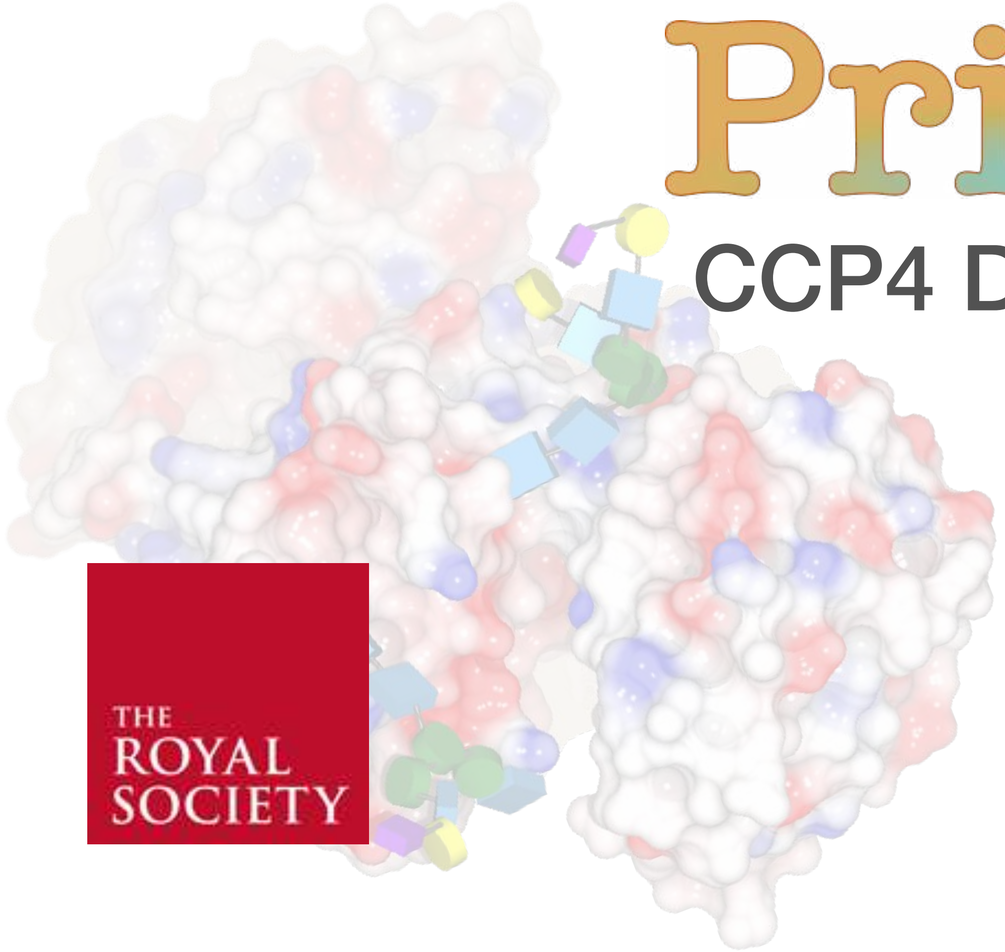


Privateer

CCP4 DLS workshop 2023

Jon Agirre
Royal Society University Research Fellow
York Structural Biology Laboratory
@glycojones@mastodon.world

Protein glycosylation

Protein glycosylation

glyco-
/ˈglʌɪkəʊ/

GREEK
glukus → glyco-

Protein glycosylation

glyco-
/ˈglʌɪkəʊ/

GREEK
glukus → glyco-

Sweet

Protein glycosylation

glyco-
/ˈglʌɪkəʊ/

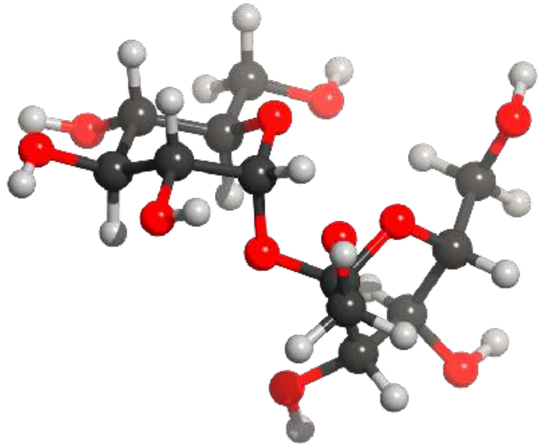
GREEK
glukus → glyco-

Sweet

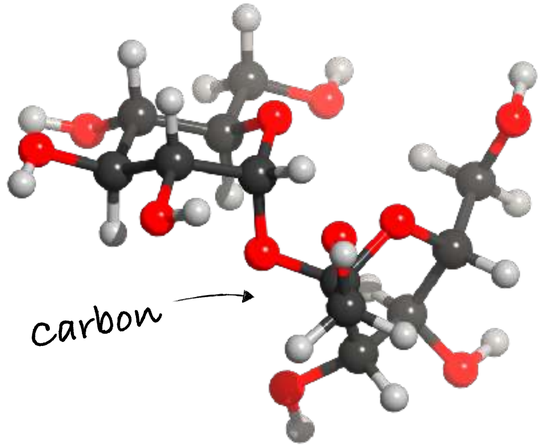
Not always sweet!

Not always sweet!

Not always sweet!



Not always sweet!



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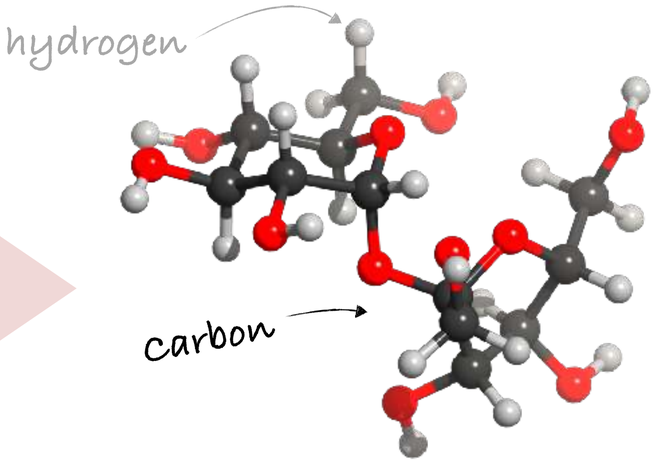

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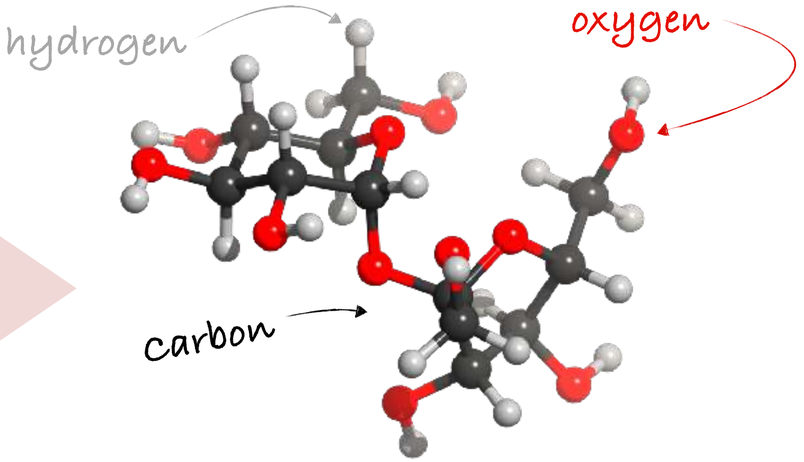

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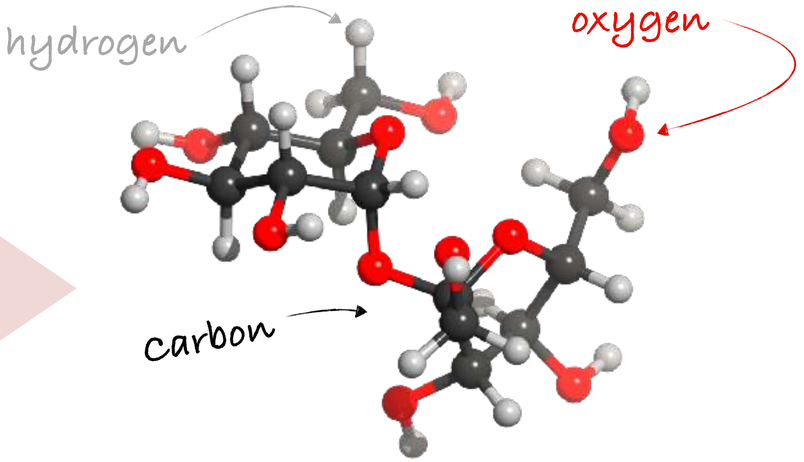

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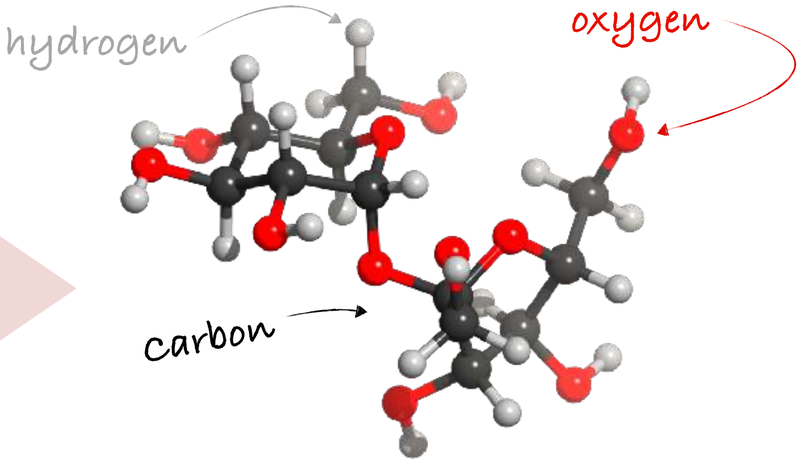

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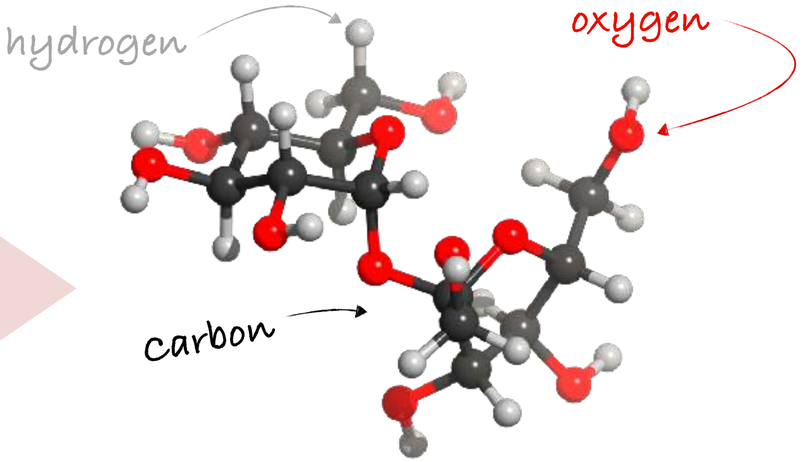

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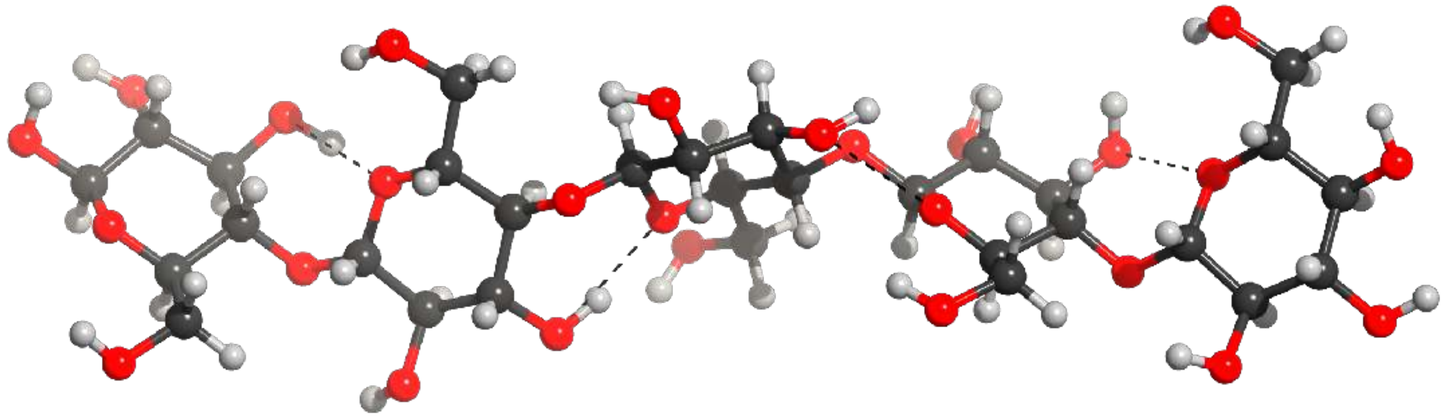

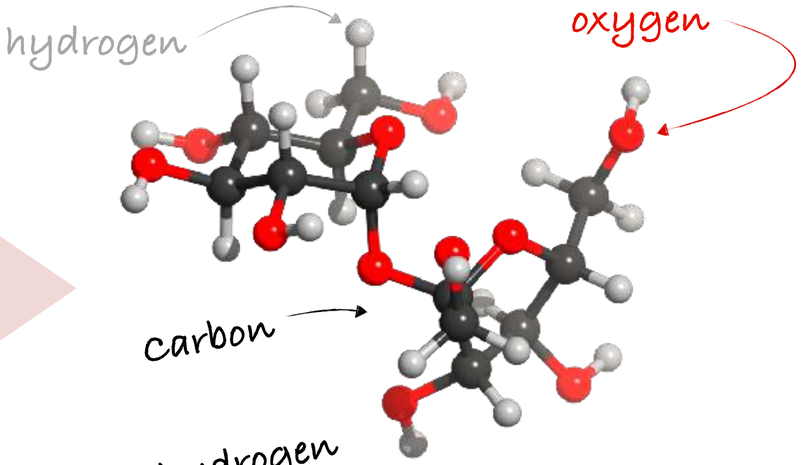

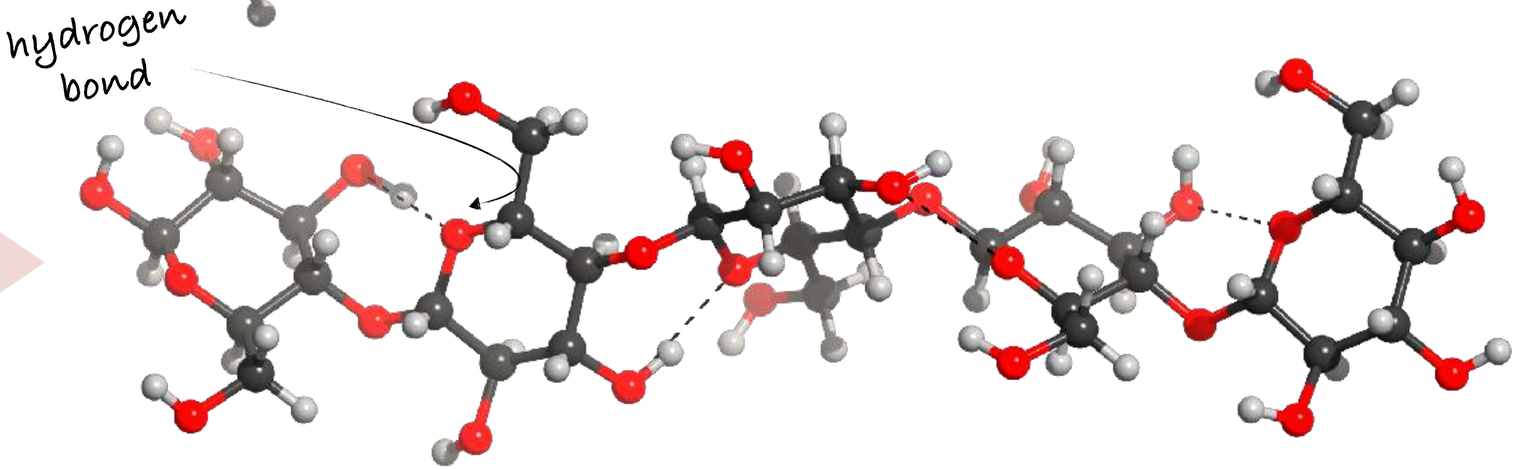

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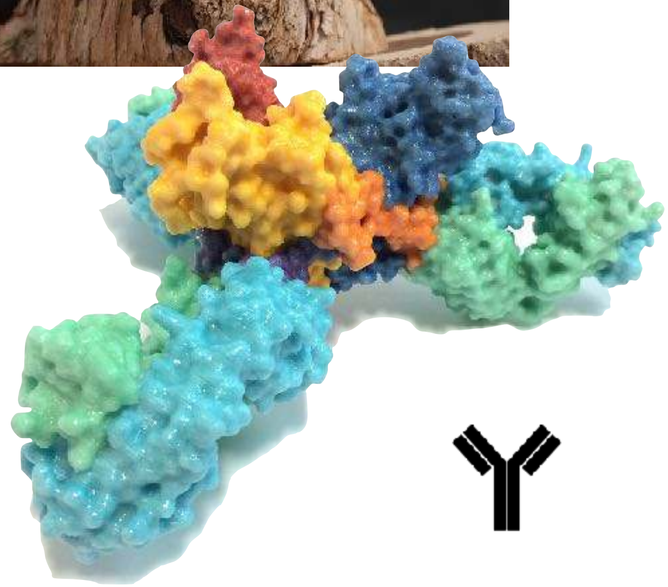


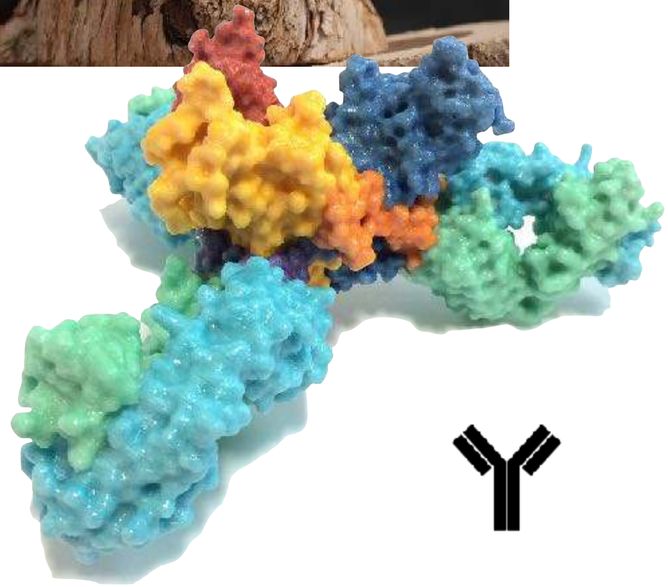
hydrogen

oxygen

carbon

hydrogen bond

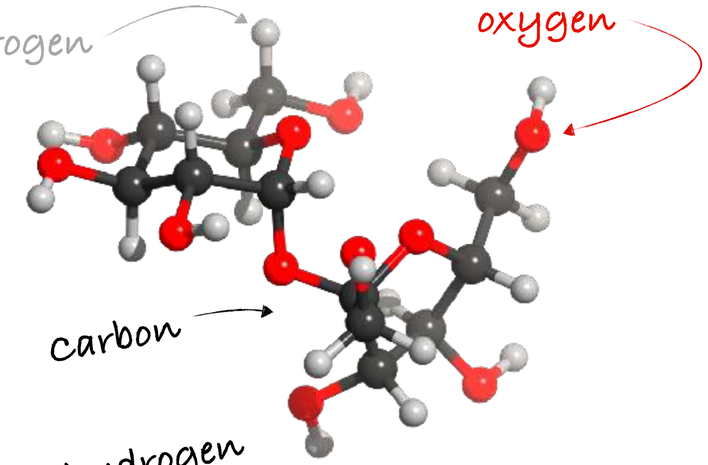
Not always sweet!



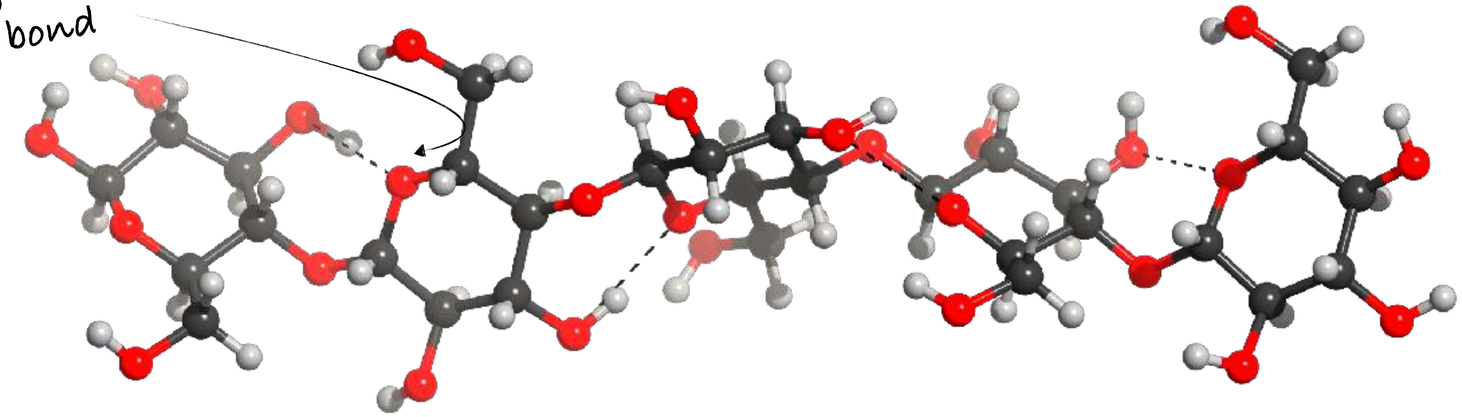
hydrogen

oxygen

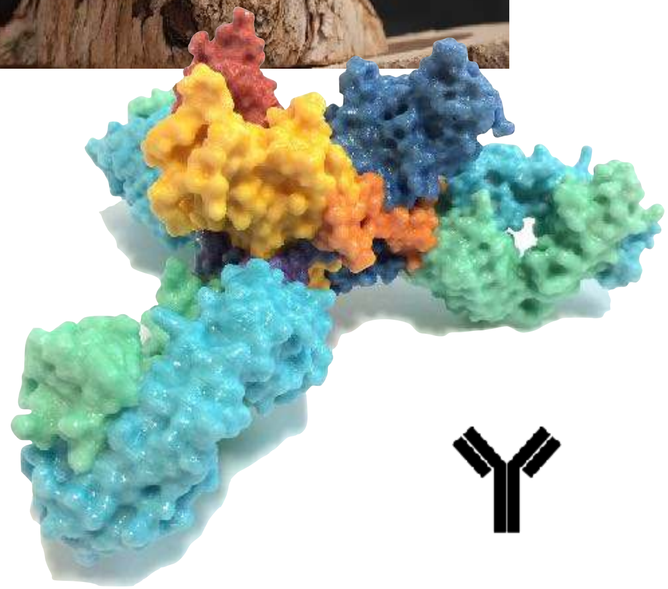
carbon



hydrogen bond



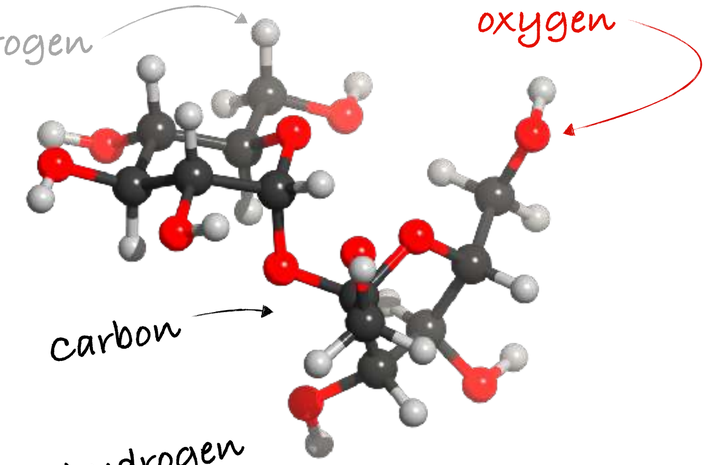
Not always sweet!



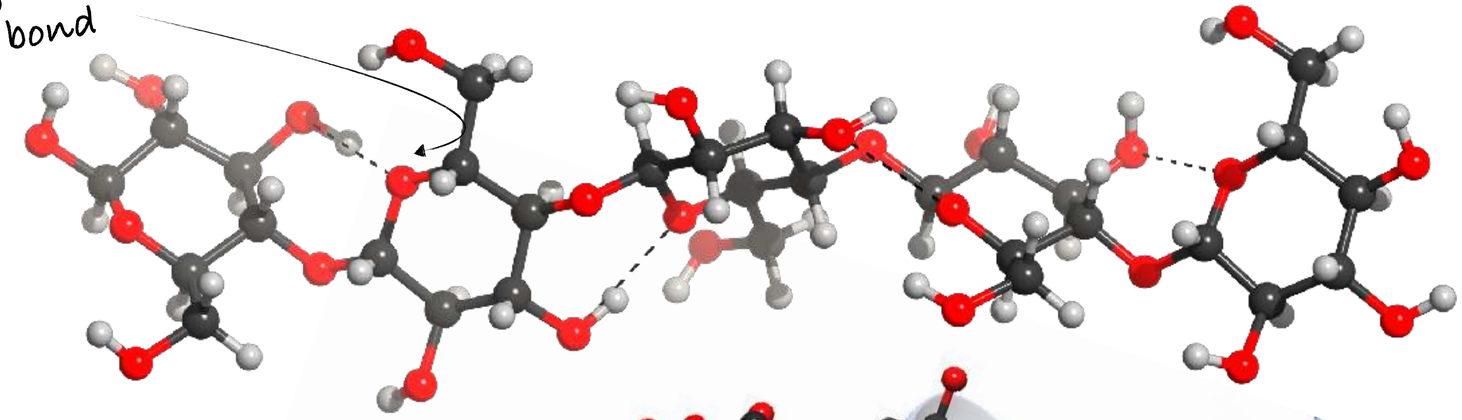
hydrogen

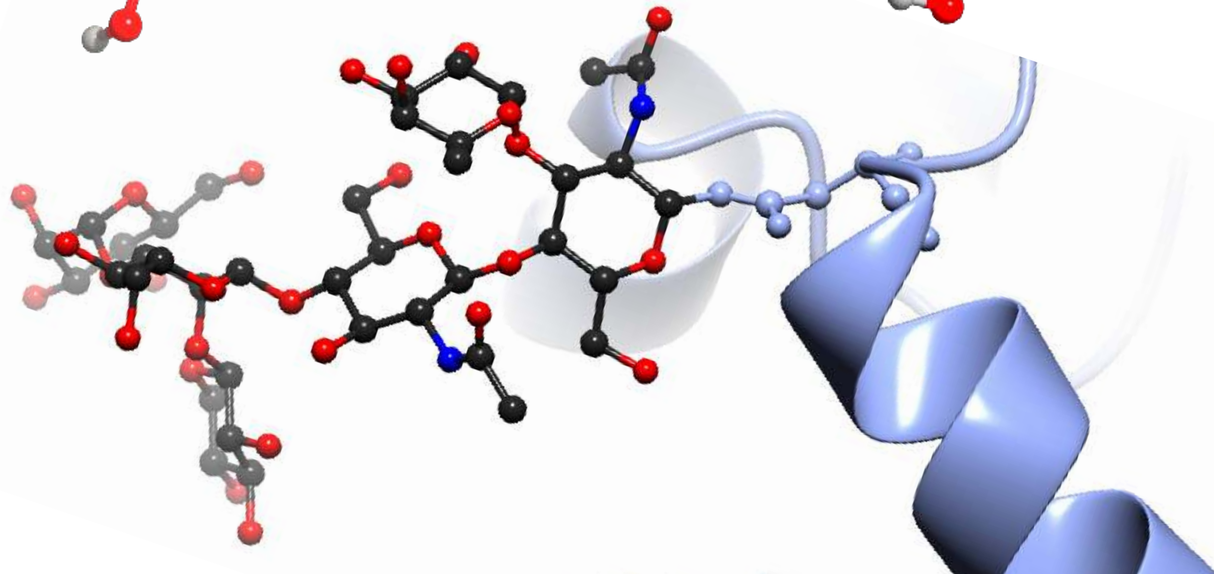
oxygen

carbon

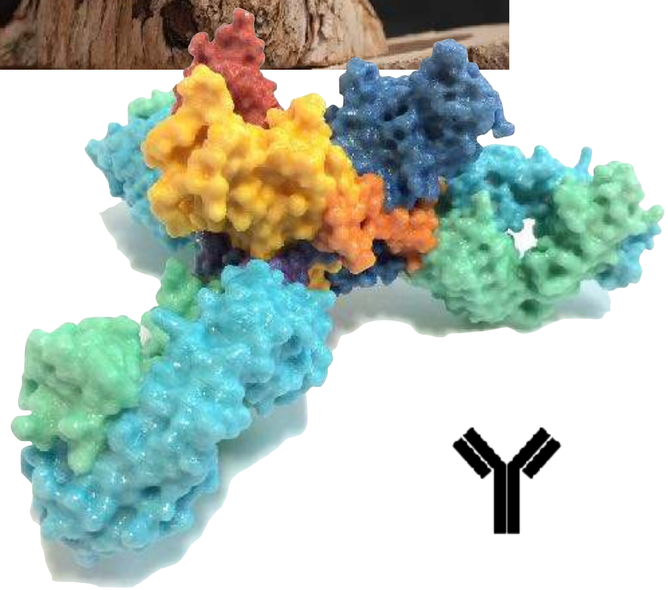


hydrogen bond





Not always sweet!



hydrogen

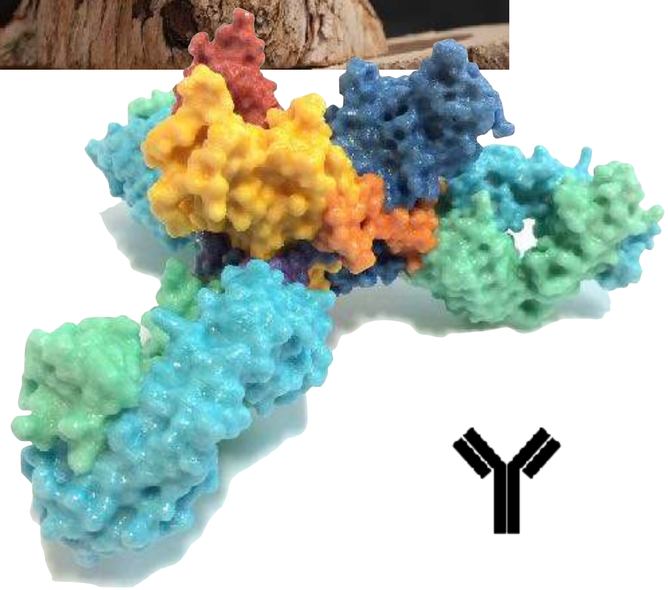
oxygen

carbon

hydrogen bond

N-glycan

Not always sweet!



hydrogen

oxygen

carbon

hydrogen bond

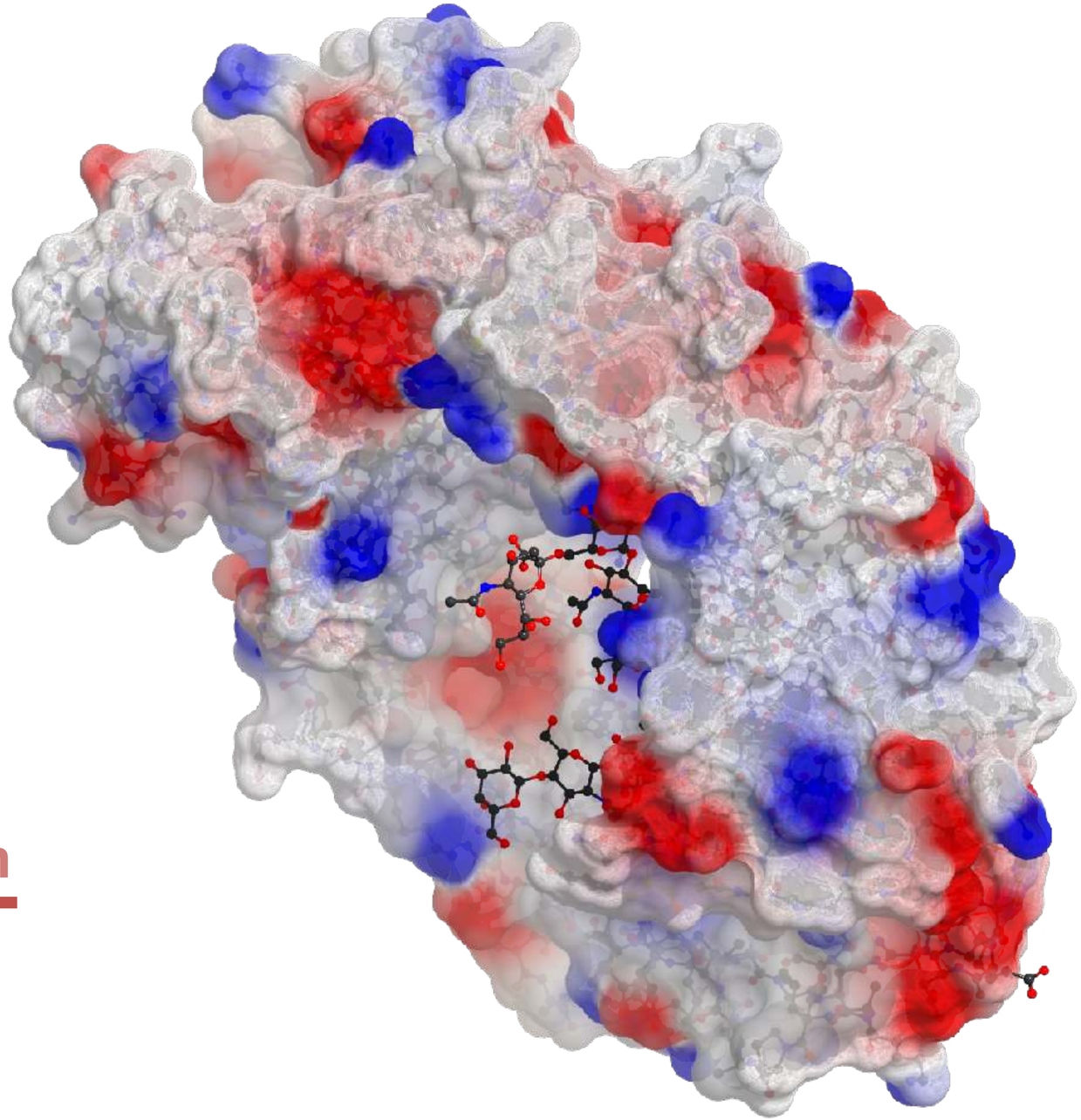
N-glycan

Protein

Not always sweet!

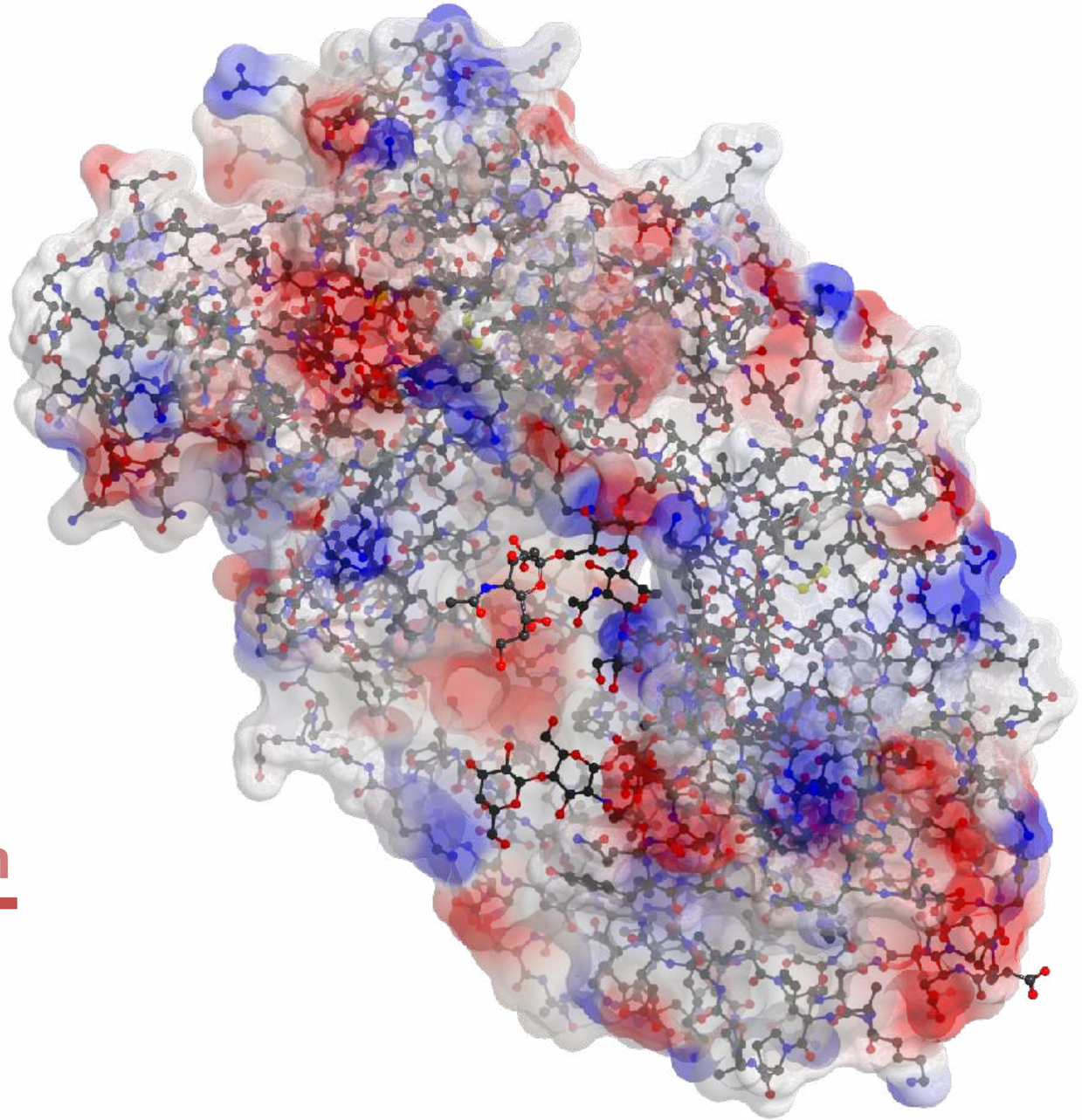
Protein glycosylation

Protein glycosylation

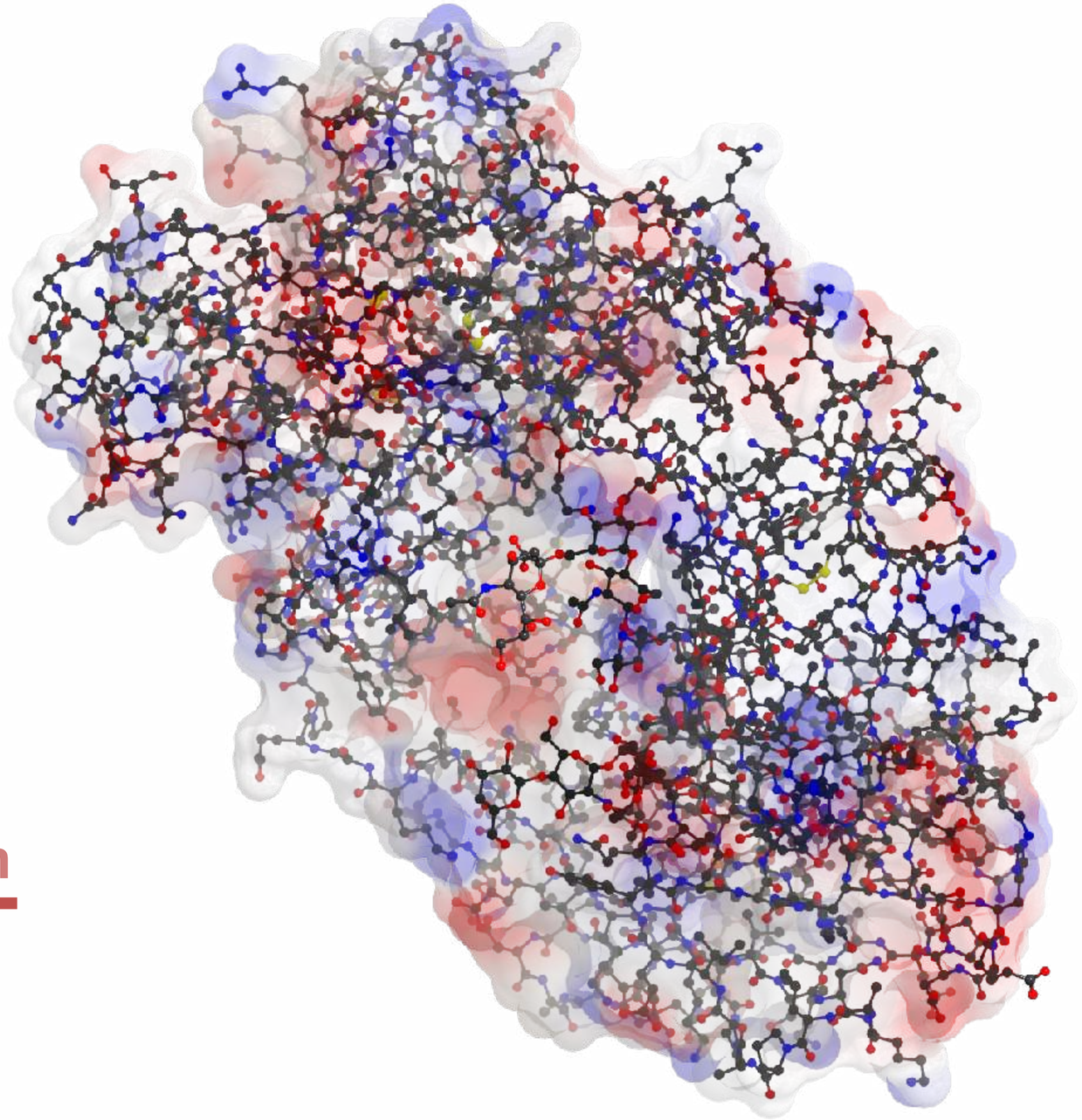


1 nm

Protein glycosylation



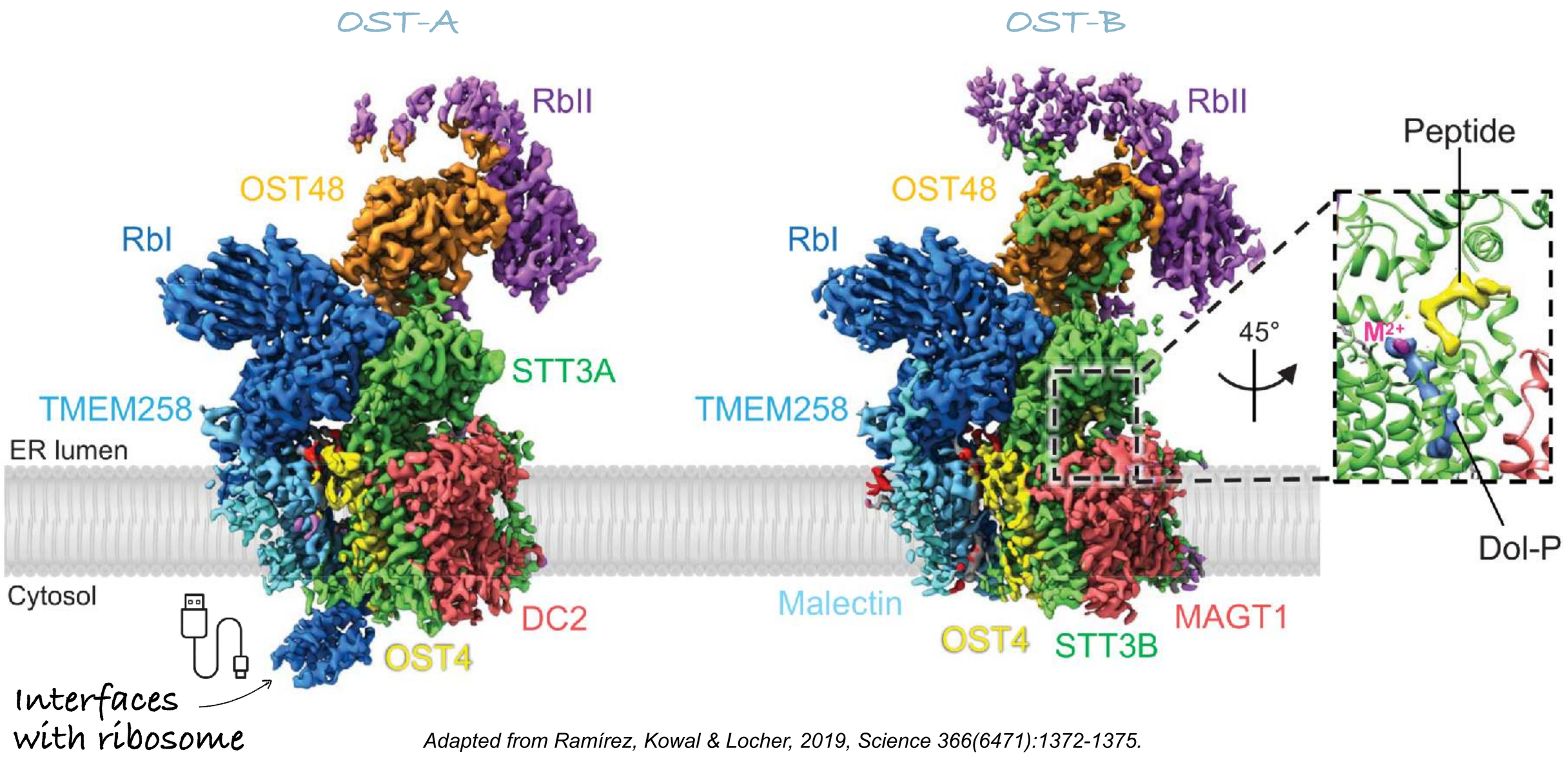
1 nm



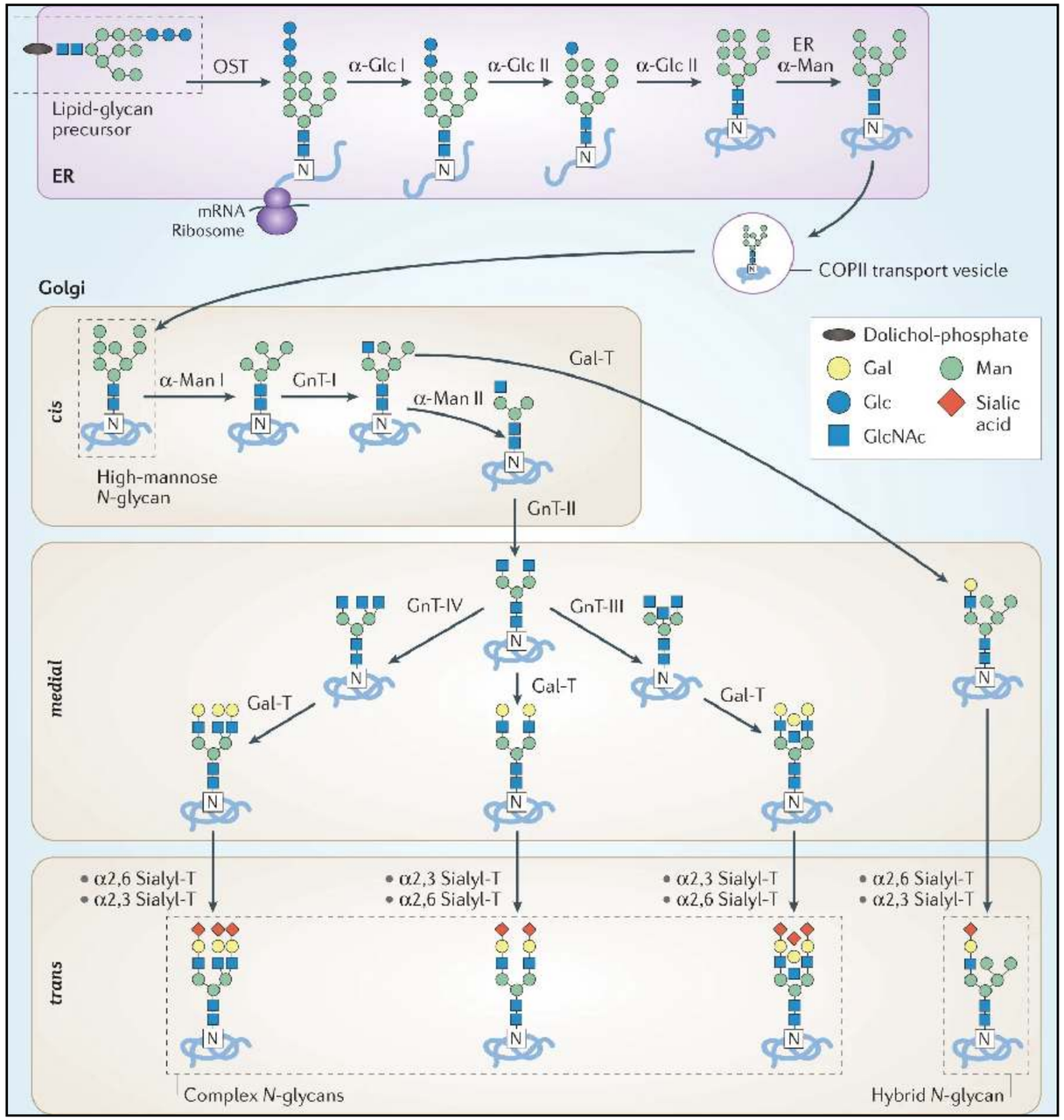
Protein glycosylation

Co-translational

Post-translational



Adapted from Ramírez, Kowal & Locher, 2019, Science 366(6471):1372-1375.

