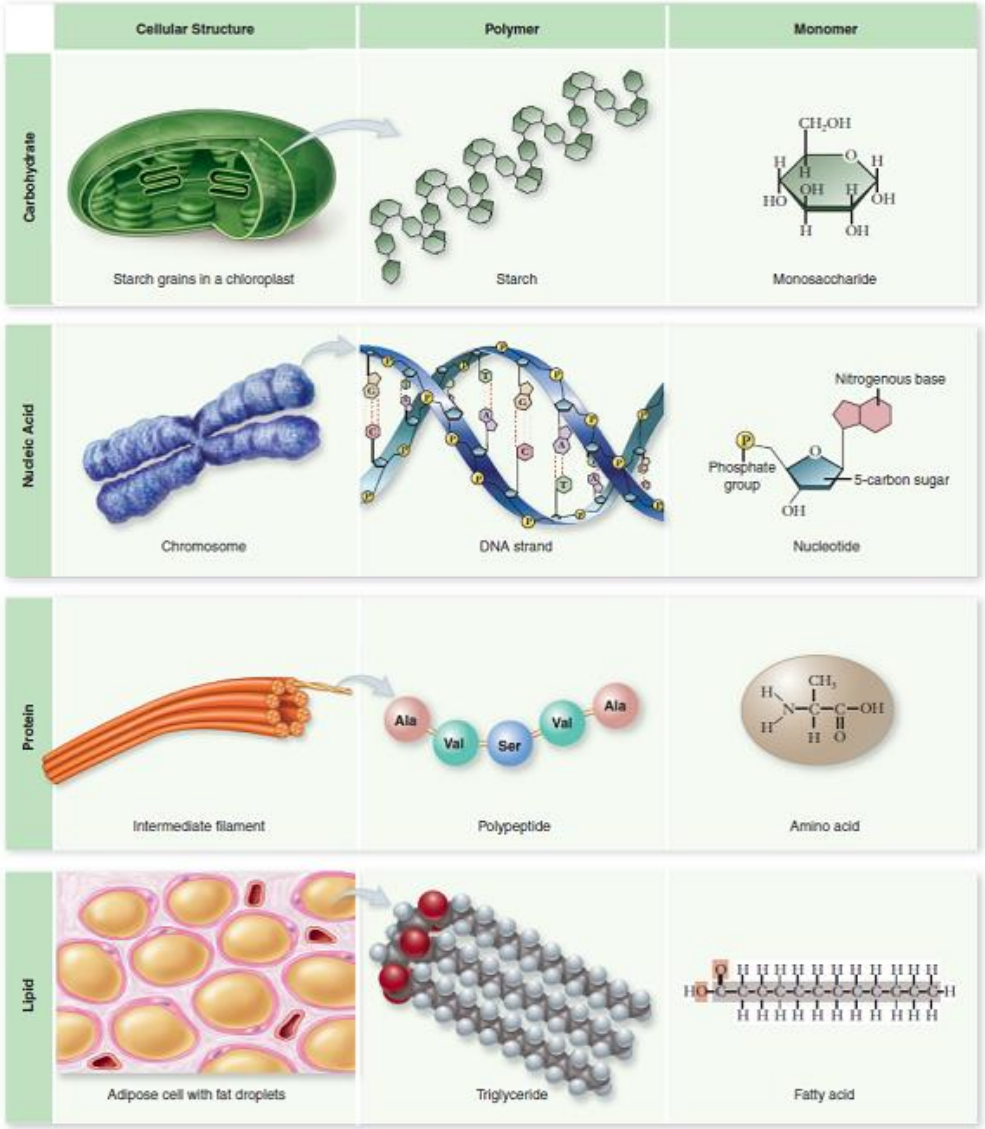
4 L-aminoacids ⟹ 256 tetra-peptides

4 D-aldohexoses ⟹ 7.602.176 tetrasaccharides



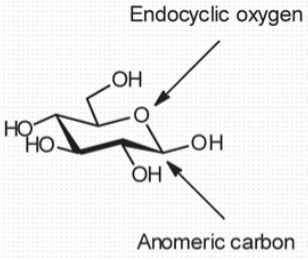
Protein and nucleic acid biopolymers are linear

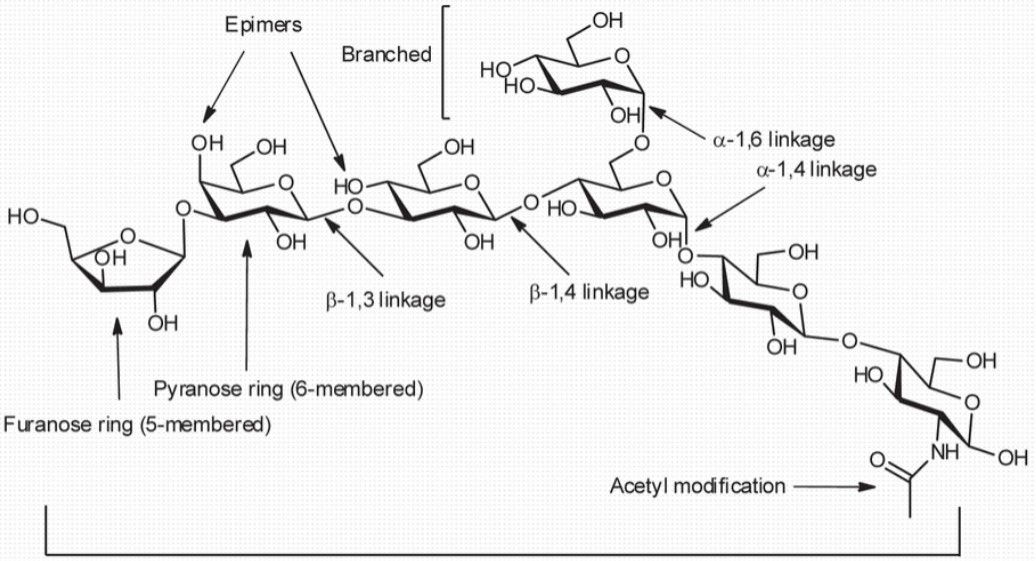
Every building block is linked to the next through the same kind of connection…



Turnbull & Field, 2007 Nature Chemical Biology 3(2), pp74I77

..sugar building blocks can be linked together at many different sites and in different spatial orientations

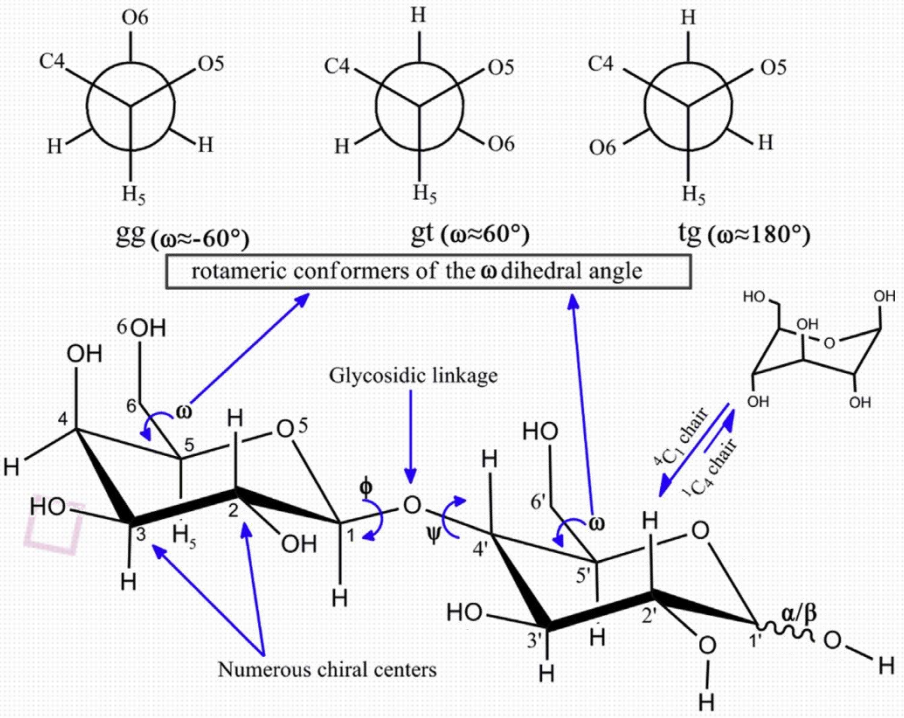




Not always linear polymers but frequently branched…

Tracey M. Gloster *Biochm. Soc. Trans*. 2012;40:913-928

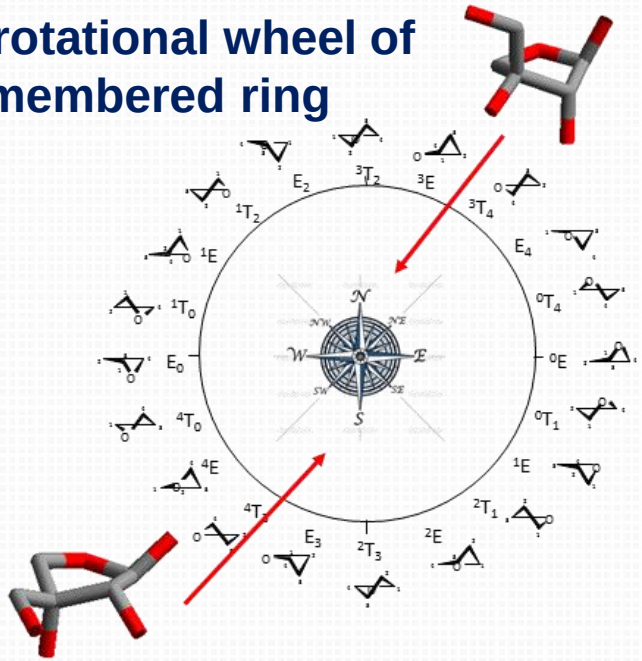
..Not to speak about conformation!



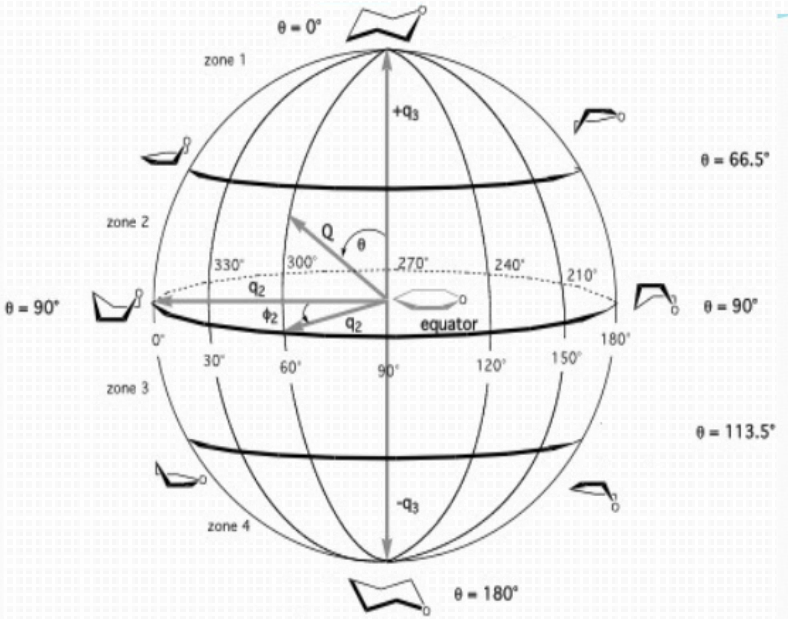
X. Xiong et al. *Carbohydrate Research* 401 (2015) 73–81

FLEXIBILITY AND SHAPES OF 5-/6- MEMBERED SUGAR RINGS

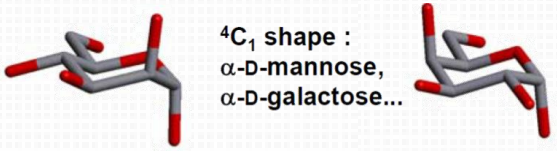
Pseudo-rotational wheel of five-membered ring



Degree of puckering of six-membered ring

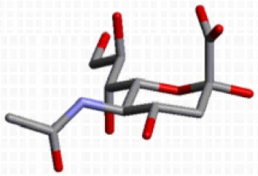


$^4C_1$ shape :
α-D-mannose,
α-D-galactose...

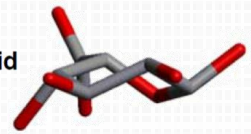


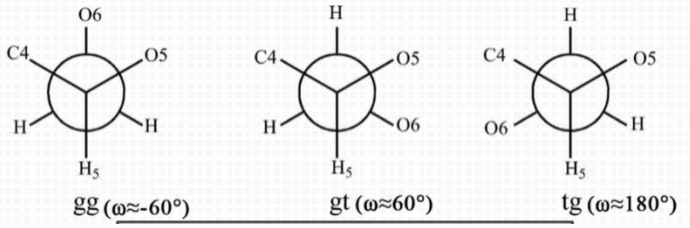
$^1C_4$ shape :
α-L-fucose

$^2C_5$ shape :
α-D-NeuAc



Flexible ring:
α-L-iduronic acid
($^1C_4$ and ^2S_O)

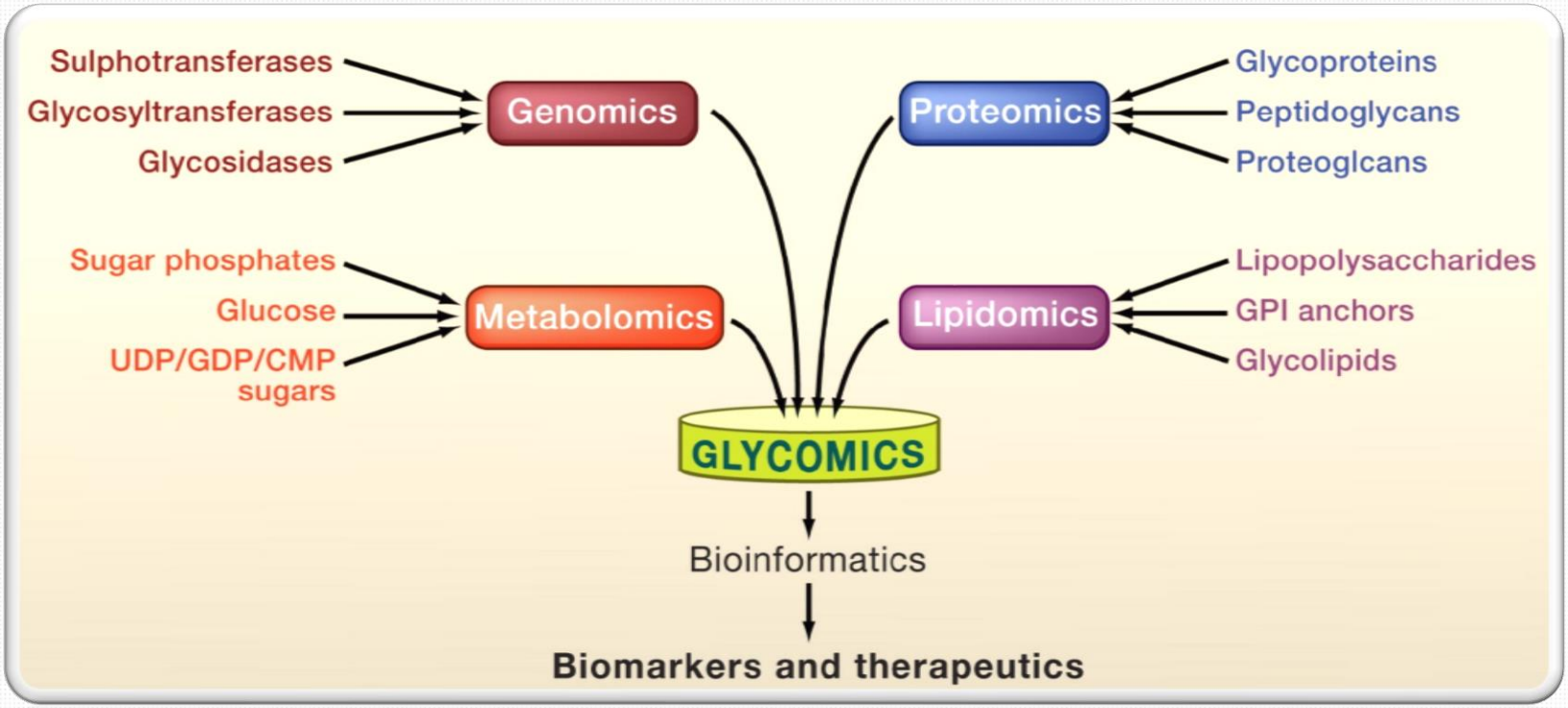




Three staggered rotameric conformers in sugar with an exocyclic hydroxymethyl group

Glycomics: the knowledge of the structure and function of glycans linked to proteins or lipids

Complex glycans ⟷ complexity of biology
target and diagnostic for human, animal and plant health

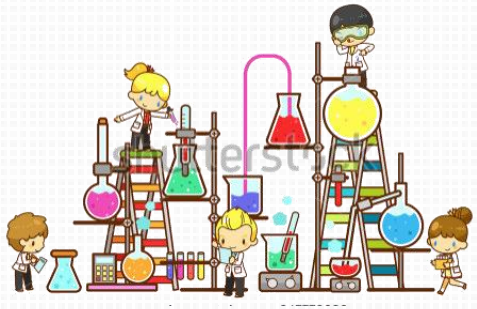


Hart, G. W. and R. J. Copeland (2010). "Glycomics Hits the Big Time." Cell 143(5): 672-676.

