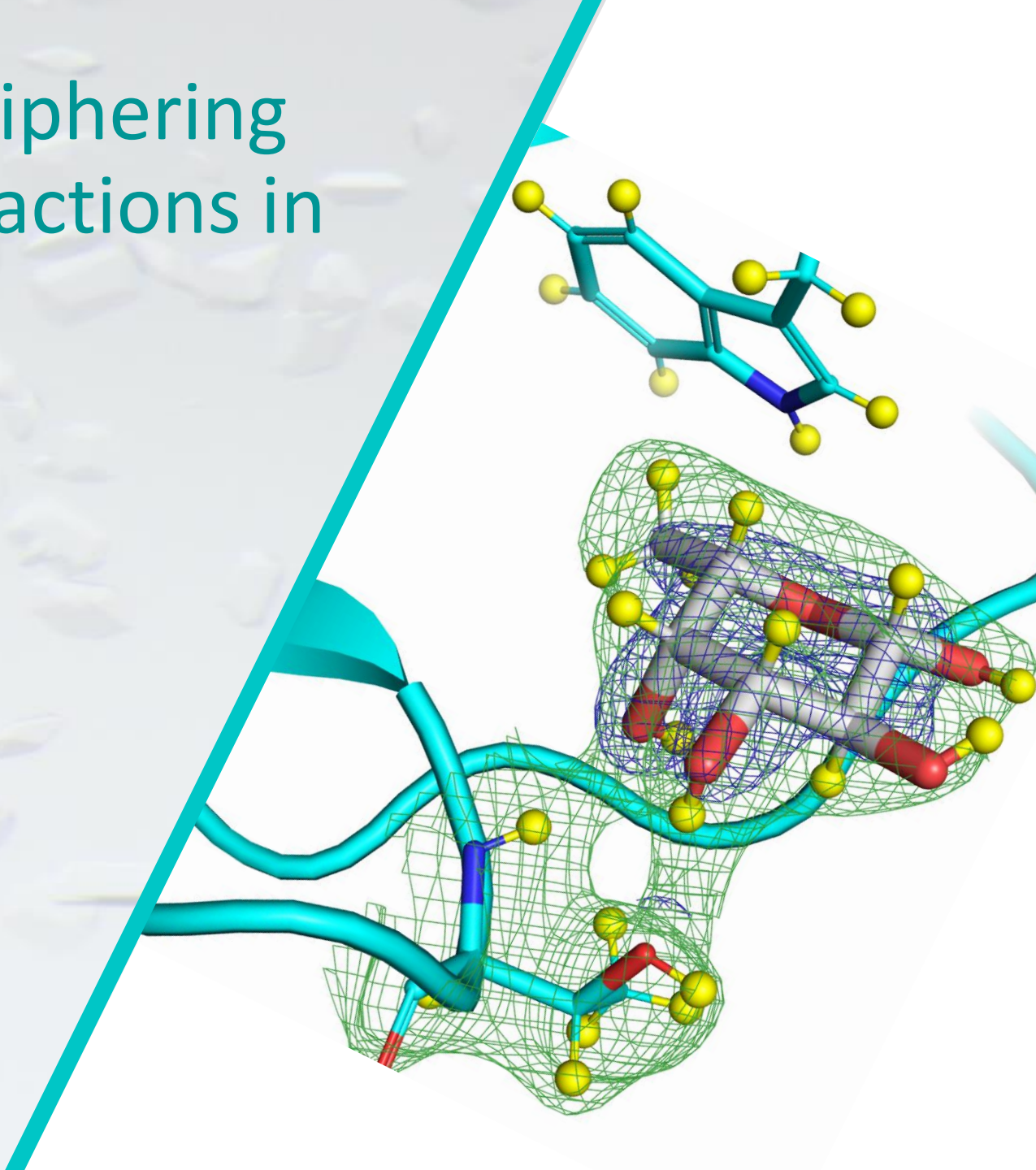
Neutron diffraction for deciphering protein-carbohydrate interactions in bacterial infection

Lukáš Gajdoš

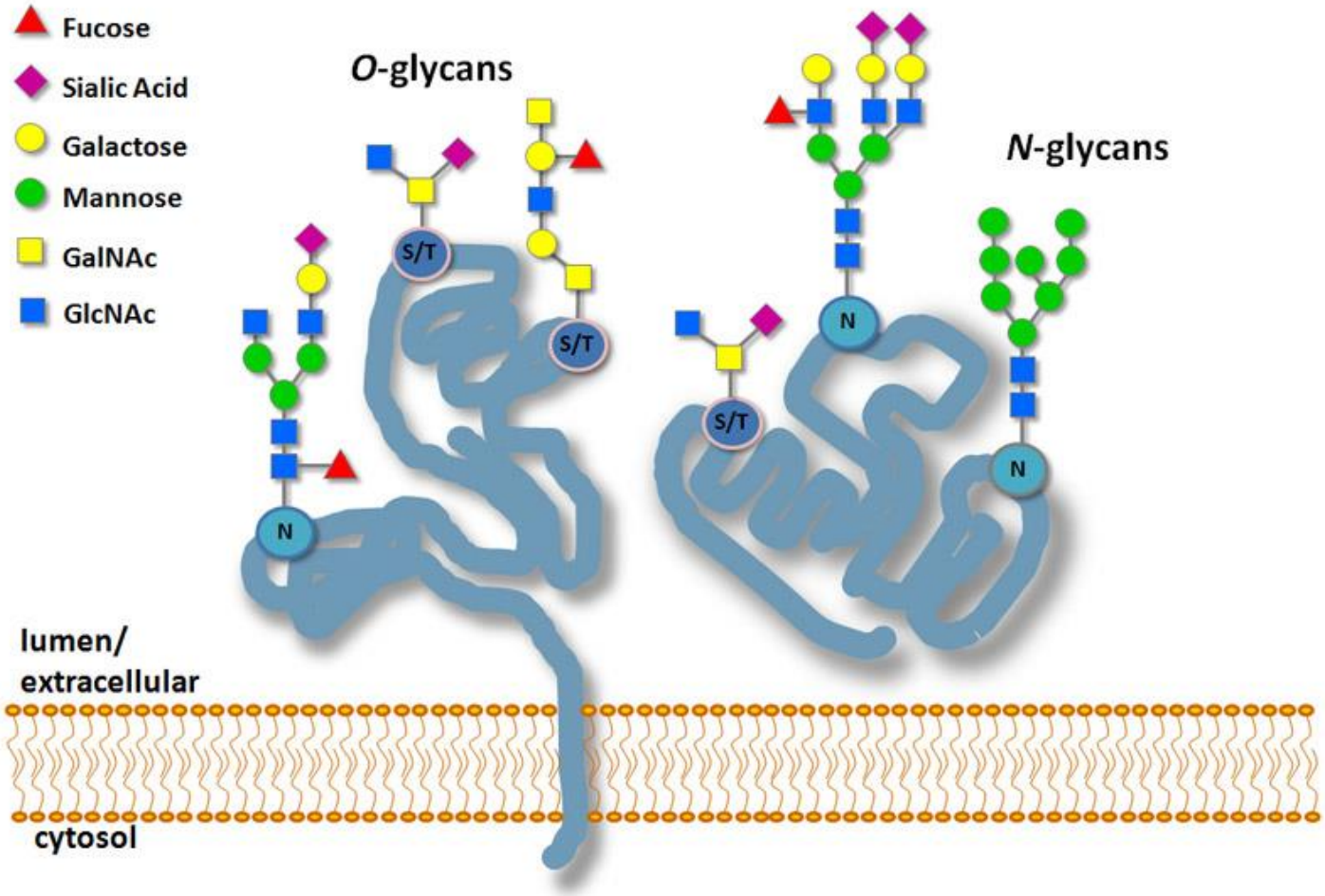
ESS/ILL User Meeting

Anne Imberty (CERMAV)
Juliette Devos (ILL)
Trevor Forsyth (ILL, LINXS)
Matthew Blakeley (ILL)



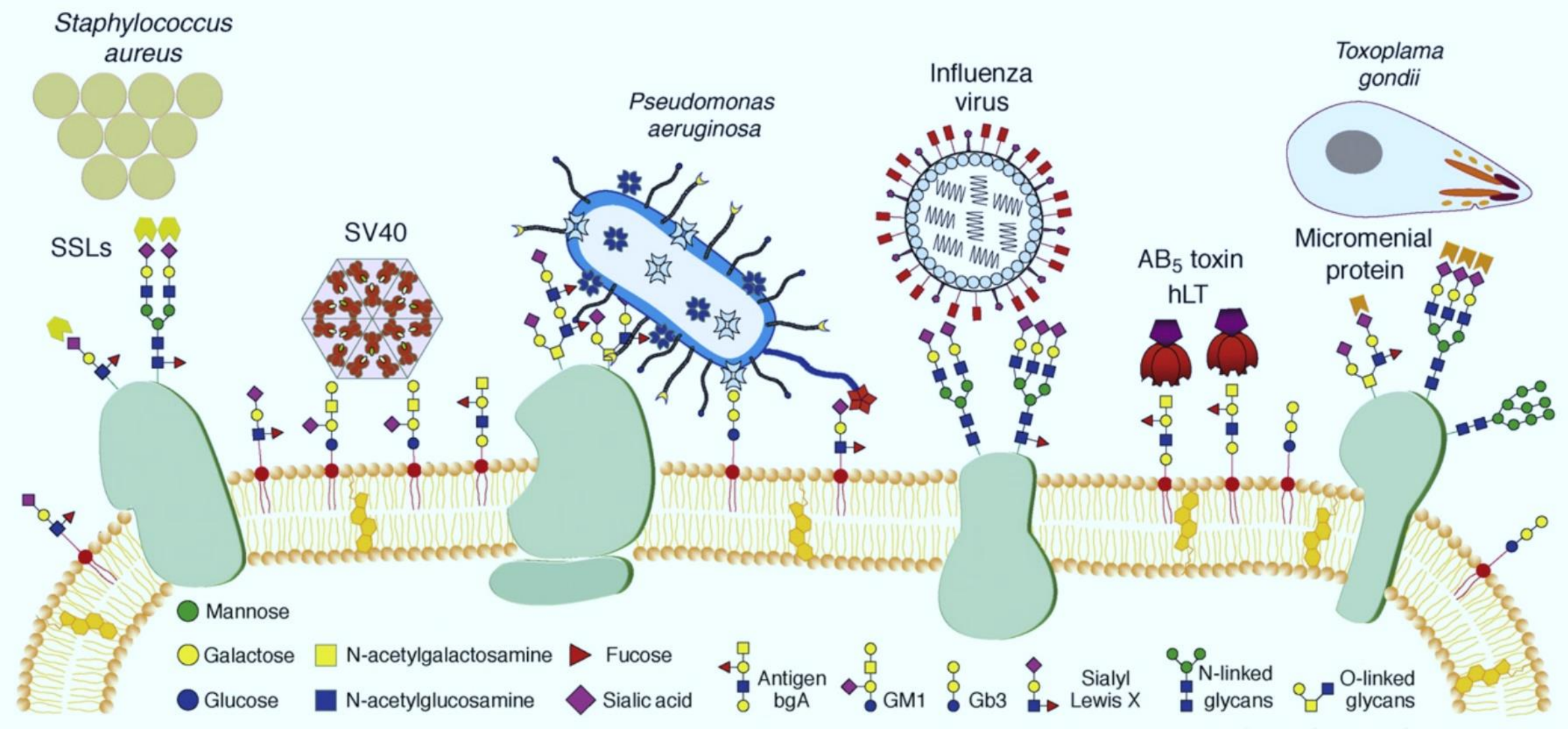
Glycans

Nieuwdorp M., et al. 2005



Lectins are proteins that can decode this complex „glycocode"

Lectins from pathogenic organisms



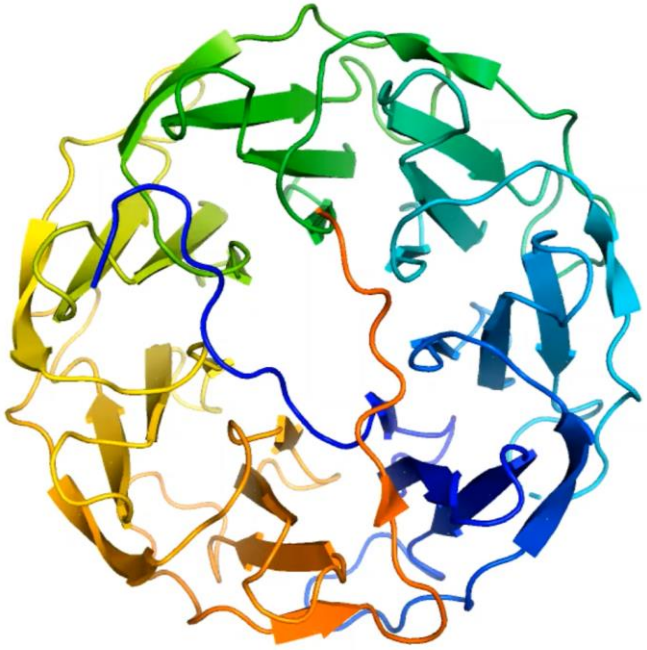
Imberty A. and Varrot A. , 2008

Photorhabdus luminescens & Pseudomonas aeruginosa

Photorhabdus luminescens & Pseudomonas aeruginosa

- **Bioluminescent** bacteria
- Insect **pathogen**
- **Symbiosis** with nematodes
- **Complex** life cycle
- Production of **toxins**
- Produces several **lectins**

Courtesy of Dr. A. Regaiolo and Prof. R. Heermann



PLL lectin
Fucose-specific
K_D= 5 mM
PDB: 6C9P

Kumar A. et al., 2016

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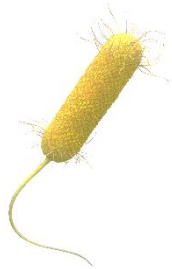
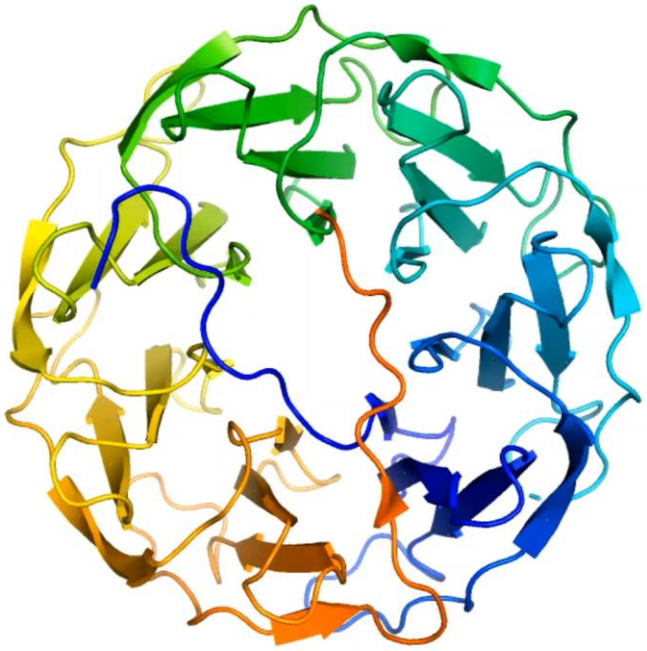


