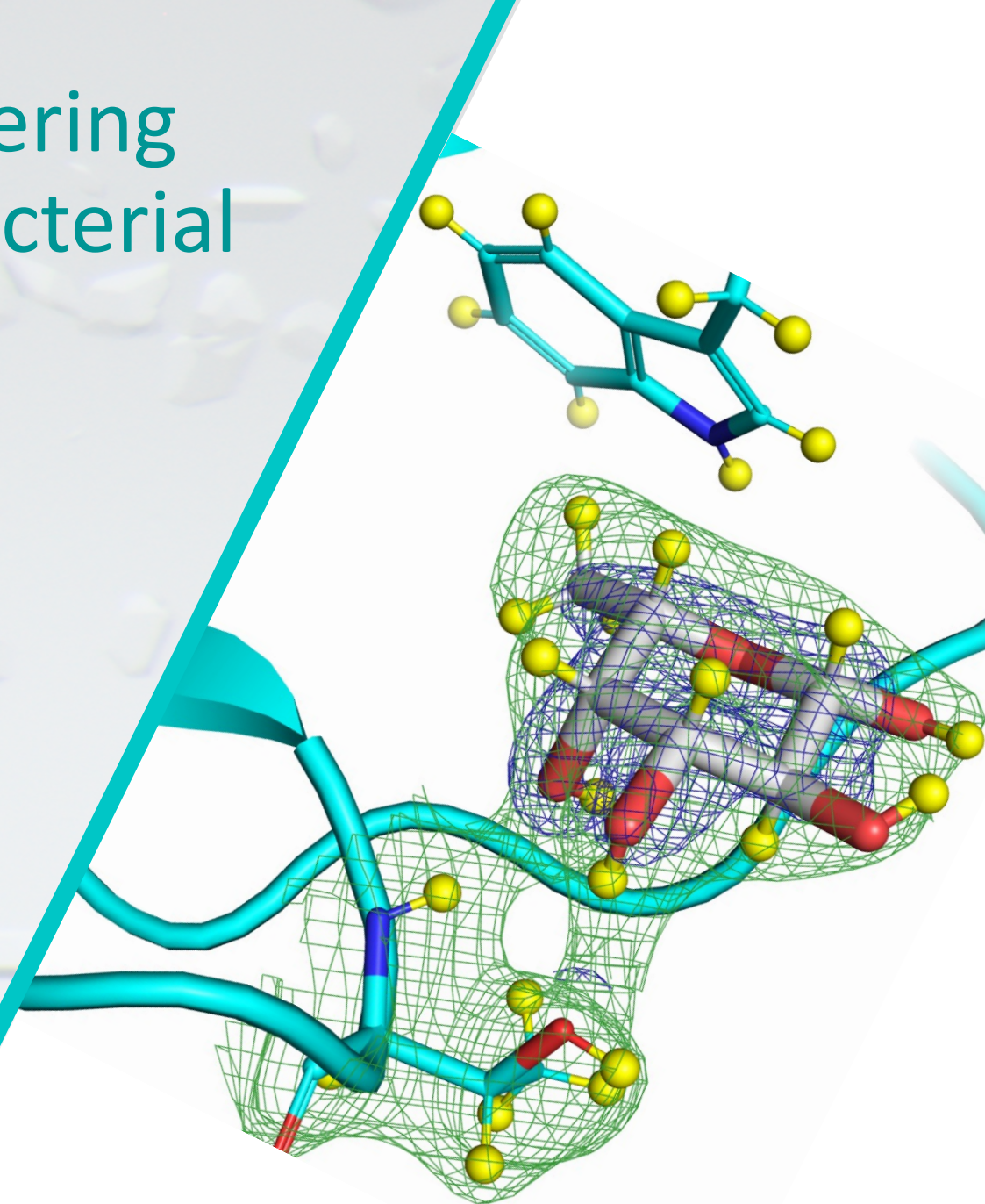


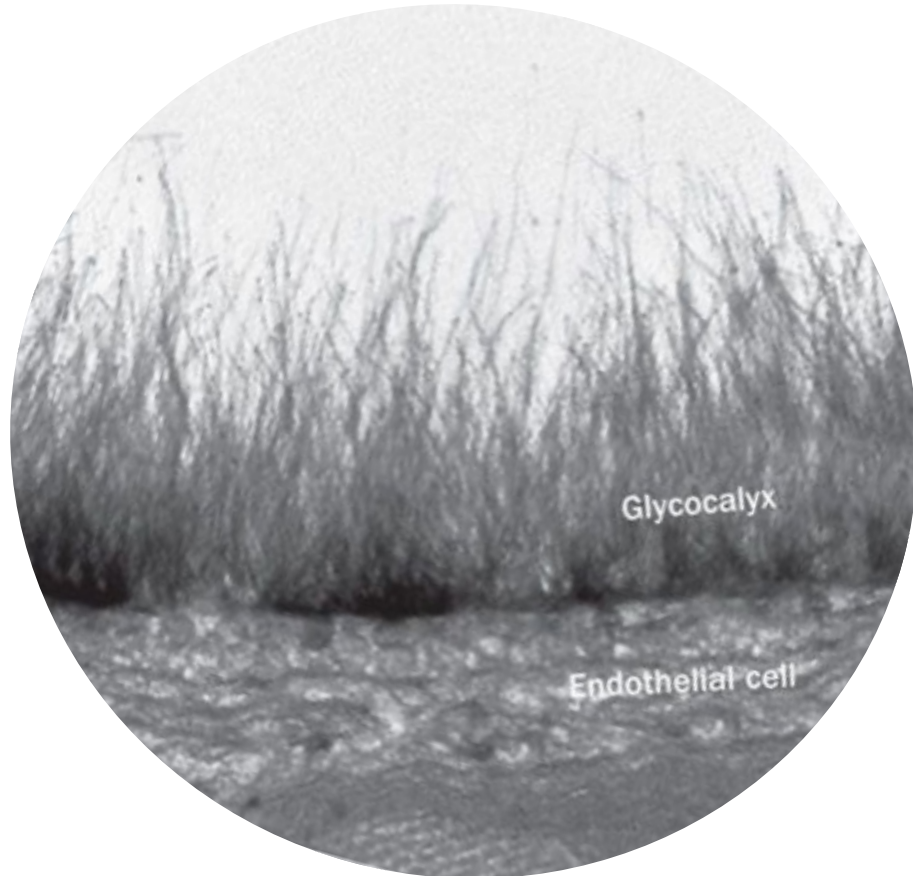
Neutron diffraction for deciphering lectin-glycan interactions in bacterial infection