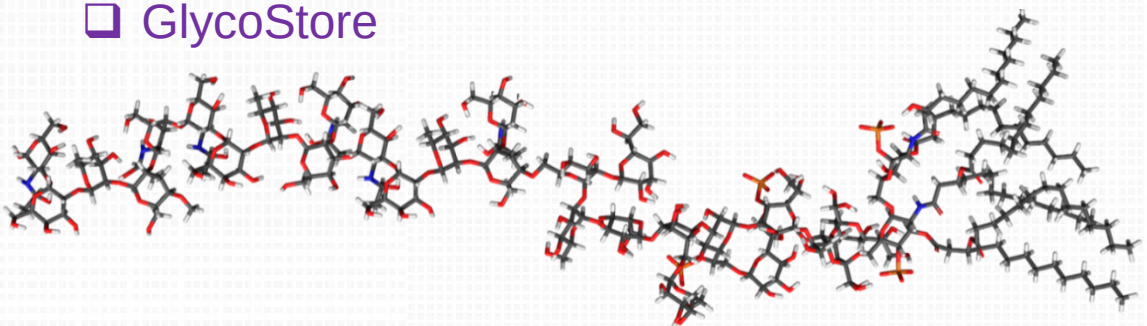
No template to predict sugar sequences from the genome

DATABASES and TOOLS USEFUL IN THE ANALYSIS OF COMPLEX GLYCAN STRUCTURE

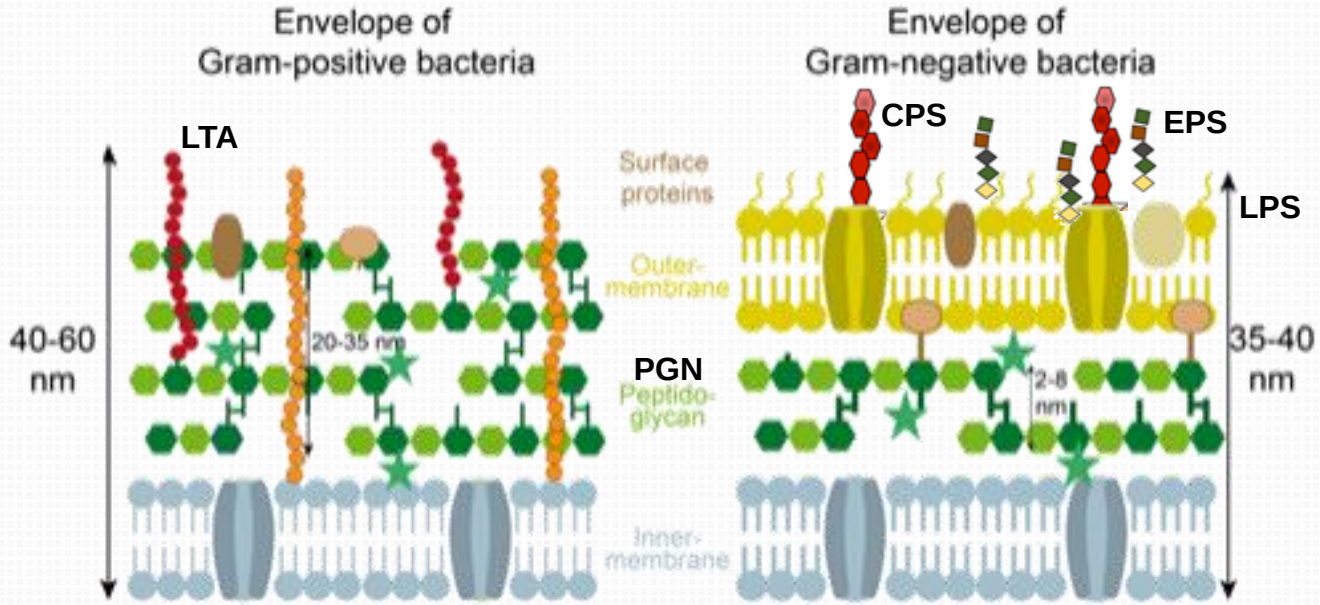
- **Glycan nomenclature**
  - Monosaccharide DB

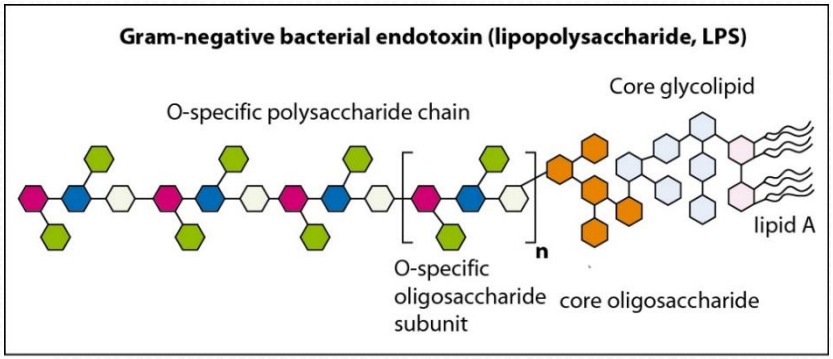


- **Carbohydrate databases**
  - GlyTouCan
  - CFG-Glycan database
  - UniCArbDB
  - CSDB
  - KEGG GLYCAN
  - EPS database
- **Glycan NMR tools**
  - MestreJ
  - CASPER
- **Glycan MS tools**
  - GlycoWorkBench
  - Glycopedia
  - CCRC spectral database
  - GlycoStore
- **Glycan 3D Modeling**
  - Glycosciences.de
  - Glyco3D
  - Glycam
  - GLYCAN
  - GlycanBuilder
- **Glycan Binding**
  - SugarBindDB
  - GlycoEpitope
- **Others..**
  - GlycoProtDB
  - www.stenutz.eu
  - GlycoGeneDatabase

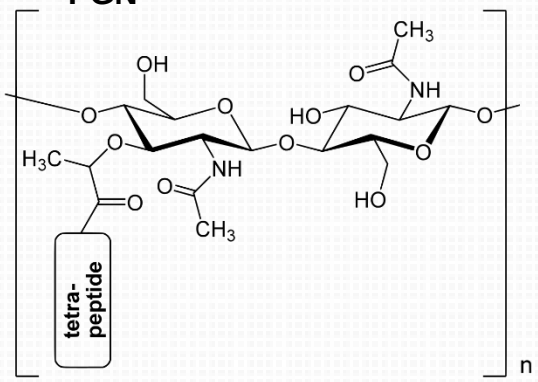


BACTERIAL POLYSACCHARIDES

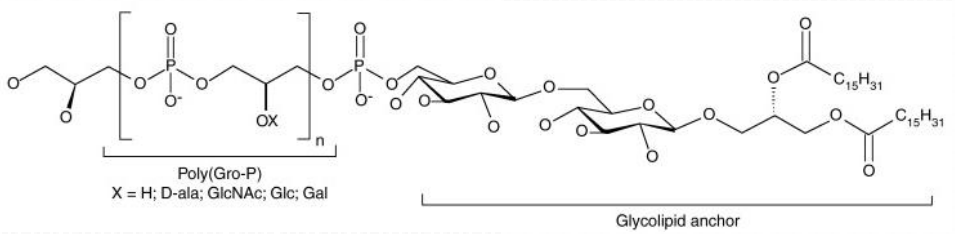


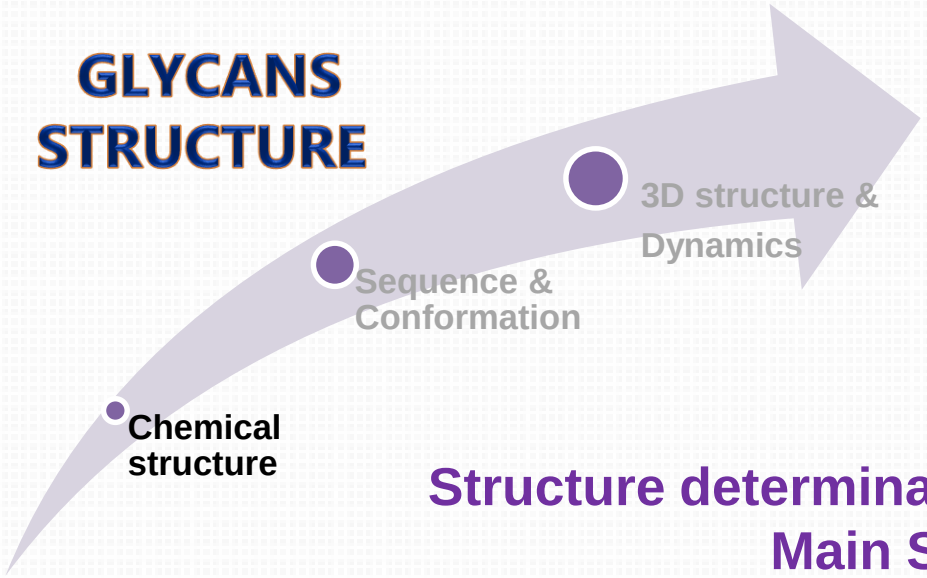


PGN



LTA





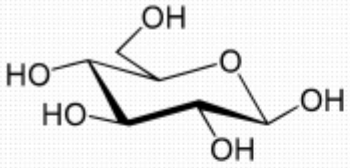
Which are the monosaccharides constituting the glycan?

How the monosaccharides are linked together?

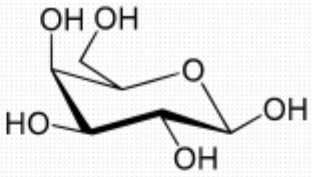
Structure determination of a glycan chain: Main Steps

- Quali-quantitative analysis (GC-MS)
- Absolute configuration (GC-MS, NMR)
- Size of the ring (GC-MS, NMR)
- Anomeric configuration (NMR)
- Linkage analysis (GC-MS, NMR)
- Monosaccharides sequence (MALDI-MS^n, ESI-MS^n, 2D NMR)
- Determination of non-carbohydrate appendages (GC-MS, MALDI-MS^n, ESI-MS^n, 2D NMR)

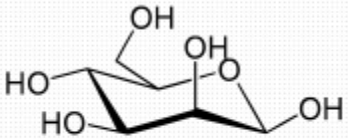
EUKARYOTIC MONOSACCHARIDES



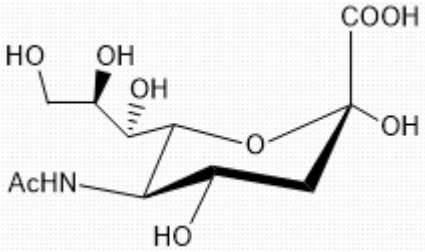
β-D-Glc



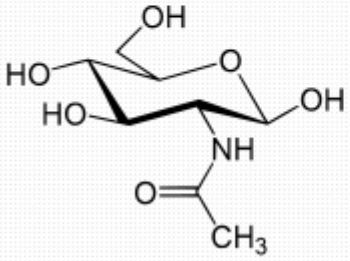
β-D-Gal



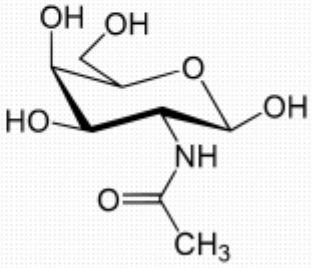
β-D-Man



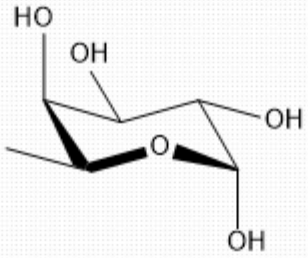
α-D-Neu5Ac



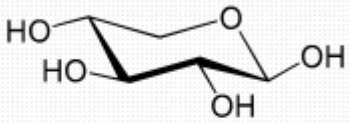
β-D-GlcNAc



β-D-GalNAc



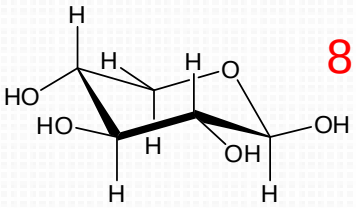
α-L-Fuc



β-D-Xyl

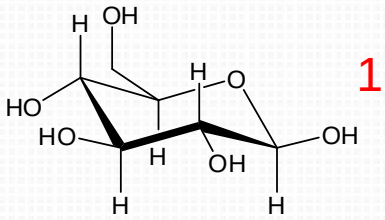
BACTERIAL MONOSACCHARIDES

Pentose



8

Hexose

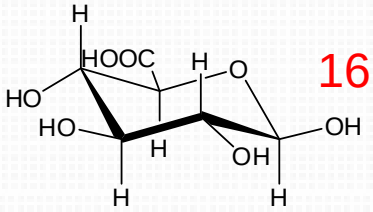


16

Ulosonic acids

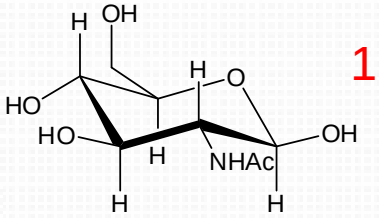
Kdo
Sialic acid
Legionamminic acid
….

Uronic Acids



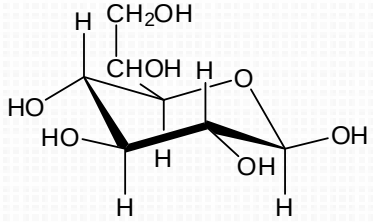
16

2-Aminosugars



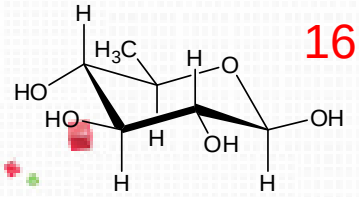
16

2-Aminuronic acids



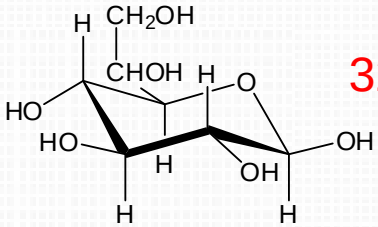
16

Deoxysugars



16

Heptose



32

Dideoxysugars
Aminodeoxysugars
Branched sugars
…..