- Human **opportunistic** pathogen
- Lung infections (**cystic fibrosis**)
- **LecA** (PA-IL), **galactose**-specific
- **LecB** (PA-IIL), **fucose**-specific
- Roles in attachement and biofilm formation
- Targets for novel glycomimetics



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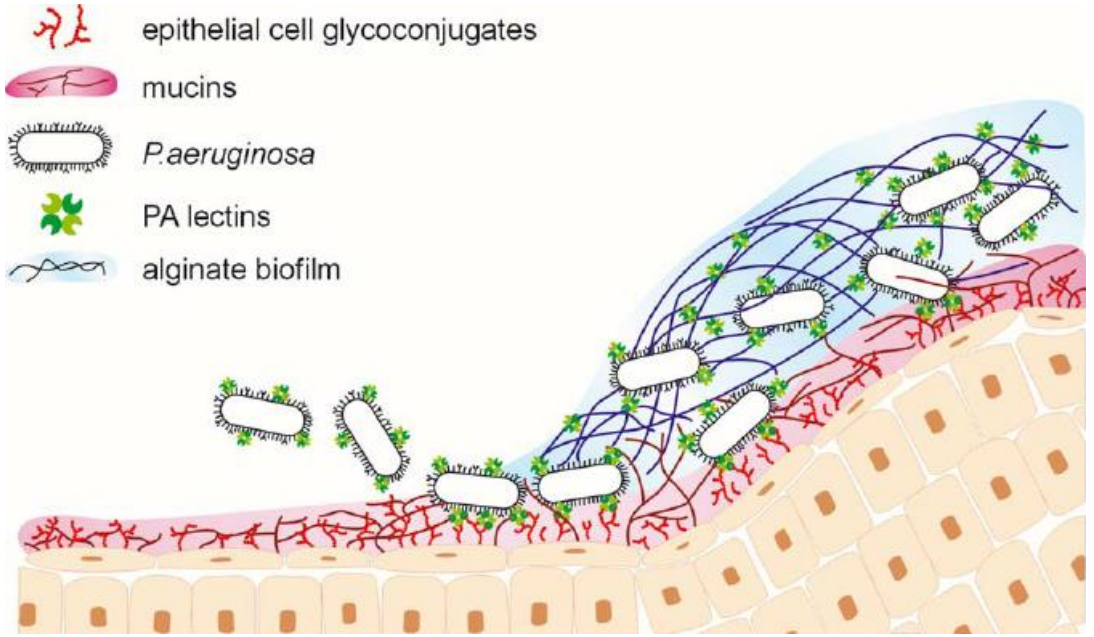
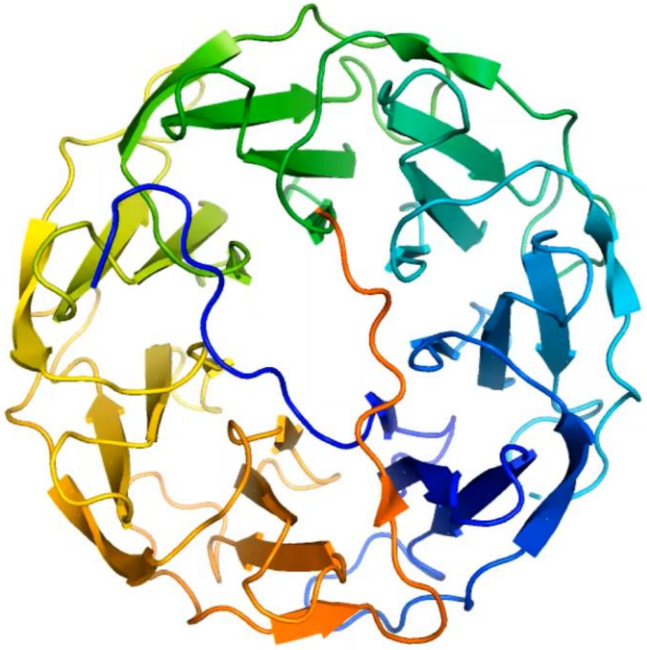


Imberty A., et al., 2014



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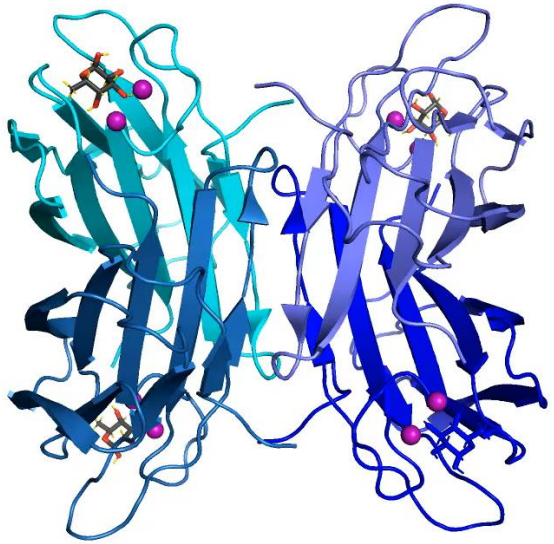
Kumar A. et al., 2016



LecB lectin
Fucose-specific
K_D= 4 µM
PDB: 1GZT

Mithell E. et al., 2002

Protein-carbohydrate interactions

Fucose in PLL

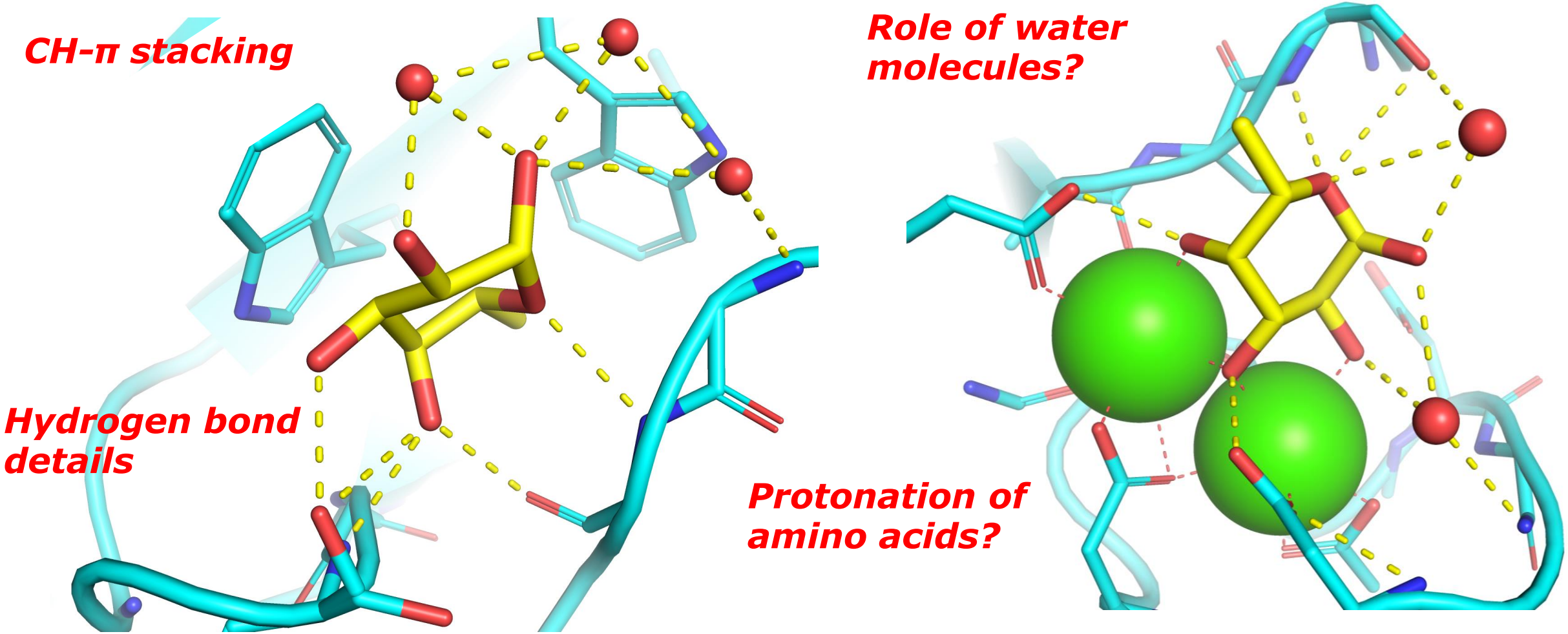
CH-π stacking

Hydrogen bond details

Fucose in LecB

Role of water molecules?

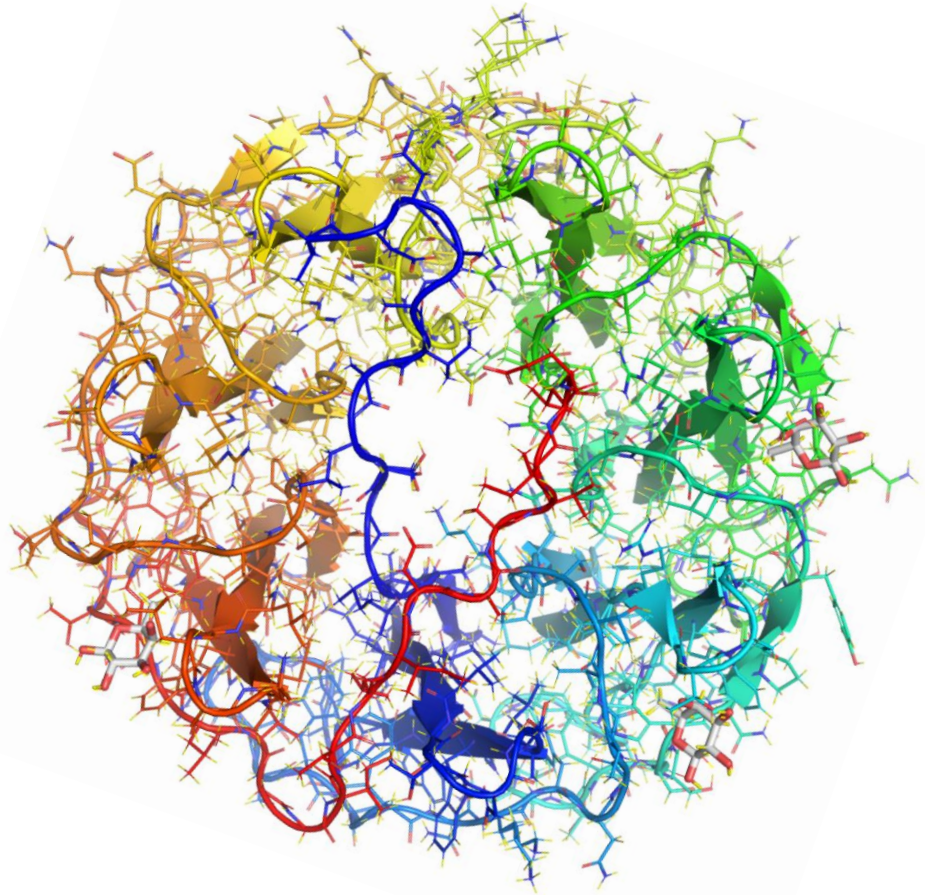
Protonation of amino acids?

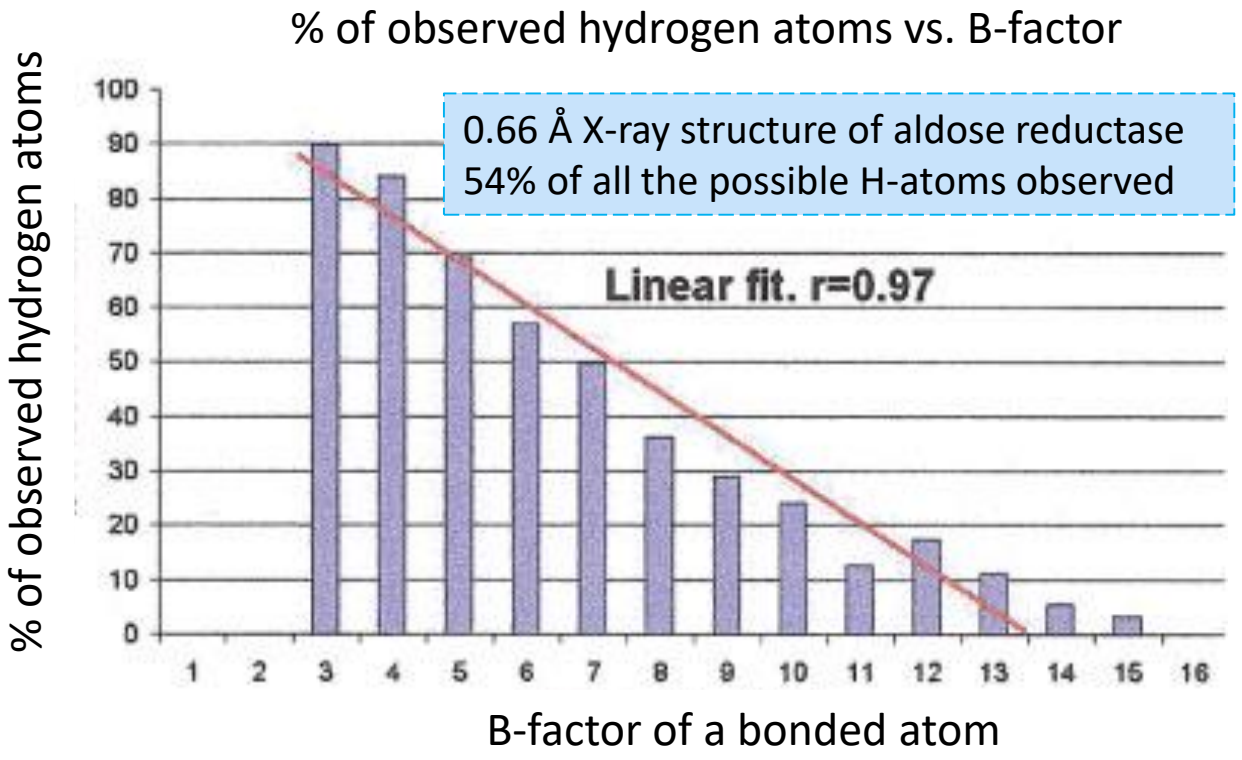


Wanted : Location of hydrogen atoms

H atoms „invisible“ in X-ray structures

- **Hydrogen atoms** account for about **half** of all the atoms in proteins
- Critical roles in **biological functions** (enzyme mechanisms, ligand binding)
- **Rarely** observable in X-ray diffraction experiments