Lukáš Gajdoš

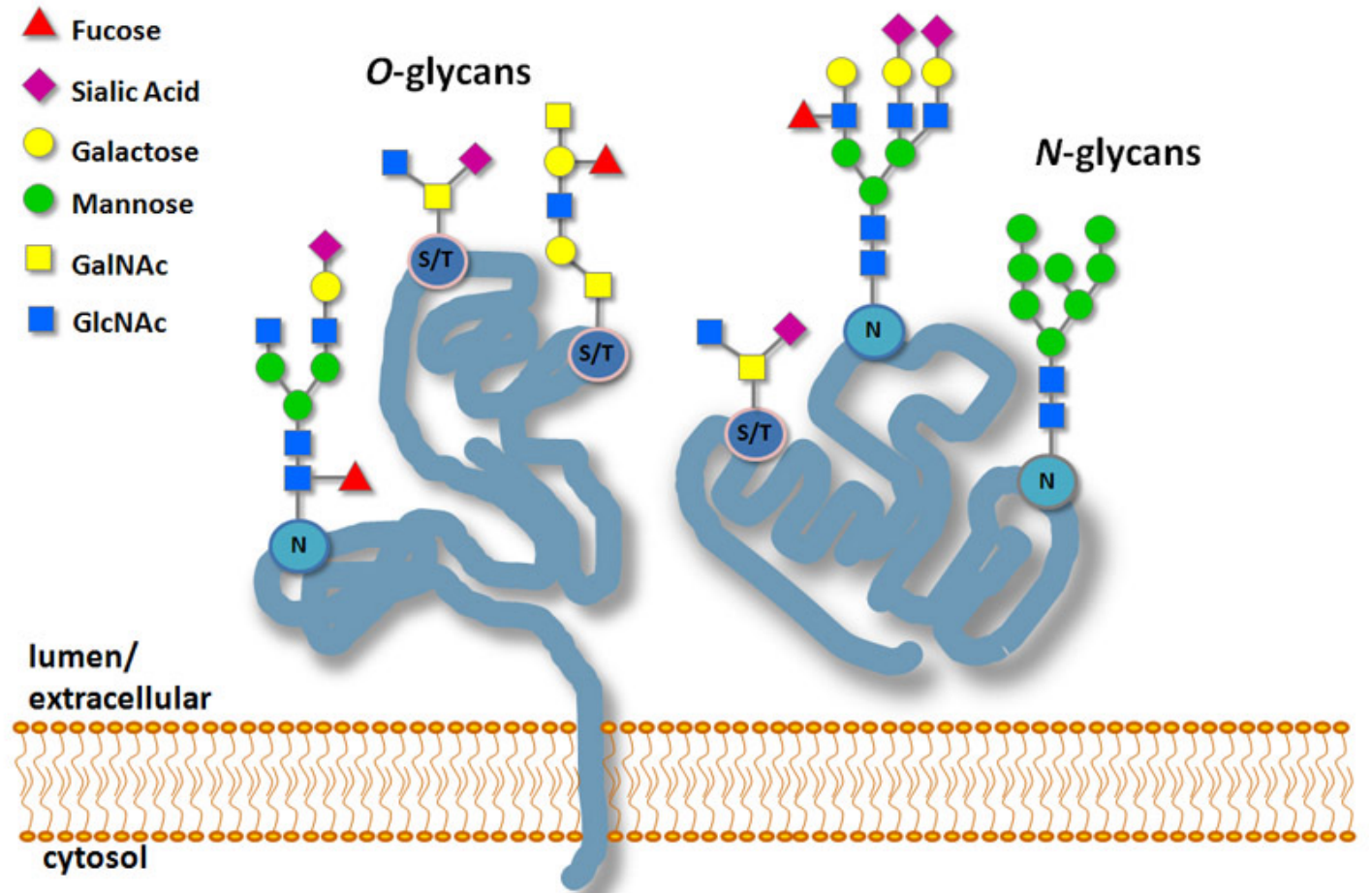
ECM34



Glycans

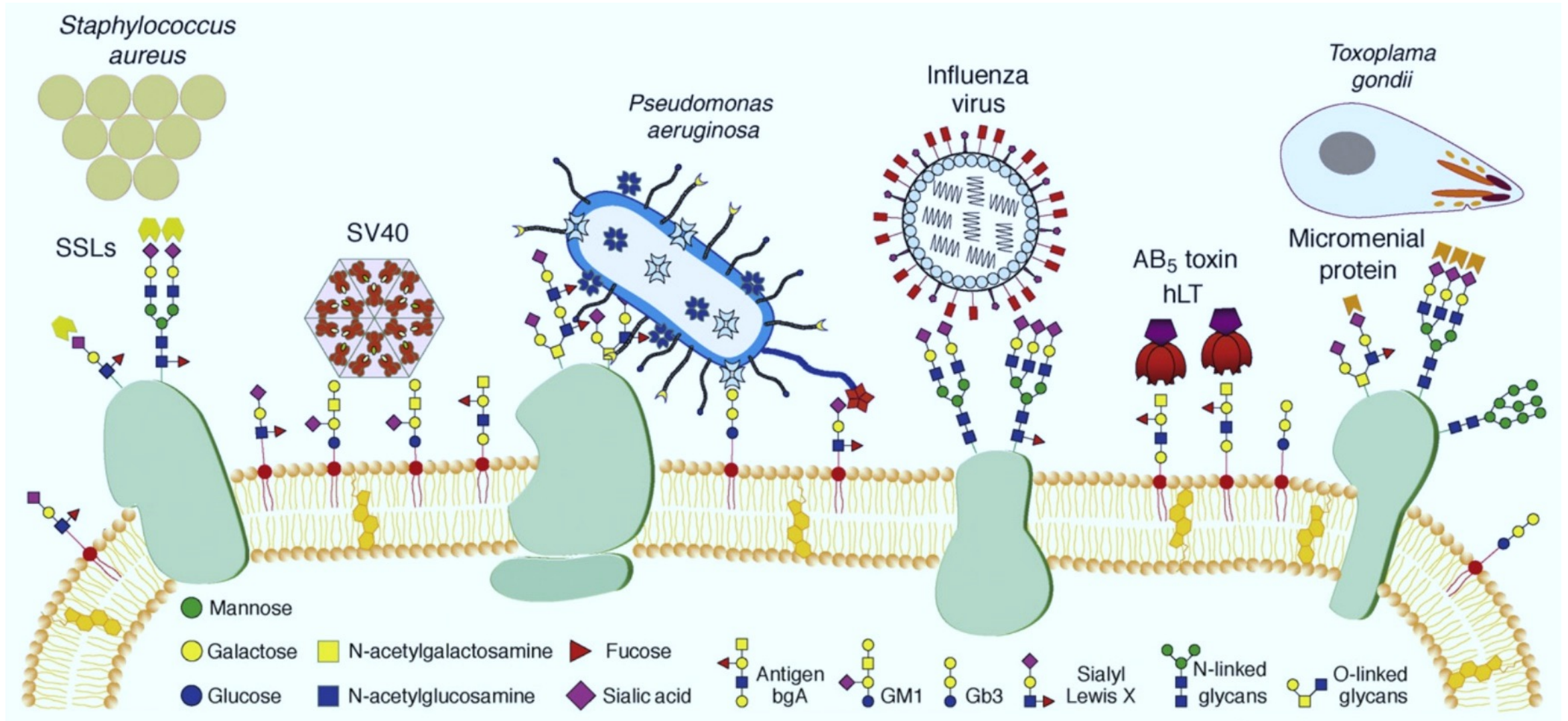


Nieuwdorp M., et al. 2005



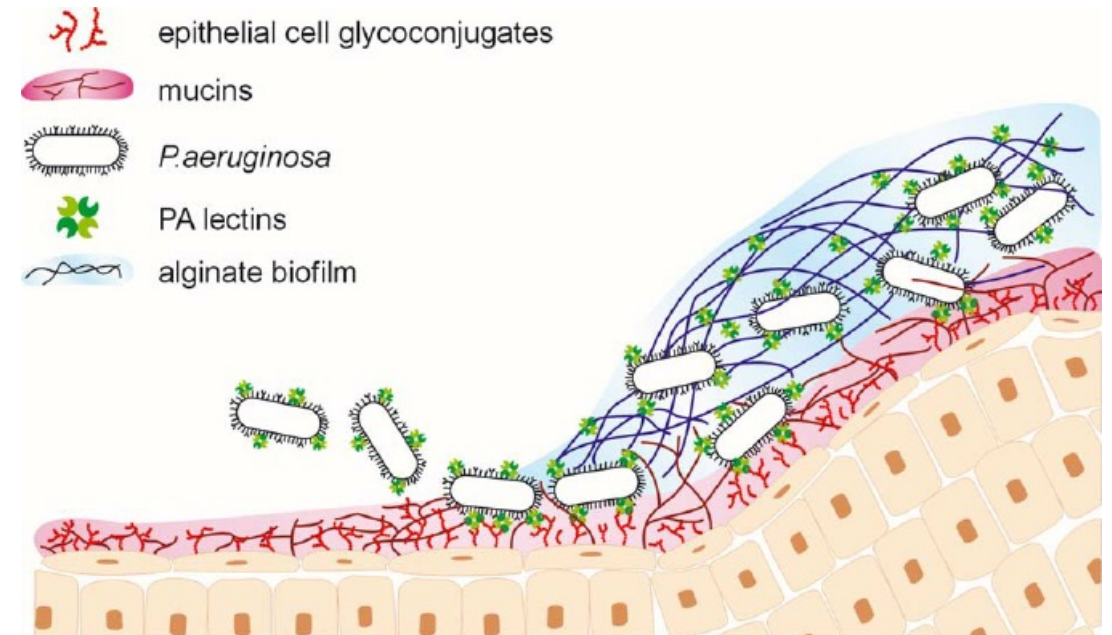
Lectins are proteins that can decode this complex „glycocode“

Lectins from pathogenic organisms

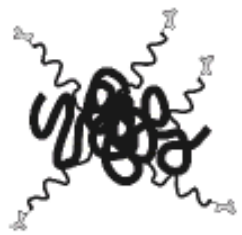


Pseudomonas aeruginosa

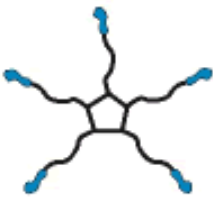
- Human **opportunistic** pathogen
- Lung infections (**cystic fibrosis** patients)
- Two soluble **Ca²⁺**-dependant lectins
- **LecA** (PA-IL), **galactose**-specific
- **LecB** (PA-III), **fucose**-specific
- Roles in attachment and biofilm formation
- Targets for novel antiadhesive glycomimetics



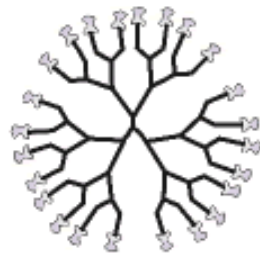
Imberty A., et al., 2014



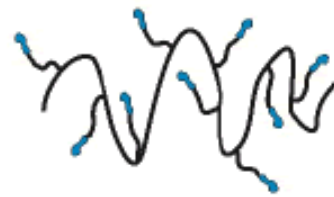
Neoglycoprotein



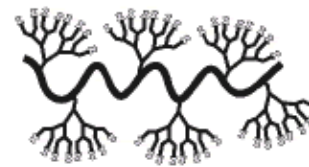
Glycocluster



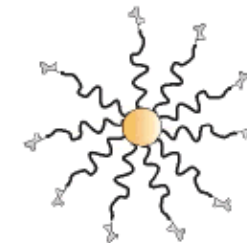
Glycodendrimer



Glycopolymer



Dendronized polymers



Glyconanoparticles

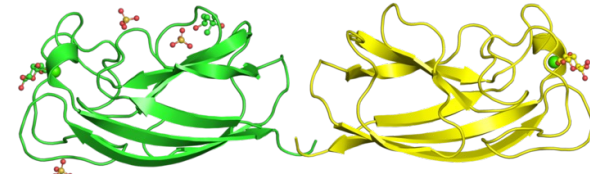
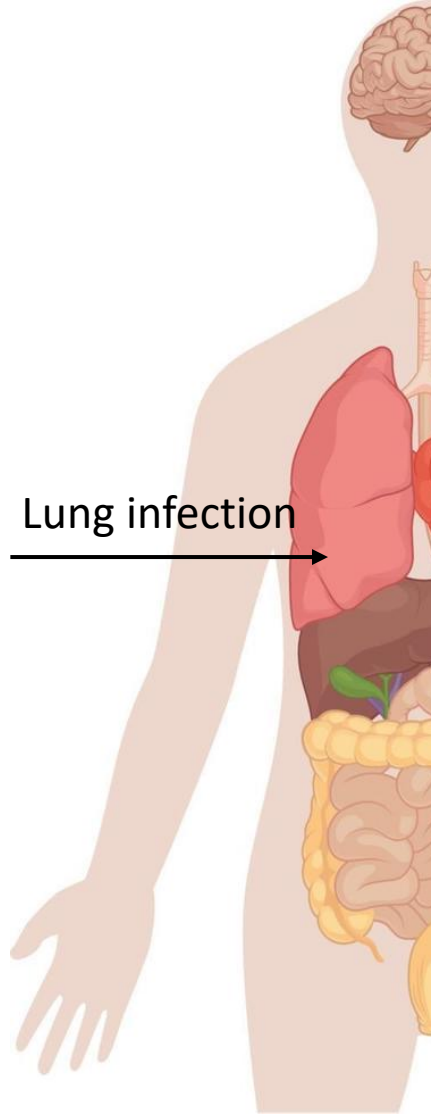


Nanoemulsions

Pseudomonas aeruginosa



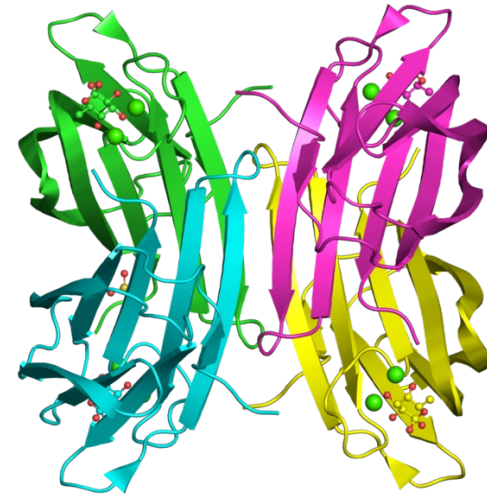
P. aeruginosa
opportunistic bacteria



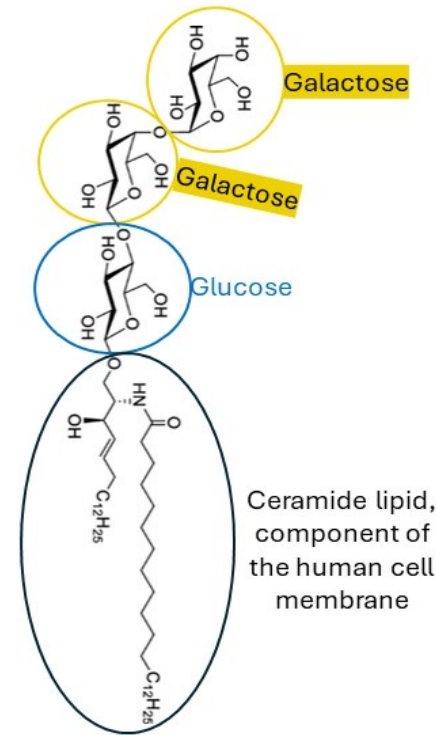
LecA lectin (PA-IL, PDB:1UOJ)



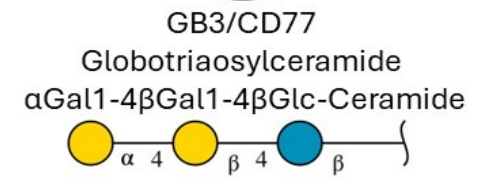
LecB lectin (PA-III, PDB: 1GZT)



