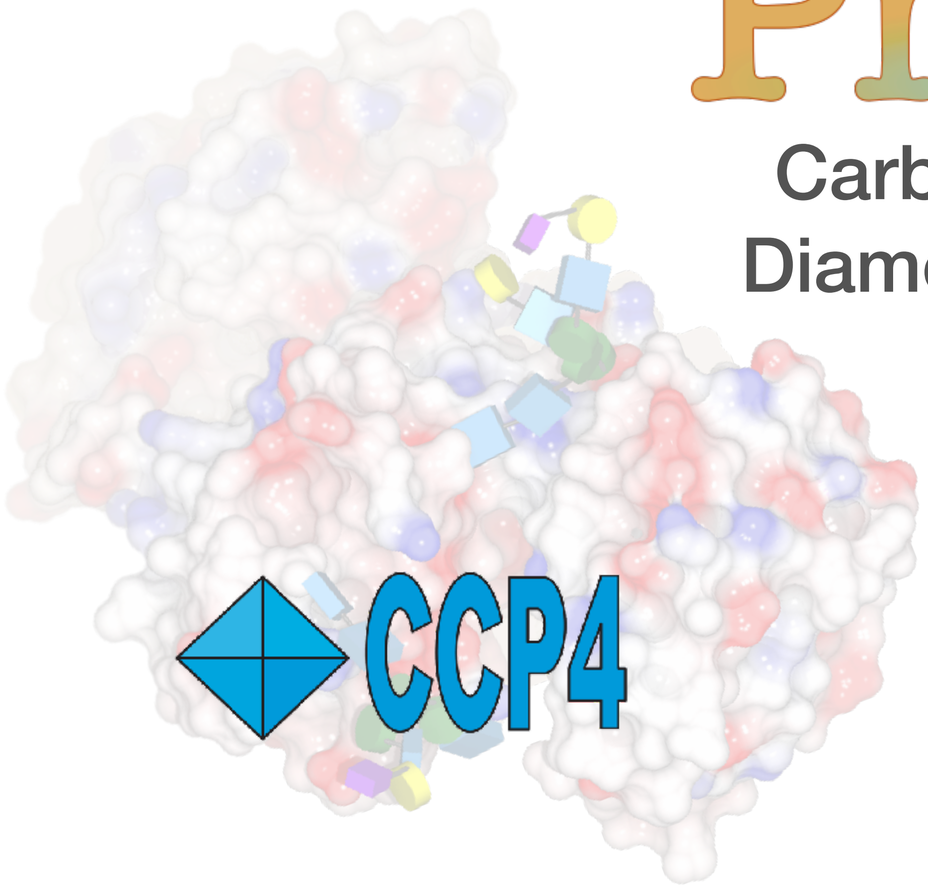
Privateer

Carbohydrate Validation Tools
Diamond-CCP4 Workshop 2024



Lucy Schofield and Jordan Dialpuri
Glycojones Team
Supervisor: Dr. Jon Agirre
York Structural Biology Laboratory

Glycoproteins

glyco-
/ˈglʌɪkəʊ/

GREEK
glukus → glyco-

Sweet

hydrogen

oxygen

carbon

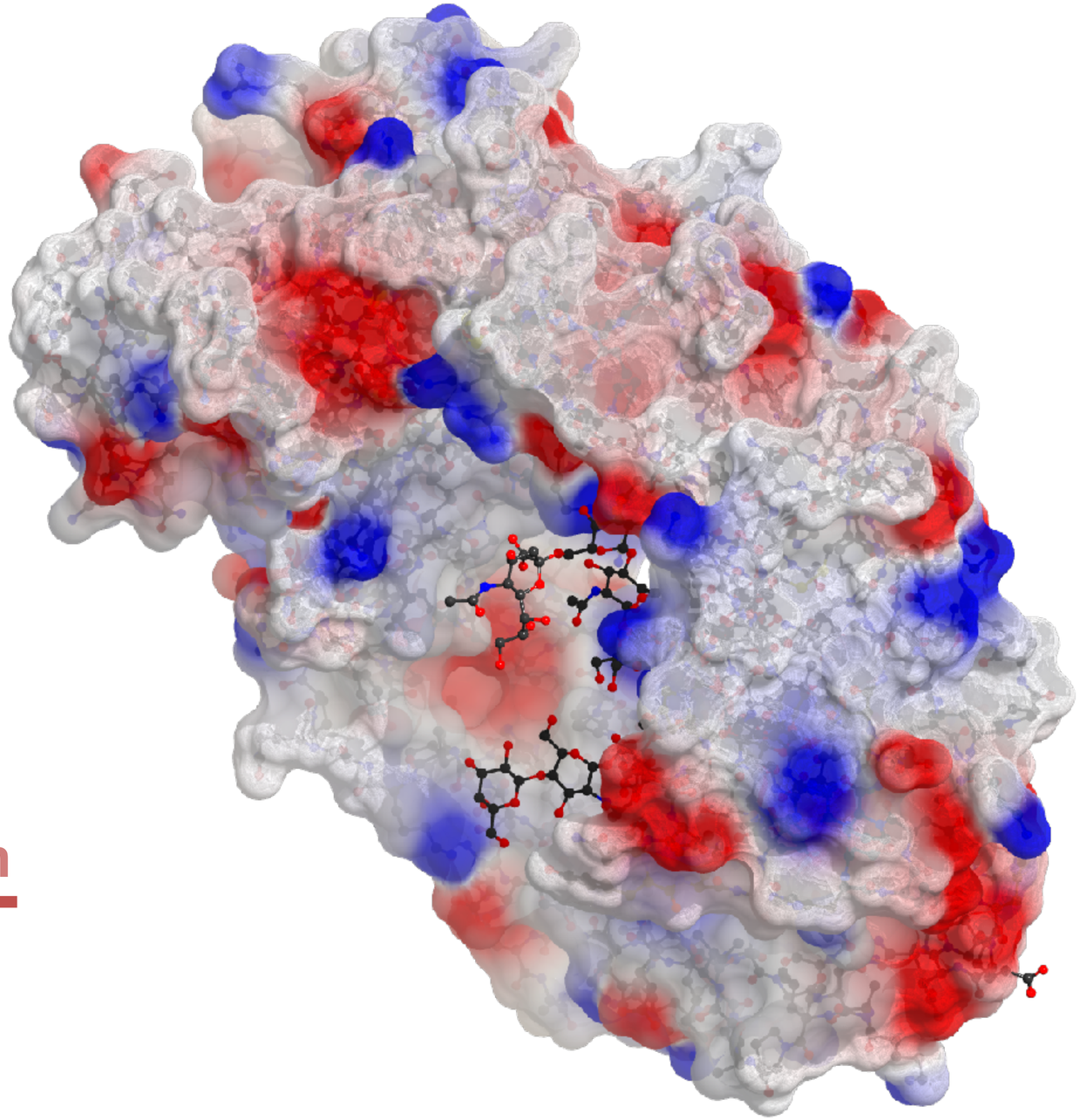
hydrogen bond

N-glycan

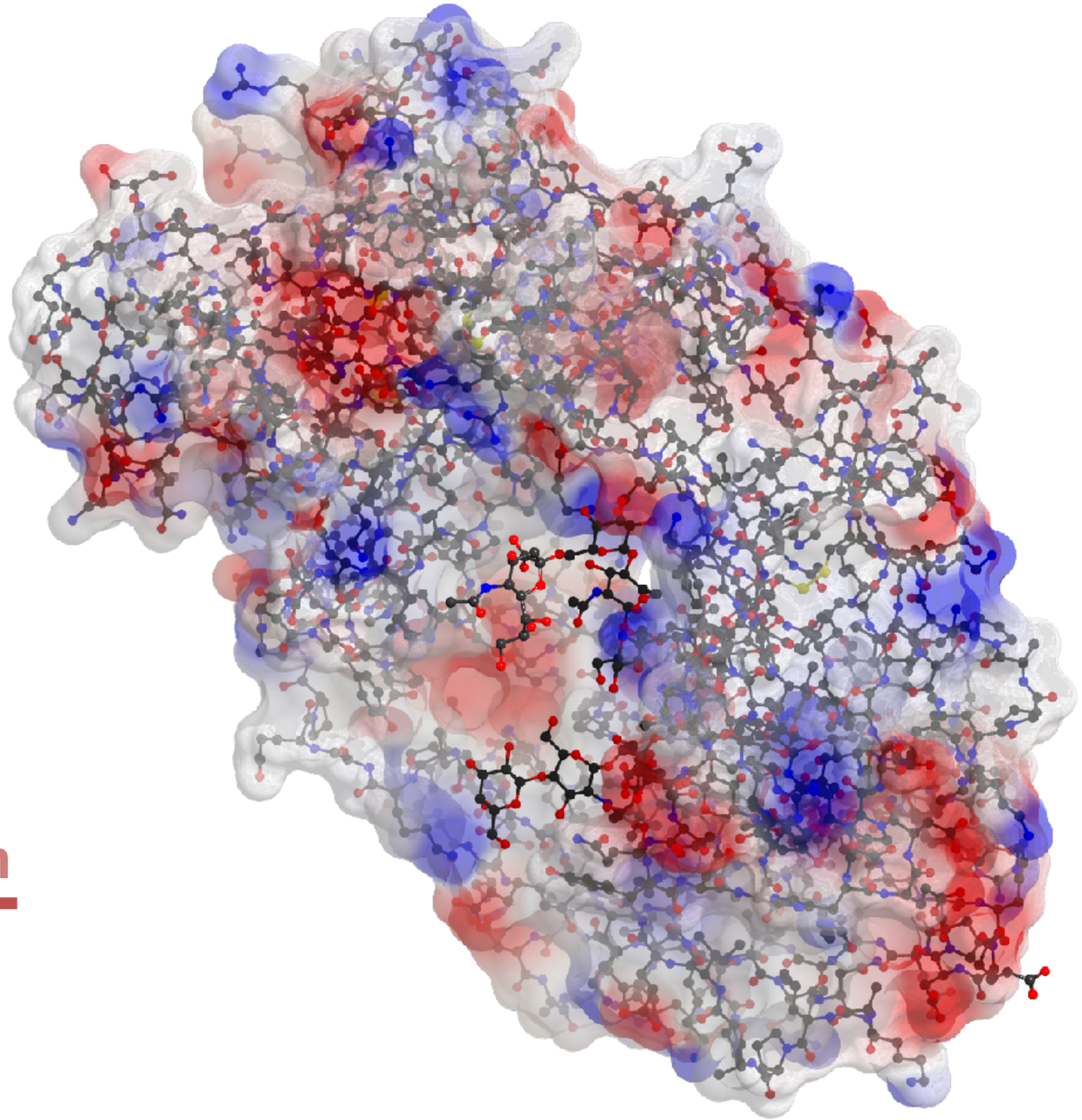
Protein

Not always sweet!