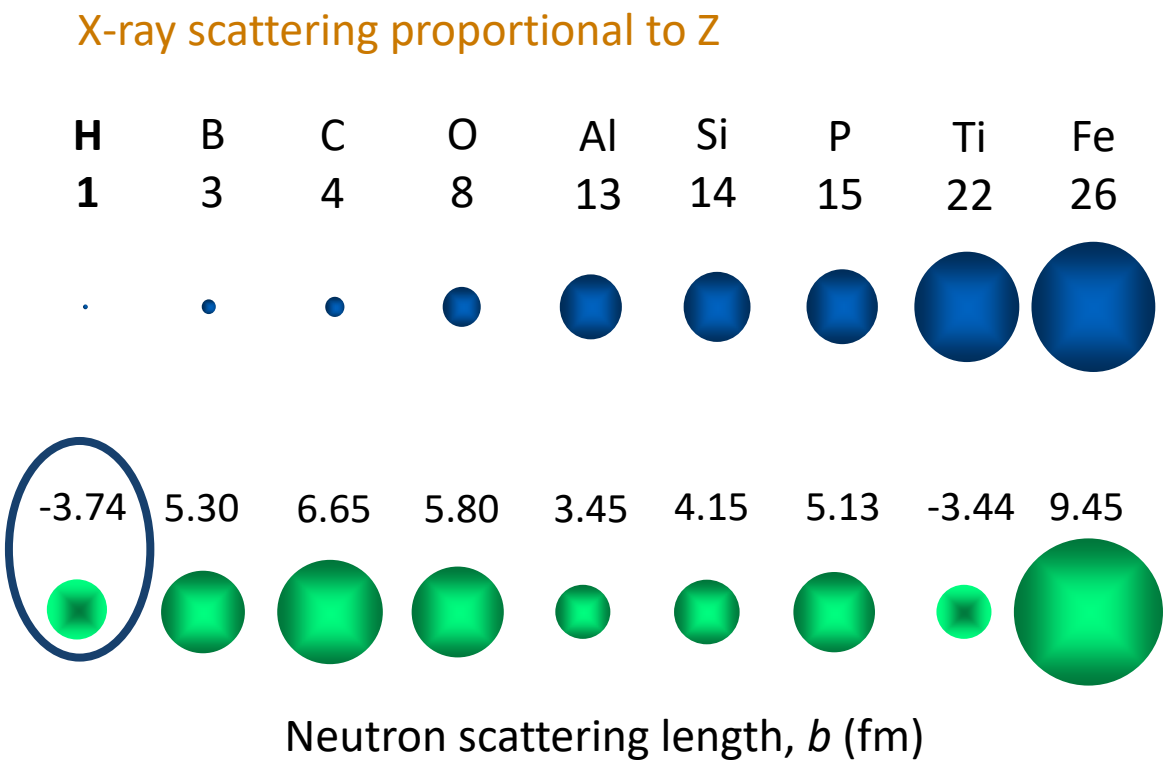


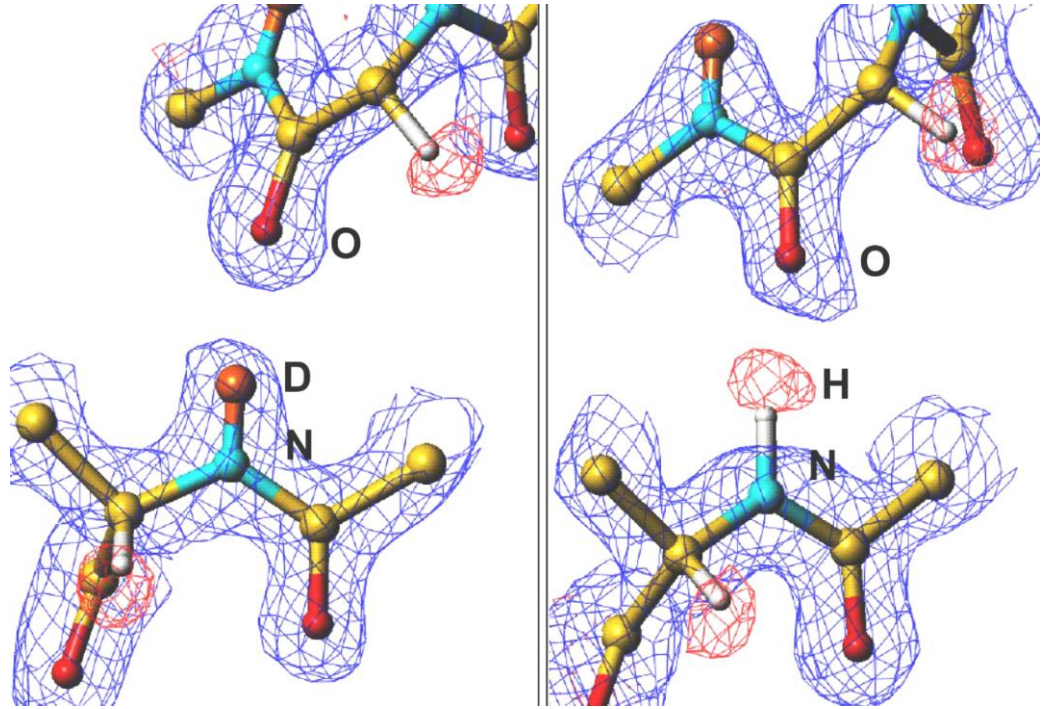


Howard E.I. et al., 2004

Neutrons as a diffraction probe

- Interaction with atomic **nuclei**
- Scattering **varies** with elements and even **isotopes** of the same element (**H/D**)
- Non-destructive probe (room-temperature data collection)





Shu F. et al., 2000

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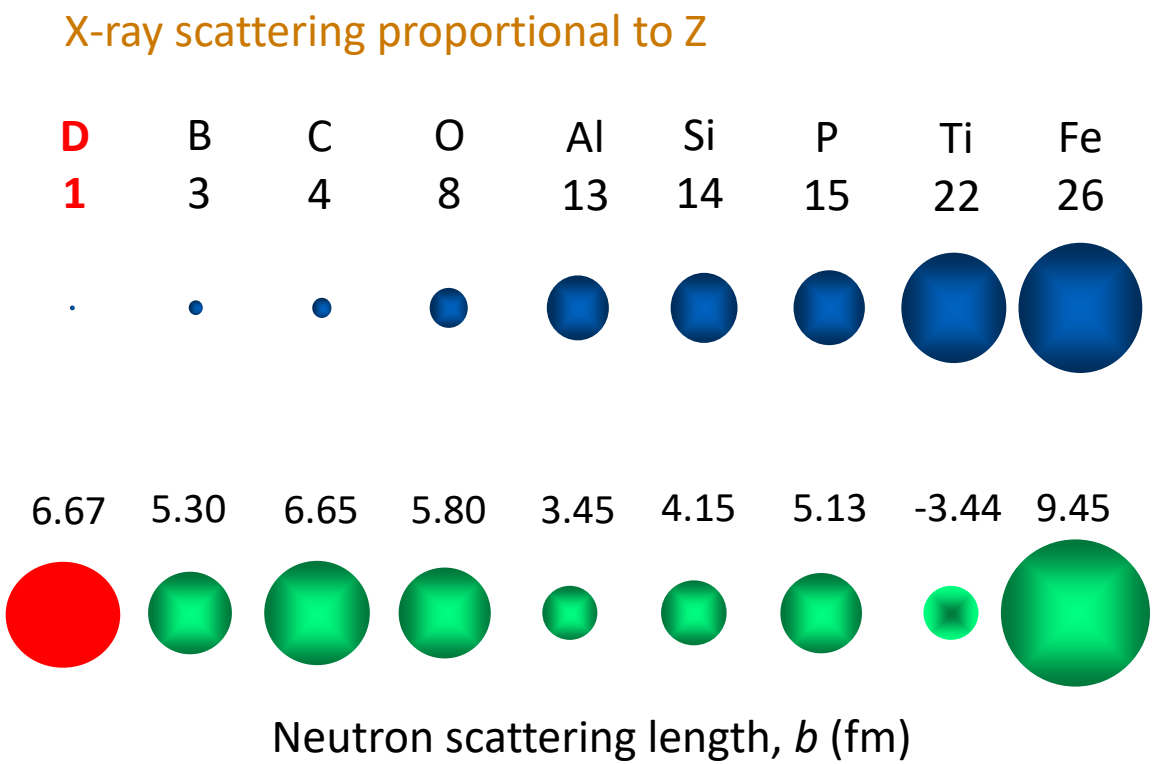
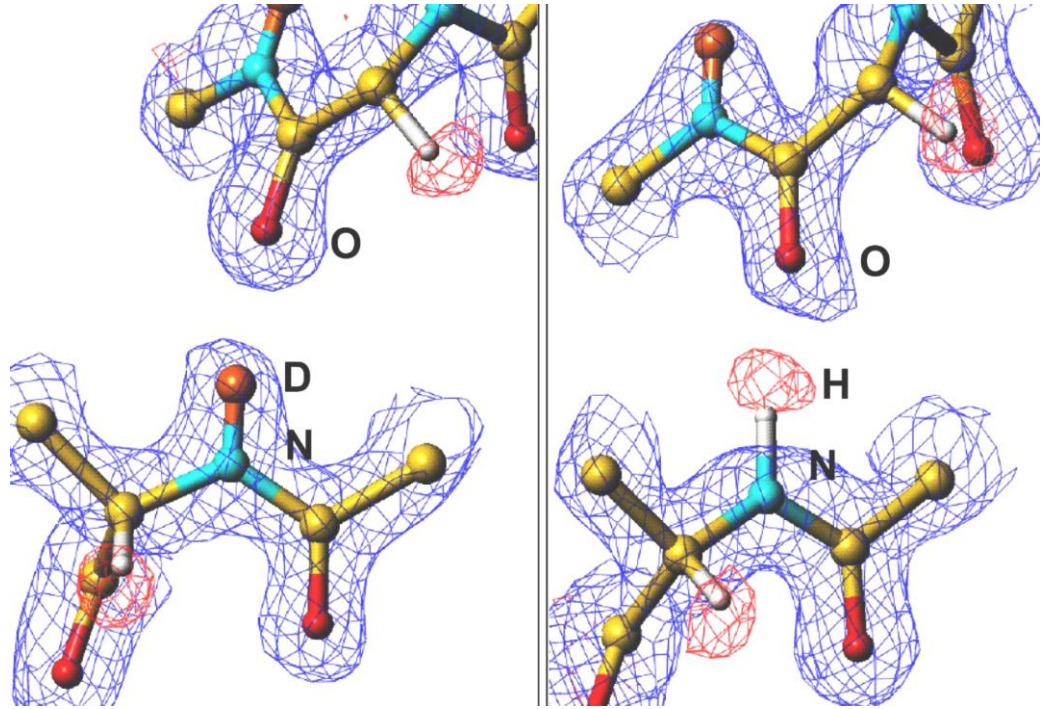
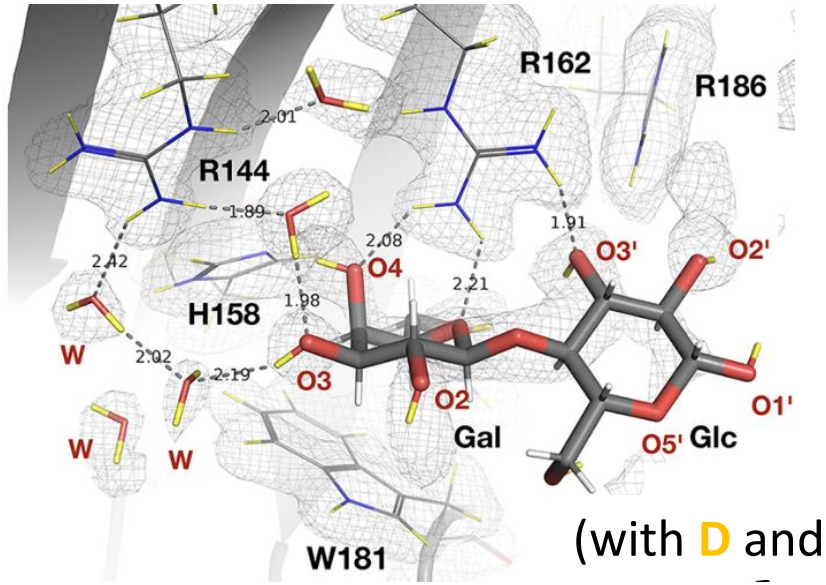




Shu F. et al., 2000

Need of perdeuteration

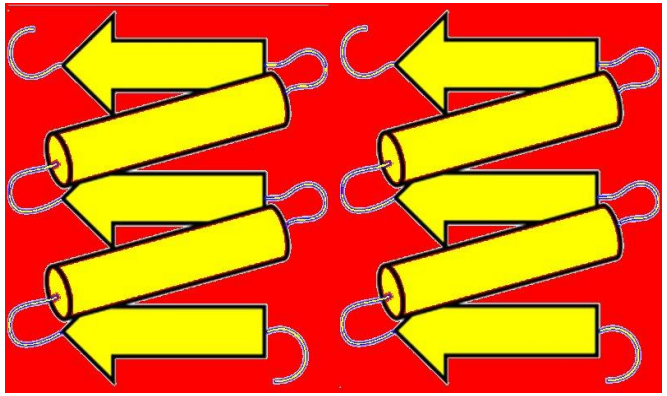
- Full replacement of all **hydrogen** (H) atoms by **deuterium** (D) atoms
- **Reduces** the large **incoherent** scattering of H (~ 40 times larger than for D)
- **Reduces the background** and **increases the signal-noise ratio**
- Clearer visualization of neutron maps
- **Cancellation effects** limit visualization of CH_n groups



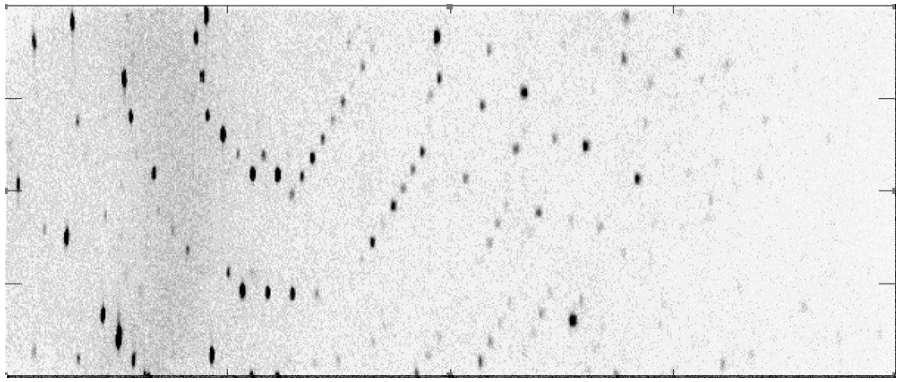
(with D and H)