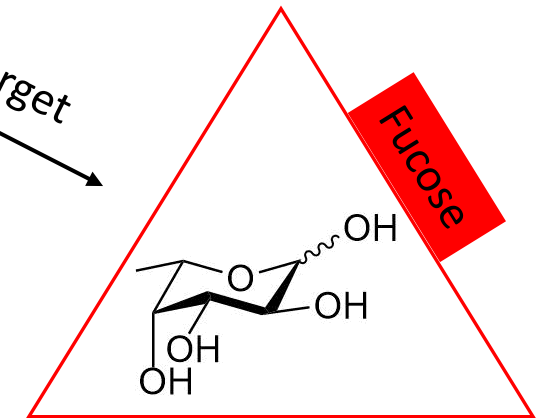
Cell target



Ceramide lipid,
component of
the human cell
membrane

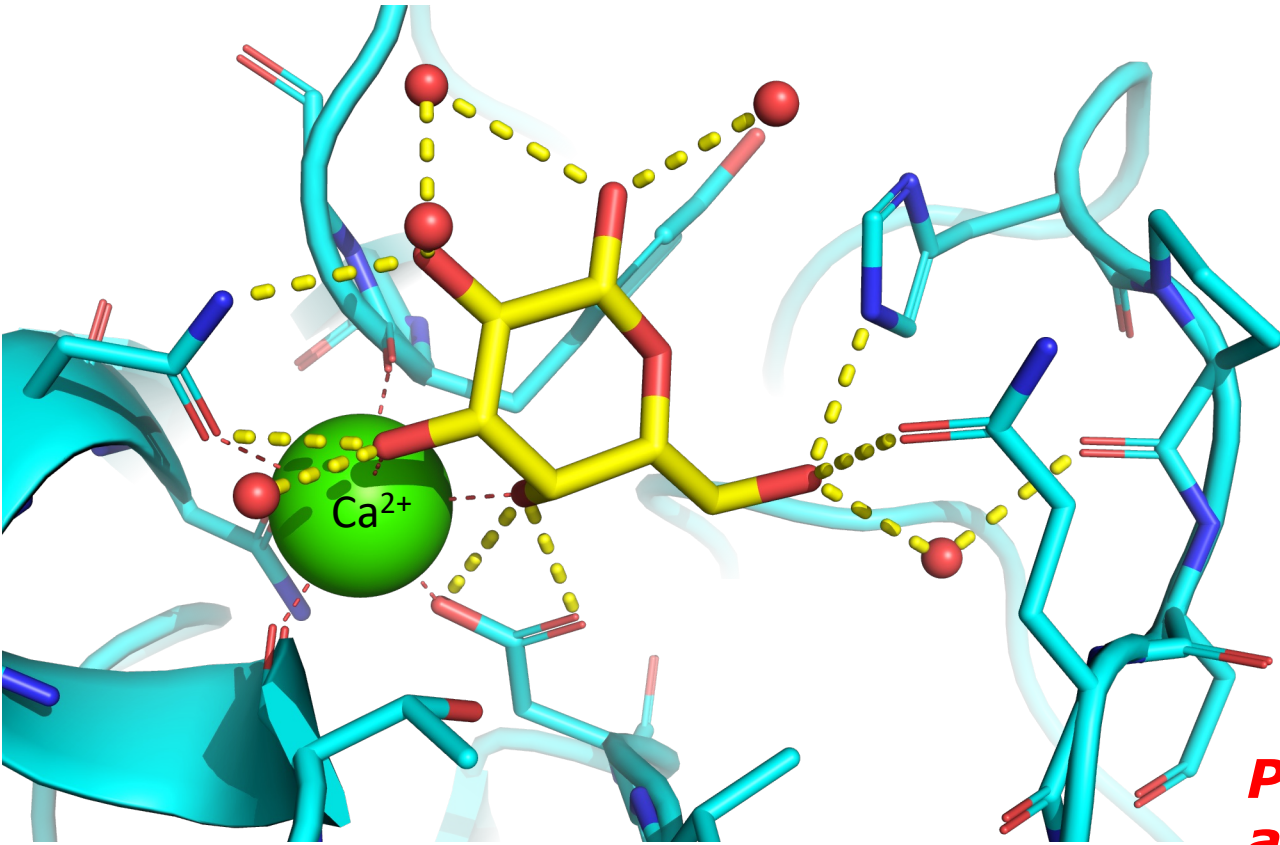


Cell target



Protein-carbohydrate interactions

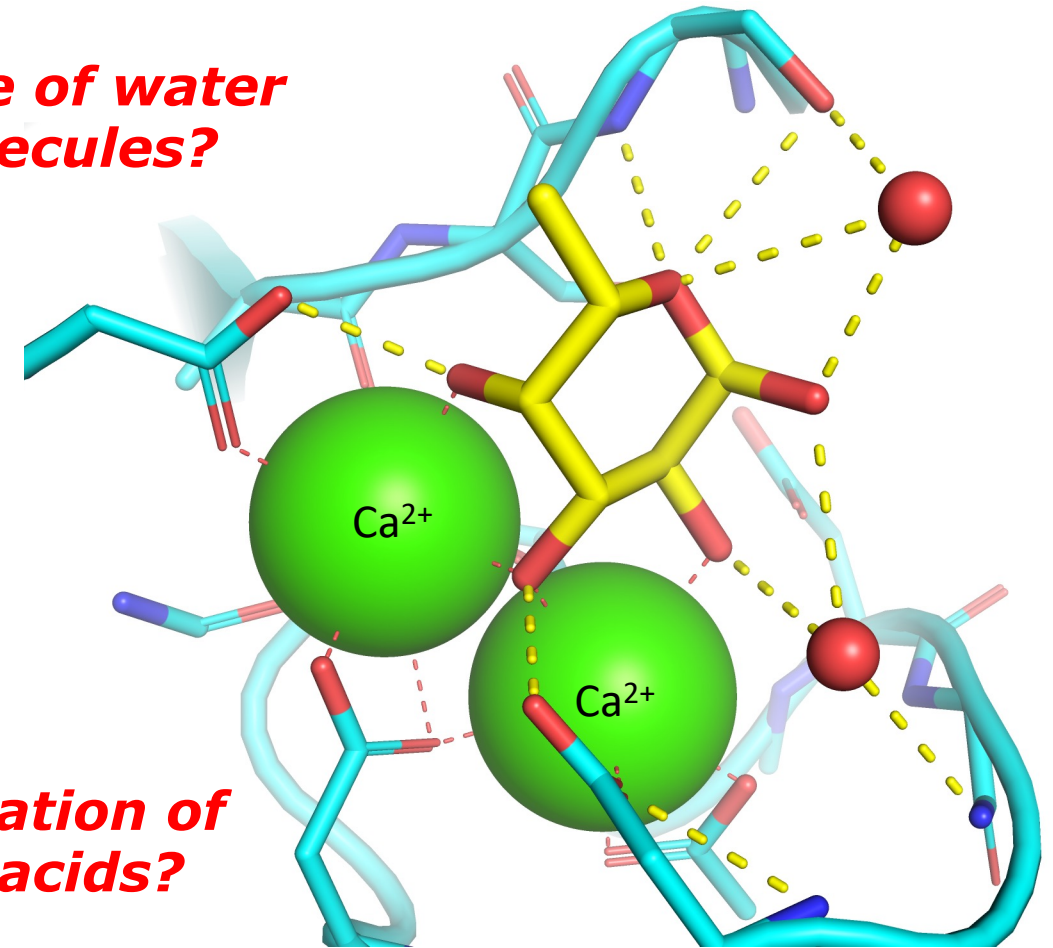
Galactose in LecA



Hydrogen bond details

Fucose in LecB

Role of water molecules?

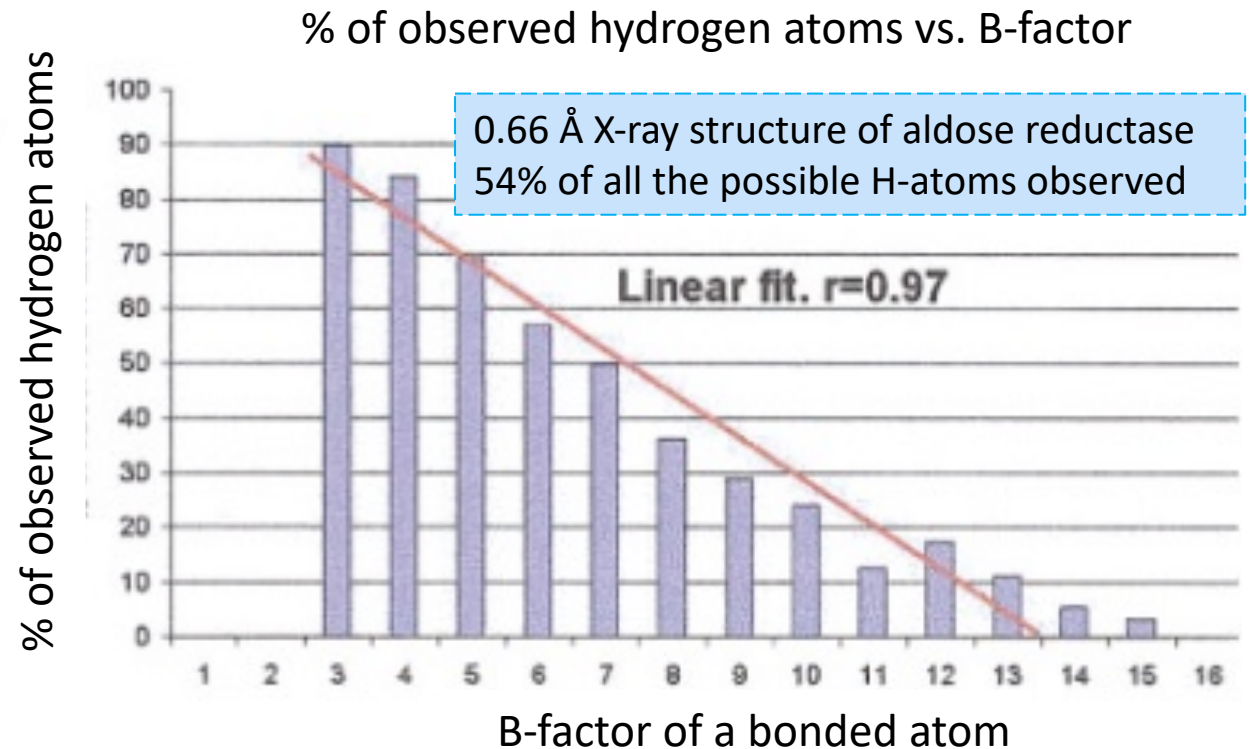
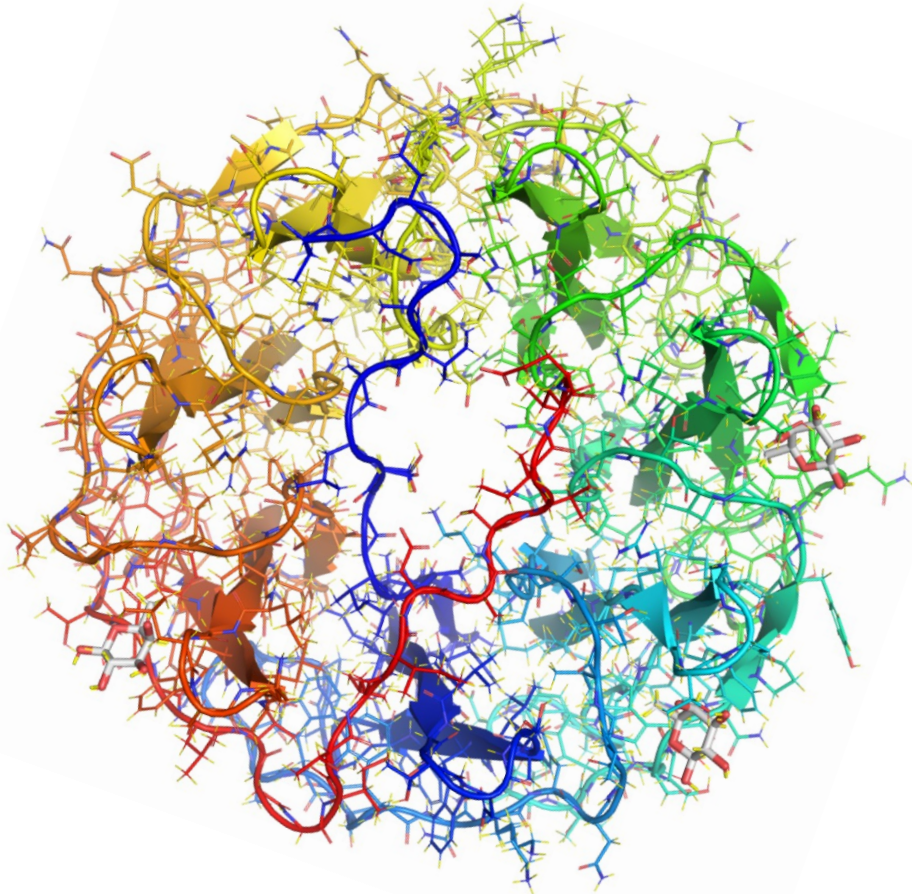


Protonation of amino acids?

Wanted : Location of hydrogen atoms

H atoms „invisible“ in X-ray structures

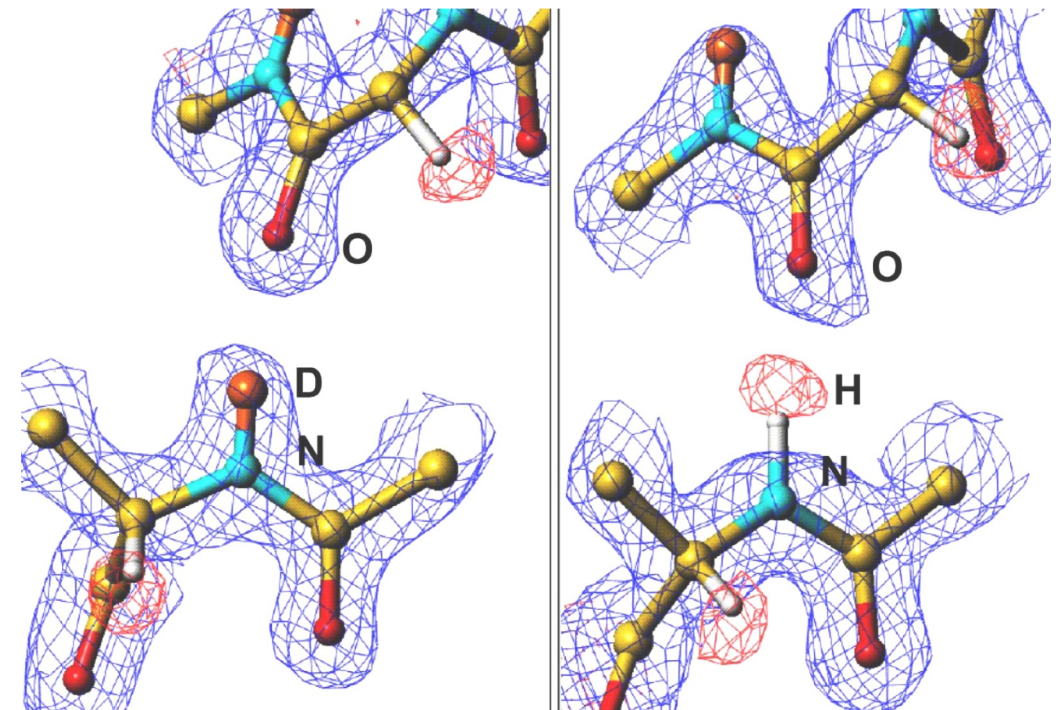
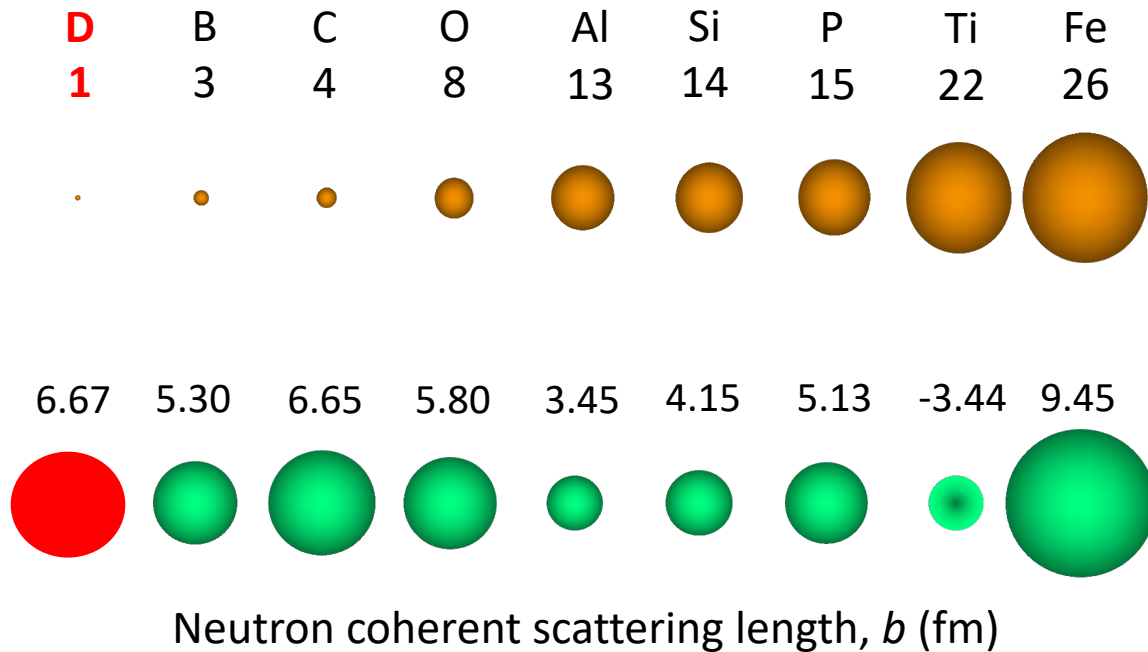
- **Hydrogen atoms** account for about **half** of all the atoms in proteins
- Critical roles in **biological functions** (enzyme mechanisms, ligand binding)
- **Rarely** observable in X-ray diffraction experiments