Protein glycosylation

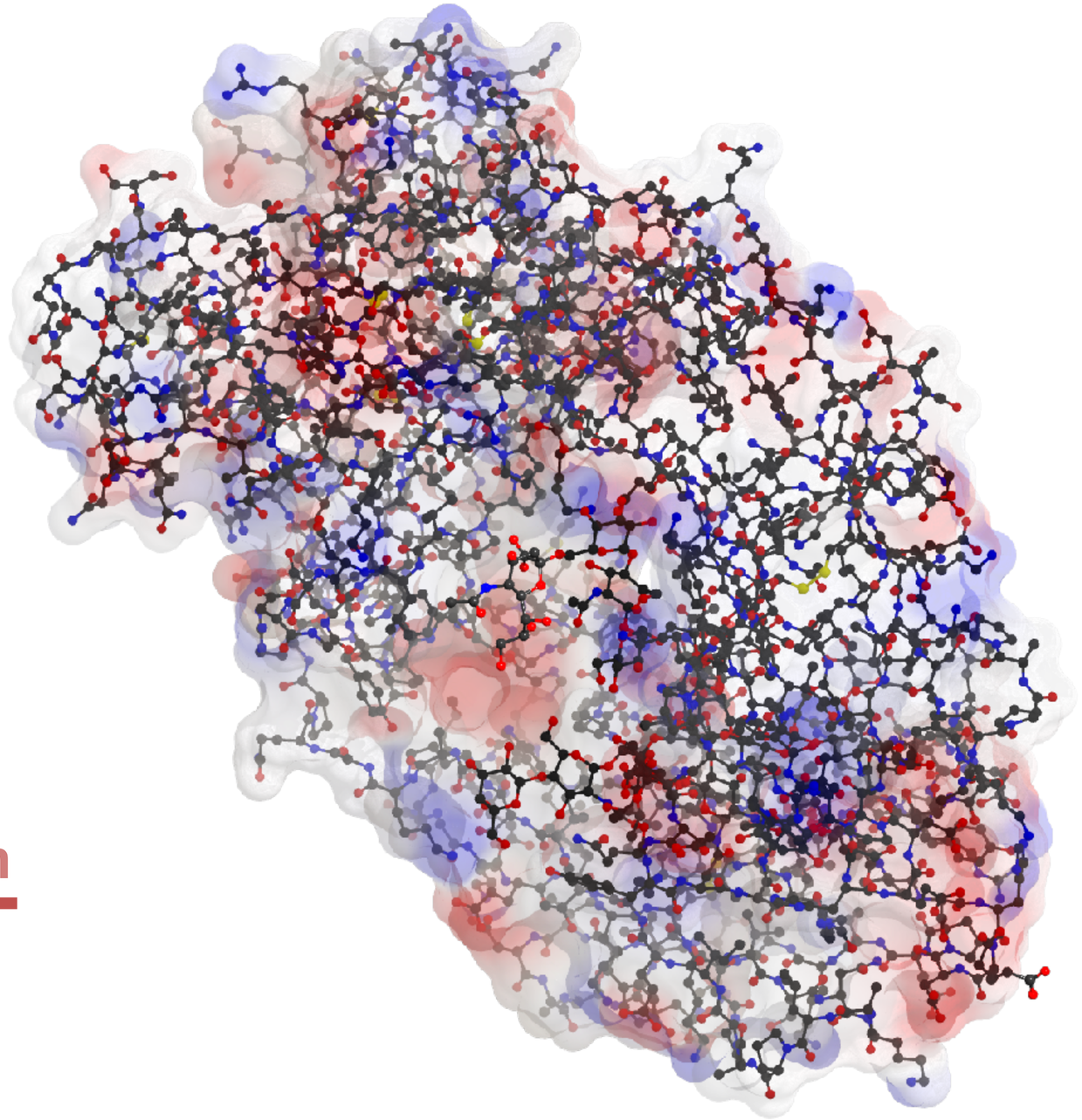


1 nm



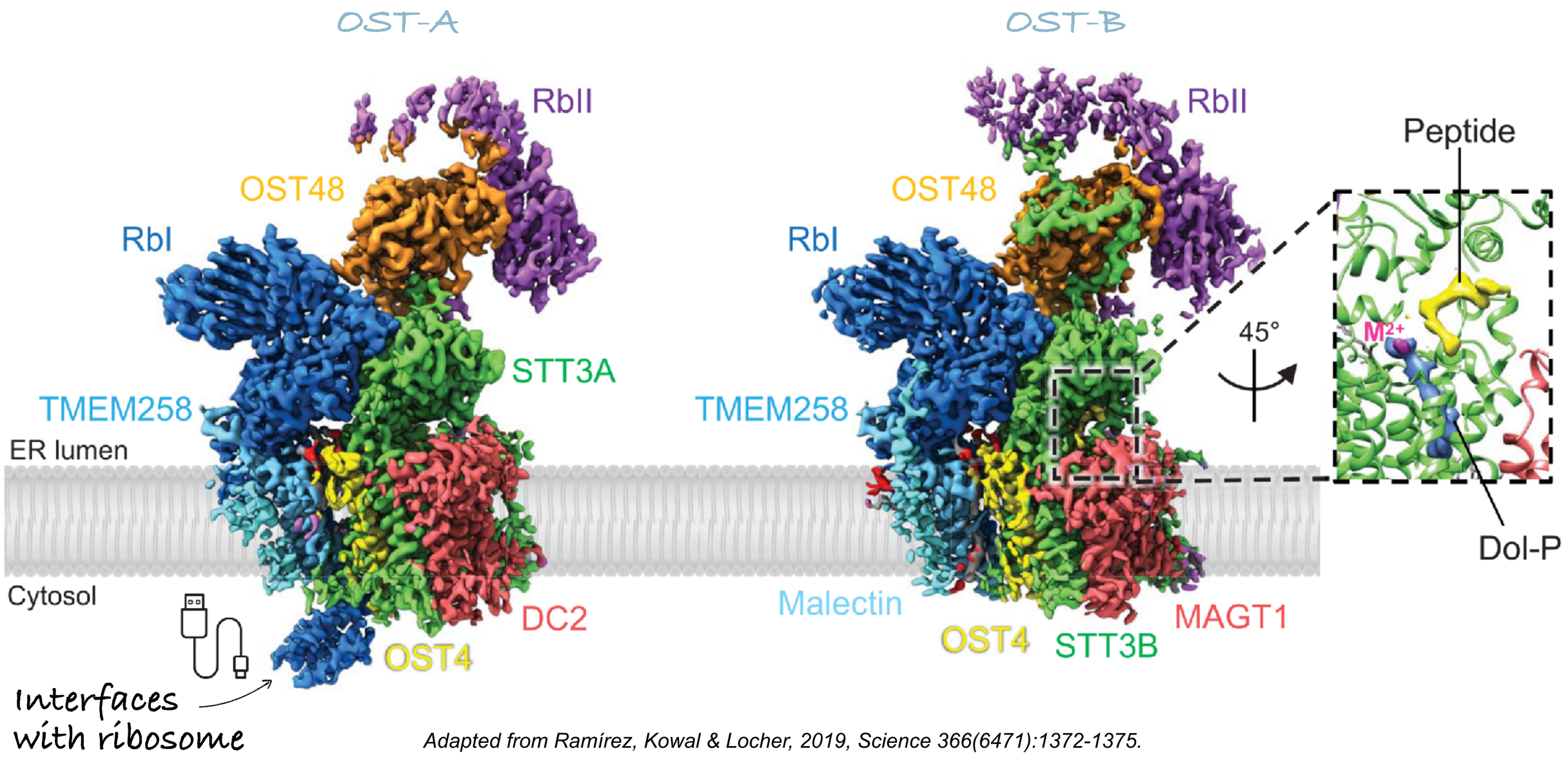
Protein glycosylation

Protein glycosylation

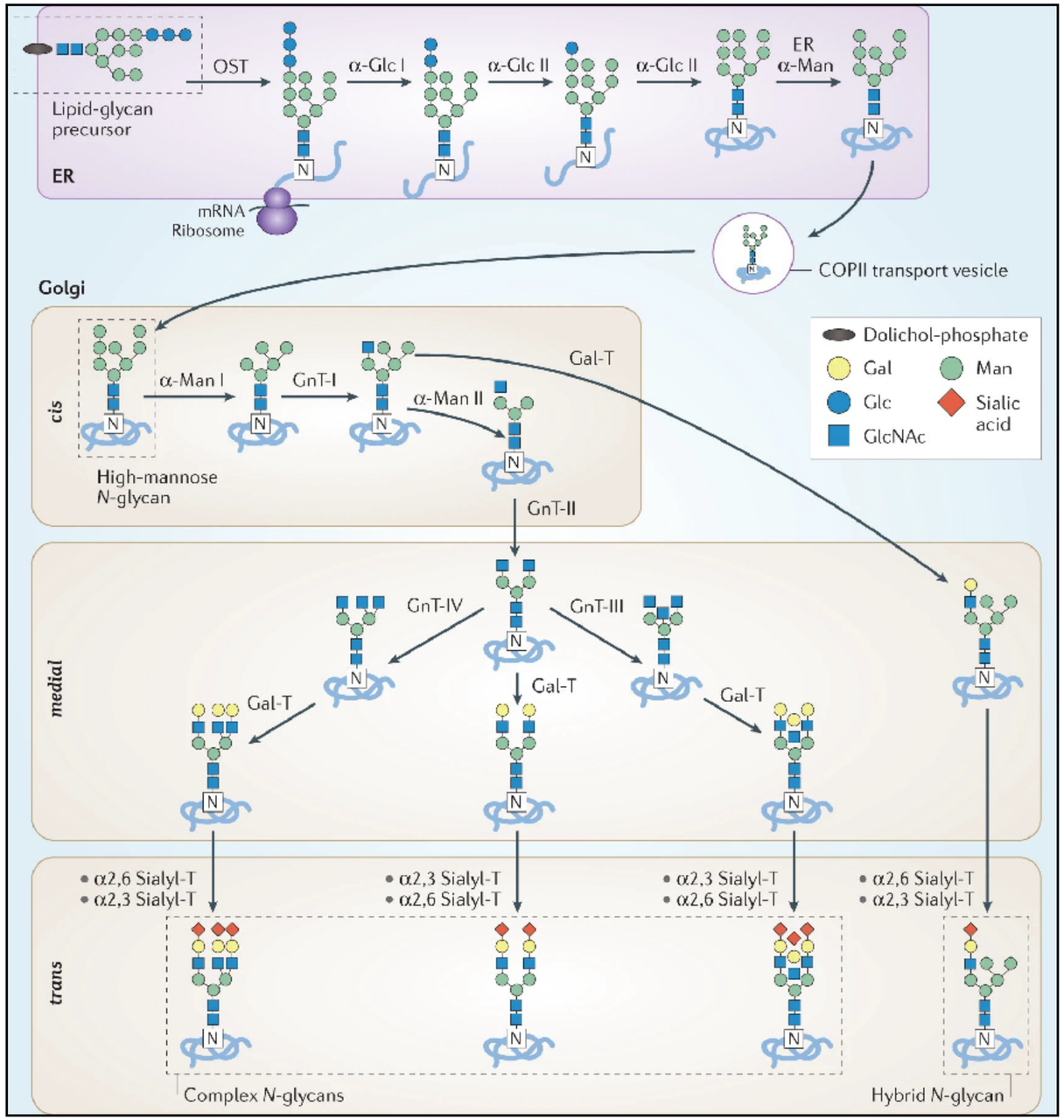


Co-translational

Post-translational



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

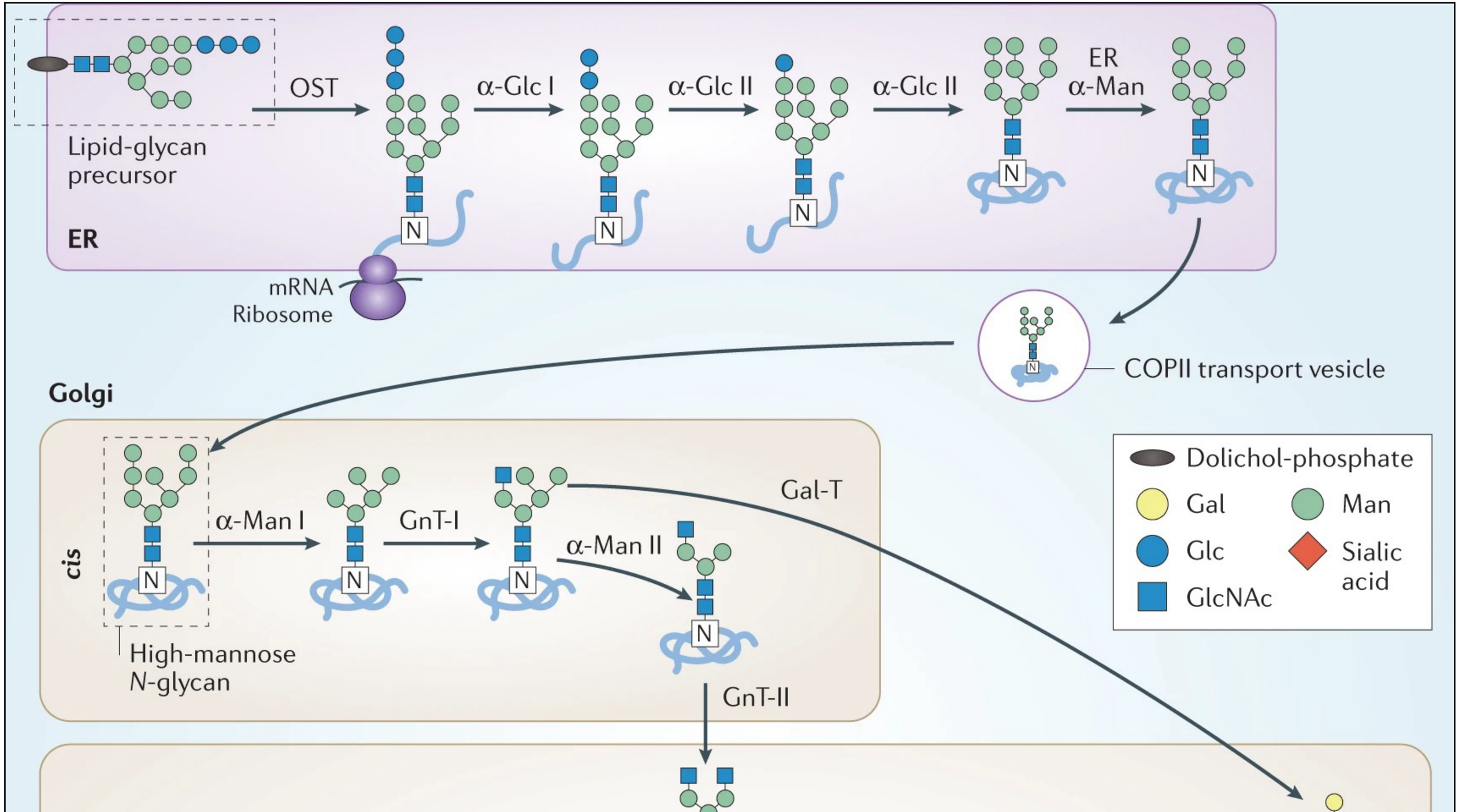


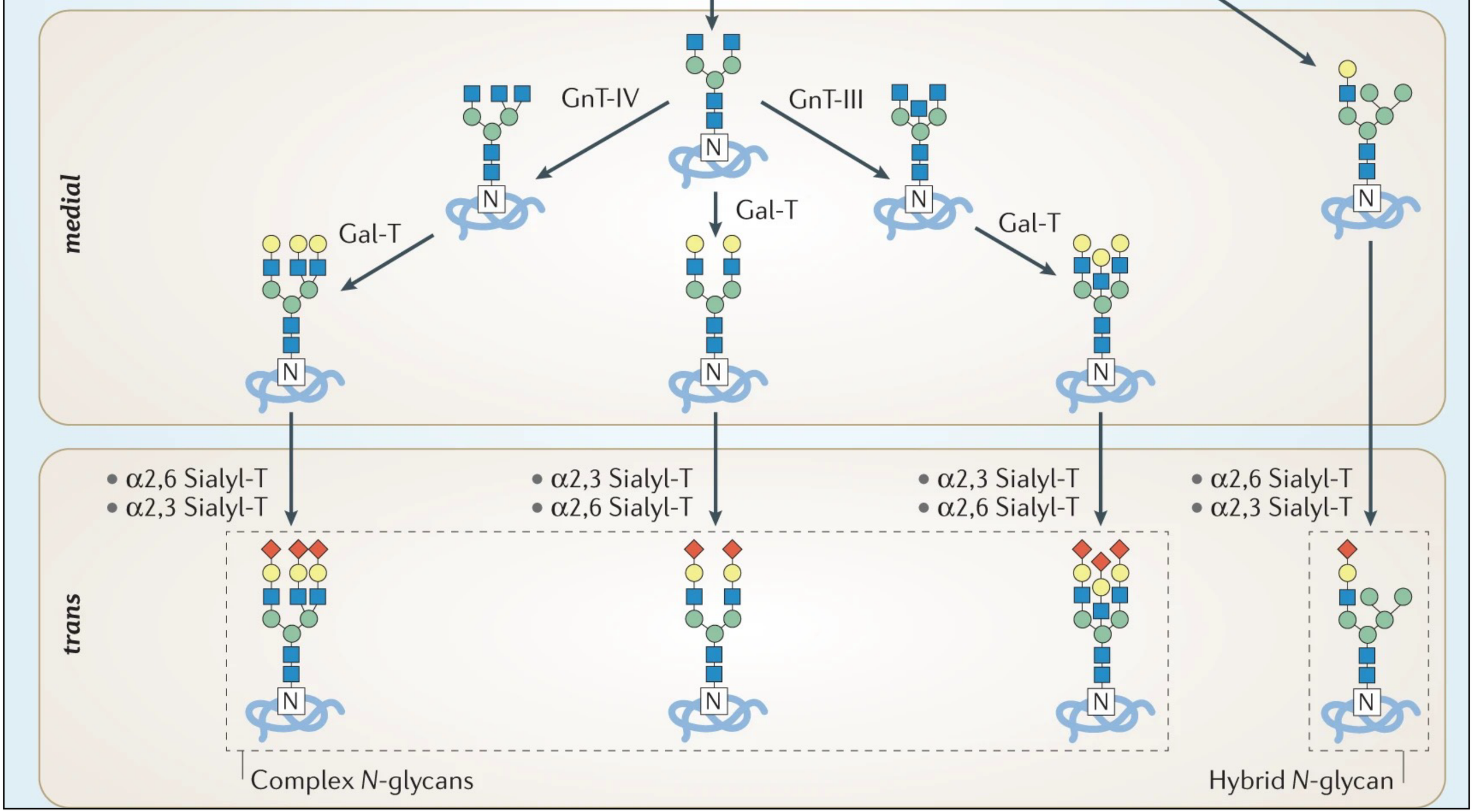
Reily, C., Stewart, T.J., Renfrow, M.B. & Novak, J., 2019, Nat Rev Nephrol 15:346–366.

Protein glycosylation

N-glycans

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

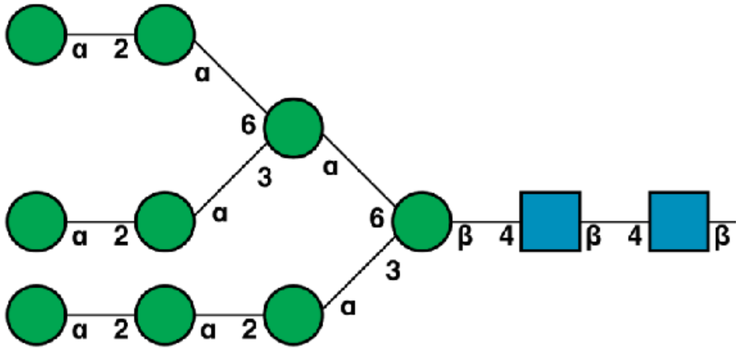

Lipid-glycan precursor
ER
OST
α-Glc I
α-Glc II
α-Glc II
ER α-Man
mRNA
Ribosome
COPII transport vesicle
Golgi
cis
α-Man I
GnT-I
α-Man II
Gal-T
High-mannose N-glycan
GnT-II
Dolichol-phosphate
Gal
Man
Glc
Sialic acid
GlcNAc


medial
trans
GnT-IV
GnT-III
Gal-T
Gal-T
Gal-T
N
• 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

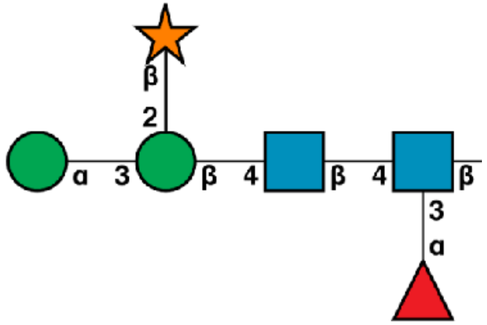
(a)

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



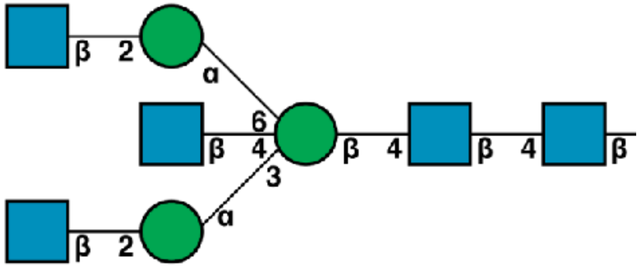
(b)

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



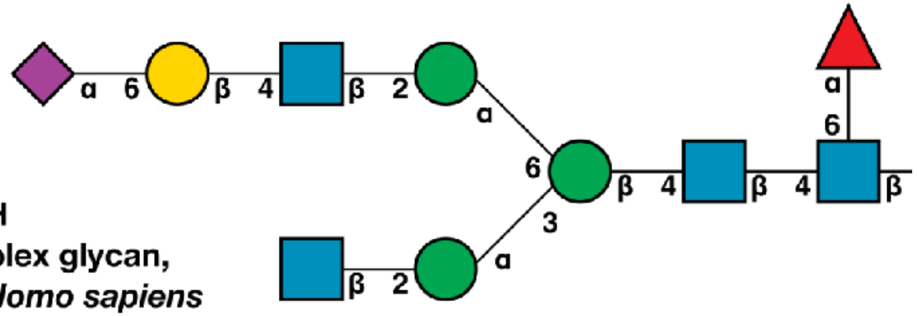
(c)