D-Galectin-3C/lactose complex

Manzoni F. et al., J. Med. Chem., 2018



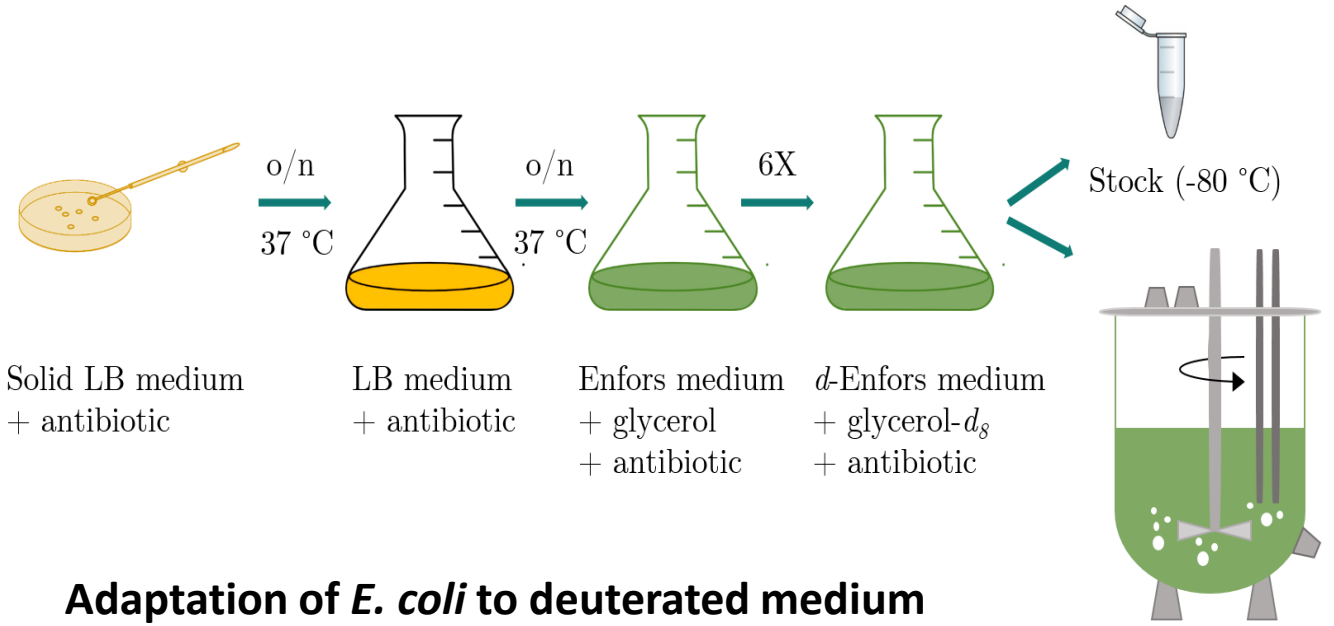
Perdeuterated protein, D_2O solvent



Courtesy of Prof. Trevor Forsyth

How to obtain perdeuterated biomolecules?

- **Adaptation** of *E.coli* cells to deuterated medium
- **Production** of recombinant proteins **in D_2O** --- > D-Lab at ILL
- **Fermentation** (high cell-density cultures) of ***E.coli***
- **Deuterated carbon source** (glycerol-d_8, glucose-d_{12})



Adaptation of *E. coli* to deuterated medium

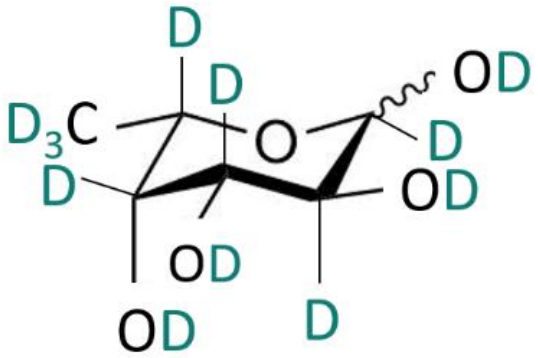
How to obtain perdeuterated biomolecules?

- Proteins:

  Production of recombinant protein in D_2O --- > D-Lab at ILL

- Sugars:

  Glucose-d_{12} : grow plant/algae in D_2O and degrade cellulose

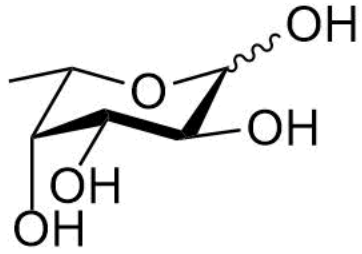


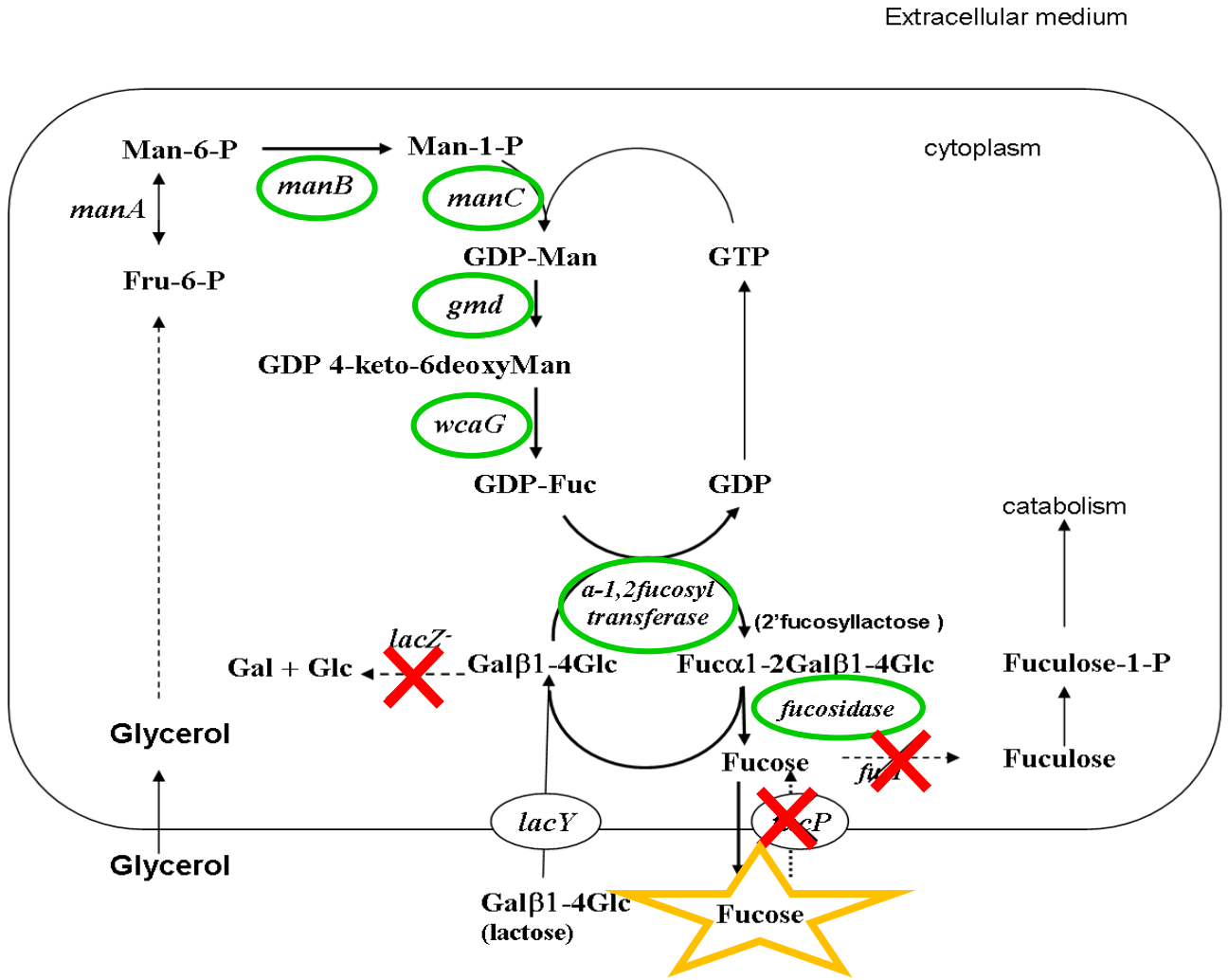
Synthetic chemistry « isotopic hydrogen-exchange technique »

Synthetic (glyco)biology

In vivo production of L-fucose-d_{12} in *E. coli*

Fucose-producing strain of *E. coli* designed and enginereed by Dr. Eric Samain at CERMAV





Overexpressed genes

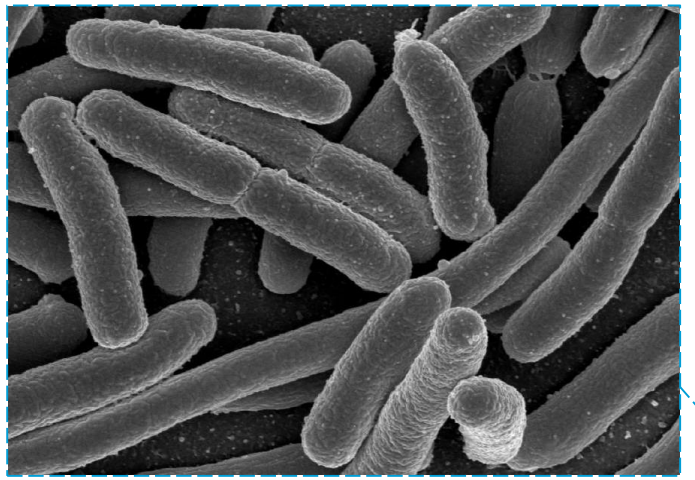
manB: phosphomannomutase
manC: Man-1-P-guanyltransferase
gmd: GDP-Man 4,6-dehydratase
wcaG: GDP-L-fucose synthase
α-1,2-fucosidase
α-1,2-fucosyltransferase

Knocked-out genes

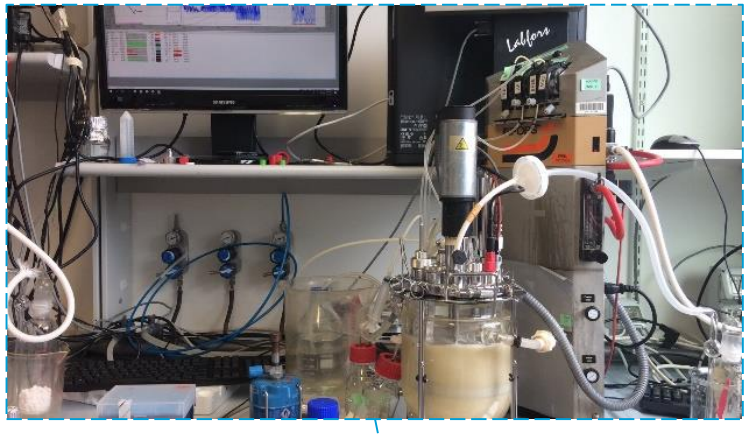
lacZ: β-galactosidase
fucI: fucose isomerase
fucP: fucose permease

Production, purification and characterization of L-fucose-d_{12}

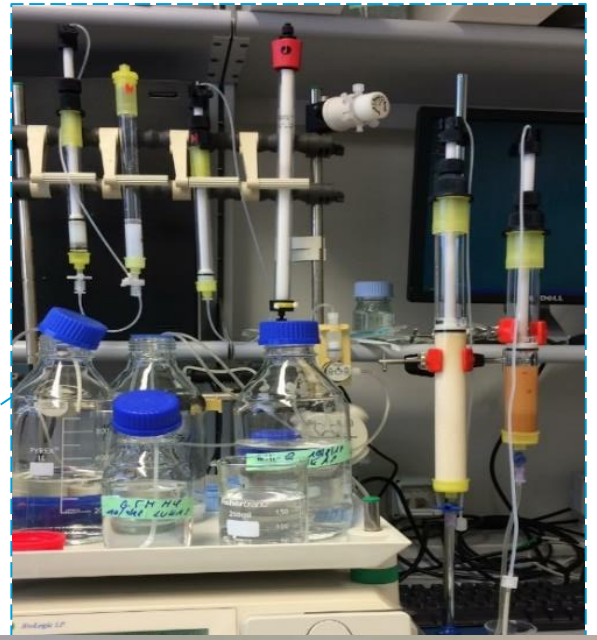
Adaptation to D_2O and glycerol-d_8



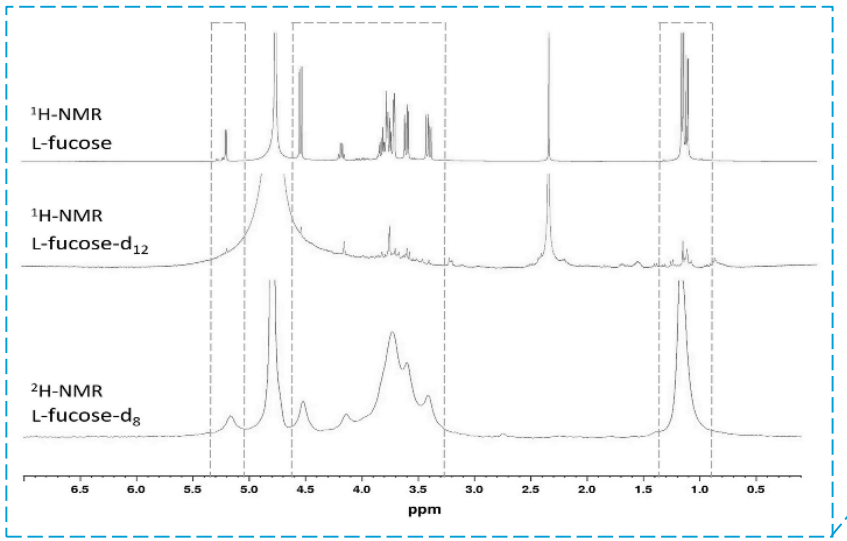
Batch production

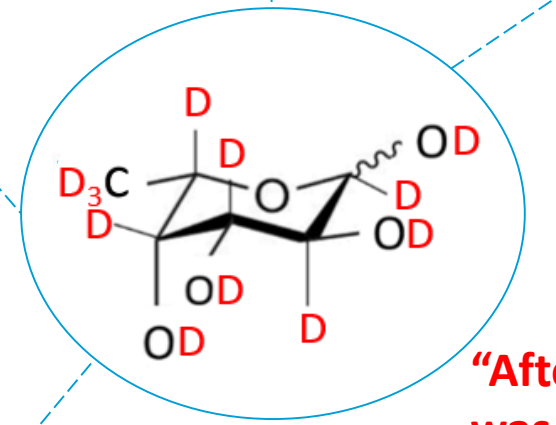


Purification



Characterization (Mass spec, NMR)

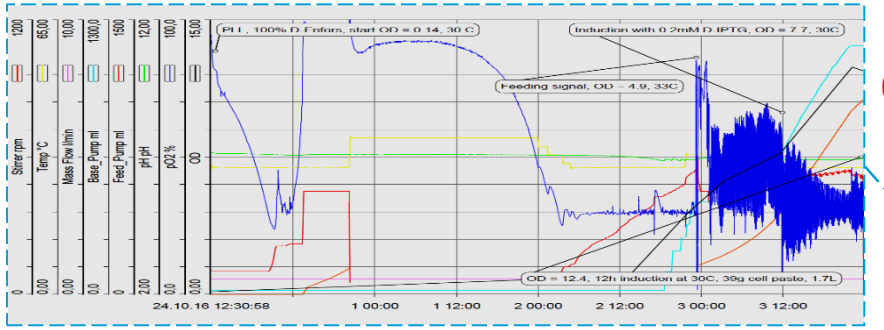




"After purification, 220 mg of lyophilized Fuc-d12 was obtained from a culture using 1.5 L of D_2O and 45 g of deuterated glycerol"