Neutrons as a diffraction probe

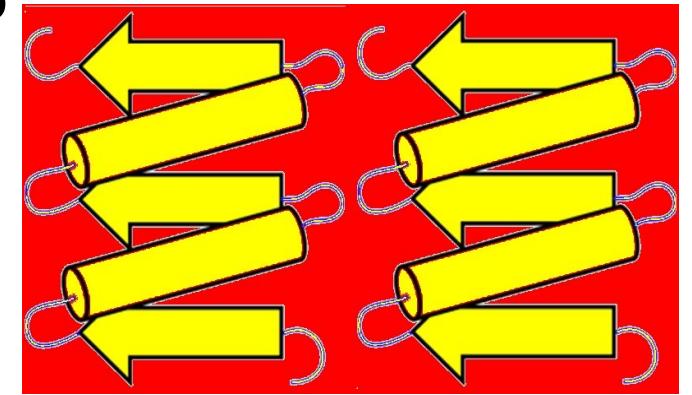
- Interaction with atomic **nuclei**
- Scattering **varies** with elements and even **isotopes** of the same element (**H/D**)
- Non-destructive probe (room-temperature data collection)

X-ray scattering proportional to Z

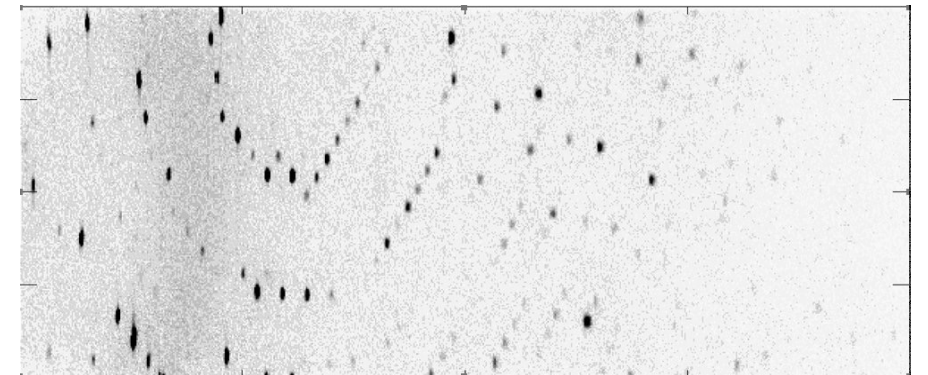


Need of perdeuteration

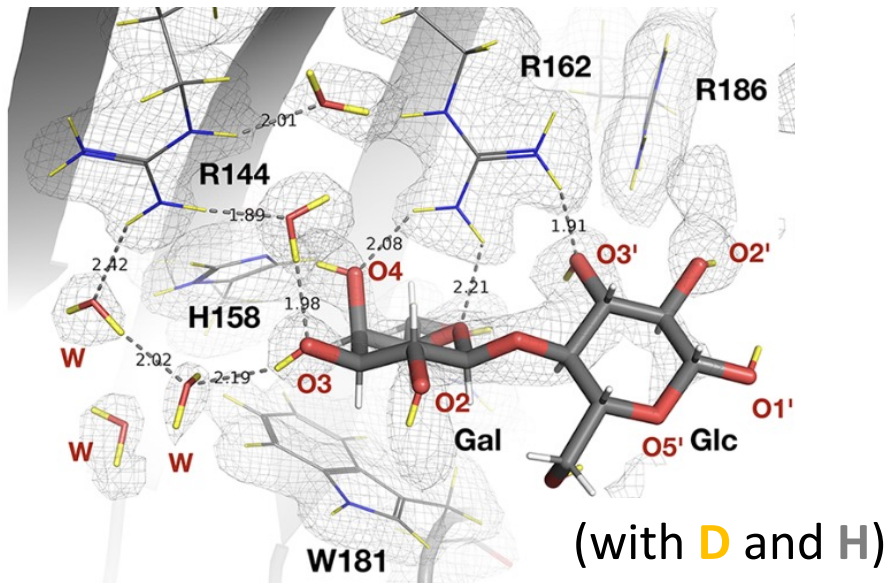
- Full replacement of all **hydrogen** (H) atoms by **deuterium** (D) atoms
- **Reduces** the large **incoherent** scattering of H (~ 40 times larger than for D)
- **Reduces the background** and **increases the signal-noise ratio**
- Clearer visualization of neutron maps
- **Cancellation effects** limit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



Perdeuterated protein, D_2O solvent



Courtesy of Prof. Trevor Forsyth

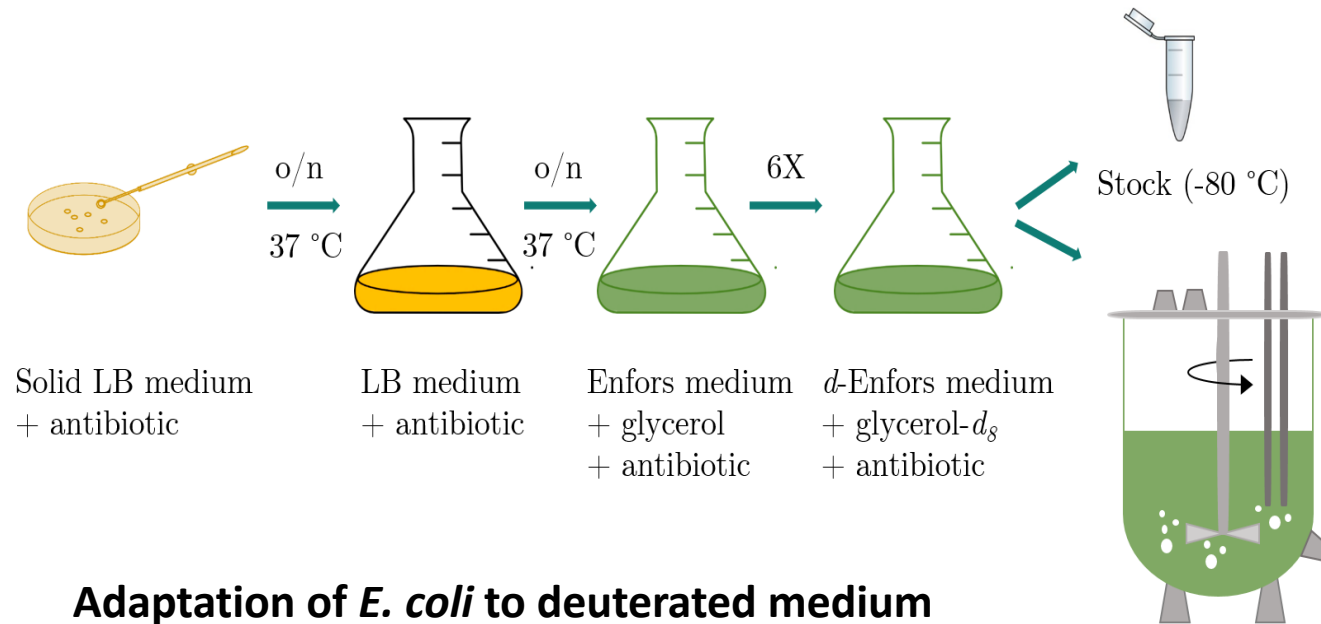


D-Galectin-3C/lactose complex

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

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

Innoculum for fermentation

How to obtain perdeuterated biomolecules?

Proteins:

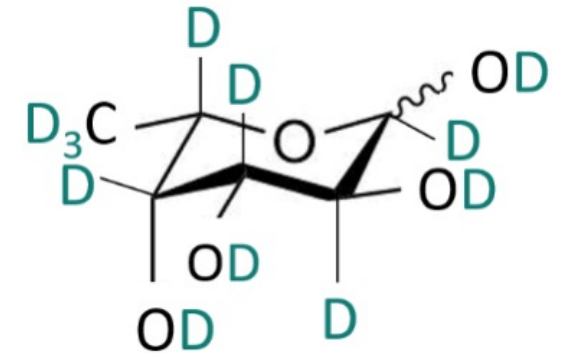
Production of recombinant proteins in D_2O --- > D-Lab at ILL
High cell-density cultures



Sugars:

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

Synthetic chemistry « isotopic hydrogen-exchange technique »

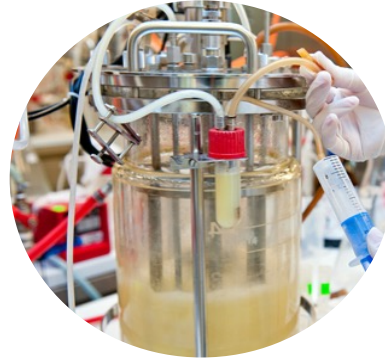


Synthetic (glyco)biology

Methodology



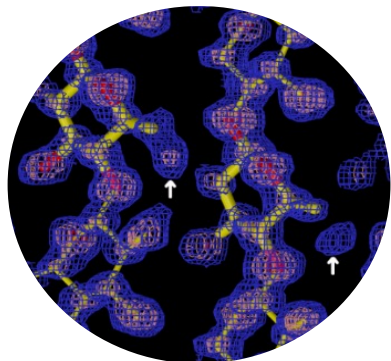
E. coli adaptation to deuterated medium



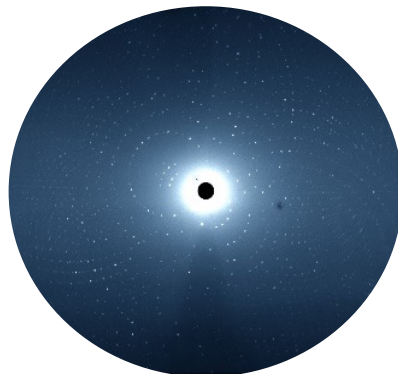
Production of perdeuterated lectins/sugars



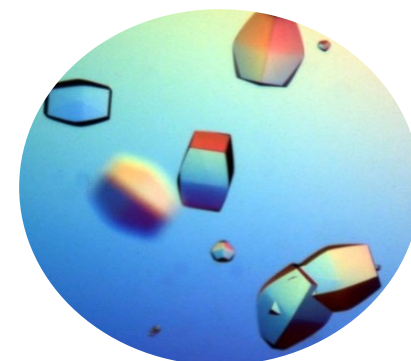
Protein/sugar purification



Structure refinement



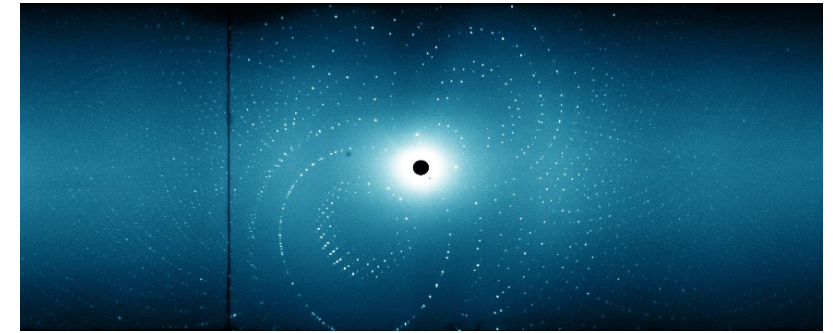
Neutron diffraction



Co-crystallization

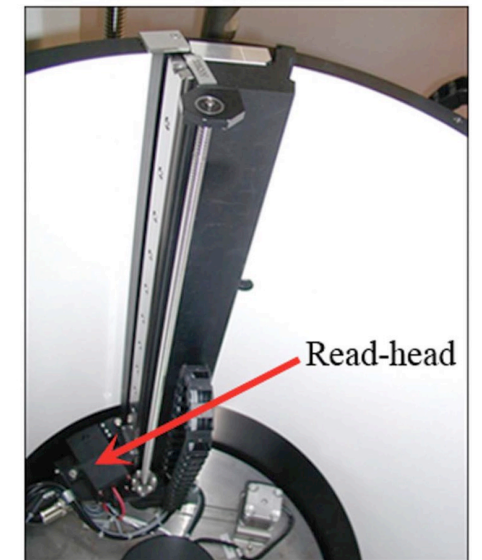
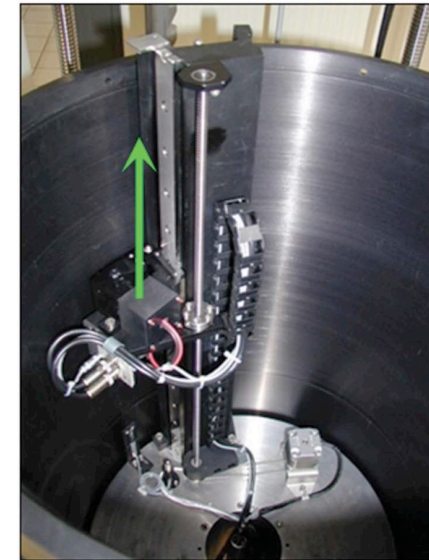
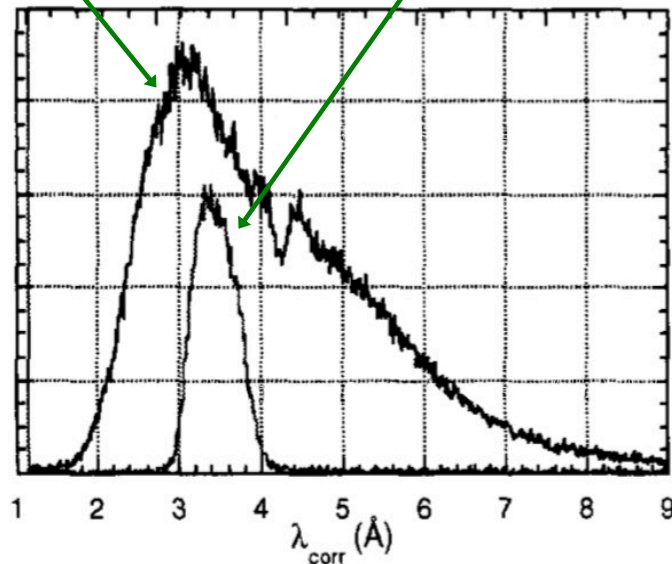
Laue diffractometer LADI-III at ILL