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



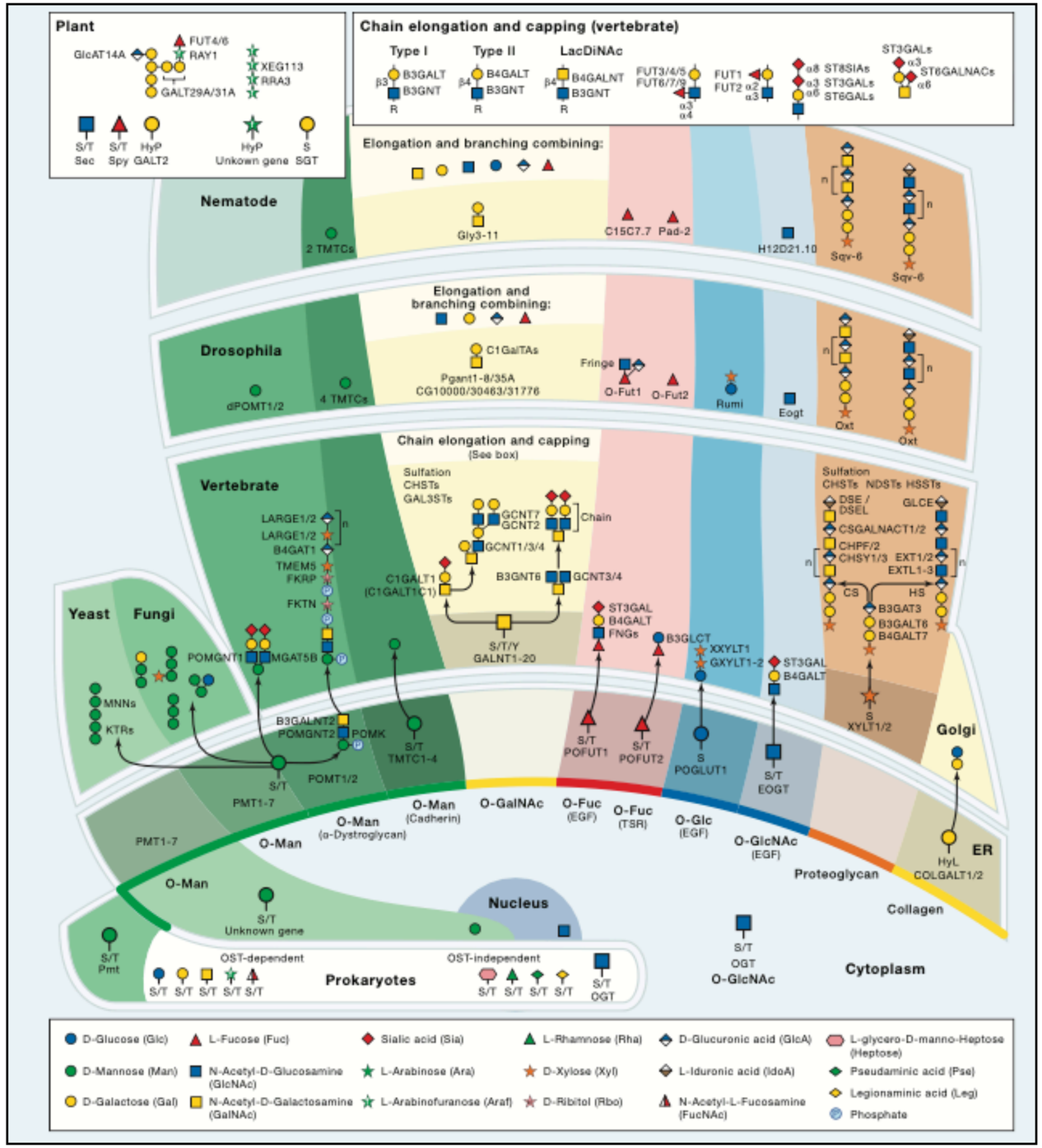
(d)

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



Symbol Nomenclature For Glycans (SNFG)

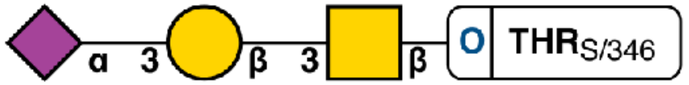
SNFG: Symbol Nomenclature for Graphical Representation of Glycans, Glycobiology 25: 1323-1324, 2015.



Protein glycosylation

O- and *C*-glycans

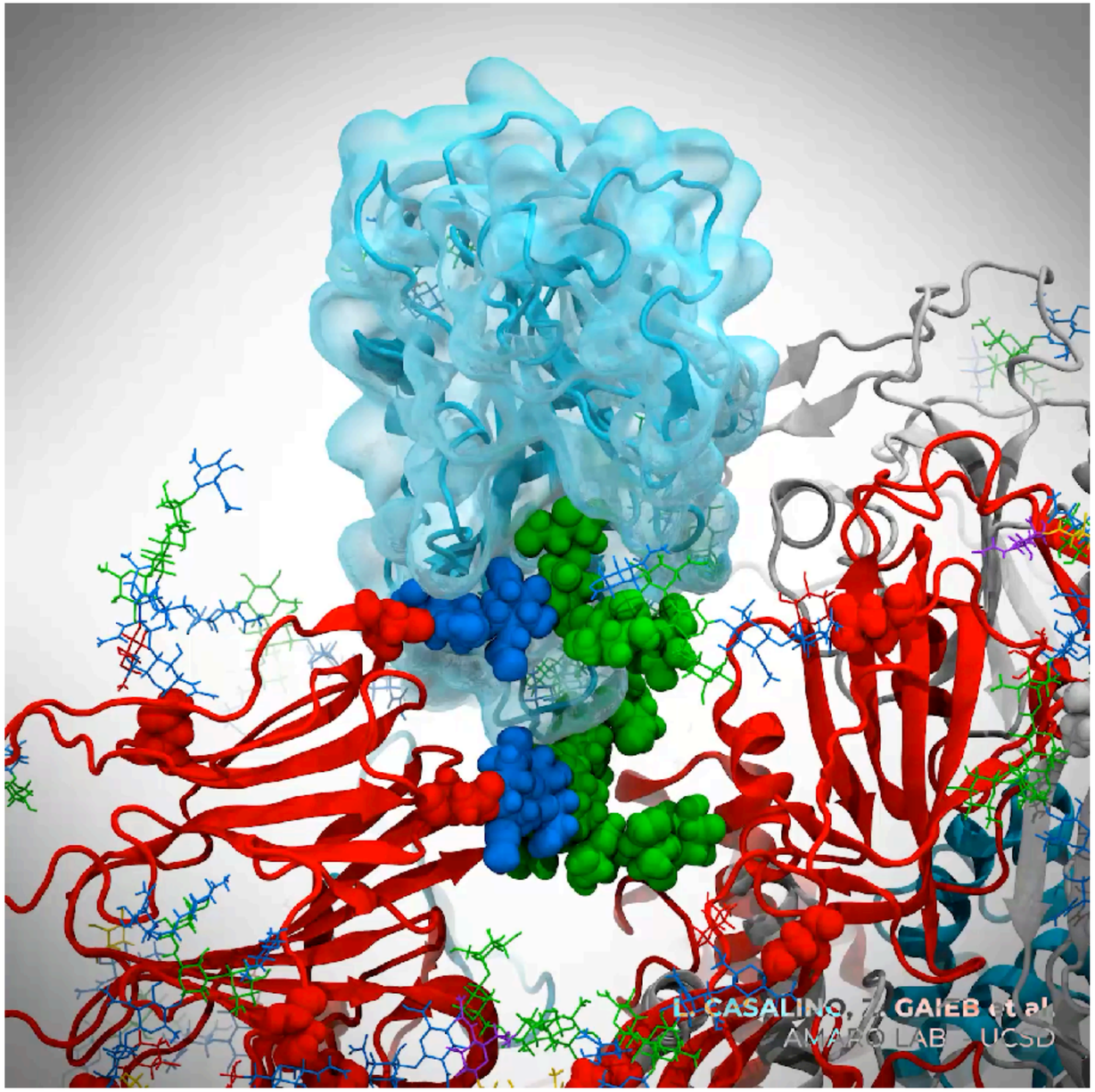
O-GalNAc (o-glycan)

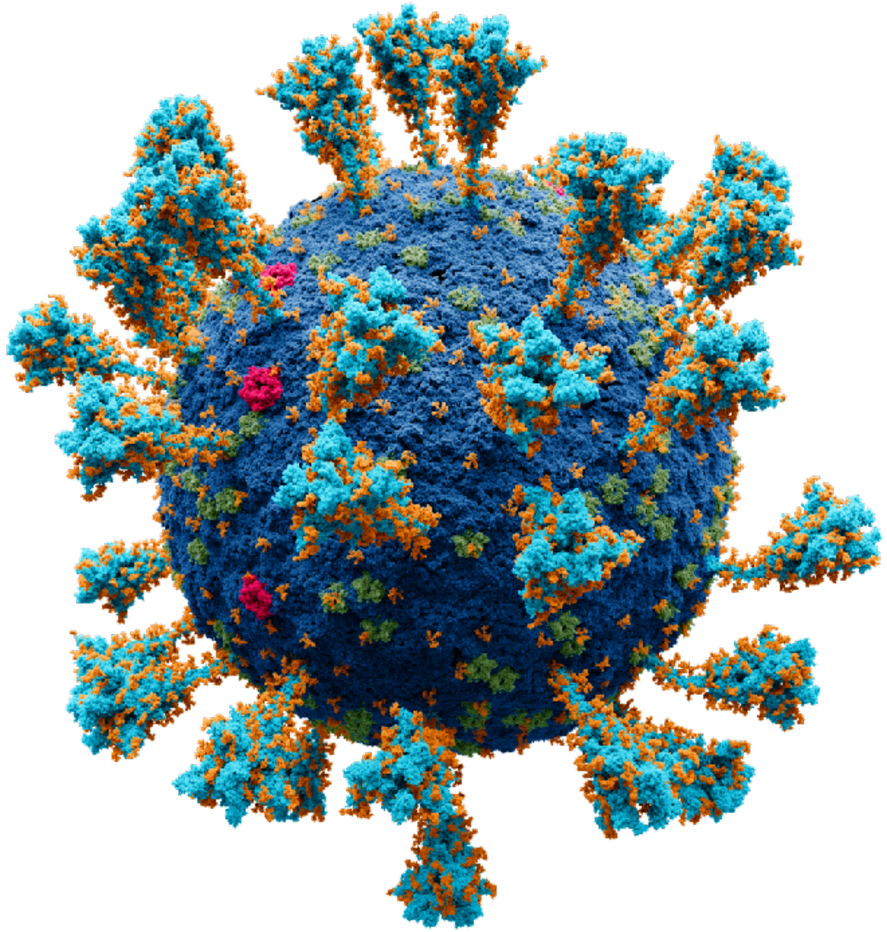


Tryptophan mannosylation (c-glycan)

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

Glycans matter





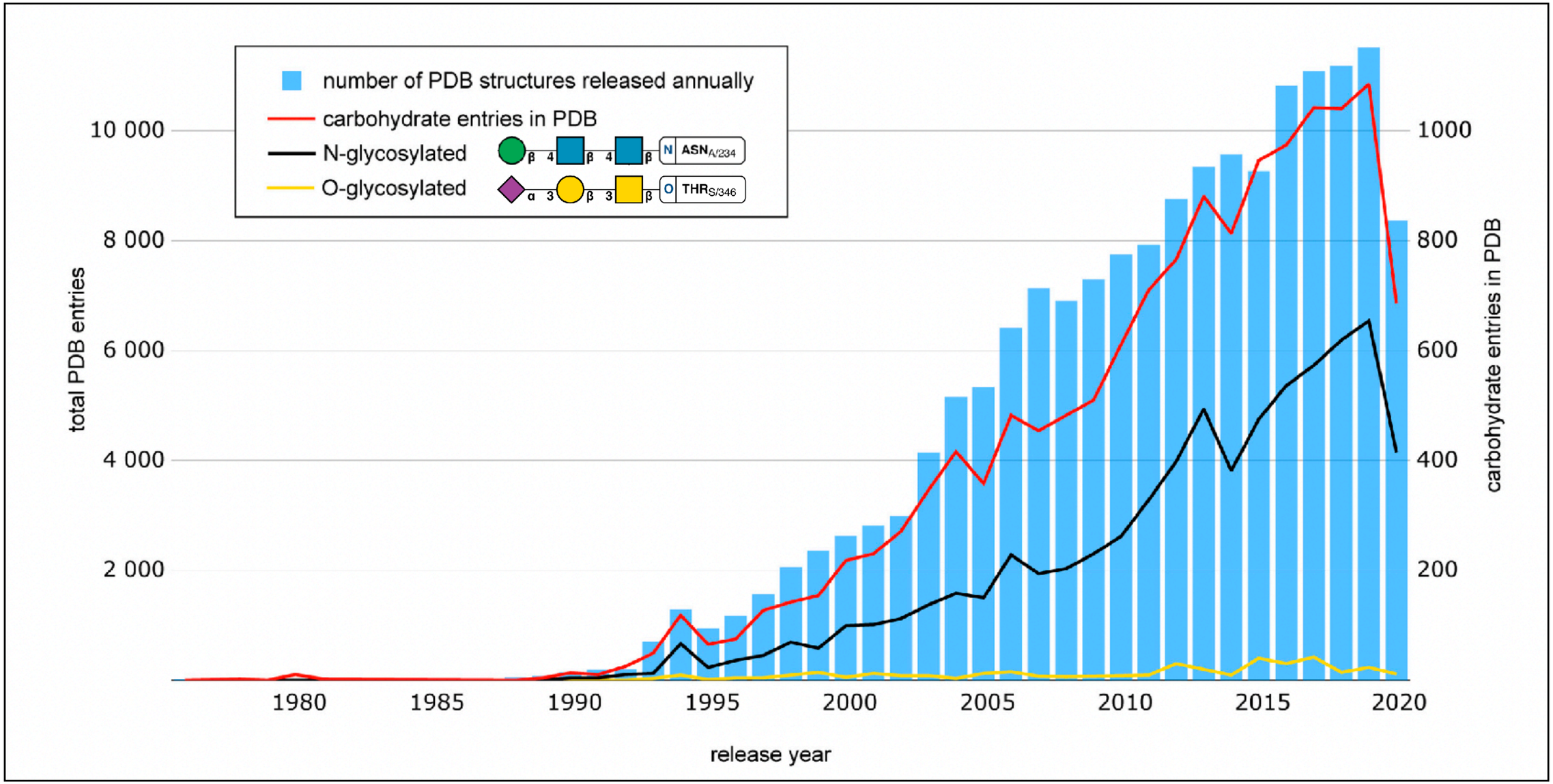
Casalino, Gaieb, Goldsmith, Hjorth, Dommer, Harbison, Fogarty, Barros, Taylor, McLellan, Fadda, 2021, ACS Central Science 6(10):1722-34.

Mechanistic studies show
how things may work

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

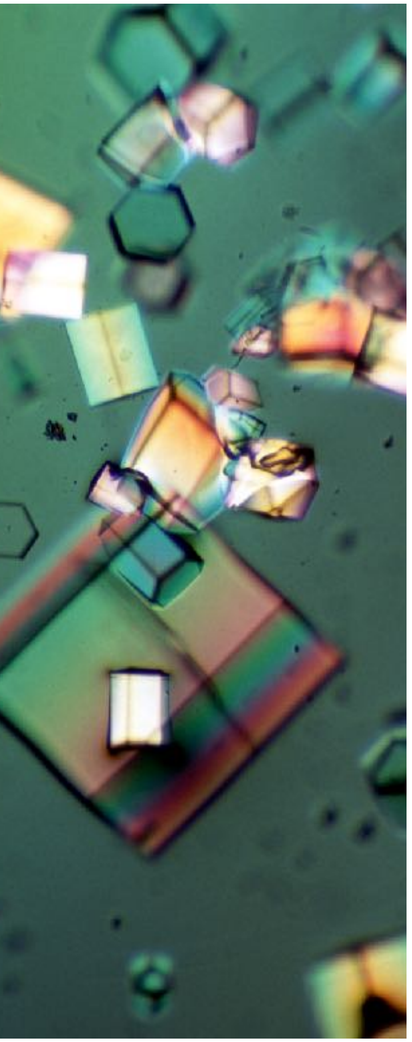
Are all atomic models correct?