Gajdos L. *et al.*, Glycobiology, 2021

Production and crystallization of lectins



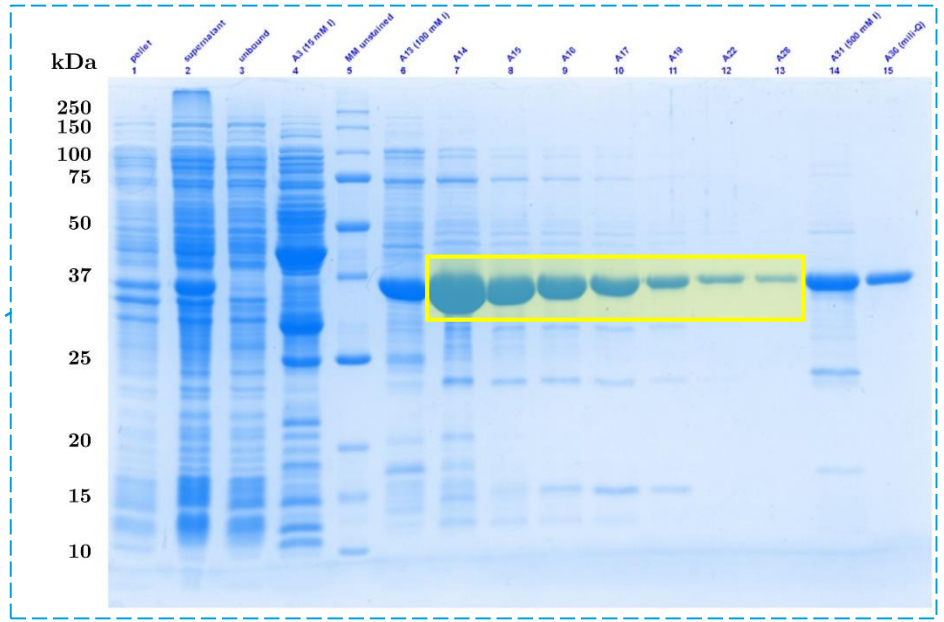
Fermentation

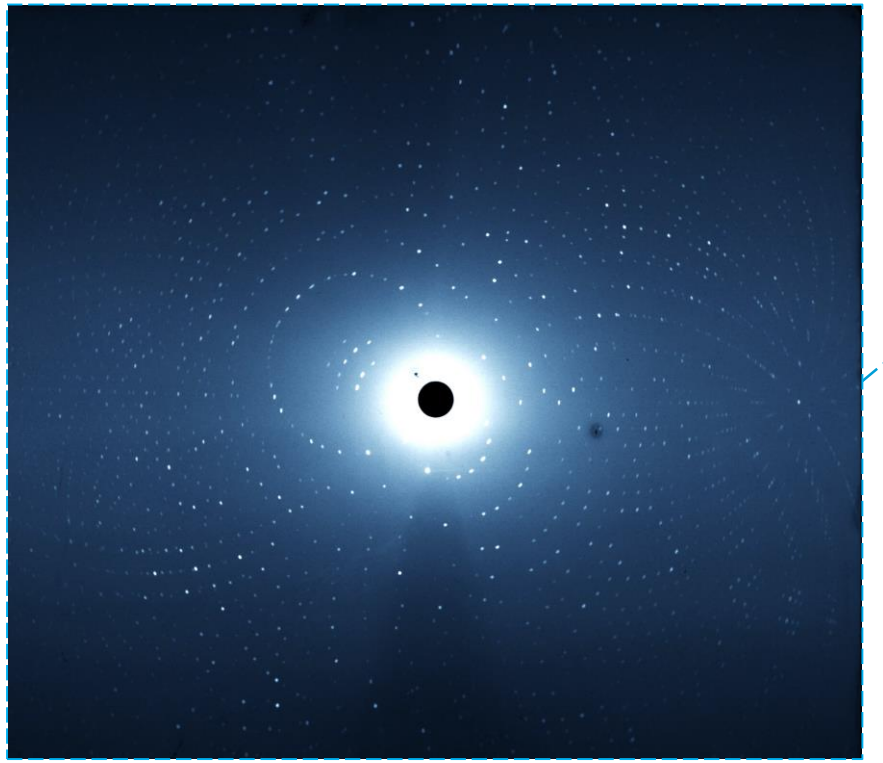
66 g of wet cell paste

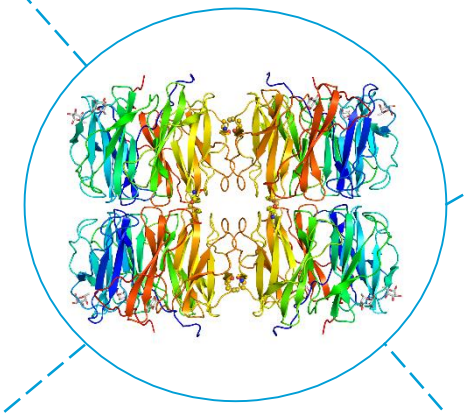
5 mg of protein /g of cell paste

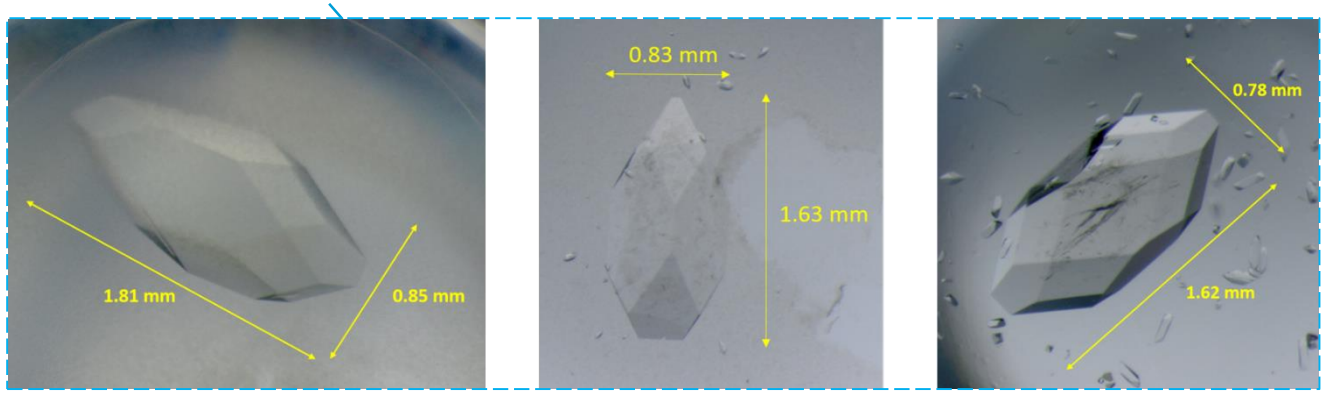
Total **330 mg** of protein

Purification



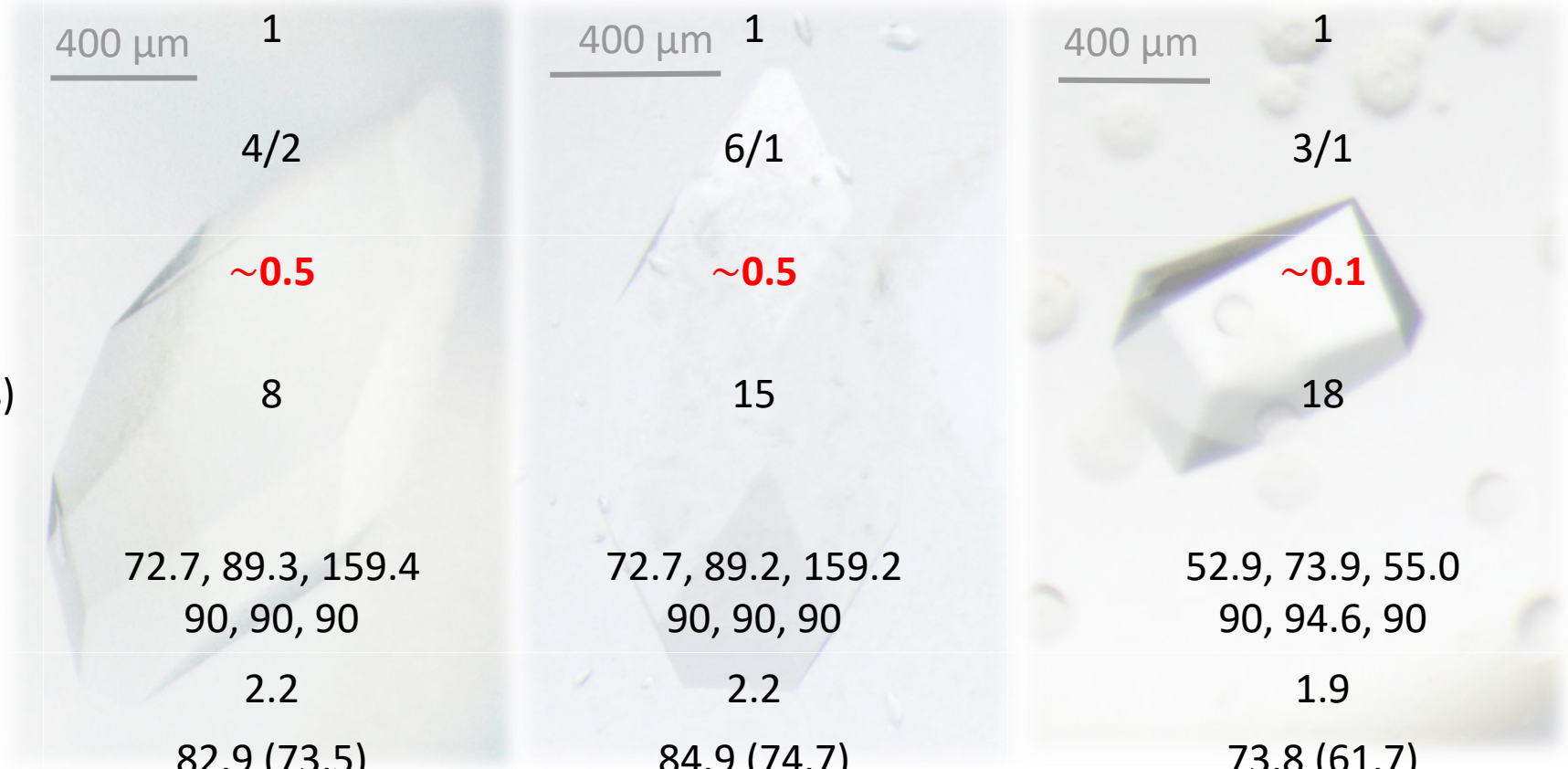






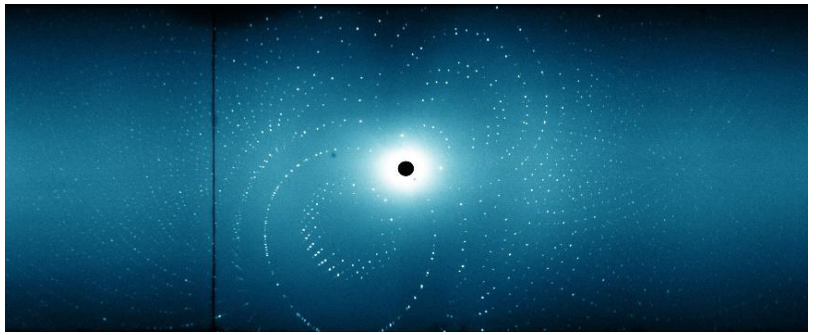
Neutron data collection summary (LADI-III at ILL)

	PLL lectin		D-LecB lectin
	H/D-exchanged apo	D-PLL/Fuc-d_{12}	Fuc-d_{12}
Number of proposals	1	1	1
Number of crystals (tested/collected)	4/2	6/1	3/1
Crystal volume (mm^3)	**~0.5**	**~0.5**	**~0.1**
Neutron data collection (days)	8	15	18
Unit cell dimensions			
a, b, c (Å)	72.7, 89.3, 159.4	72.7, 89.2, 159.2	52.9, 73.9, 55.0
α, β, γ	90, 90, 90	90, 90, 90	90, 94.6, 90
Resolution (Å)	2.2	2.2	1.9
Completeness (%)	82.9 (73.5)	84.9 (74.7)	73.8 (61.7)



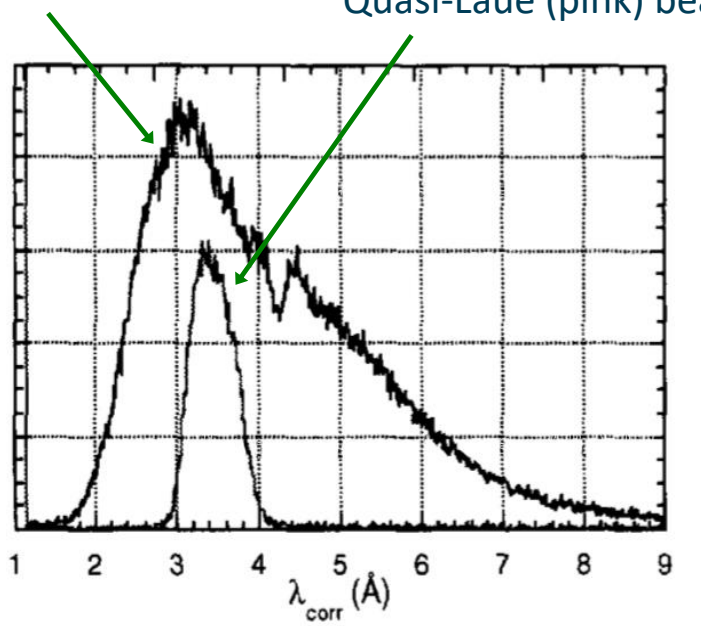
Laue diffractometer LADI-III at ILL

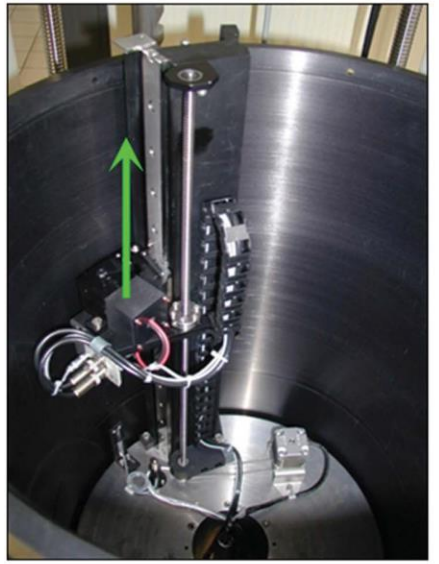
- Institut Laue-Langevin (ILL) in Grenoble, France
- Quasi-Laue diffraction method (pink beam of neutrons)
- Large cylindrical neutron-sensitive image plate detector
- Data collection at room temperature