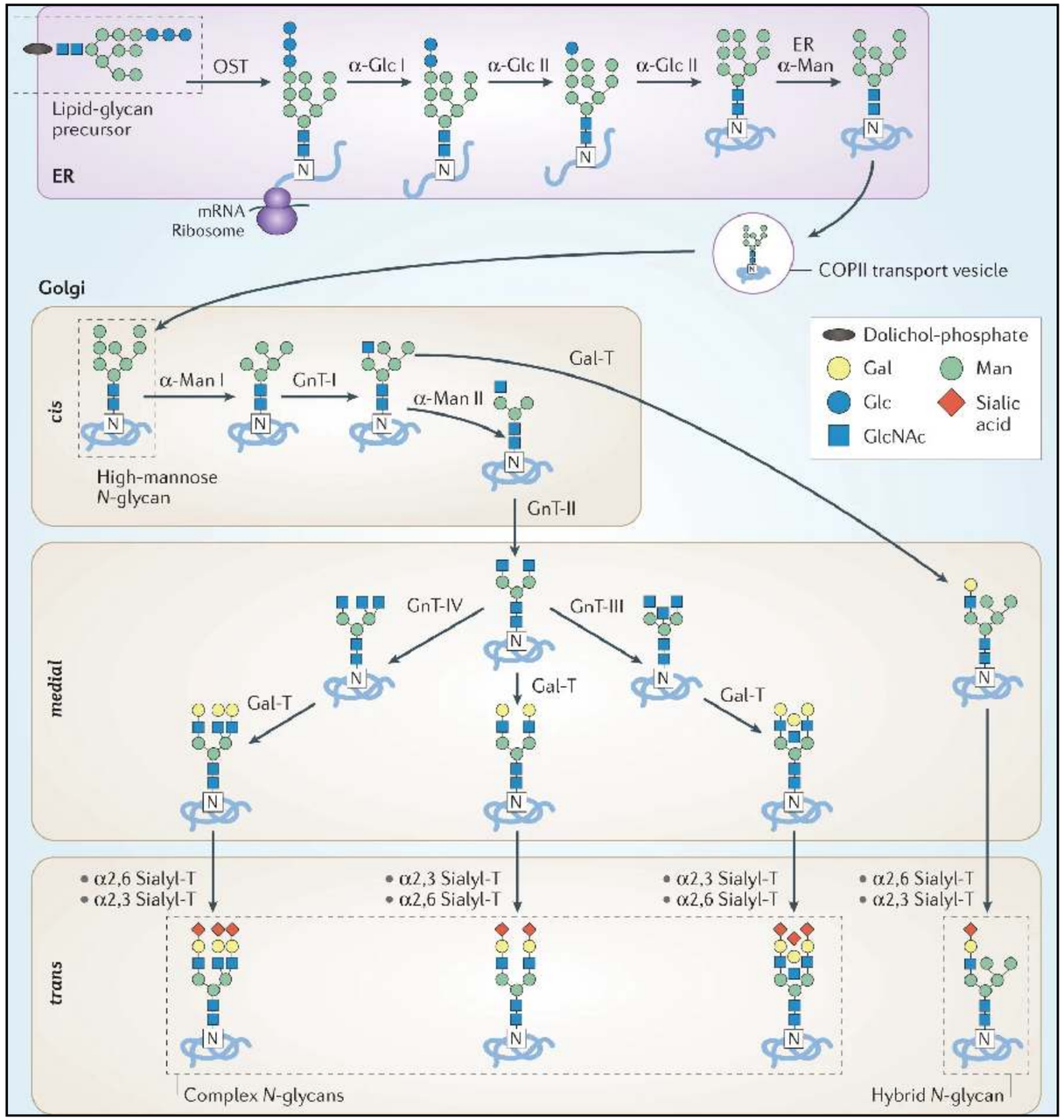


Protein glycosylation

N-glycans

Consensus sequence (sequon)
Asn – not Pro – Ser/Thr

Reily, C., Stewart, T.J., Renfrow, M.B. & Novak, J., 2019, Nat Rev Nephrol 15:346–366.



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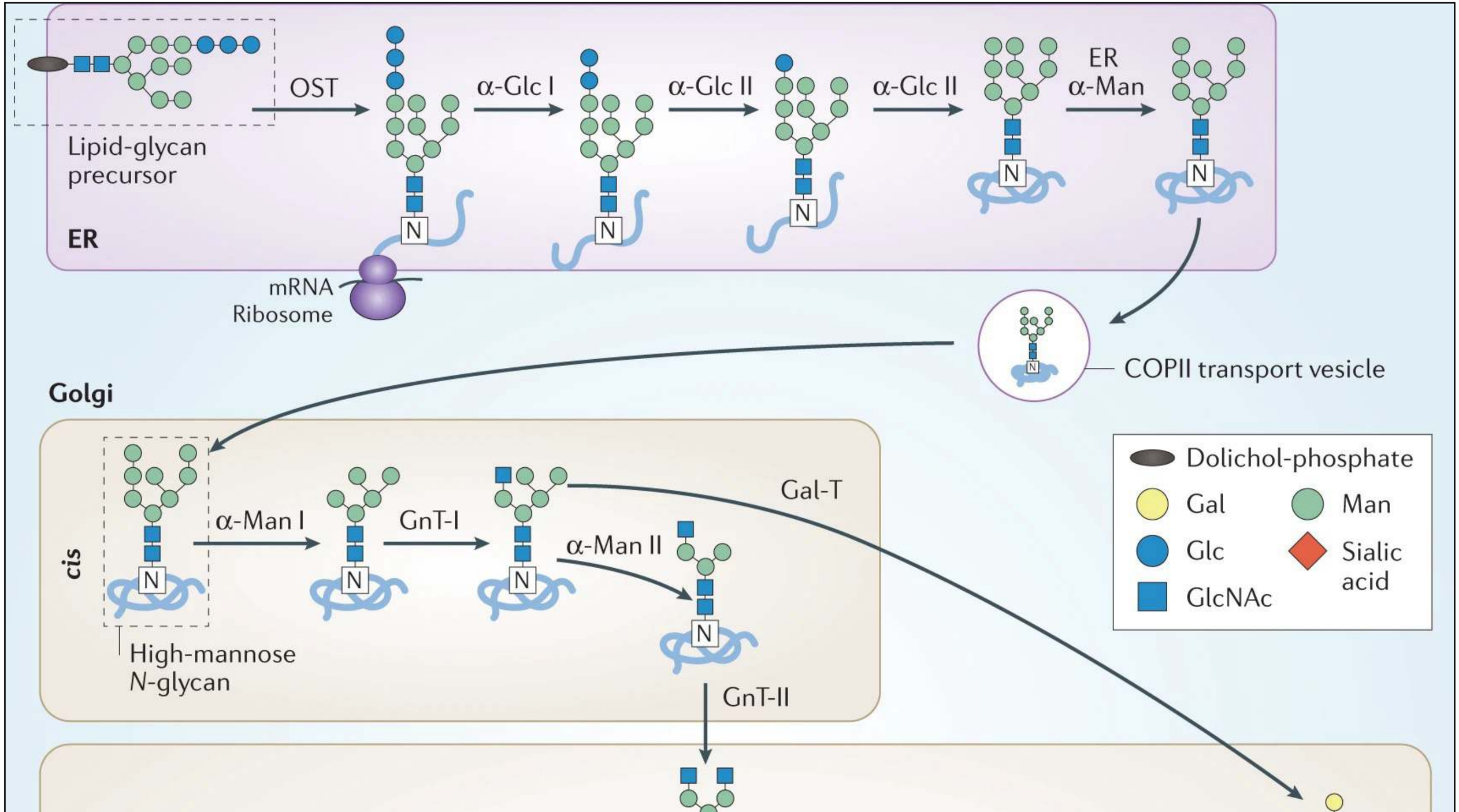
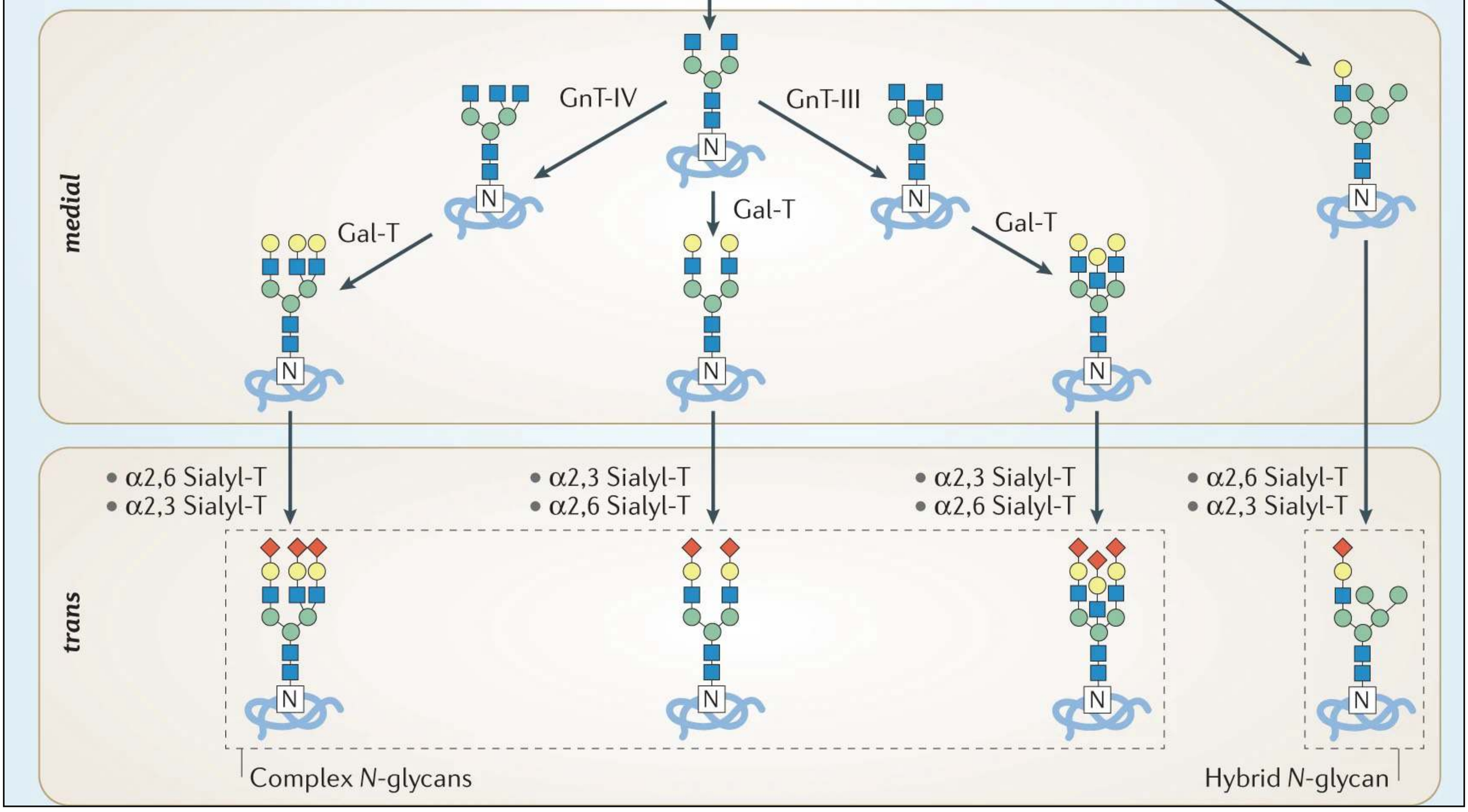
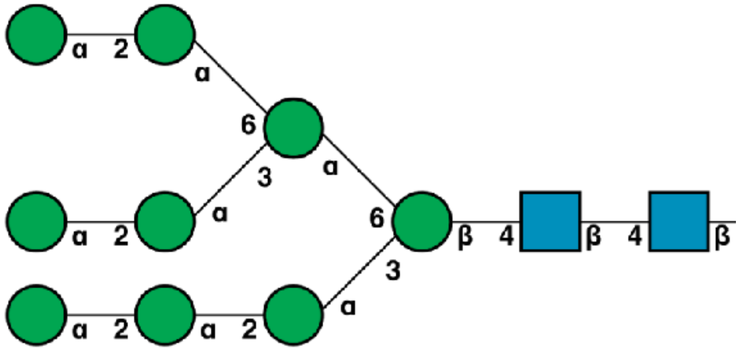

Lipid-glycan precursor
ER
OST
α-Glc I
α-Glc II
α-Glc II
ER α-Man
N
mRNA
Ribosome
COPII transport vesicle
Golgi
cis
α-Man I
GnT-I
α-Man II
Gal-T
High-mannose N-glycan
GnT-II
Dolichol-phosphate
Gal
Man
Glc
Sialic acid
GlcNAc


medial
GnT-IV
GnT-III
Gal-T
Gal-T
Gal-T
N
trans
• α2,6 Sialyl-T
• α2,3 Sialyl-T
• α2,3 Sialyl-T
• α2,6 Sialyl-T
• α2,3 Sialyl-T
• α2,6 Sialyl-T
Complex N-glycans
Hybrid N-glycan

N-glycan diversity

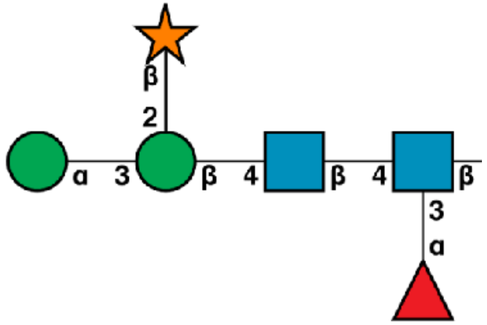
(a)

PDB code 5FJI
High mannose,
GH3 enzyme from
Aspergillus fumigatus



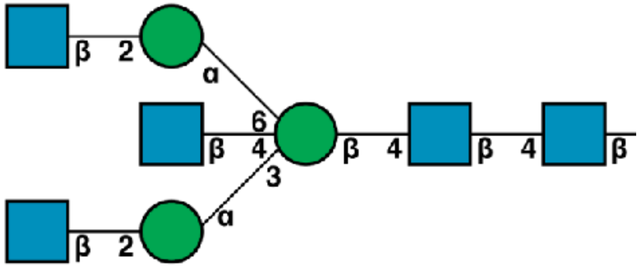
(b)

PDB code 5AOG
Plant glycan,
peroxidase enzyme from
Sorghum bicolor



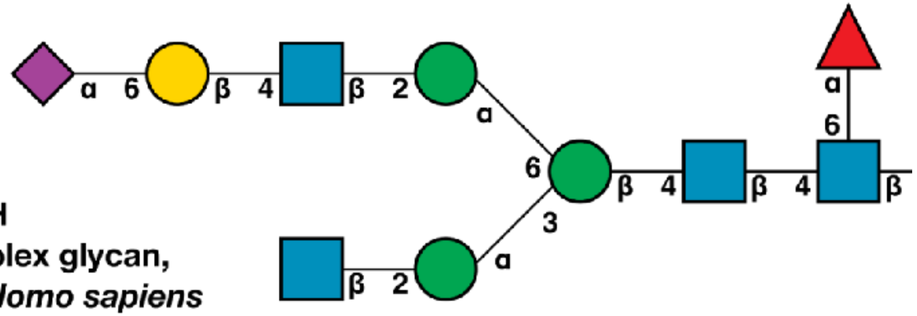
(c)

PDB code 3SGK
Complex glycan,
antibody from *Homo sapiens*,
expressed in *Cricetulus griseus*



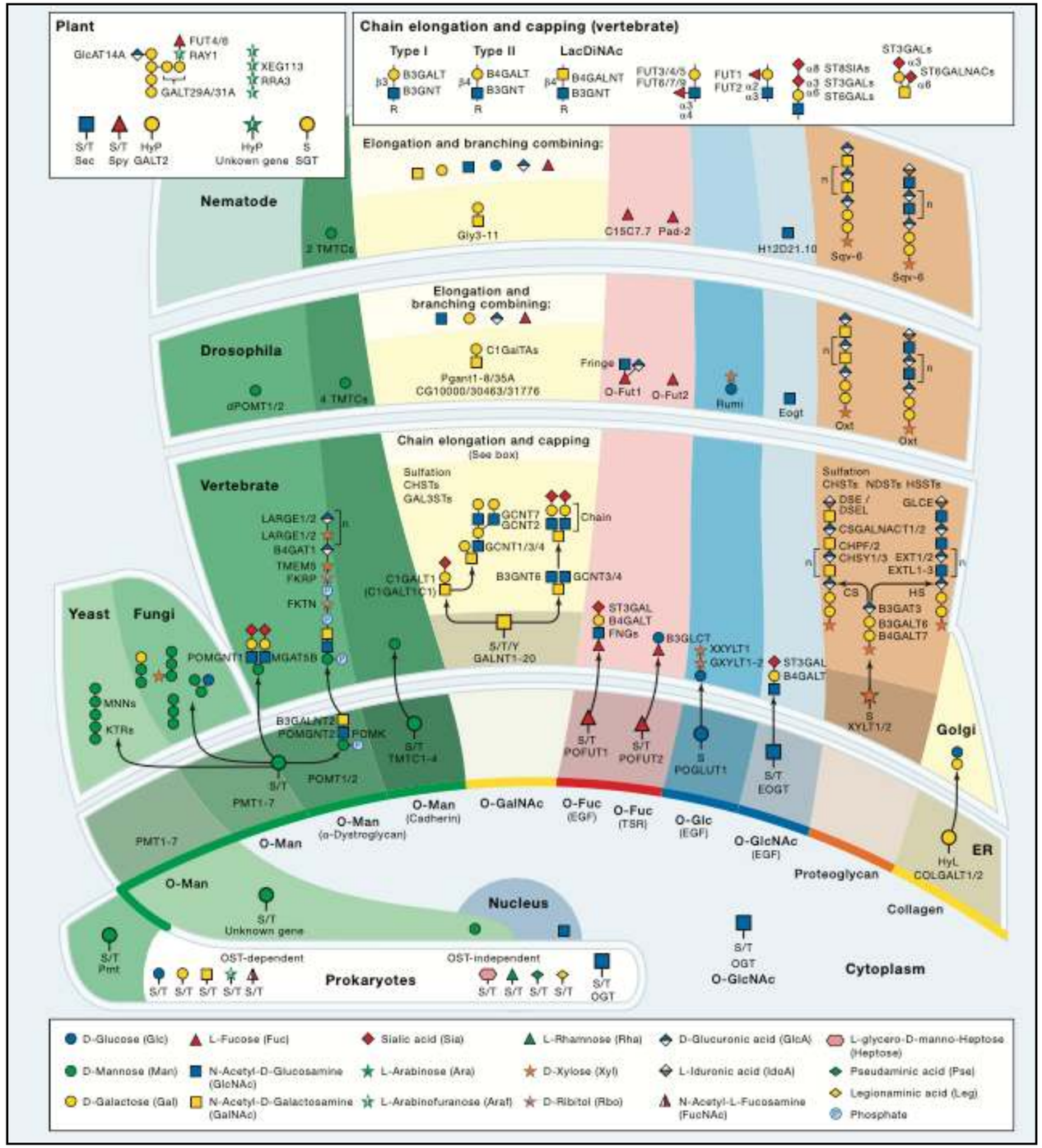
(d)

PDB code 4BYH
Sialylated complex glycan,
antibody from *Homo sapiens*



Symbol Nomenclature For Glycans (SNFG)

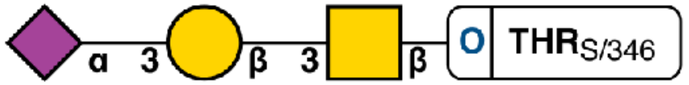
SNFG: Symbol Nomenclature for Graphical Representation of Glycans, Glycobiology 25: 1323-1324, 2015.



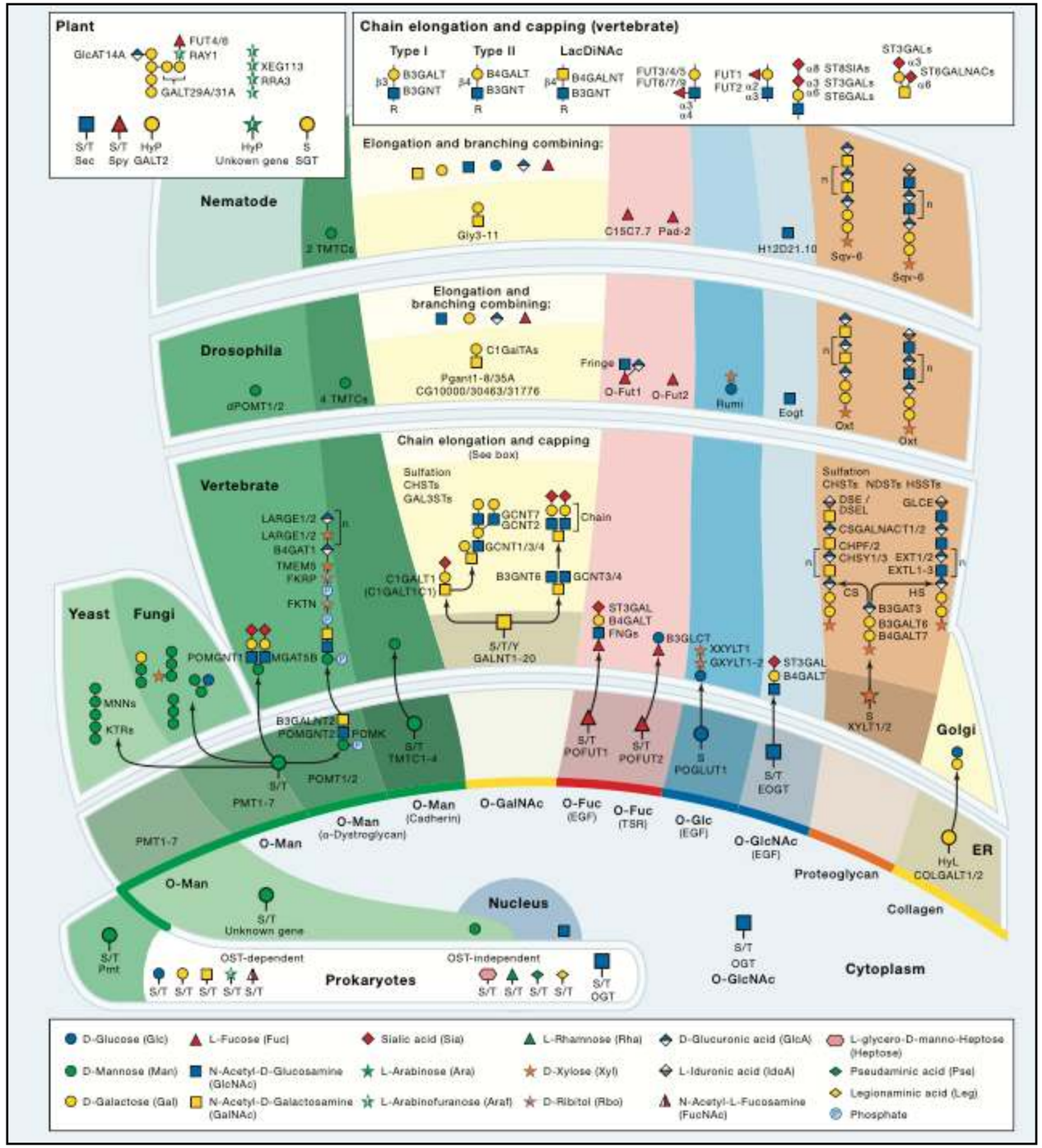
Protein glycosylation

O- and C-glycans

O-GalNAc (o-glycan)



Tryptophan mannosylation (c-glycan)



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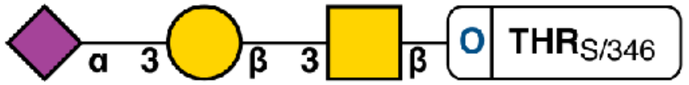
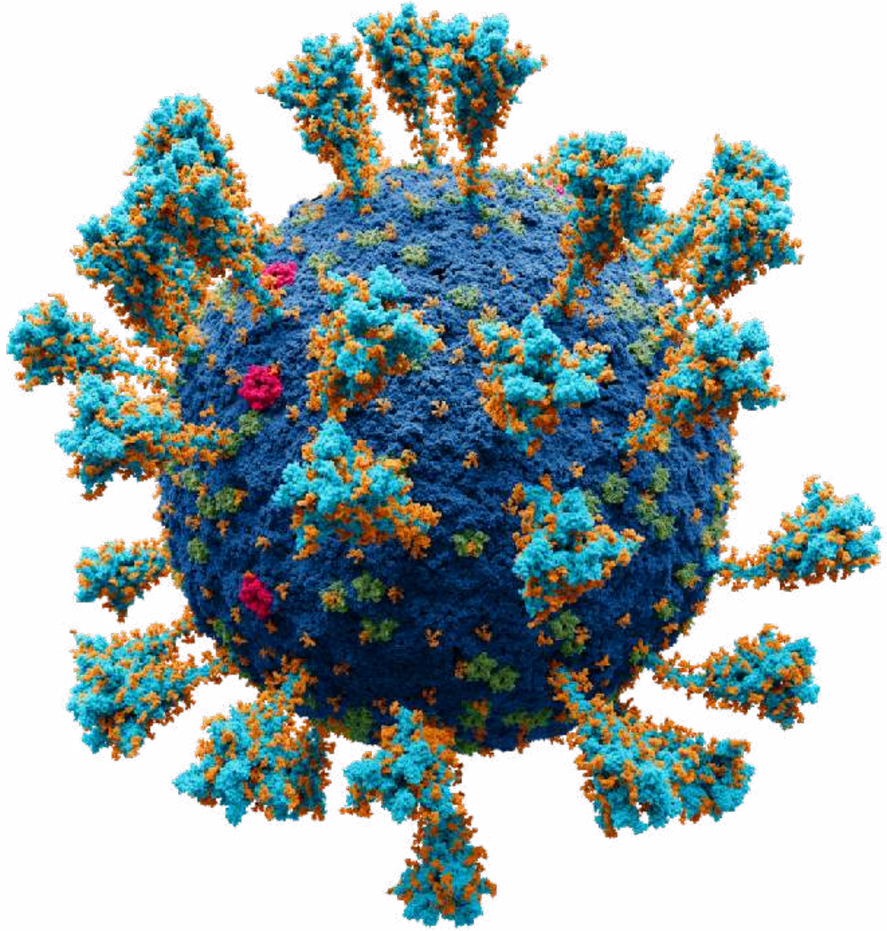


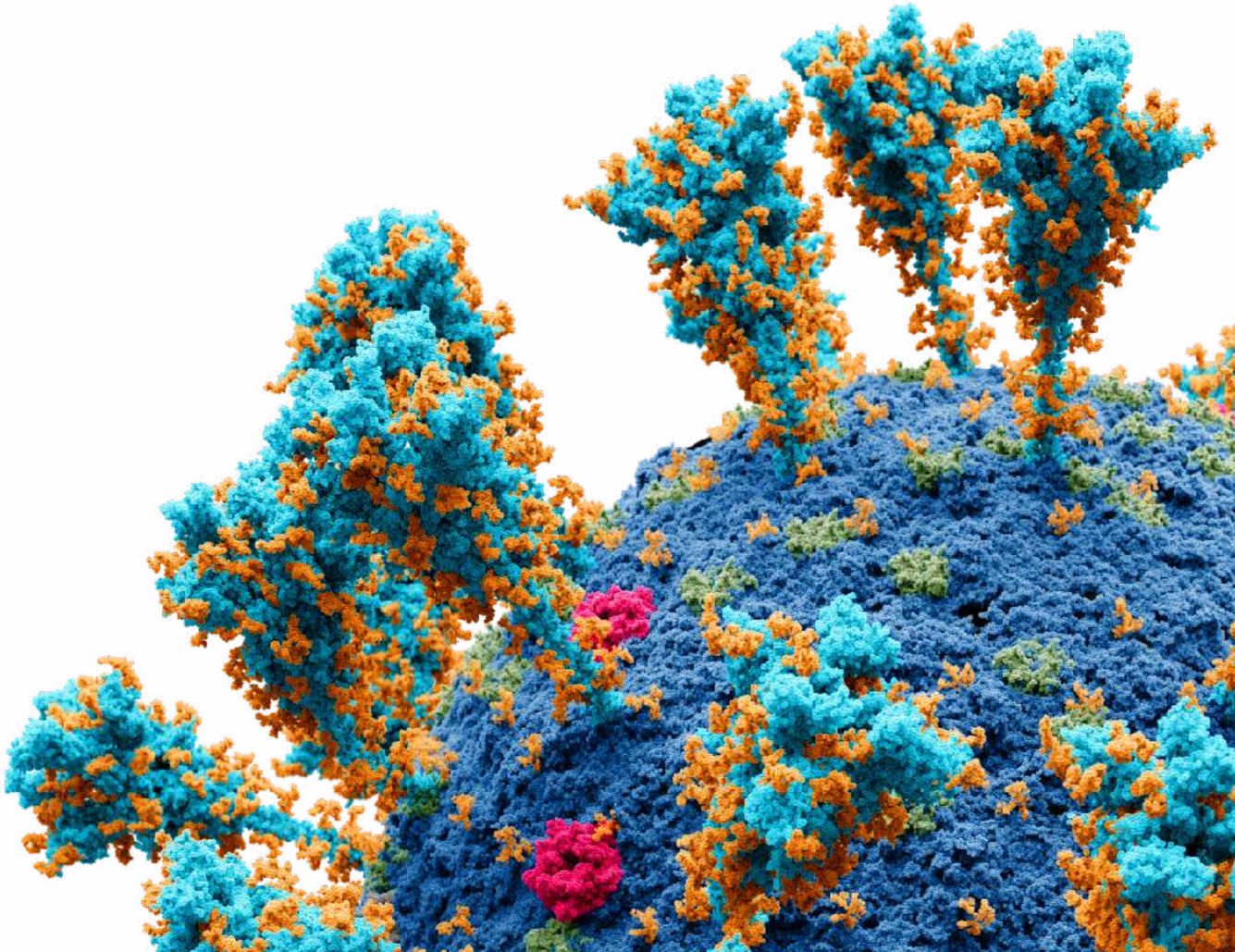
Tryptophan mannosylation (c-glycan)

Joshi, Narimatsu, Schjoldager, Tytgat, Aebi, Clausen & Halim, 2018, Cell 172.

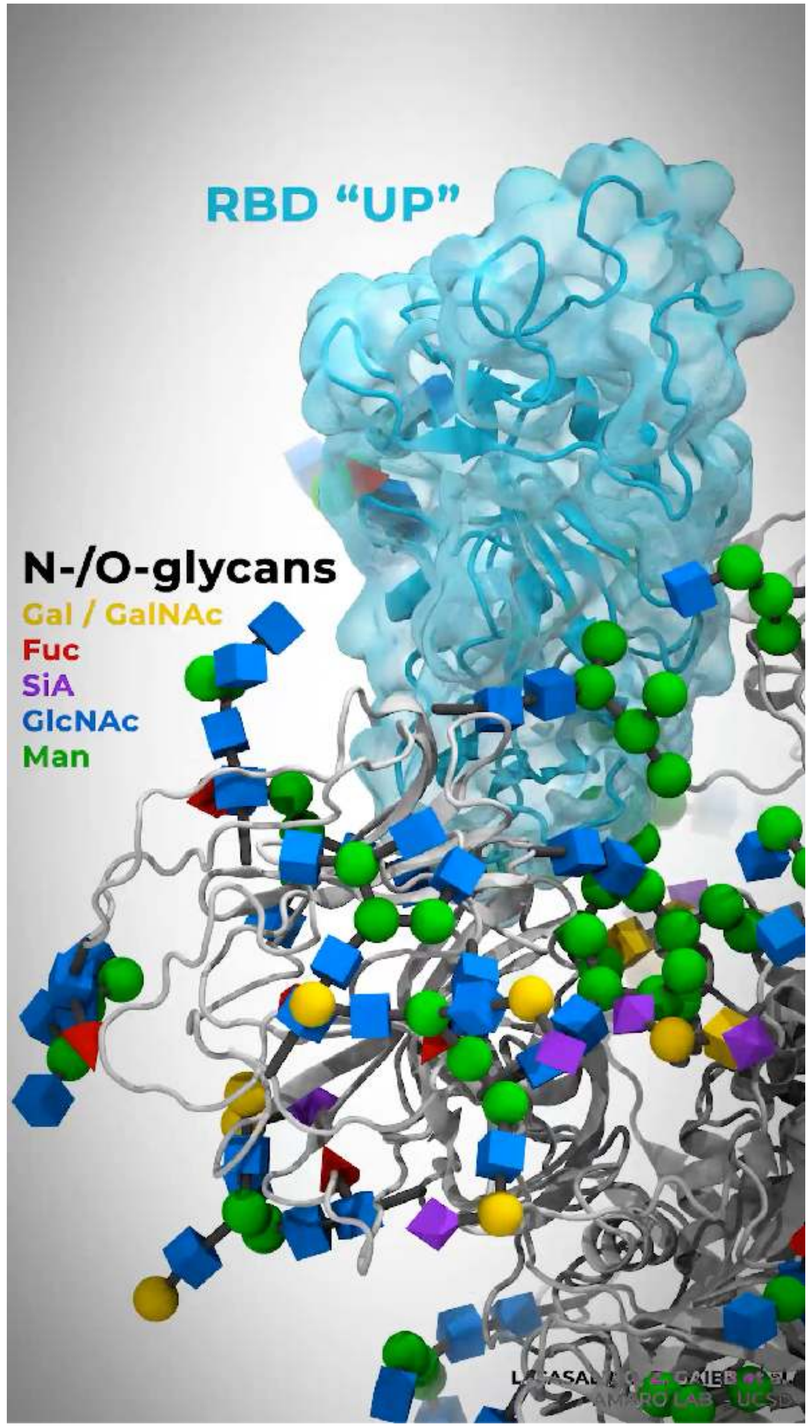
Glycans matter



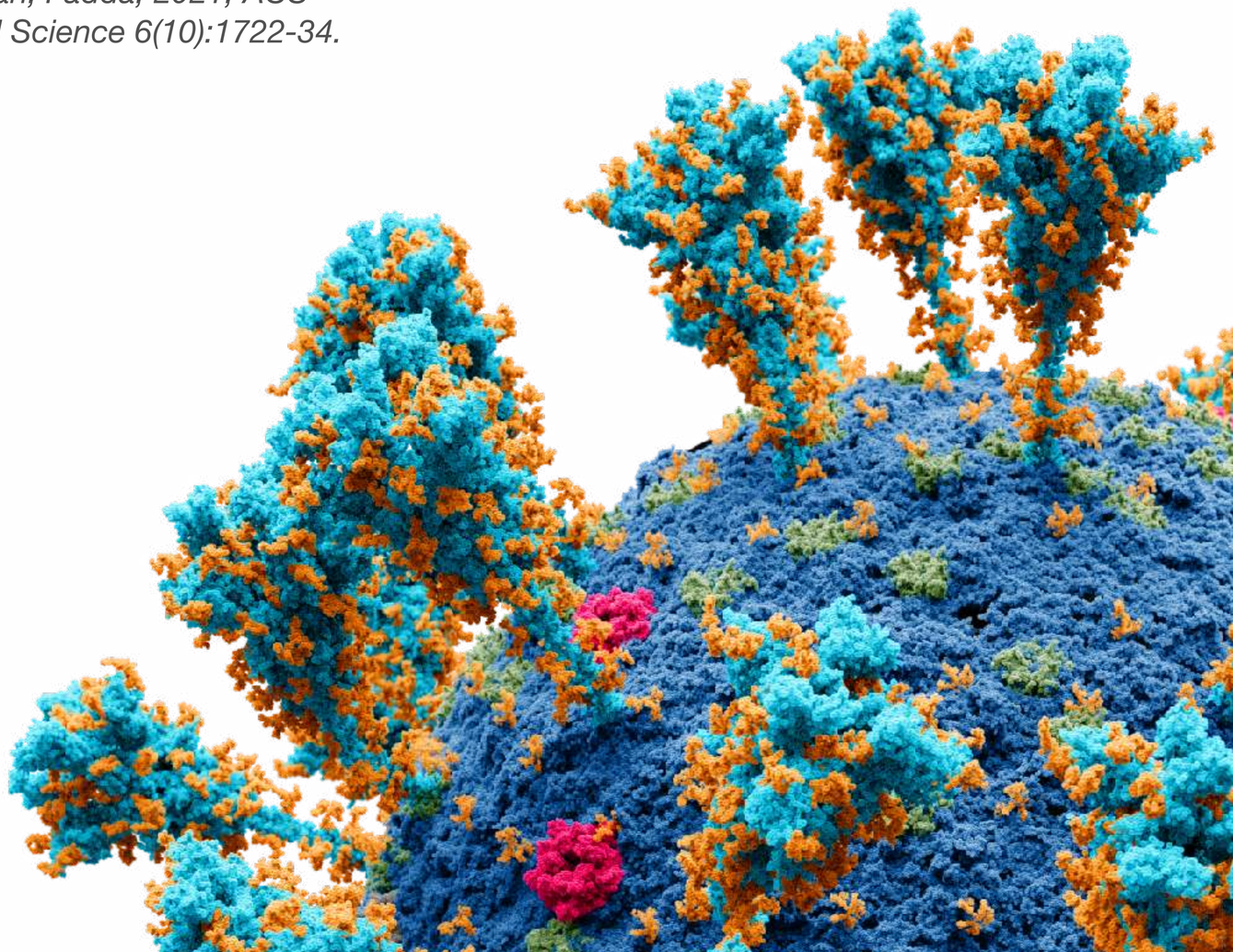
Glycans matter



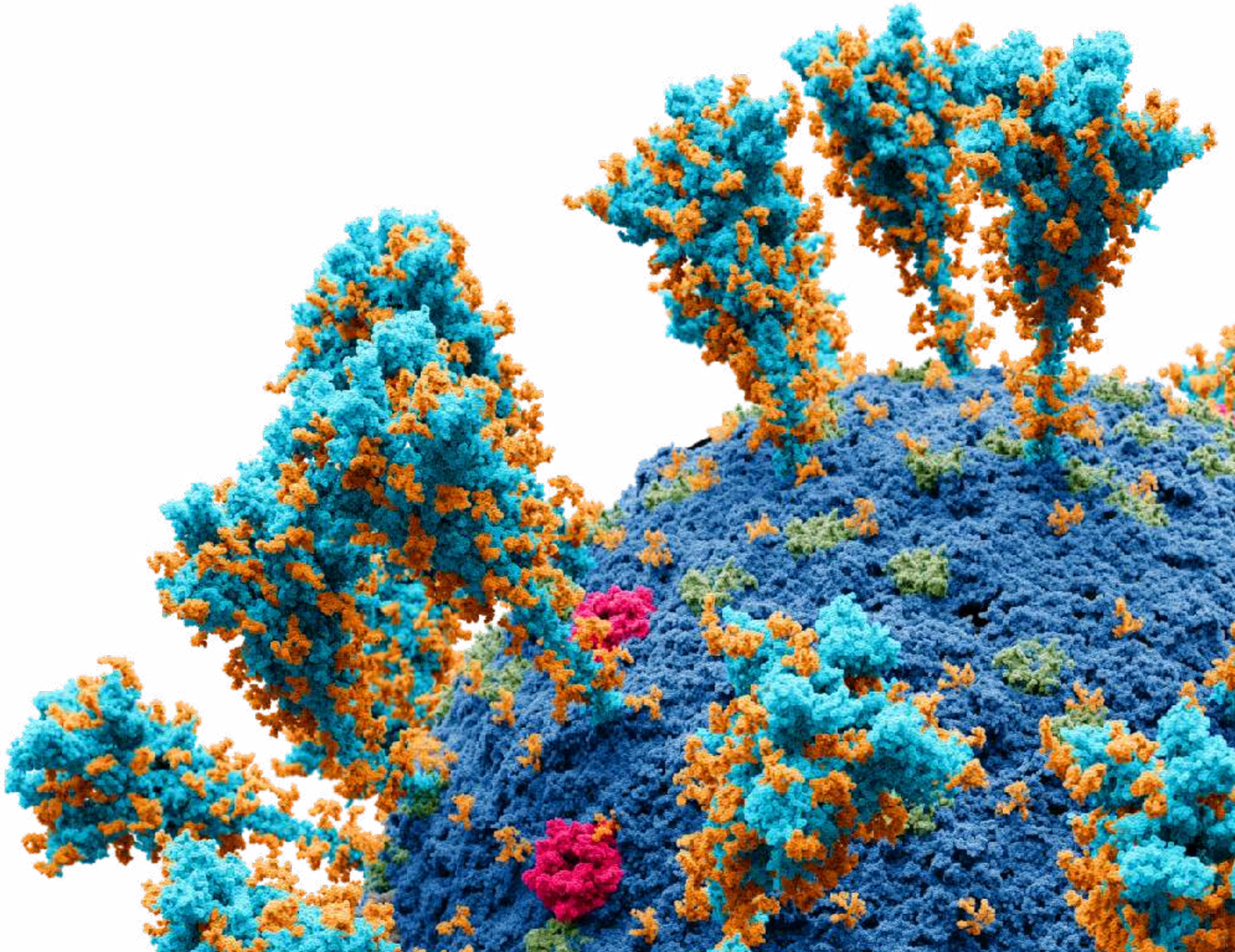
Glycans matter



Casalino, Gaieb, Goldsmith, Hjorth, Dommer, Harbison, Fogarty, Barros, Taylor, McLellan, Fadda, 2021, ACS Central Science 6(10):1722-34.



Glycans matter



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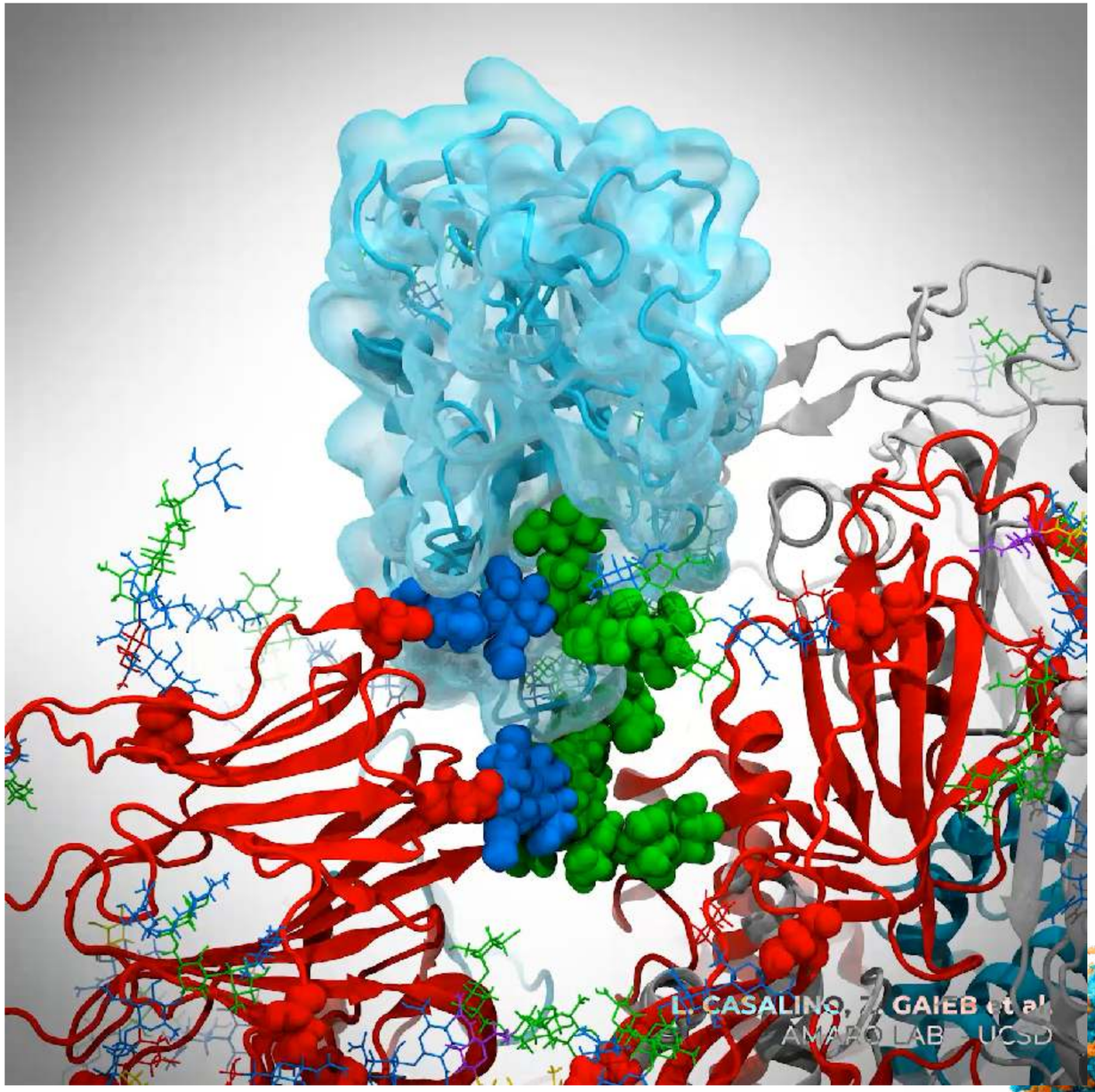

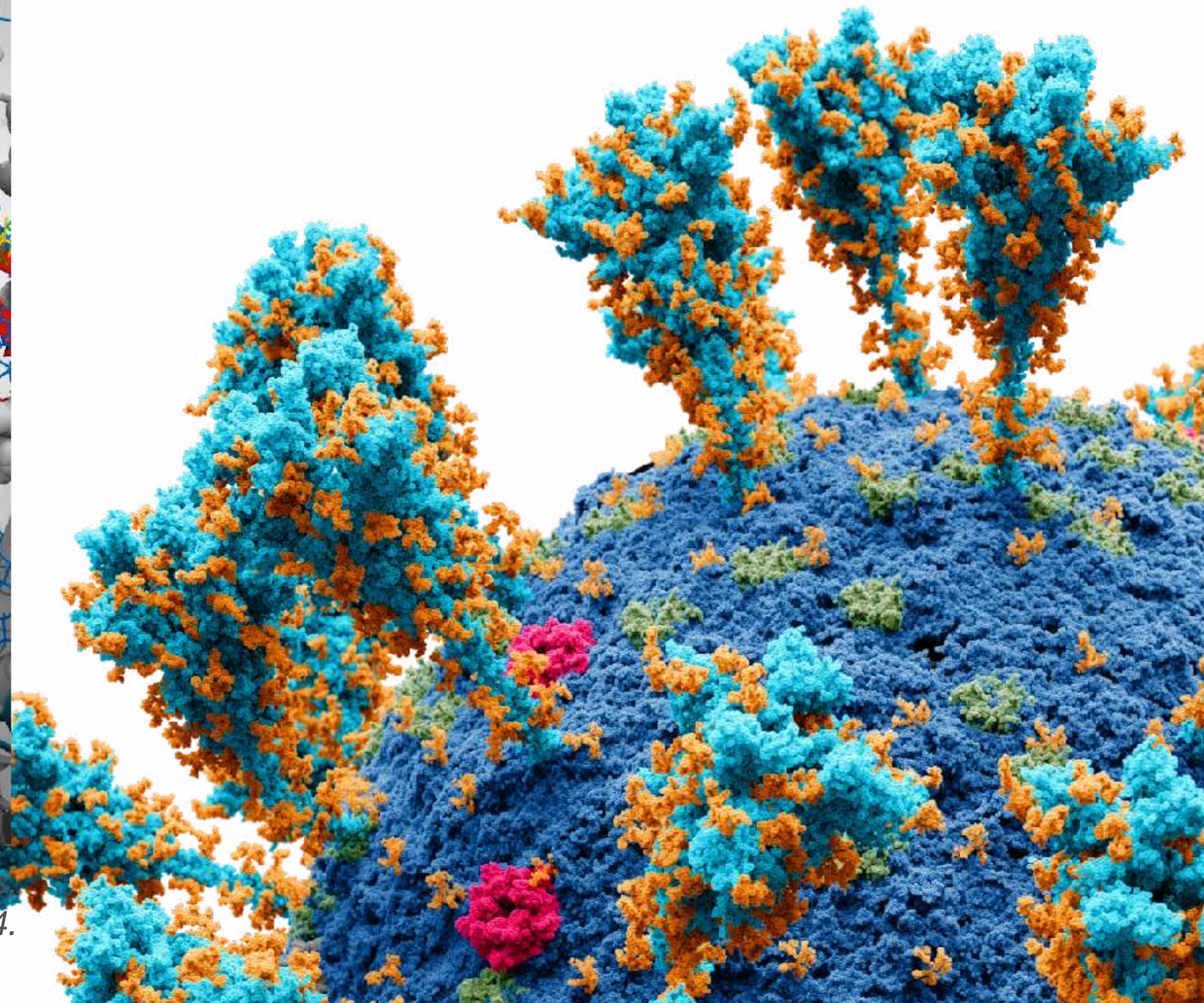

Casalino, Gaieb, Goldsmith, Hjorth, Dommer, Harbison, Fogarty, Barros, Taylor, McLellan, Fadda, 2021, ACS Central Science 6(10):1722-34.

Mechanistic studies show how things may work

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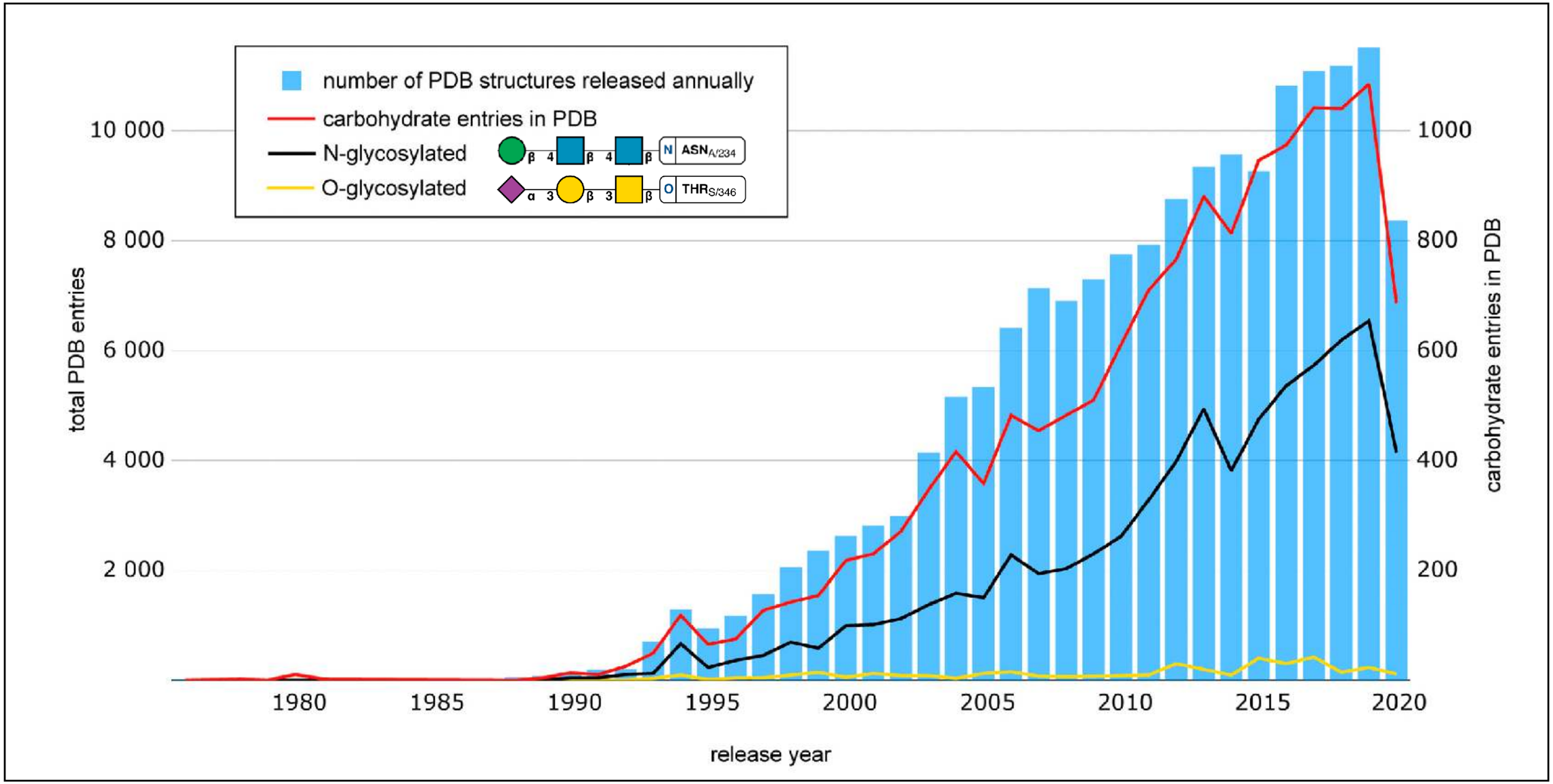
But simulations will only make sense if the atomic models used are correct

Mechanistic studies show
how things may work

But simulations will only make sense
if the atomic models used are correct

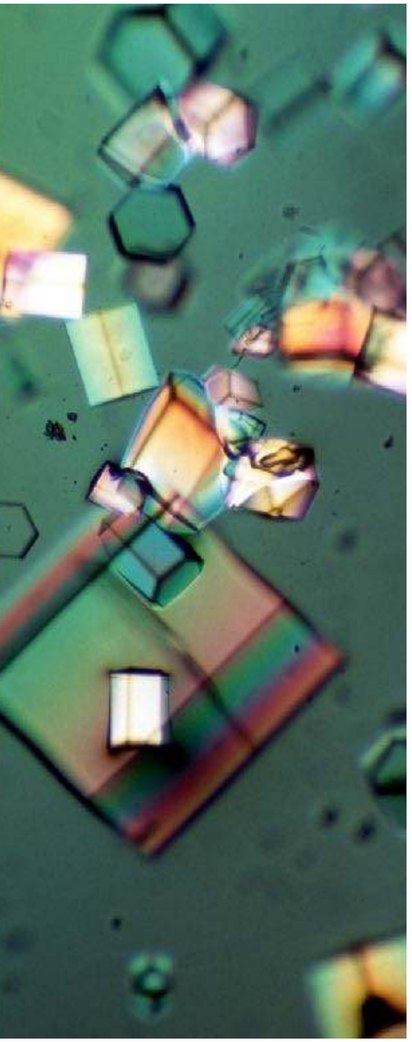
Are all atomic models correct?

