Privateer

DLS/CCP4 Workshop 2025

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Protein glycosylation

glyco-
/ˈglʌɪkəʊ/

GREEK
glukus → glyco-

Sweet

N-glycan

Protein

Protein glycosylation

Protein glycosylation

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.

Lipid-glycan precursor
OST
α-Glc I
α-Glc II
α-Glc II
ER α-Man
ER
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
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.

Other types of protein glycosylation

O- and *C*-glycans

***O*-GalNAc (*O*-glycan)**

Tryptophan mannosylation (*C*-glycan)

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.

Privateer

- 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)

Glycomics powered validation

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

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.

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.

Anomaly!

Well supported by map and interactions

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.

TRYPTOPHAN MANNOSYLATION (MAN-TRP)

$^4C_1$ 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.

TRYPTOPHAN MANNOSYLATION (MAN-TRP)

$^1C_4$ chair alpha link

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

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.

SPOTTING OR FIXING MAN-TRP

Holland, L., Pham, P. T., Bagdonas, H., Dialpuri, J. S., Schofield, L. C., & Agirre, J. (2025). Methods for detecting, building, and improving tryptophan mannosylation in glycoprotein structures. Protein Science, 34(2), e70025.

Privateer Overview

- **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

Carbohydrate building and validation workshop

Workshop