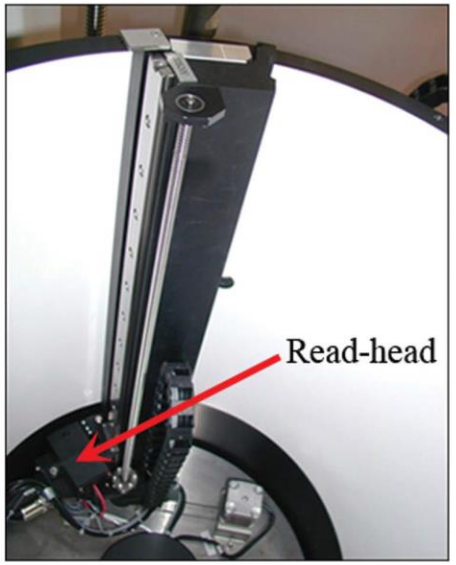


Laue (white) beam

Quasi-Laue (pink) beam



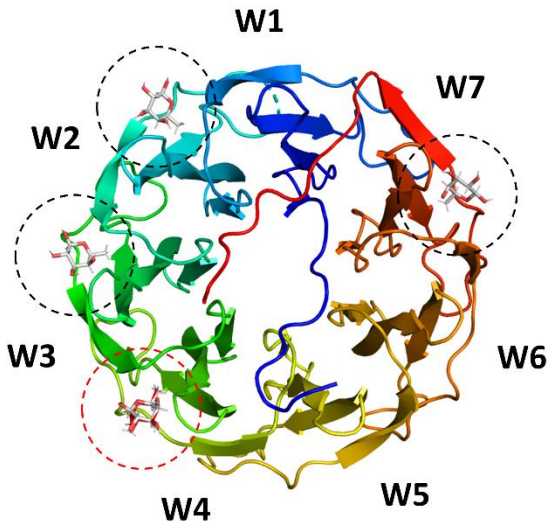


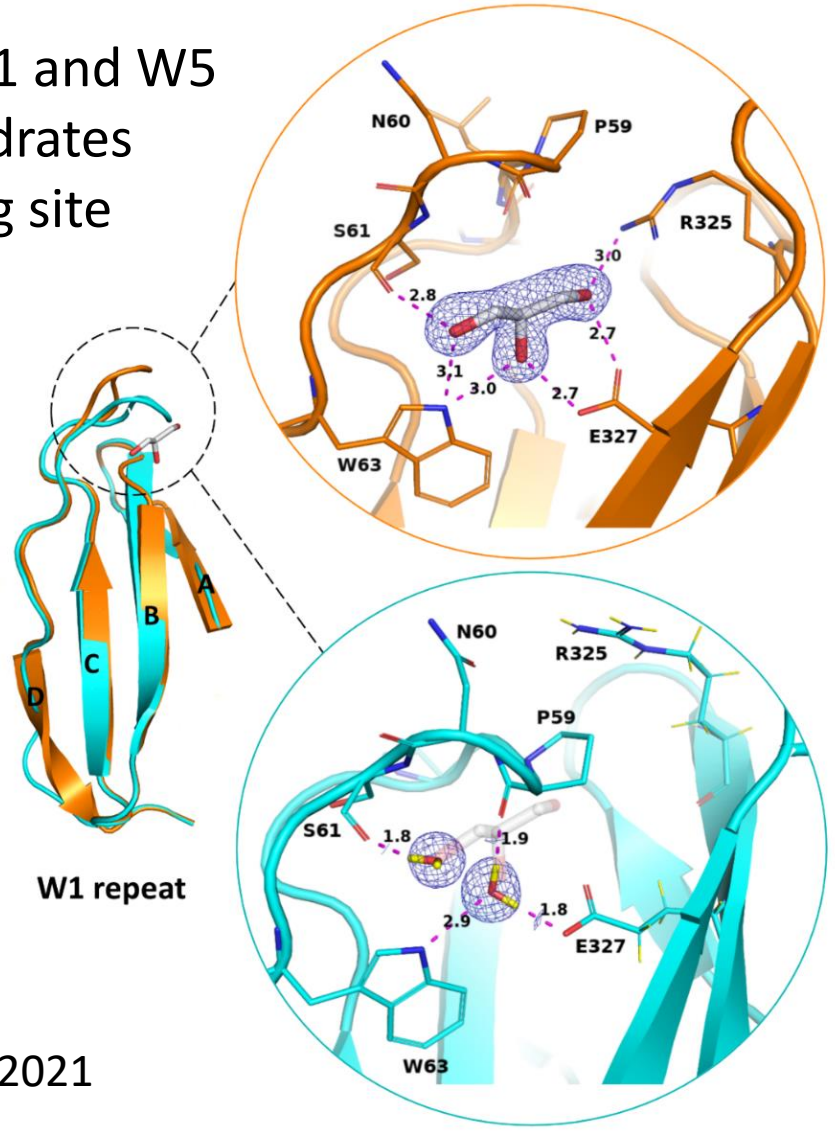


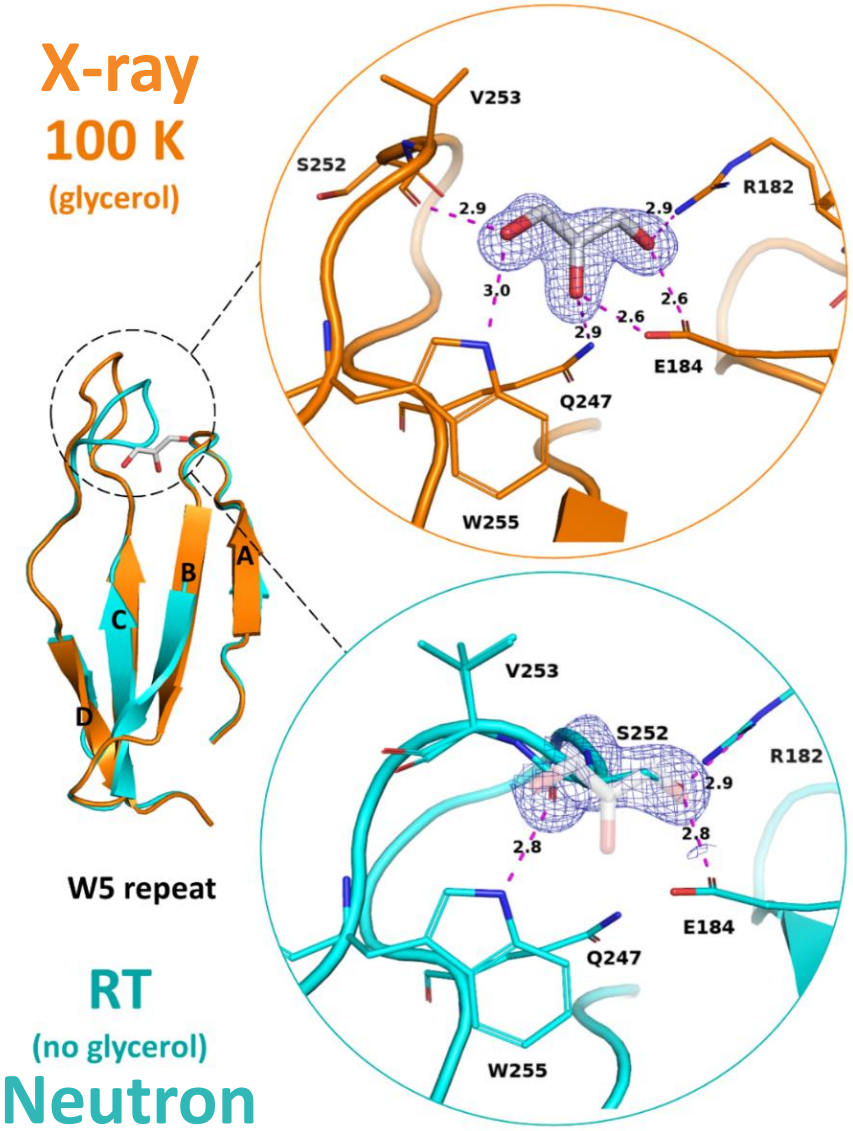
Blakeley M.P., et al, 2010

Room temperature (RT) versus cryo (100K) data

- Differences in repeats W1 and W5
- Glycerol mimics carbohydrates
- Additional fucose-binding site



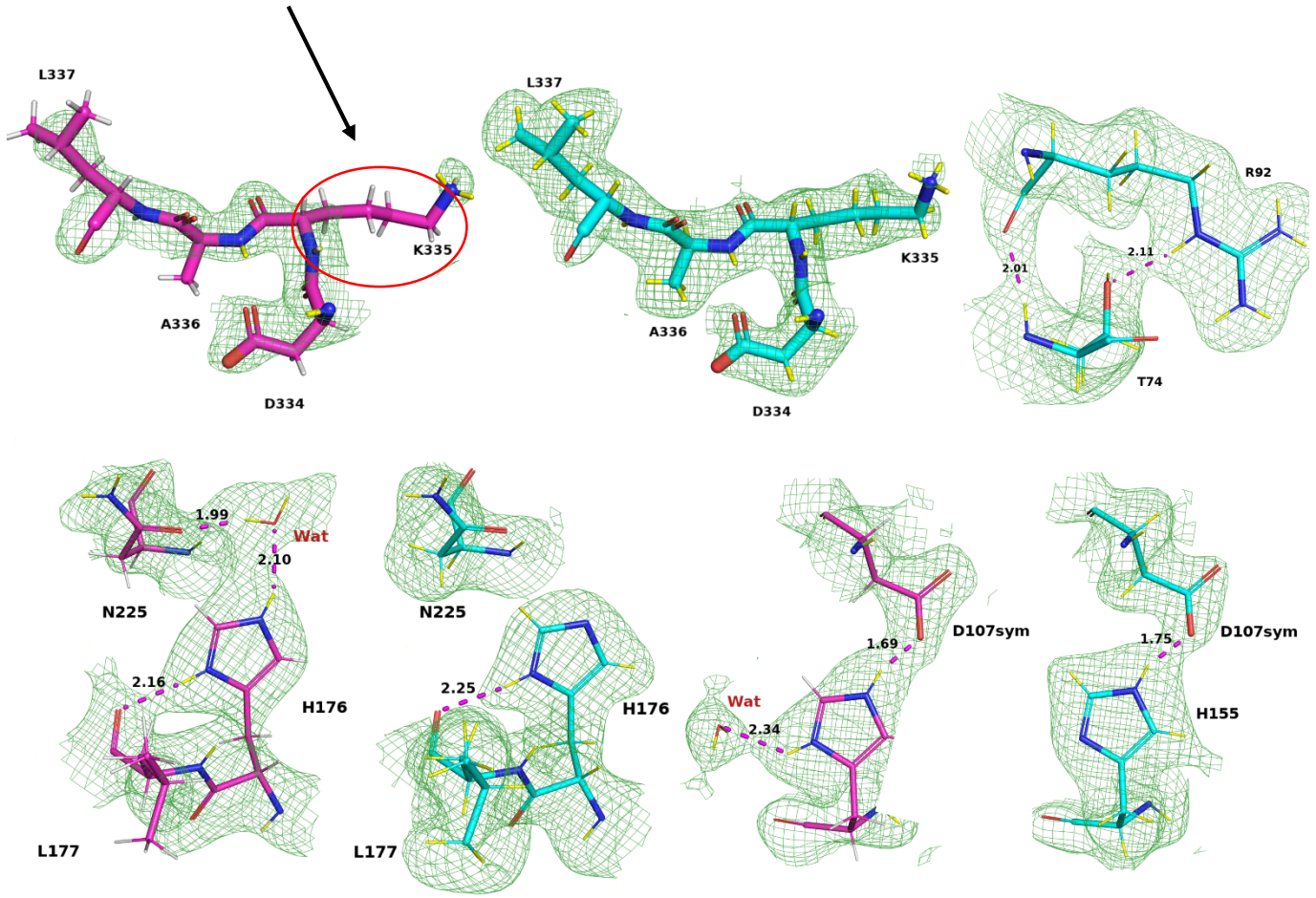




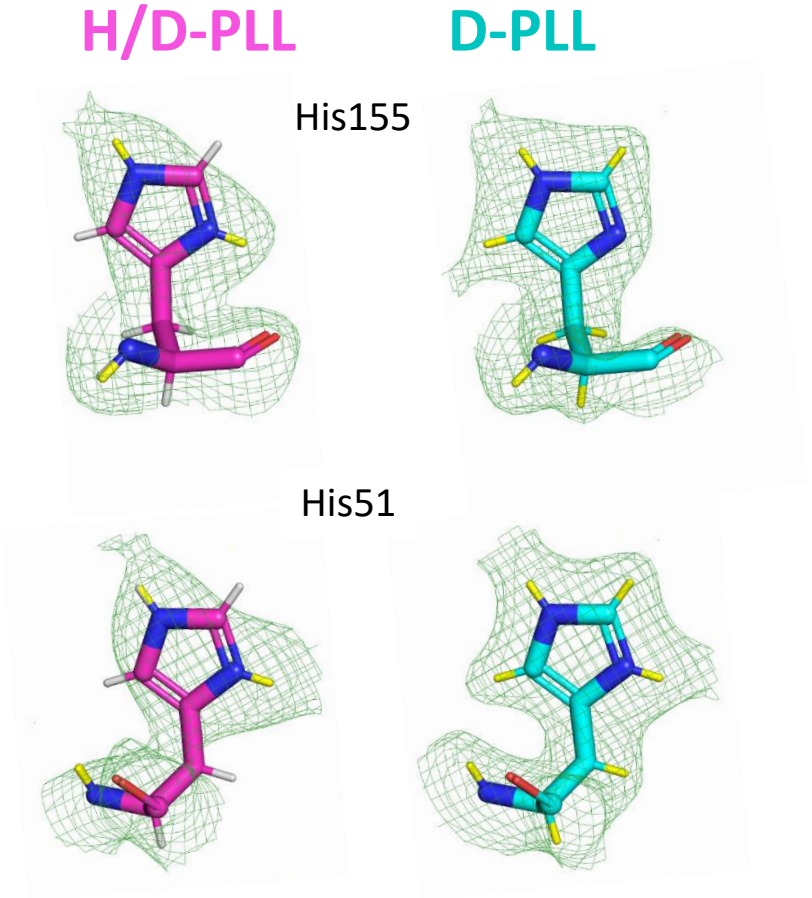
Gajdos L. *et al*., Structure, 2021

Advantages of perdeuteration in neutron structures

- **H/D-exchanged apo PLL** and **D-PLL**
- Cancellation effects around CH_2 groups