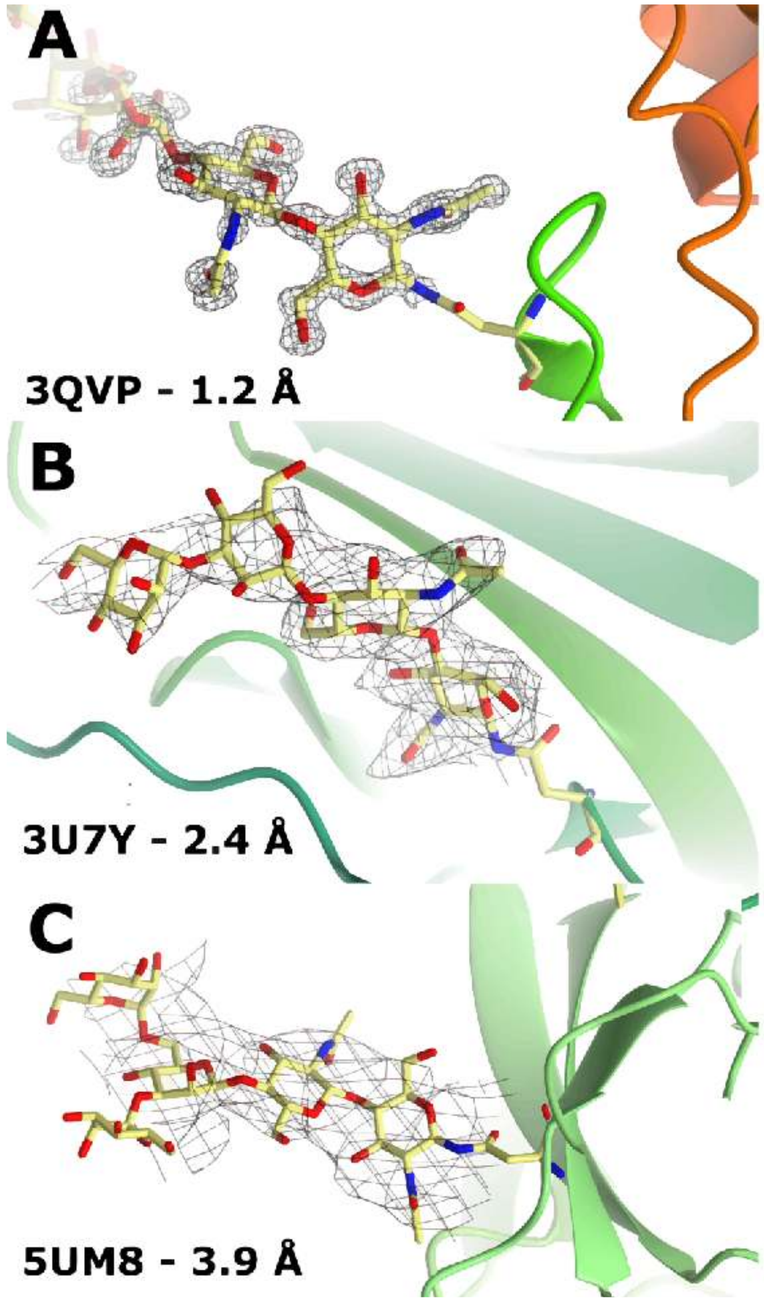
Carbohydrates in the PDB

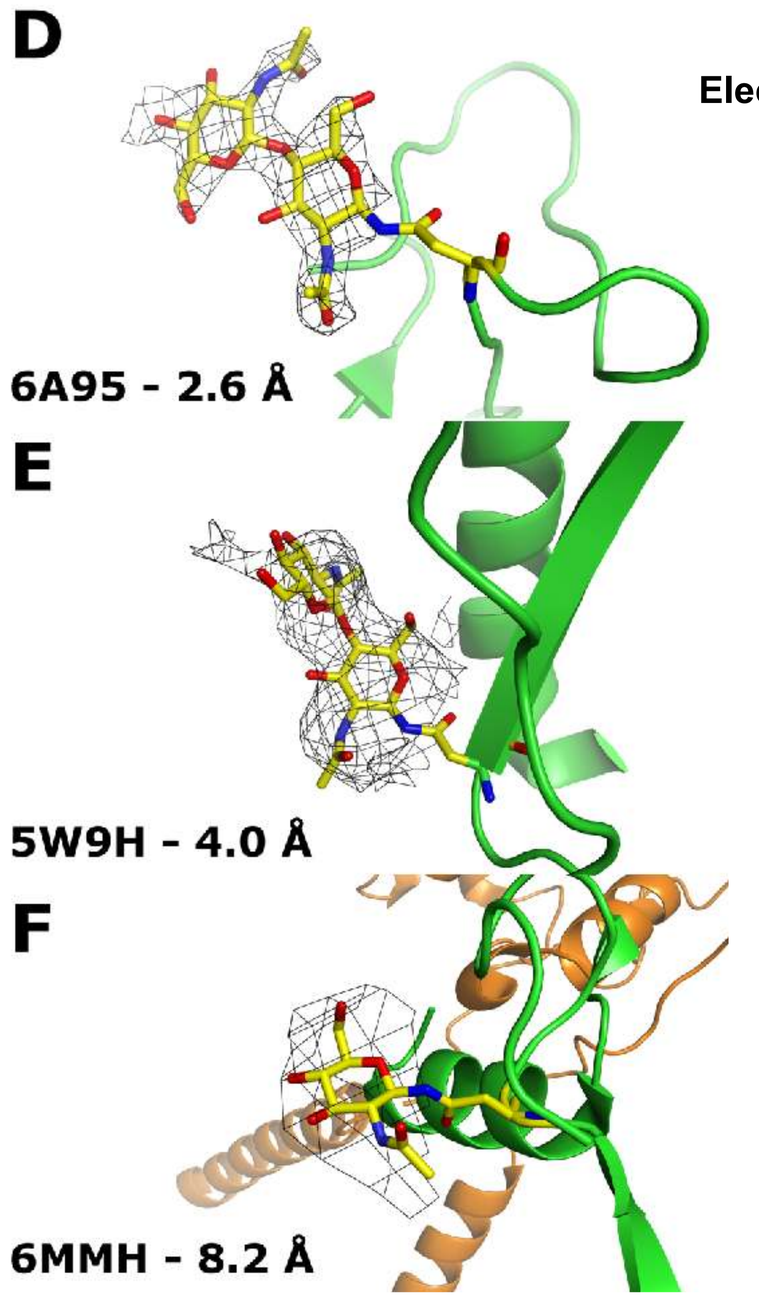


Scherbinina and Toukach, 2020 IJMS 21, 7702

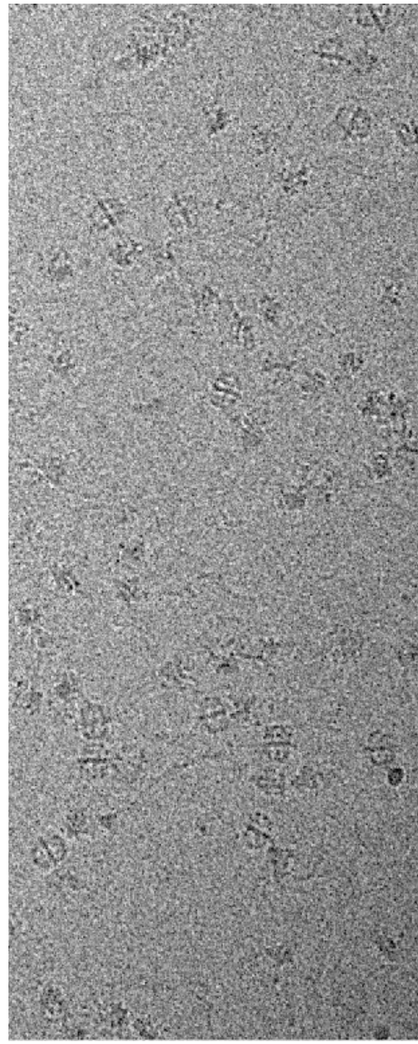
X-ray Crystallography







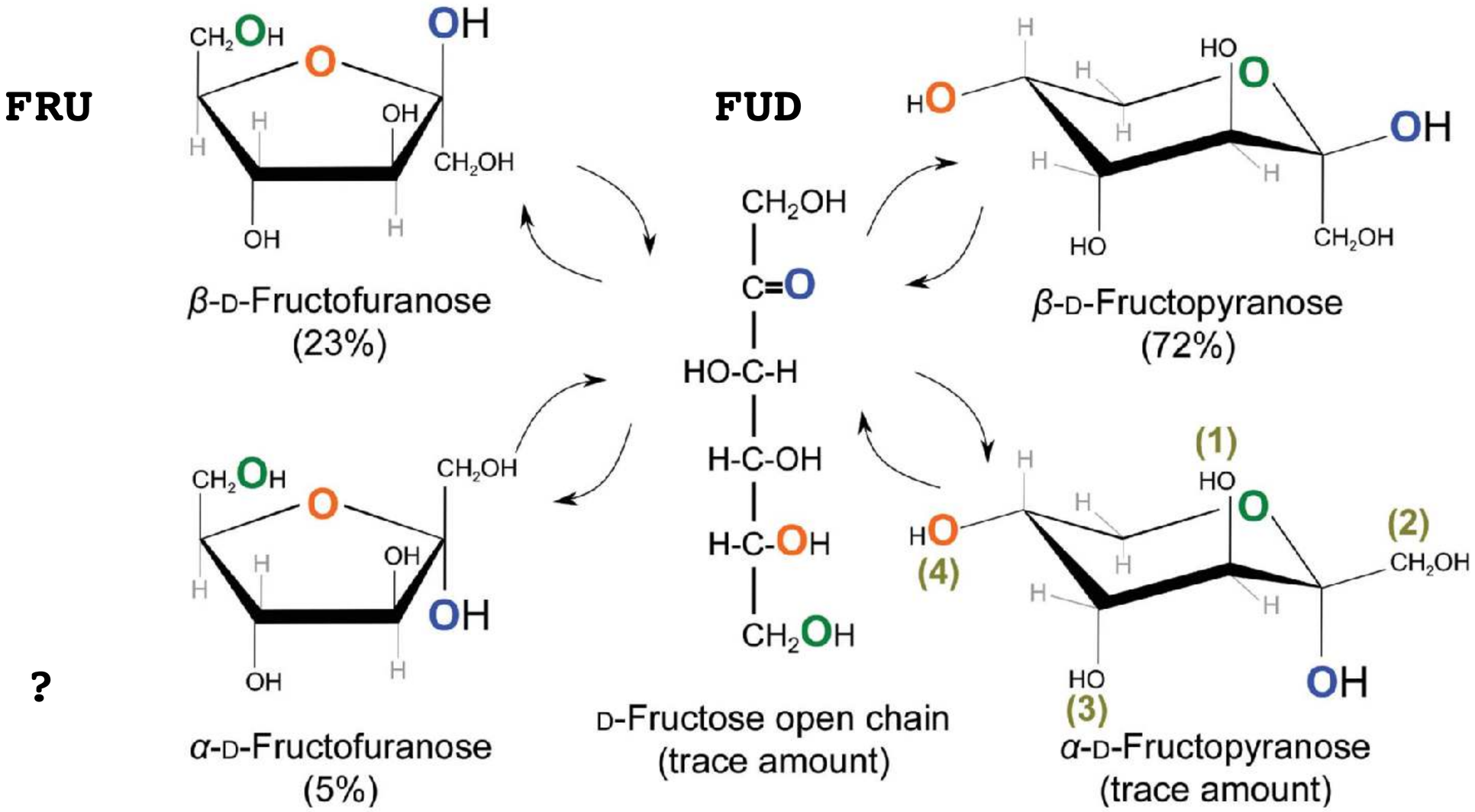
Electron Cryo-Microscopy



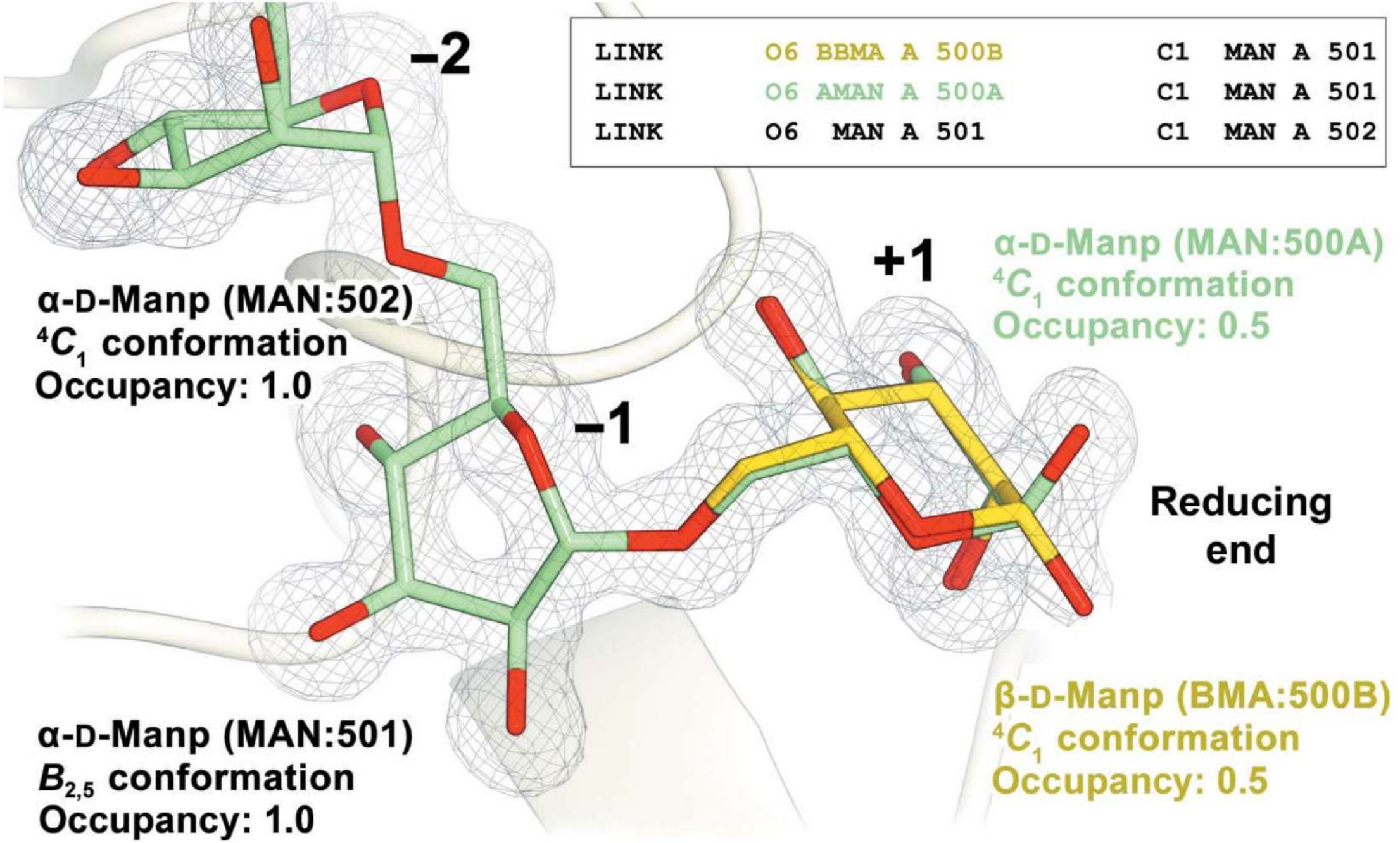
PDBe
Protein Data Bank in Europe

Adapted from Atanasova, Bagdonas & Agirre, 2020, Current Opinion in Structural Biology 62:70-78.

5 forms = 5 codes 👀

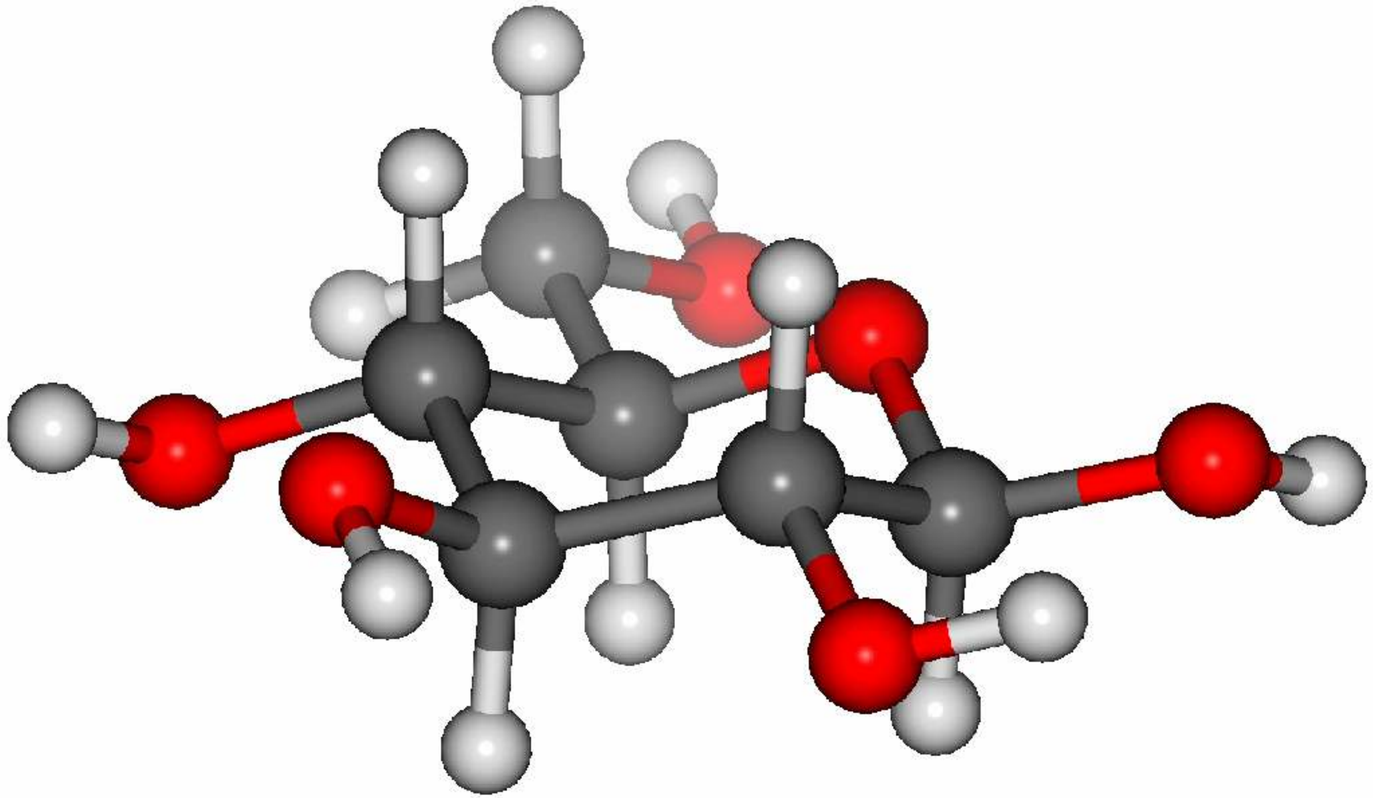


Agirre, 2017, Acta Cryst D73(2):171-186



Adapted from Agirre, 2017, Acta Cryst D73(2):171-186

Ring conformation



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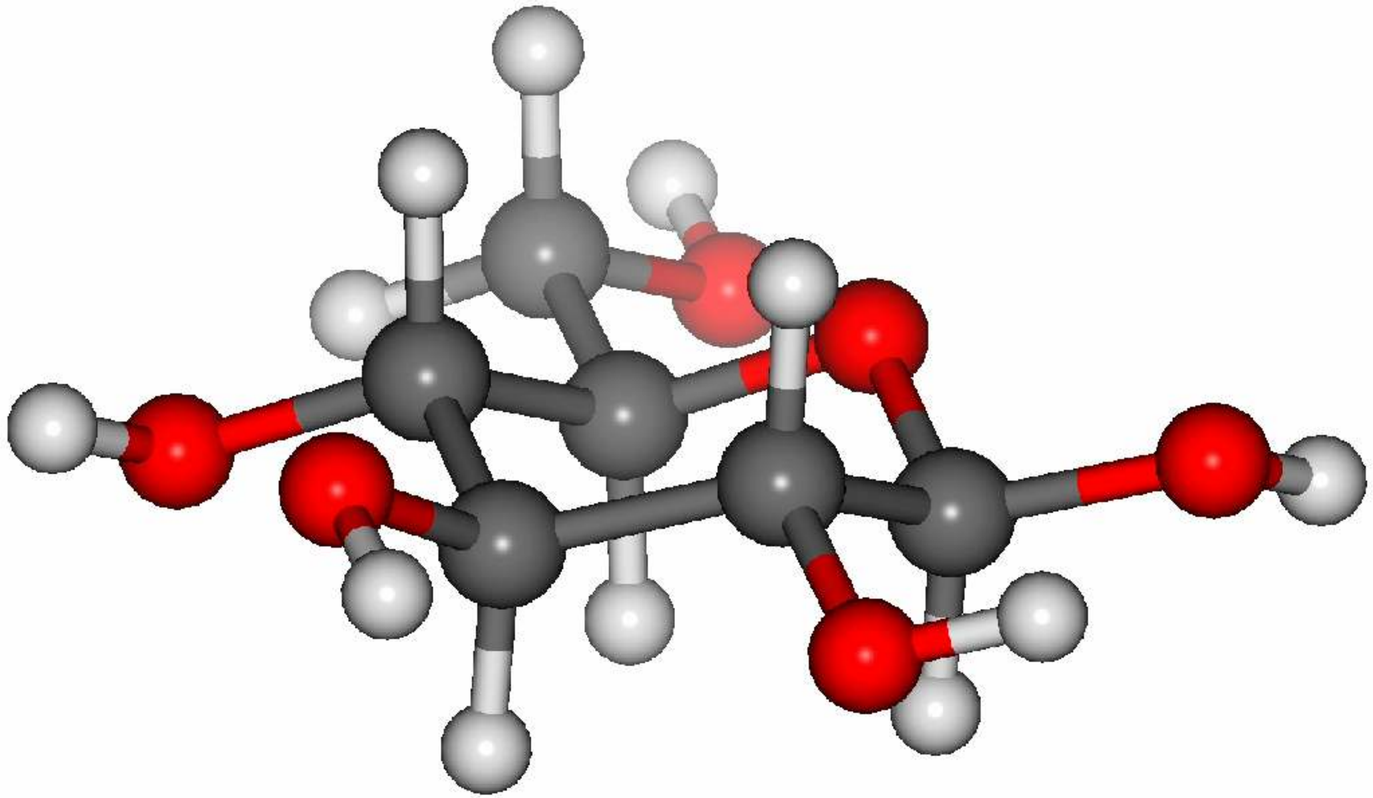

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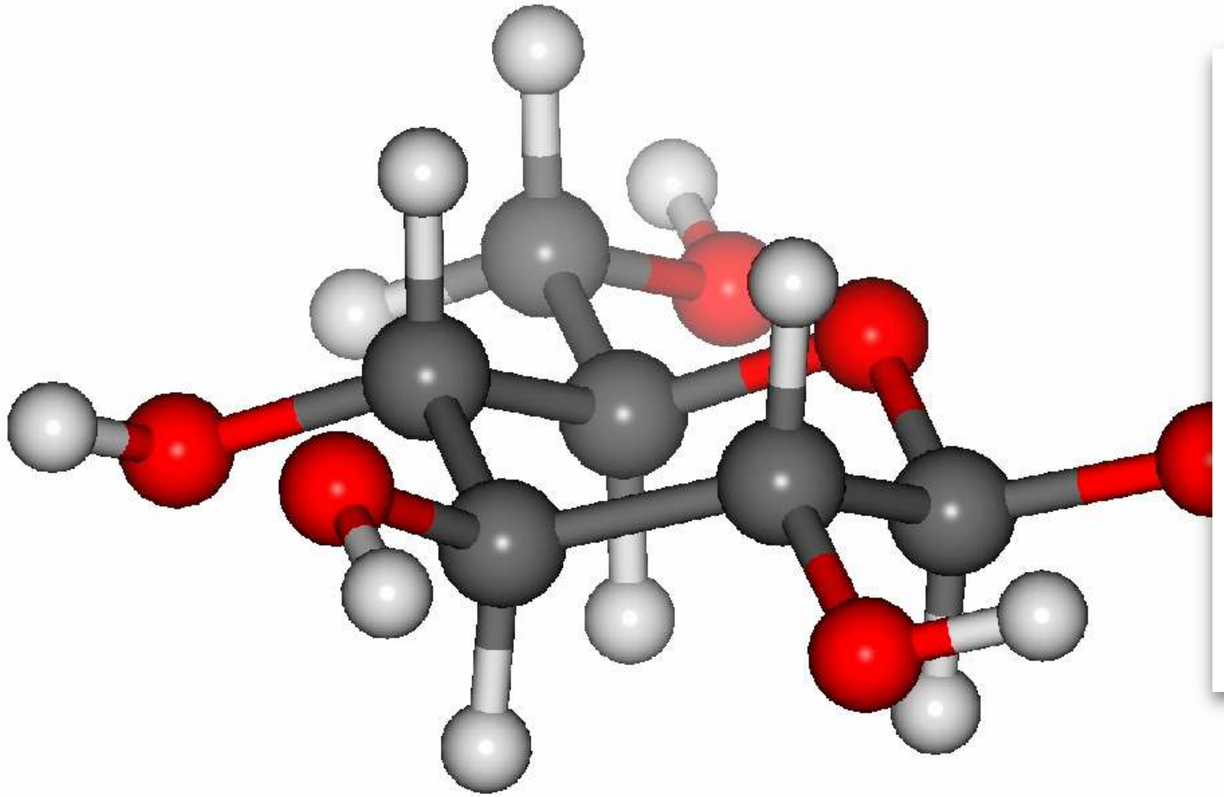

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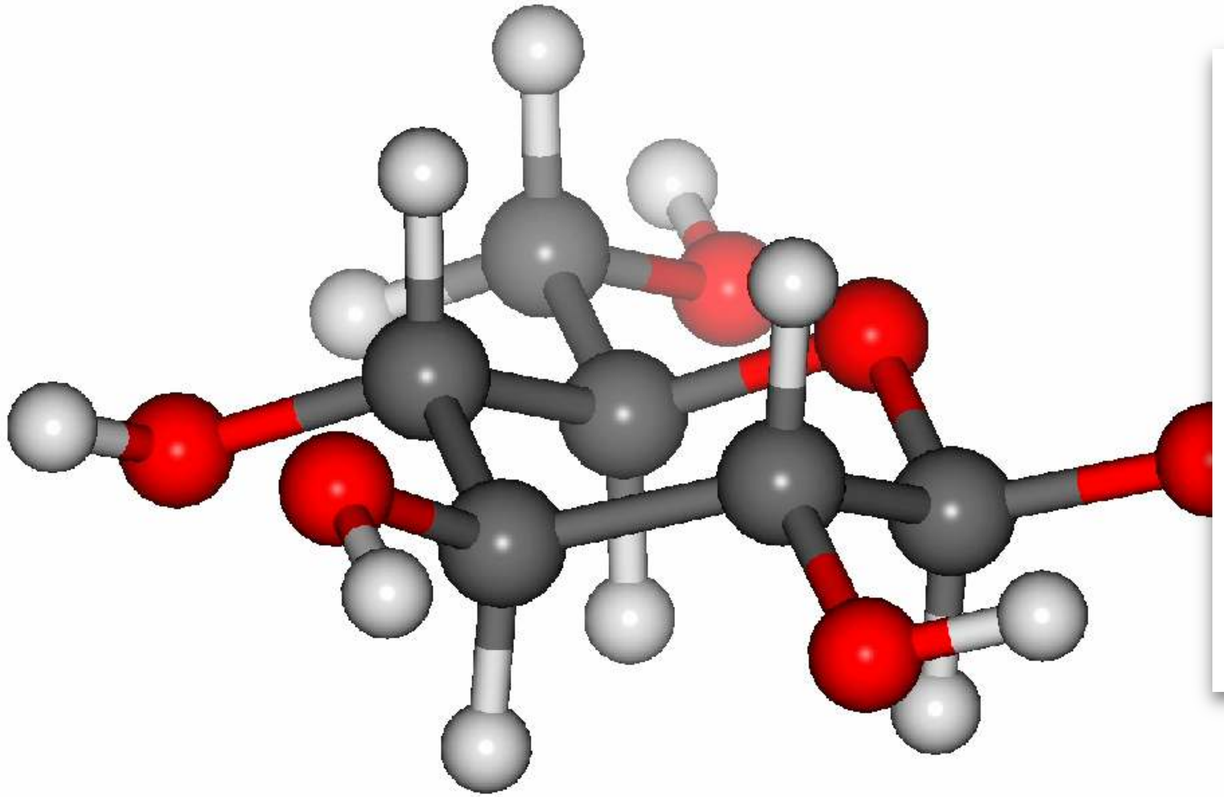
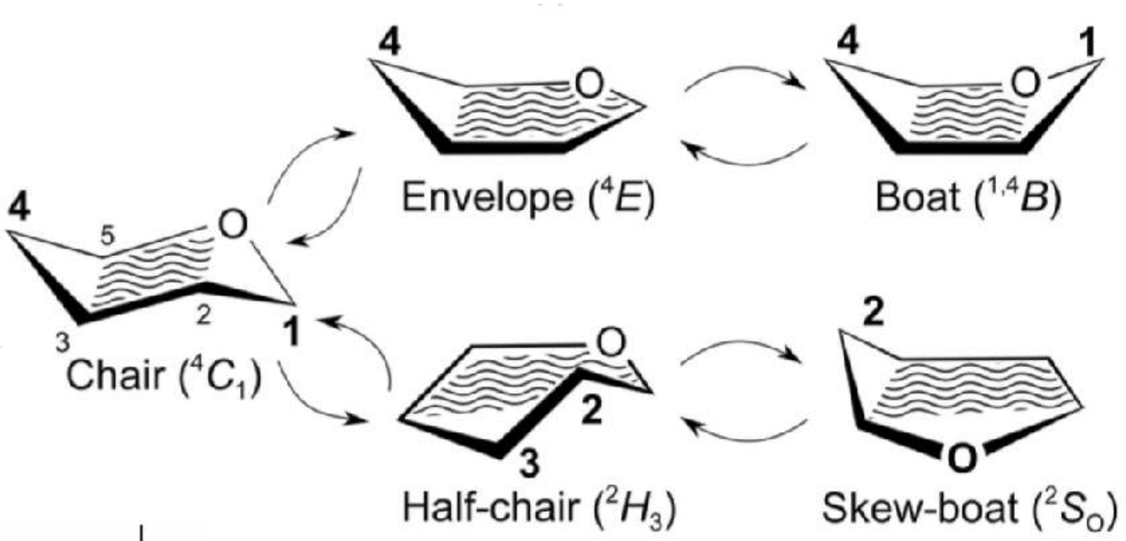


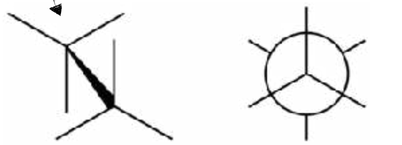
a $^{4}C_{1}$ chair

chairs are comfortable for sugars

Ring conformation

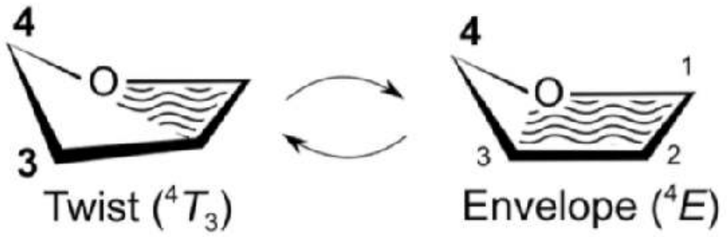
Wavy lines indicate which atoms are roughly coplanar





Staggered

Furanose forms
- 5-membered rings
- 2 ring puckers
- 20 conformations



Eclipsed

Pyranose forms
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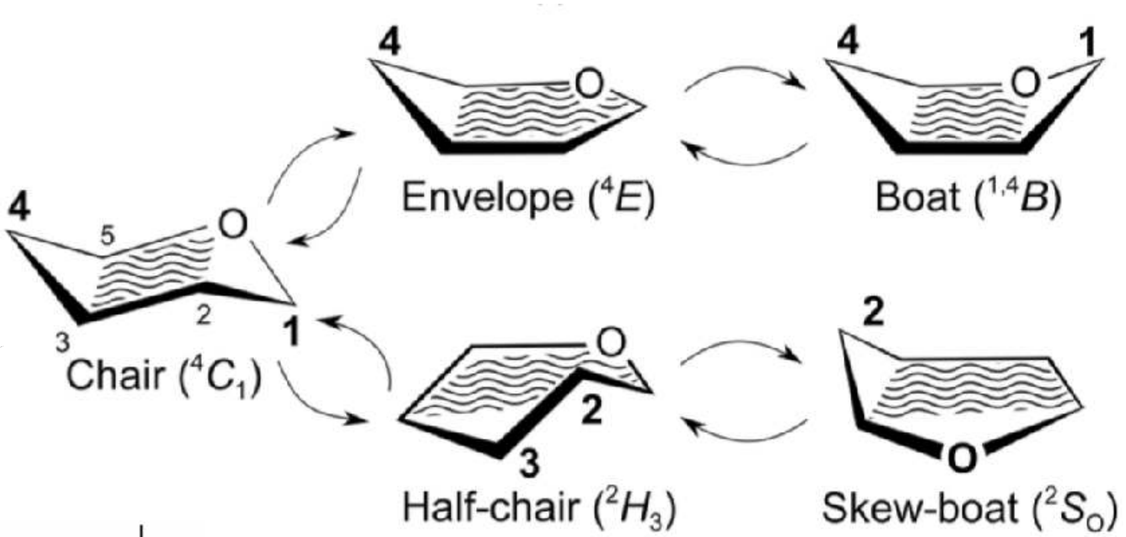

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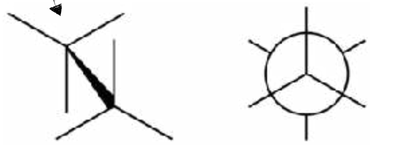

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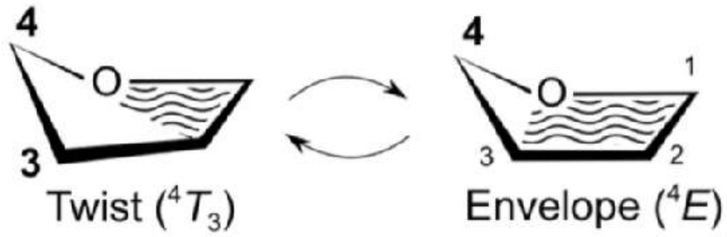
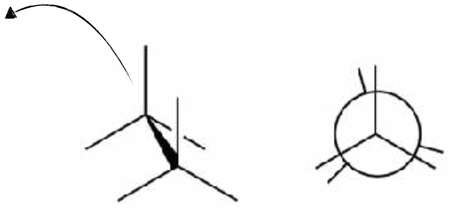


Very strained :'(



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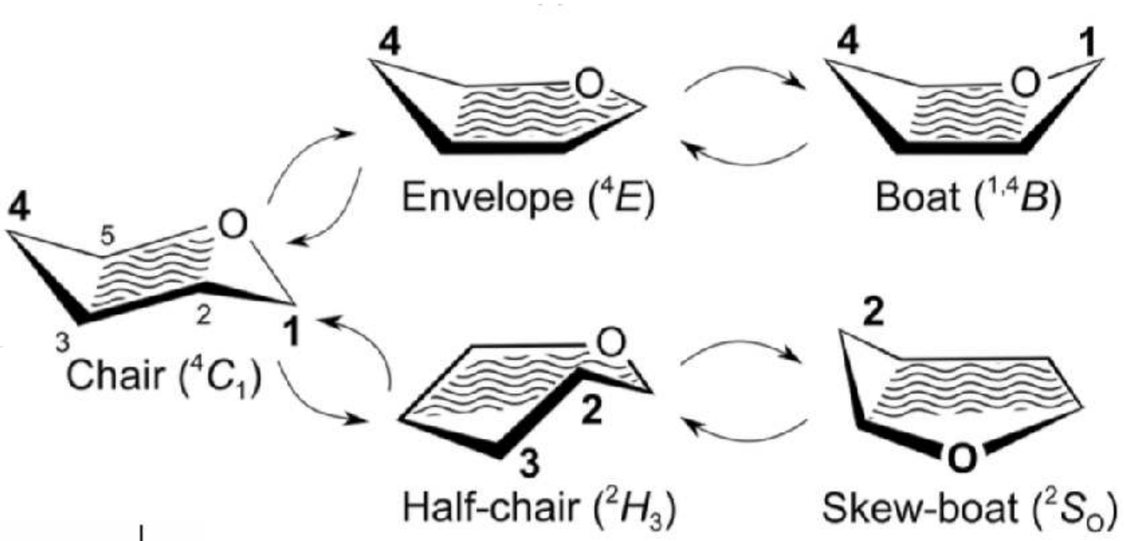

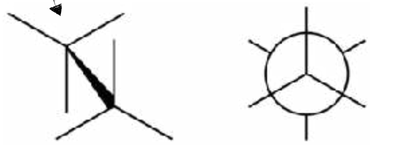

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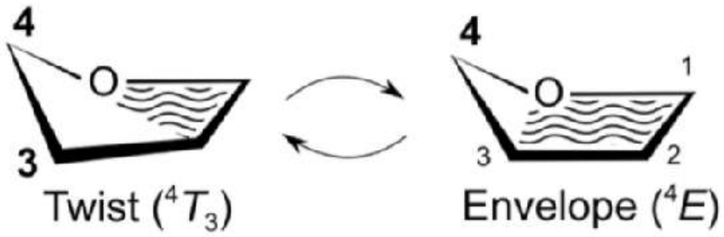

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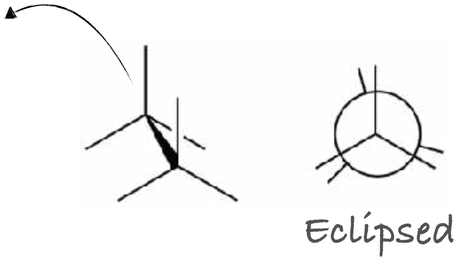

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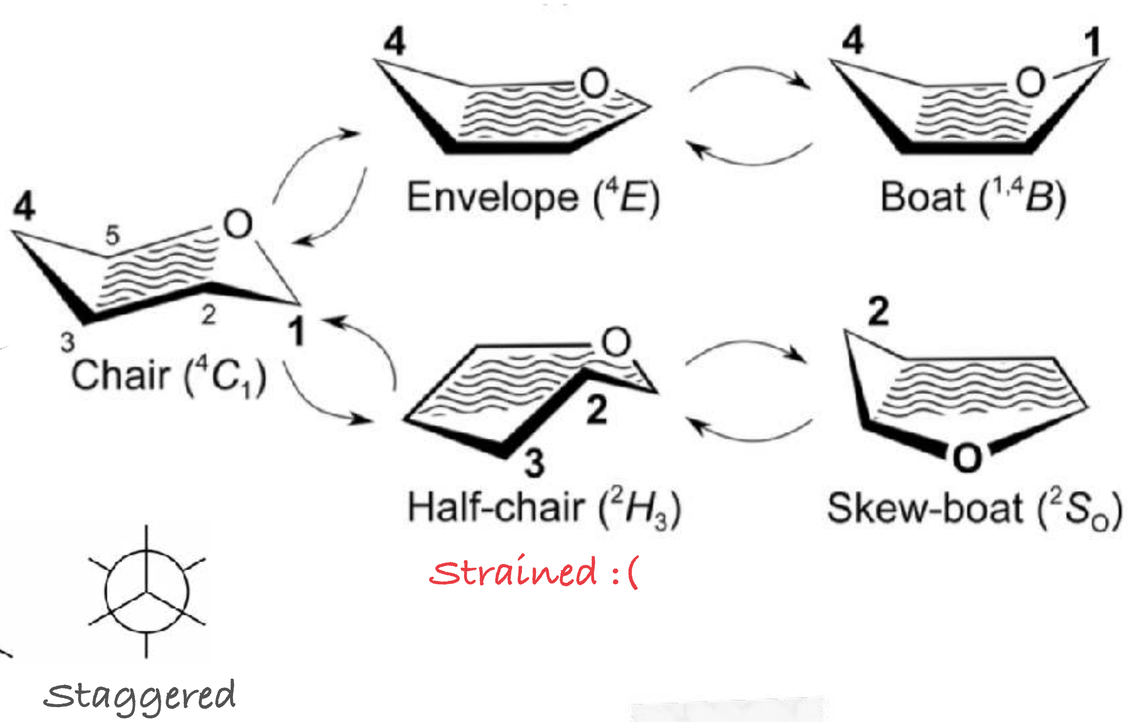

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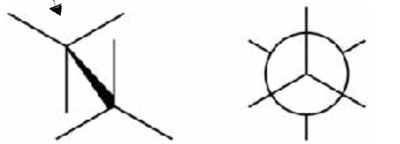

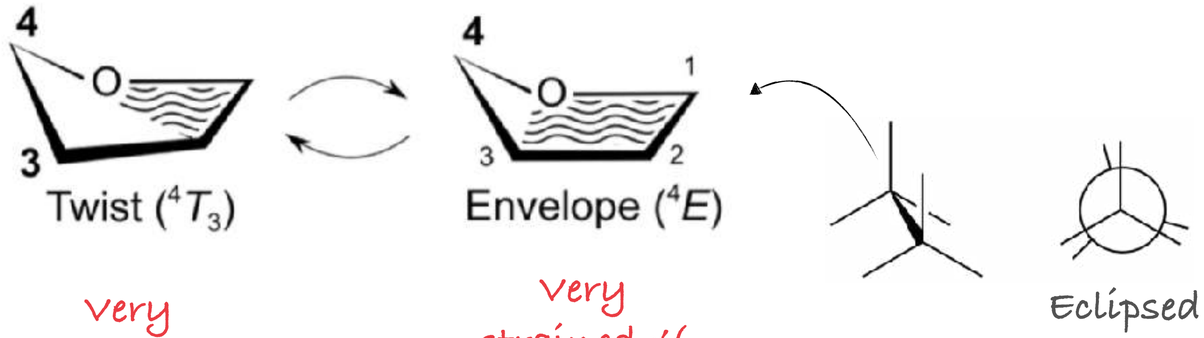

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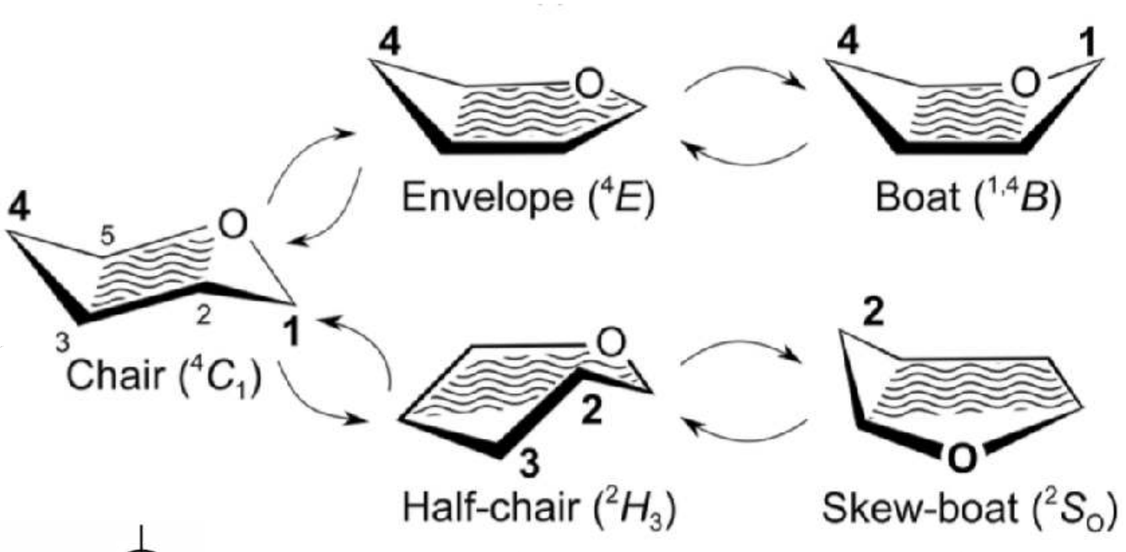

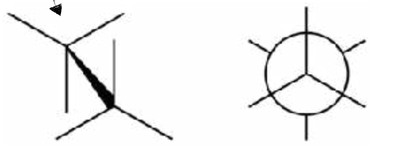

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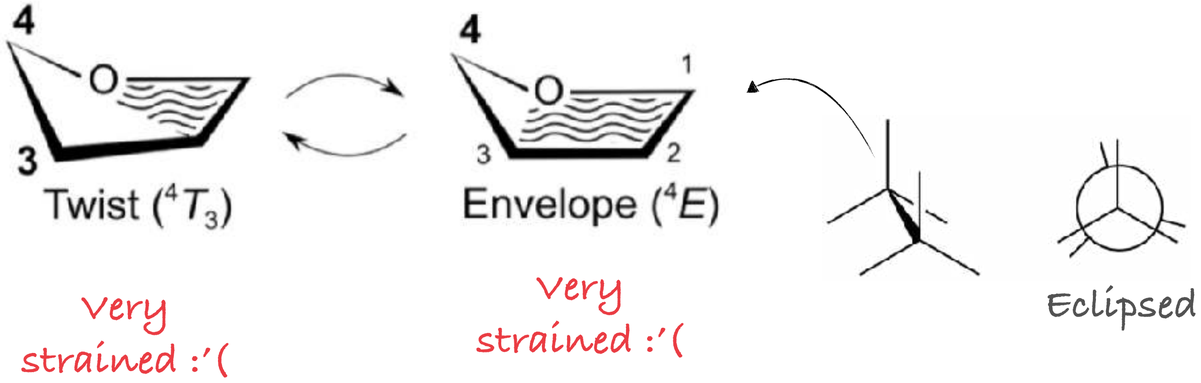

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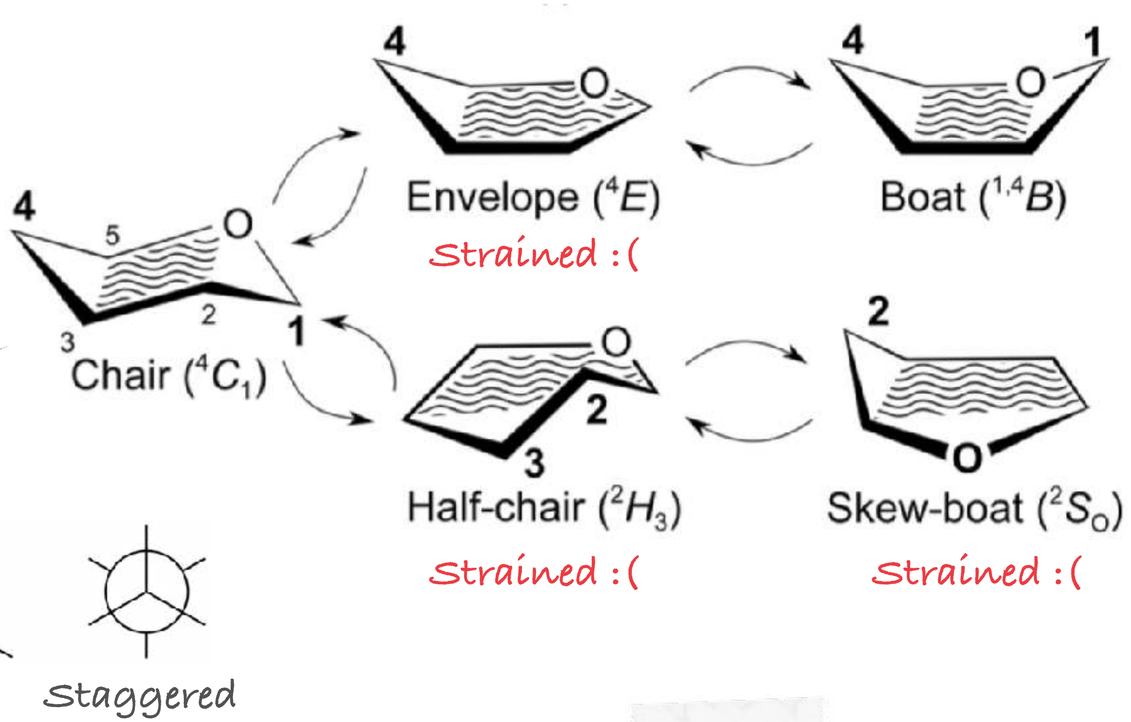

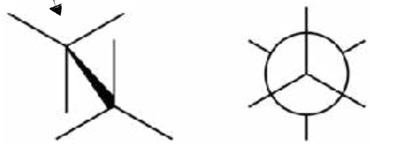

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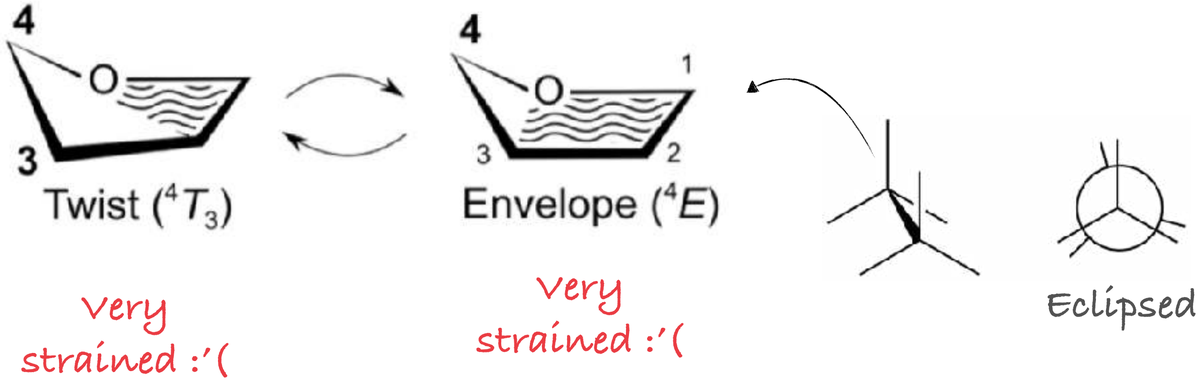