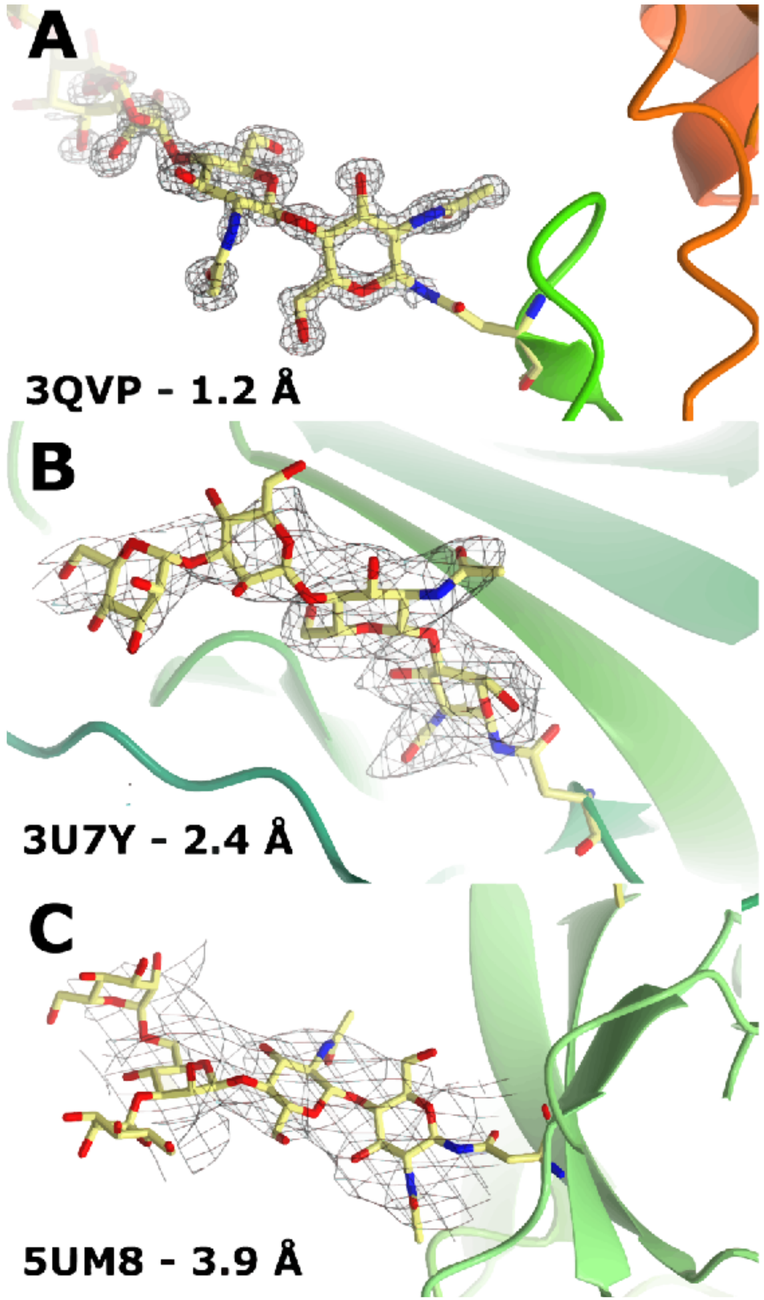
Carbohydrates in the PDB

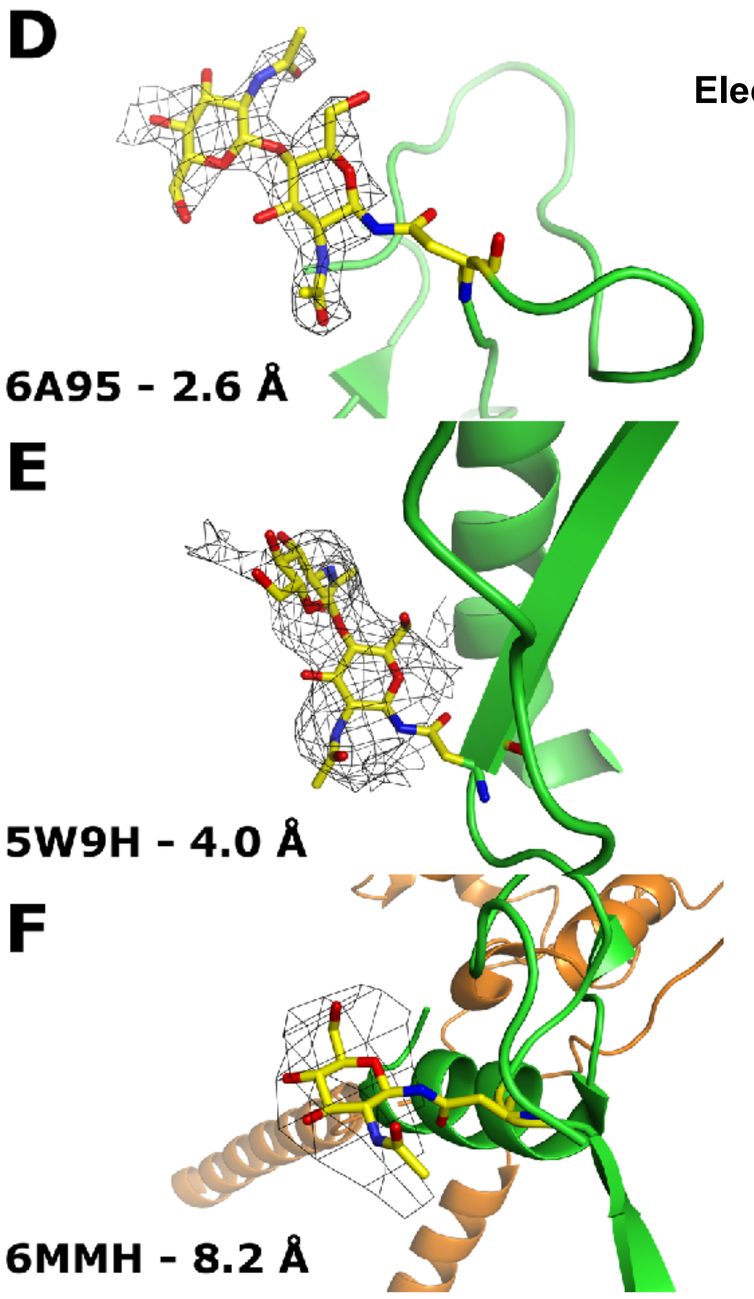


Scherbinina and Toukach, 2020 IJMS 21, 7702

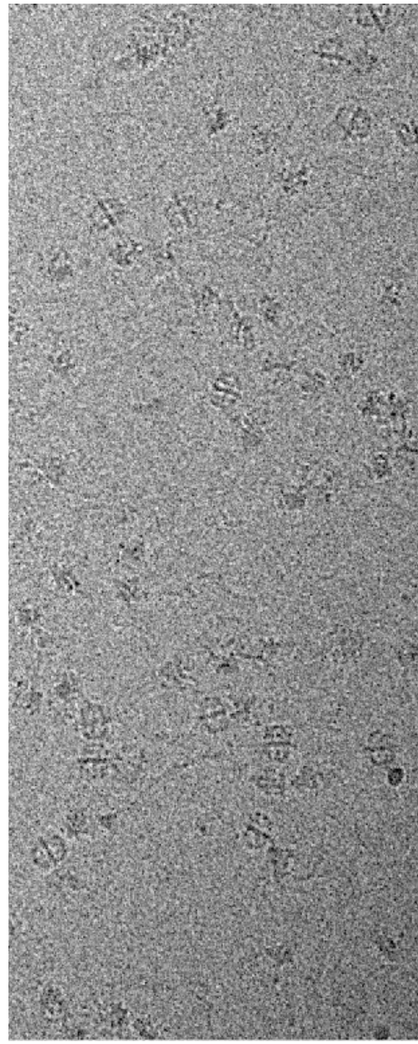
X-ray Crystallography







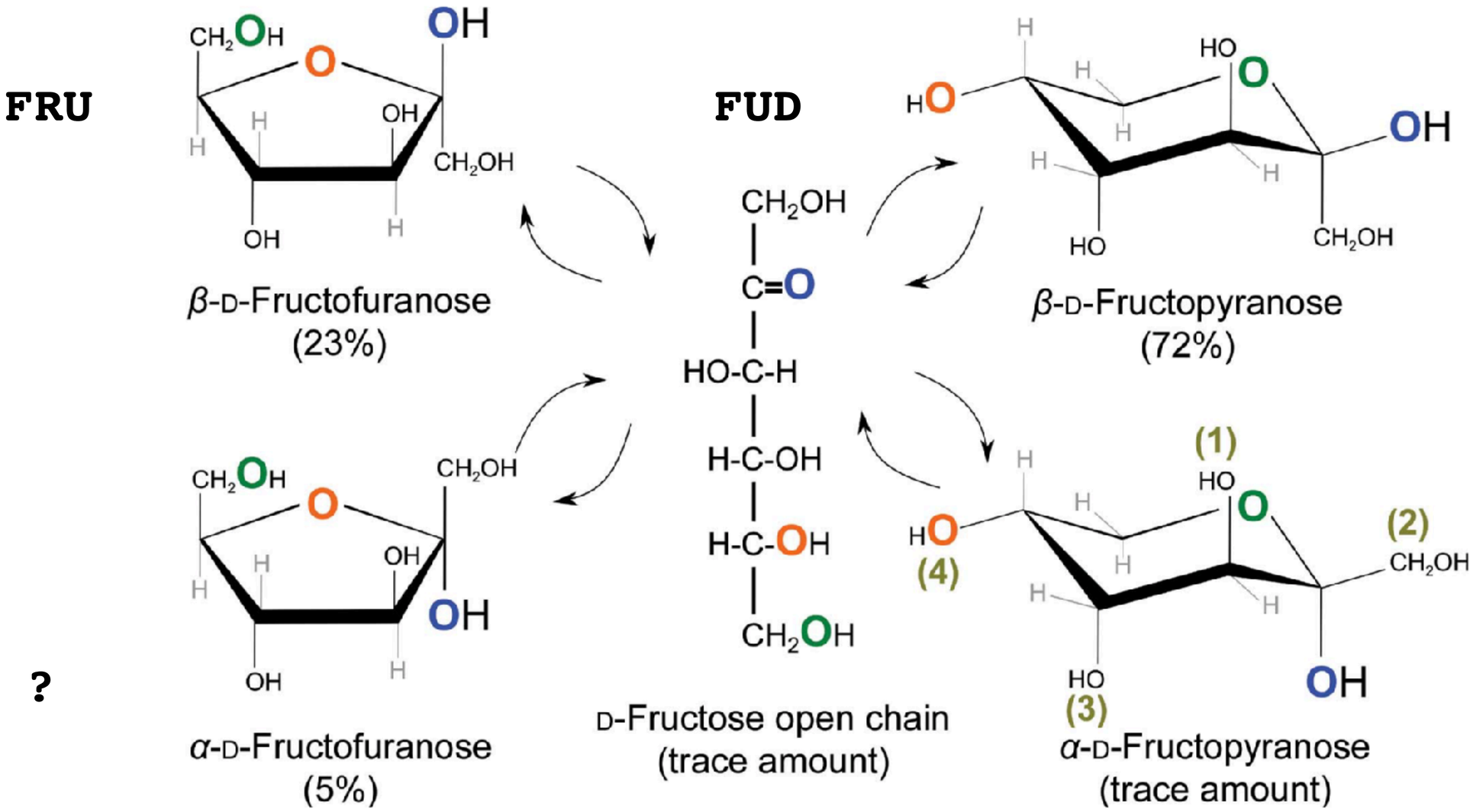
Electron Cryo-Microscopy



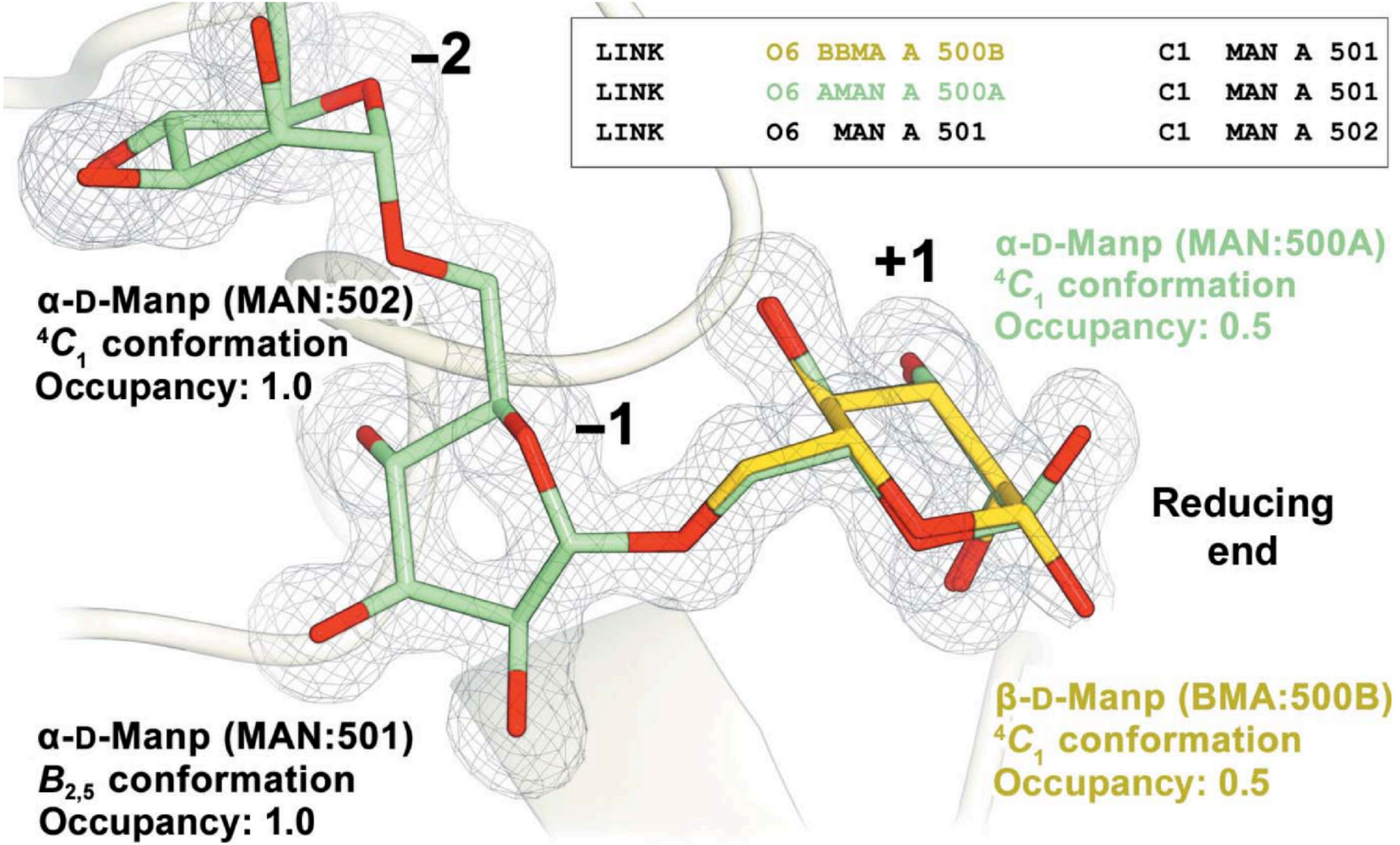
PDBe
Protein Data Bank in Europe

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

5 forms = 5 codes 👀

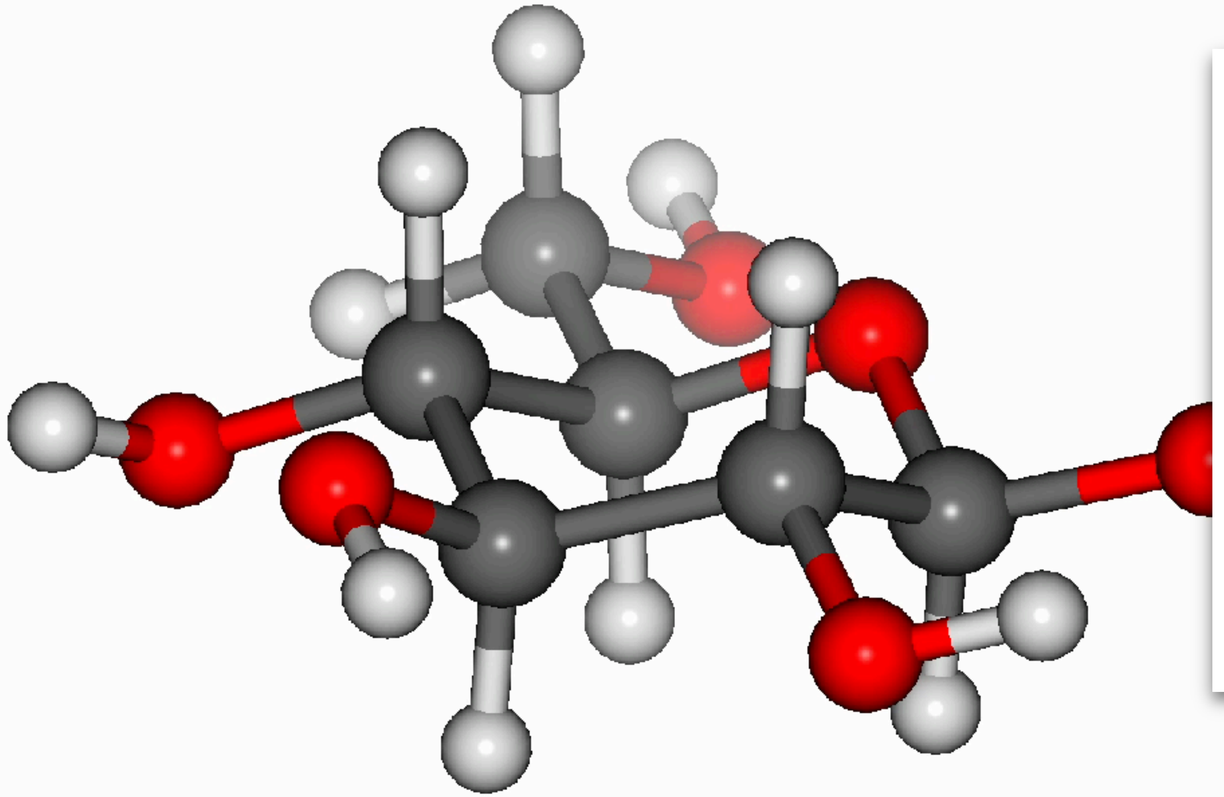


Agirre, 2017, Acta Cryst D73(2):171-186



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

Ring conformation