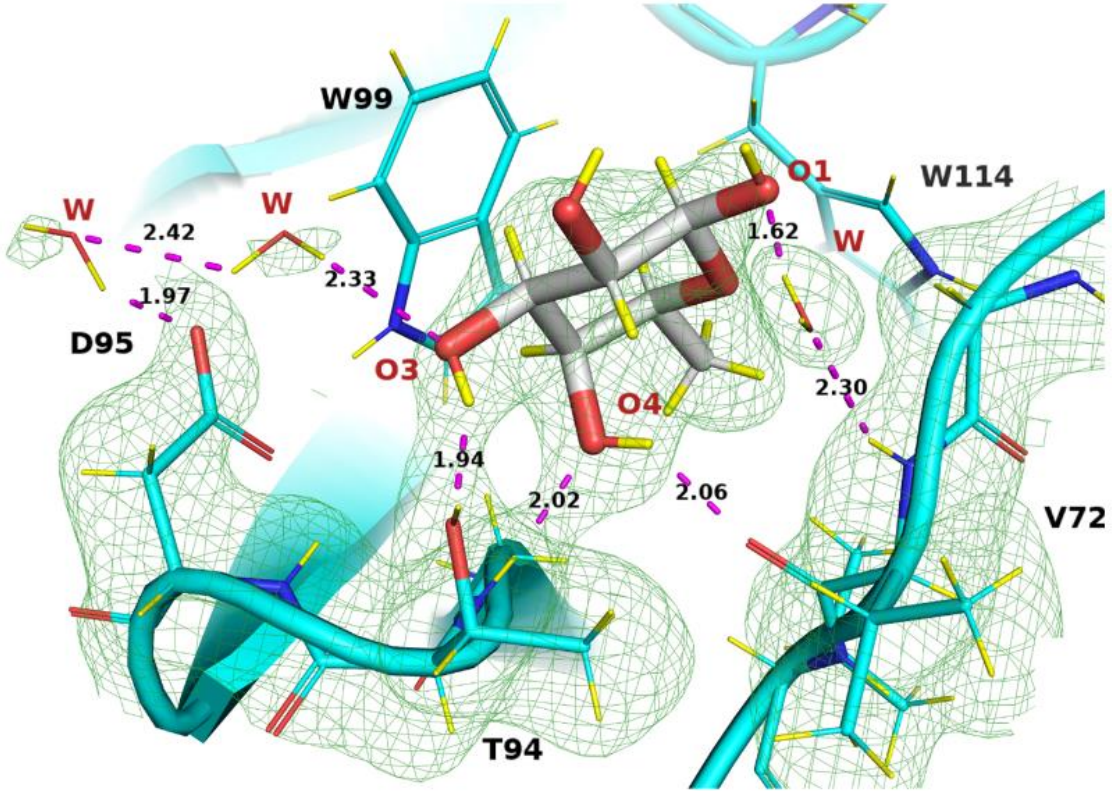
Protonation state of histidines



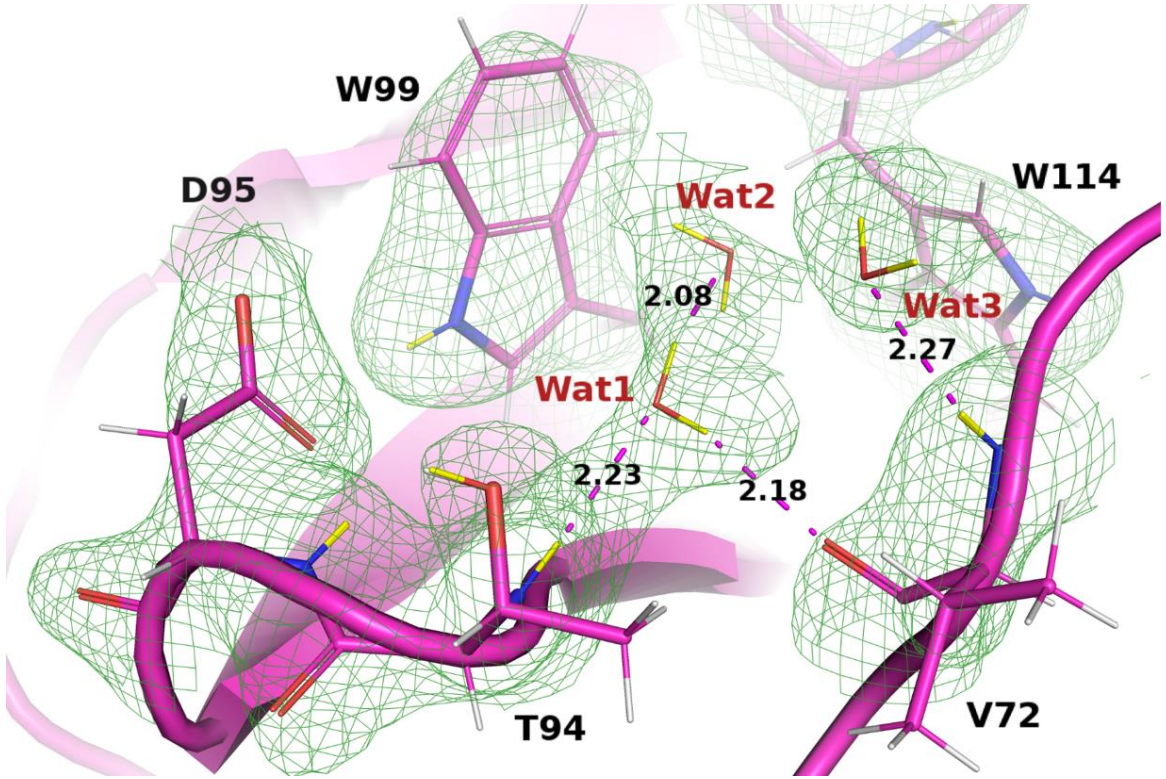
Gajdos L. *et al.*, Structure, 2021

Binding site details of the D-PLL/Fuc-d_{12} complex

- Direct hydrogen bonds
- Water-mediated in iteractions
- CD-π stacking interaction between the apolar face of fucose and tryptophan residues



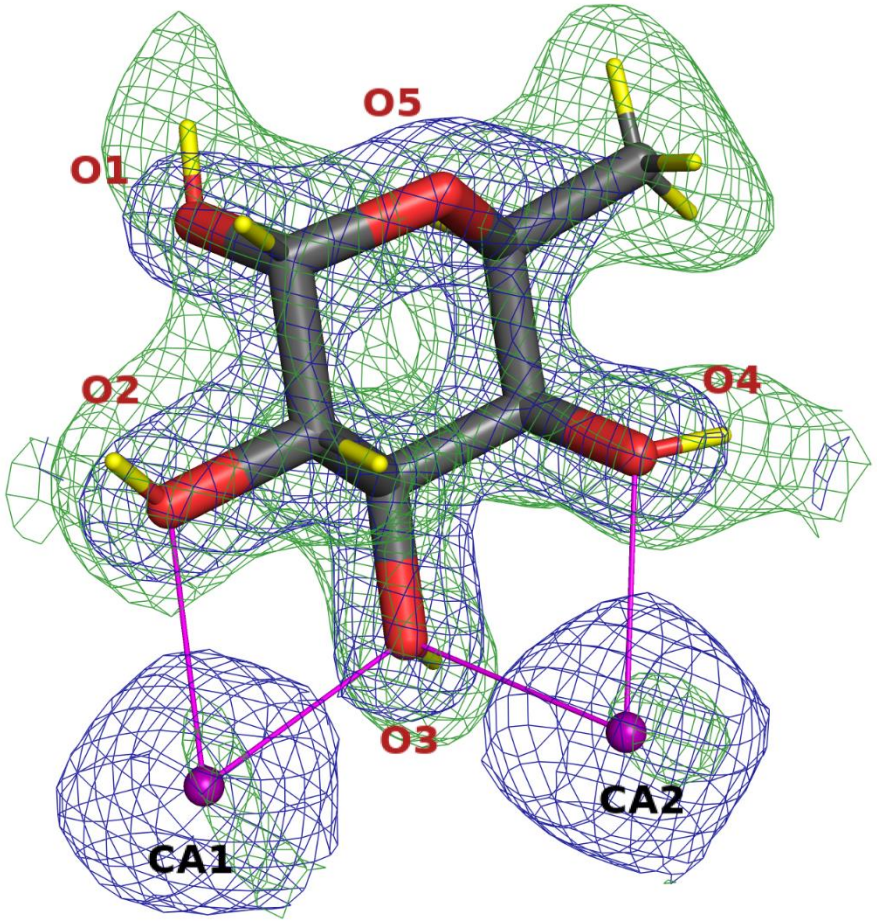
D-PLL/Fuc-d_{12}

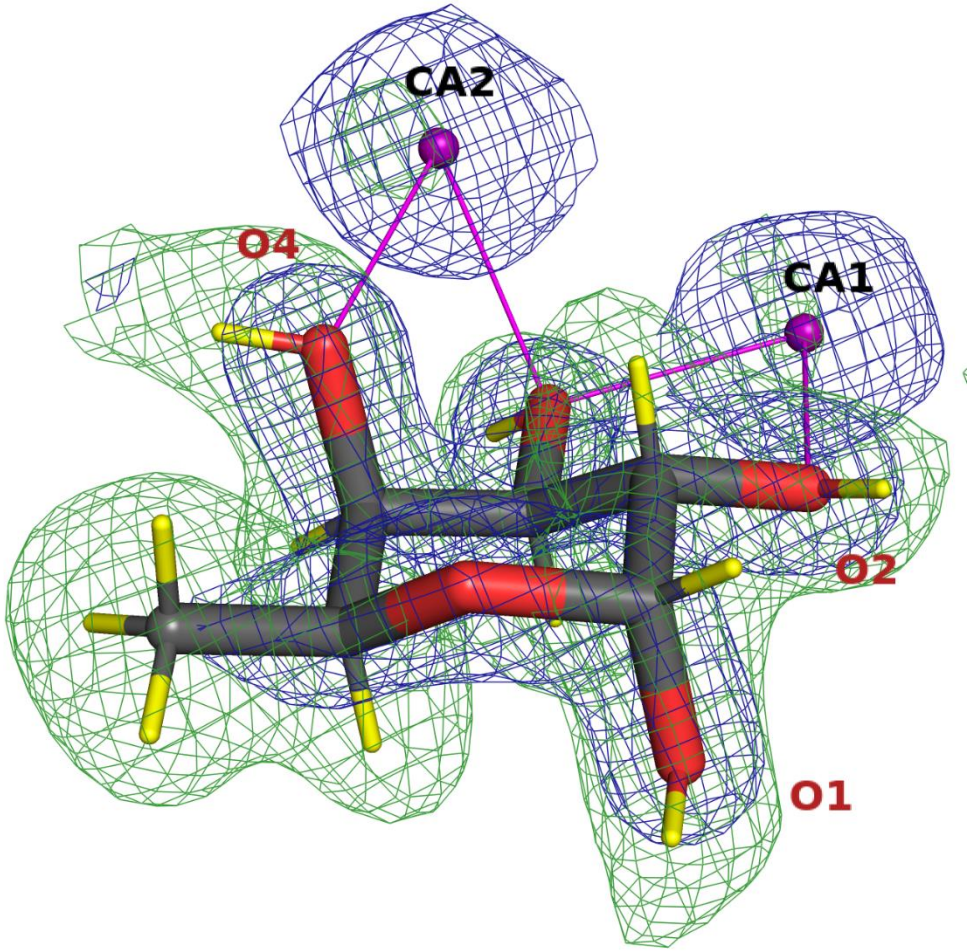


H/D-PLL apo

Perdeuterated fucose in the LecB binding site

- Electron density (1.4σ)
- Neutron density (2.2σ)

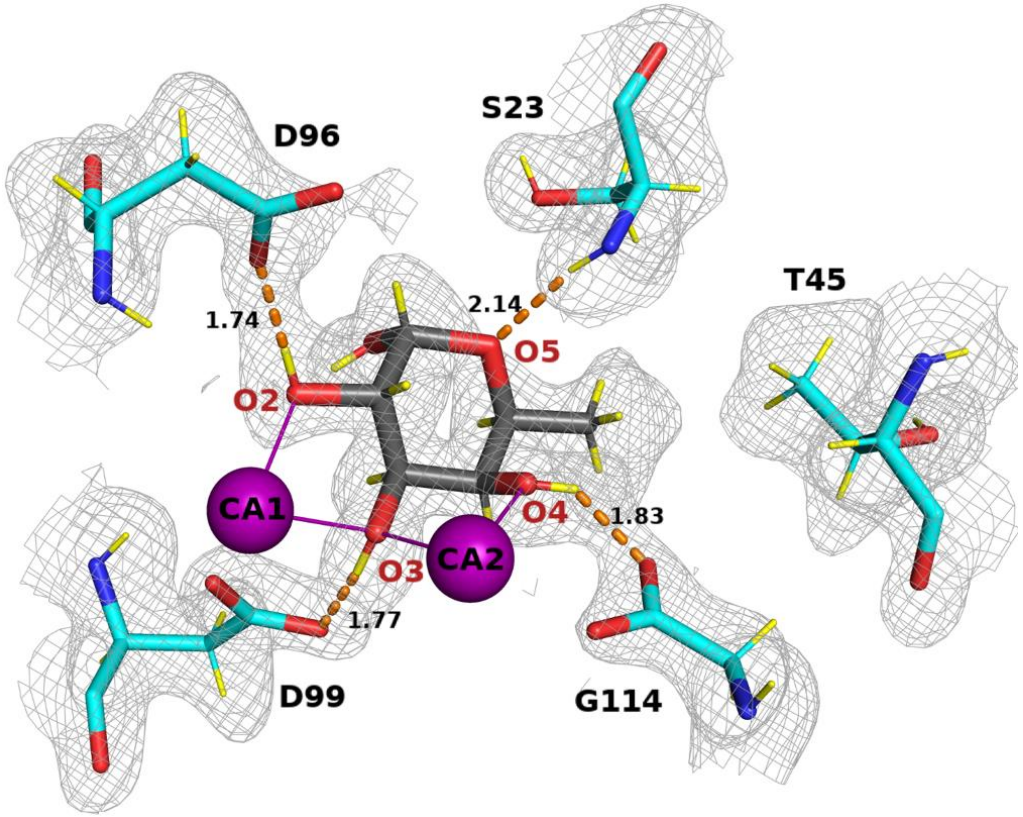


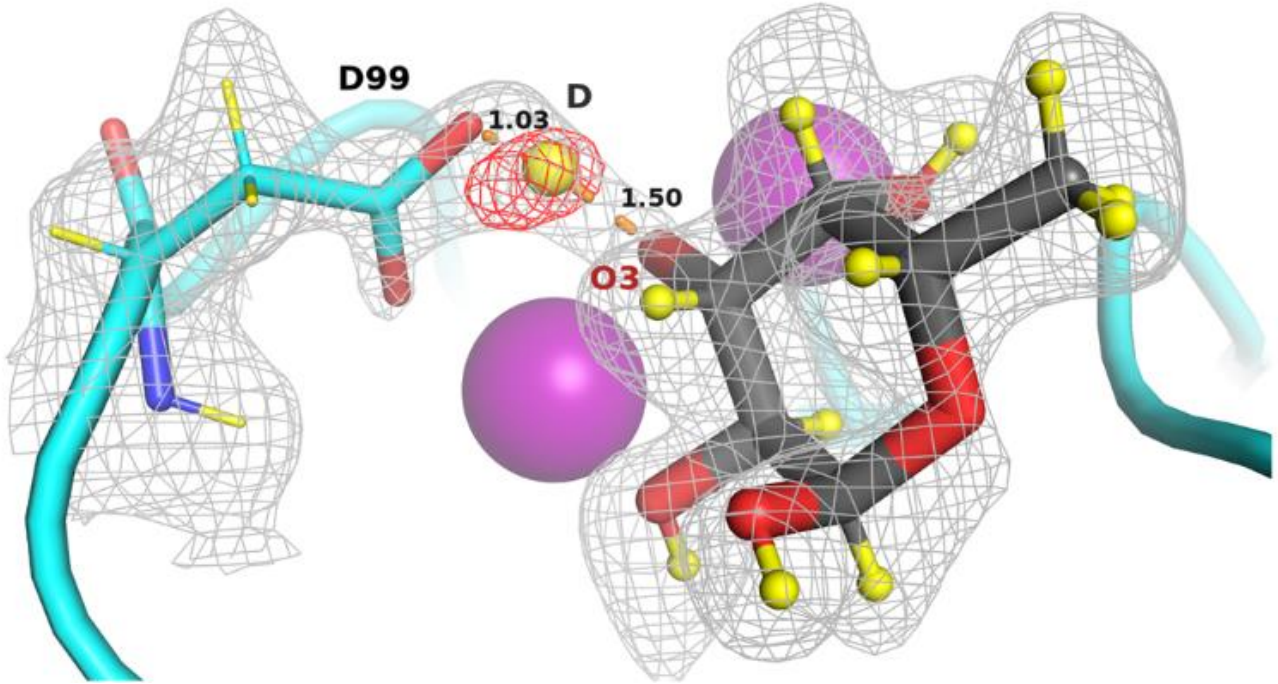


Gajdos L. *et al.,* (2022) *Nat Commun*

H-bonding network and a low-barrier H-bond

- 4 direct H-bonds (orange dashed lines) with the protein + hydrophobic interaction
- Charged amino acid residues are non-protonated
- Delocalized electrons contribute to the overall net charge -2
- A low-barrier hydrogen bond formed in the proximity of calcium ions