- 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

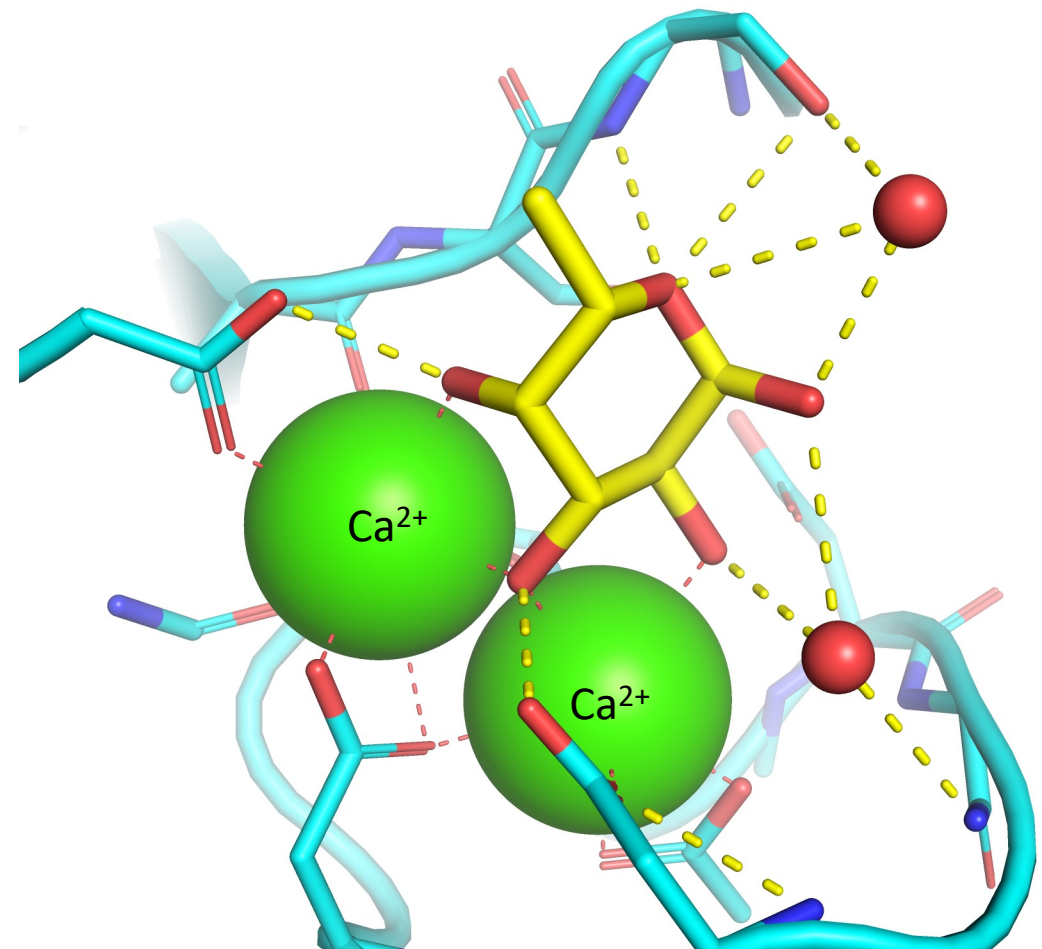
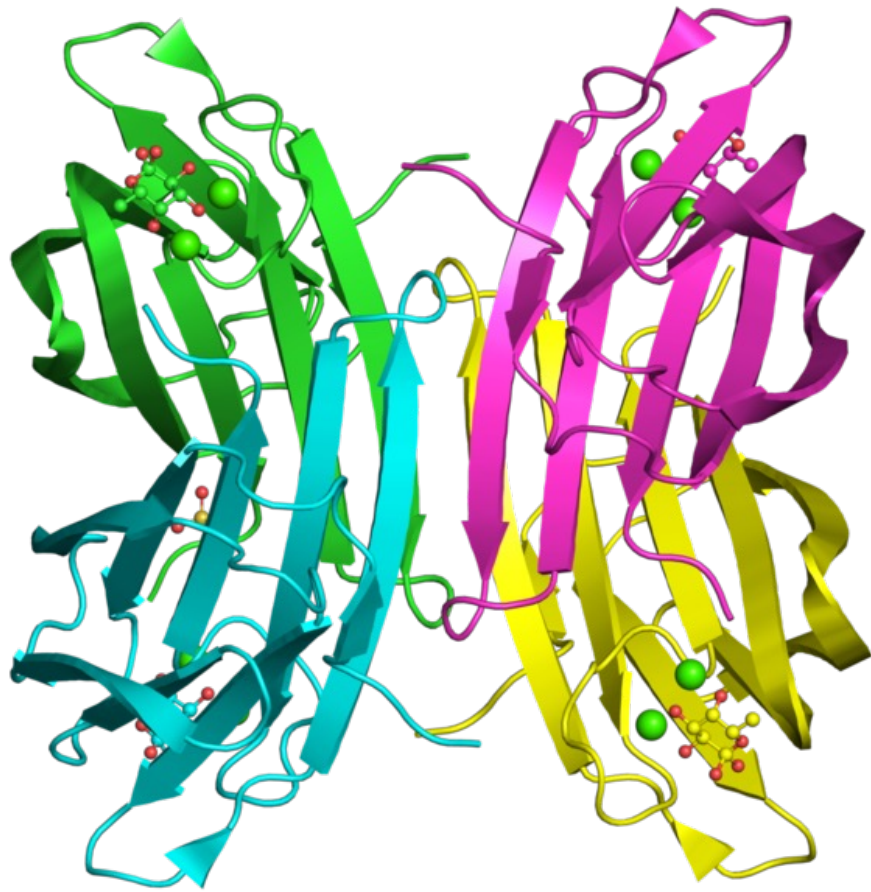


Laue (white) beam

Quasi-Laue (pink) beam

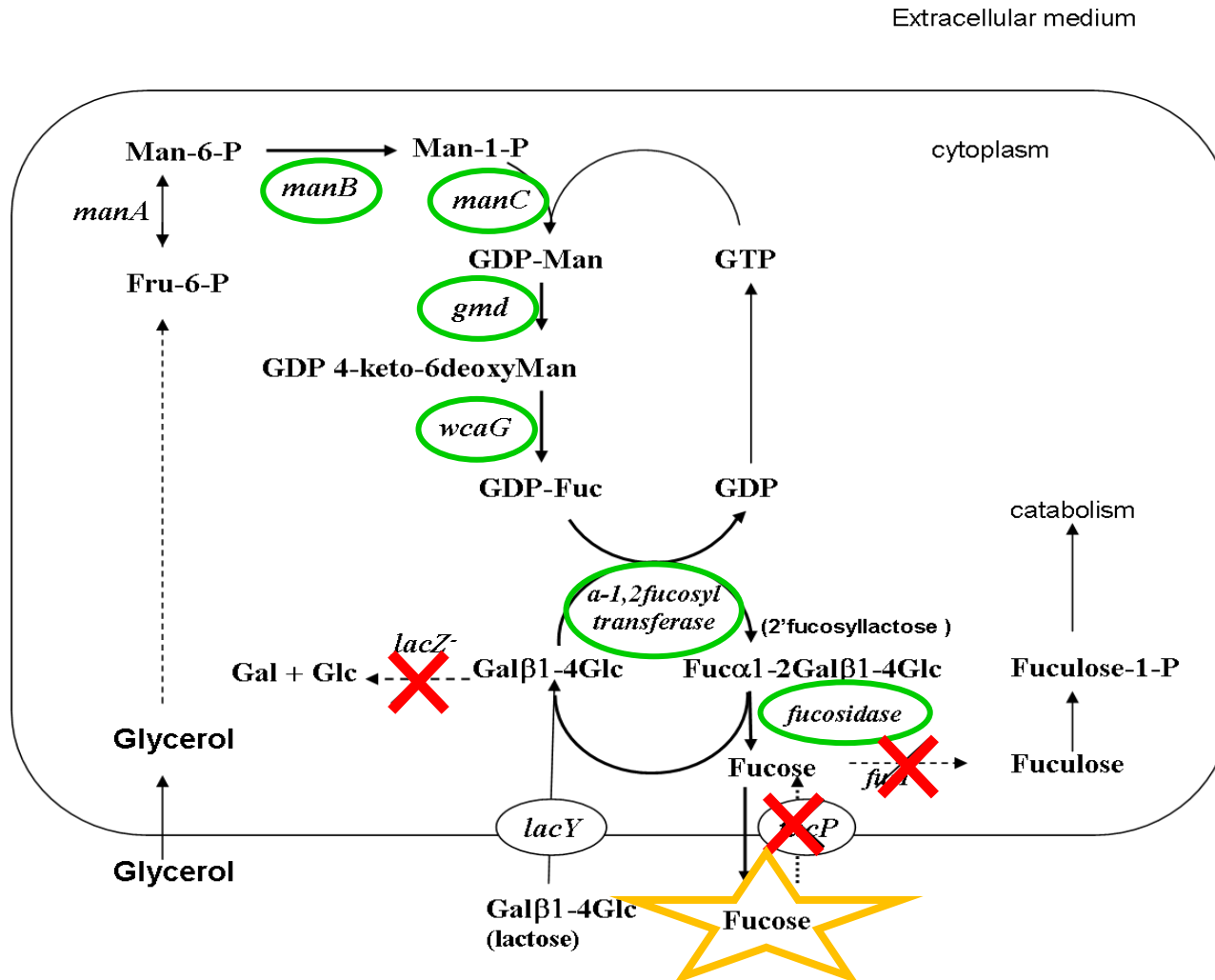
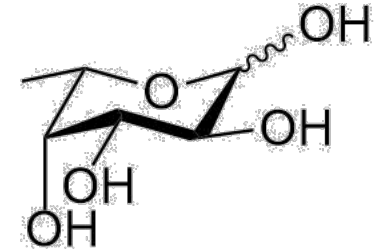


LecB-fucose neutron study



In vivo production of L-fucose-d₁₂ in *E. coli*

Fucose-producing strain of *E. coli* designed and engineered 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**

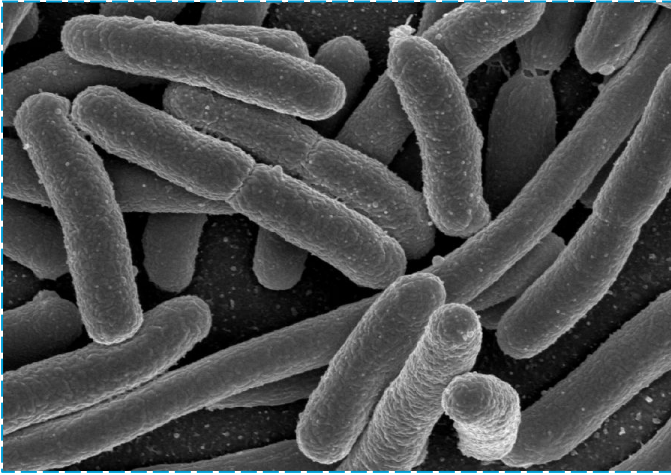


Knocked-out genes

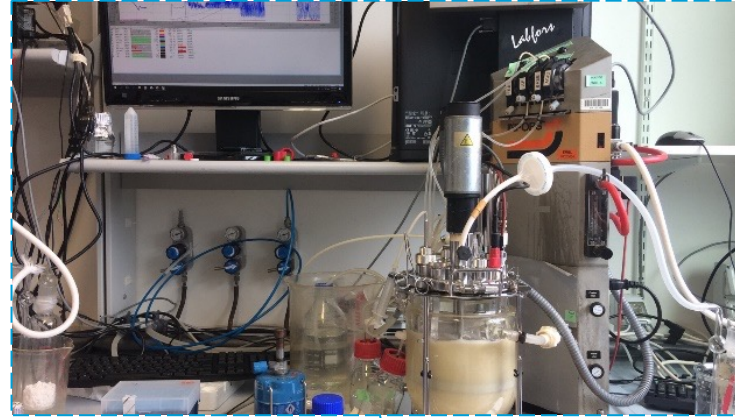
- lacZ: β-galactosidase
- fucI: fucose isomerase
- fucP: fucose permease