∞

GlycoRDF Resource Description Framework

http://www.glycoinfo.org/GlycoRDF/

Common ontology for representing glycan structures and their pertinent metadata

Representation format	Pros	Cons
IUPAC condensed/IUPAC extended	Fairly human readable; standardized by the IUPAC commission	Somewhat computer readable, but variations exist between databases
LINUCS	Somewhat human readable	Can only represent completely defined structures (no ambiguity allowed)
CarbBank 2D notation	Human readable	Difficult for computer to process
BCSDB linear code	Can represent rare and complex oligosaccharides including those found in bacteria and plants	Difficult for humans to read
KCF	Uses graph notation; can represent ambiguous structures	Monosaccharide representation is ambiguous, making integration with other databases difficult
Linear code ®	Compact representation	Not human readable
GlycoCT	Uses graph notation; has strict rules to represent monosaccharides	Uses a library to represent substituents, which makes it difficult to update and integrate with other databases
GLYDE-II	Uses XML notation, making database integration and exchange easier	Not human readable
WURCS	Can uniquely represent any sequence, including those containing rare monosaccharides and ambiguous linkages	Not human readable

MonosaccharideDB

http://www.monosaccharidedb.org/

- Comprehensive resource of monosaccharides
- Dynamic dictionary
- Translate residue names from one notation to another

Notation		
Scheme: ?	Name:	Separate Substituents:
MonosaccharideDB	b-dglc-HEX-1:5|(2d:1)n-acetyl	
CarbBank	b-D-GlcpNAc	
Glycosciences.de	b-D-GlcpNAc	
GlycoCT	b-dglc-HEX-1:5	▪ (2d-1) n-acetyl
CFG	GNb	
BCSDB	bDGlcpN	▪ (2-1) Ac
GLYCAM	0YB	
Protein Data Bank	NAG	
IUPAC	2-acetamido-2-deoxy-beta-D-Glucopyranose	

Ranzinger R. et al. *Bioinformatics, 2015. 31(6):919-925.*

GLYCANS STRUCTURE

3D structure & Dynamics

Sequence & Conformation

Chemical structure

Complex operation of bench organic and analitical organic chemistry are needed!

Bacterial cells → Extractions → Purifications → Derivatizations

www.stenutz.eu

A practical guide to structural analysis of carbohydrates

Phenol-water extraction

Phenol-water extraction separates proteins from bacterial extracts. The principle for the separation is that a 50/50 mixture of phenol and water is heated to 65-68 °C at which temperature only one homogenous phase is present. After cooling two phases are obtained and the polysaccharides and nucleic acids are in the water phase and the proteins in the phenol phase. A totally insoluble fraction, mostly cell remains, is obtained either at the interface or at the bottom if centrifuged hard. Sometimes the polysaccharide, in particular rough LPS, goes to the phenol phase which should be analysed for carbohydrates before being discarded.

Reagents

- Stirrer ca 300 rpm, propeller
- 1L Duran bottle with wide neck.
- 90% aqueous phenol

Procedure

1. Suspend 20 g bacteria in 350 mL of hot water (65-68 °C).
2. Add 350 mL of 90% phenol (65-68 °C) under vigorous stirring.
3. Stir for 1h at 65-68 °C.
4. Cool in an ice bath to *ca* 10 °C, or leave overnight in refrigerator.
5. Separate in separatory funnel if possible, or centrifuge at 3000 rpm 30 for 45 min, or pour everything in dialysis bag.
6. The upper, water layer is saved (not the interphase).
7. Treat the residual phenol phase and interphase, if any, with another volume of hot water and proceed as described.
8. Dialyse the combined water extracts 2-4 days in first tap water and then distilled water.
9. Concentrate to a volume suitable for freeze-drying and freeze-dry.

Comments

1. Sodium salts of LPS do not dissolve in H_2O but form micelles and give a colloidal mixture.
2. 90% aqueous phenol is a liquid and there much more convenient to handle than solid phenol. The separation of LPS from cell wall often requires stirring at high speed. If the stirring is too efficient foam will be formed and the total volume increase. In many cases loss of material will result.
3. Sometimes only 15-30 minutes is required for the extraction.

References

- *Meth. Carbohydr. Chem.* **V** (1965) 83-91

GLYCANS STRUCTURE

Chemical structure

Sequence & Conformation

3D structure & Dynamics

Once extracted and purified the polysaccharide material, it is necessary to define several items..

Toward the determination of glycan structure..

- **Monosaccharide composition**: nature of each constituting monosaccharide along with absolute configuration.
- **Linkage patterns**: monosaccharide positions involved in the glycosidic linkages. Determination of possible branching points.
- **Ring size**: distinction between pyranose or furanose ring.
- **Anomeric configuration**: α- or β -configuration at the glycosidic linkage.
- **Sequences of monosaccharide** residues and, if the glycan has a regular structure, determination of the repeating unit.
- Identification, localization and distribution of **possible substituents** on hydroxyl groups.
- **Molecular size**.

GLYCANS STRUCTURE

Chemical structure

Sequence & Conformation

3D structure & Dynamics

The use of different analytical techniques, complementing them with bioinformatic tools, is necessary!!

Structure determination of a glycan chain: Main Steps

- Quali-quantitative analysis (GC-MS)
- Absolute configuration (GC-MS, NMR)
- Size of the ring (GC-MS, NMR)
- Anomeric configuration (NMR)
- Linkage analysis (GC-MS, NMR)
- Monosaccharides sequence (MALDI-MS^n, ESI-MS^n, 2D NMR)
- Determination of non-carbohydrate appendages (GC-MS, MALDI-MS^n, ESI-MS^n, 2D NMR)

MS TOOLS for GLYCANS STRUCTURE DETERMINATION

MS spectrometry MALDI & ESI MS methods

- Chemical structure
- Sequence & Conformation
- 3D structure & Dynamics

GlycoWorkBench

Widespread open source software containing an extensive library of fragmentation types

MS Tools

GlycoSearchMS

Compares each peak of a measured mass spectrum with the calculated fragments of all structures contained in the SweetDB database

GlycoMod
Glyco-Peakfinder

Predicts the possible oligosaccharide structures that occur on proteins from their experimentally determined masses

Glycofragment
Glycoforest

Partial de novo algorithm for sequencing glycan structures based on MS/MS spectra

Glycostore

Chromatographic, electrophoretic and mass-spectrometry composition database of N-, O-, glycosphingolipid and free oligosaccharides

NIST Glycan MS library (on line)

Collection of tandem mass spectral data of glycans (experimentally acquired)

GlycosidIQ

Generates a theoretical peak list for each structure in the database by computing all its theoretical fragments

Cartoonist tool

Generates only the N-linked glycans possibly synthesized by mammalian cells using a set of archetypal structures

MultiGlycan

Helps user to gather glycan profile information from LC-MS Spectra

GS-align

Computational method for glycan structure alignment and similarity measurement

STAT / Oscar

Generate candidate structures from an estimated composition selected by the user amongst those compatible with the precursor mass

GRITS Toolbox

Modular software suite for processing, annotating and archiving of glycomics data

Campbell MP et al. *Biochim Biophys Acta*. 2014.1844:108-16.

TOOLS for N-GLYCANS STRUCTURE DETERMINATION

MS spectrometry

3D structure & Dynamics

Sequence & Conformation

Chemical structure

GlycoWorkBench

Environment in which structure models can be assembled and their mass computed

In silico fragmentation engines

↓

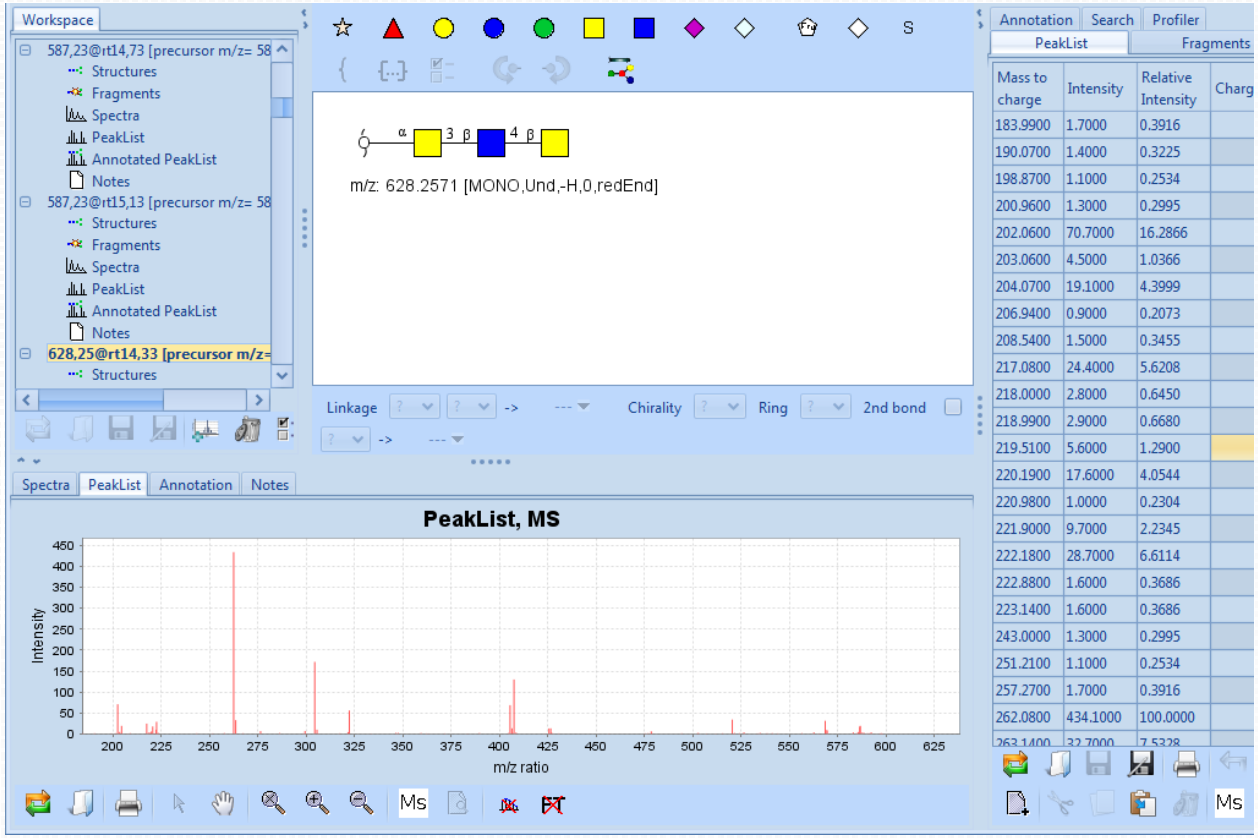
Computation of theoretical fragments (so glycosidic and cross-ring)

↓

Matching with MS/MS data

↓

Glycan structure



https://download.cnet.com/GlycoWorkbench-64-bit/3000-2383_4-75758804.html

Ceroni A. et al *J. Proteome Res*., 2008, 7 (4), pp 1650–1659.

MS spectrometry MALDI & ESI MS methods

3D structure & Dynamics

Sequence & Conformation

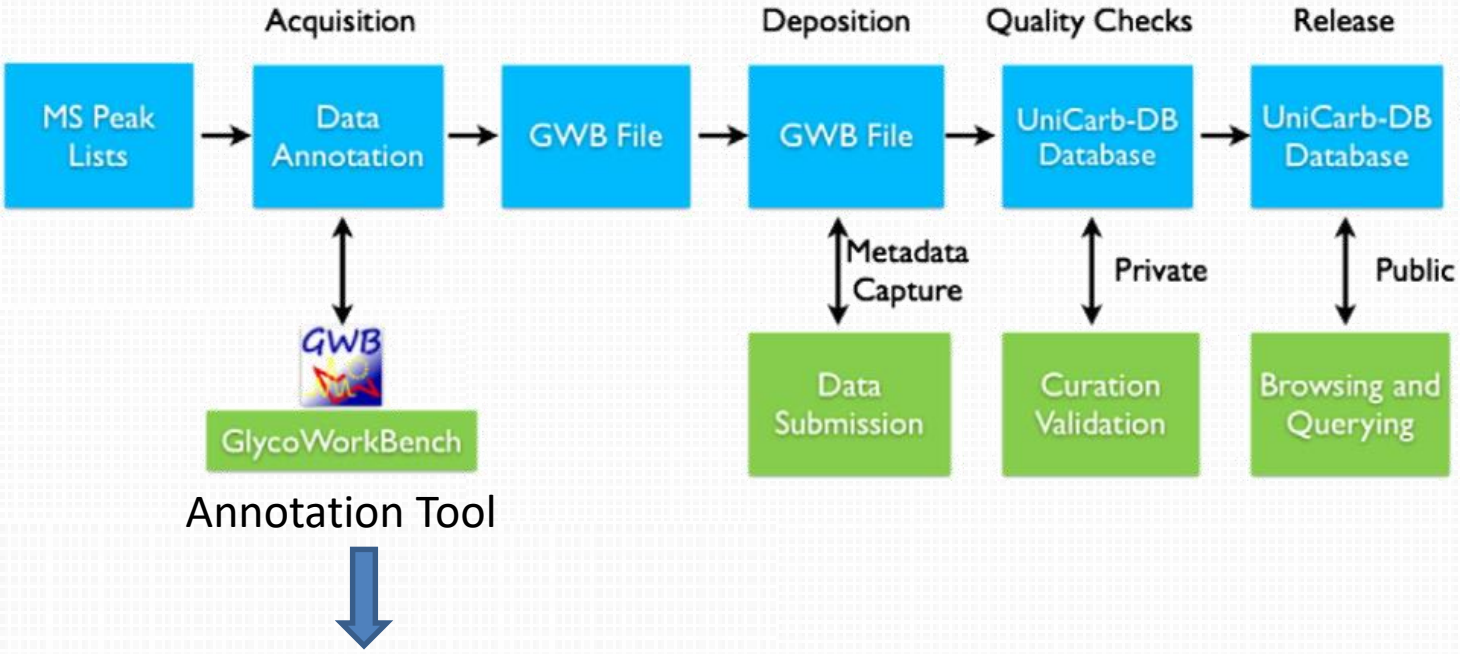
Chemical structure

TOOLS for N-GLYCANS STRUCTURE DETERMINATION

UNICARB_DB

http://unicarb-db.expasy.org/

Archival database of open-access LC MS/MS library of N- and O- linked glycans released from glycoproteins



Annotation Tool

- ✓ Glycosidic and cross-ring fragmentation ions
- ✓ Retention times
- ✓ Associated experimental metadata descriptions

Hayes CA et al *Bioinformatics.* 2011 May. 27(9): 1343-4.