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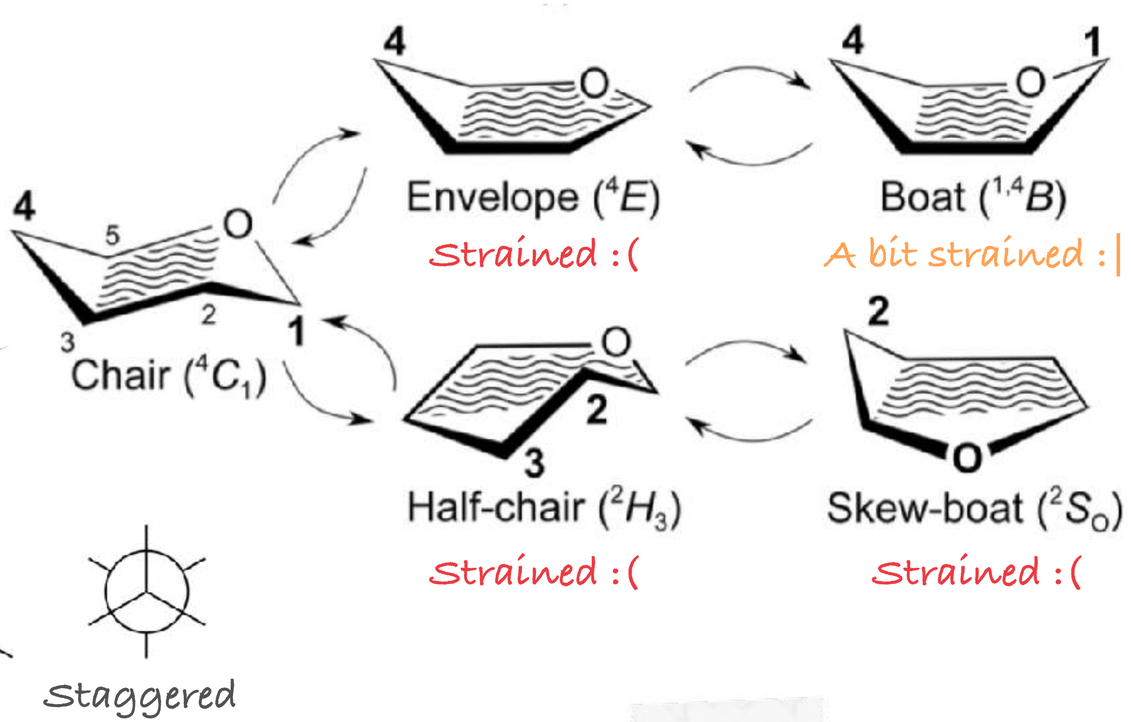

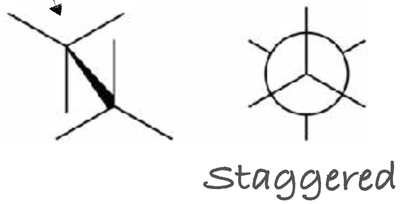

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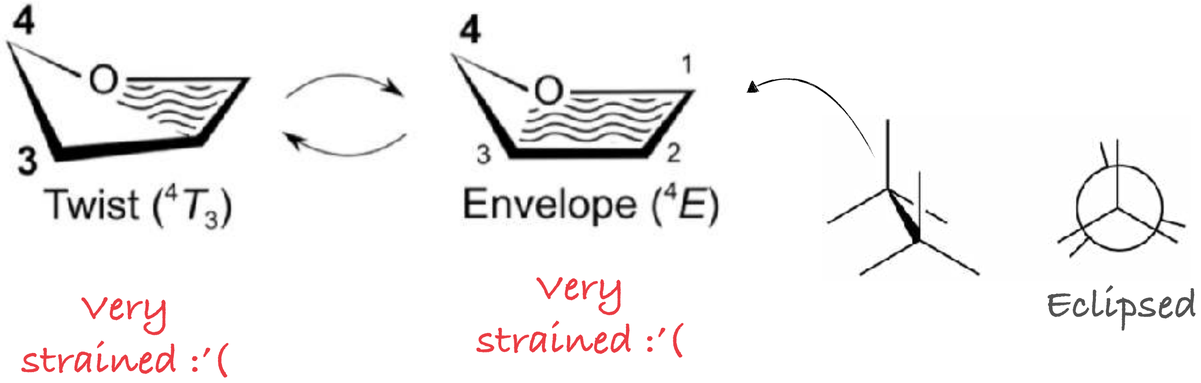

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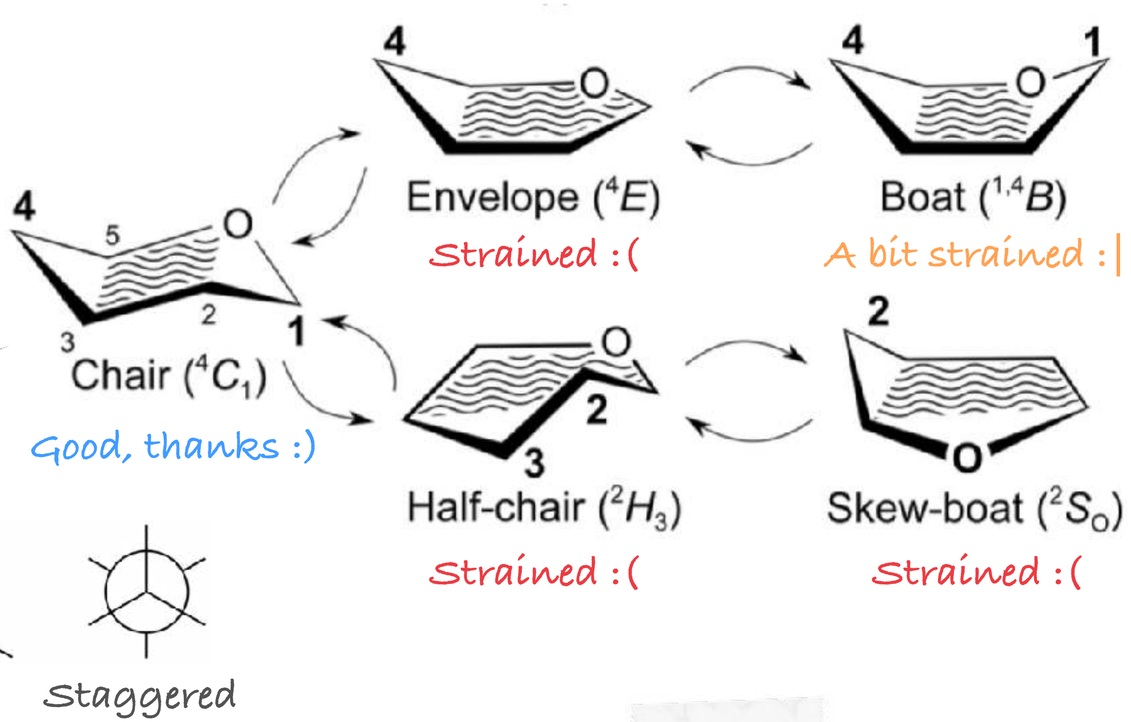

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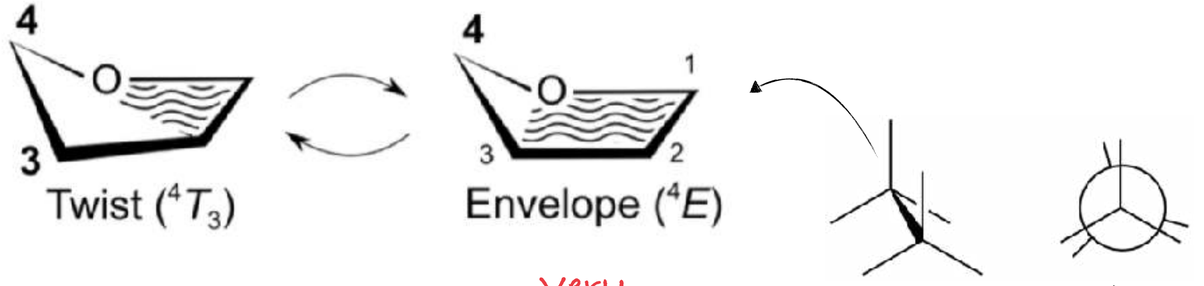

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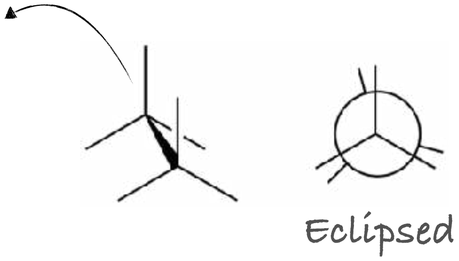

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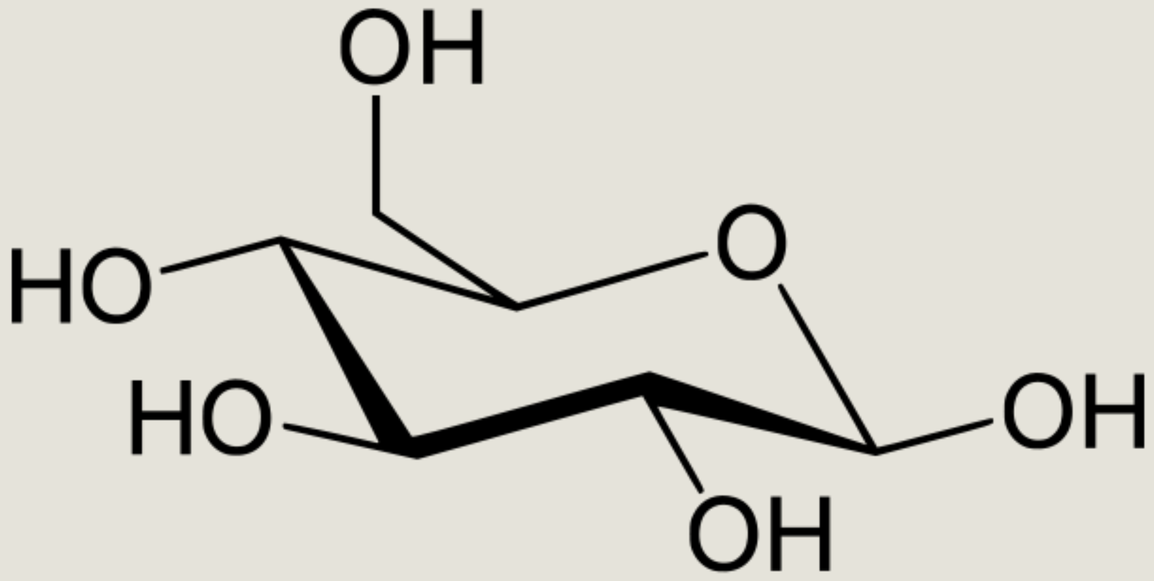
Ring conformation

The Cremer-Pople algorithm

Θ and Φ tell us which atoms move away from the mean ring plane, describing the conformation



Q tells us by how much:

$$Q = \sqrt{\sum_{i=0}^{5} Z_i^2}$$

"total puckering amplitude"

Q = 0.54Å for an ideal Glucose $^4C_1$ chair

Cremer & Pople, 1975, JACS 97(6)

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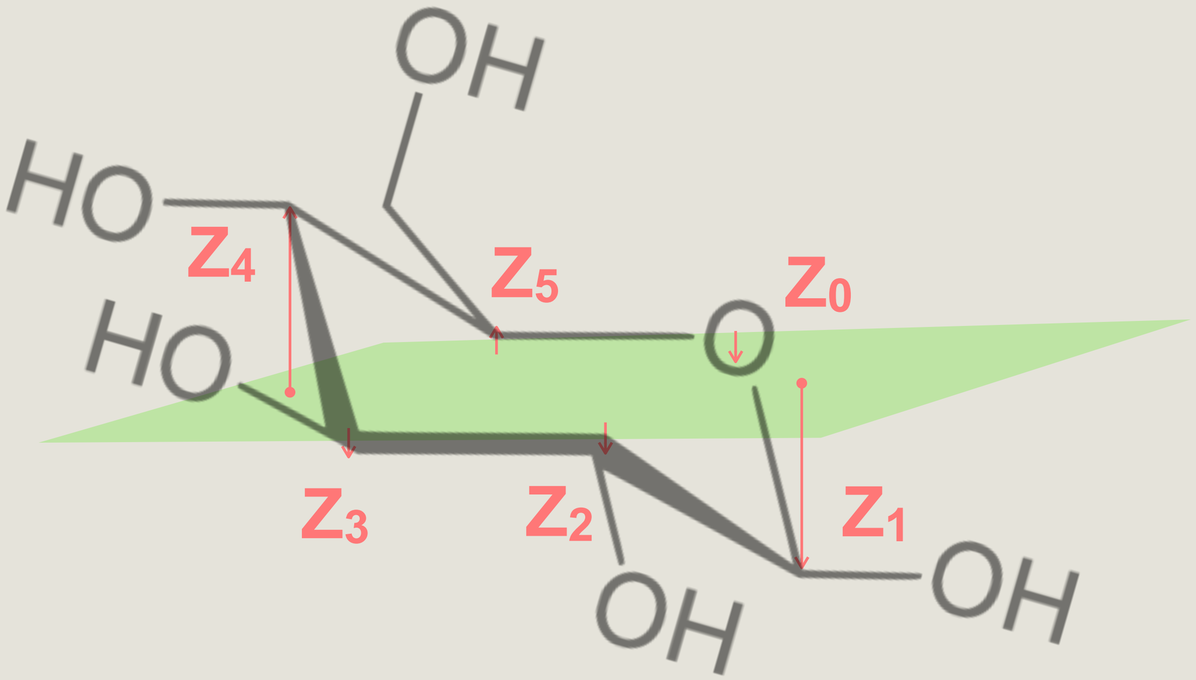

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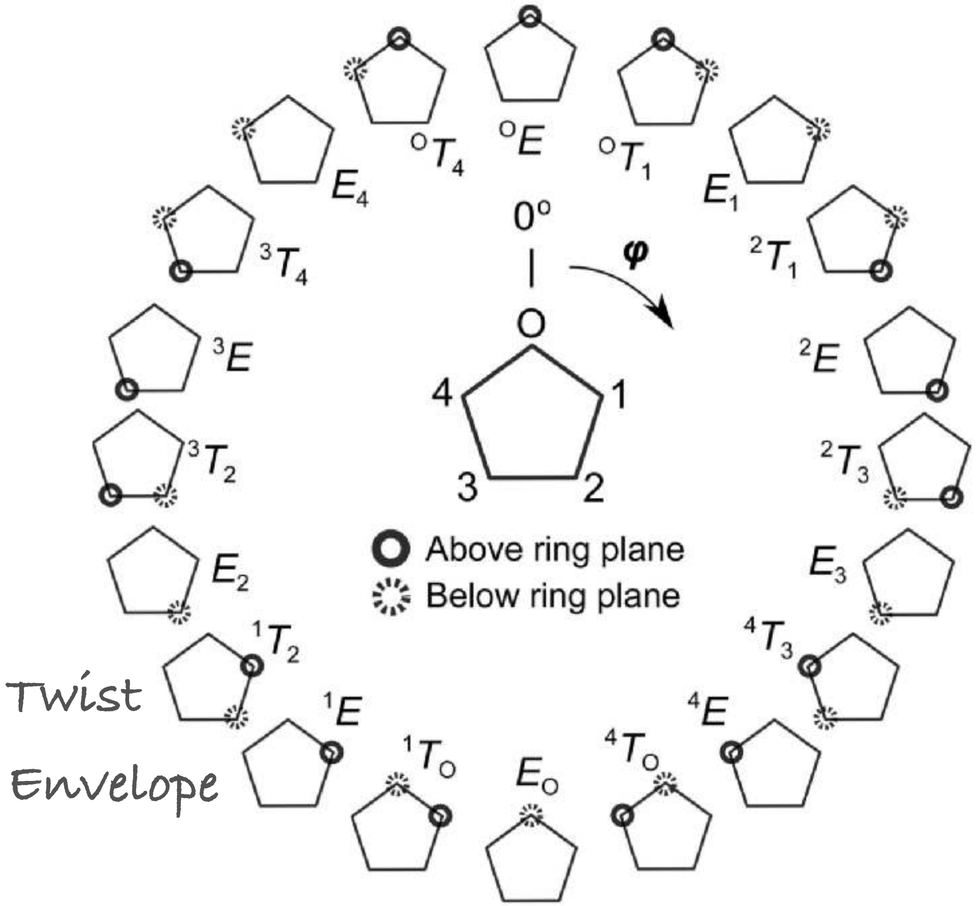

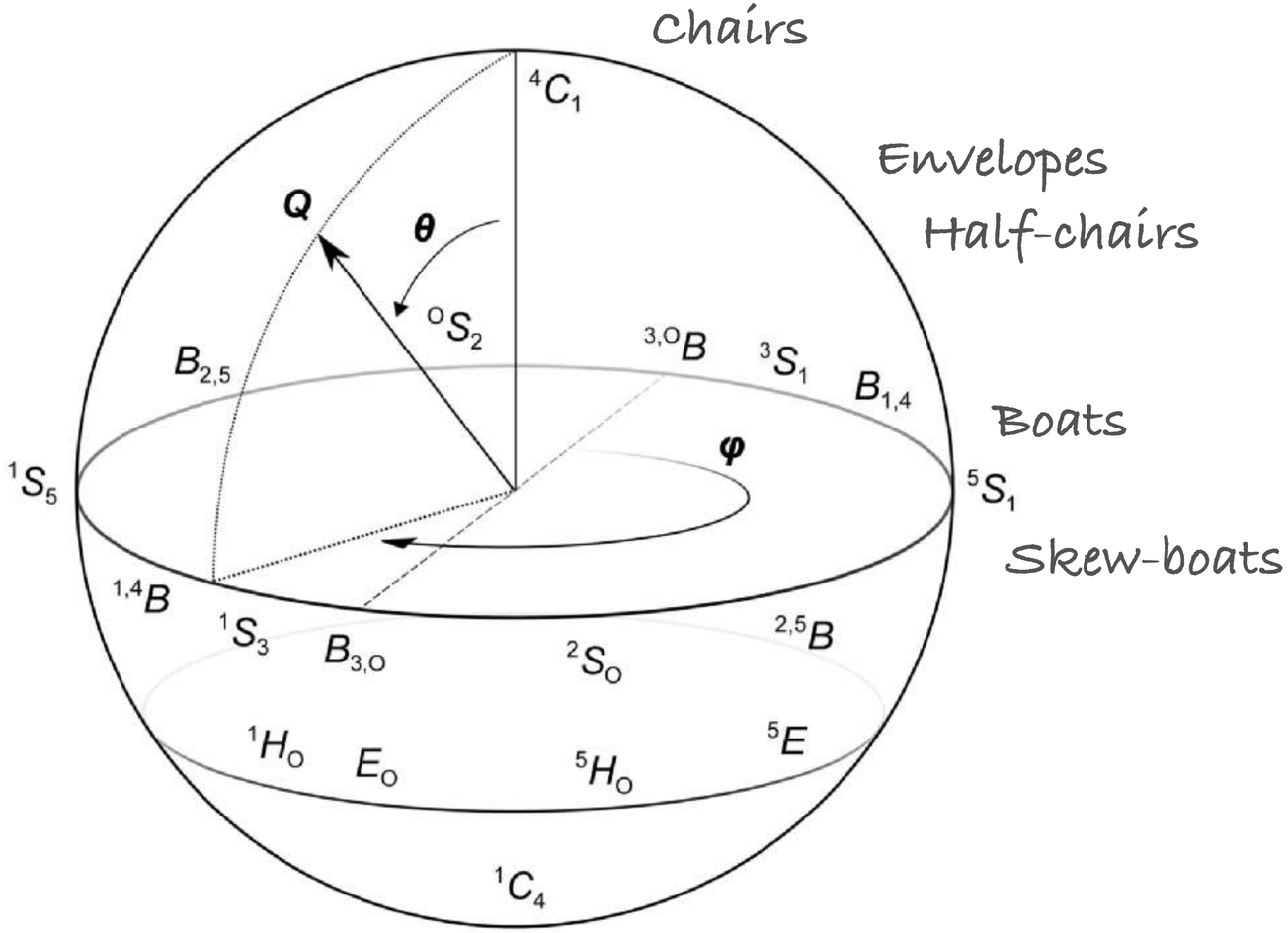

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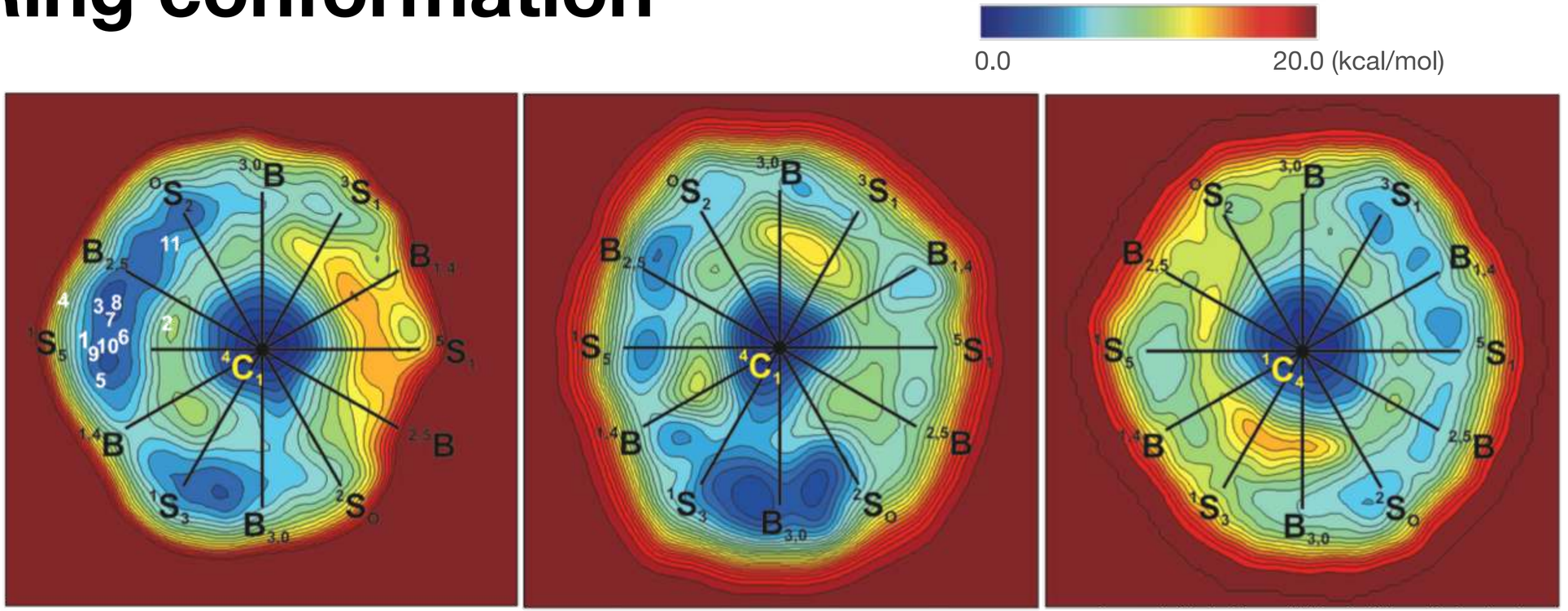
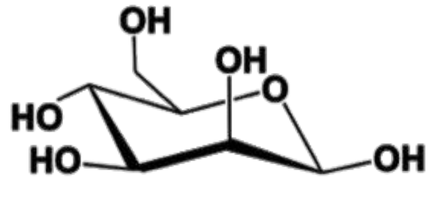
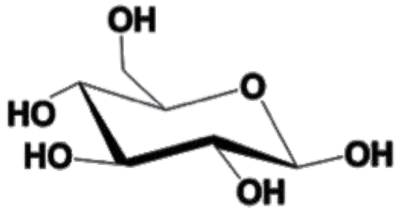
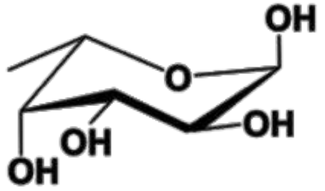




β-D-Mannopyranose



β-D-Glucopyranose

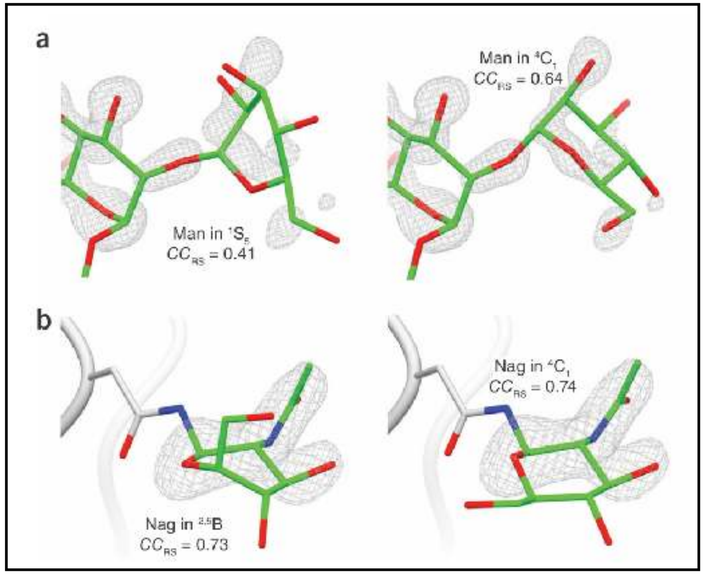


α-L-Fucopyranose

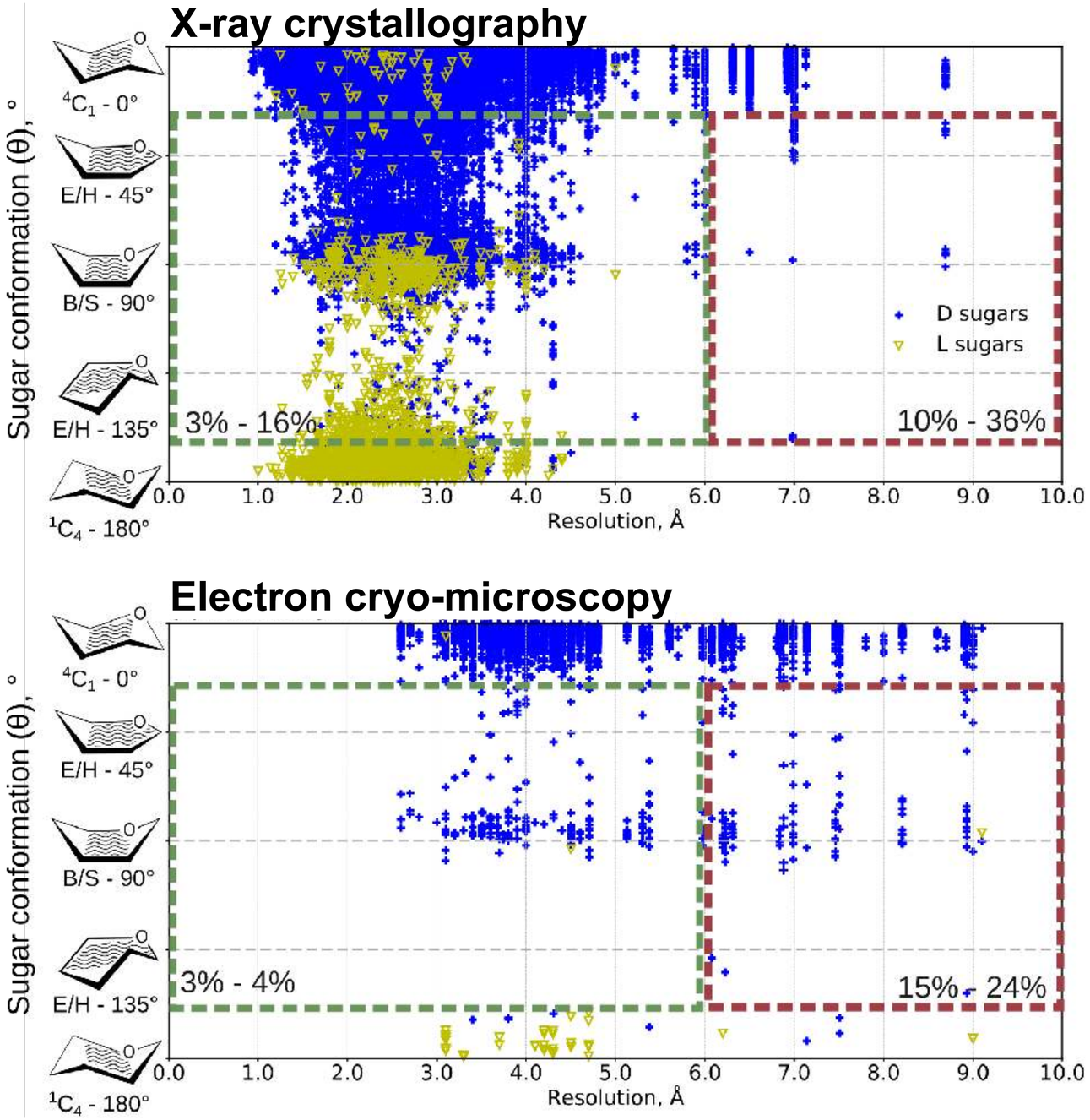
Adapted from Ardevol, Biarnes, Planas & Rovira, 2010, JACS 132(45).

Ring conformation in protein glycosylation

- Many more **high-energy** conformations than expected.
- Clear need **for carbohydrate specific methodology**.

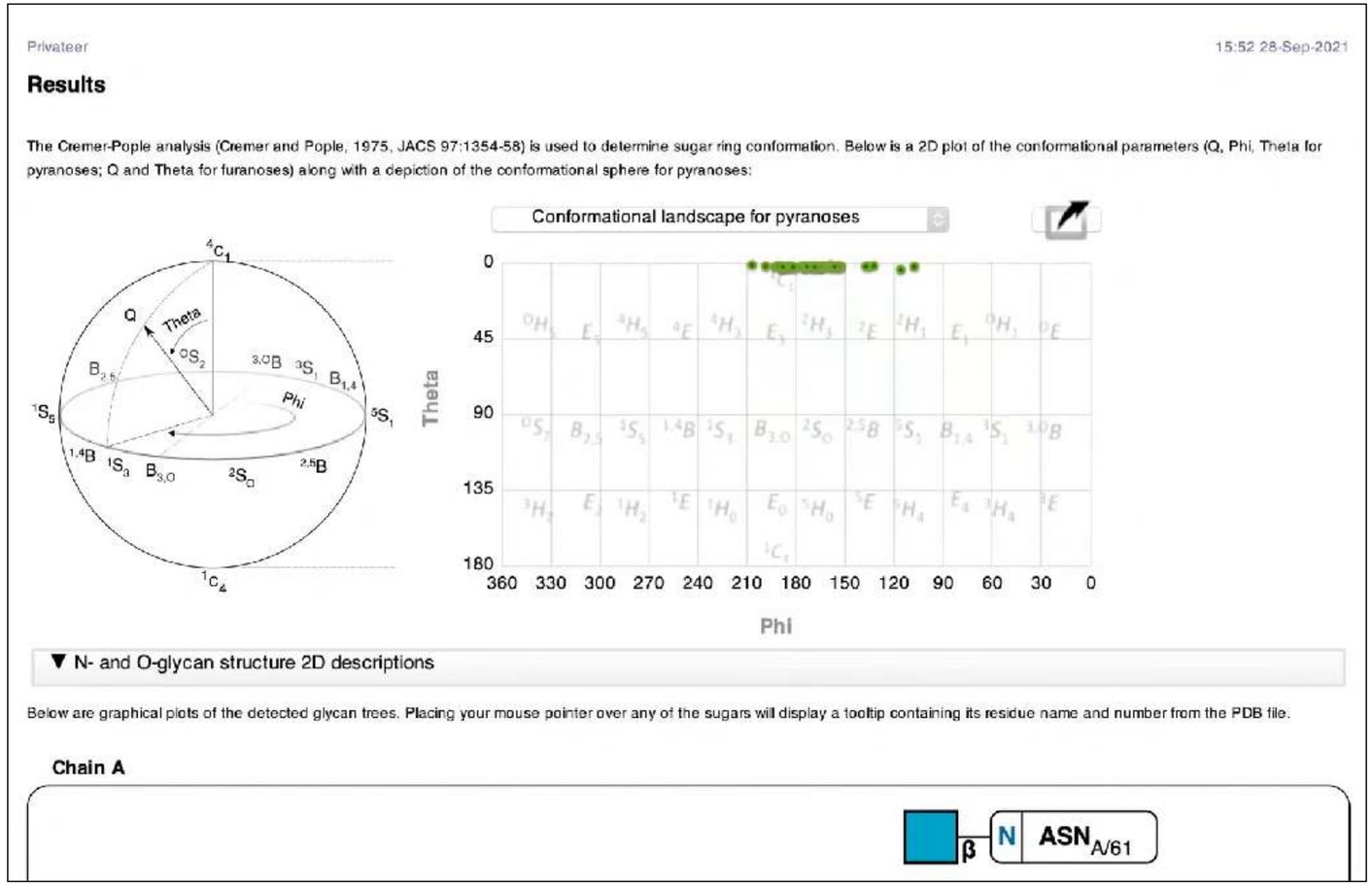


Agirre, Davies, Wilson & Cowtan, 2015, Nature Structural & Molecular Biology 22(11):833-834.



Atanasova, Bagdonas & Agirre, 2020, Current Opinion in Structural Biology 62:70-78.

Validation of structure, ring and link conformation of carbohydrate structures (MKIV)



Bagdonas, Ungar & Agirre, 2020, Beilstein Journal of Organic Chemistry, 16(1):2523-2533.

Privateer

Available in CCP4 8.0 and CCP-EM
Web server: http://privateer.hosted.york.ac.uk
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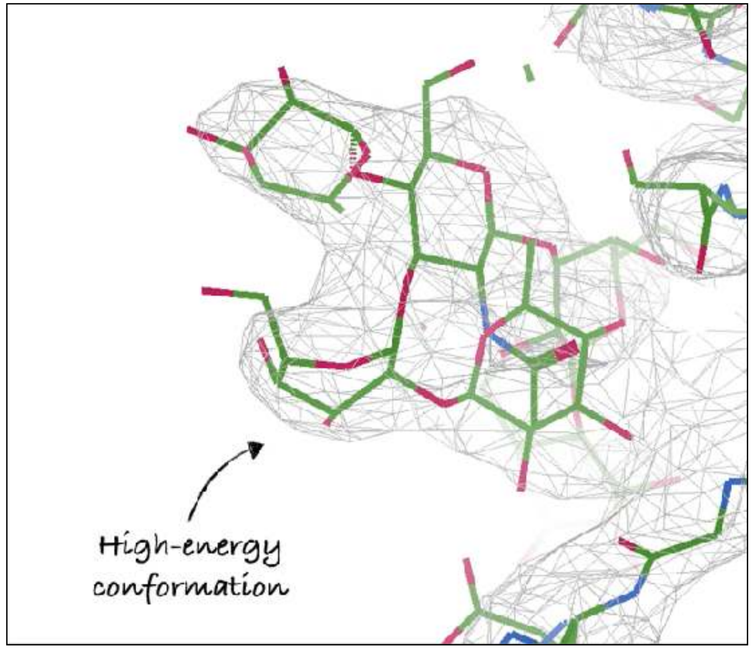
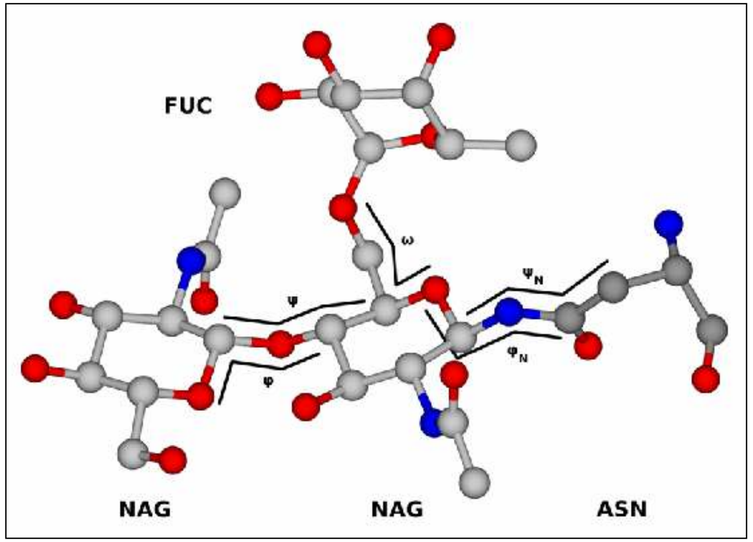
Dictionaries for model improvement (MKIV)



Linkage torsion validation (MKV)



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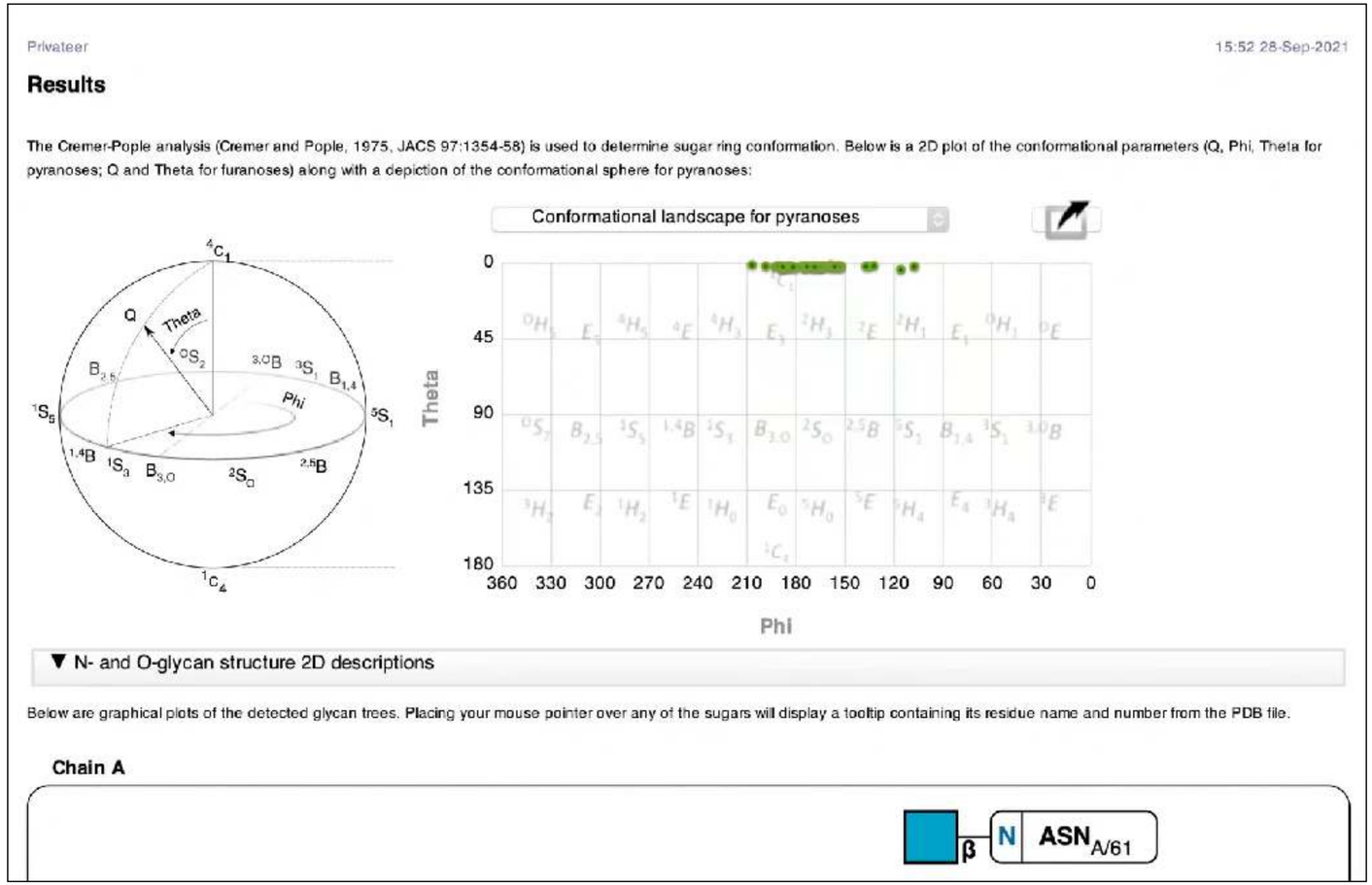

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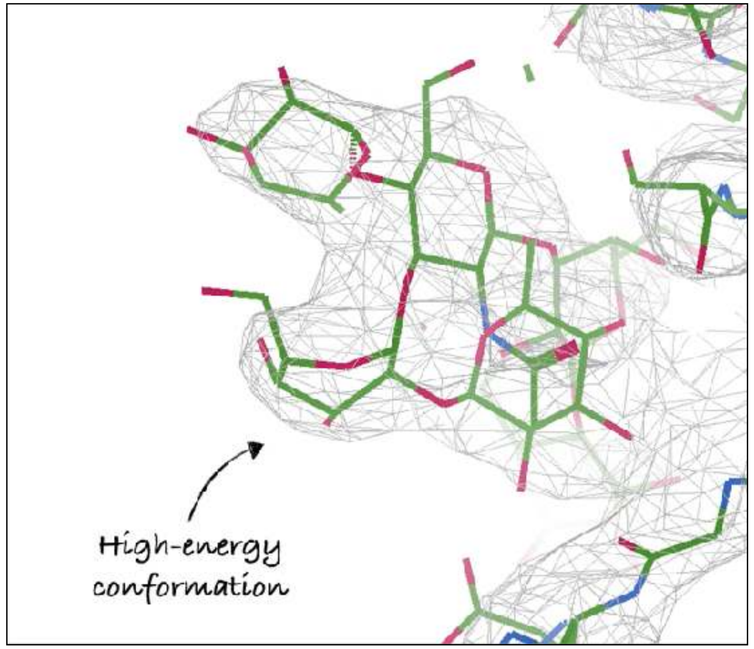

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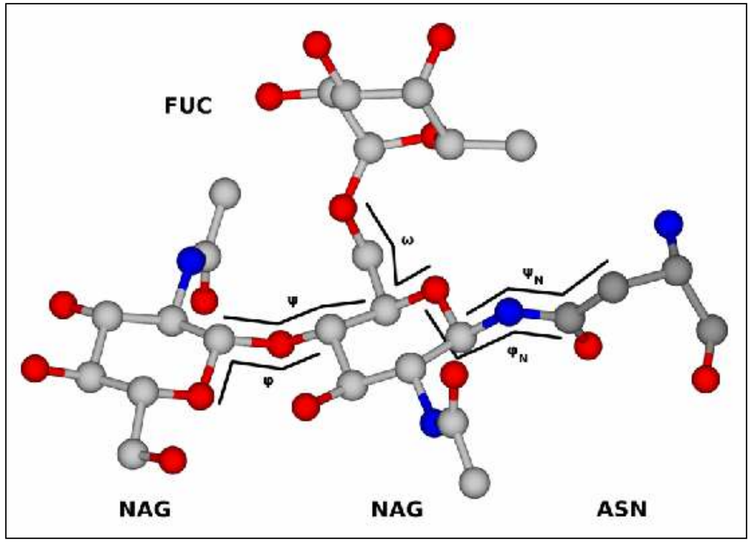

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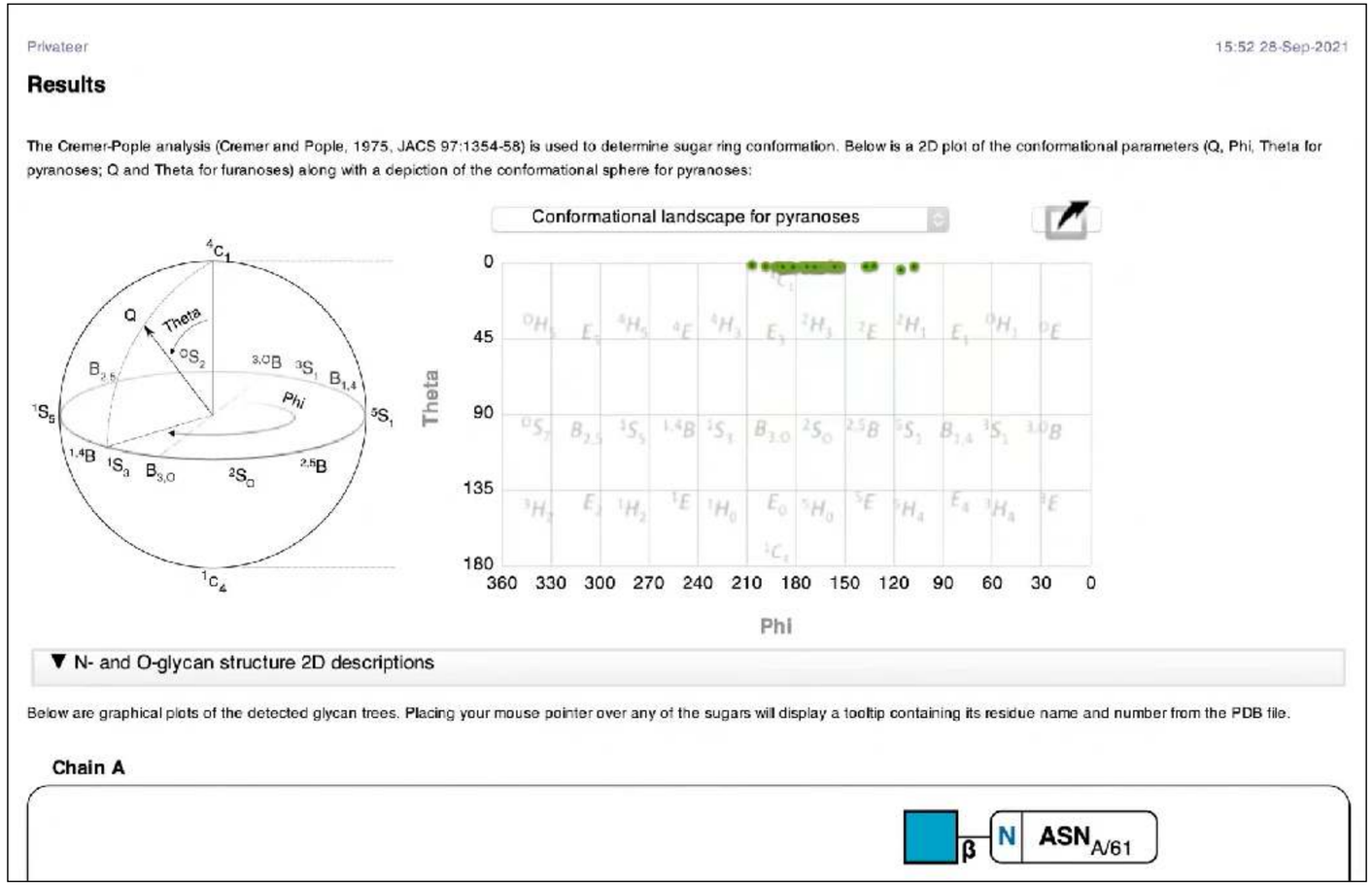

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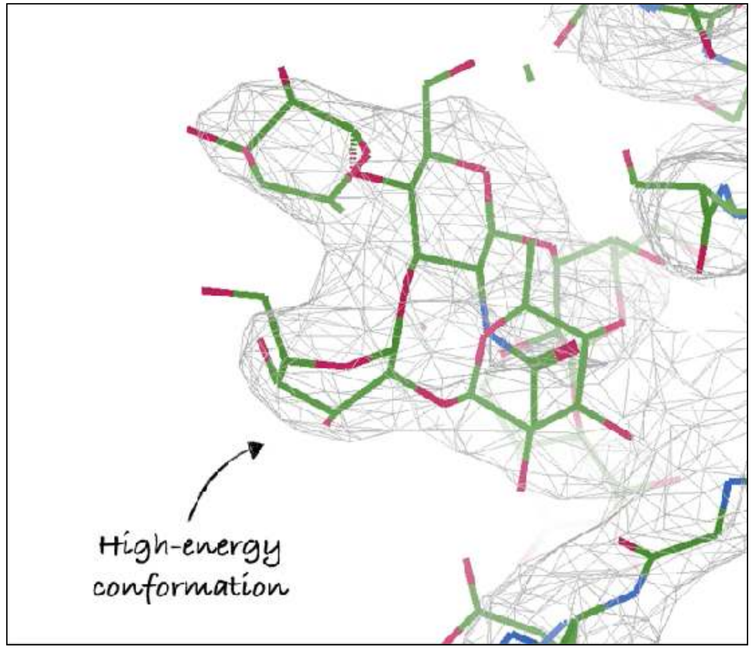
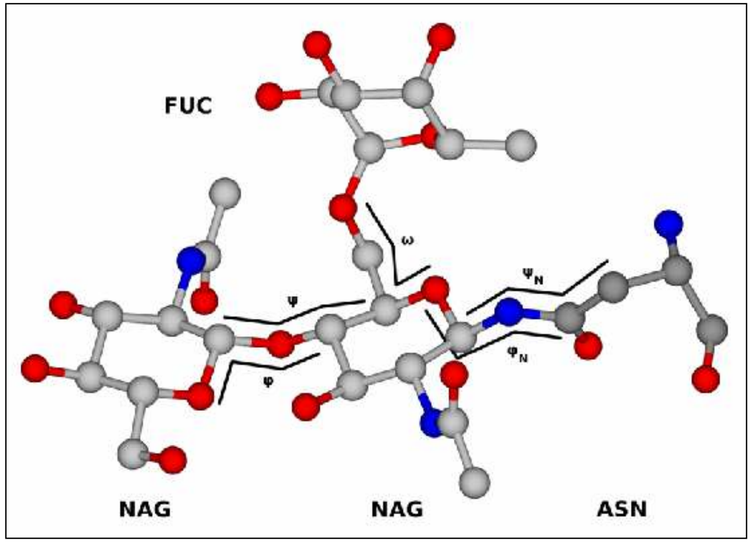


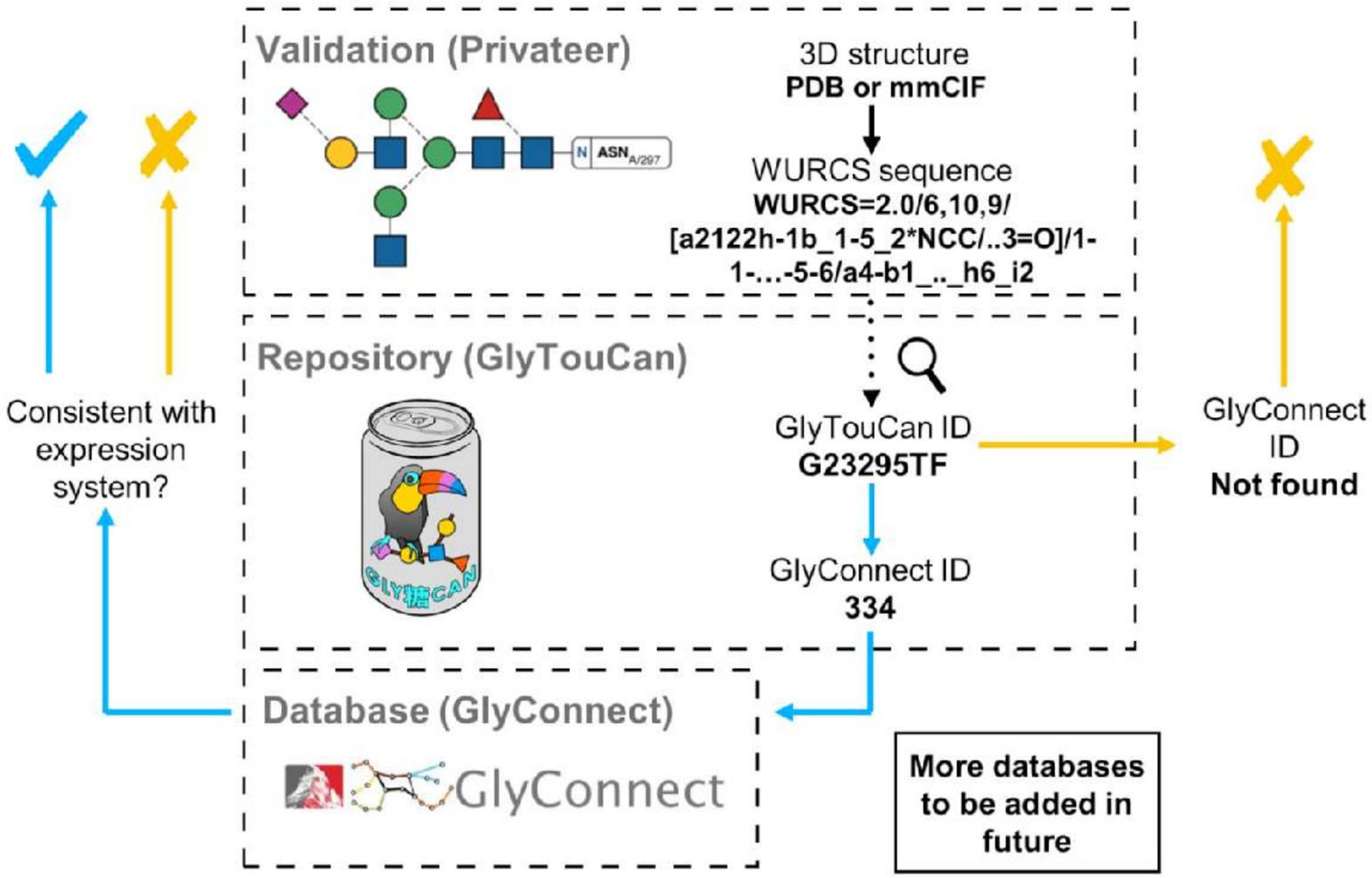
Linkage torsion validation (MKV)

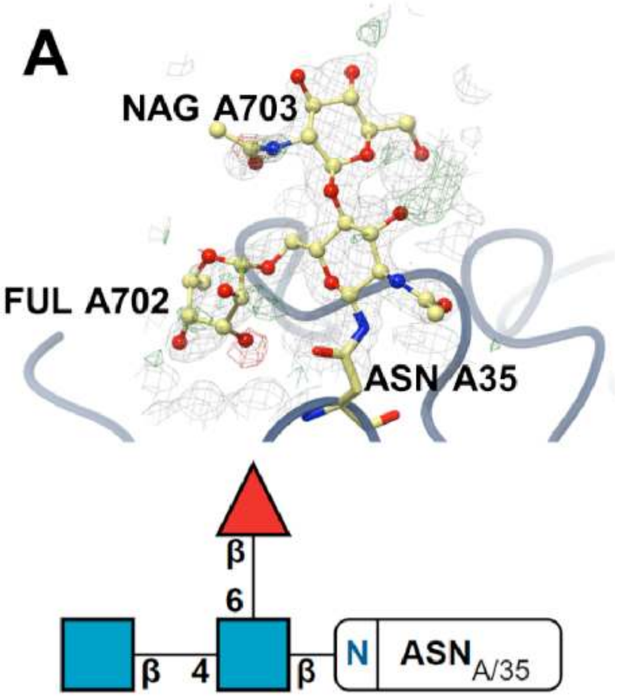


The Privateer software

- Has been useful in **every relevant context** where structural glycobiology has played a role: biofuels, immunology, cancer, fecundation and, of course, viral glycoproteins (HIV, Ebolavirus, Influenza, MERS, SARS-CoV-1 & 2).
- COVID-19 pandemic: used in constructs informing **mRNA vaccine design**, structures of **spike-antibody complexes** and **neutralising drugs**. Integrated in several analysis and validation pipelines.
- Released with main UK structural biology software suites: CCP4 (MKIV available in 8.0, MKV soon via update) and CCP-EM. New Python bindings (MKV) will bring fully interactive graphical interfaces for Coot and ChimeraX. A web server for *Expasy* is planned too.