Production, purification and characterization of L-fucose-d₁₂

Adaptation to D₂O and glycerol-d₈



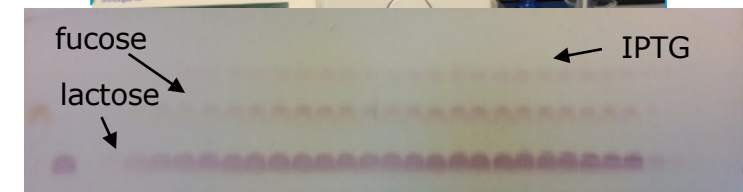
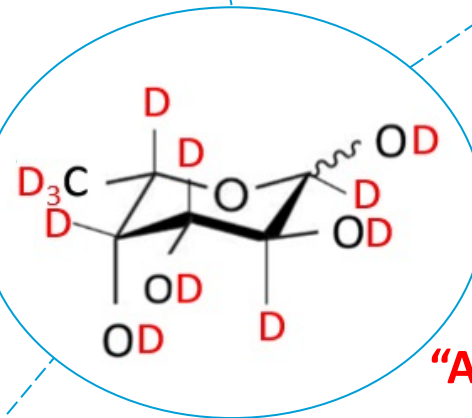
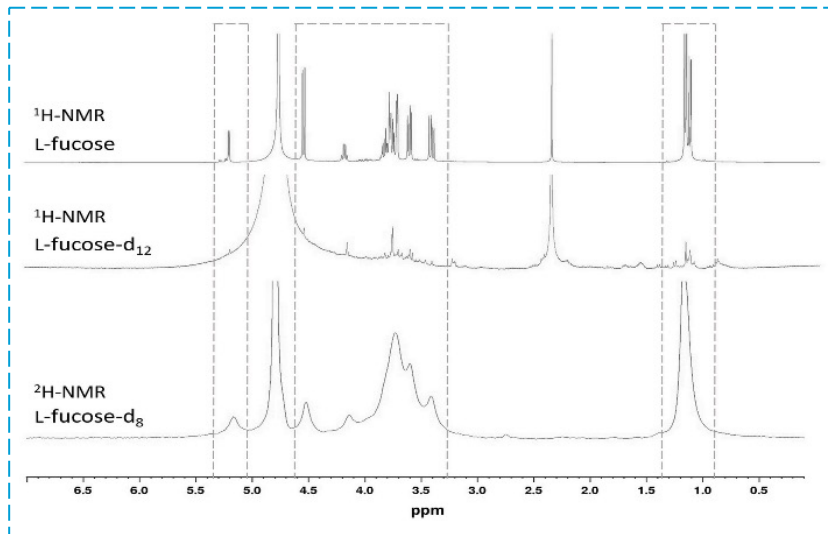
Batch production



Purification



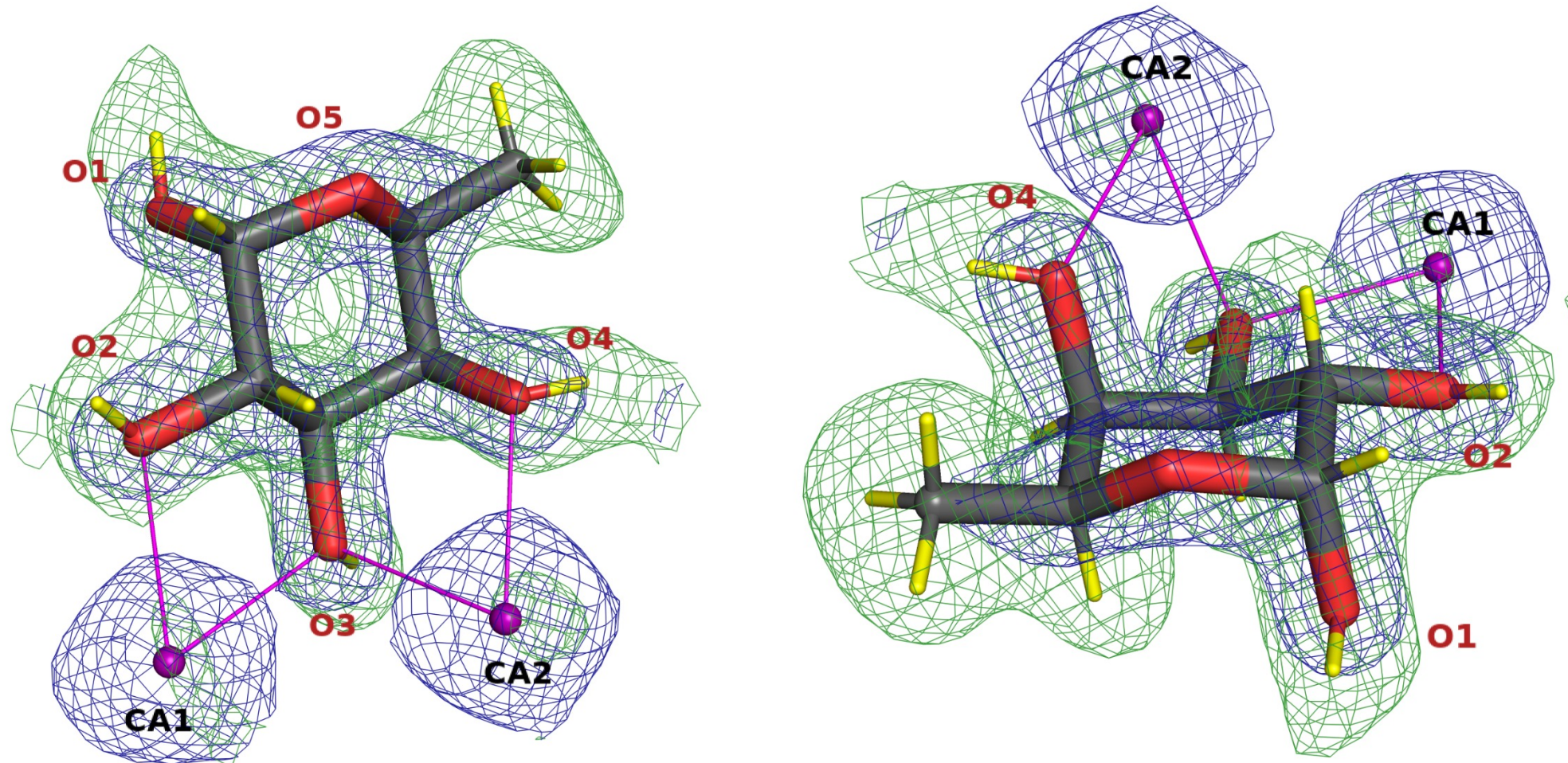
Characterization (Mass spec, NMR)



“After purification, 220 mg of lyophilized Fuc-d₁₂ was obtained from a culture using 1.5 L of D₂O and 45 g of deuterated glycerol”

Perdeuterated fucose in the LecB binding site

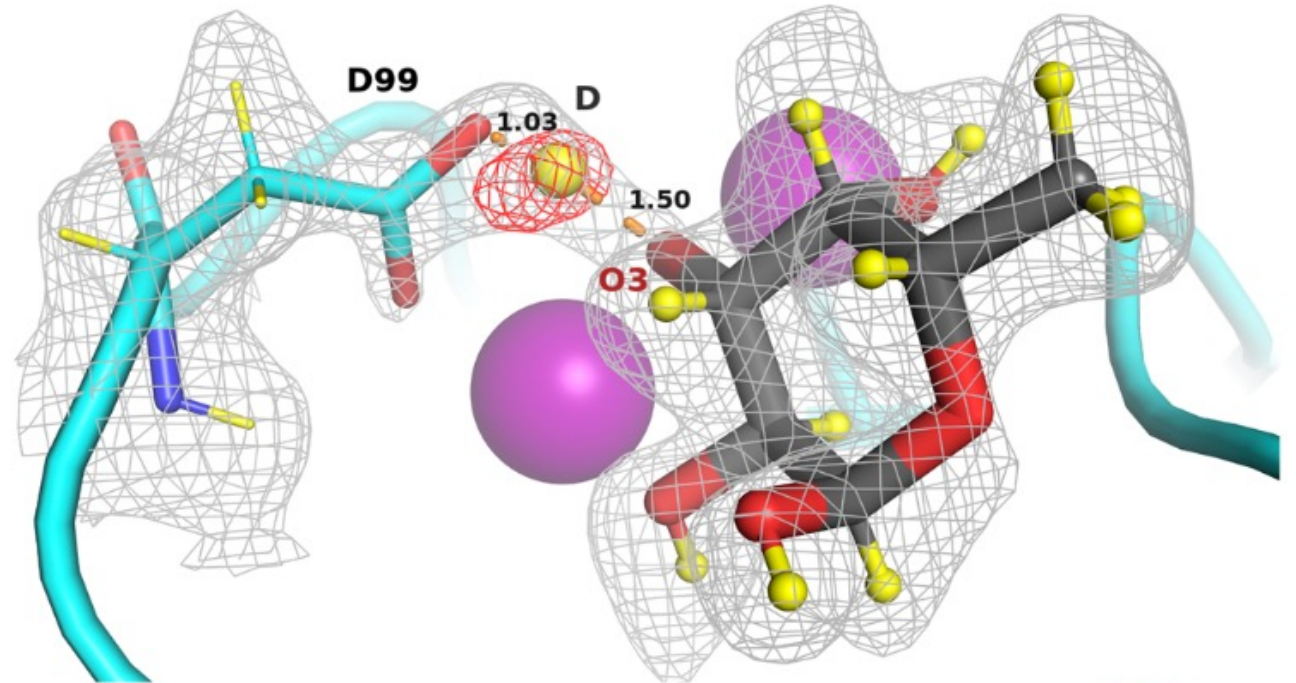
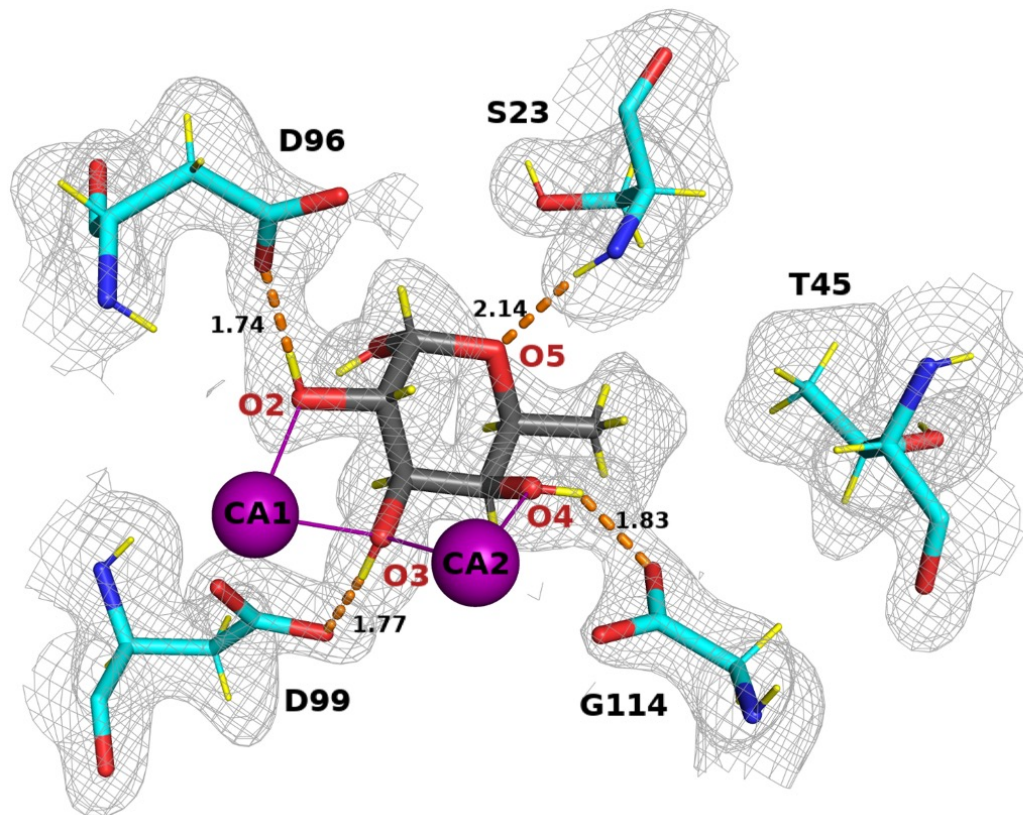
- Electron density (1.4σ)
- Neutron density (2.2σ)