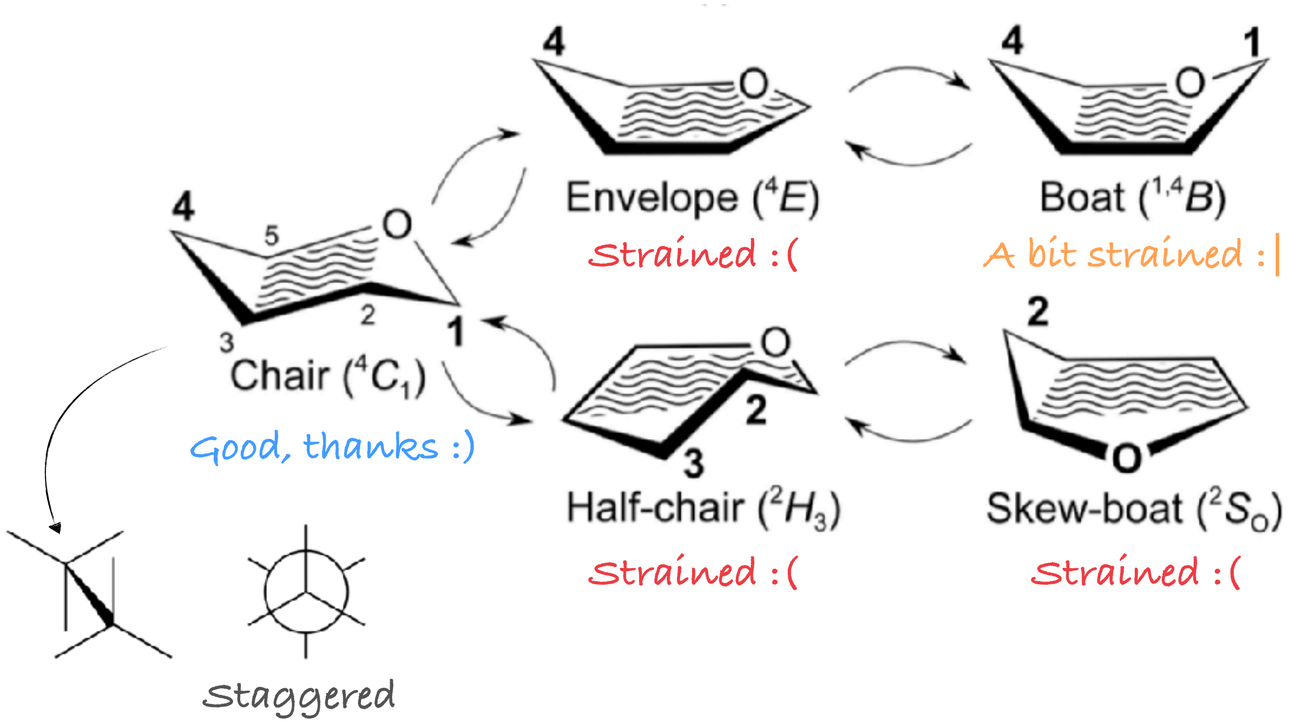


a $^{4}C_{1}$ chair

chairs are comfortable for sugars

Ring conformation

Wavy lines indicate which atoms are roughly coplanar



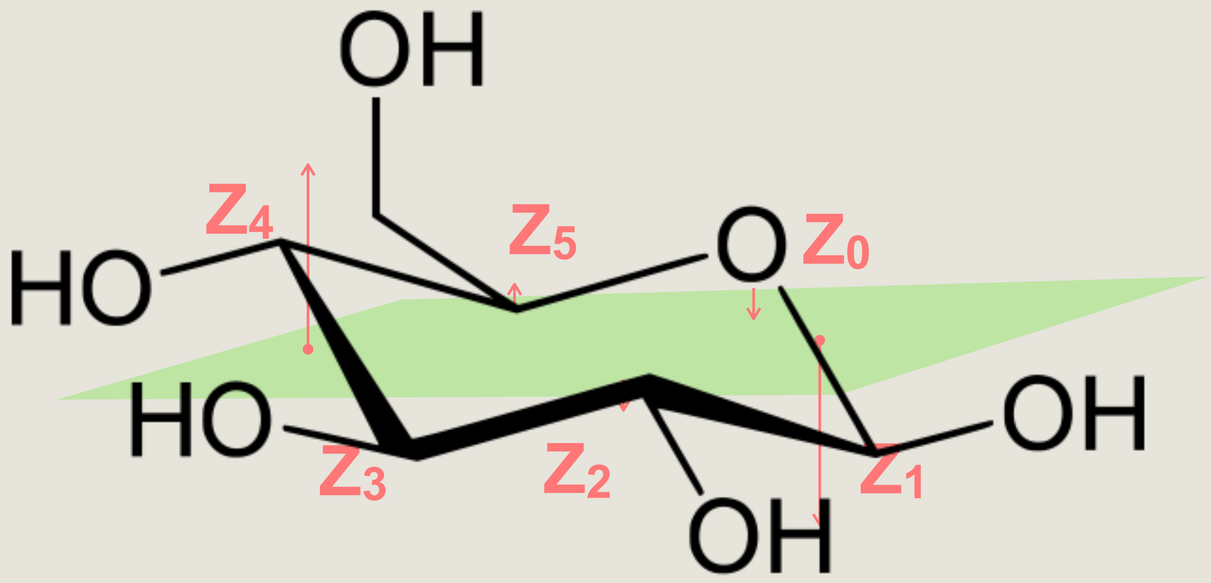
Pyranose forms
- 6-membered rings
- 5 ring puckers
- 38 conformations

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

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:

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

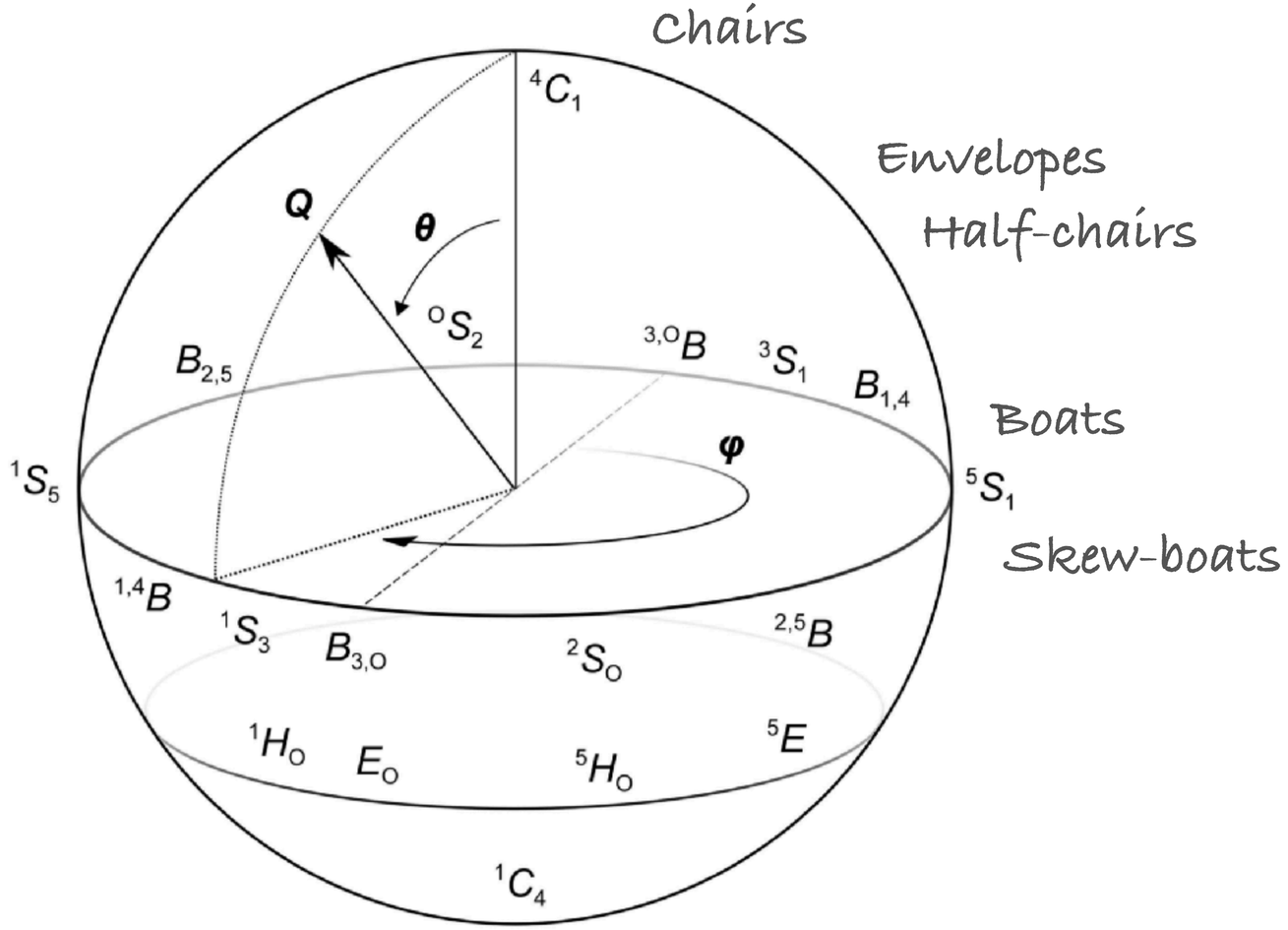
"total puckering amplitude"

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

Cremer & Pople, 1975, JACS 97(6)