Glycomics powered validation





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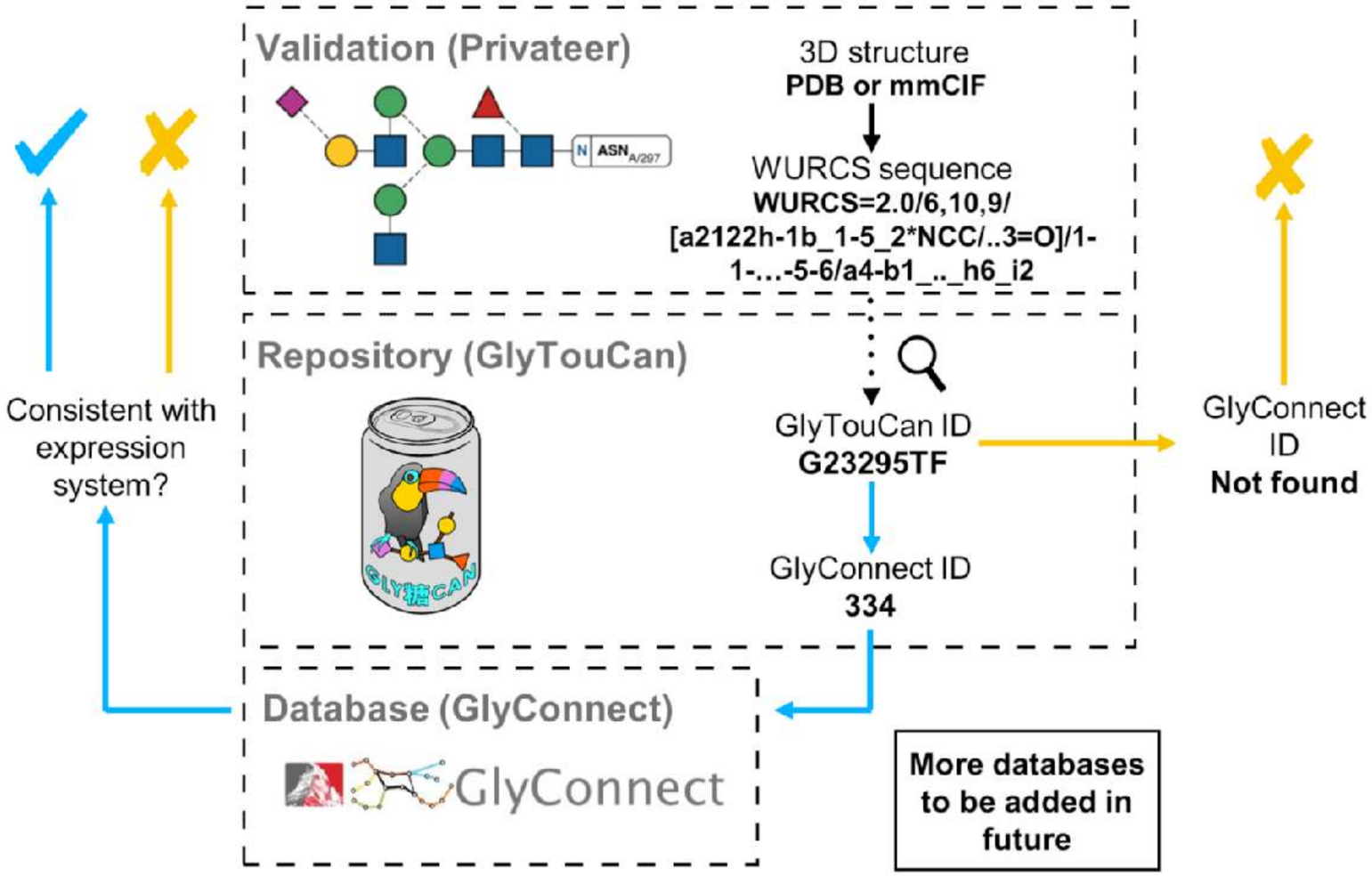
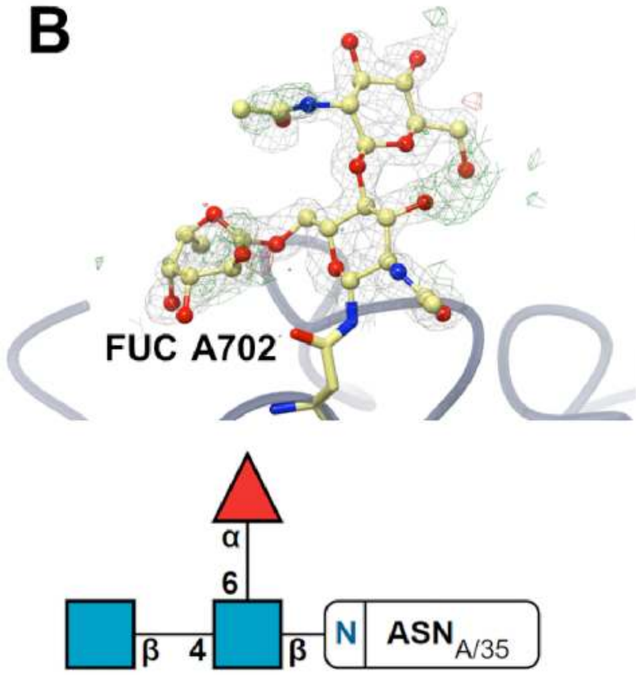
GlyTouCan ID: G28454KX

GlyConnect ID: Not Found.

Bagdonas, Ungar & Agirre, 2020, Beilstein Journal of Organic Chemistry, 16(1):2523-2533.

Glycomics powered validation





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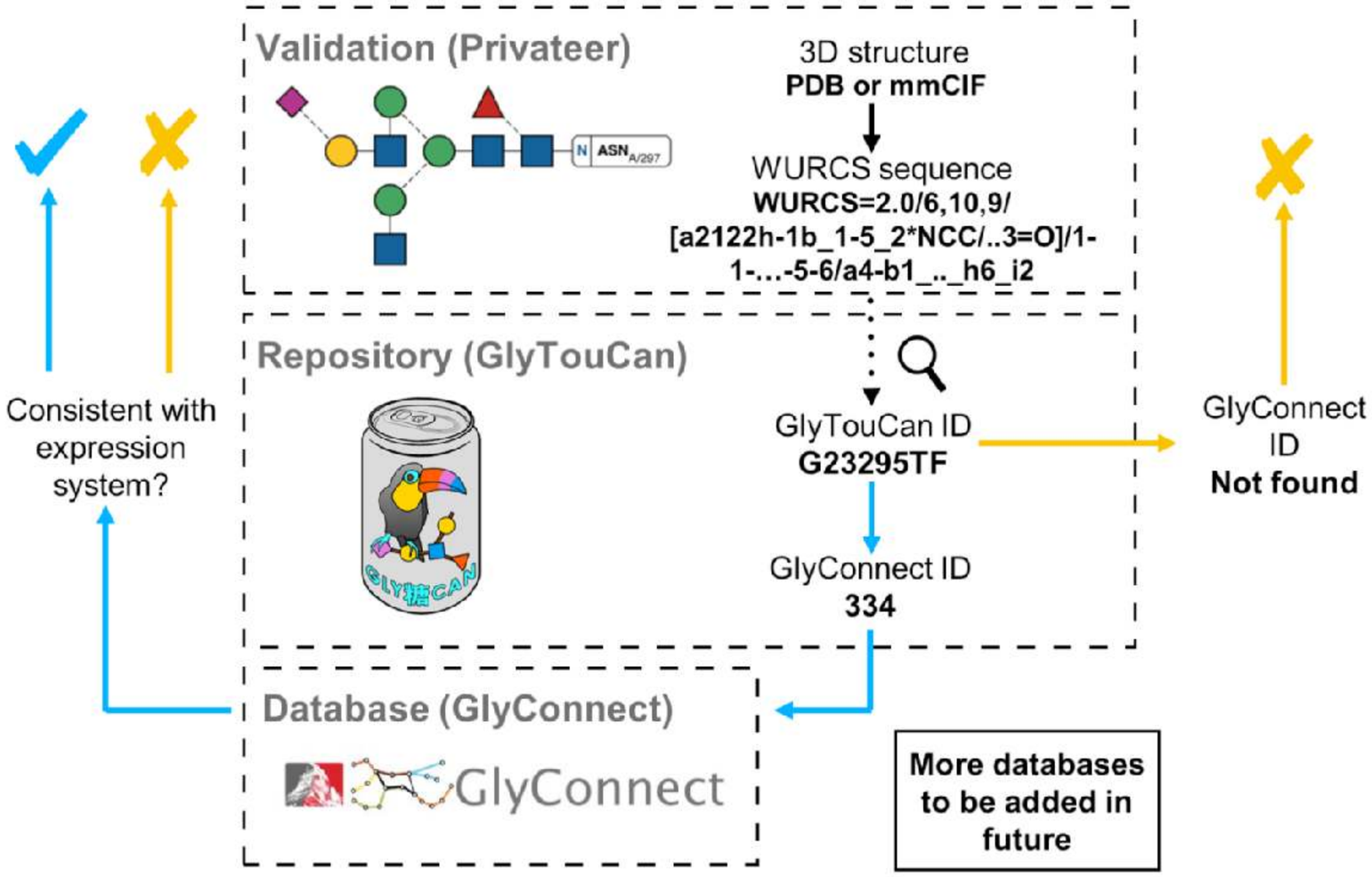
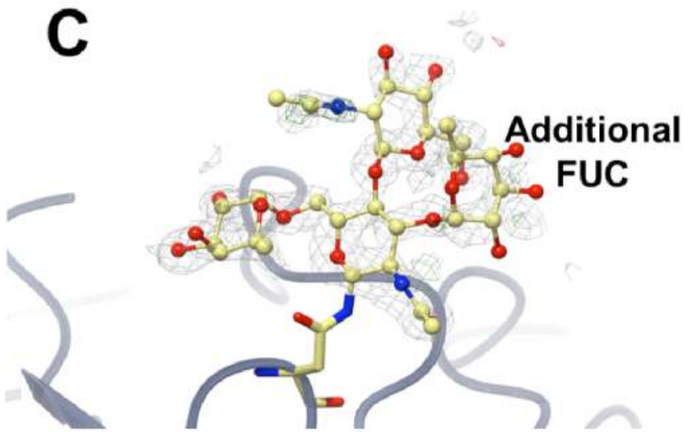
GlyTouCan ID: G21290RB

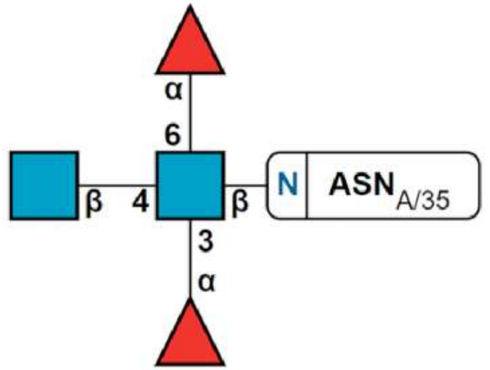
GlyConnect ID: 54

Bagdonas, Ungar & Agirre, 2020, Beilstein Journal of Organic Chemistry, 16(1):2523-2533.

Glycomics powered validation







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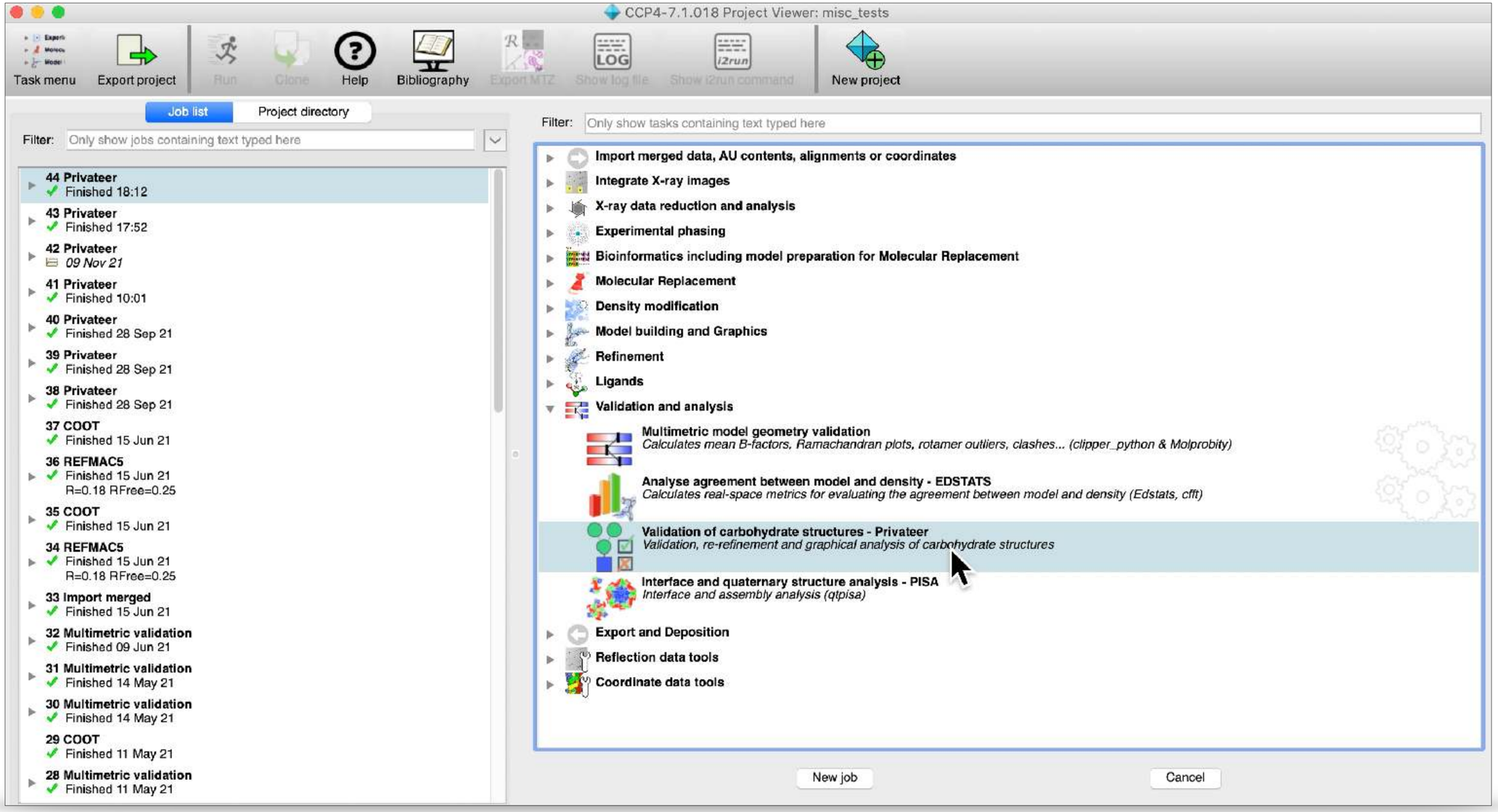
GlyTouCan ID: G63564LA

GlyConnect ID: 145

Bagdonas, Ungar & Agirre, 2020, Beilstein Journal of Organic Chemistry, 16(1):2523-2533.

Running Privateer MKIV (CCP4i2)



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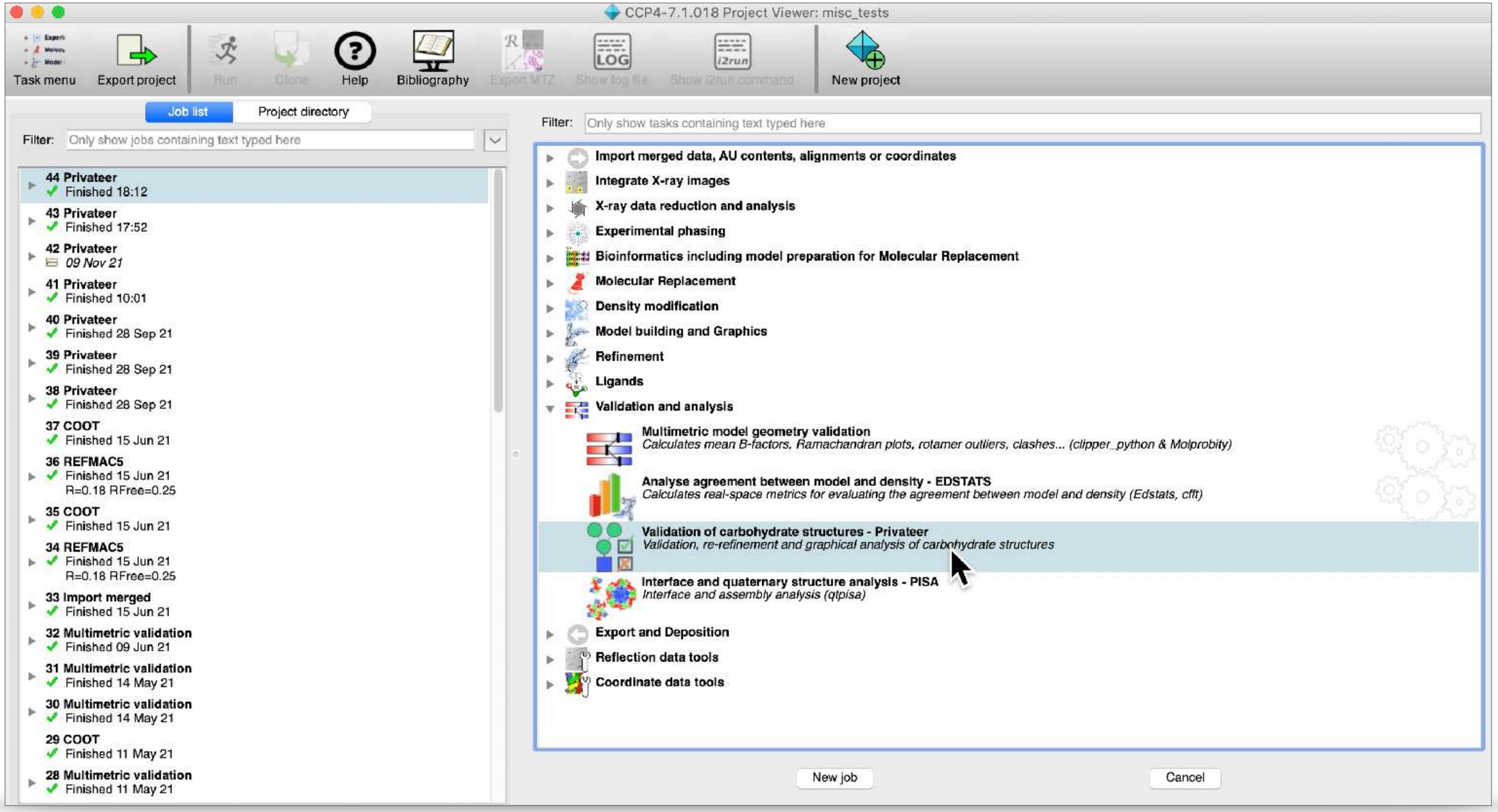
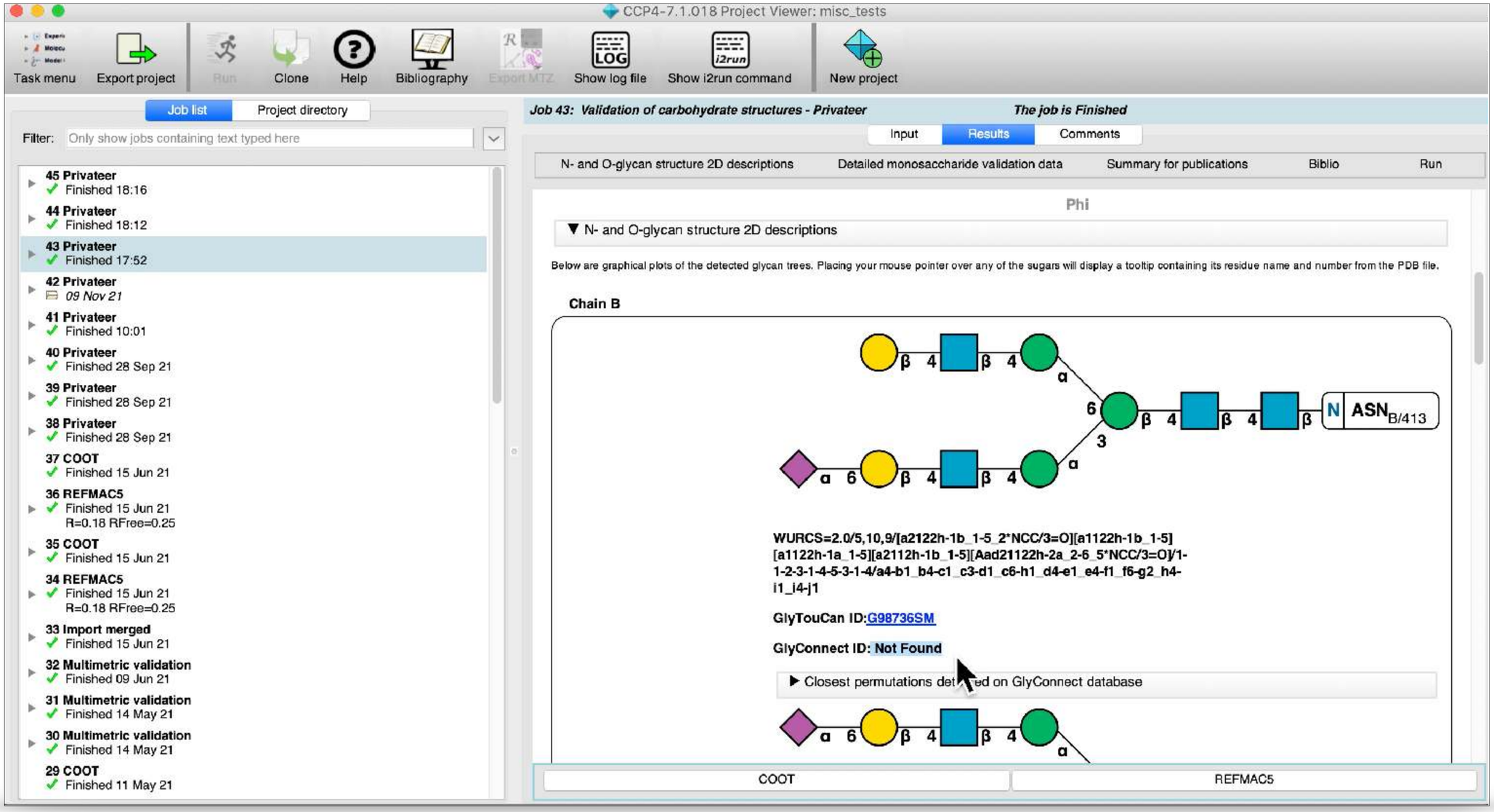
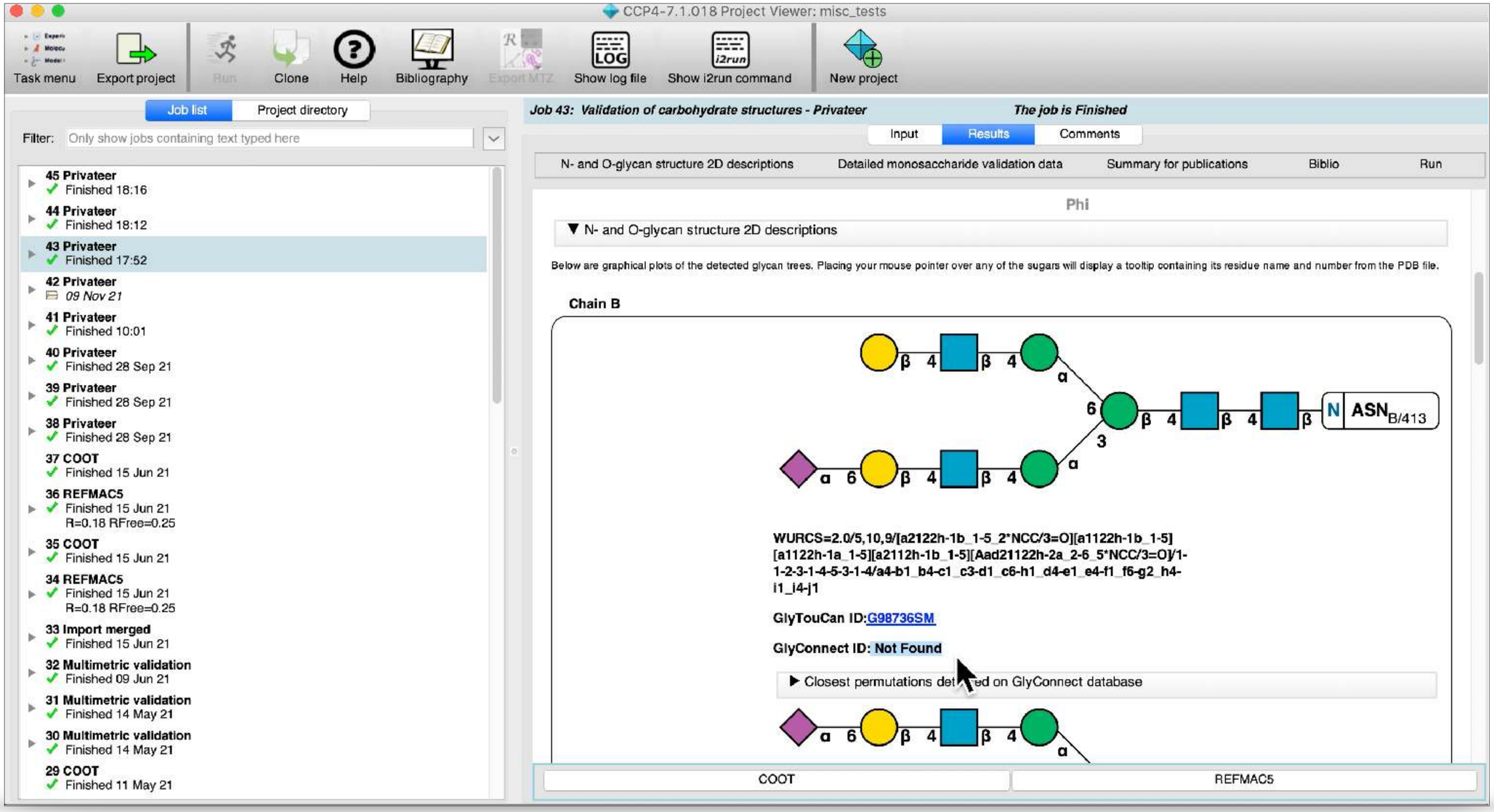


Running Privateer MKIV (CCP4i2)



Running Privateer MKIV (CCP4i2)



Running Privateer MKIV (CCP-EM)

Running Privateer MKIV (CCP-EM)

CCP4-7.0.071 Project Viewer: 3qvp

Task menu | Export project | Run | Run on server | Clone job | Help | Bibliography | Export MTZ | Show log file

Job list | Project directory

Filter: Only show jobs containing text typed here

Job/File	Eva
5 Privateer	
4 REFMAC5	R=
3 COOT	
2 Privateer	
1 Import merged	

Job 2: Validation of carbohydrate structures - Privateer **The job is Finished**

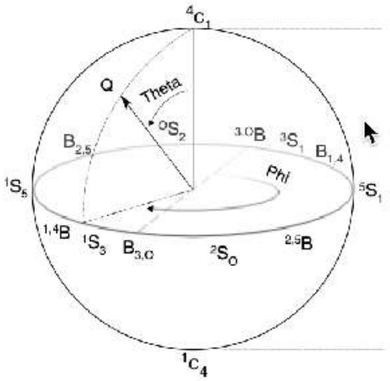
Input | Results | Comments

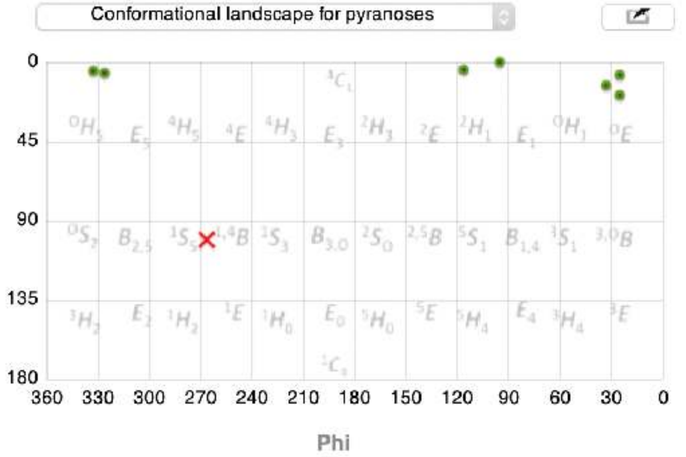
N- and O-glycan structure 2D descriptions | Detailed monosaccharide validation data | Summary for publications | Biblio | Run

20:21 27-Feb-2019

Results

The Cremer-Pople analysis (Cremer and Pople, 1975, JACS 97:1354-58) is used to determine sugar ring conformation. Below is a 2D plot of the conformational parameters (Q, Phi, Theta for pyranoses; Q and Theta for furanoses) along with a depiction of the conformational sphere for pyranoses:





▶ N- and O-glycan structure 2D descriptions

▼ Detailed monosaccharide validation data

Validation results for pyranose sugars:

Chain	Name	Q[1]	Phi	Theta	Anomer	D/L[2]	Conformation	RSCC[3]	<Bfactor>	Diagnostic
A	NAG	0.554	116.329	4.41419	beta	D	$^4C_1$	0.92	14.4	Ok
A	NAG	0.550	33.2673	13.2611	beta	D	$^4C_1$	0.91	15.3079	Ok

COOT | REFMAC5

CCP4-7.0.071 Project Viewer: 3qvp

Task menu | Export project | Run | Run on server | Clone job | Help | Bibliography | Export MTZ | Show log file

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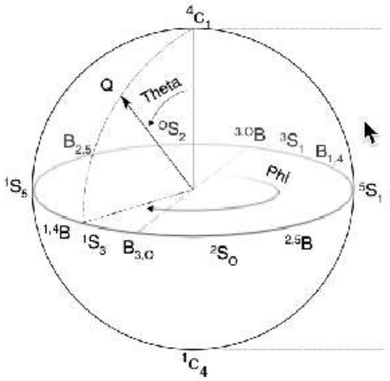

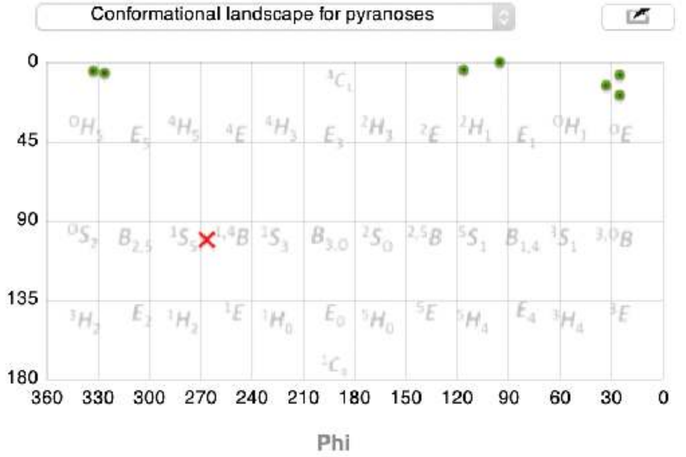

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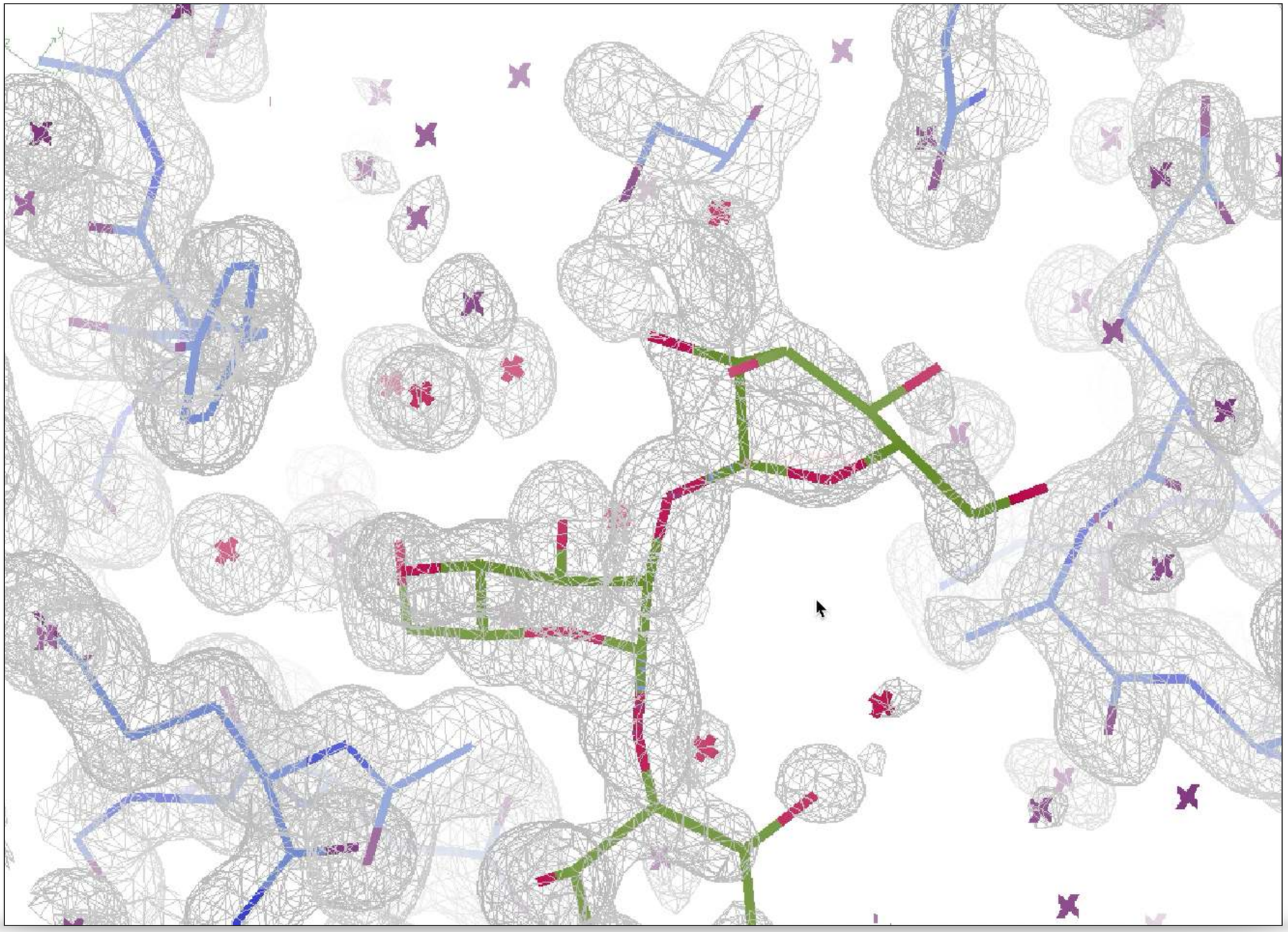

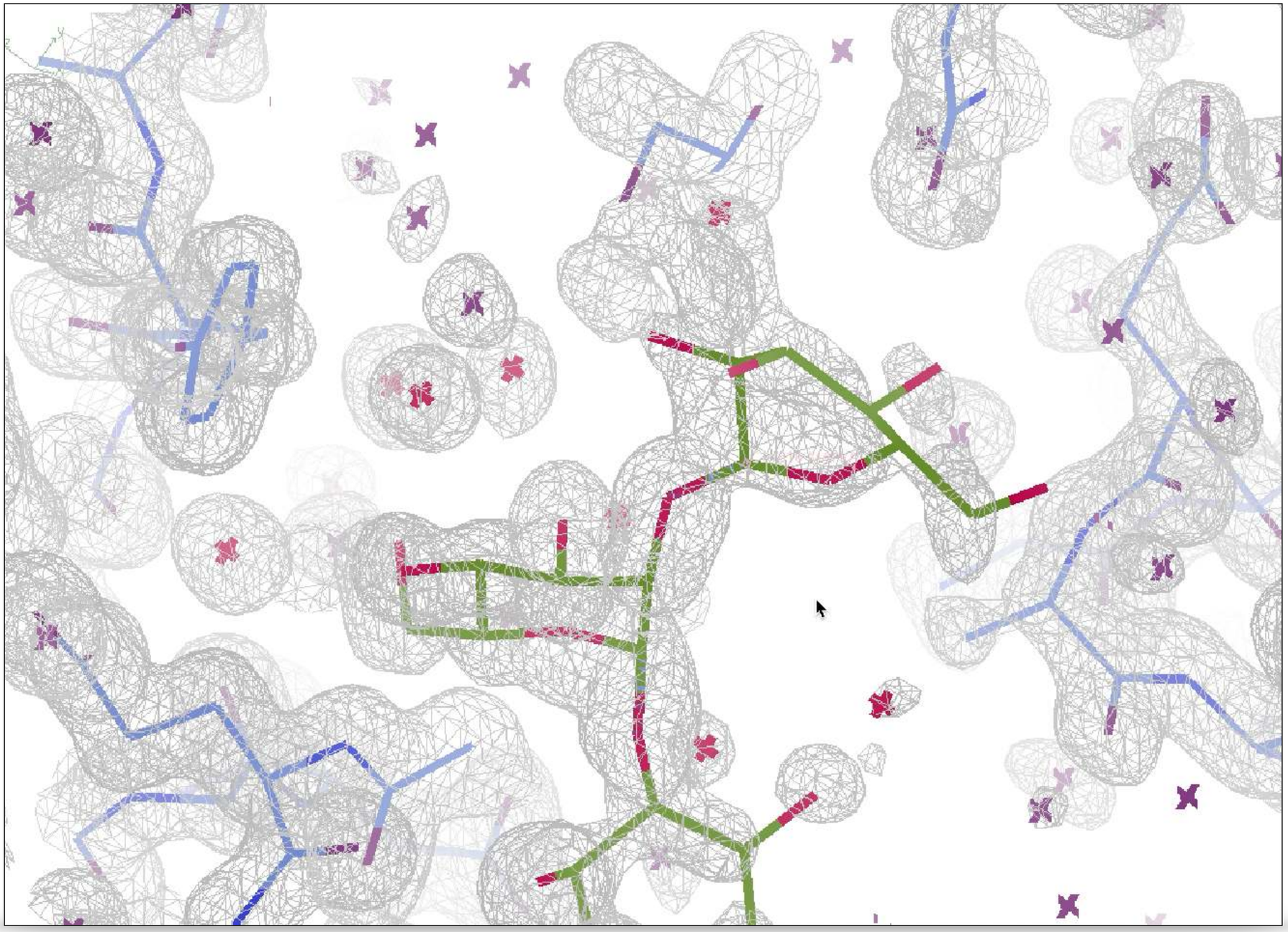

CCP4-7.0.071 Project Viewer: 3qvp

Task menu | Export project | Run | Run on server | Clone job | Help | Bibliography | Export MTZ | Show log file

Job list | Project directory

Filter: Only show jobs containing text typed here

Job/File	Eva
5 Privateer	
4 REFMAC5	R=
3 COOT	
2 Privateer	
1 Import merged	

Job 5: Validation of carbohydrate structures - Privateer **The job is Finished**

Input | Results | Comments

N- and O-glycan structure 2D descriptions | Detailed monosaccharide validation data | Summary for publications | Biblio | Run

10:37 28-Feb-2019

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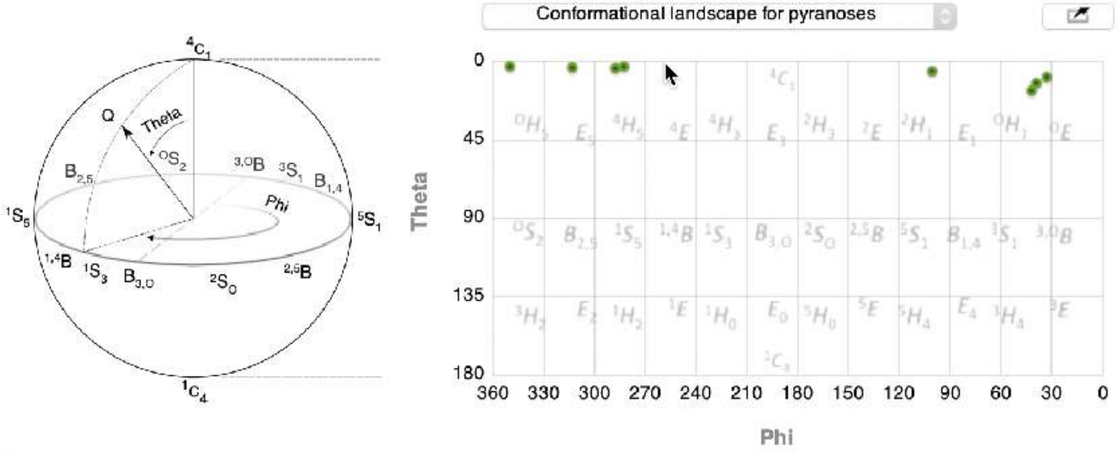

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COOT | REFMAC5

CCP4-7.0.071 Project Viewer: 3qvp

Task menu | Export project | Run | Run on server | Clone job | Help | Bibliography | Export MTZ | Show log file

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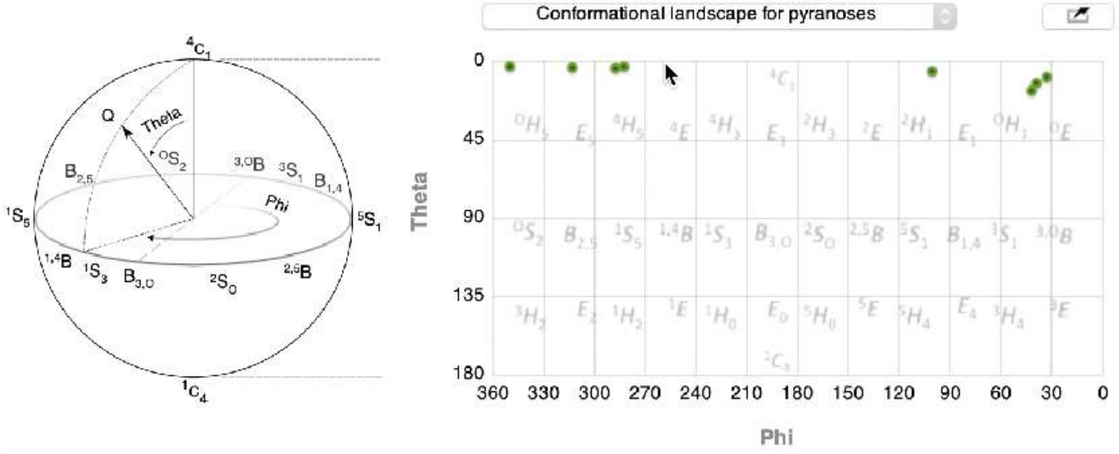

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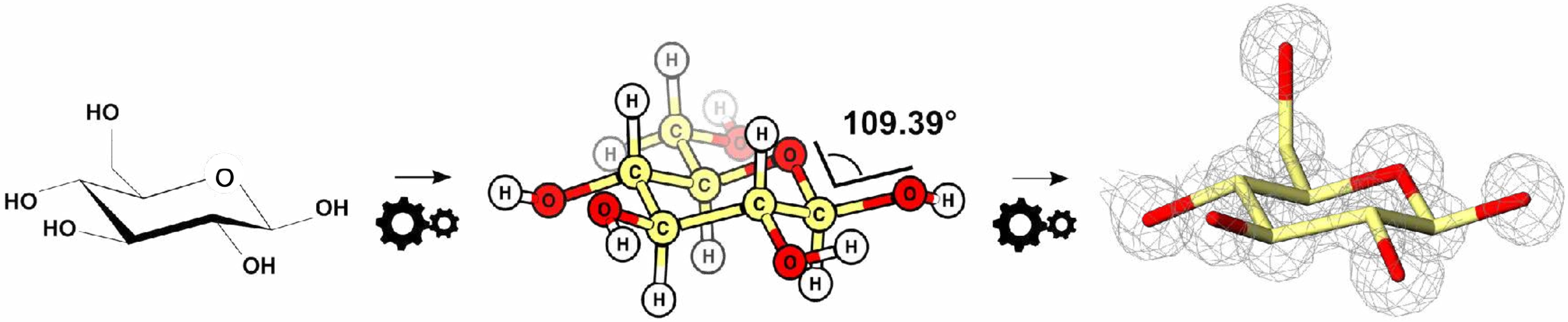
COOT | REFMAC5