TOOLS for N-GLYCANS STRUCTURE DETERMINATION

MS spectrometry
MALDI & ESI MS methods

- Chemical structure
- Sequence & Conformation
- 3D structure & Dynamics

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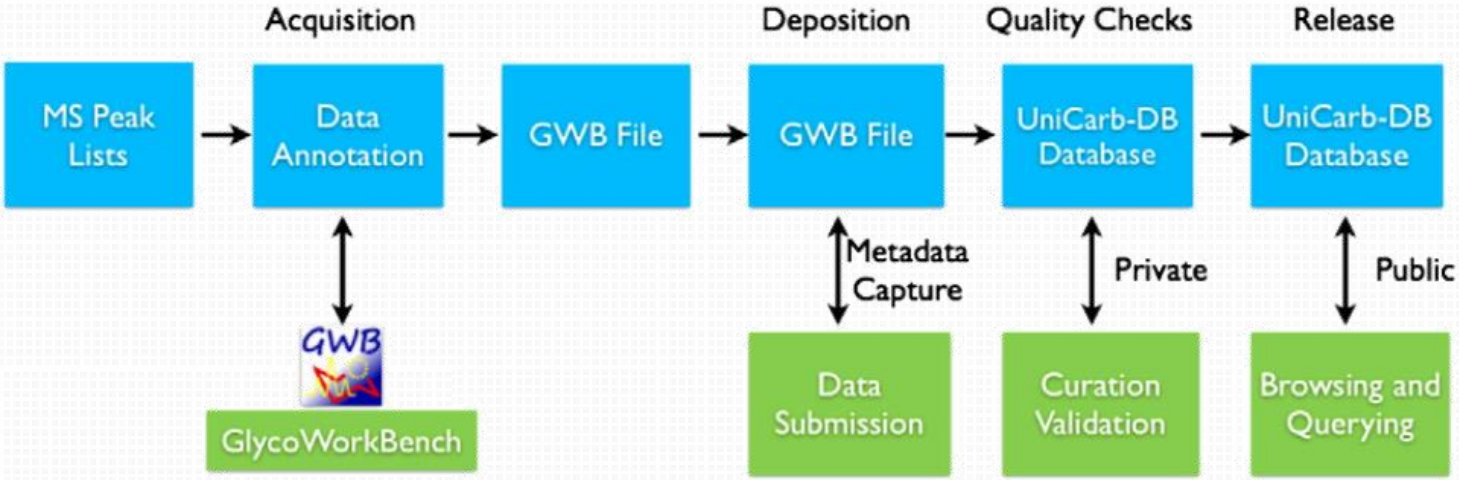


http://www.beilstein-institut.de/en/projects/mirage

MIRAGE: Minimum Information Required for A Glycomic Experiment

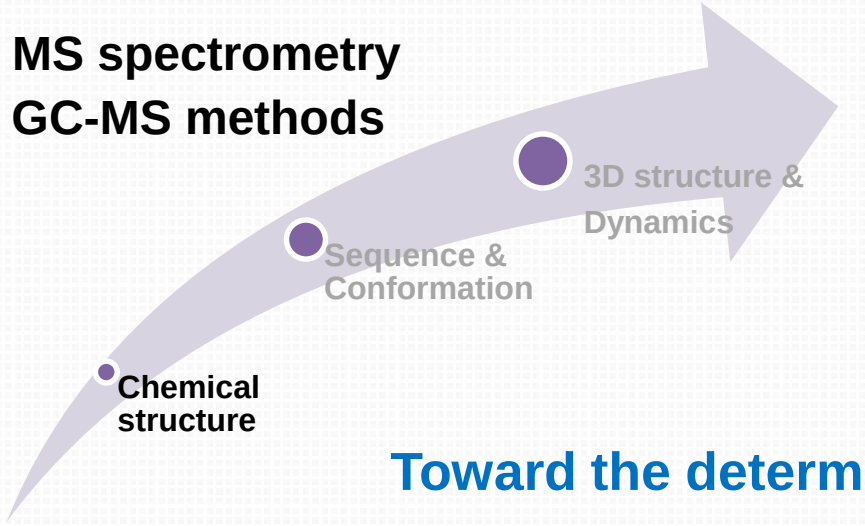
Guidelines for sample preparation, analysis and publication

GlyTouCan: International glycan structure repository

Central register & portal for search glycan-related publications

https://glytoucan.org/

MS spectrometry
GC-MS methods



Toward the determination of glycan structure..

- **Monosaccharide composition**: nature of each constituting monosaccharide along with absolute configuration.
- **Linkage patterns**: monosaccharide positions involved in the glycosidic linkages. Determination of possible branching points.
- **Ring size**: distinction between pyranose or furanose ring.
- **Anomeric configuration**: α- or β -configuration at the glycosidic linkage.
- **Sequences of monosaccharide** residues and, if the glycan has a regular structure, determination of the repeating unit.
- Identification, localization and distribution of **possible substituents** on hydroxyl groups.
- **Molecular size**.

MS spectrometry GC methods

Chemical structure

Sequence & Conformation

3D structure & Dynamics

GlycoPedia

news | e-chapters | resources | search

Gas Chromatography - Mass Spectrometry for Glycosciences

Cristina de Castro

Mini DB of MS spectra of four types of derivatives:

- Acetylated alditols
- Acetylated methyl glycosides
- Partially methylated and acetylated alditols
- Acetylated octyl (or butyl) glycosides

Box 3: Acetylated Methyl Glycosides (AMG)

- Advantages:
  - Less reactions' step compared to Acetylated Alditols
  - O.N. reaction yields to almost complete methanolysis of the product
  - No free aldehyde group is produced during methanolysis ⇒ monosaccharide degradation is minimized
  - Suitable for most type of sugars
    - Hexoses
    - Aminosugars
    - Uronic acid
    - Ulosonic acids
- Limits:
  - One sugar ⇒ more peaks
  - Respect anhydrous conditions during methanolysis
  - Ketose residues are lost

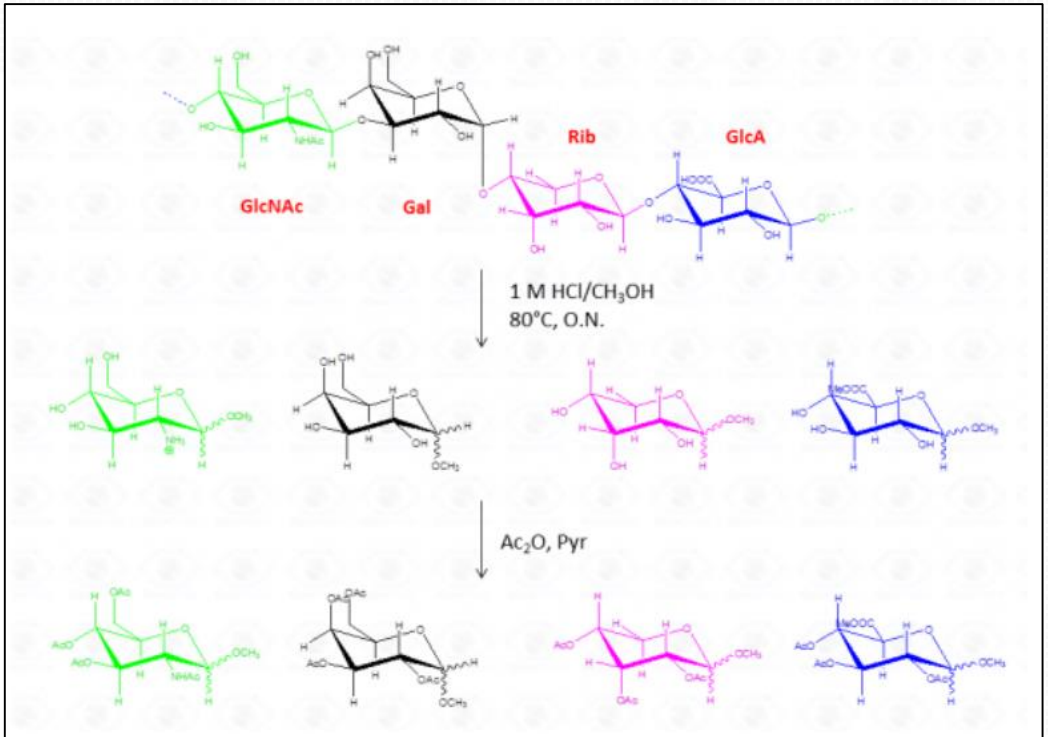


Figure 10 : Scheme of the reactions occurring during transformation of a glycan in the corresponding Acetylated Methyl Glycosides (AMG)

MS spectrometry
GC methods

Chemical structure

Sequence & Conformation

3D structure & Dynamics

GlycoPedia

news | e-chapters | resources | search

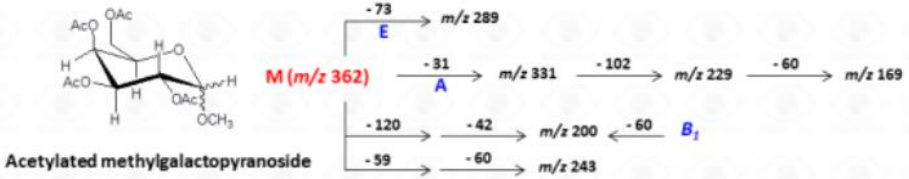
Gas Chromatography - Mass Spectrometry for Glycosciences

Cristina de Castro

Acetylated alditols
Acetylated methyl glycosides
Partially methylated and acetylated alditols
Acetylated octyl (or butyl) glycosides

Box 4: AMG fragmentation rules

- The most stable ions will be observed in the EI-MS spectrum
- Isomeric sugars (as Glc and Gal) give the same EI-MS spectrum
- The radical cation of the methylglycosides undergoes several pathways:
  - A, B, C, D, E, F, H, J, and K (example given for an hexose)
  - Fragments gives a series of daughter ions by loss of neutral molecules (AcOH, Ac_2O, $AcO^{\bullet}$, $Ac^{\bullet}$, $CH_2{=}C{=}O$)
- Occurrence of acetamido, deoxy, methylester groups, may direct the fragmentation pathway to a preferential route.
- Along with the ions from the fragmentation pathways, triacetoxonium (*m/z* 145) and diacetoxonium (*m/z* 103) ions maybe observed.



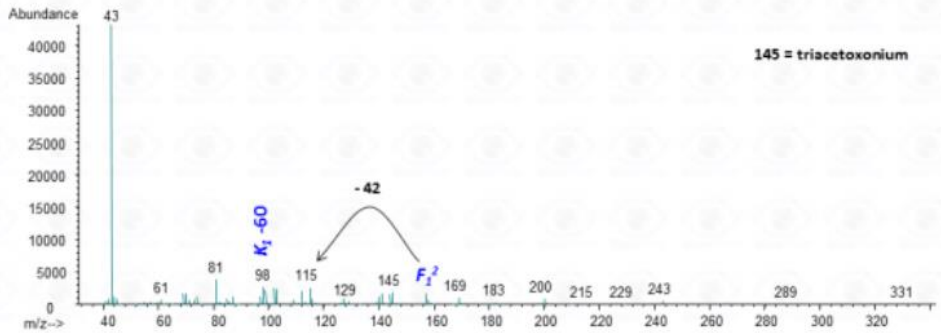


Figure 13 : EI-MS spectrum of a fully acetylated methylhexopyranose.

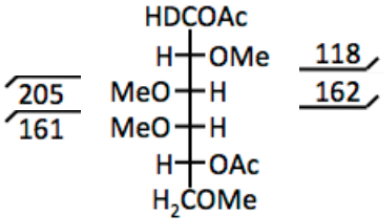
MS spectrometry
GC methods

- Chemical structure
- Sequence & Conformation
- 3D structure & Dynamics

The CCRC Spectral Database for PMAA's

https://www.ccrc.uga.edu/specdb/ms/pmaa/pframe.html

Partially methylated and acetylated alditols



The CCRC Spectral Database for PMAA's

- To see the Partially Methylated Alditol Acetate (PMAA) derived from a glycosidically linked sugar residue, *scroll down* the PMAA table until you find its entry and mouse over it.
- Click on the entry to see the electron-impact mass spectrum (EI-MS) of the PMAA.

Help | View the PMAA Table

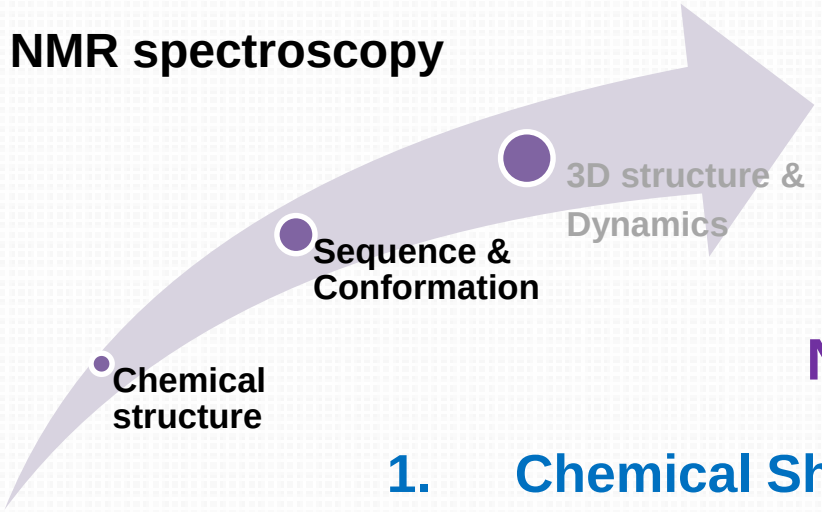
Non-Branched Hexopyranosyl Residues

Linkage	Gal*p*	Glc*p*	Man*p*	Fuc*p*	Rha*p*	Gal*p*NAc	Glc*p*NAc	Man*p*NAc
T								
2						-	-	-
3								
4								
6				-	-			

Singly Branched Hexopyranosyl Residues

Linkage	Gal*p*	Glc*p*	Man*p*	Fuc*p*	Rha*p*	Gal*p*NAc	Glc*p*NAc	Man*p*NAc
2,3						-	-	-
2,4						-	-	-
2,6				-	-	-	-	-
3,4								
3.6				-	-			

NMR spectroscopy



Non-destructive method!

NMR parameters

1. **Chemical Shifts**
   Monosaccharide composition, anomeric configuration, identification, localization and distribution of possible substituents on hydroxyl groups,
2. **Area of peaks**
   Relative proportion of nuclei
3. **Coupling constant (J) ➔ Hz**
   Structural and Conformational information
4. **Nuclear Overhauser Effect**
   Distance between nuclei ➔ monosaccharide sequence and conformational information
5. **Molecular Motion**
   Information is contained in relaxation, NOE and diffusion experiments

NMR ANALYSIS of COMPLEX CARBOHYDRATES

NMR spectroscopy

- Chemical structure
- Sequence & Conformation
- 3D structure & Dynamics

Assigning saccharides 3D structure implies identifying and quantifying an ensemble of conformations!