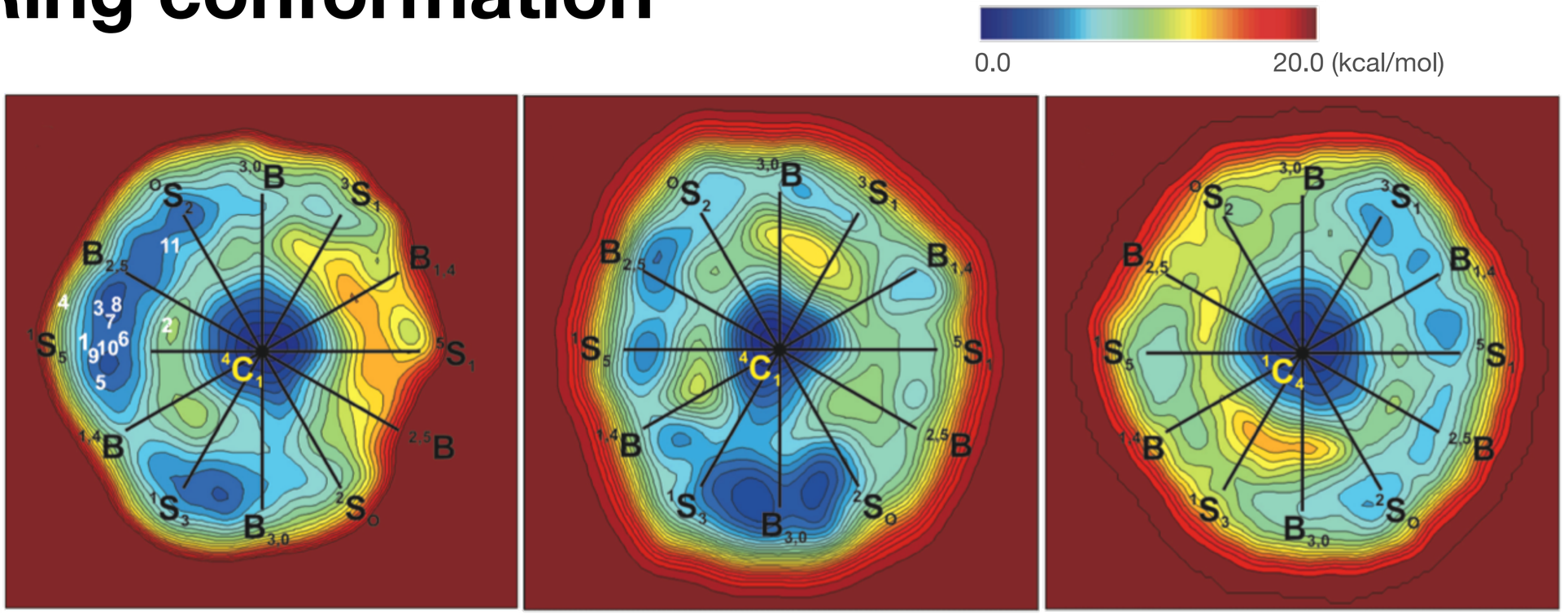
Ring conformation

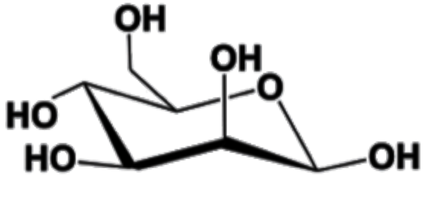
The Cremer-Pople algorithm



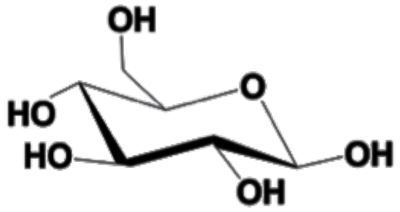
Agirre, 2017, Acta Cryst D73(2):171-186

Ring conformation

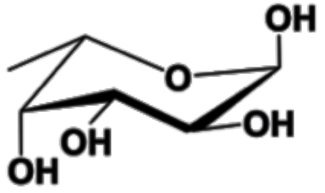




β-D-Mannopyranose



β-D-Glucopyranose

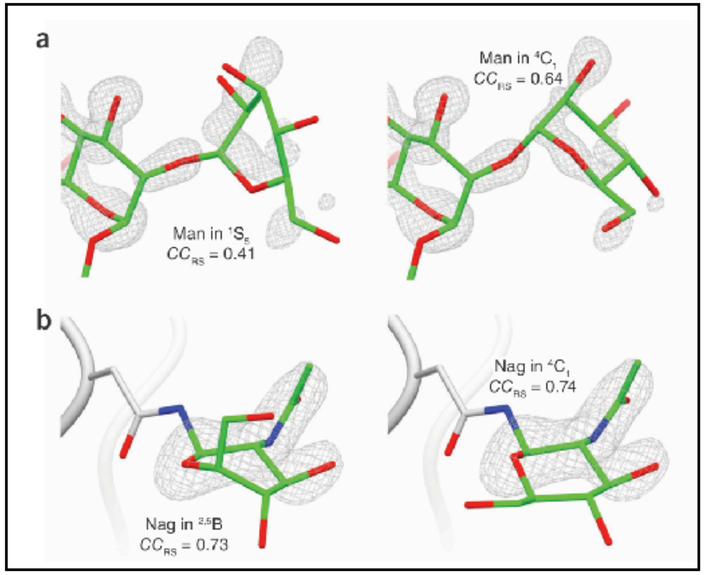


α-L-Fucopyranose

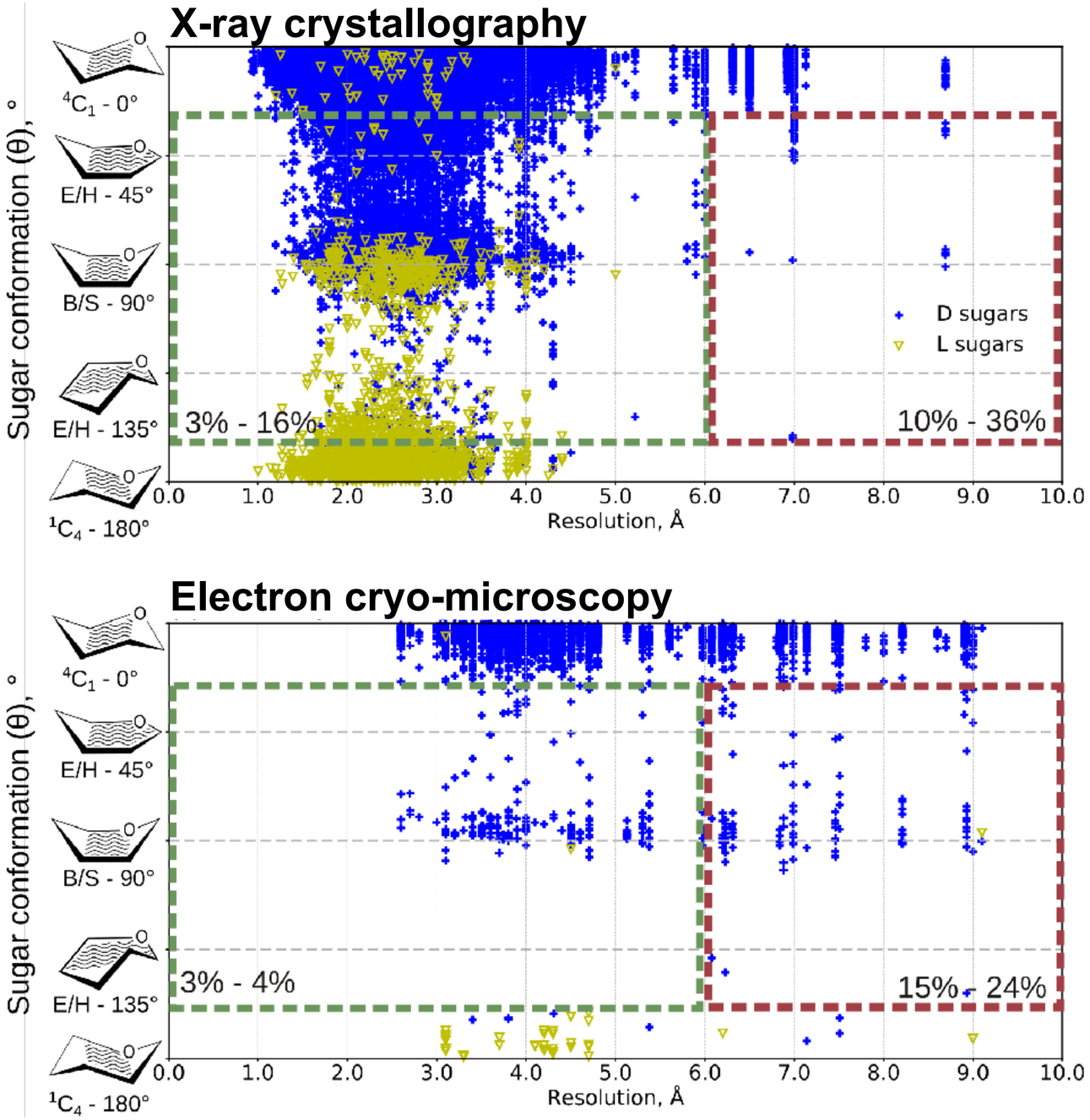
Adapted from Ardevol, Biarnes, Planas & Rovira, 2010, JACS 132(45).

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.

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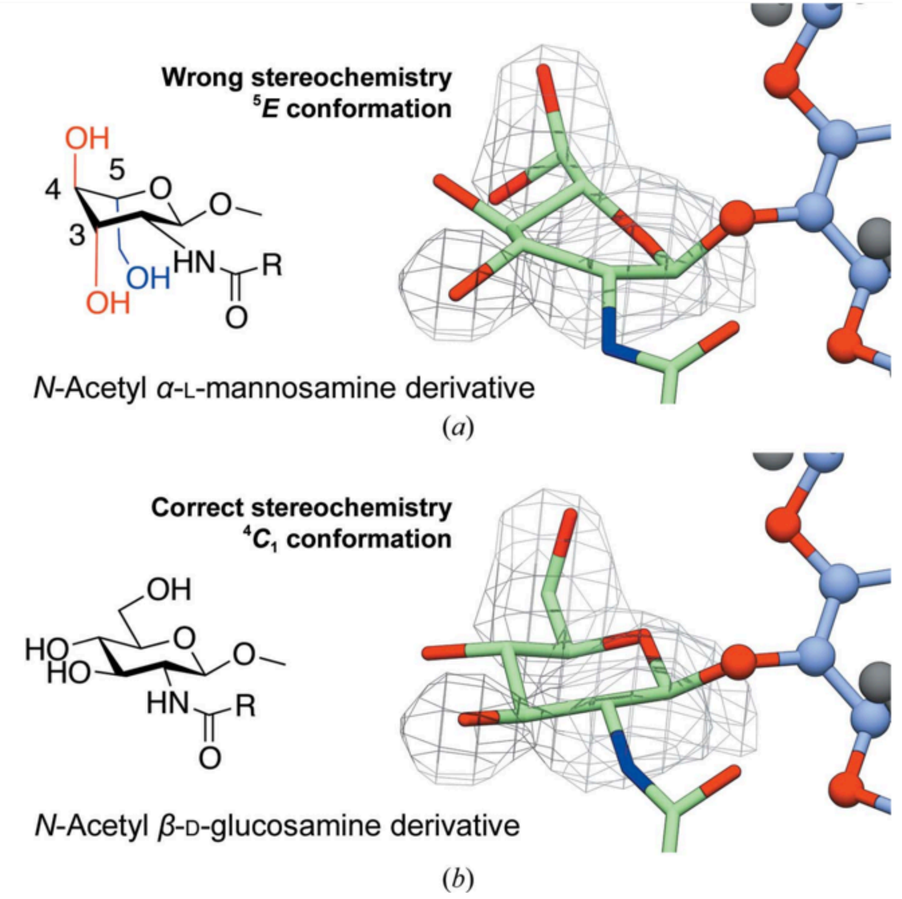
- Software for **validation** of carbohydrates for both crystallography and electron microscopy.
- Detects issues with regiochemistry, stereochemistry and conformation.

Available in:
- CCP4
- CCP-EM
- Web App

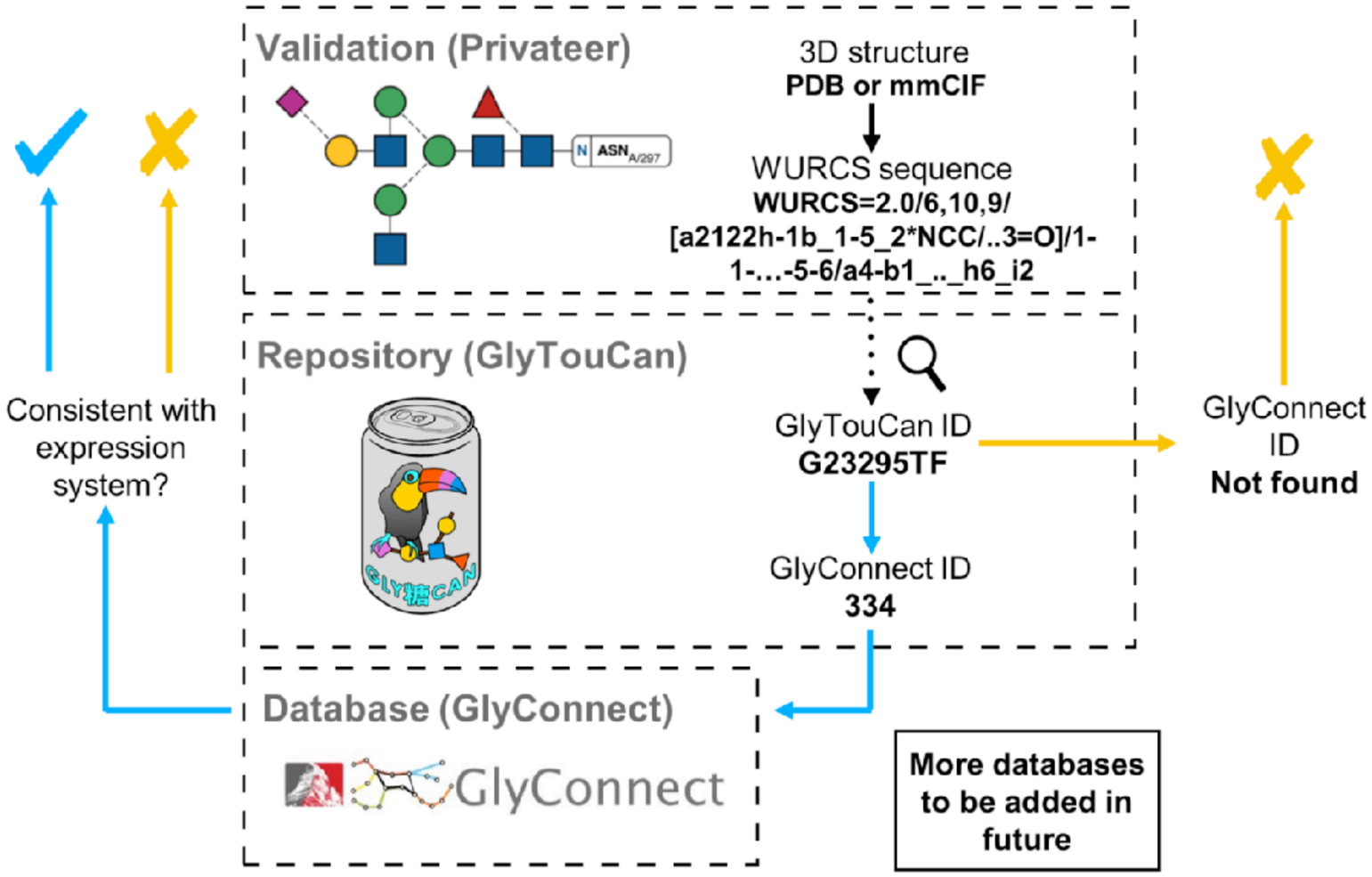


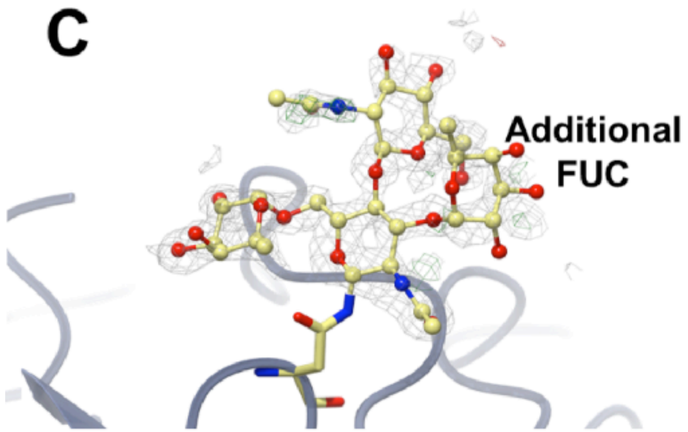
Errors in sugar stereochemistry. From Agirre, J. Strategies for carbohydrate model building, refinement and validation. *Acta Crysta D.* **D73**, 178-186 (2017)

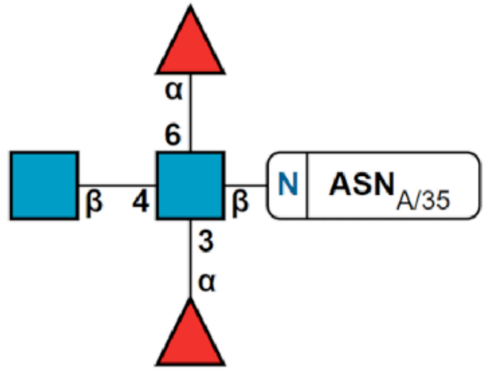
Privateer

- 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.

Glycomics powered validation







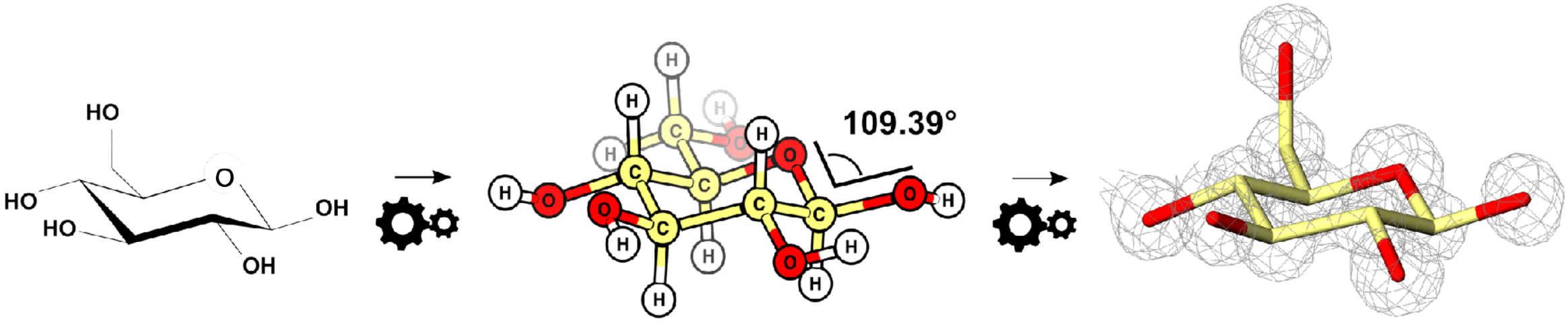
WURCS=2.0/2,4,3/[a2122h-1b_1-5_2*NCC/3=O][a1221m-1a_1-5]/1-2-1-2/a3-b1_a4-c1_a6-d1

GlyTouCan ID: G63564LA

GlyConnect ID: 145

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

New carbohydrate dictionaries



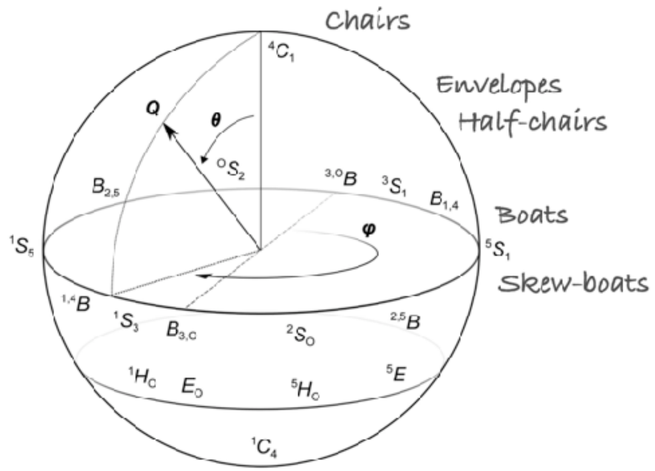
chemical description

restraints & coordinates

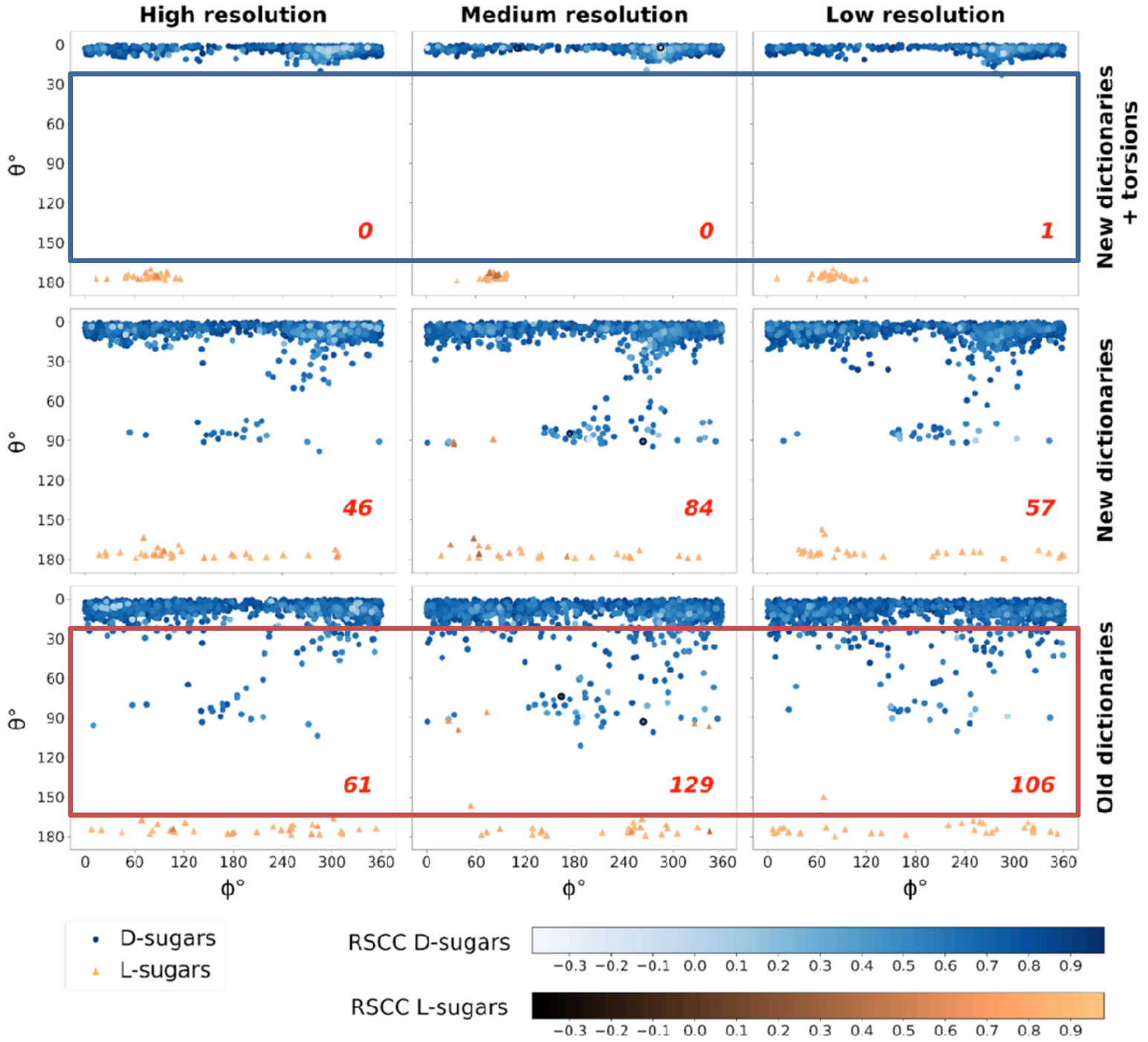
fitted model

Conformational restraints
N-glycosylation

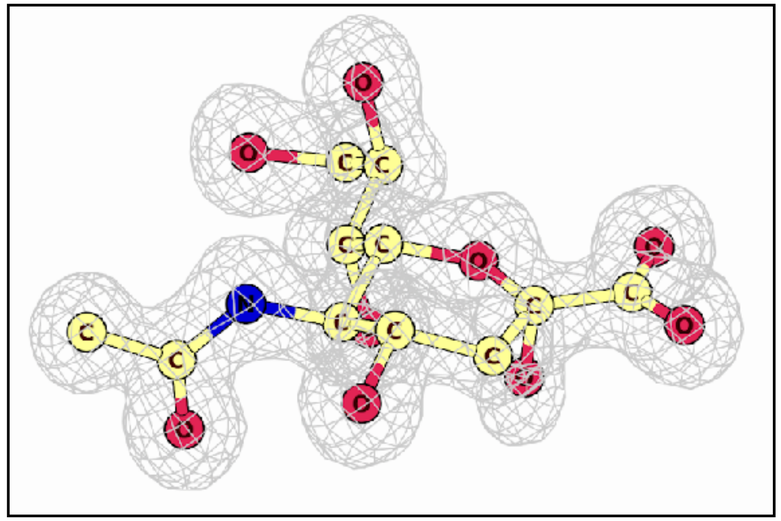
No conformational distortions expected!