New carbohydrate dictionaries

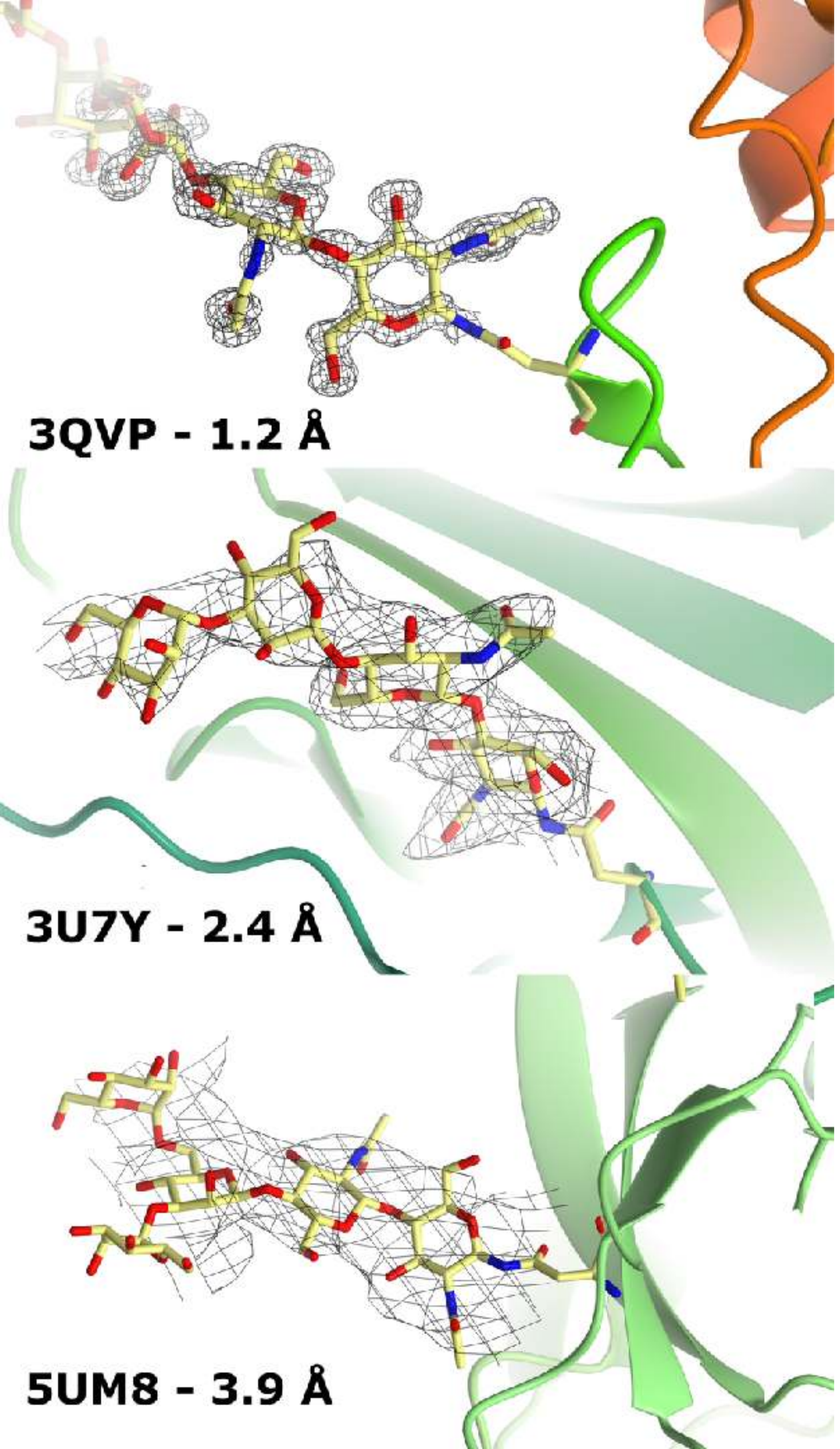


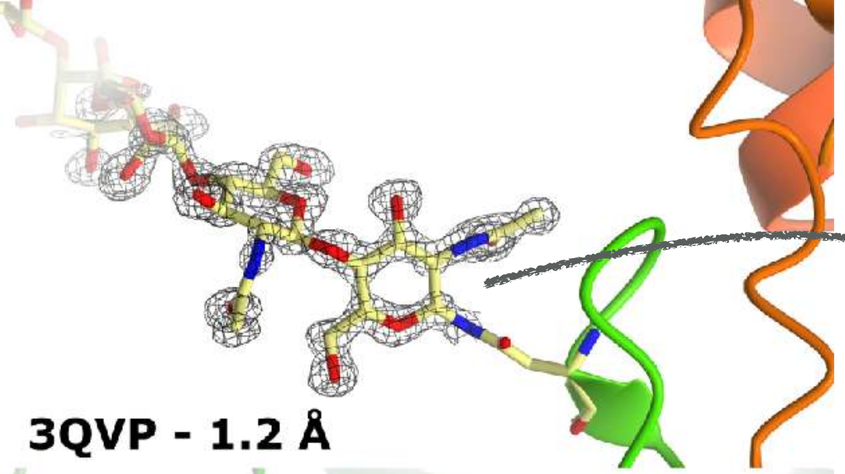
chemical description

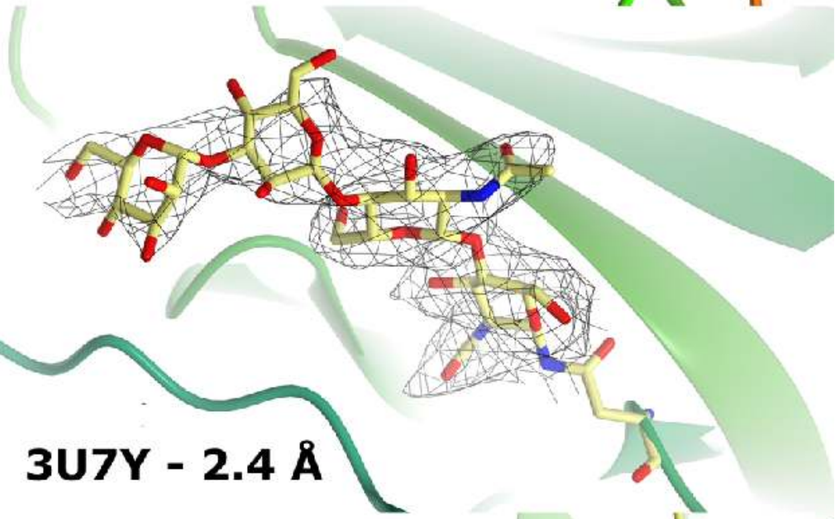
restraints & coordinates

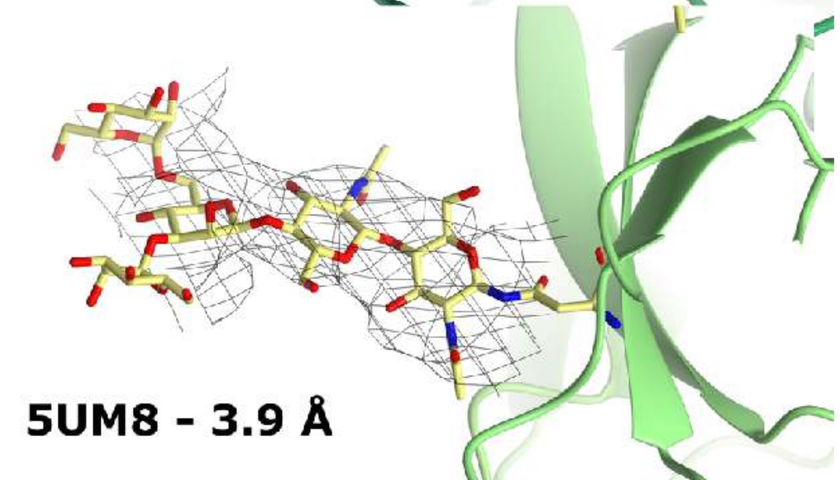
fitted model

New carbohydrate dictionaries









New carbohydrate dictionaries

All this information to restrain one GlcNAc

Compound definition, with **3-letter code**, **full name**, **type** and number of atoms, **all** and **non-hydrogen**

```
NAG        NAG  'N-ACETYL-D-GLUCOSAMINE                  '  pyranose              30   15  .
```

Atom by atom definition of the conformer, with **name**, symbol, charge and **cartesian coordinates**

```
NAG        O1       O      OH1         0.000       1.203      0.420      0.648
```

A bond between a **pair of atoms**, **cardinality**, **expected distance** and **standard deviation**

```
NAG        O1       C1           single        1.432      0.020
```

An angle between **three atoms**, with **expected value** and **standard deviation**

```
NAG        O1       C1       C2         109.470      3.000
```

A **torsion angle** between **four atoms**, with **expected value**, **standard deviation** and **periodicity**

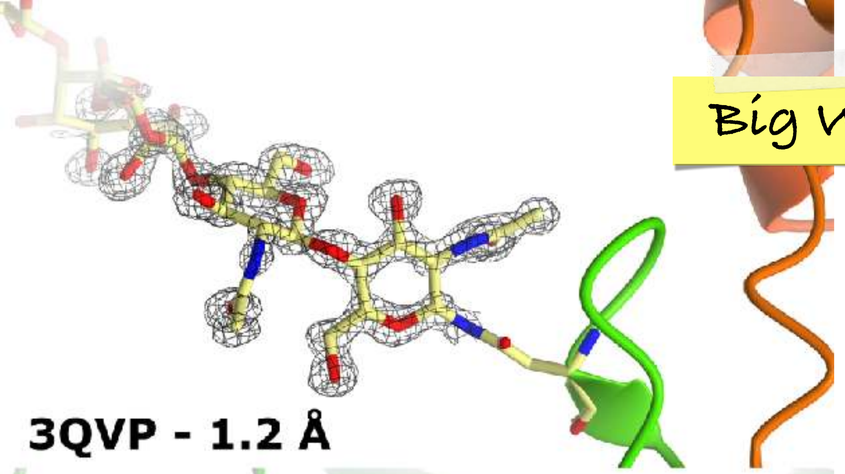
```
NAG        var_7      C5       C4       C3       C2           60.000     20.000     3
```

A **chiral volume**, defined by **four atoms** in a tetrahedral arrangement, and a **sign defining orientation**

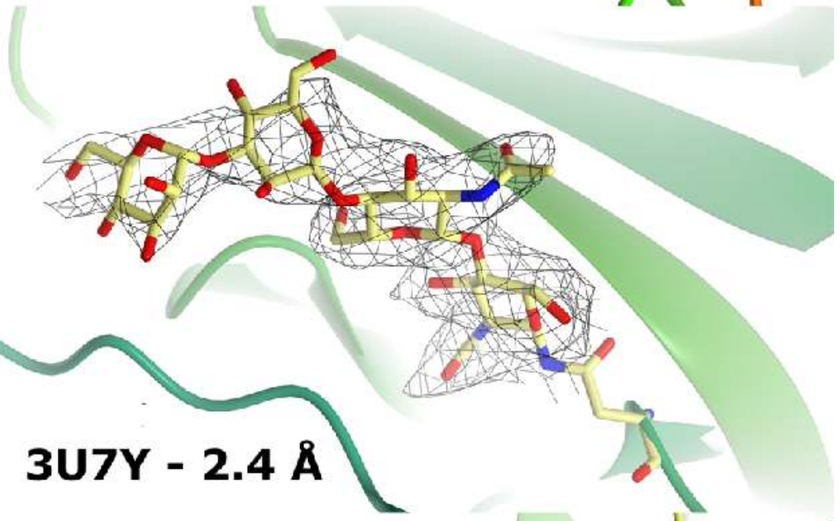
```
NAG        chir_01  C1       C2       O1       O5           positiv
```

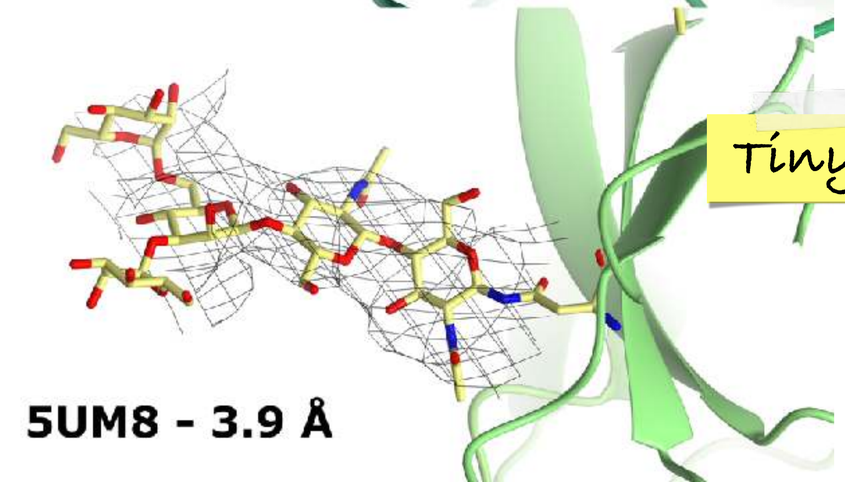
A **series of atoms** taking part of a **planar arrangement**, plus **standard deviation**

```
NAG        plan-2     N2           0.020
NAG        plan-2     C2           0.020
NAG        plan-2     C7           0.020
NAG        plan-2     HN2          0.020
```



Big W



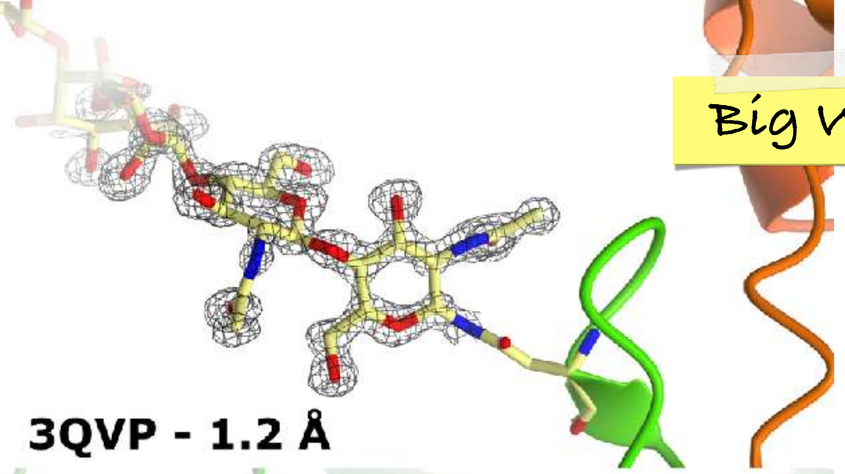


Tiny W

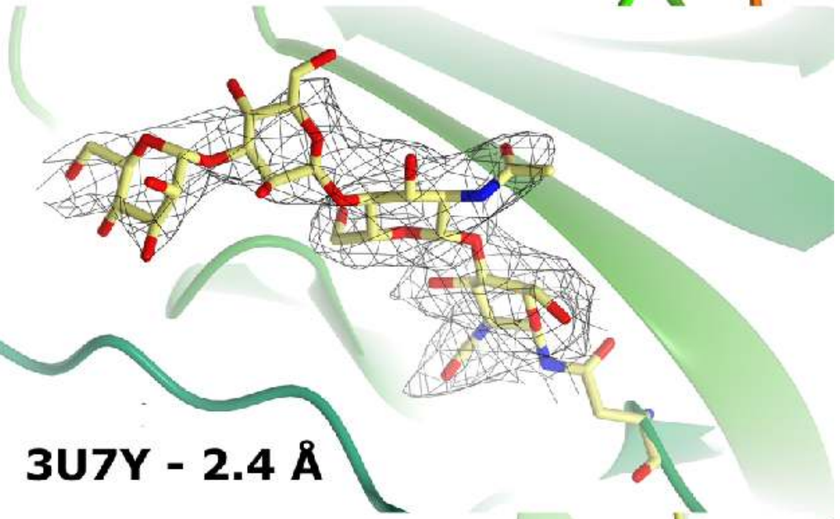
What about ring conformation?

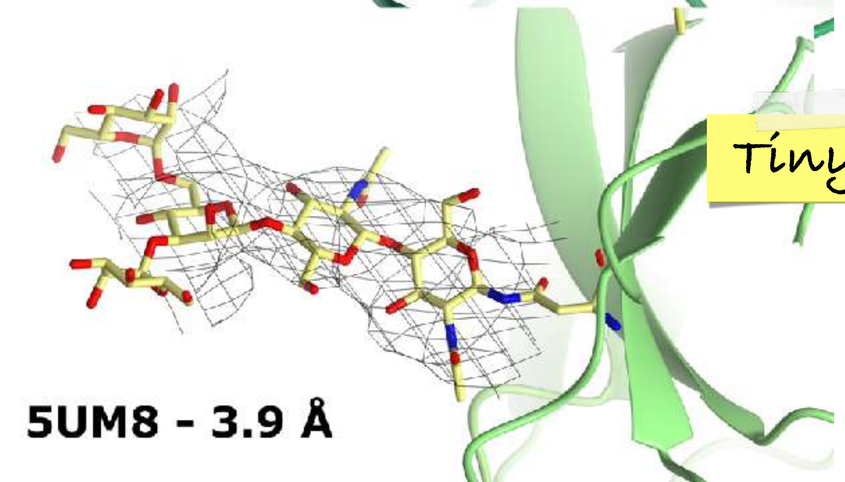
With weak/incomplete density, a ring's lowest-energy conformation may have to be explicitly restrained:

$$L(p) = wLX(p) + LG(p)$$



Big W





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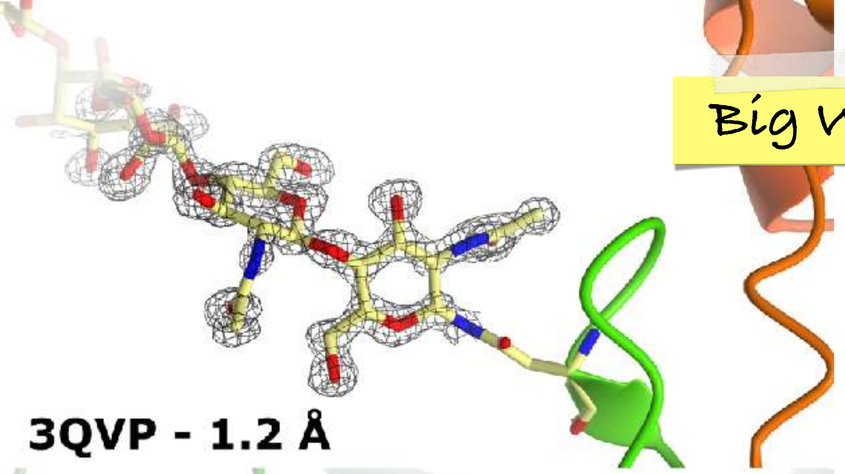
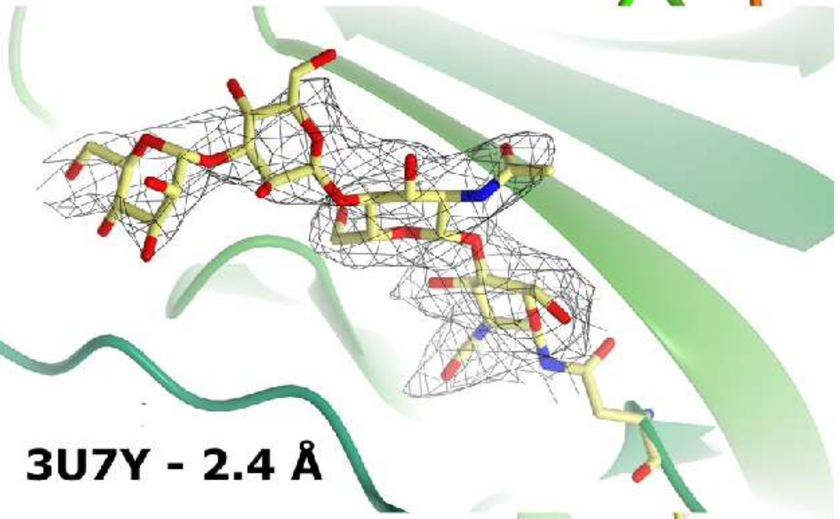
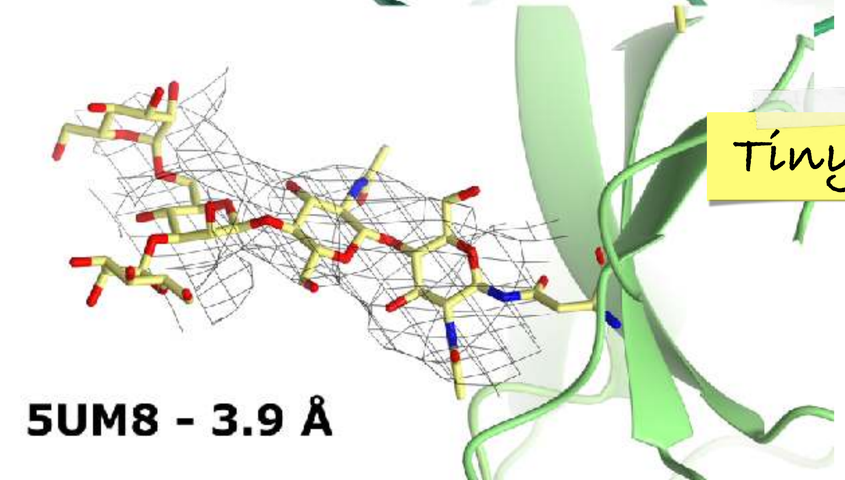
Yes!

bond lengths



Big W





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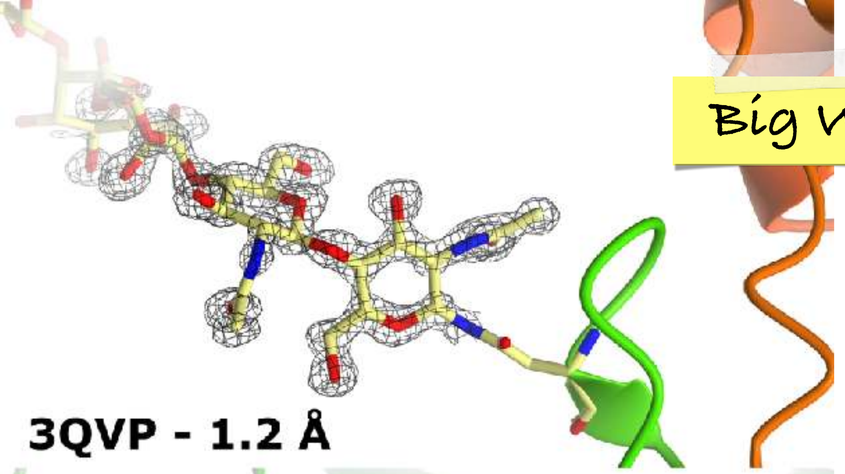
Yes!
bond lengths

Yes!
bond angles



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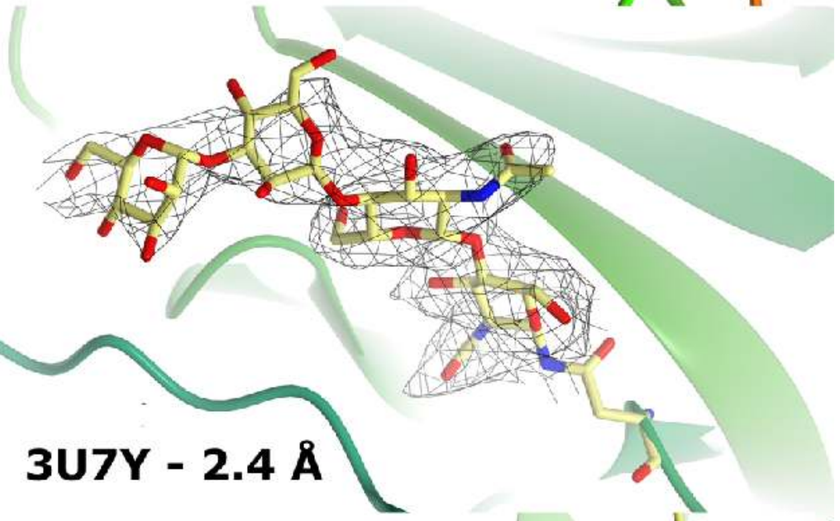

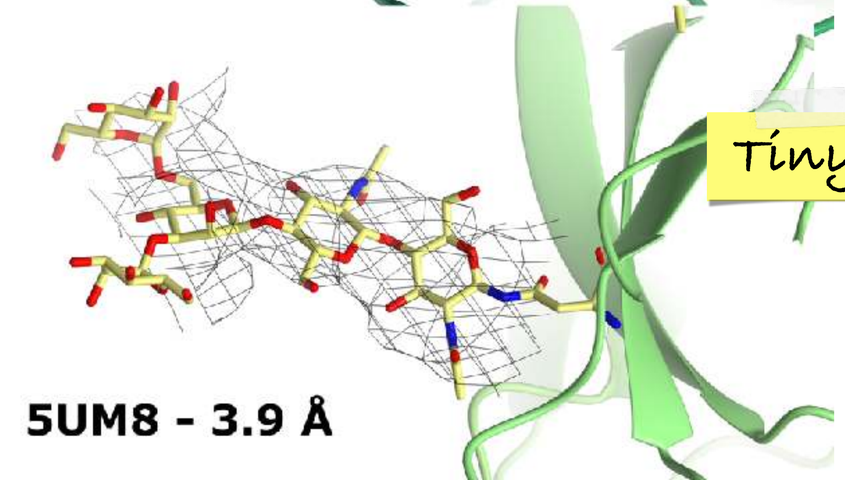

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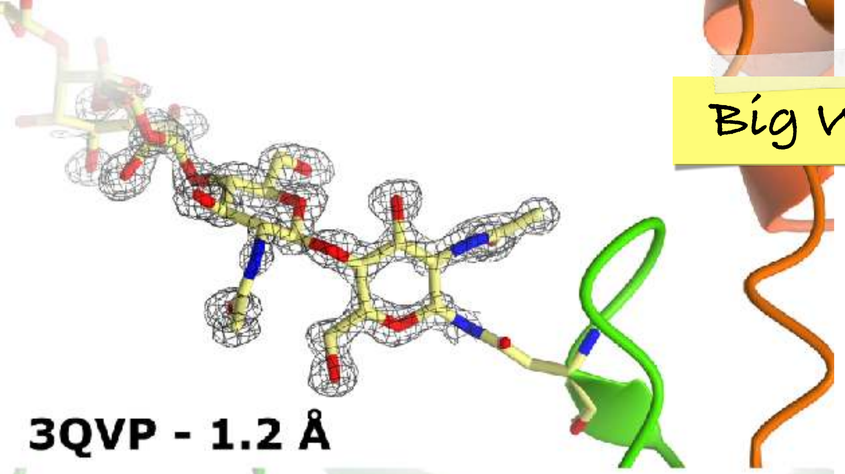
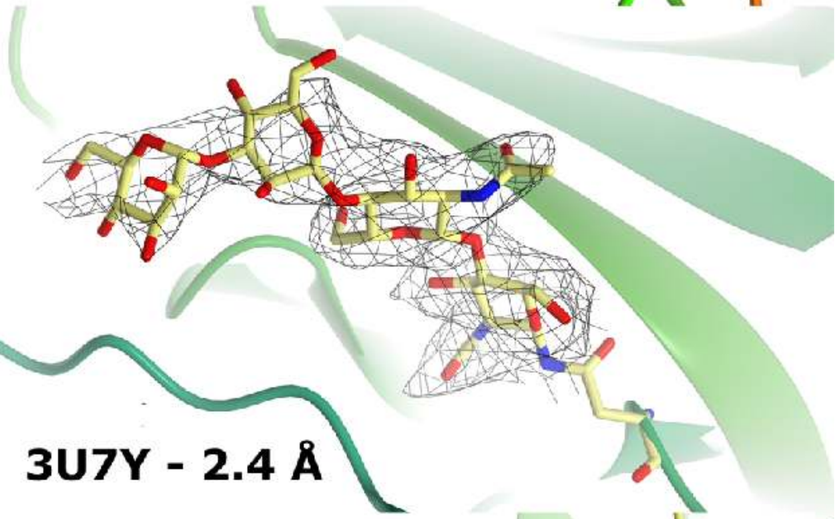
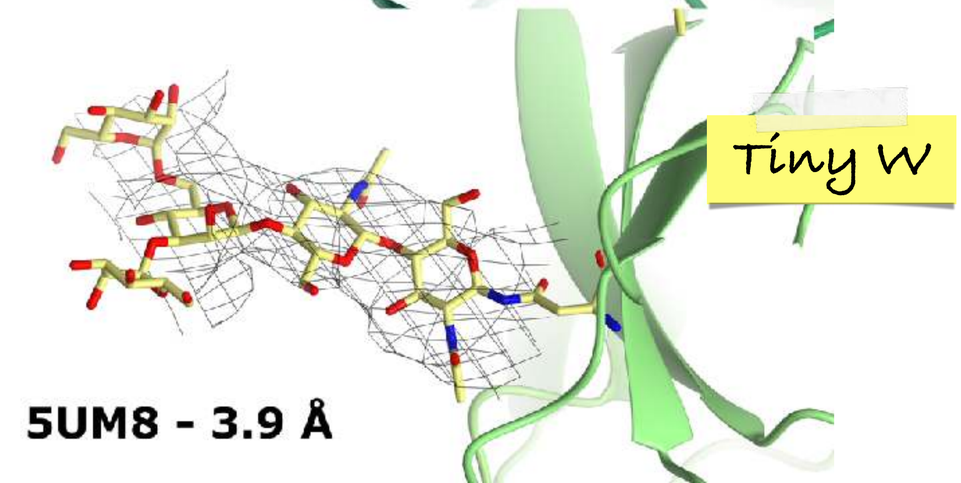
Yes! bond lengths

Yes! bond angles

Yes! Planar groups



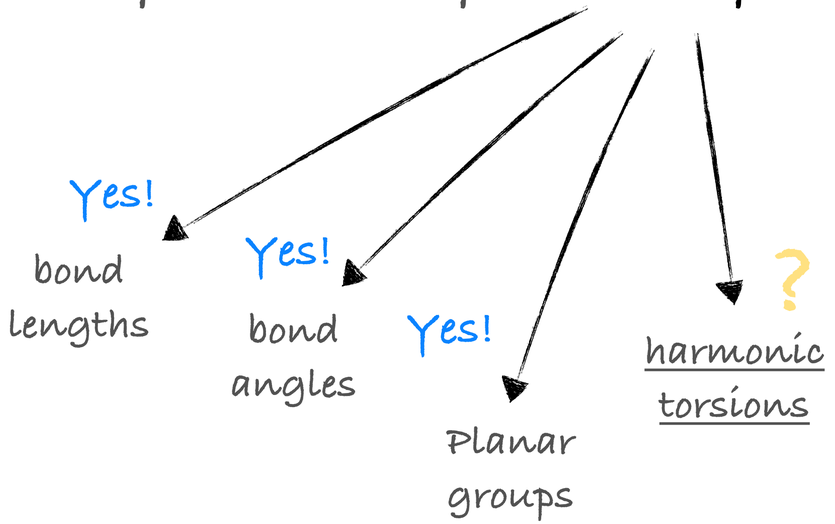


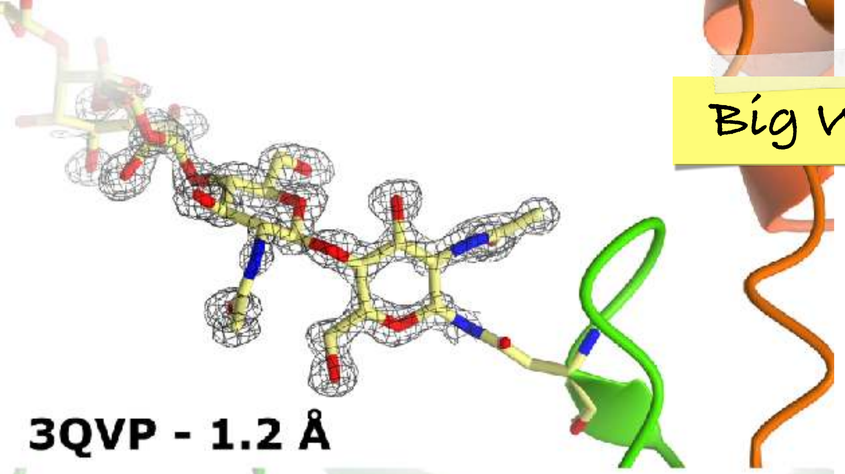


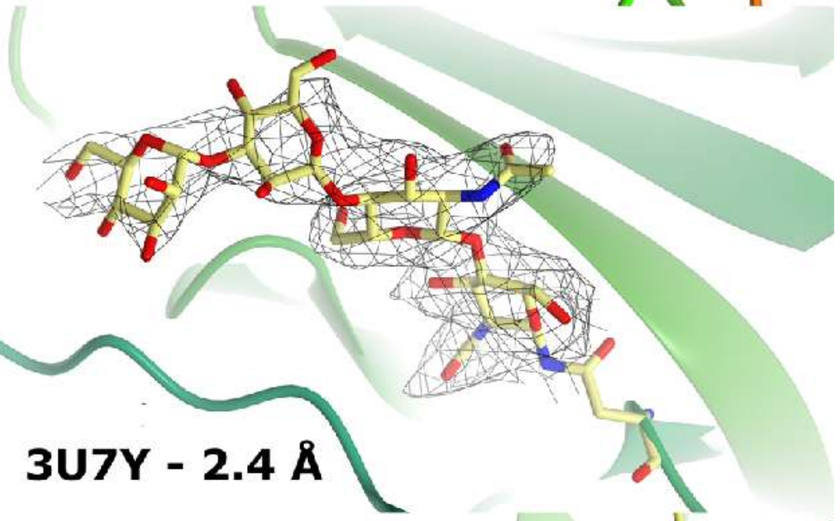
What about ring conformation?

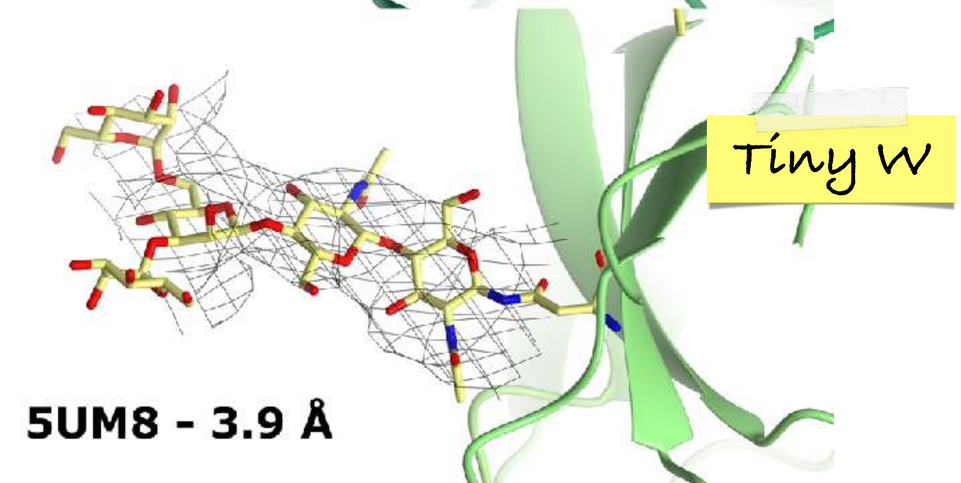
With weak/incomplete density, a ring's lowest-energy conformation may have to be explicitly restrained:

$$L(p) = wLX(p) + LG(p)$$









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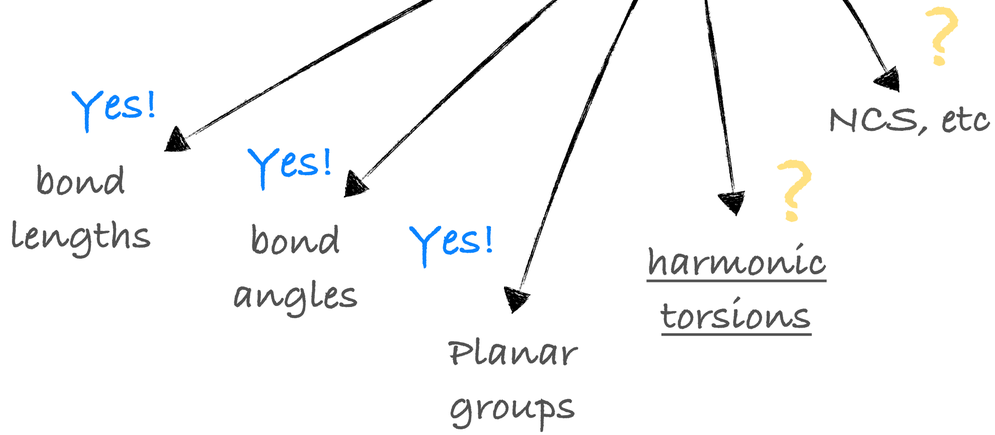

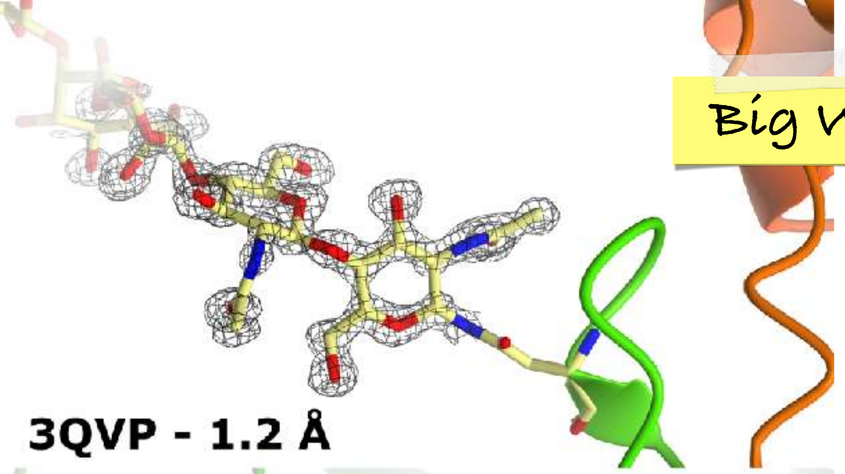

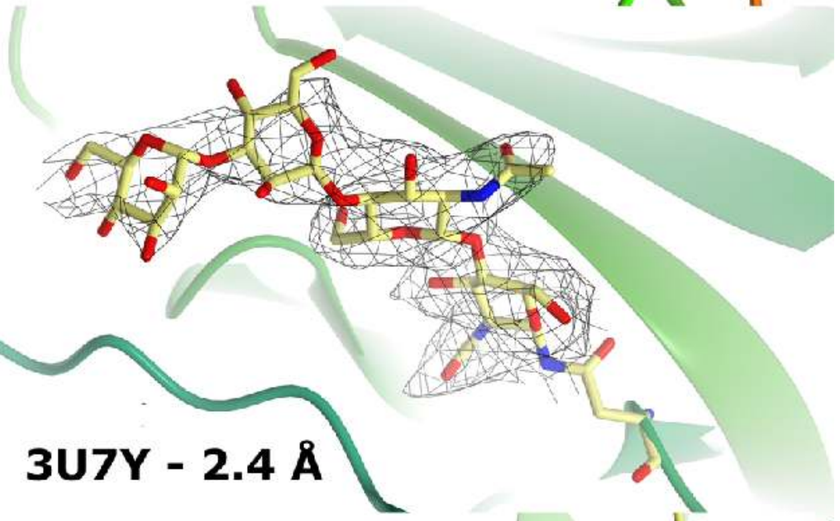

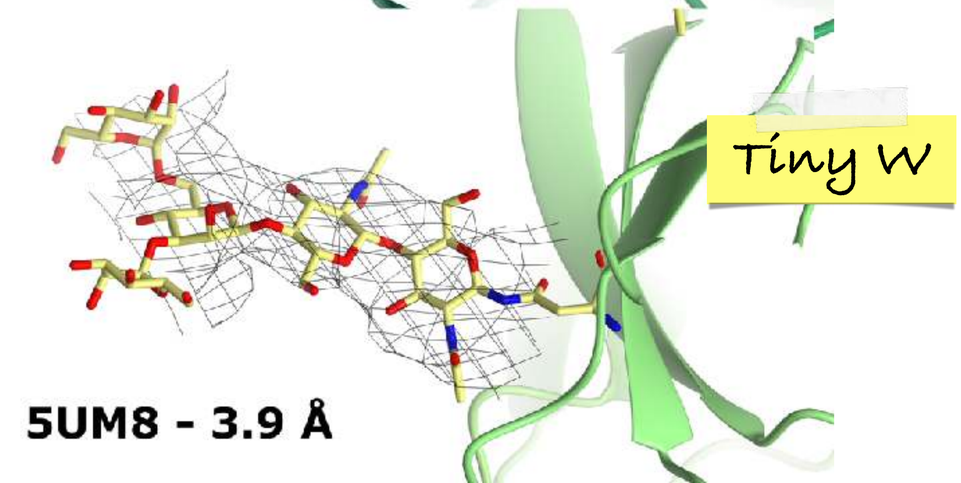

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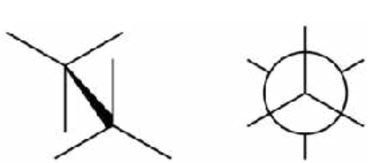
With weak/incomplete density, a ring's lowest-energy conformation may have to be explicitly restrained:

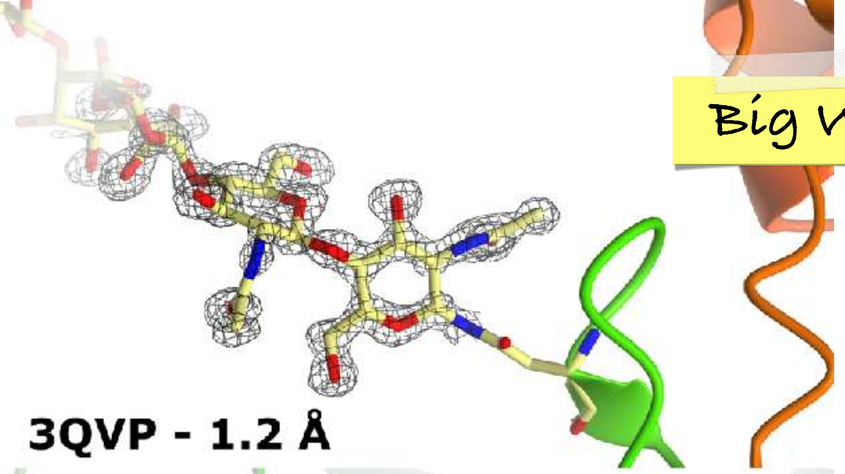
$$L(p) = wL_X(p) + L_G(p)$$

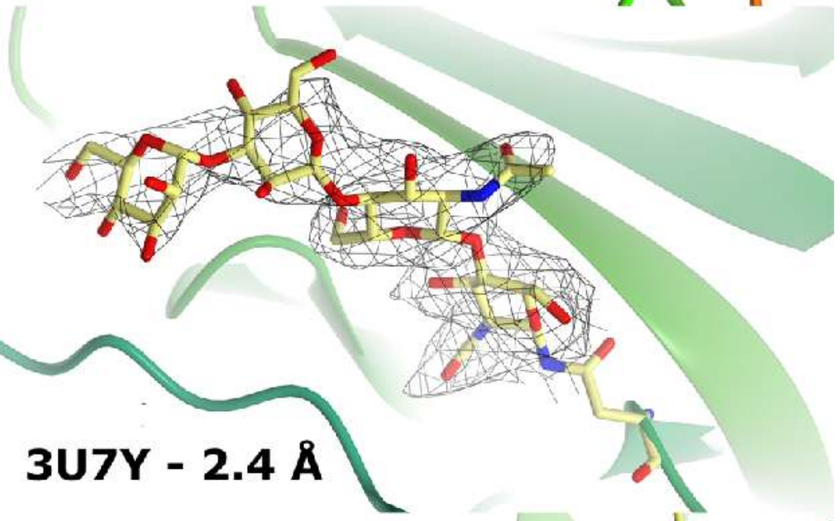
- Yes! bond lengths
- Yes! bond angles
- Yes! Planar groups
- ? harmonic torsions
- ? NCS, etc

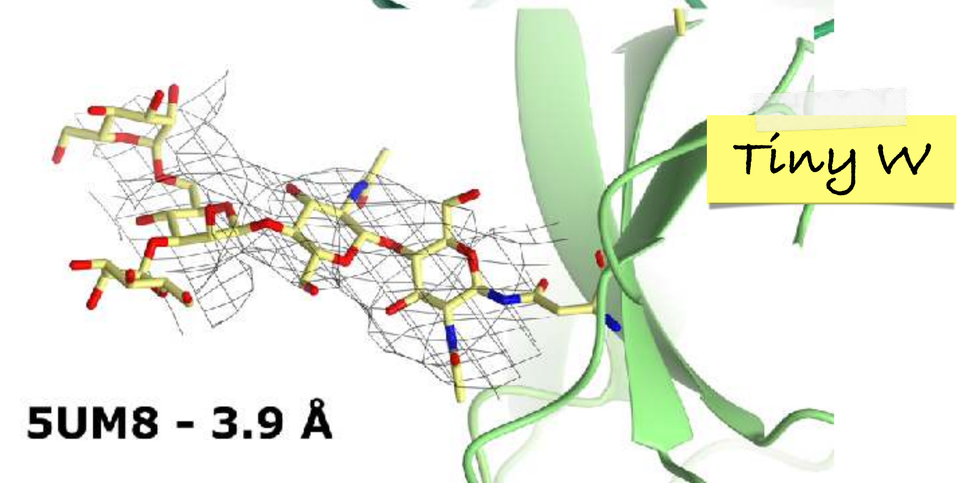


Staggered

Eclipsed







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$$L(p) = wL_X(p) + L_G(p)$$

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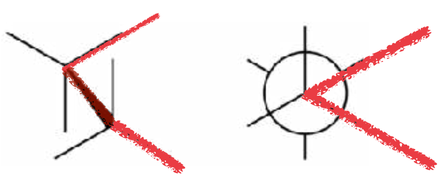
- Yes! Planar groups
- ? harmonic torsions
- ? NCS, etc

~60°/-60°



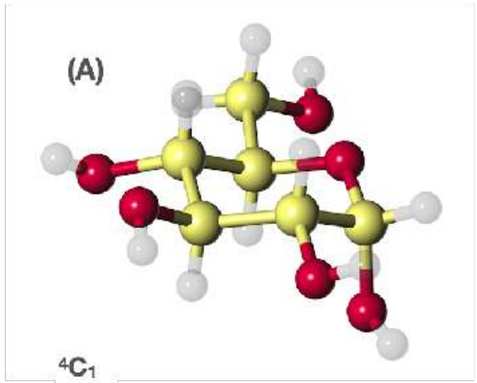
Staggered

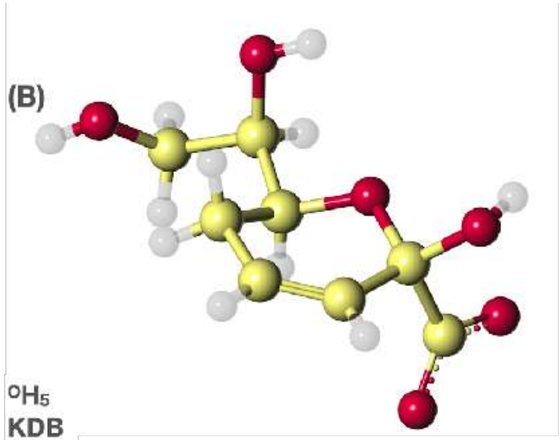
Eclipsed

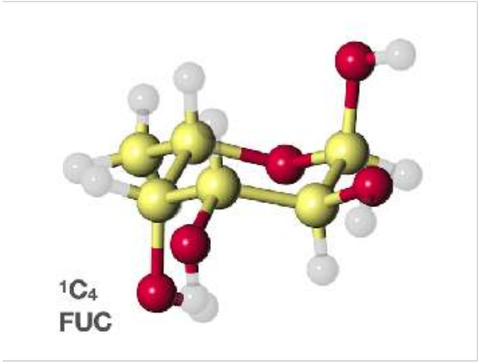
Our new restraint dictionaries *understand* ring conformation

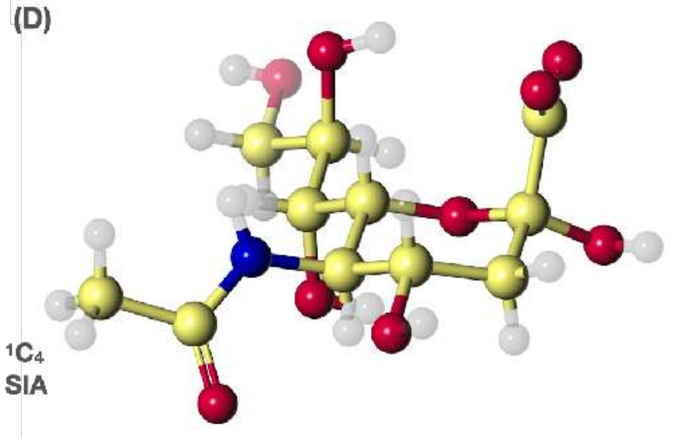
Patched _chem_comp_tor section of a restraint dictionary separating **ring torsion angles** from the **rest**, as specified by **four atoms**, **target value**, **uncertainty** and **periodicity**

NAG	ring_1	C5	O5	C1	C2	-59.675385	3.0	1
NAG	ring_2	O5	C1	C2	C3	53.650513	3.0	1
NAG	ring_3	C1	C2	C3	C4	-52.014420	3.0	1
NAG	ring_4	C2	C3	C4	C5	54.096725	3.0	1
NAG	ring_5	C3	C4	C5	O5	-56.921230	3.0	1
NAG	ring_6	C4	C5	O5	C1	61.200516	3.0	1
NAG	tors_1	C8	C7	N2	C2	-175.114227	10.0	2
NAG	tors_2	N2	C7	C8	H81	-13.703261	10.0	6
NAG	tors_3	C5	C6	O6	HO6	-177.520996	10.0	3
NAG	tors_4	C4	C5	C6	O6	61.135471	10.0	3
NAG	tors_5	C6	C5	O5	C1	-175.561295	10.0	3
NAG	tors_6	O4	C4	C5	C6	63.707928	10.0	3
NAG	tors_7	C3	C4	O4	HO4	-61.268230	10.0	3
NAG	tors_8	O3	C3	C4	O4	-63.830528	10.0	3
NAG	tors_9	C2	C3	O3	HO3	-169.485916	10.0	3
NAG	tors_10	C7	N2	C2	C1	124.894669	10.0	6
NAG	tors_11	N2	C2	C3	O3	61.918137	10.0	3
NAG	tors_12	C2	C1	O1	HO1	163.115189	10.0	3
NAG	tors_13	O1	C1	O5	C5	179.557251	10.0	3
NAG	tors_14	O1	C1	C2	N2	-62.214077	10.0	3





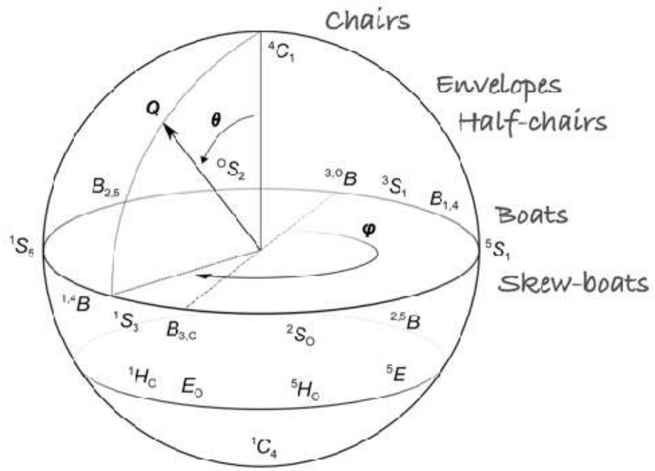




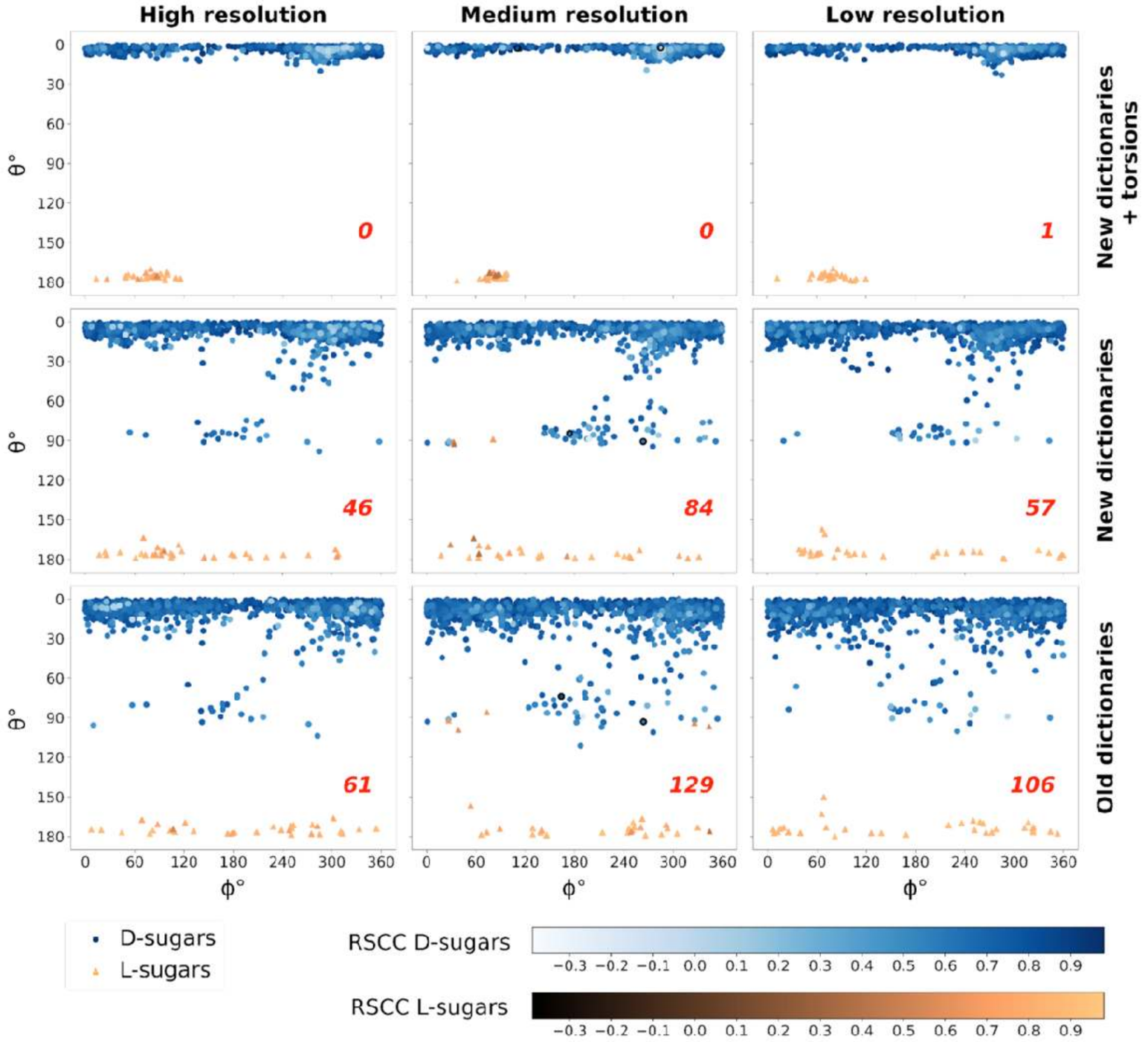
Atanasova, Joosten, Nicholls & Agirre, 2022, Acta Crystallographica D(78):455-465

Conformational restraints
N-glycosylation

No conformational distortions expected!