Websites:

www.stenutz.eu Chemical Shifts & Coupling constants libraries

http://www.organ.su.se/gw/doku.php Calculation of NMR chemical shifts
Determining the primary structures from experimental NMR data

http://www.nmrdb.org/ Prediction of ^{13}C and ^{1}H NMR spectra

Databases:

BCSD Carbohydrate Structure Databases

Glyco3D

Glycoscience.de

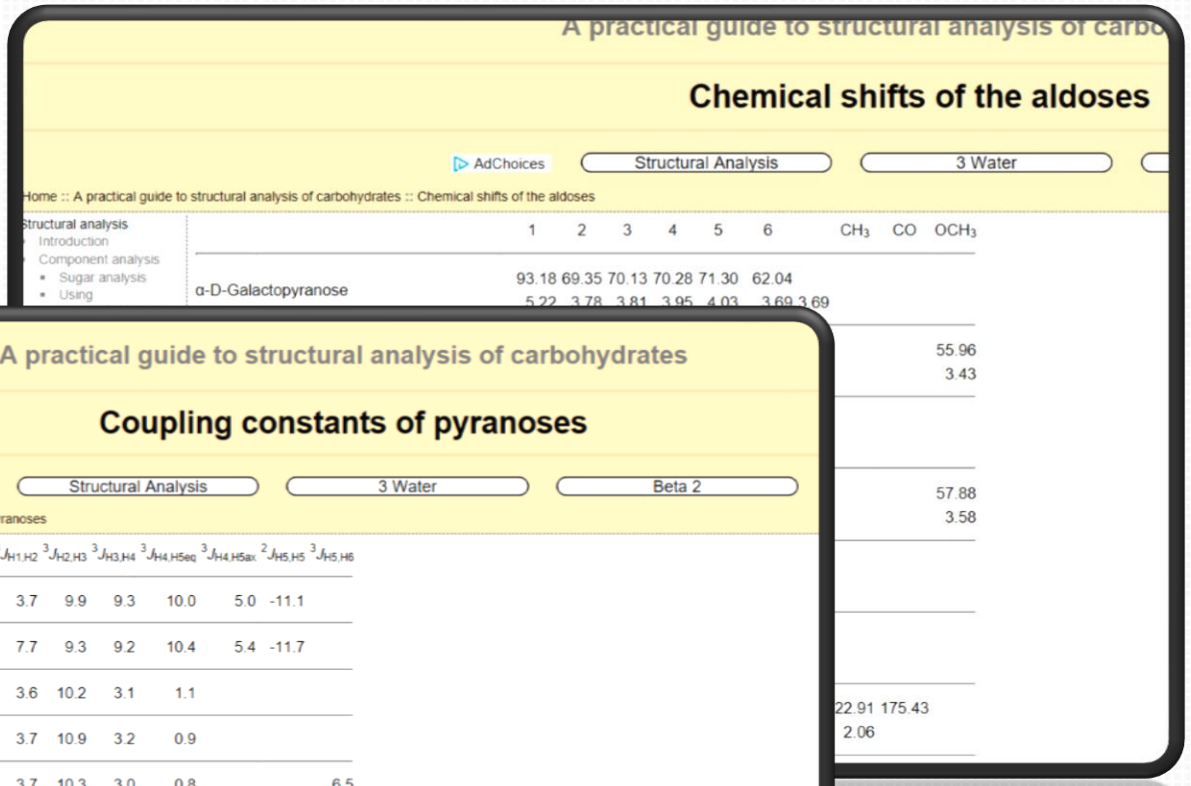
NMR spectroscopy chemical shift

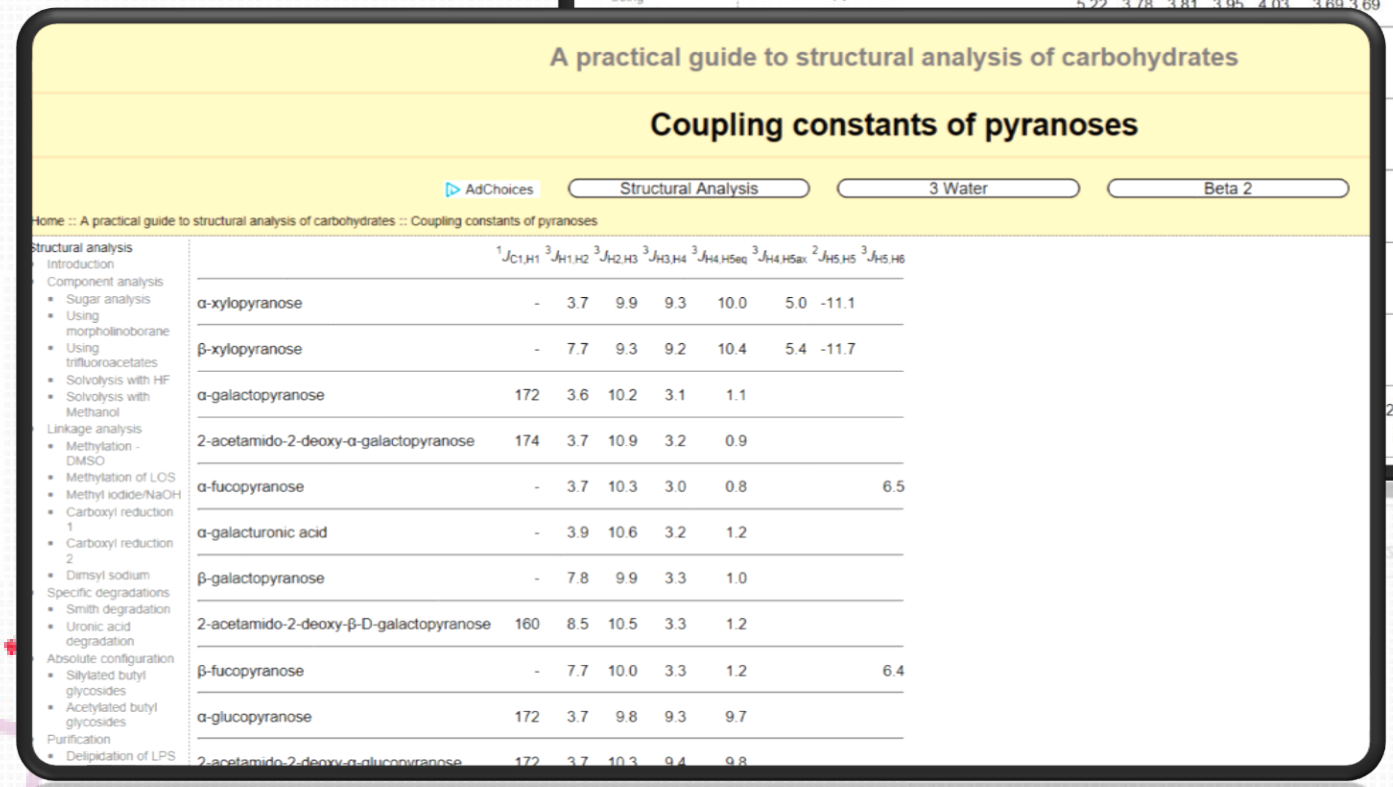
www.stenutz.eu

3D structure & Dynamics

Sequence & Conformation

Chemical structure





NMR spectroscopy coupling constants

www.stenutz.eu

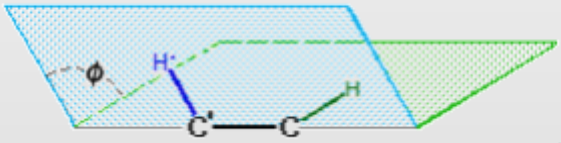
3D structure & Dynamics

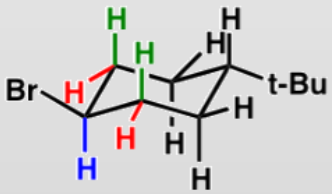
Sequence & Conformation

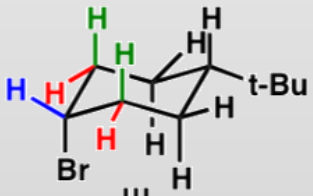
Chemical structure

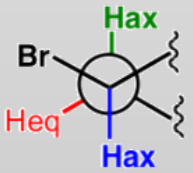
Karplus equation

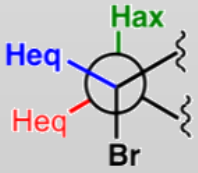
$$^3J_{H,H} = A + B\cos\phi + C\cos 2\phi$$











$J_{Hax,Hax}$ = 10-13 Hz (~180°)

$J_{Hax,Heq}$ = 2-5 Hz (~60°)

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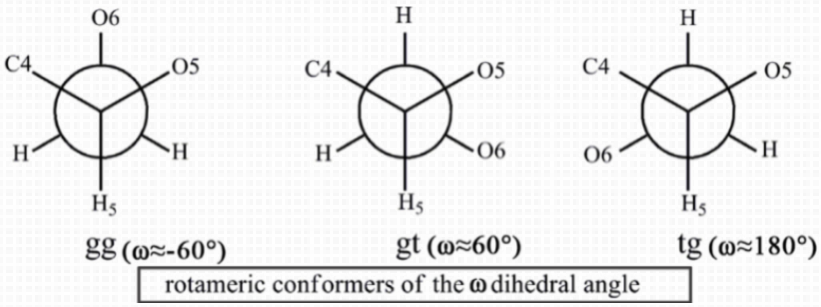
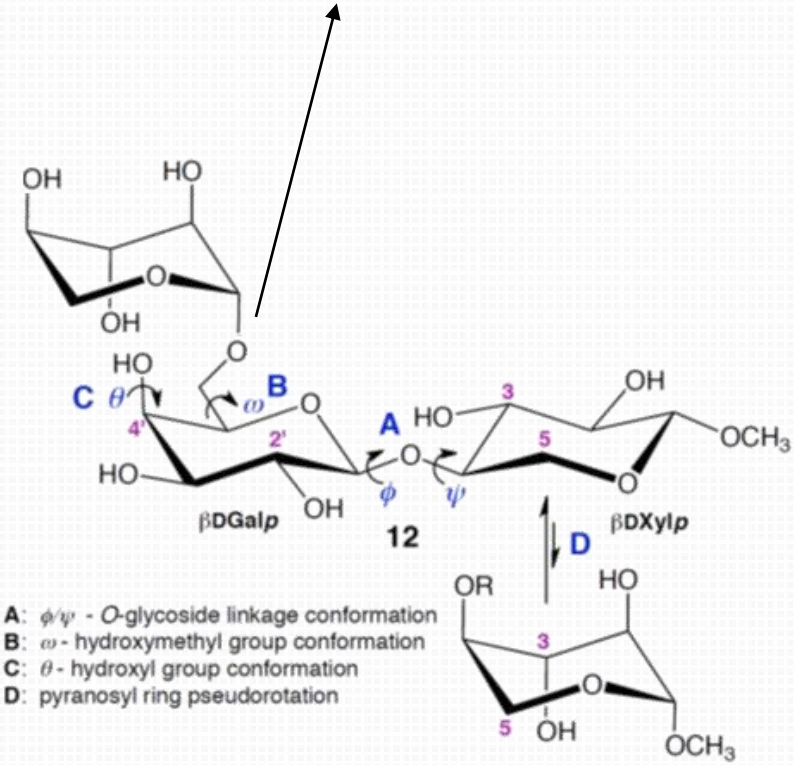
NMR J couplings help to define the glycan stereochemistry





NMR spectroscopy coupling constants

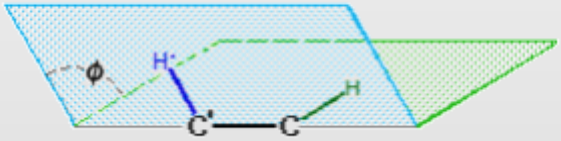
3D structure & Dynamics

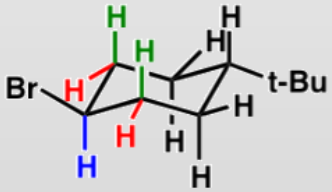
Sequence & Conformation

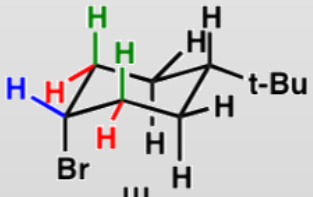
Chemical structure

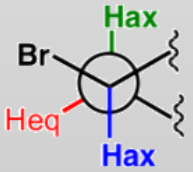
Karplus equation

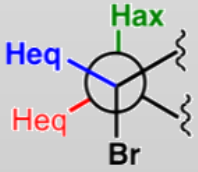
$$^3J_{H,H} = A + B\cos\phi + C\cos 2\phi$$











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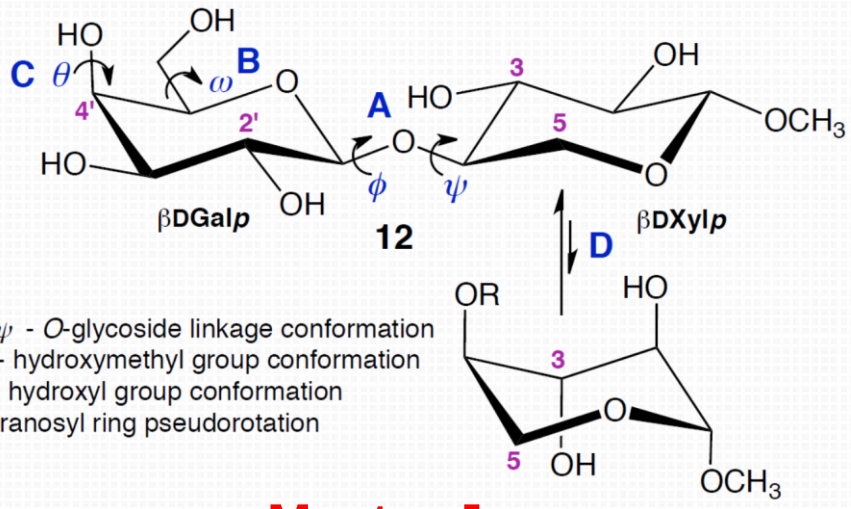
www.stenutz.eu

NMR J couplings help to define the glycan stereochemistry

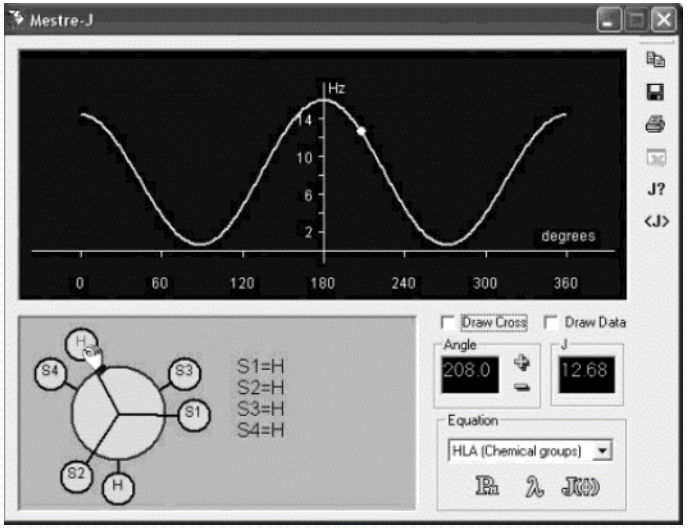


A: ϕ/ψ - *O*-glycoside linkage conformation
B: ω - hydroxymethyl group conformation
C: θ - hydroxyl group conformation
D: pyranosyl ring pseudorotation

Mestre J

http://mestrelab.com/software/freeware/

Prediction of vicinal proton-proton couplings



Navarro-Vazquez A. et al *J. Chem. Inf. Comput. Sci.* 44 (5), 2004.

NMR spectroscopy

chemical shift

3D structure & Dynamics

Sequence & Conformation

Chemical structure

Website useful for the structural and conformational analysis of glycans by NMR

http://www.organ.su.se/gw/doku.php

Stockholm University

Bioorganic Chemistry

The Widmalm Research Group

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- CASPER
- ECODAB
- CarbBuilder
- NMR Pulse Sequences

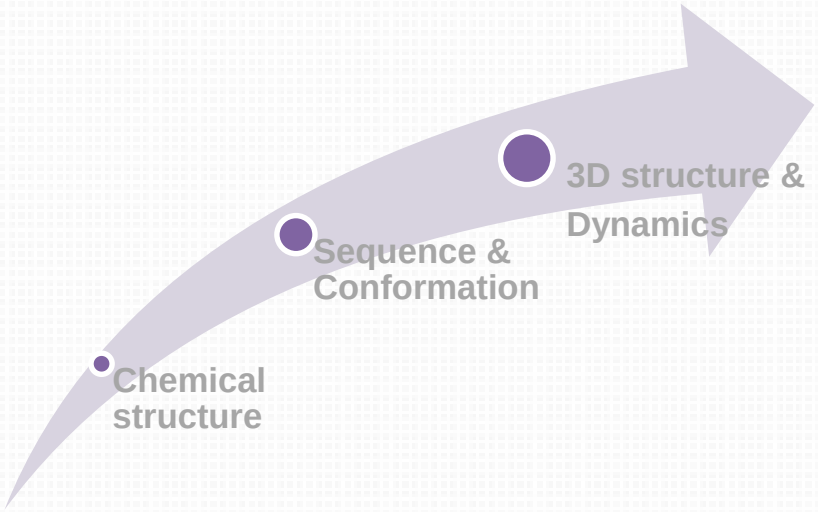
Research in Bioorganic Chemistry

The research is focused on understanding structure-dynamics-interaction relationships of carbohydrates in general and glycan-protein interactions in particular. Three different avenues are chosen to this end, viz., (i) organic synthesis; (ii) experimental biophysical techniques, especially NMR spectroscopy; and (iii) molecular simulations, in particular molecular dynamics simulations. We carry out investigations on the following subjects:

- Development of rapid and robust methodology for the determination of carbohydrate structure, i.e., toward fully automatic structure determination by NMR
- Chemical synthesis of oligosaccharides, glycoconjugates, and of protein inhibitors
- In depth studies of conformational dynamics of glycoconjugates found in biologically and medically relevant systems
- Interaction and docking studies of glycoconjugates and of small organic molecules with proteins in general and enzymes in particular with the aim of improving antibiotic uptake in gram-negative bacteria

The research spans from Glycobiology to Biophysics.