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

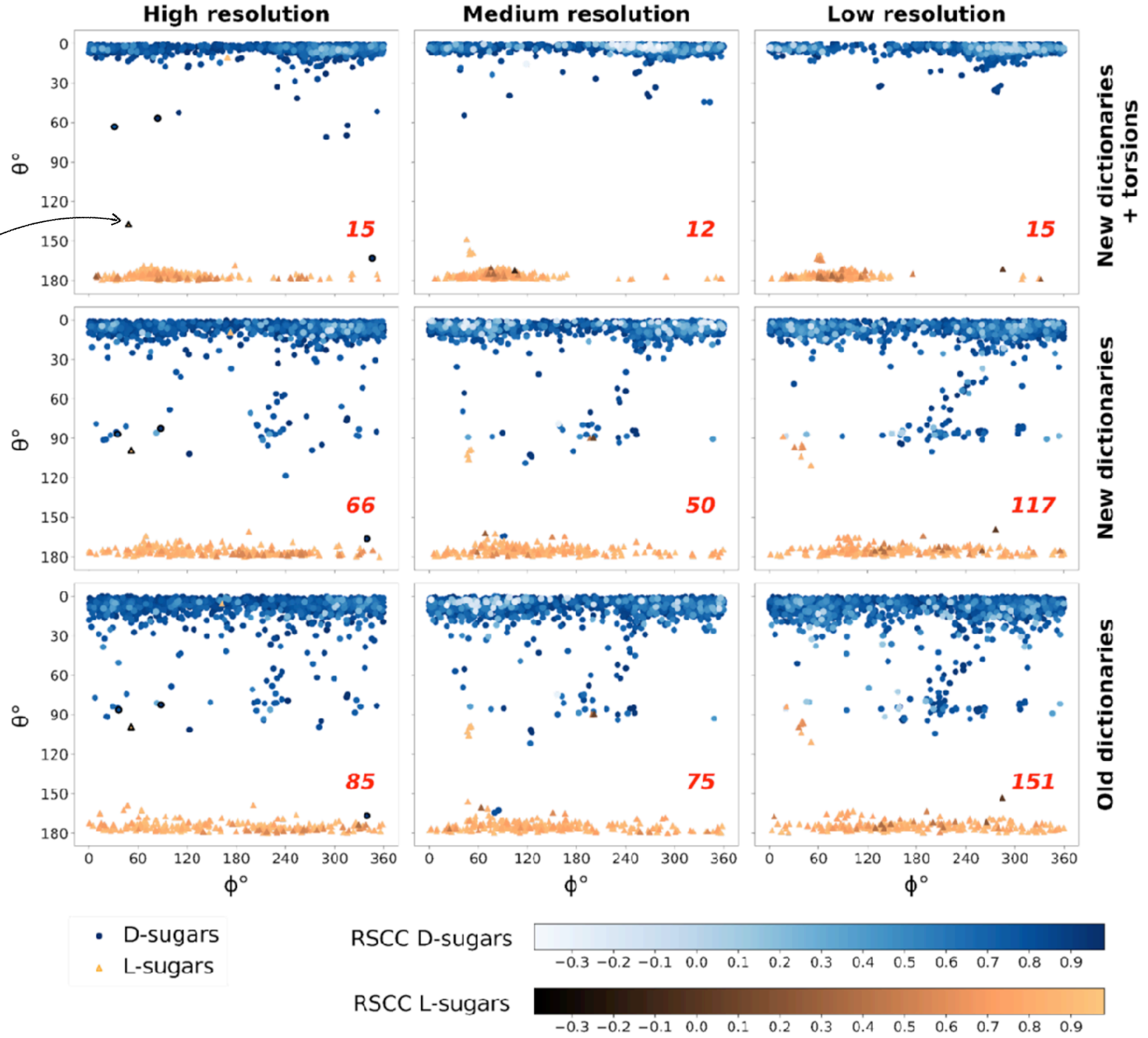


Conformational restraints
Ligands

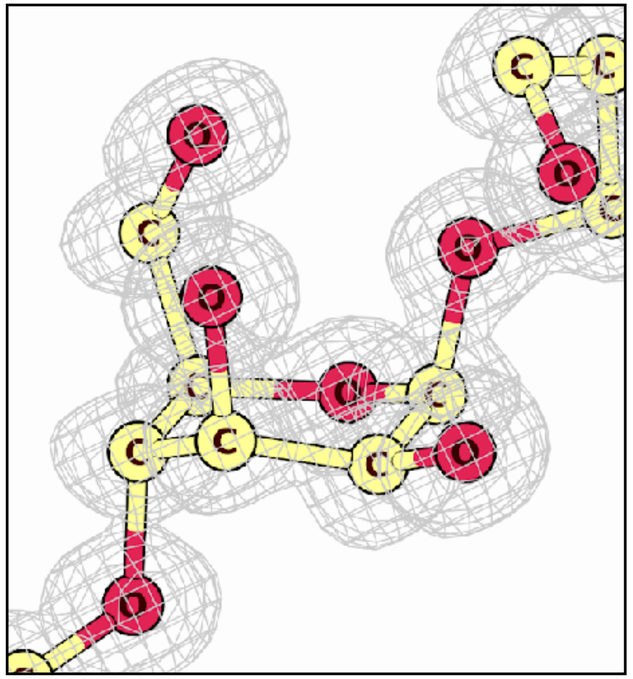


6HG0 (SIA-A-522) in ^{4}E
1.30 Å resolution, RSCC=0.94

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

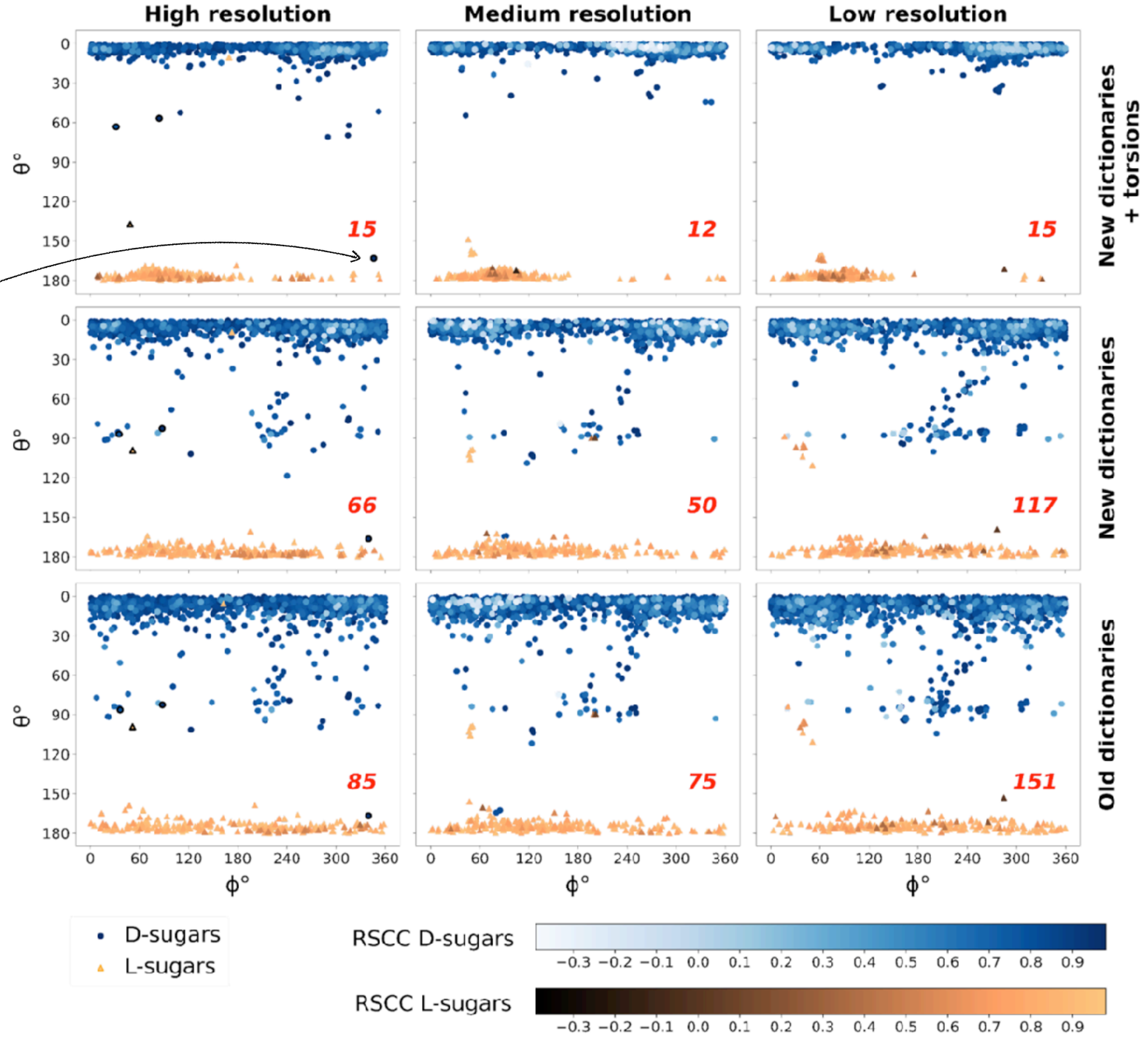


Conformational restraints
Ligands

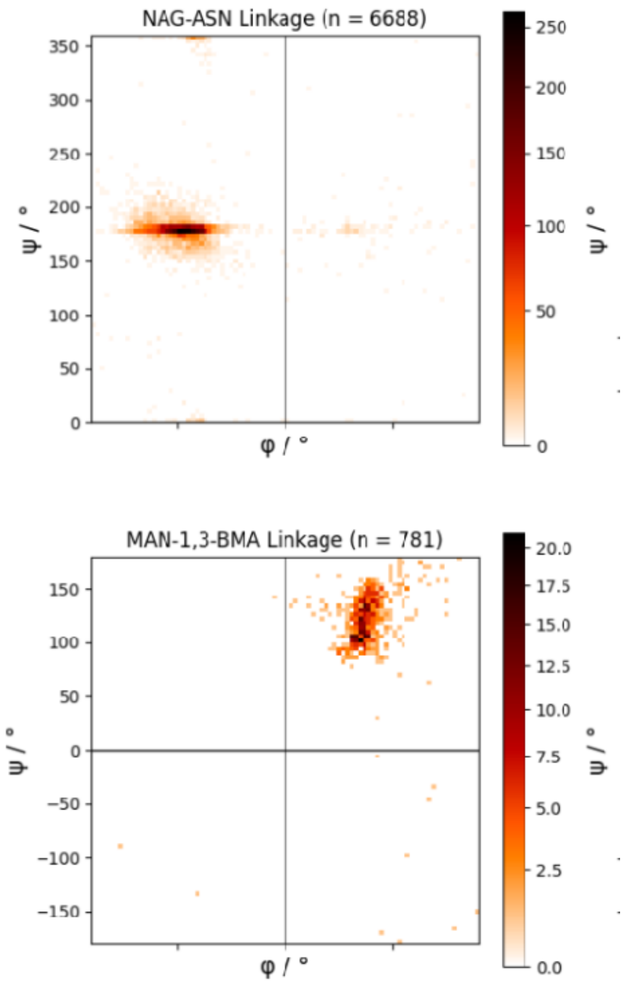


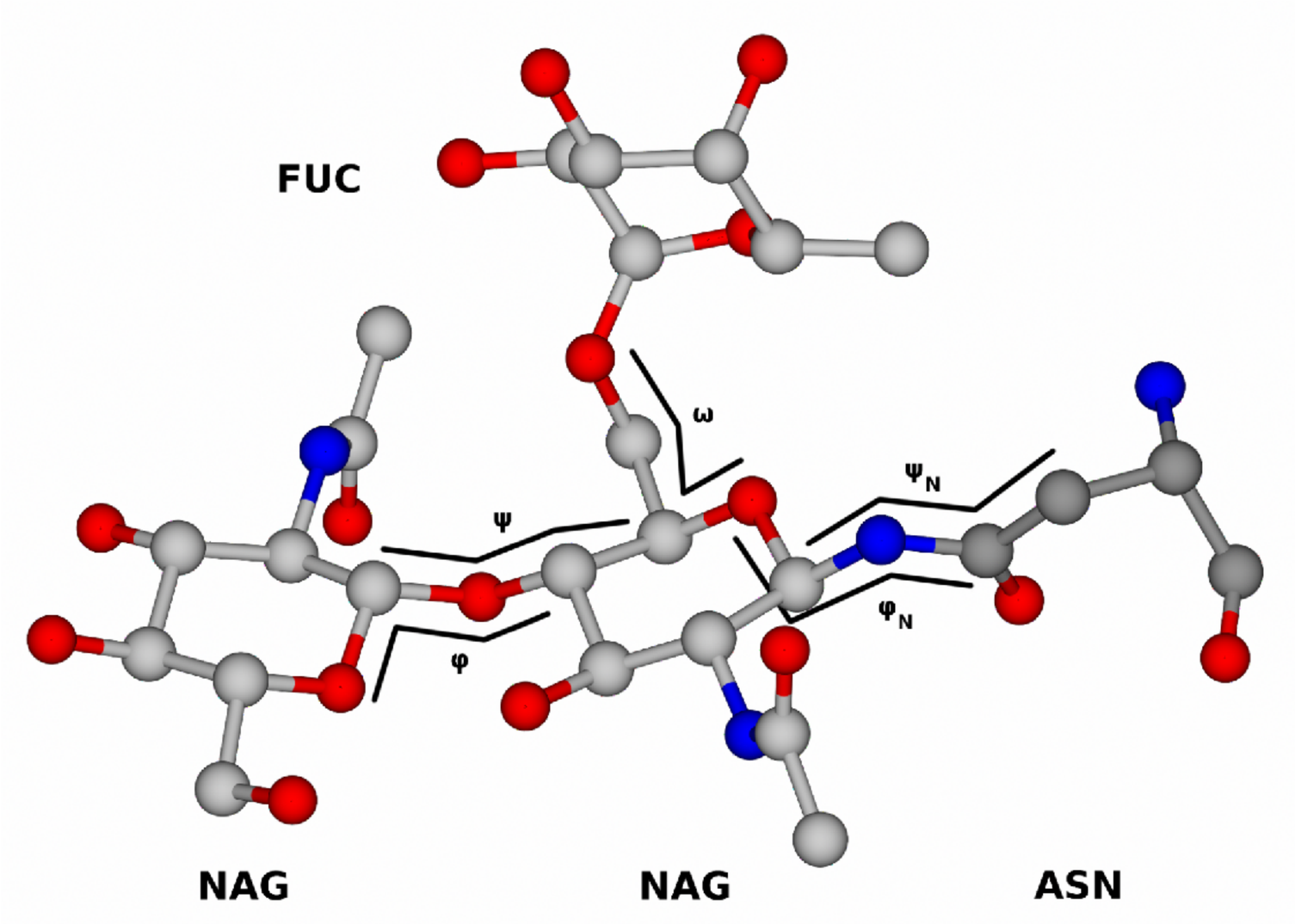
5JUG (BMA-B-3) in $^1C_4$
0.95 Å resolution, RSCC=0.95