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

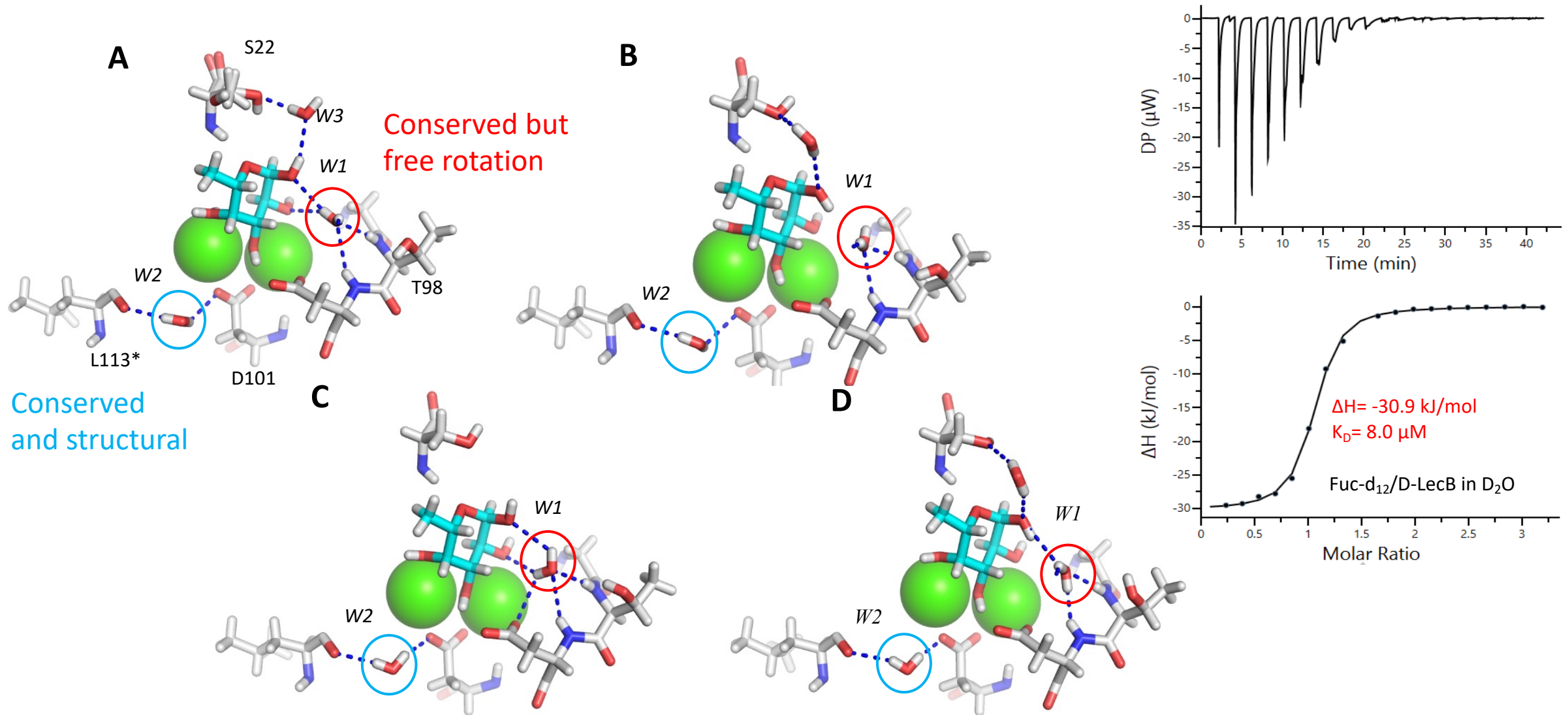
H-bonding network and a low-barrier H-bond

- 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



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

Water network in the fucose binding site of LecB



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

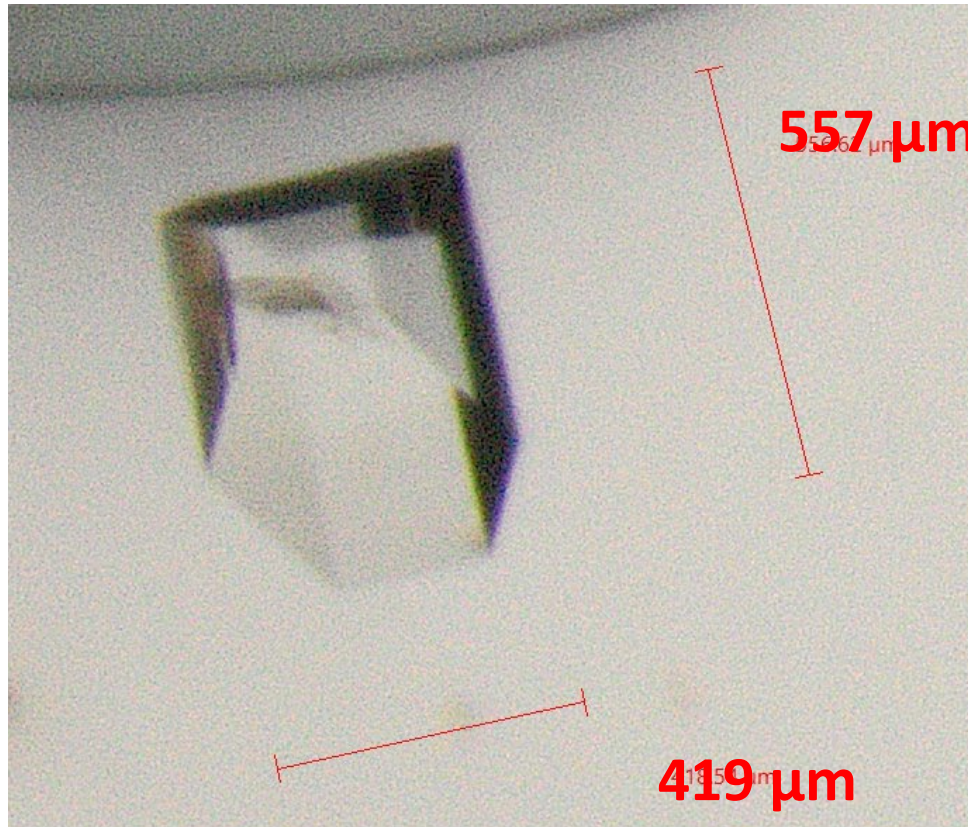
Gajdos L. *et al.*, (2022) *Nat Commun*

Neutron structure of the apo LecB lectin

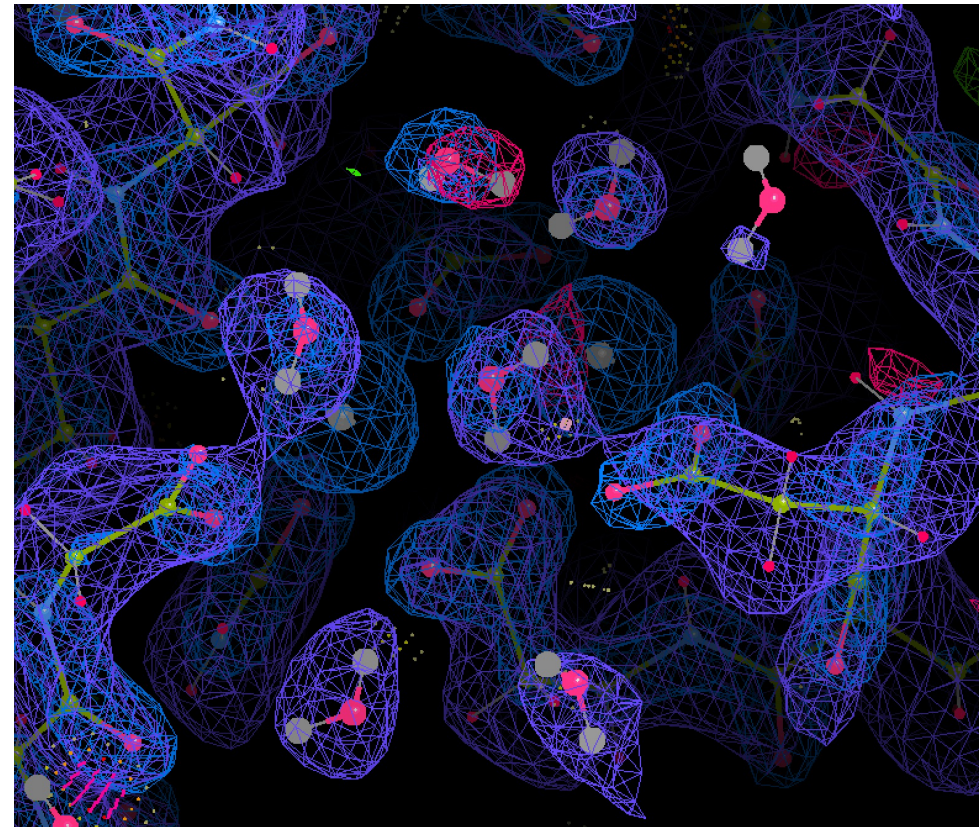
Neutron data collected on LADI instrument at the ILL (24h exposures, 2.1 Å resolution)

X-ray data collected on BM-07 beamline at the ESRF

Joint X-ray/neutron refinement undergoing

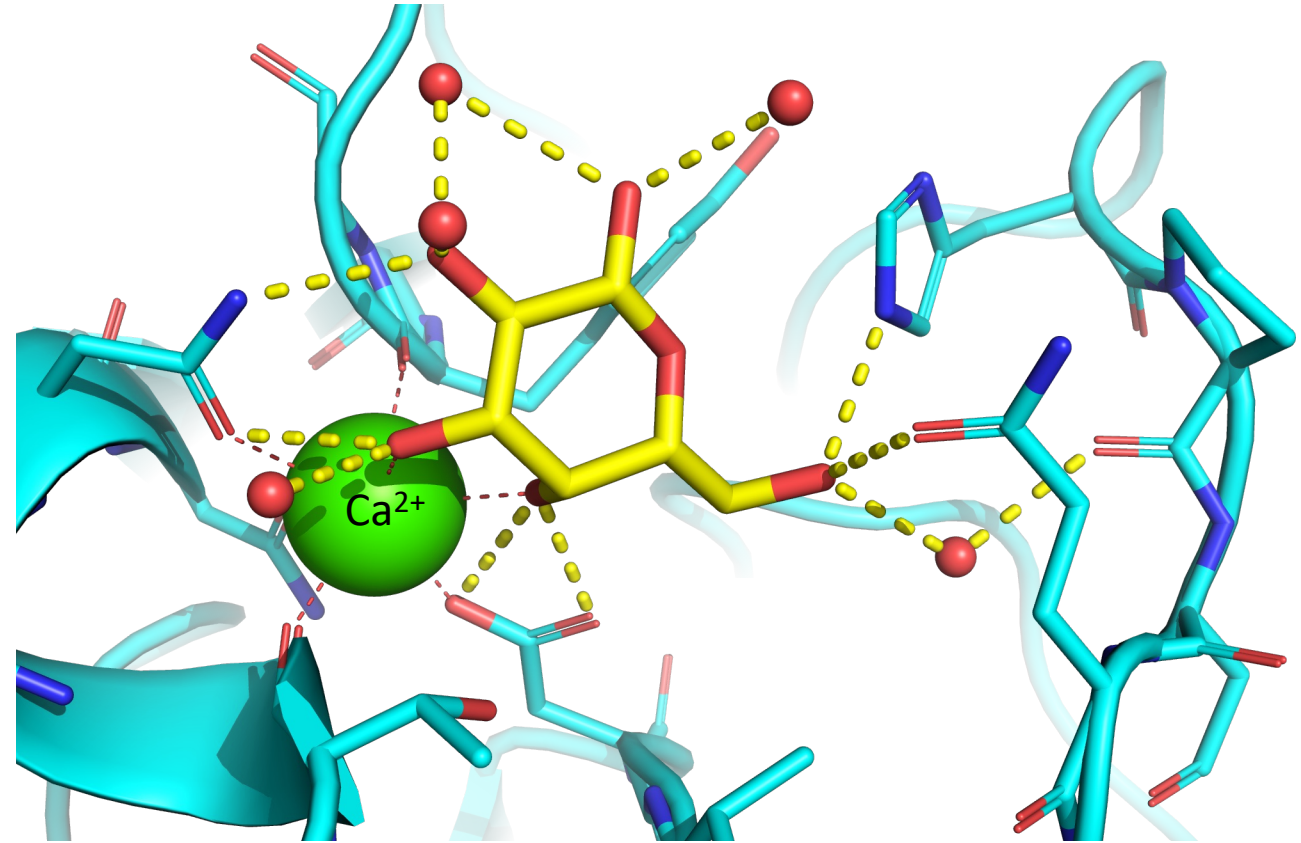
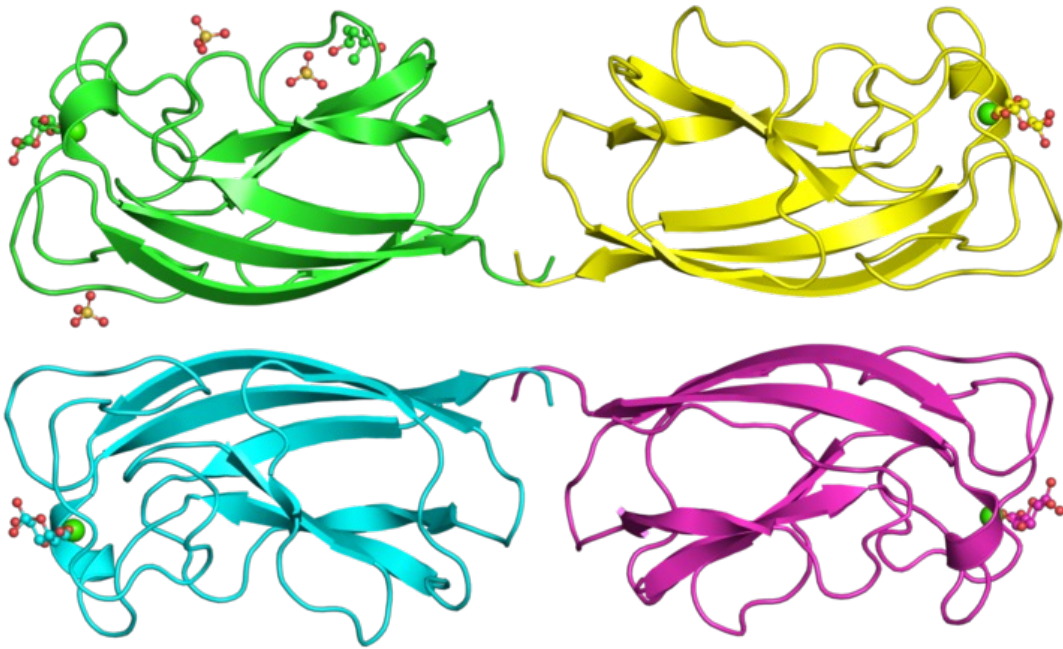