Atanasova, Joosten, Nicholls & Agirre, 2022, Acta Crystallographica D(78):455-465



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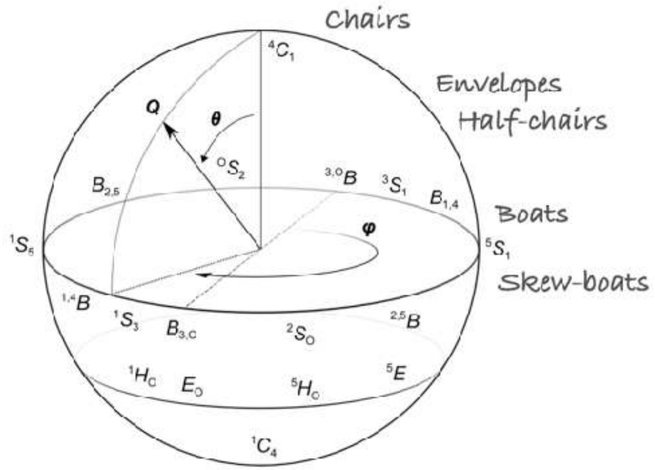

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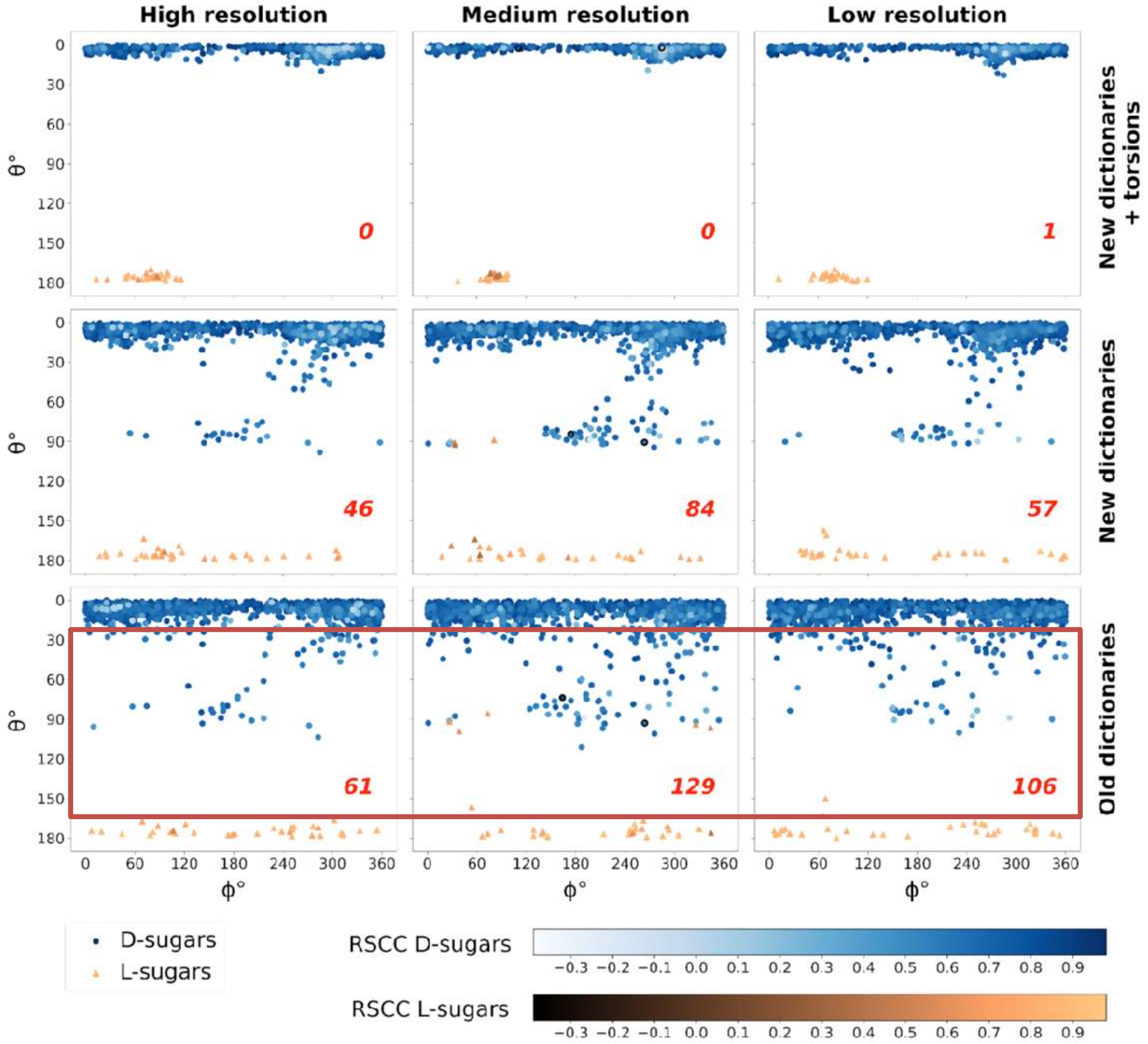

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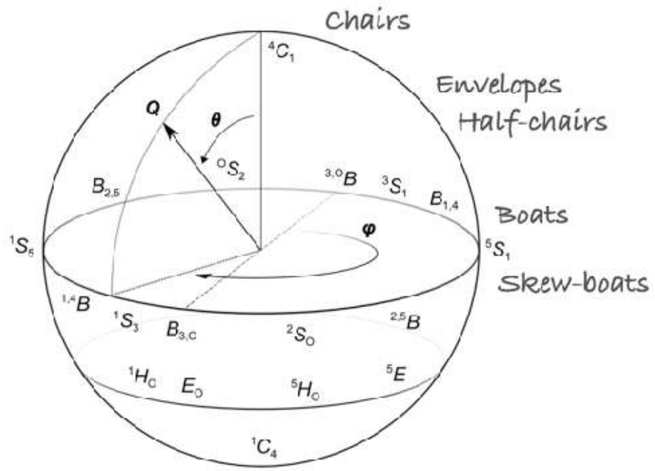

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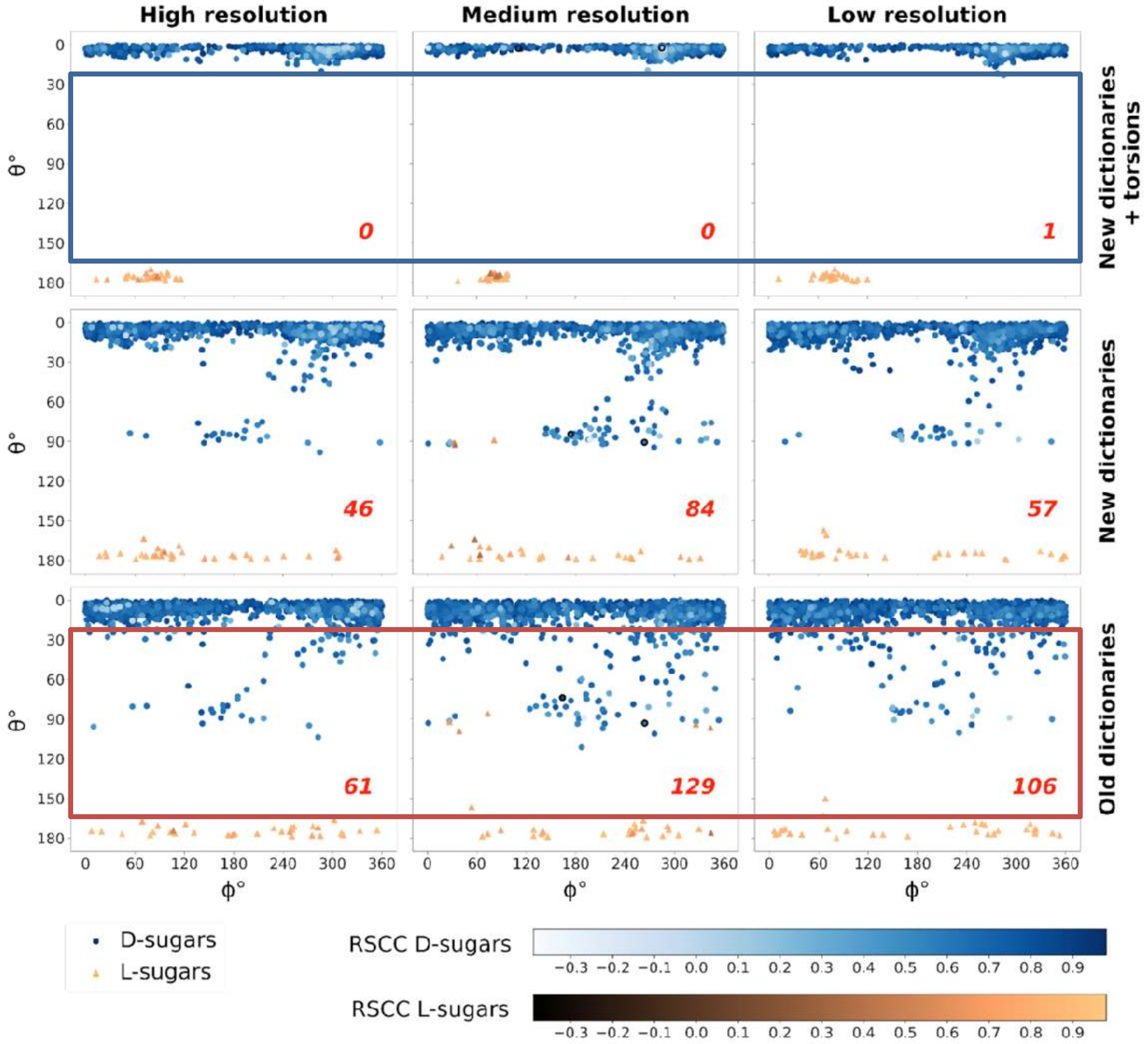
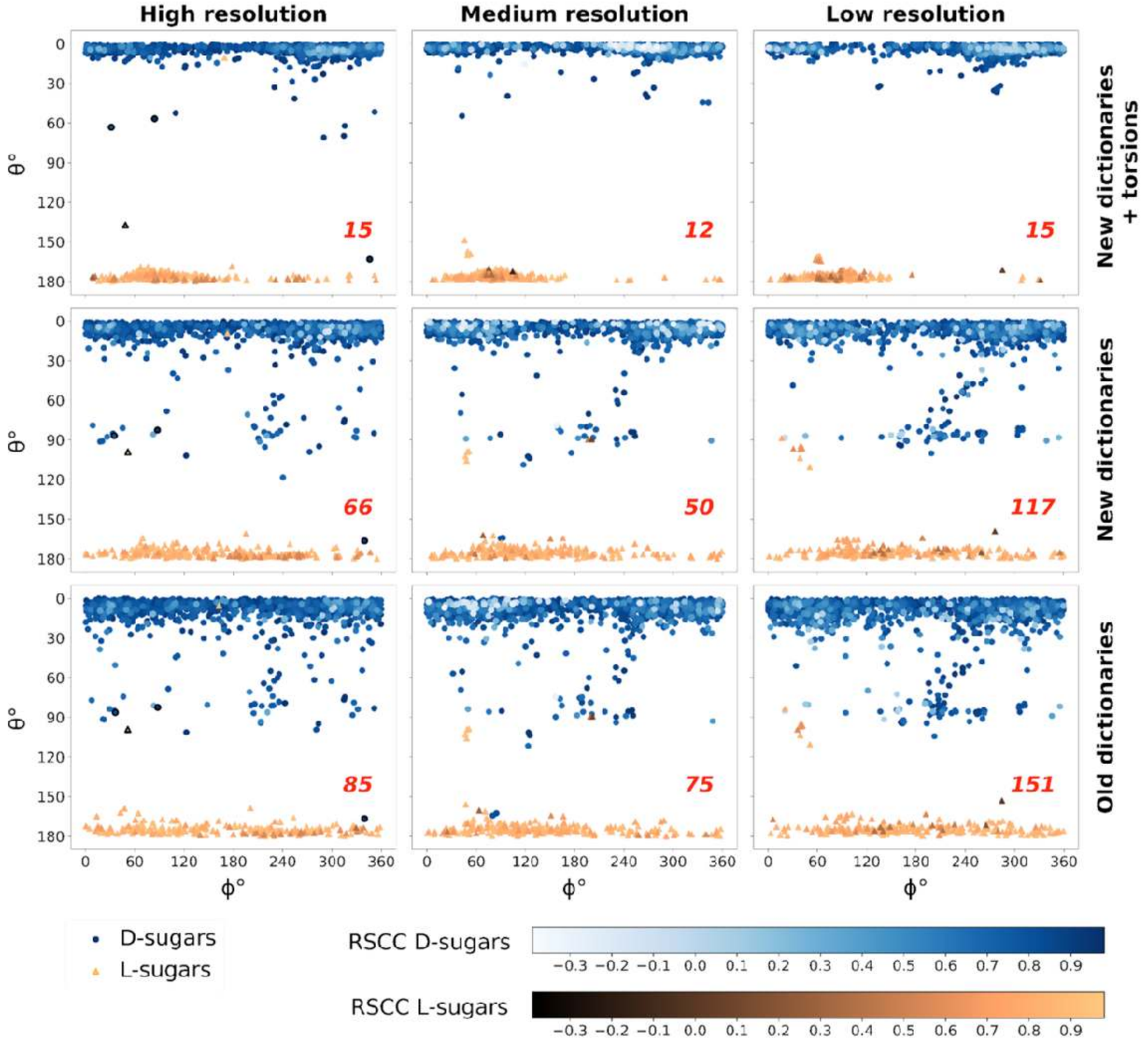
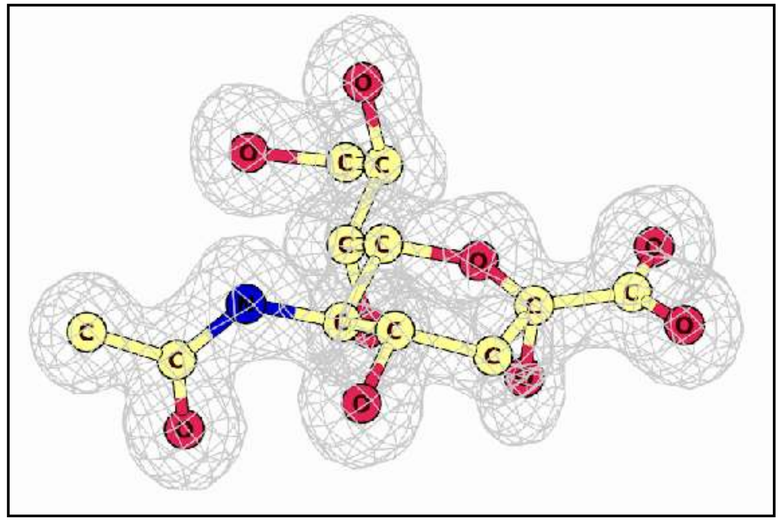


Conformational restraints
Ligands



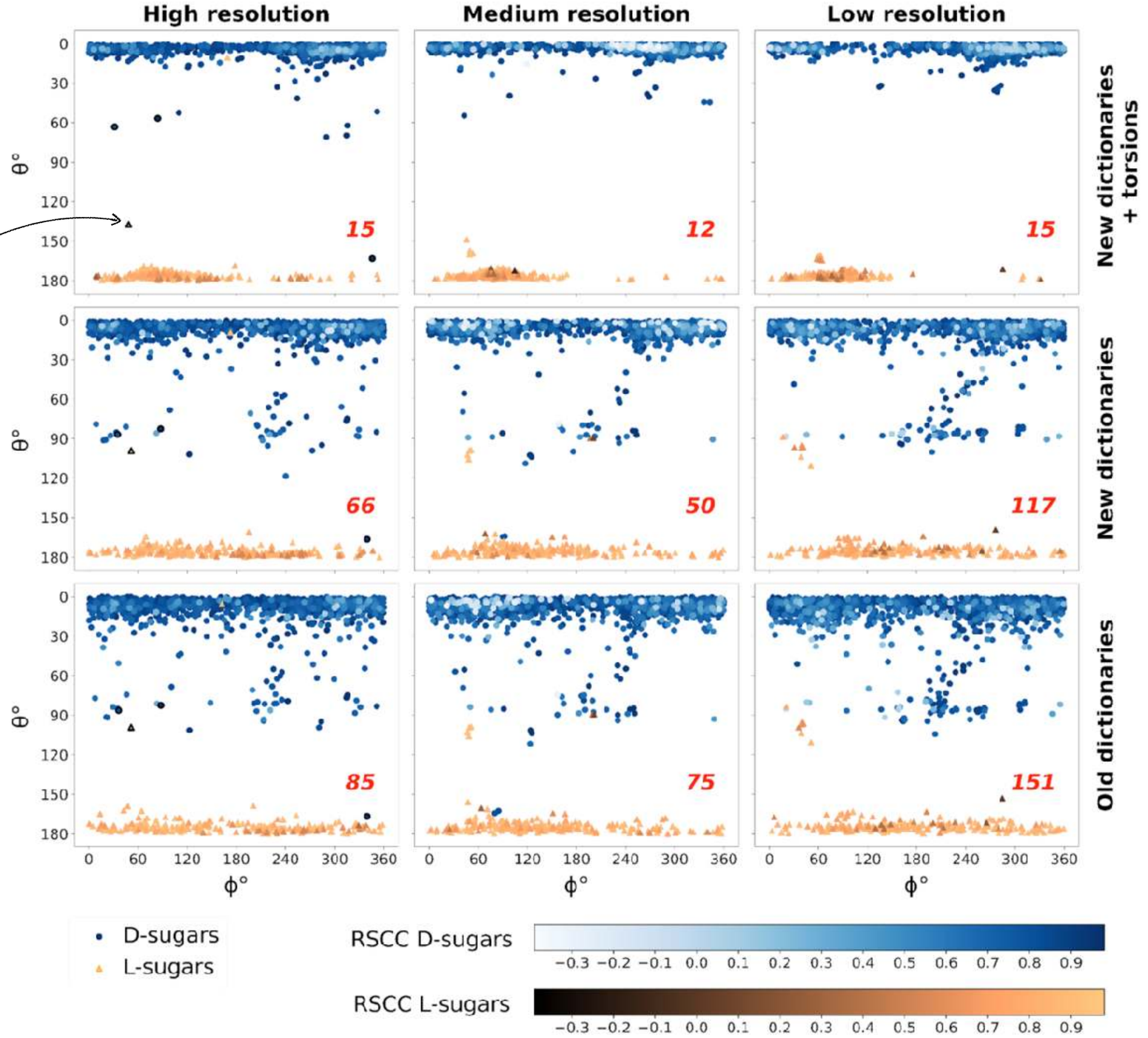
Atanasova, Joosten, Nicholls & Agirre, 2022, Acta Crystallographica D(78):455-465

Conformational restraints
Ligands

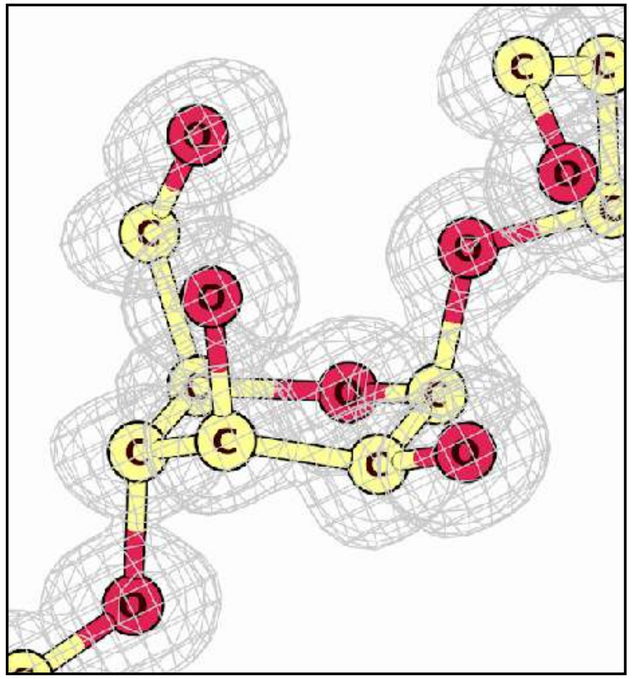


6HG0 (SIA-A-522) in ^{4}E
1.30 Å resolution, RSCC=0.94

Atanasova, Joosten, Nicholls & Agirre, 2022,
Acta Crystallographica D(78):455-465

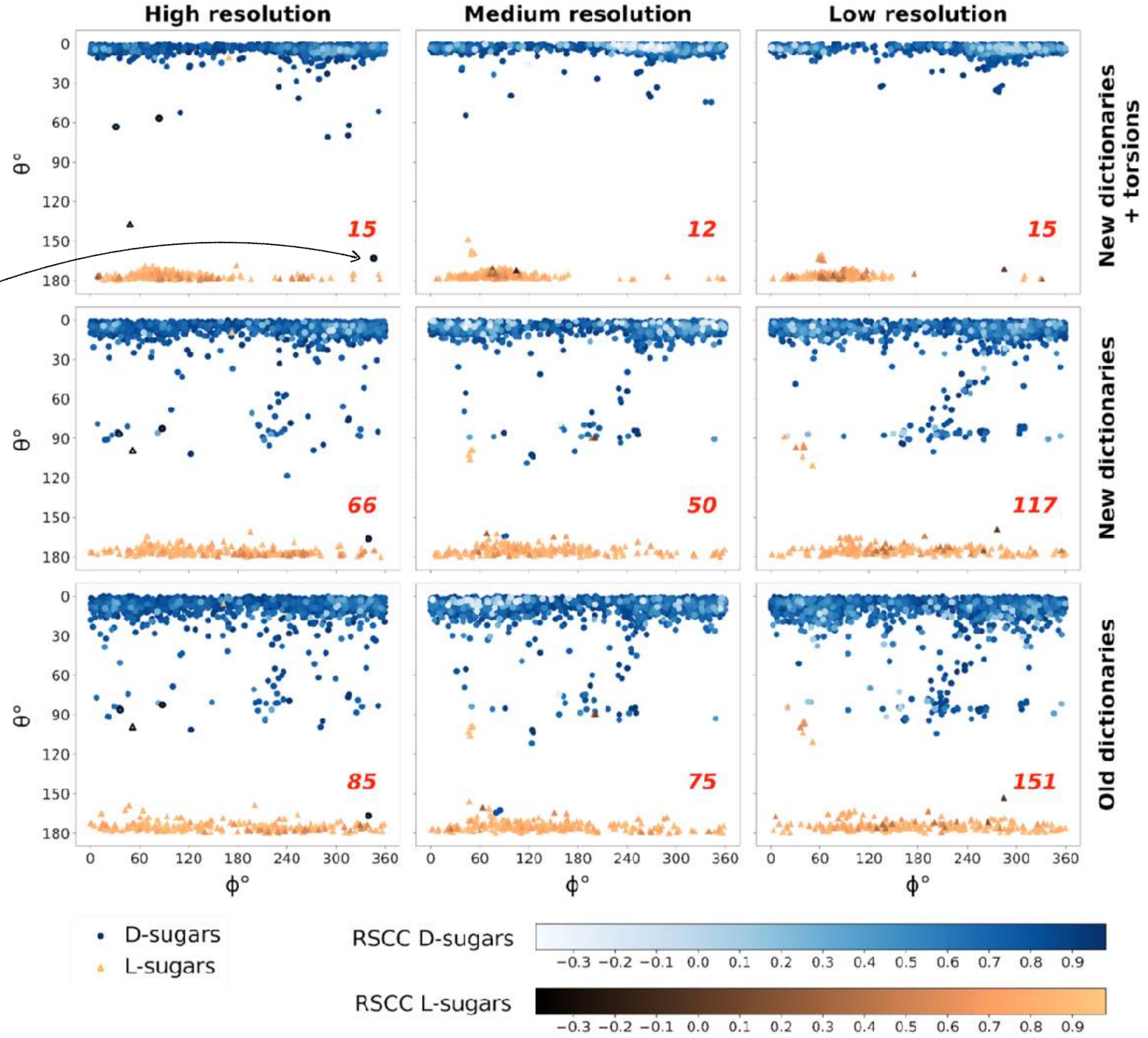


Conformational restraints
Ligands

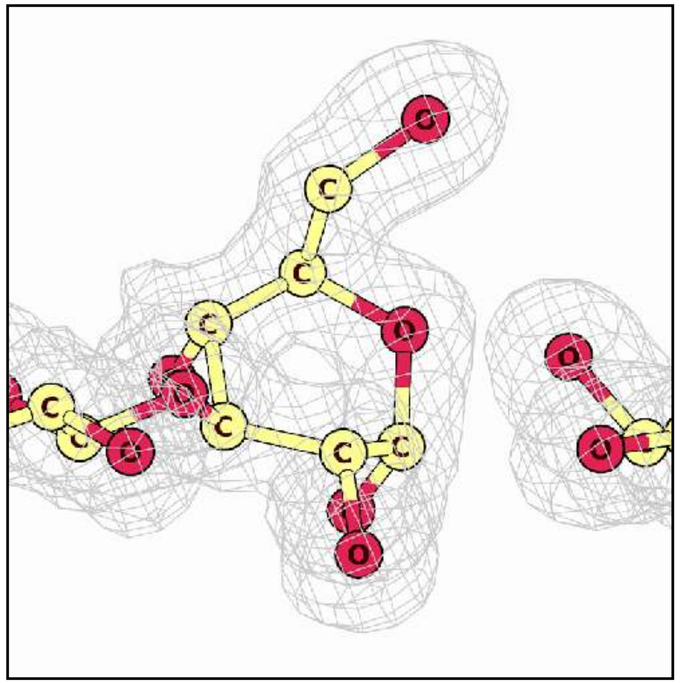


5JUG (BMA-B-3) in $^1C_4$
0.95 Å resolution, RSCC=0.95

Atanasova, Joosten, Nicholls & Agirre, 2022,
Acta Crystallographica D(78):455-465

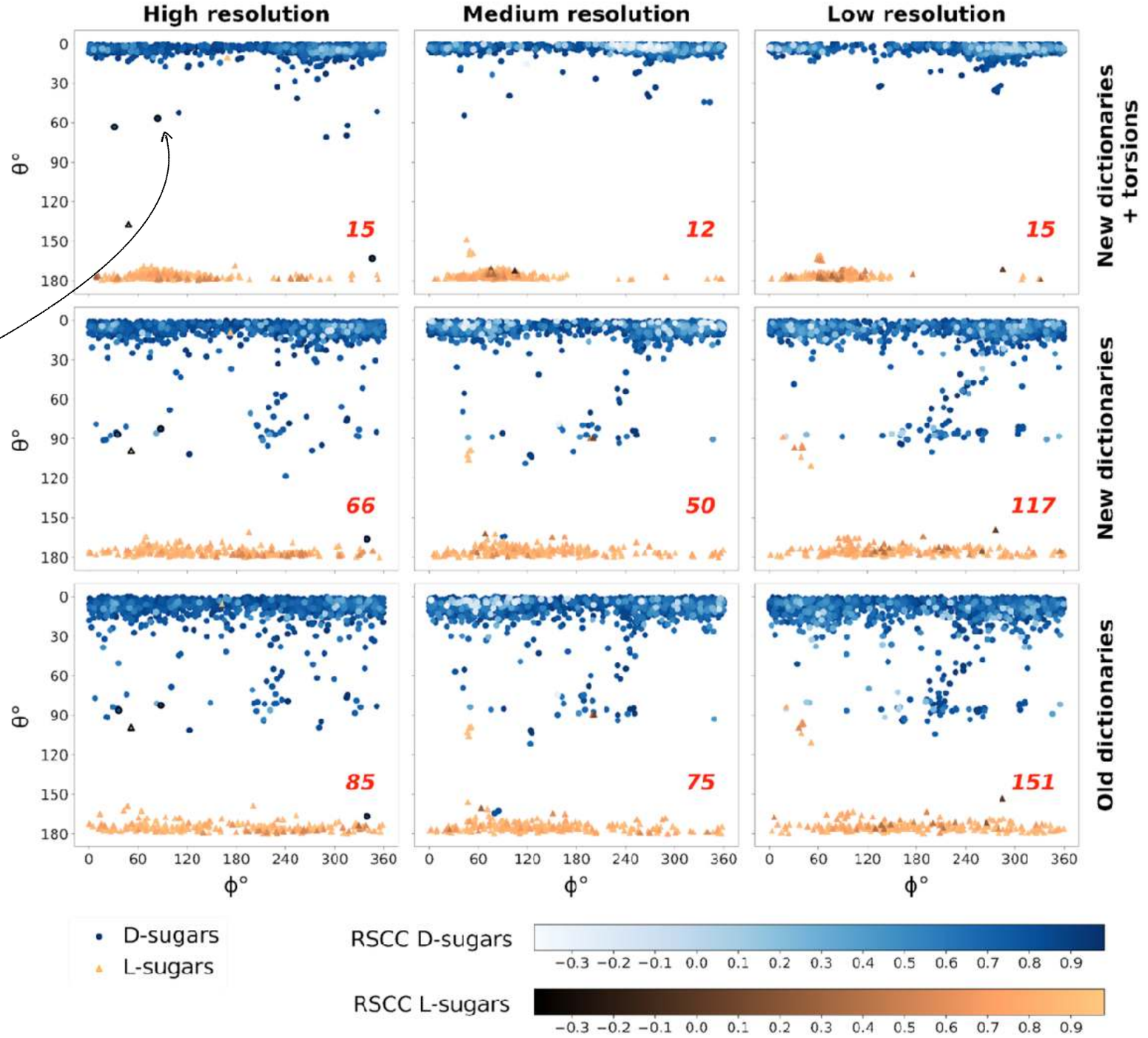


Conformational restraints
Ligands



5UPM (GLC-C-1) in $^{2}H_{1}$
1.70 Å resolution, RSCC=0.96

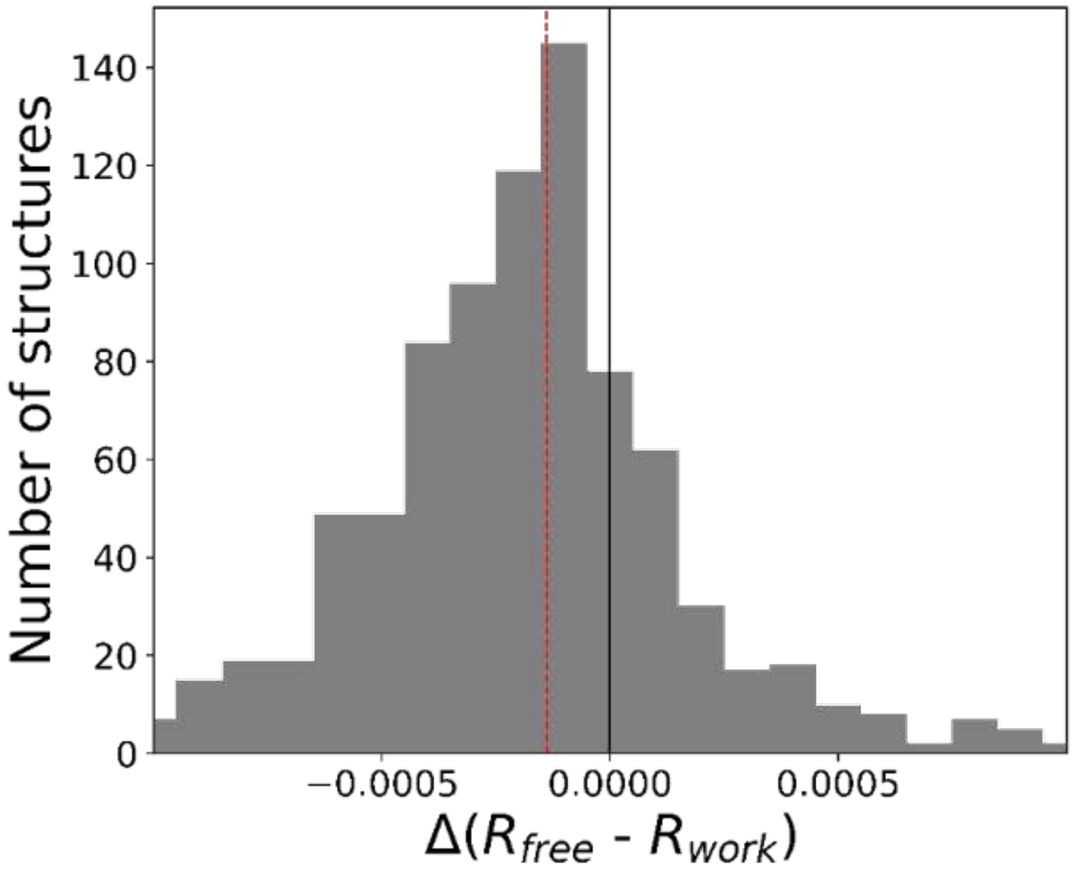
Atanasova, Joosten, Nicholls & Agirre, 2022,
Acta Crystallographica D(78):455-465



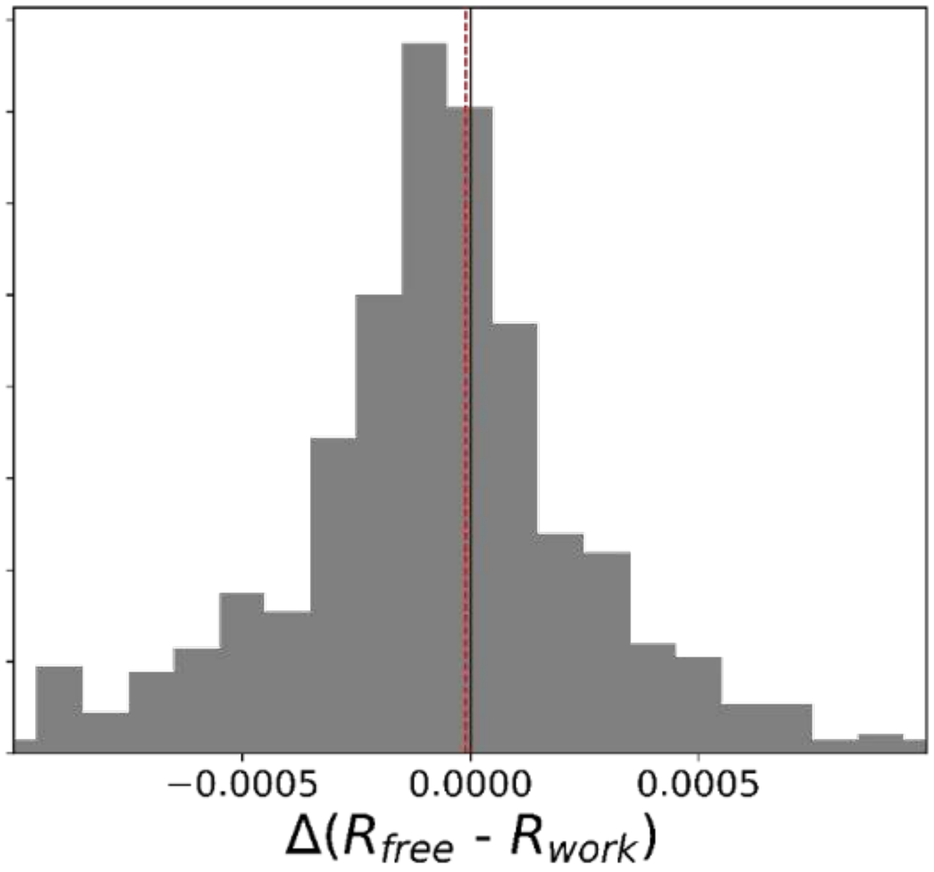
New dictionaries with conformational restraints

Reduction in R_{gap} = less overfitting

New dictionaries - old dictionaries

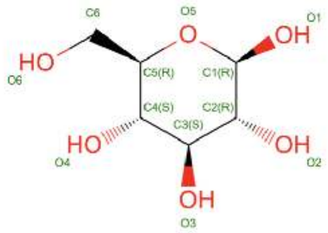


New dictionaries (torsions) - new dictionaries

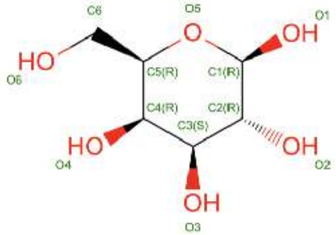


Atanasova, Joosten, Nicholls & Agirre, 2022, Acta Crystallographica D(78):455-465

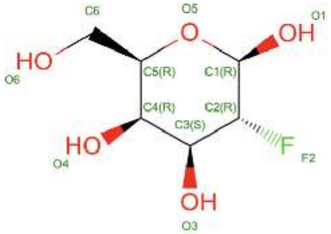
Improving restraint consistency across software: getting in touch with developers



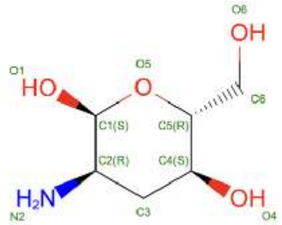
BGC
β-D-glucose



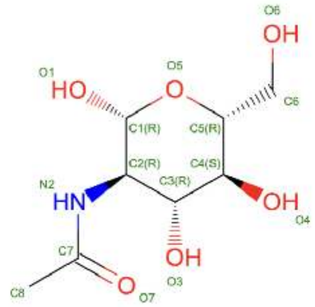
GAL
β-D-galactose



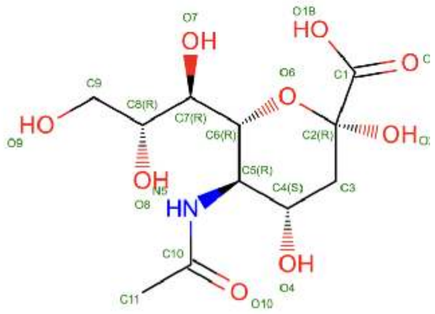
2FG
2-deoxy-2-fluoro-β-D-galactose



GCN
3-deoxy α-D-glucosamine

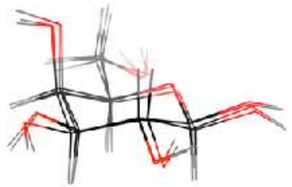


NAG
N-acetyl β-D-glucosamine



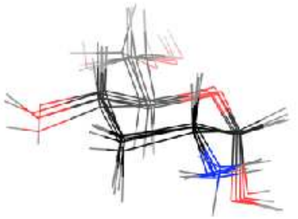
SIA
N-acetyl α-neuraminic acid

BGC ($^4C_1$)
β-D-glucose



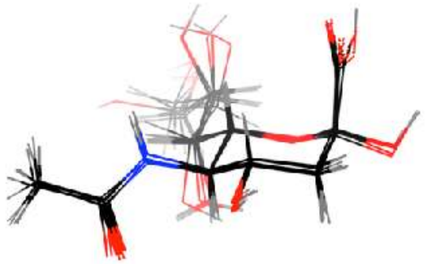
GAL ($^4C_1$)
β-D-galactose

2FG ($^4C_1$)
2-deoxy-2-fluoro-β-D-galactose



GCN ($^4C_1$)
3-deoxy α-D-glucosamine

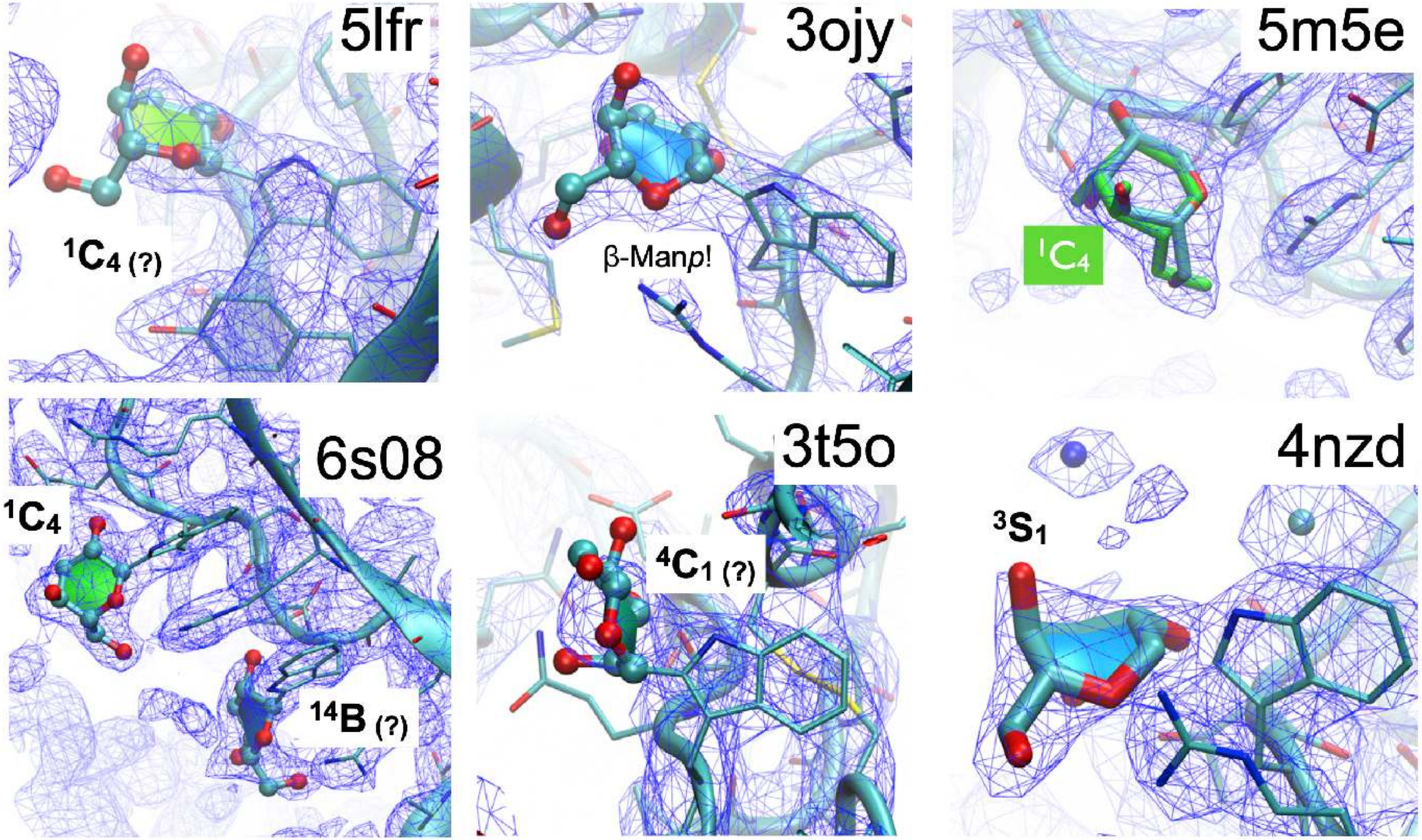
NAG ($^4C_1$)
N-acetyl β-D-glucosamine



SIA ($^1C_4$)
N-acetyl α-neuraminic acid

Joosten, Nicholls & Agirre, 2022, Current Medicinal Chemistry 29(4).

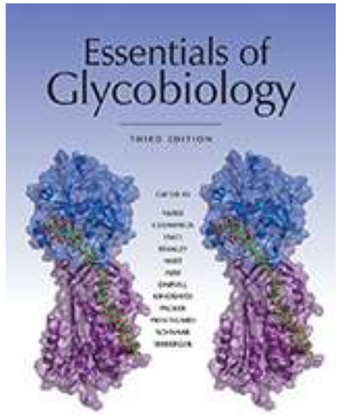
Restraints for C-mannosylation (MKV)

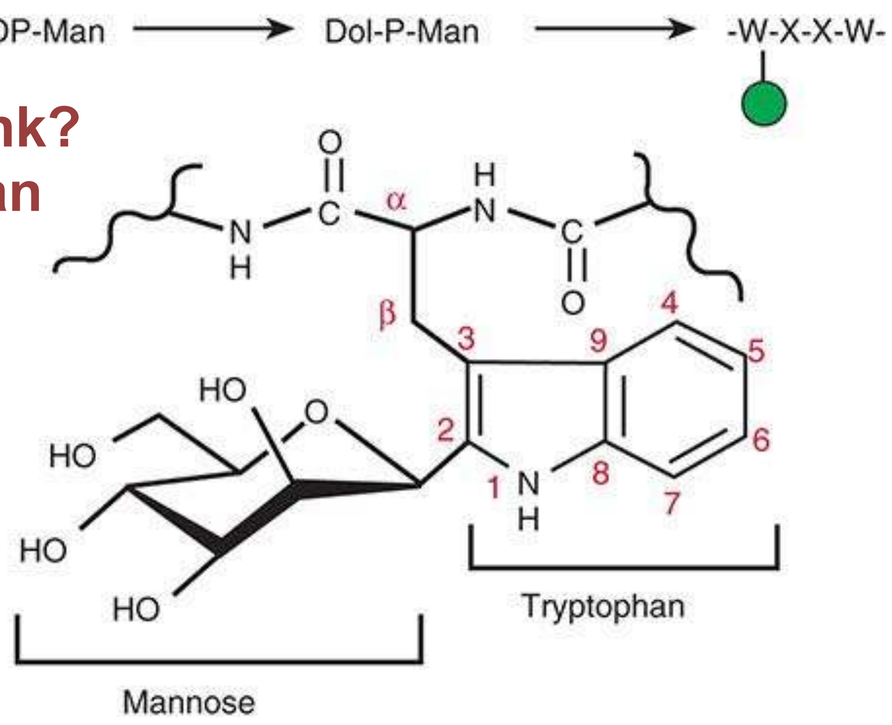


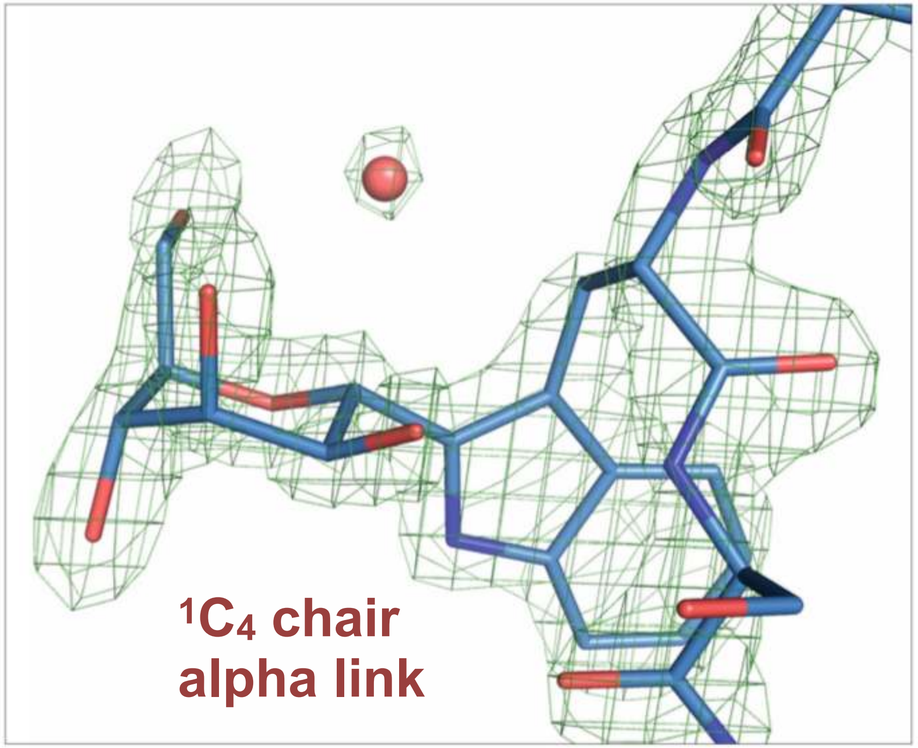
Frank, Beccati, Leeflang, Vliegenthart, 2020, iScience 23(8):101371.

Restraints for C-mannosylation (MKV)

$^4C_1$ chair, beta link?
Enzyme makes an alpha link…







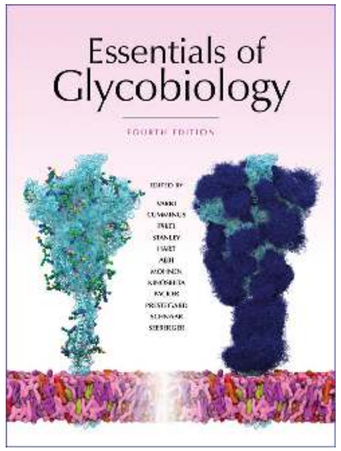
PDB 6PLH, omit mFo-DFc map at 1.5σ, 1.6 Å data

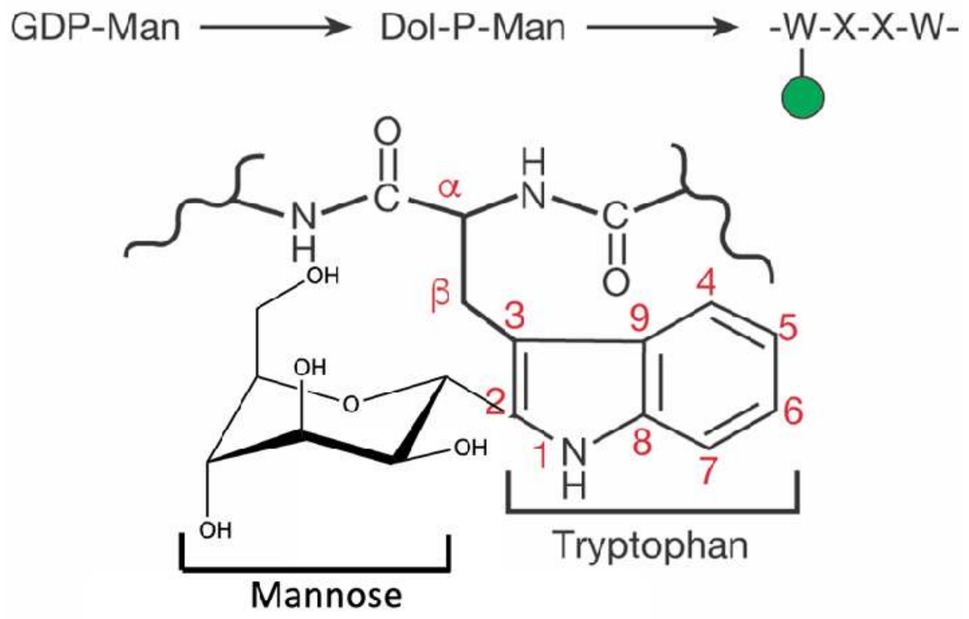
John, Jarva, Shah, Mao, Chappaz, Birkinshaw, Czabotar, Lo, Scott & Goddard-Borger, 2021, Nature Chemical Biology 17: 428-437

Essentials of Glycobiology [Internet]. 3rd edition.
Varki A, Cummings RD, Esko JD, et al., editors.
Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015-2017.