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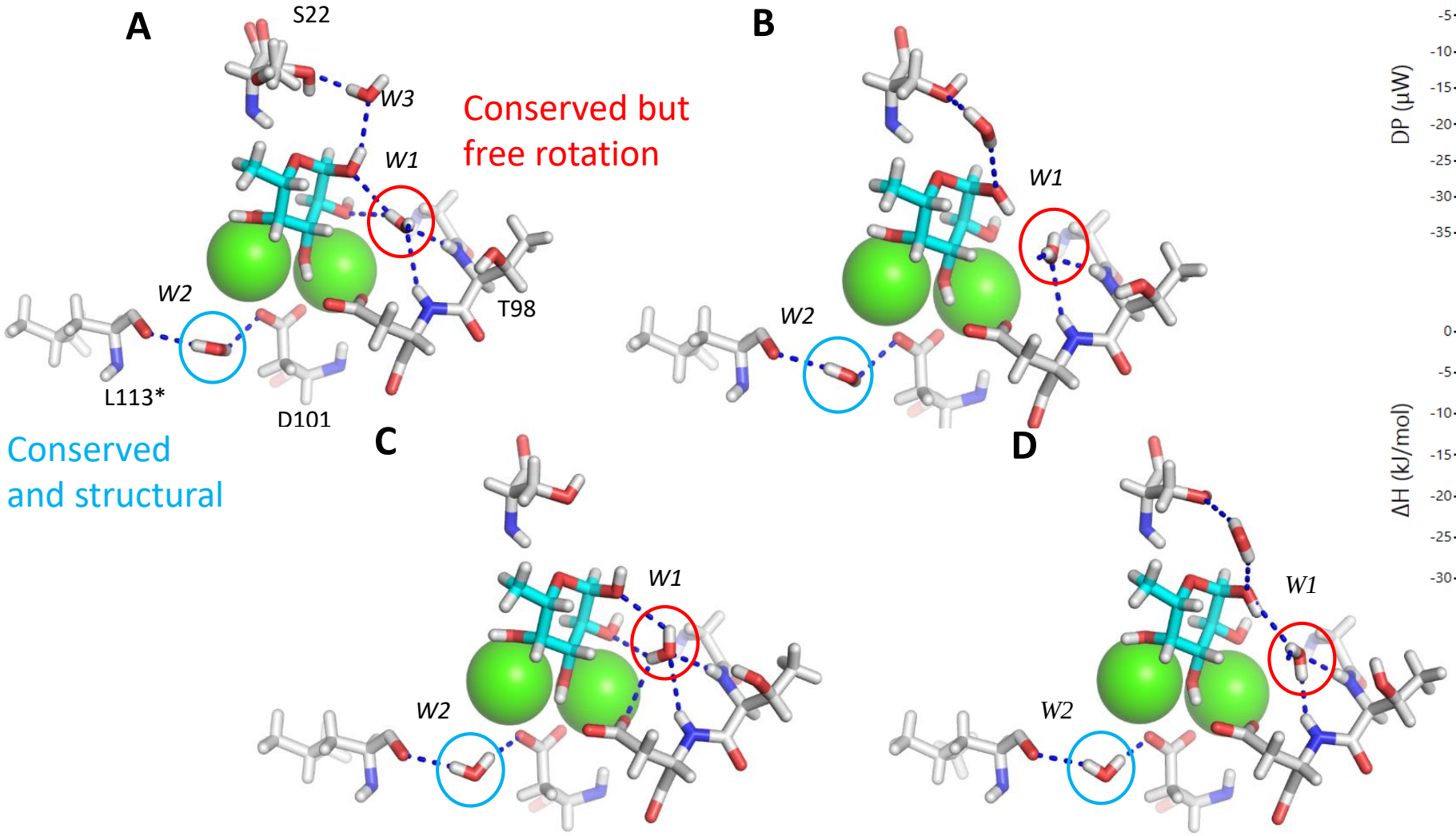
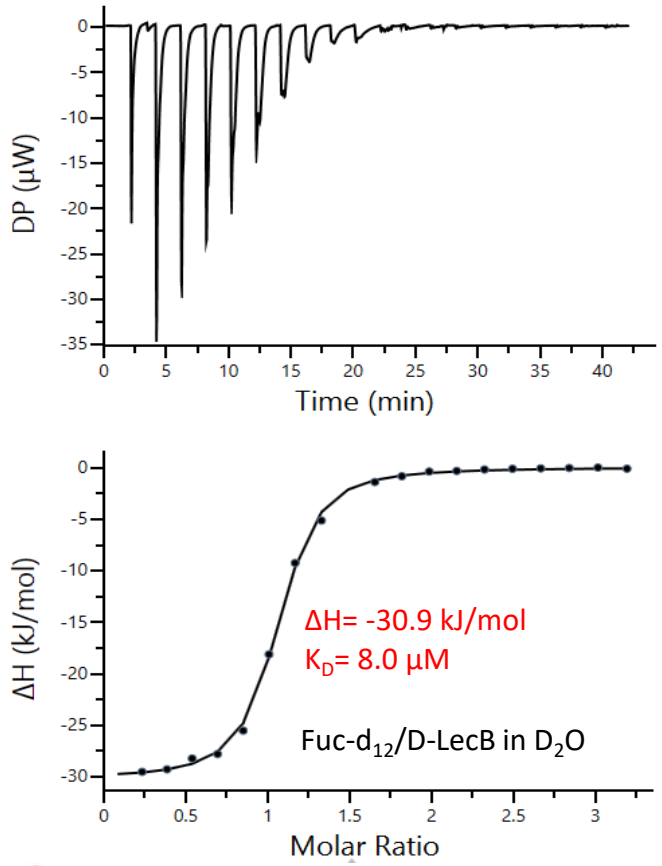
Water network in the fucose binding site of LecB





Water molecules in four chains (A, B, C, D) in the **neutron structure**

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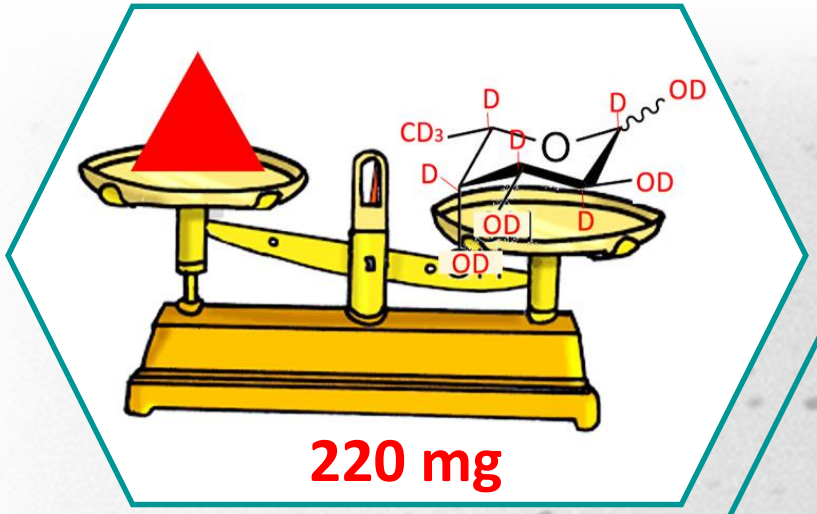
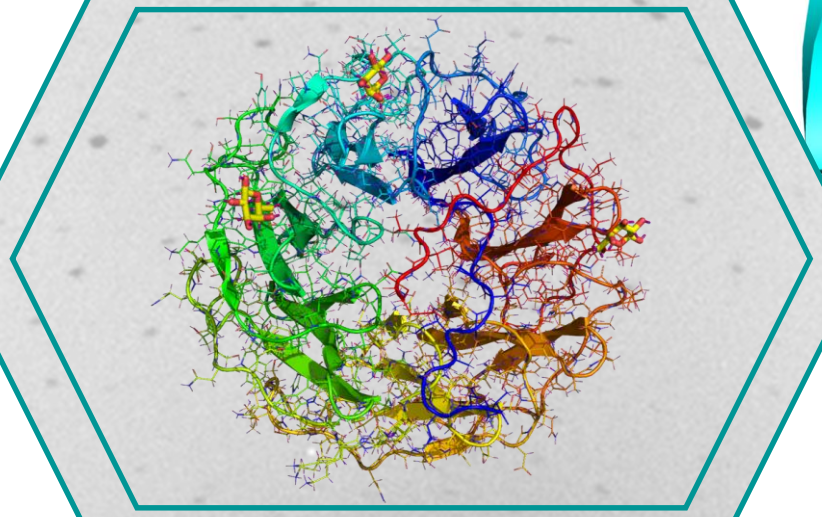
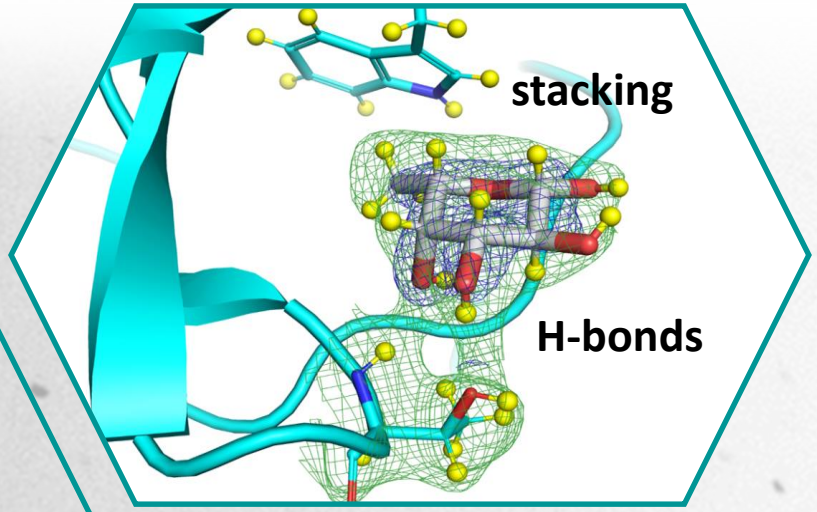
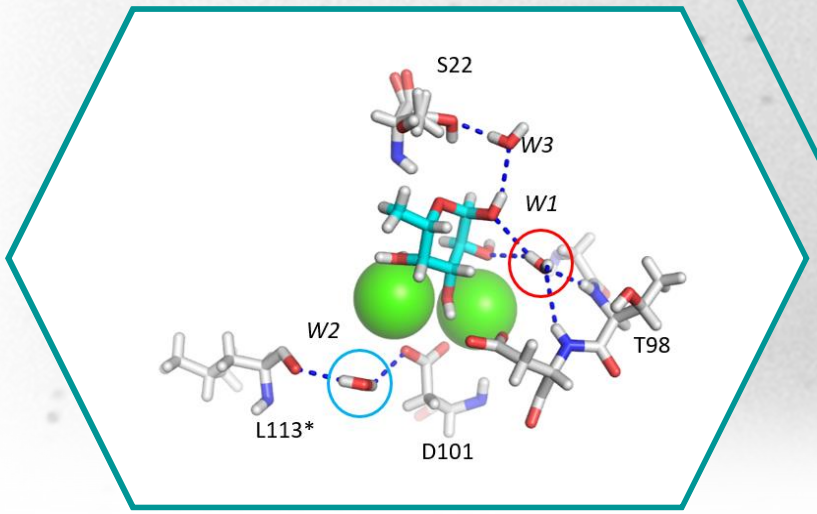
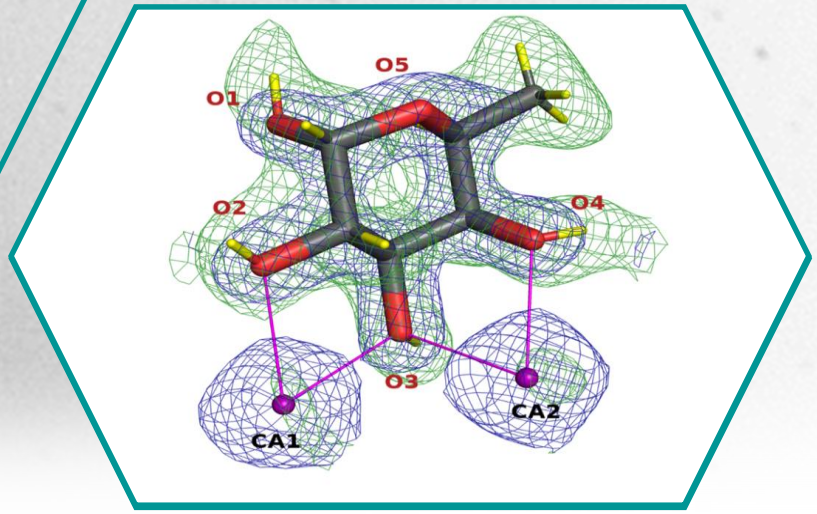
Summary


D
CD3
D
D
OD
D
O
D
OD
OD
D
OD
220 mg




stacking
H-bonds


S22
W3
W1
W2
T98
L113*
D101


O5
O1
O2
O4
O3
CA1
CA2

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Atul Kumar

Glycobiochemistry group