Welcome



ECODAB

Database containing structures of the repeating units that comprise the O-antigen from *E. coli.*

Information about glycosyltransferases involved in the assembly of the O-antigen polysaccharide are also included.

http://nevyn.organ.su.se/ECODAB/

EcoDAB
Escherichia coli O-Antigen Database

Main | Components | List | Summary | Search | Other Bacteria

Summary

1	0/0/4	1/3/4	0/4/4	0/3/3	0/2/3	0/4/4	2/4/4	0/2/2	2/2/4	0/4/4	10
11	0/4/4	0/3/3	1/2/4		0/2/2	0/3/3	0/2/4	0/4/4	0/3/4	0/0/1	20
21	0/4/4	0/4/4	0/3/5	3/3/3	0/4/4	1/2/2	0/4/5	0/3/2	0/5/4	0/3/3	30
31		0/4/4	0/3/0	0/3/0	0/4/5	0/3/4	0/3/3	0/4/4	0/4/4	0/3/3	40
41	0/4/5	3/3/3	0/4/4	0/2/4	0/2/2	0/4/3		0/4/4	0/3/3	0/3/0	50
51	0/4/0	0/3/1	0/3/4	0/3/0	0/4/0	2/3/3		0/3/3	0/3/4	0/4/0	60
61	0/2/2	0/3/4	0/3/0	0/5/4	0/4/4	0/4/4		0/2/6	0/3/4	0/3/4	70
71	3/3/3		2/2/5	0/3/3	0/3/3	0/3/3	2/2/3	1/3/3	0/5/5	0/4/0	80
81	0/4/0	0/3/3	0/4/4	0/3/0	1/4/4	2/4/4	0/3/3	0/3/3	0/6/0	0/3/3	90
91	0/4/4	0/2/0			0/3/0	0/4/4	0/5/3	3/3/3	3/3/5	0/2/3	100
101	0/6/1	4/4/4	0/4/4	3/3/3	1/5/5	0/2/0	2/4/4	0/3/3	3/3/3	0/3/3	110

Structure(s) of this serogroup O-antigen exist in ECODAB.

The O-antigen structure does not exist in ECODAB.

The O-antigen structure does not exist, but there is GT information.

This serogroup has been removed.

The numbers in the table (of each entry) are interpreted as following:

- The first number is the number of GTs with known function.
- The second number is the number of GTs in this entry.
- The third number is the anticipated number of GTs based on the structure.

NMR spectroscopy

chemical shift

3D structure & Dynamics

Sequence & Conformation

Chemical structure

CASPER

http://www.casper.organ.su.se/casper/

CASPER: computer assisted spectrum evaluation of regular polysaccharides

- ✓ Calculating NMR chemical shifts
- ✓ Determining the structure of unknown glycans from experimental data

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Welcome

NMR spectroscopy

3D structure & Dynamics

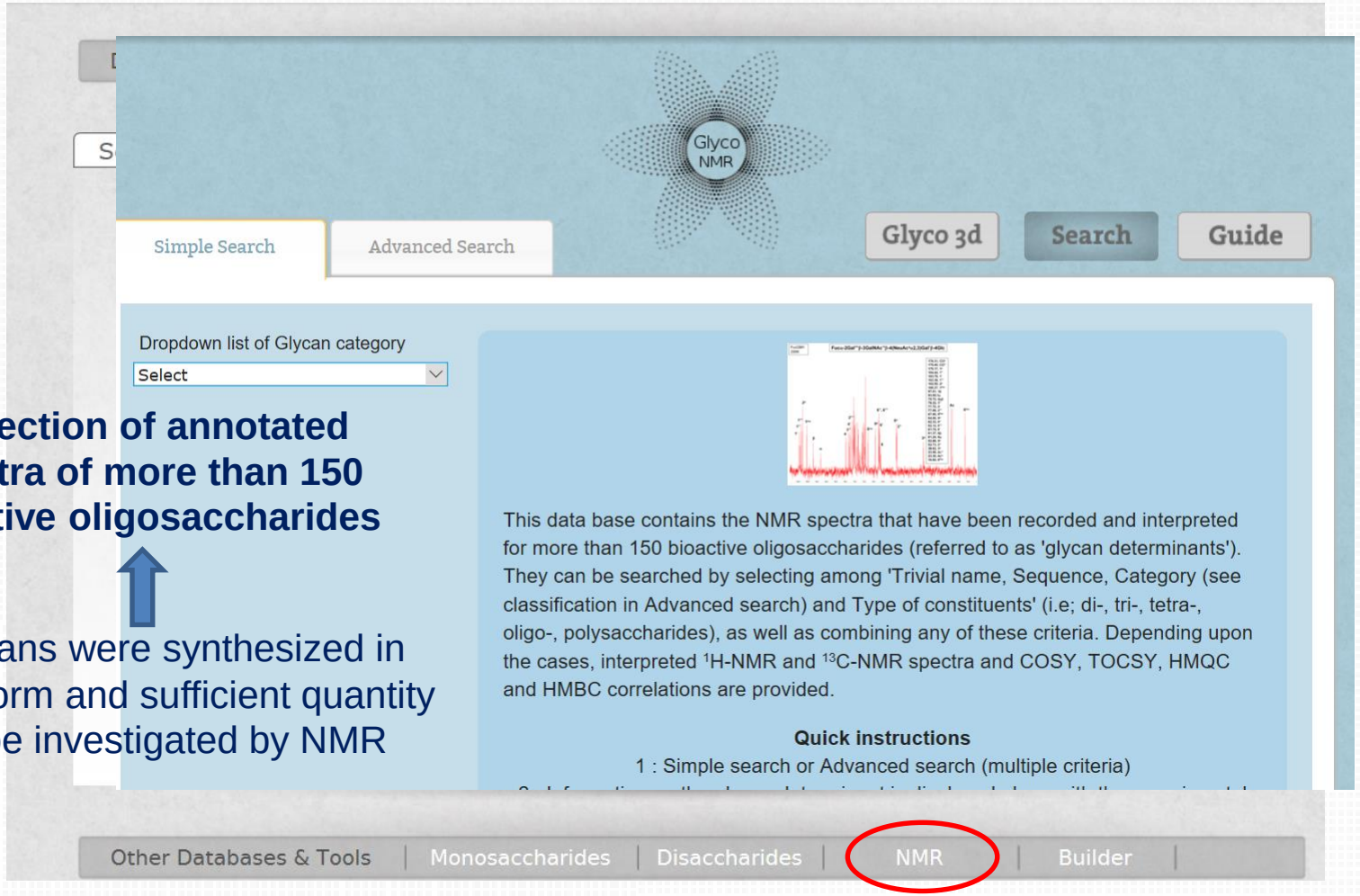
Sequence & Conformation

Chemical structure

Glyco3D

http://glyco3d.cermav.cnrs.fr/home.php

Portal for structural glycobiology of several interlinked databases covering the 3D features of glycans and proteins.



Collection of annotated spectra of more than 150 bioactive oligosaccharides

Glycans were synthesized in pure form and sufficient quantity to be investigated by NMR

NMR spectroscopy

Chemical structure

Sequence & Conformation

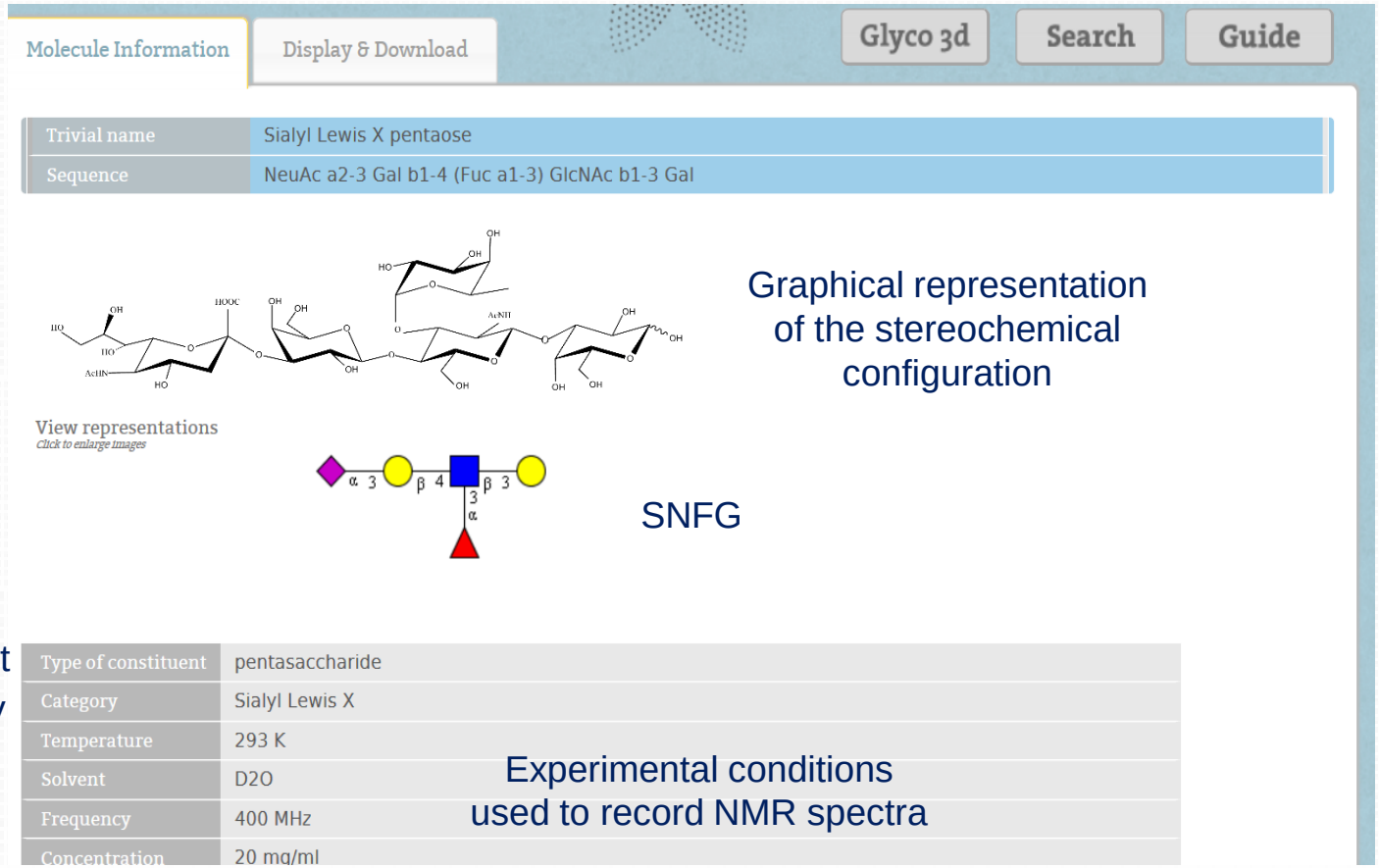
3D structure & Dynamics

Glyco3D

http://glyco3d.cermav.cnrs.fr/home.php

The detailed results are organized under two different tabs:

1. **Molecule Information**
2. **Display & Download**



Trivial name

Sequence

Graphical representation of the stereochemical configuration

SNFG

Type of constituent

Category

Experimental conditions used to record NMR spectra

NMR spectroscopy

3D structure & Dynamics

Sequence & Conformation

Chemical structure

Glyco3D

http://glyco3d.cermav.cnrs.fr/home.php

The detailed results are organized under two different tabs:

1. **Molecule Information**
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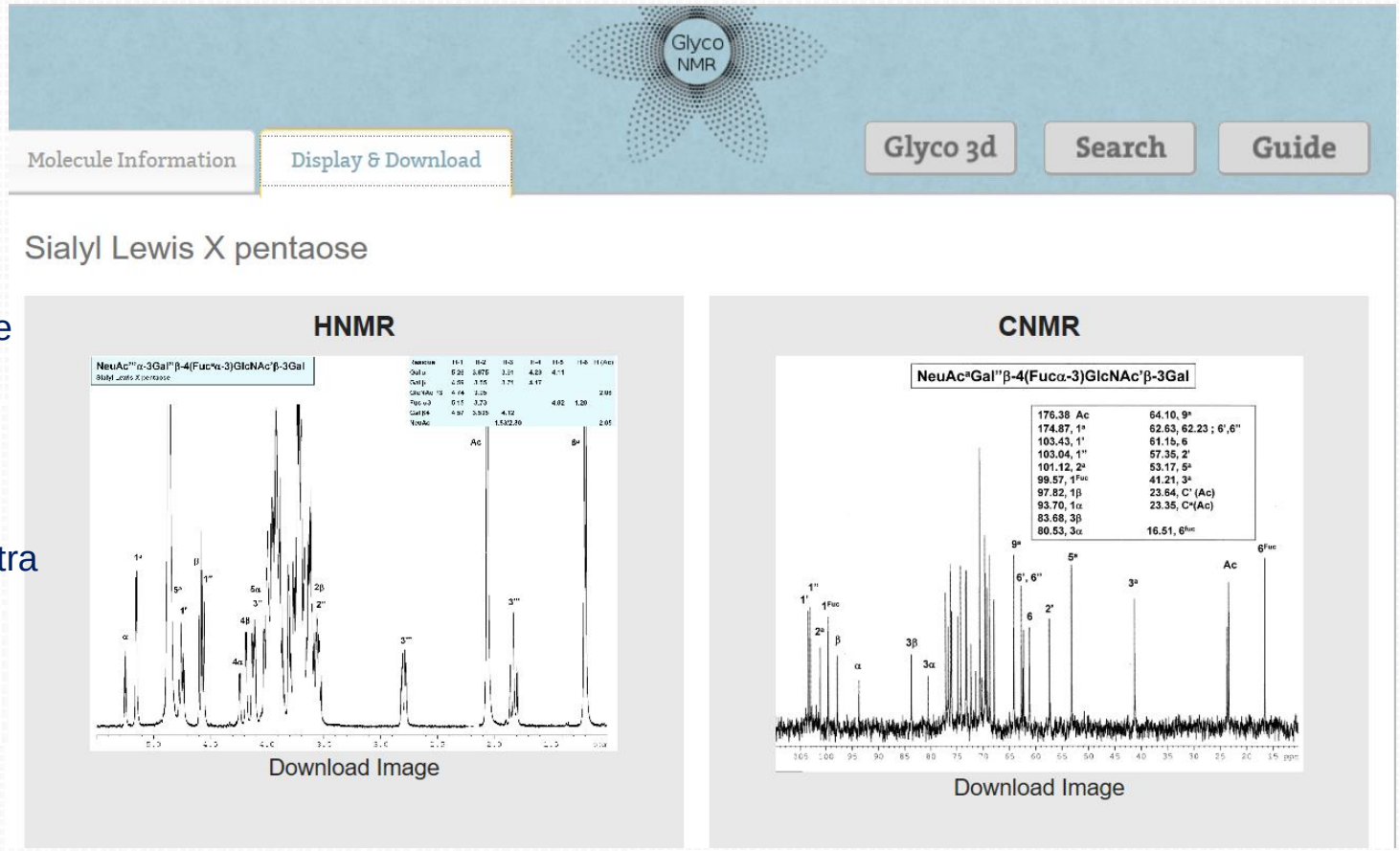
Representation of the chemical repeat