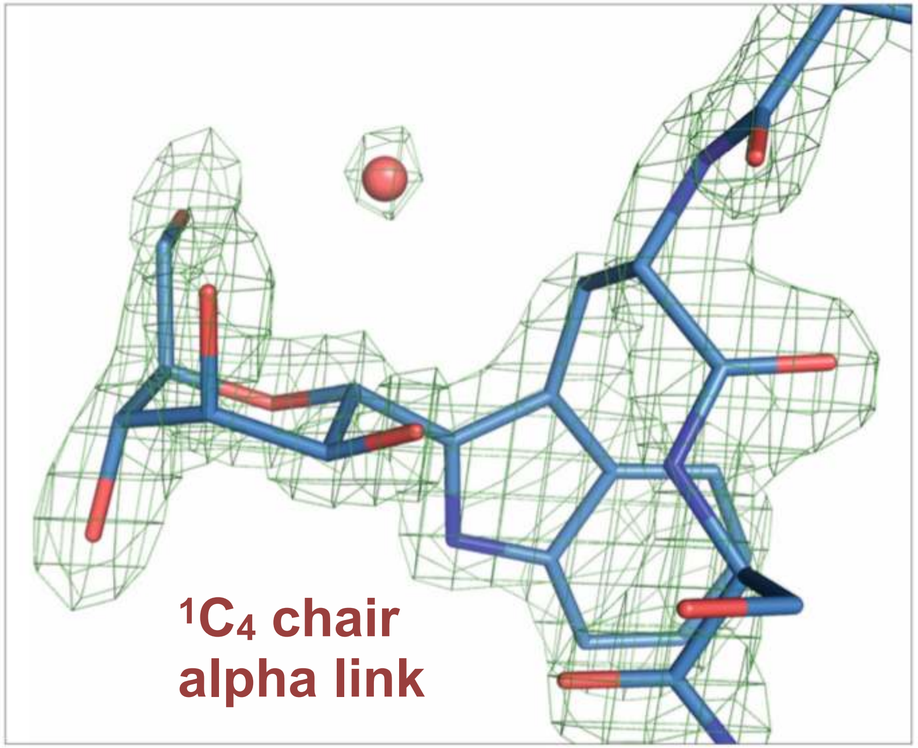
Restraints for C-mannosylation (MKV)

$^1C_4$ chair alpha link





Updated figure courtesy of Prof. Pamela Stanley (Einstein College, USA)



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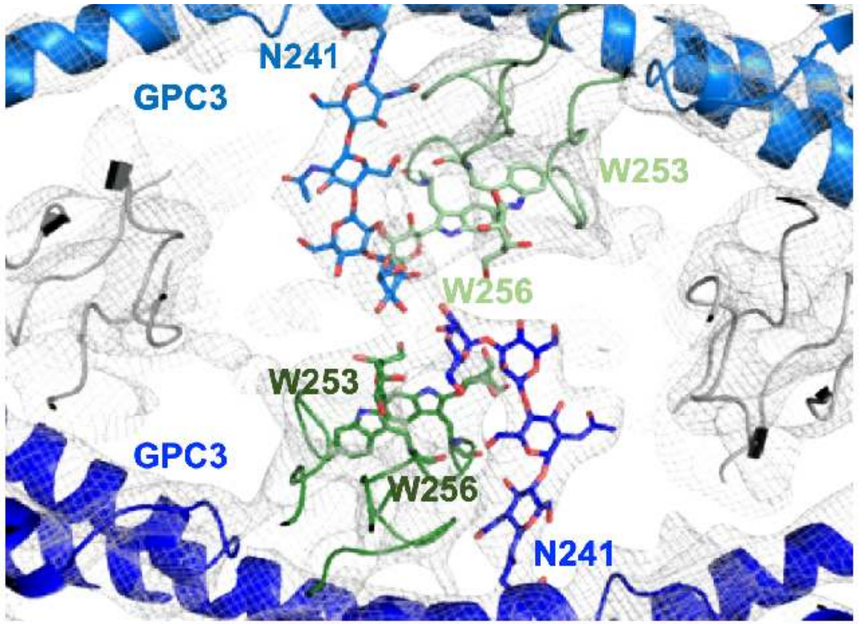
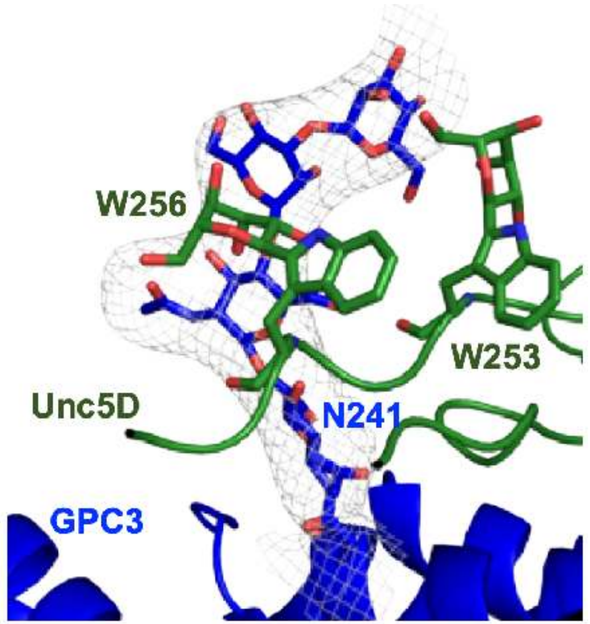
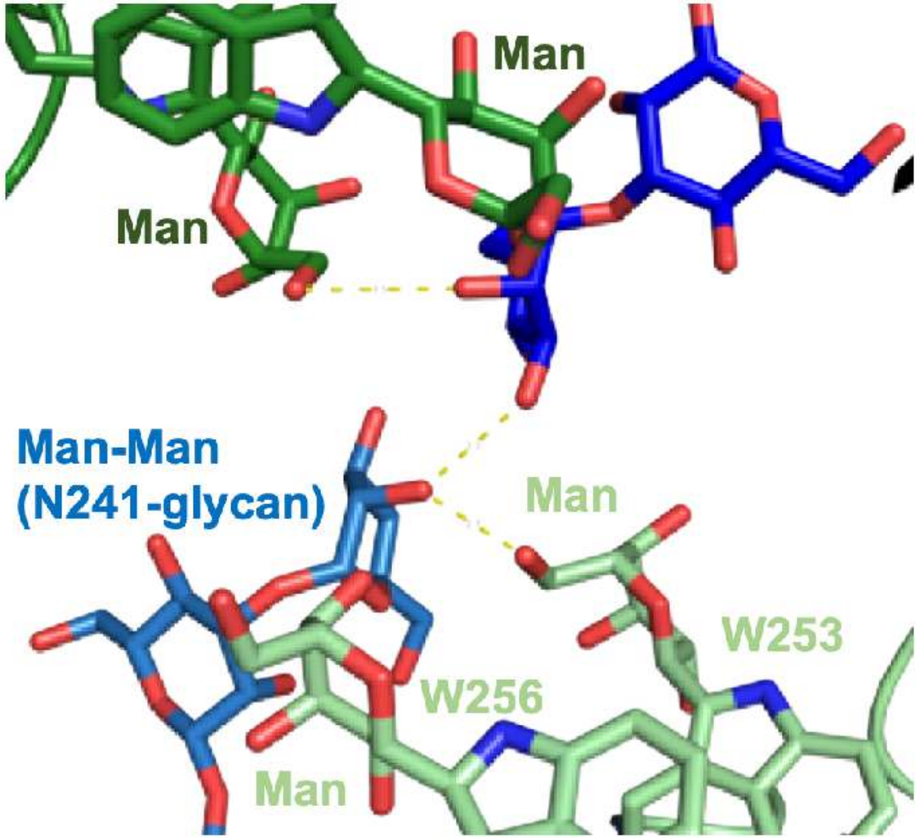
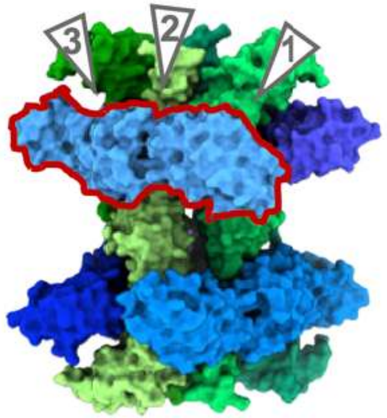
Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2022.

Restraints for C-mannosylation (MKV)









GPC3

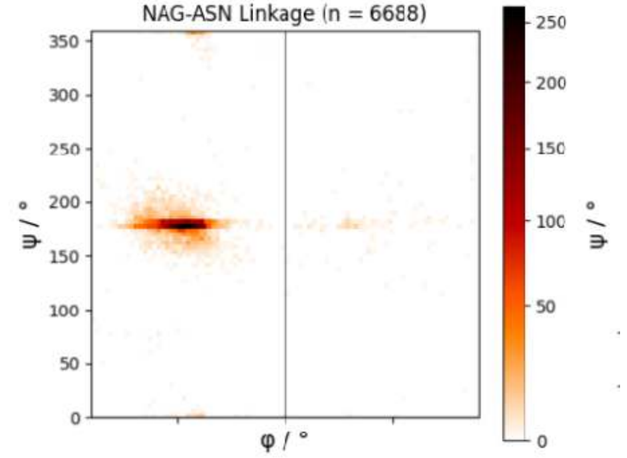
Unc5D

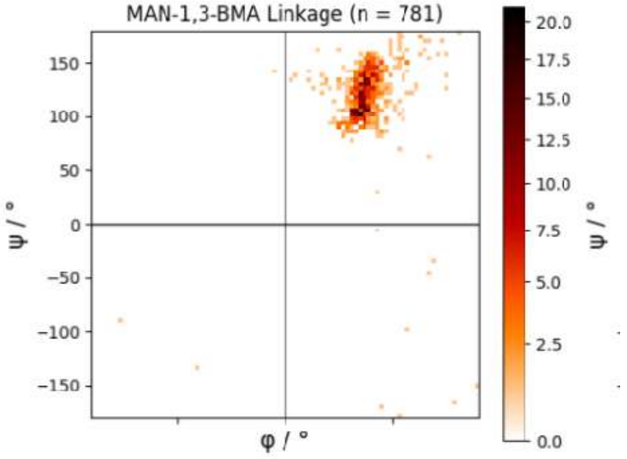
Akkermans, Delloye-Bourgeois, Peregrina, Carrasquero, Kokolaki, Santana, Chavent, Reynaud, Raj, Agirre, Aksu, White, Lowe, Ben Amar, Zaballa, Huo, McCubbin, Comoletti, Owens, Robinson, Castellani, del Toro & Seiradake, 2022, Cell 185(21): 3931-3949.

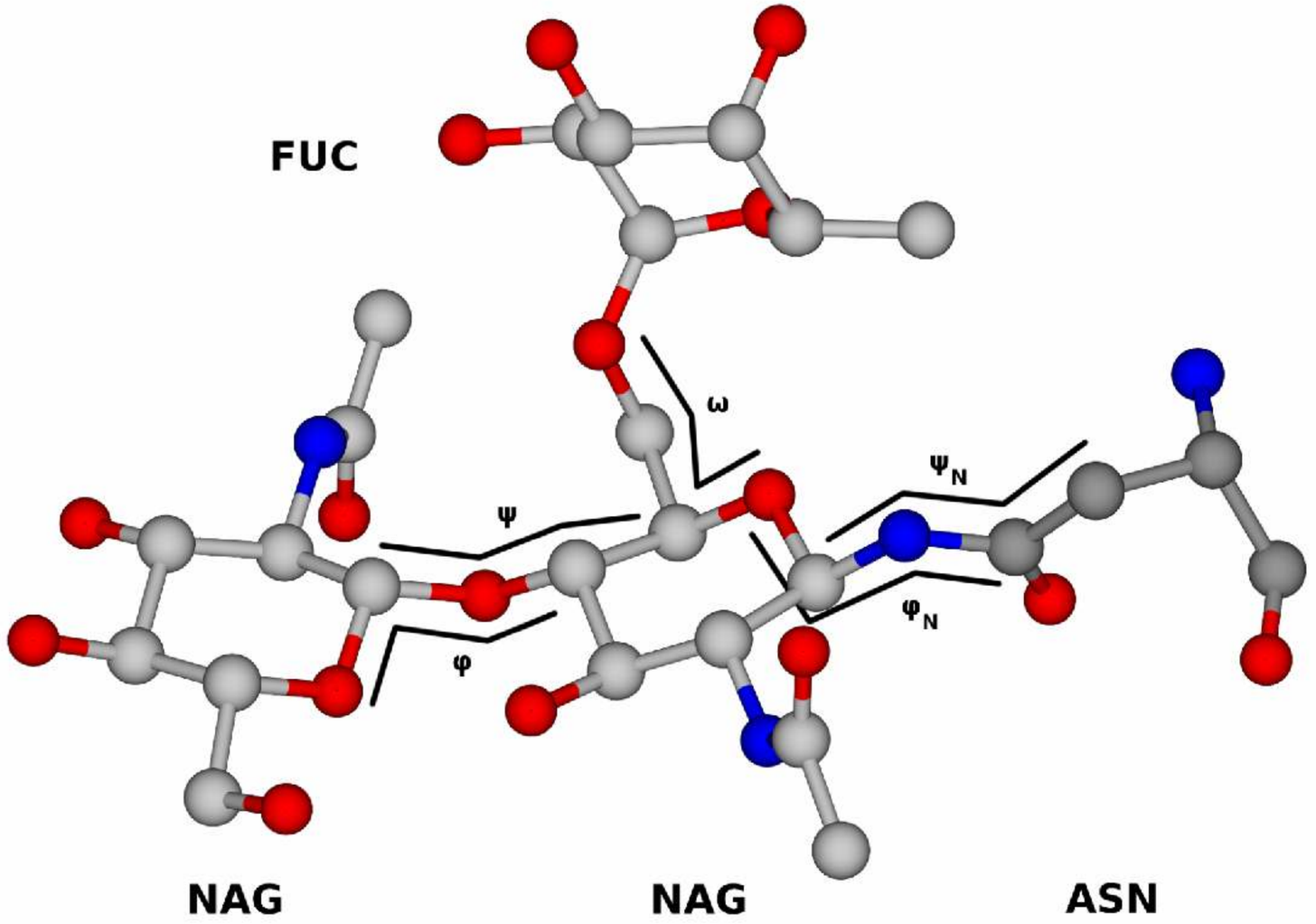
Support for C-mannosylation (MKV)

- **Privateer MKV (CCP4 8.0 via update)**
  - checks that mannopyranose ring is $^{1}C_{4}$
  - makes sure linkage is alpha, shouts if it isn't
- For refinement at **low resolution with refmac5**
  - External torsion restraints for link and $^{1}C_{4}$ conformation
  - Creates modified linkage dictionary to impose torsions for $^{1}C_{4}$ conformation on the mannose

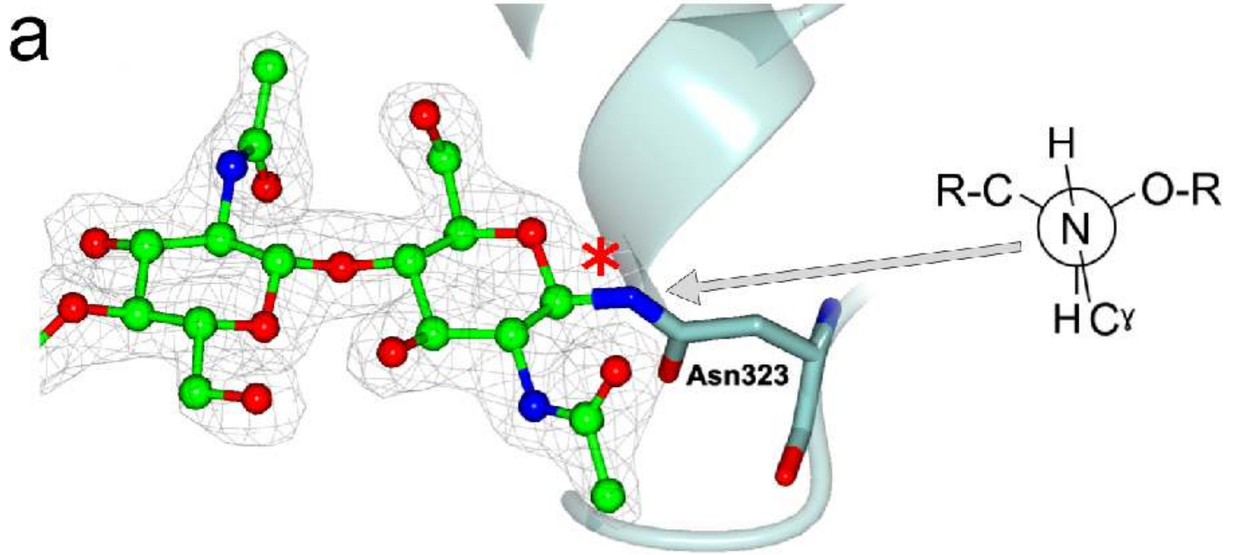
Analysis of linkage torsions

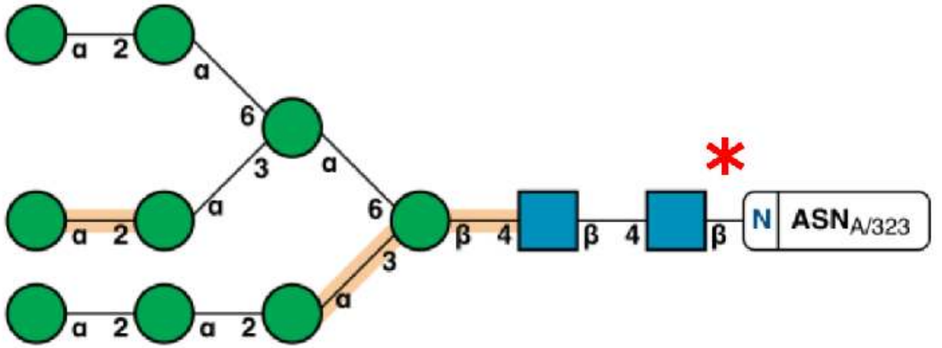


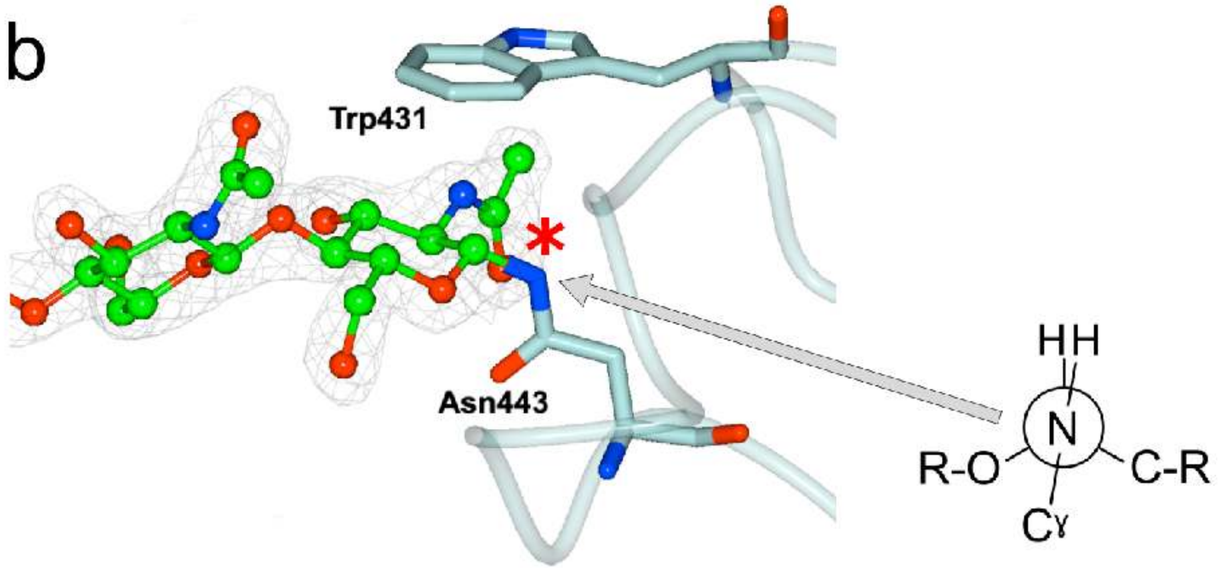


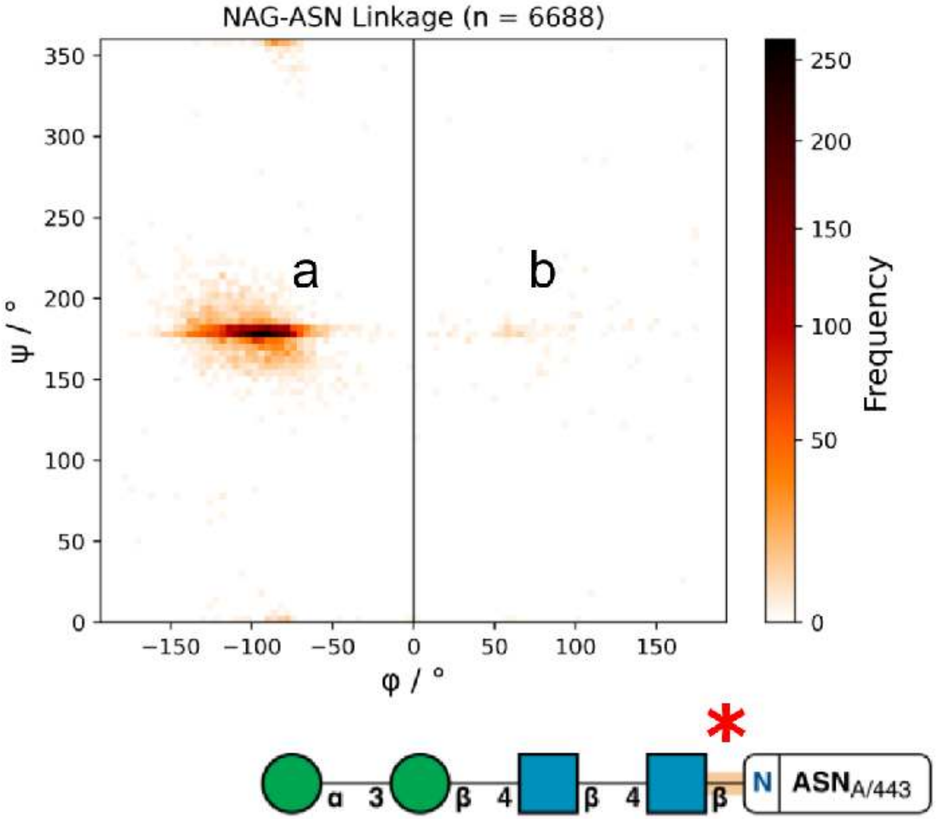


Dialpuri, Bagdonas, Atanasova, Schofield, Hekkelman, Joosten & Agirre, 2023, Acta Cryst D 79(6):462-472









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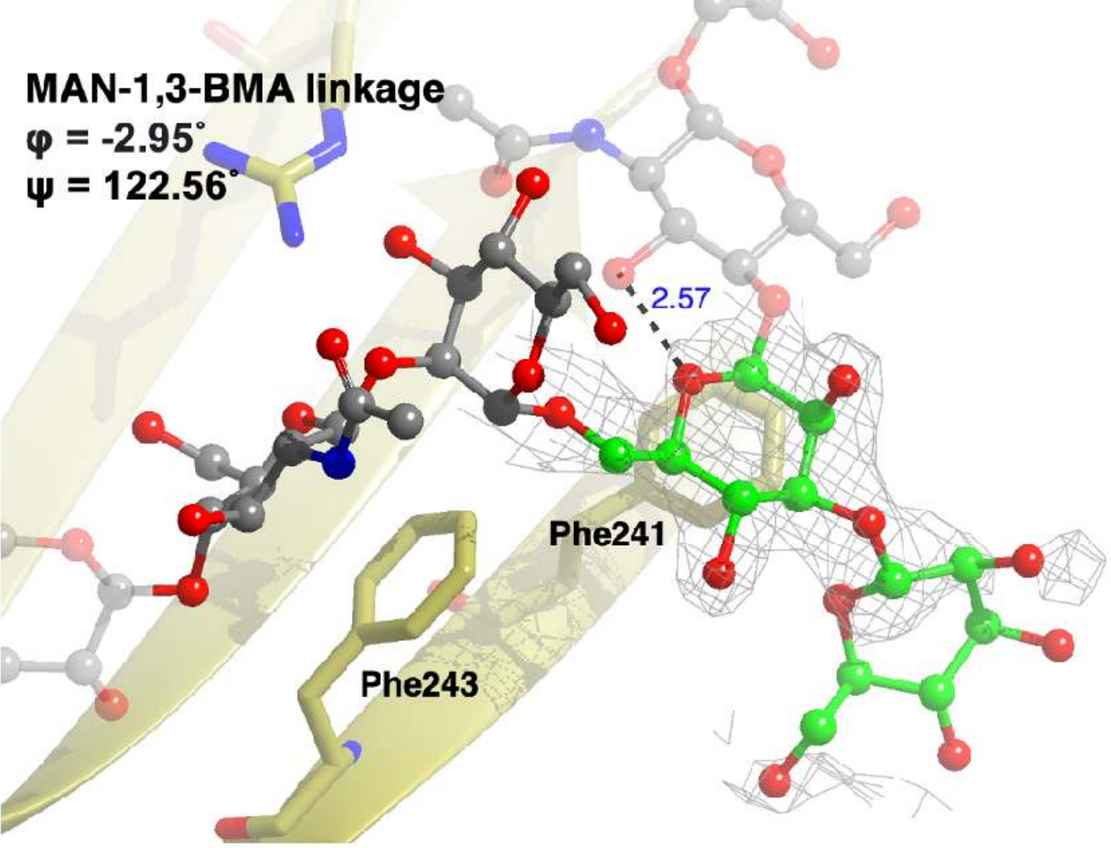
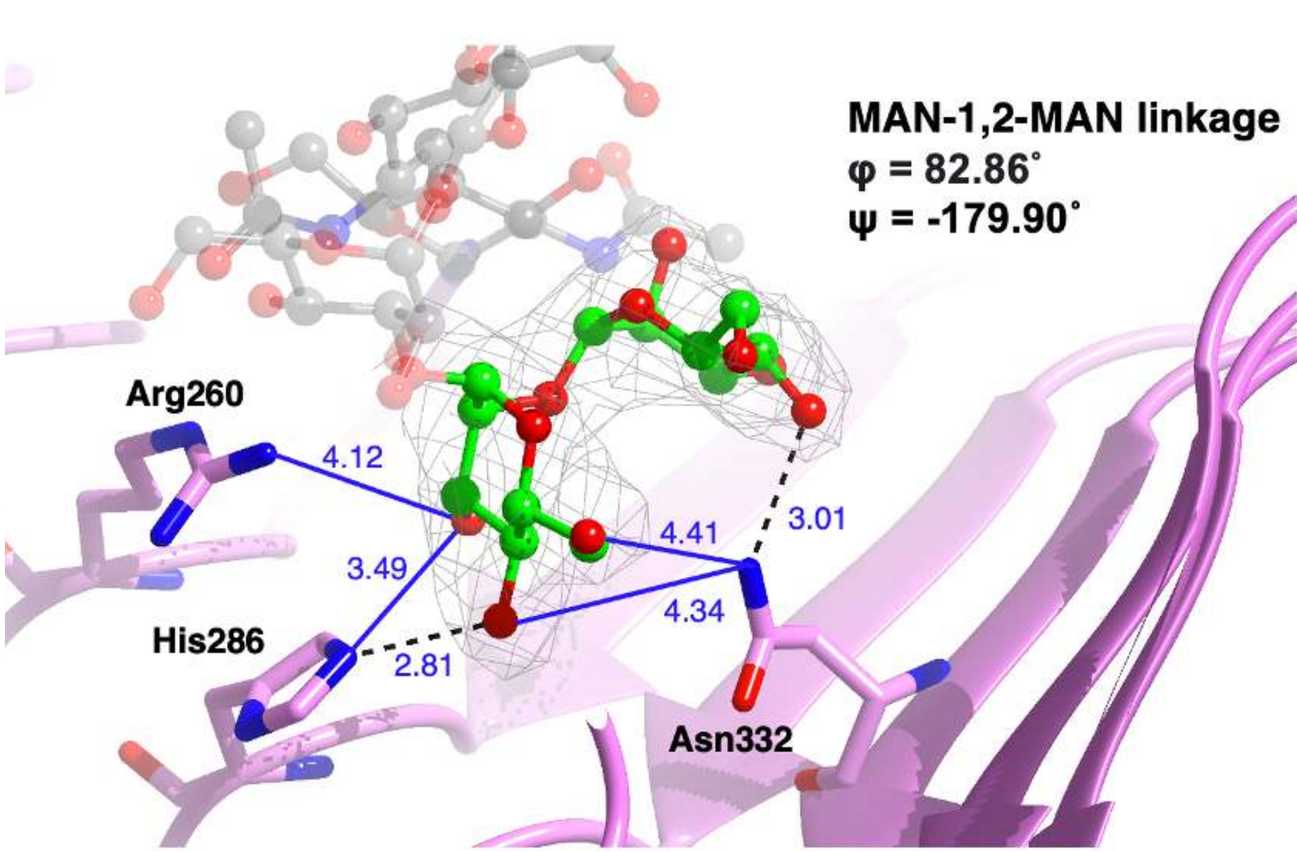
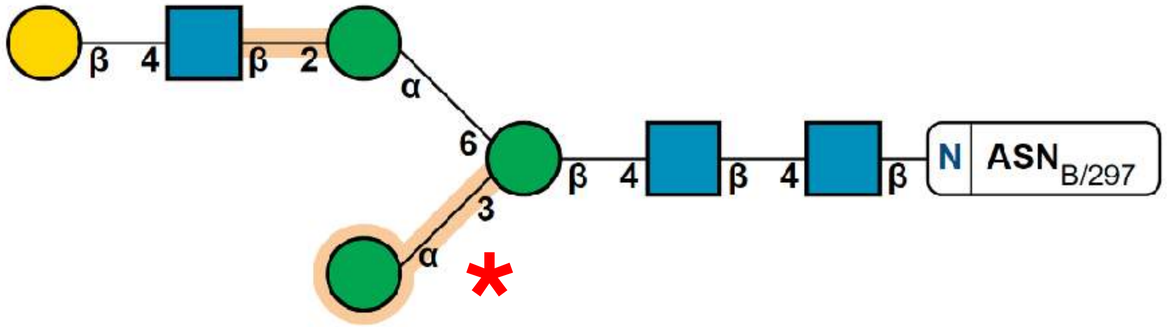
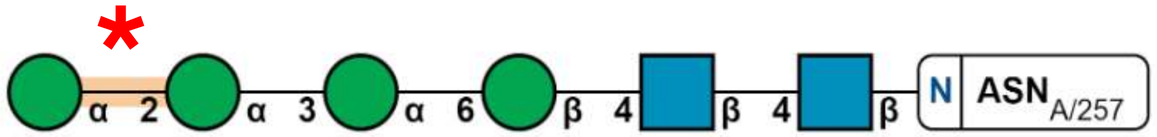






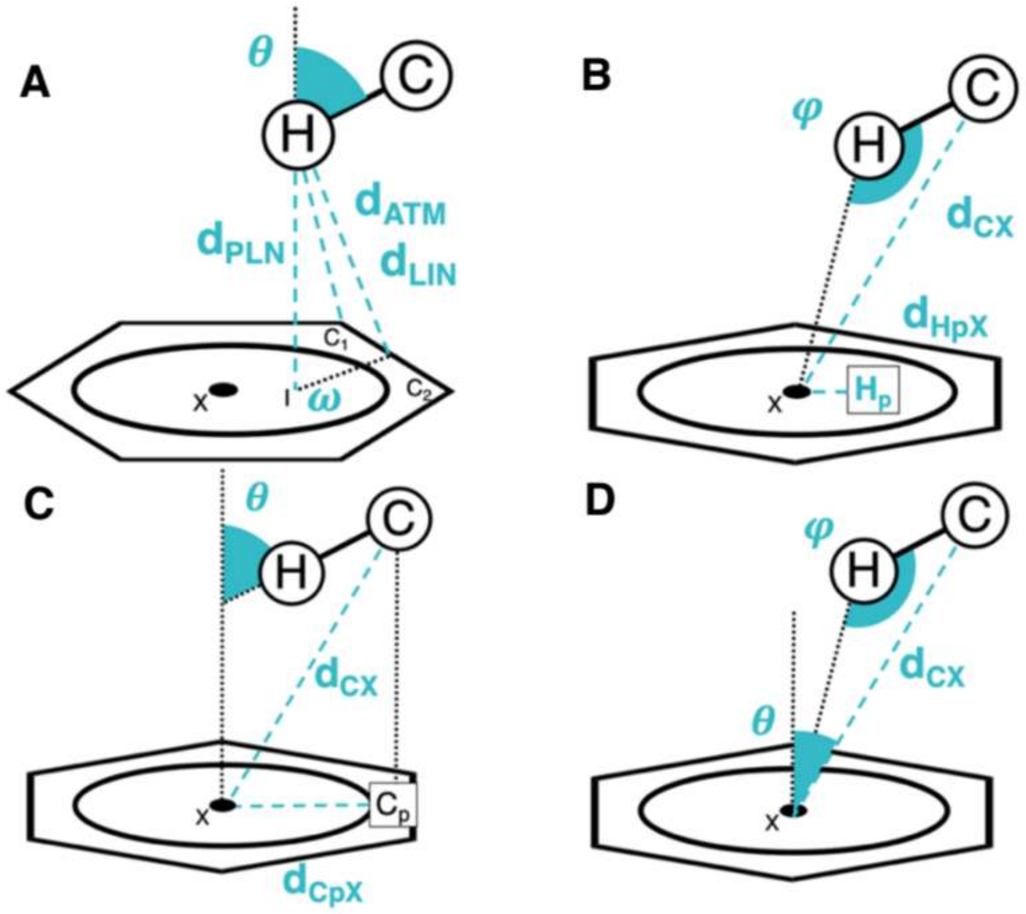


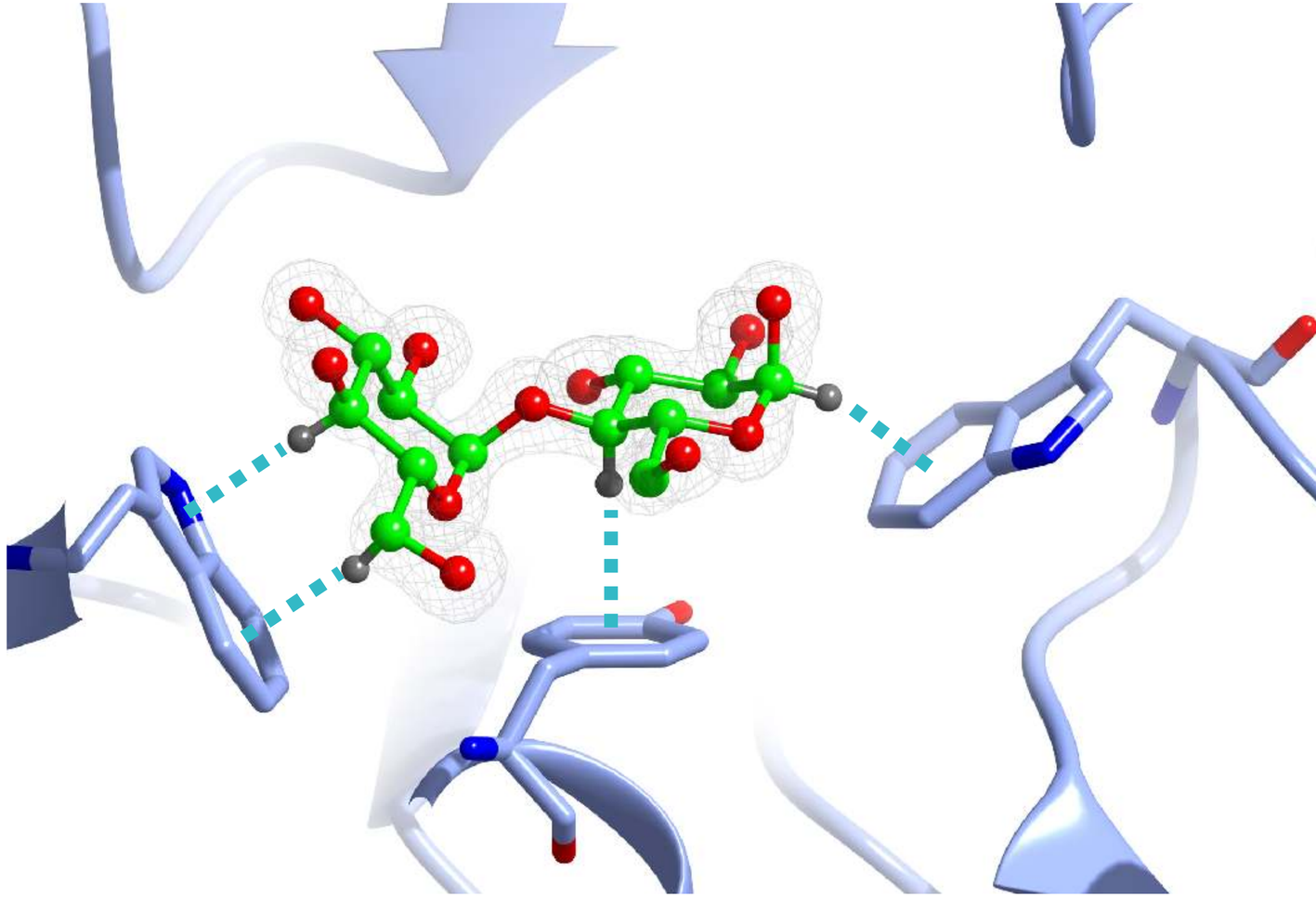
Anomaly!

Well supported by map and interactions

Dialpuri, Bagdonas, Atanasova, Schofield, Hekkelman, Joosten & Agirre, 2023, Acta Cryst D 79(6):462-472

Detection of HX-Pi interactions

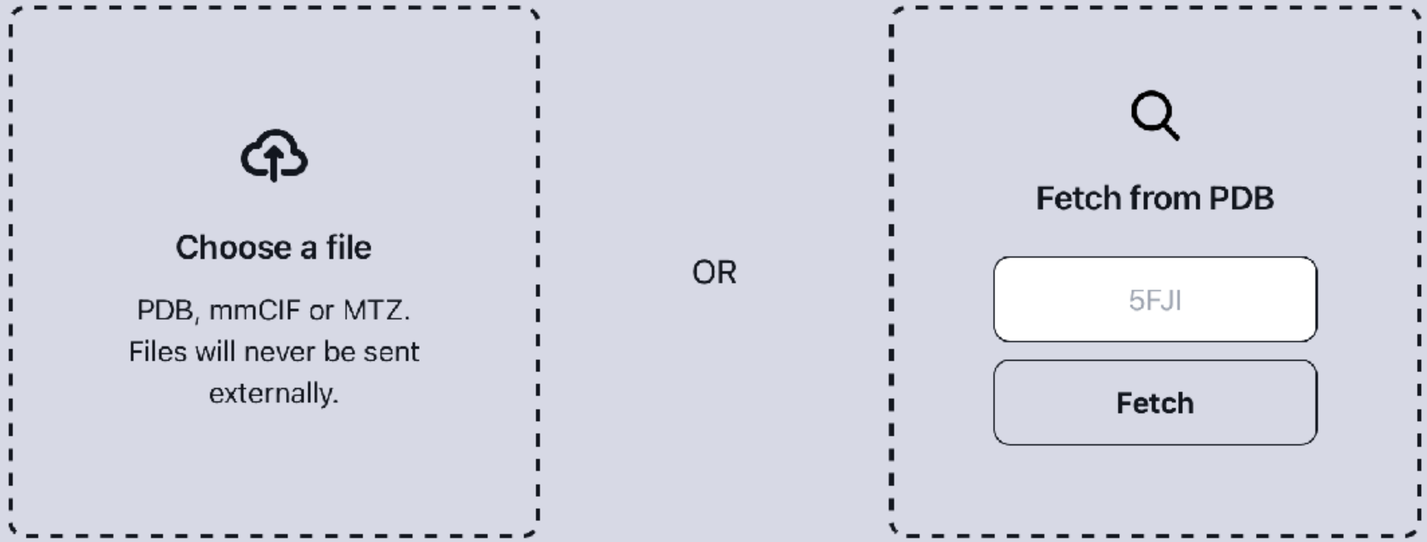




Validate your carbohydrates online with

Privateer

The Swiss Army knife for carbohydrate structure validation, refinement and analysis



https://privateer.york.ac.uk

Dialpuri, Bagdonas, Schofield, Pham, Holland, Bond, Sanchez Rodriguez, McNicholas & Agirre, under review

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Glycan Details

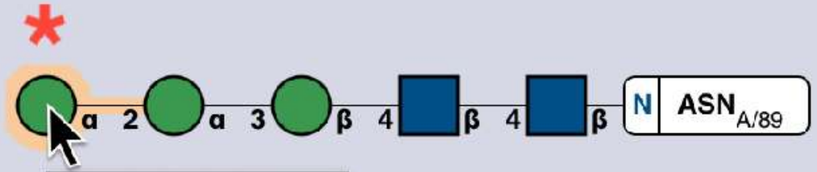
Validation Report

Glycan ID: **NAG-1/B_ASN-89/A**

Glytoucan ID: **G42227JK**

Number of conformation issues: 1
Number of anomer issues: 0
Number of torsion issues: 1
Number of pucker issues: 0
Number of chirality issues: 0

SNFG

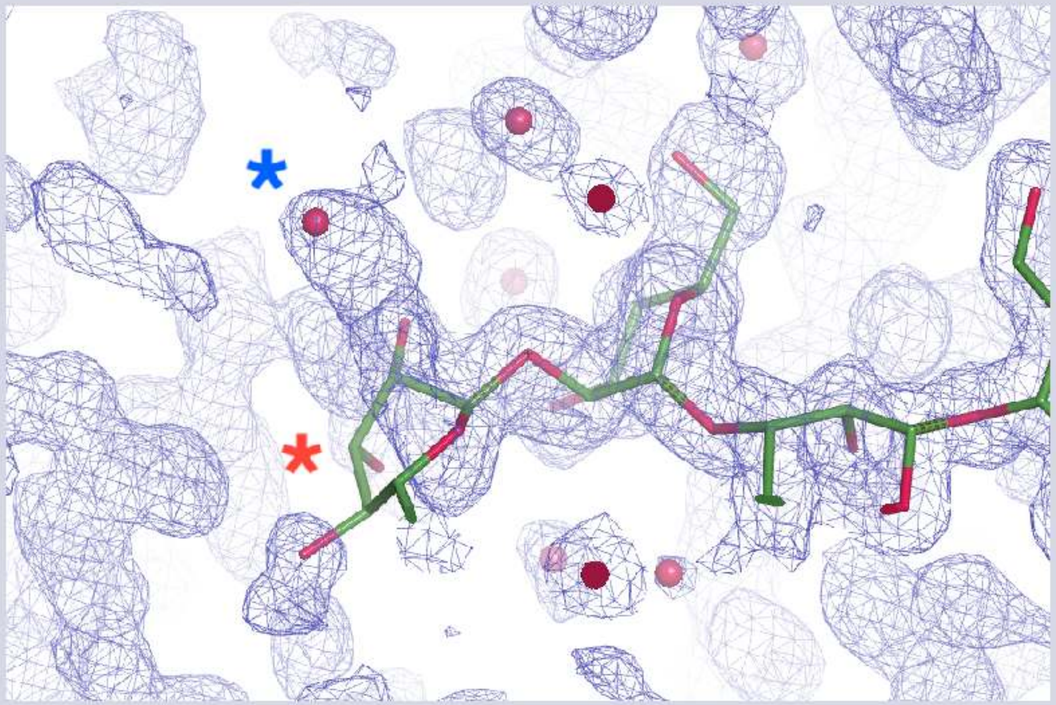


VAN G in 1sG conformation. Mean B-factor: 52.31. Detected type: alpha-D-aldopyranose. Detected issues: The sugar is in a high-energy conformation;

a linkage to see a summary

Visualise

Map Contour



← Back To Table

Glycan Details

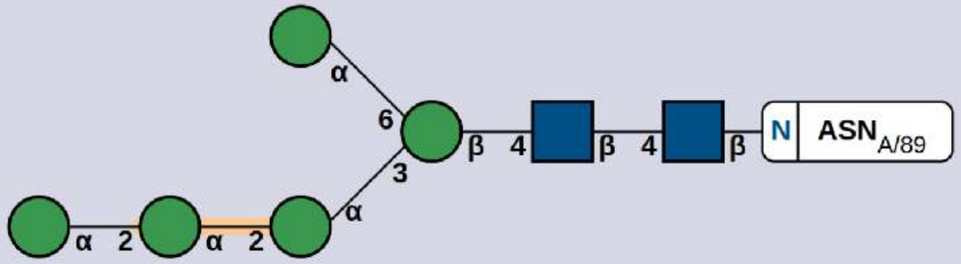
Validation Report

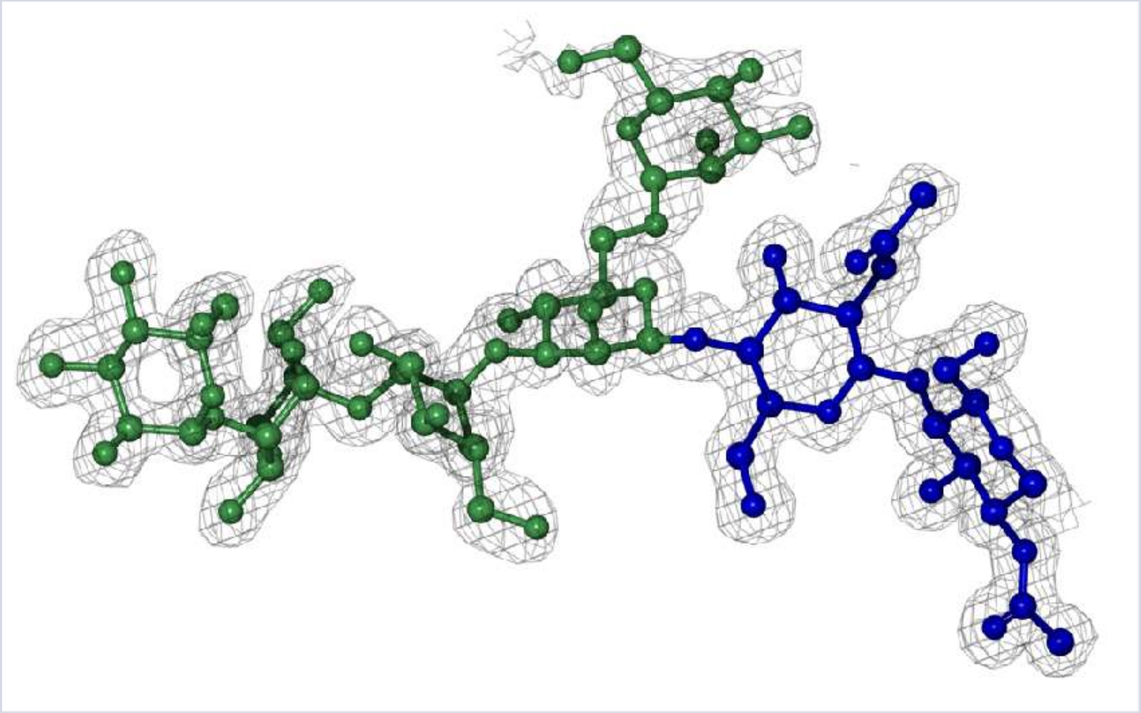
Glycan ID: **NAG-704/A_ASN-89/A**

Glytoucan ID: **G46836GH**

Number of conformation issues: 0
Number of anomer issues: 0
Number of torsion issues: 1
Number of pucker issues: 0
Number of chirality issues: 0

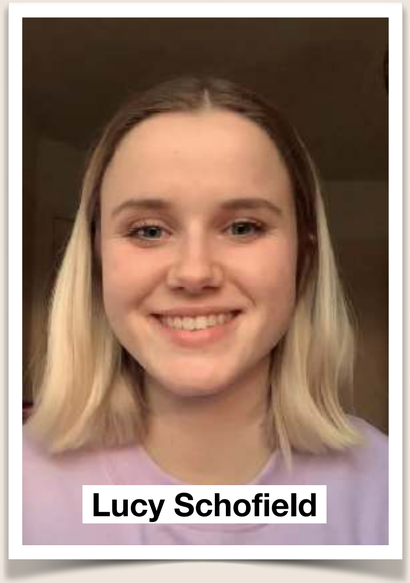
SNFG





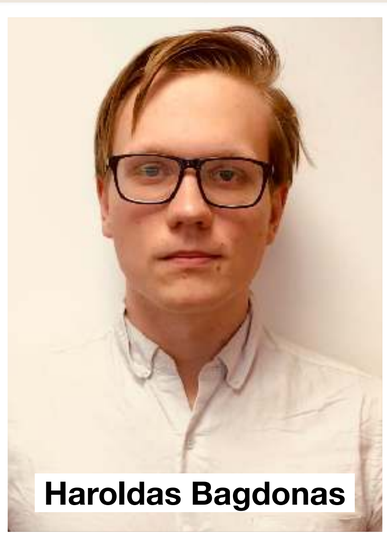
Conclusions

- **Glycan composition**
  - Always make sure your glycans **match biosynthetic pathways**
  - Privateer will check your glycans against glycomics data and suggest **alternatives** if there are inconsistencies
- **Ring conformation**
  - High-energy puckers are **almost never true**
  - These are usually the result of **modelling errors** or **refinement against poor density**, and need to be corrected
- **Glycosidic link torsions**
  - Modelling errors may force links into surprising conformations
  - Not all standout conformations are wrong – **check interactions**!



Glycojones team

Alex · Ali · Harold · Jake · Jon · Jordan · Lou · Lucy · Manal · Thao



Acknowledgements

Collaborators

Robbie Joosten (NKI, The Netherlands)
Garib Murshudov (MRC-LMB, Cambridge)
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Elisa Fadda (Maynooth University, Ireland)
Elena Seiradake (University of Oxford)
Martin Frank (Biognos, Sweden)
Frédérique Lisacek (SIB, Switzerland)
Sameer Velankar & Gerard Kleywegt (PDBe & AFDB)
CCP4 & CCP-EM core teams

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