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

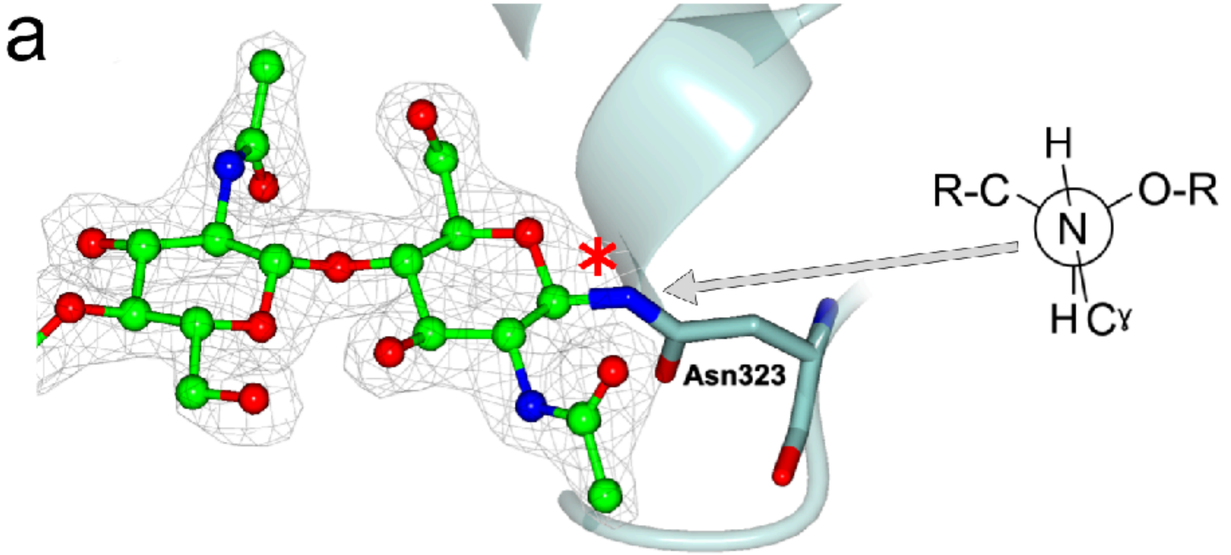


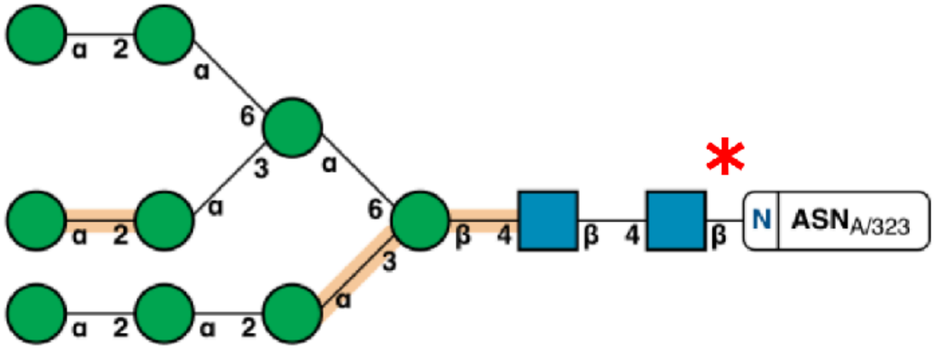
Analysis of linkage torsions

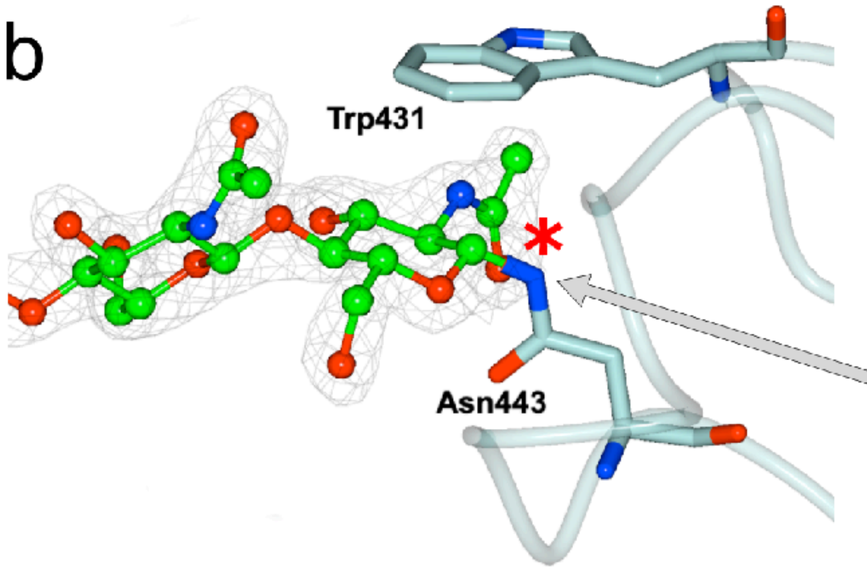


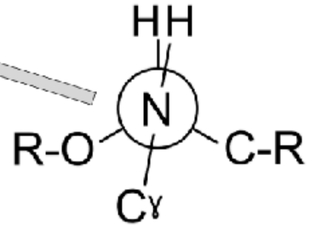


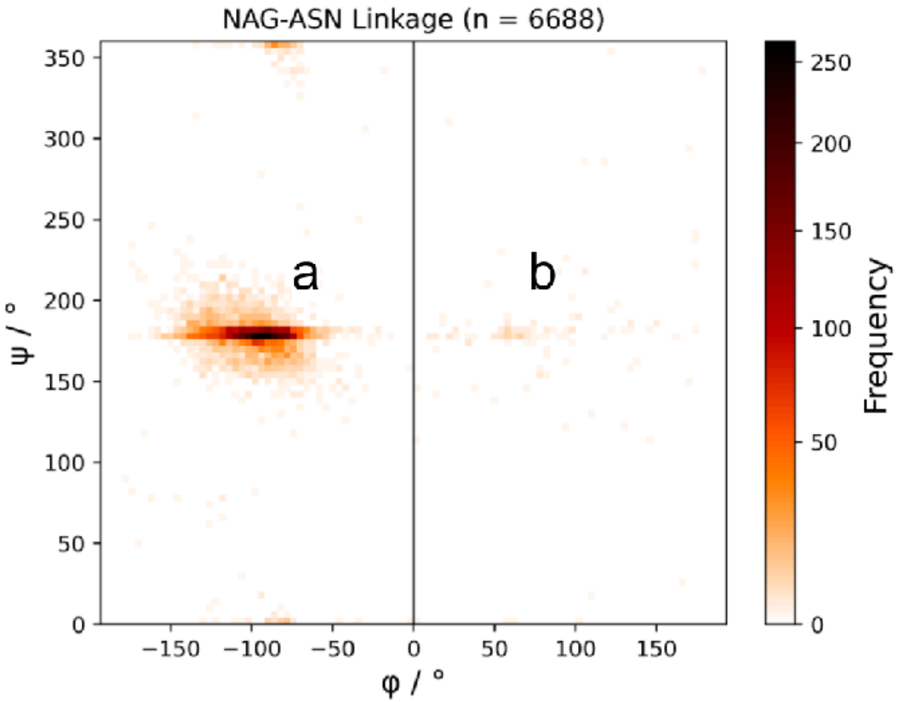
Dialpuri, J. S., Bagdonas, H., Atanasova, M., Schofield, L. C., Hekkelman, M. L., Joosten, R. P. & Agirre, J., 2023. Analysis and validation of overall N-glycan conformation in Privateer. Acta Crystallographica Section D, 79, 462-472.

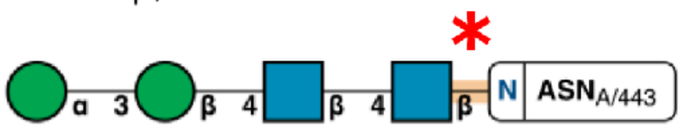












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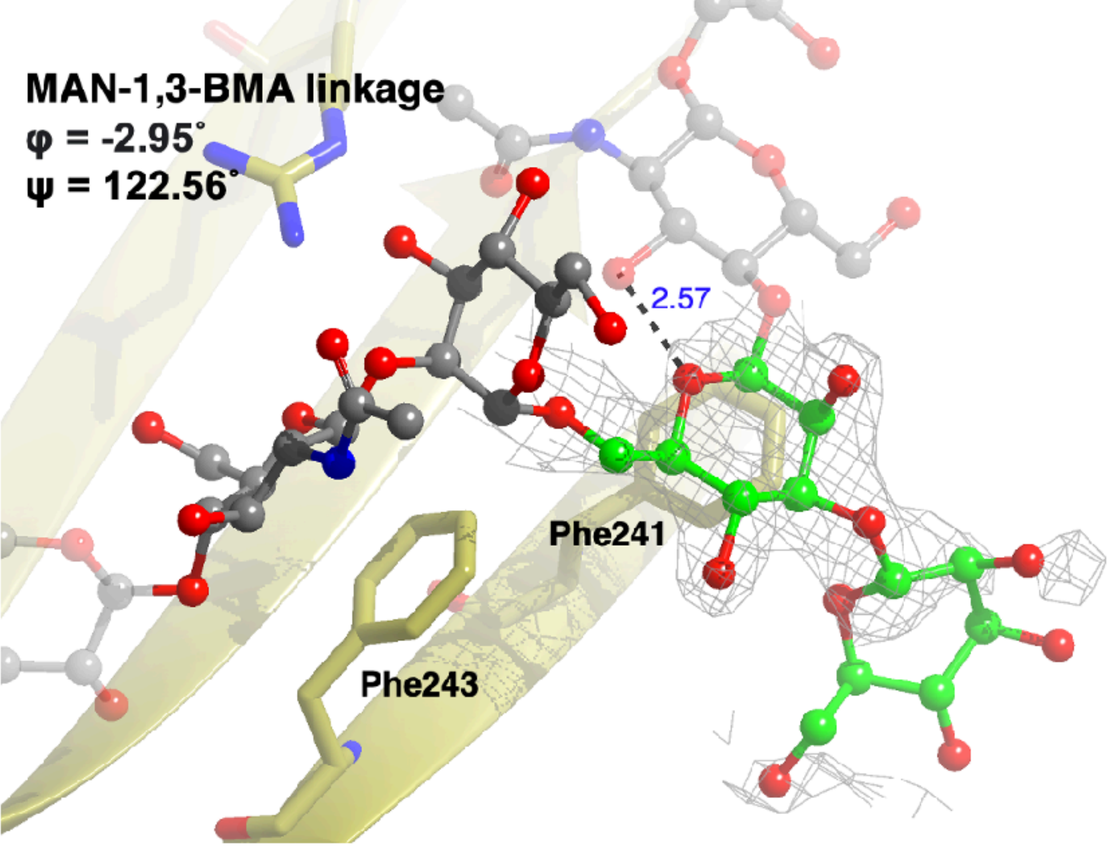
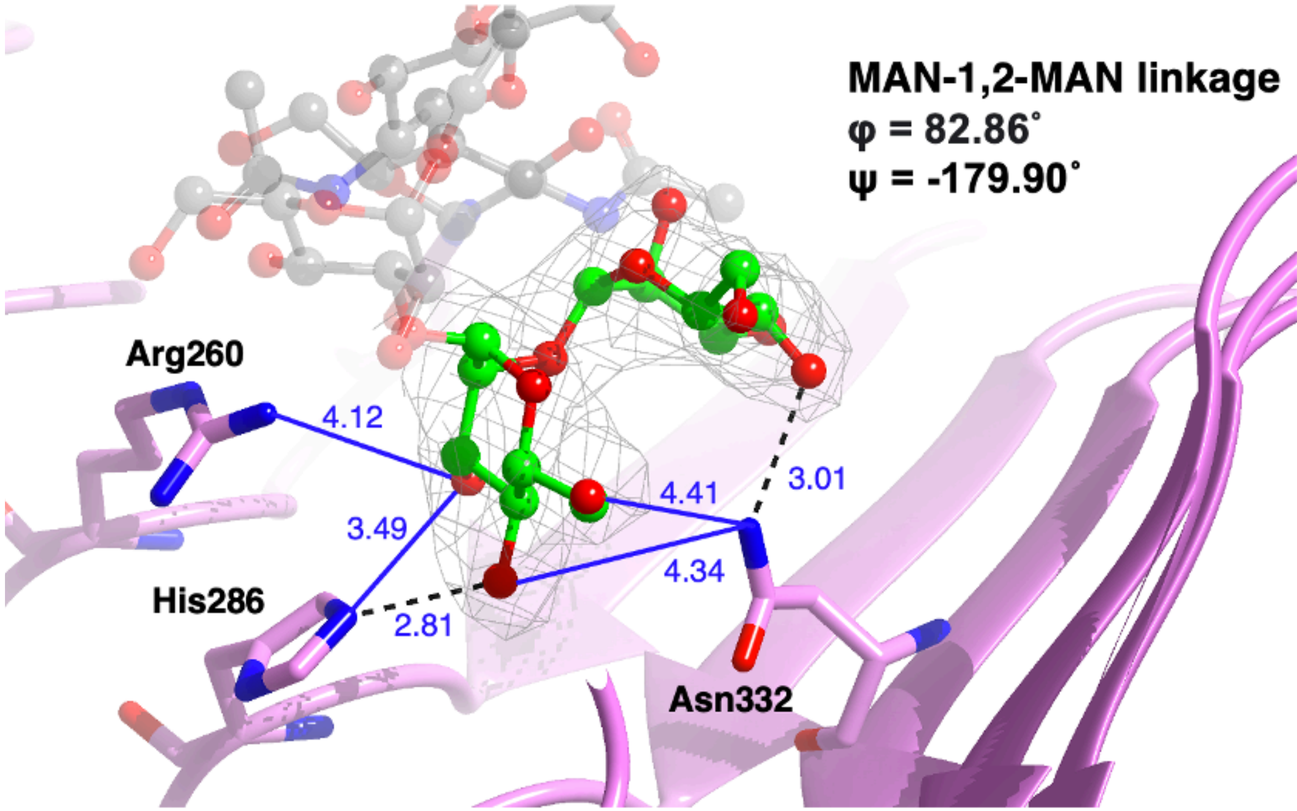
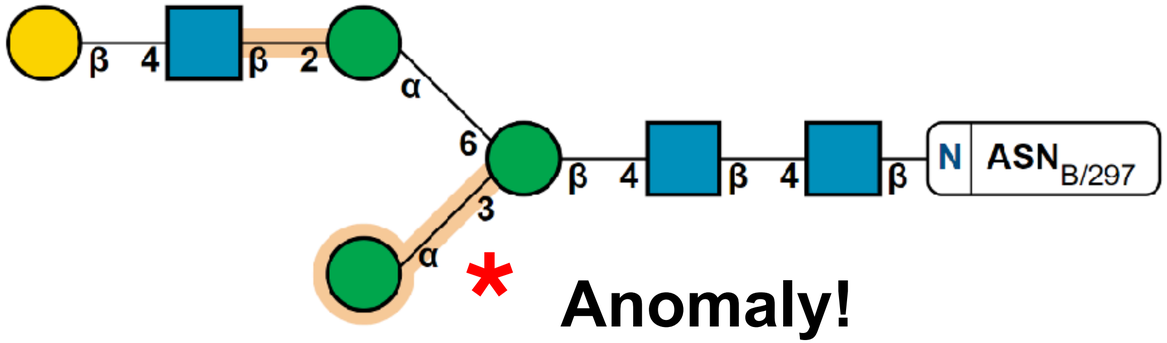
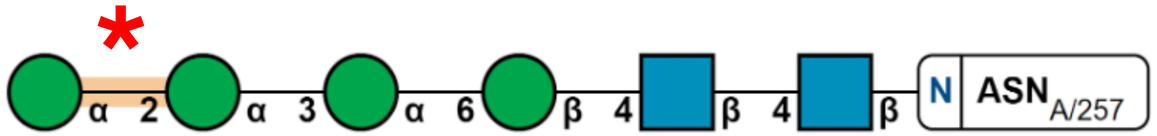






Anomaly!



Well supported by map and interactions

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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


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CCP4 & CCP-EM core teams

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Workshop

https://tinyurl.com/carbohydrateworkshop

Workshop Feedback

We would appreciate it if you could leave us fill out this short feedback form about this workshop.