Interpreted NMR spectra



NMR spectroscopy

3D structure

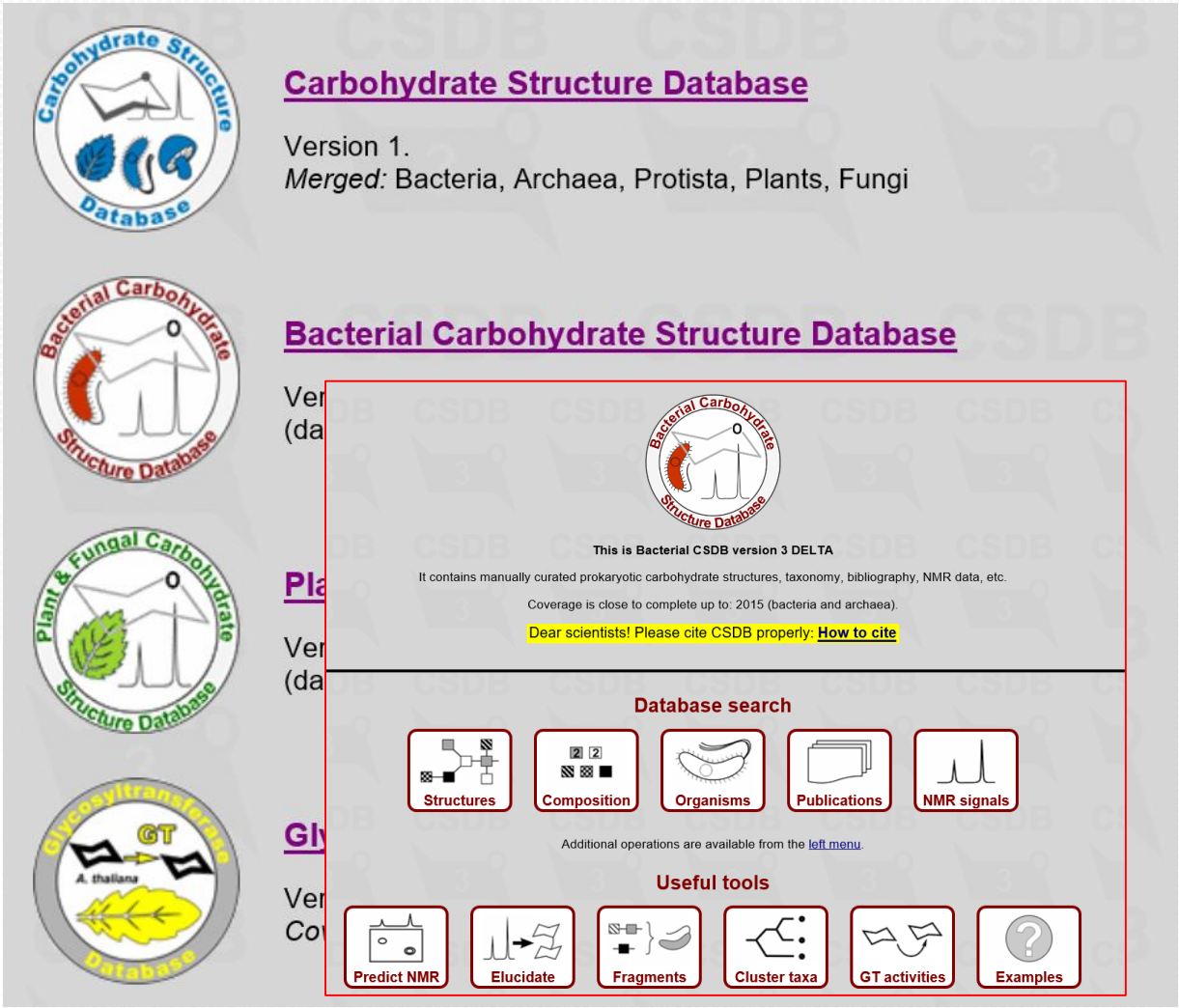
Sequence & Conformation

Chemical structure

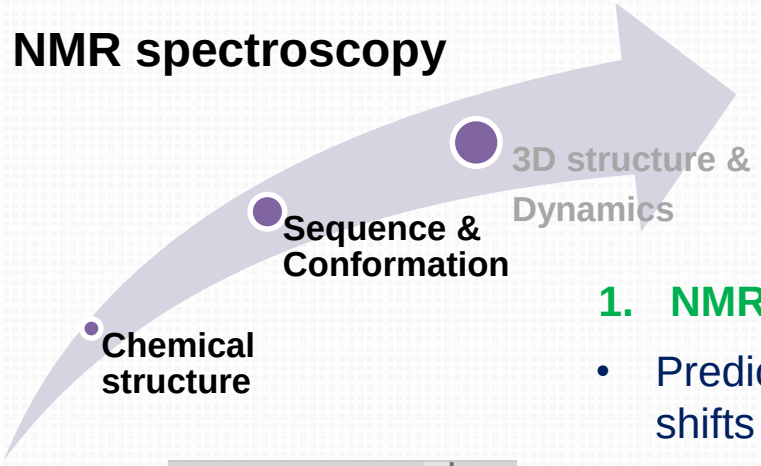
CARBOHYDRATE STRUCTURE BATABASE

http://csdb.glycoscience.ru/

Database providing structural, bibliographic, taxonomic, NMR spectroscopic information on published carbohydrates.



NMR spectroscopy



CSDB: BACTERIAL CARBOHYDRATE STRUCTURE DATABASE

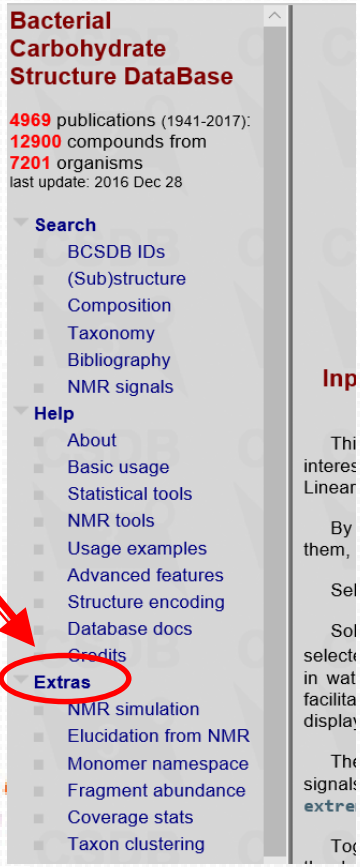
The database provides two NMR services:

1. NMR simulation tool

- Predicts ^{13}C and ^{1}H NMR chemical shifts for a specified compound.
- Ability to process almost all structural features occurring in natural glycans, including atypical and non-carbohydrate moieties.

2. NMR-based structure ranking tool

- Generates all possible structures matching the given constraints and matches them against an experimental ^{13}C NMR spectrum.



Generation
Ranking
Assignment
Saccharide
Structures

According to the fit between the simulated and experimental NMR spectra, the tool ranks structural hypotheses. To do that, it iterates through all possible carbohydrates and their derivatives limited by specified constraints.

NMR spectroscopy

3D structure & Dynamics

Sequence & Conformation

Chemical structure

CSDB: BACTERIAL CARBOHYDRATE STRUCTURE BATABASE

Rank Structural Hypotheses
Generation, Ranking and Assignment of Saccharide Structures algorithm

Bacterial Carbohydrate Structure DataBase

4969 publications (1941-2017):
12900 compounds from
7201 organisms
last update: 2016 Dec 28

- **Search**
  - BCSDB IDs
  - (Sub)structure
  - Composition
  - Taxonomy
  - Bibliography
  - NMR signals
- **Help**
  - About
  - Basic usage
  - Statistical tools
  - NMR tools
  - Usage examples
  - Advanced features
  - Structure encoding
  - Database docs
  - Credits
- **Extras**
  - NMR simulation
  - Elucidation from NMR
  - Monomer namespace
  - Fragment abundance
  - Coverage stats
  - Taxon clustering

Complete residue list - Microsoft Edge

csdb.glycoscience.ru/bacterial/core/complete.php?0#glc

Complete residue list

Selected residue: DQuiN4N-ol Retrieve & close

Absolute: D

Fast navigation:

Glucose derivatives	Threose derivatives
Mannose derivatives	Erythrose derivatives
Galactose derivatives	Glycerol derivatives
Allose derivatives	Higher sugars
Altrose derivatives	Amino acids
Talose derivatives	Aliphatic acids
Gulose derivatives	Sphingoids
Idose derivatives	Nucleosides
Xylose derivatives	Inositol derivatives
Ribose derivatives	Other monovalent residues
Arabinose derivatives	Other residues
Lyxose derivatives	

Glucose derivatives

Exclude rarely-o
structural fea

Input signals c
intensity m

Other possible structural scope limitations

The more constraints you specify → The lower number of structures is iterated → The higher the deviation between them → The more reliable the result

CSDB: BACTERIAL CARBOHYDRATE STRUCTURE BATABASE

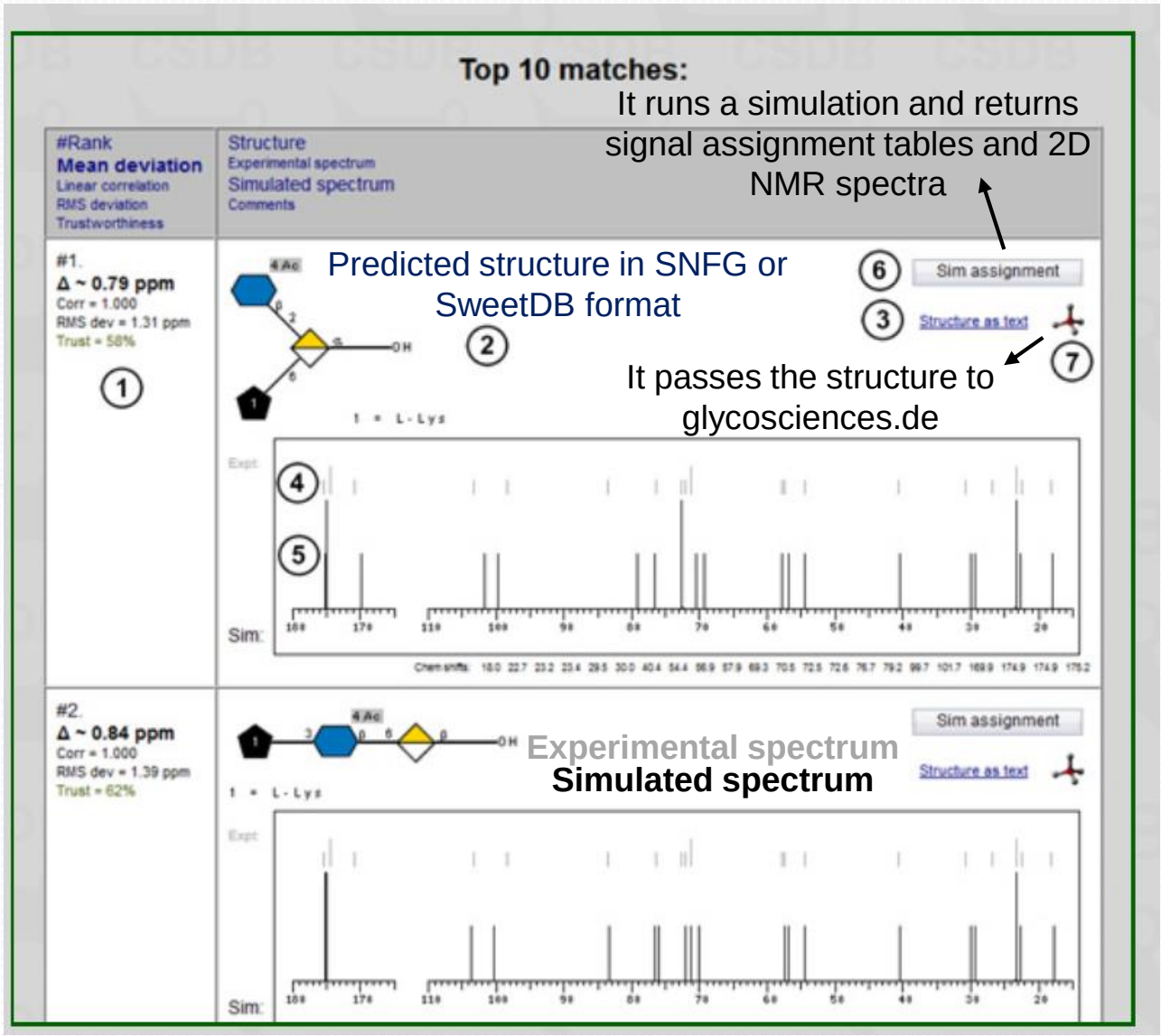
Table of structural hypotheses sorted by similarity between the simulated and the experimental NMR spectra

NMR spectroscopy

- Chemical structure
- Sequence & Conformation
- 3D structure& Dynamics

Info on spectrum similarity

- Structure rank in the top-list of structural hypotheses
- Average deviation
- Linear correlation factor
- Root-mean-square deviation in ppm
- Color-coded spectrum simulation trustworthiness level from 0% (red) to 100% (green)



Molecular Modeling

3D structure& Dynamics

Sequence & Conformation

Chemical structure

GLYCOSCIENCES.de

http://www.glycosciences.de/

Web portal that combines several tools and databases related to glycobiology and glycomics

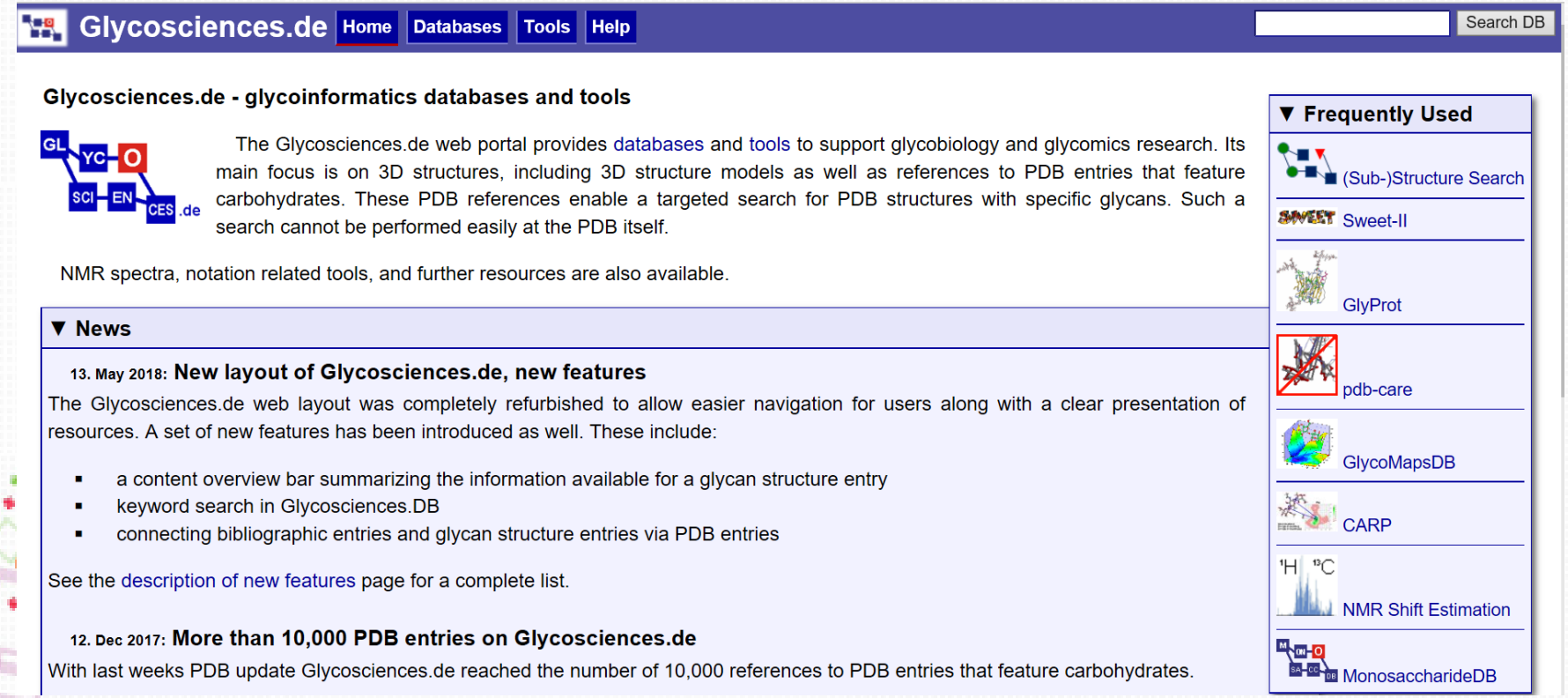
- It provides access to glycan 3D structures via cross-links to PDB entries that feature specific carbohydrates.

Errors within the carbohydrate moiety in pdb

Tools for 3D structure validation:

1. pdb-care PDB CArbohydrate REsidue

2. CARP CArbohydrate Ramachandran Plot



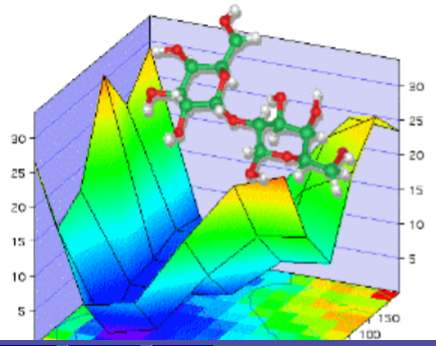
Molecular Modeling

- Chemical structure
- Sequence & Conformation
- 3D structure& Dynamics

GLYCOSCIENCES.de
http://www.glycosciences.de/

- It offers tools for 3D structure modeling of glycans
- It provides tools and databases to analyze the adopted conformations

Analysis of the conformational space adopted by a glycan



Glycosciences.de | Home | Databases | Tools | Help

[home] [search database] [difference map] [create maps] [submit data]

GlycoMaps Database

This database currently contains 2585 conformational maps.

Glycosciences.de | Home | Databases | Tools | Help

[home] [search database] [difference map] [create maps] [submit data]

Please enter the disaccharide fragment you are searching for:

b-D-Galp 1-4

B-D-GALP -(1-4)-

☑ show map previews

Results per page: 10

Submit

*
a-D-Manp
a-L-Fucp
b-D-Galp
b-D-Manp
a-D-Glcp
b-D-Glcp2NAc
a-D-Neup5Ac

Select monosaccharide units and linkage from the pull down menus or enter directly in the fields below.

Wildcards:
? matches any single character
* matches any run of text

Molecular Modelling

3D structure& Dynamics

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Chemical structure

GLYCOSCIENCES.de

http://www.glycosciences.de/

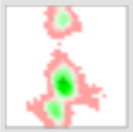
List of the conformational maps, plotting ϕ/ψ torsional angles, indicating the energy landscape of the input glycosidic linkage

Glycosciences.de | Home | Databases | Tools | Help

[home] [search database] [difference map] [create maps] [submit data]

Calculate Difference Map: Enter MapIDs of Maps to Compare: Map 1: [ ] - Map 2: [ ] Calculate

Maps 1 - 10 of 76 (click on a MapID to view map details)

MapID	Preview	Disaccaride Fragment	Complete Structure	Method
7761		b-D-Galp-(1-4)-b-D-GlcpNAc	b-D-Galp-(1-4)-b-D-GlcpNAc	MD MM3(1996) Tinker 4
8167		b-D-Galp-(1-4)-b-D-GlcpNAc	a-D-Galp-(1-3)+ | b-D-Galp-(1-4)-b-D-GlcpNAc | a-L-Fucp-(1-2)+	MD MM3(1996) Tinker 4
8173		b-D-Galp-(1-4)-b-D-GlcpNAc	b-D-Galp-(1-4)-b-D-GlcpNAc-(1-3)-a-D-GalpNAc	MD MM3(1996) Tinker 4
8179		b-D-Galp-(1-4)-b-D-GlcpNAc	a-D-Neup5NAc-(2-6)+ | b-D-Galp-(1-4)-b-D-GlcpNAc | a-L-Fucp-(1-2)+	MD MM3(1996) Tinker 4
8181		b-D-Galp-(1-4)-b-D-GlcpNAc	b-D-GalpNAc-(1-4)+ |	MD MM3(1996)

Molecular Modeling

- Chemical structure
- Sequence & Conformation
- 3D structure& Dynamics

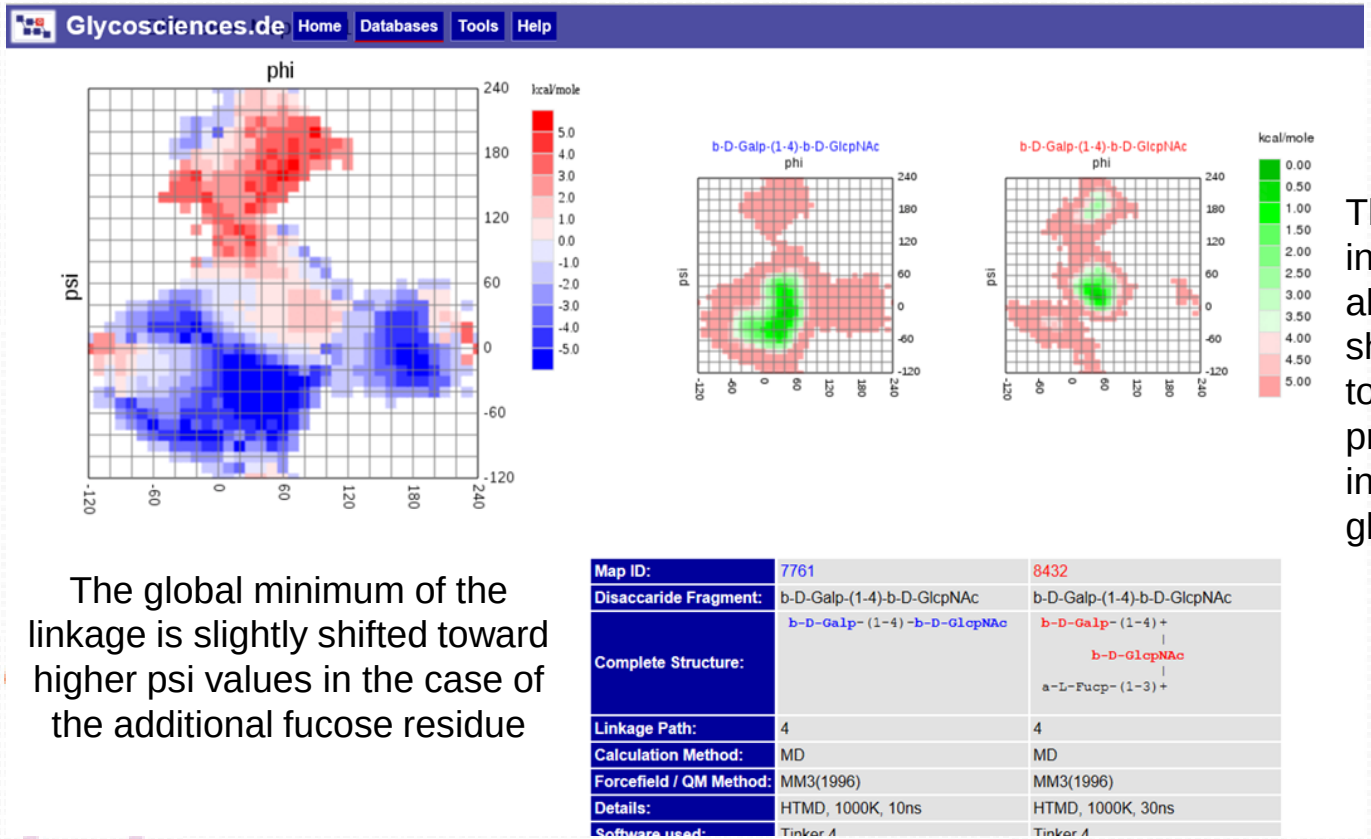
GLYCOSCIENCES.de

http://www.glycosciences.de/

Further residues that are added to the disaccharide often result in limitations of the possible conformations of an individual glycosidic linkage

↓

Calculate difference map



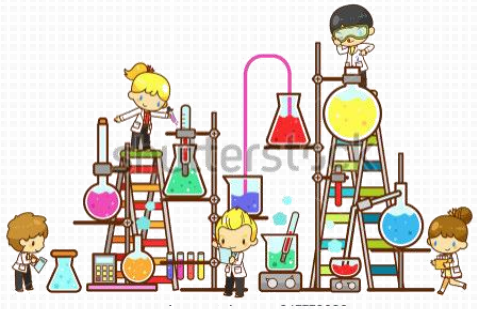
The energy difference of two individual maps is plotted, allowing so to evaluate the shifts in the values of the torsional angles due to the presence of additional residues in the neighbourhood of the glycosidic linkage.

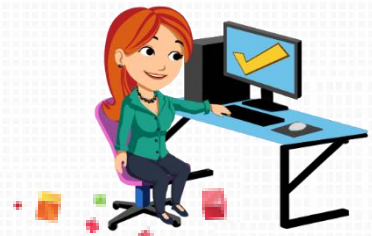
The global minimum of the linkage is slightly shifted toward higher psi values in the case of the additional fucose residue

Map ID:	7761	8432
Disaccaride Fragment:	b-D-Galp-(1-4)-b-D-GlcpNAc	b-D-Galp-(1-4)-b-D-GlcpNAc
Complete Structure:	b-D-Galp-(1-4)-b-D-GlcpNAc	b-D-Galp-(1-4)+ | b-D-GlcpNAc | a-L-Fucp-(1-3)+
Linkage Path:	4	4
Calculation Method:	MD	MD
Forcefield / QM Method:	MM3(1996)	MM3(1996)
Details:	HTMD, 1000K, 10ns	HTMD, 1000K, 30ns
Software used:	Tinker 4	Tinker 4

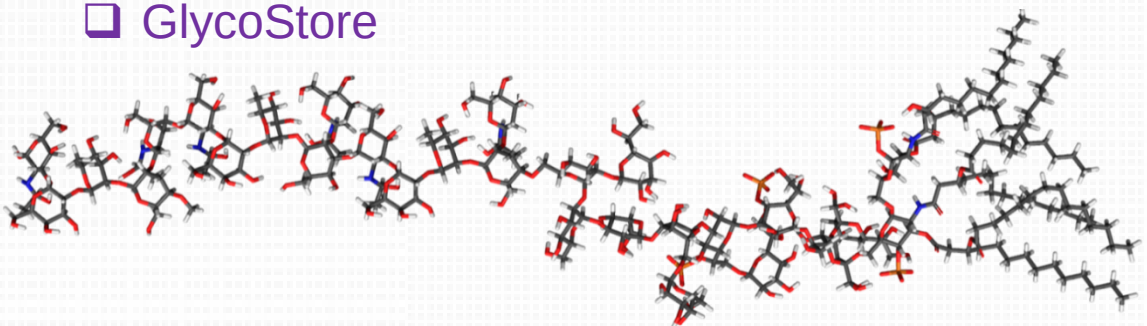
DATABASES and TOOLS USEFUL IN THE ANALYSIS OF COMPLEX GLYCAN STRUCTURE

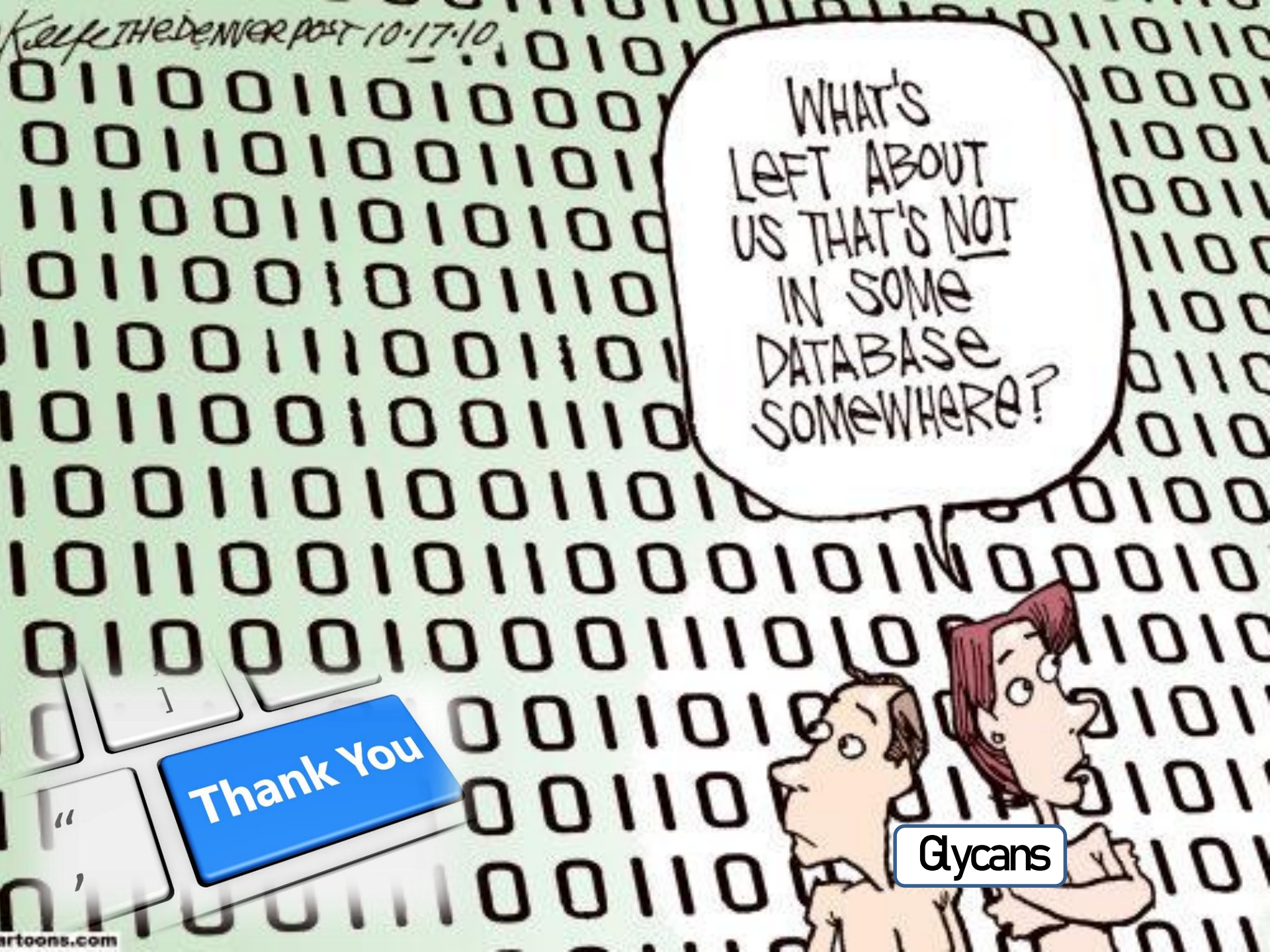
- **Glycan nomenclature**
  - Monosaccharide DB





- **Carbohydrate databases**
  - GlyTouCan
  - CFG-Glycan database
  - UniCArbDB
  - CSDB
  - KEGG GLYCAN
  - EPS database
- **Glycan NMR tools**
  - MestreJ
  - CASPER
- **Glycan MS tools**
  - GlycoWorkBench
  - Glycopedia
  - CCRC spectral database
  - GlycoStore
- **Glycan 3D Modeling**
  - Glycosciences.de
  - Glyco3D
  - Glycam
  - GLYCAN
  - GlycanBuilder
- **Glycan Binding**
  - SugarBindDB
  - GlycoEpitope
- **Others..**
  - GlycoProtDB
  - www.stenutz.eu
  - GlycoGeneDatabase




KELLY THE DENVER POST 10-17-10
WHAT'S LEFT ABOUT US THAT'S NOT IN SOME DATABASE SOMEWHERE?
Thank You
Glycans