Perdeuterated LecB crystal

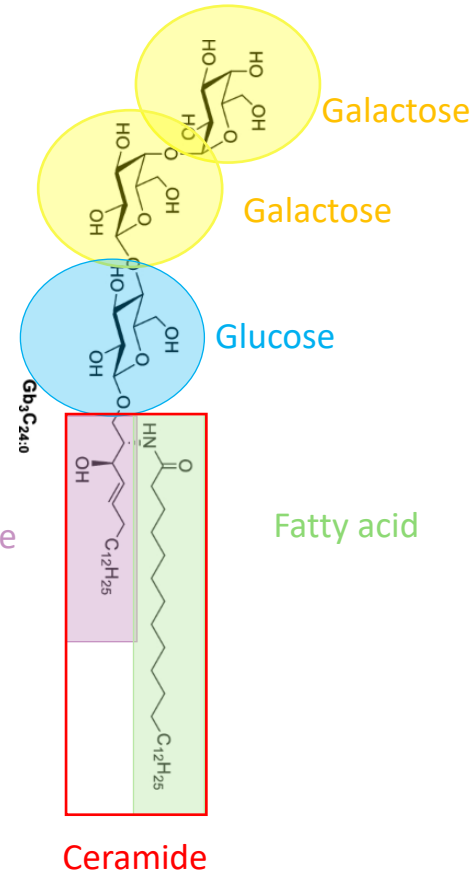
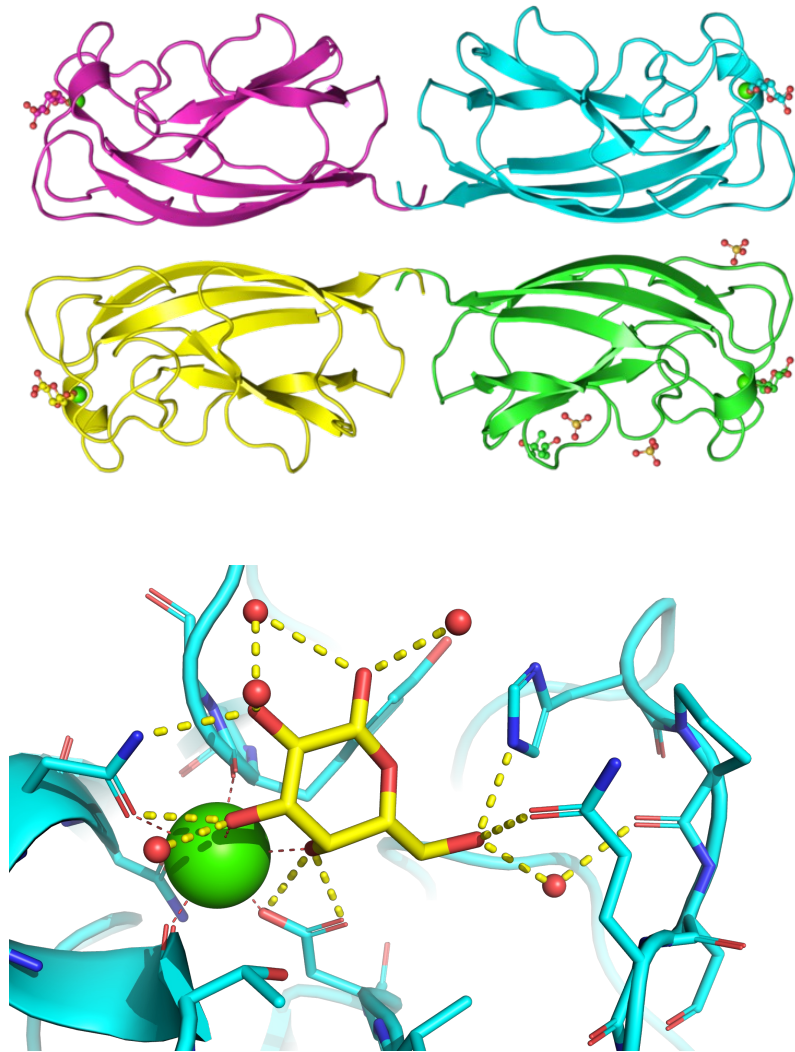


2Fo-Fc electron density map (blue) and 2Fo-Fc neutron scattering length density map (purple)

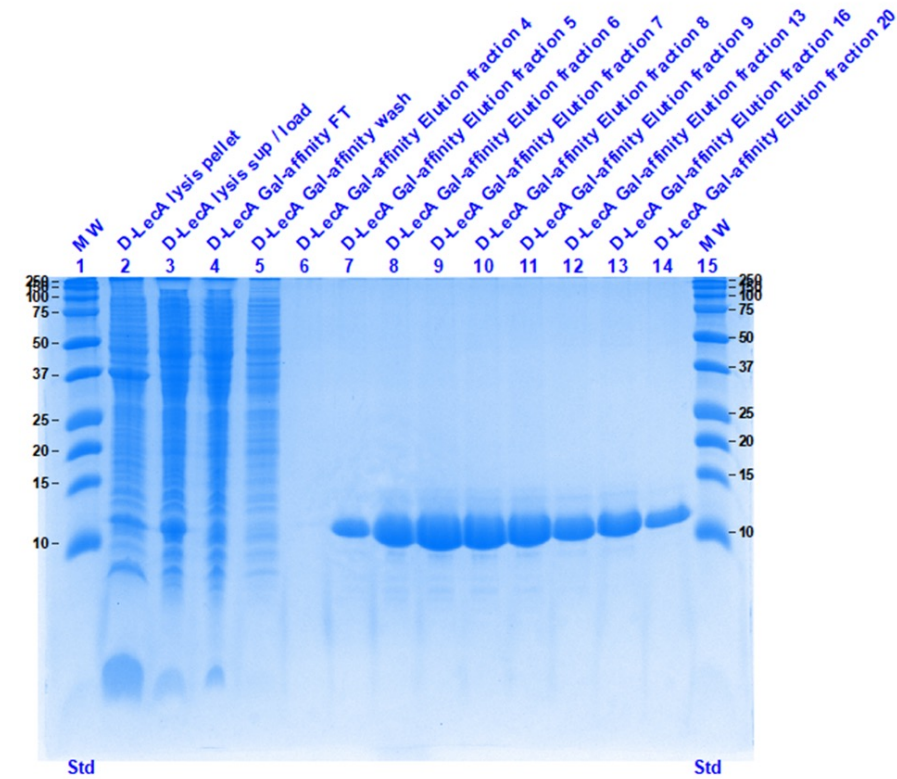
LecA-glycolipids neutron studies



Neutron diffraction studies to investigate how pathogens interact with human glycolipids

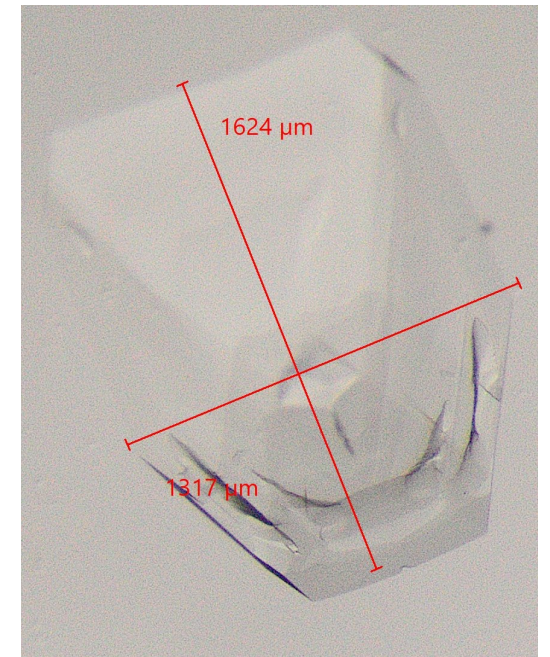
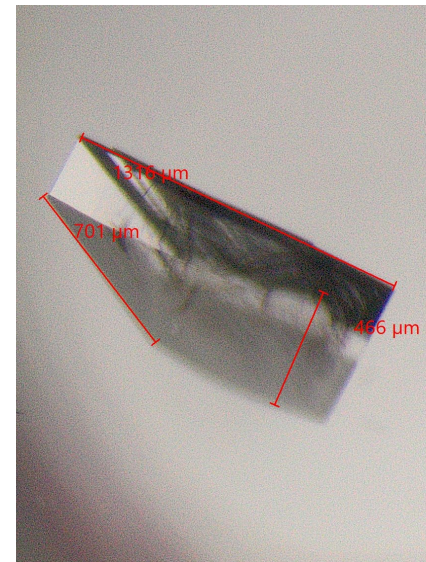
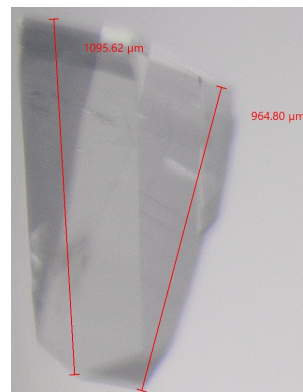
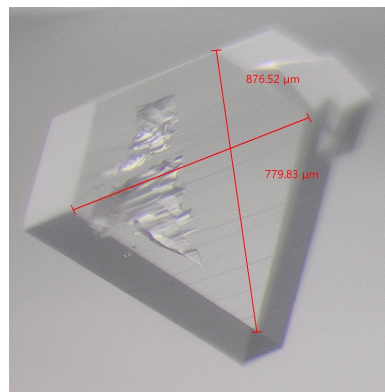
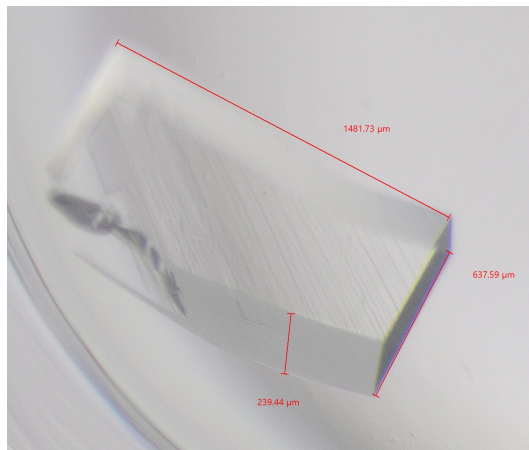
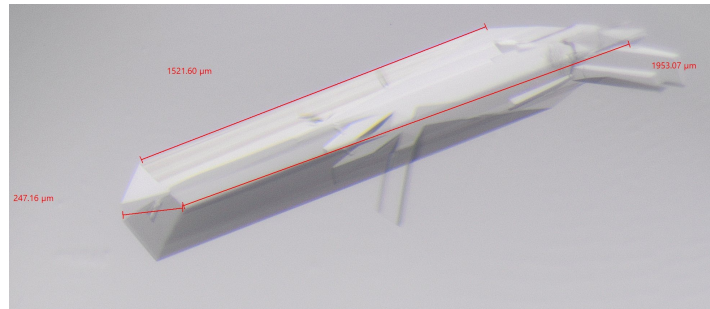
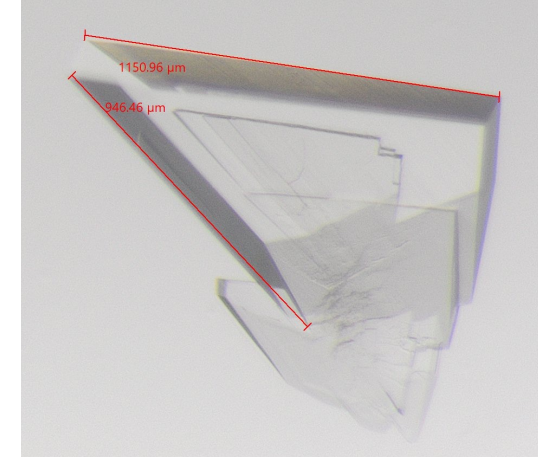
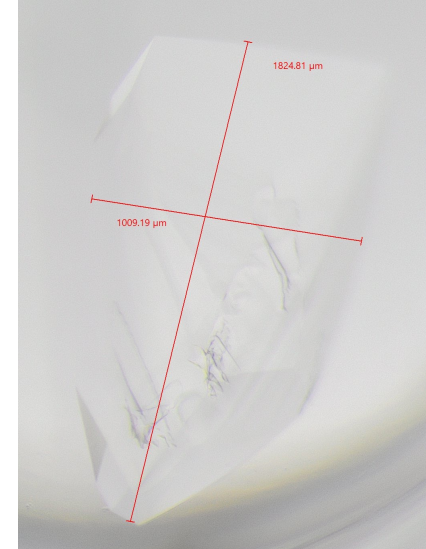
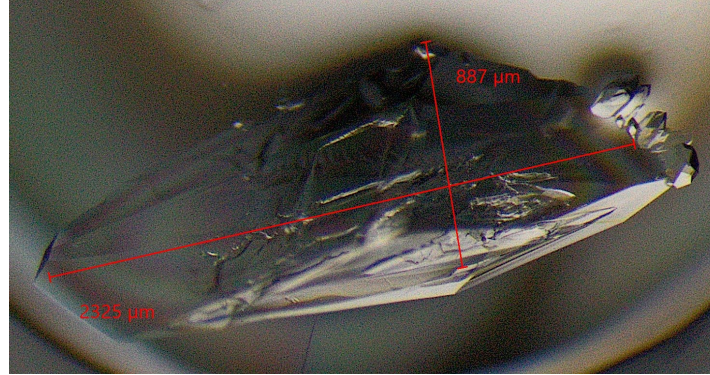
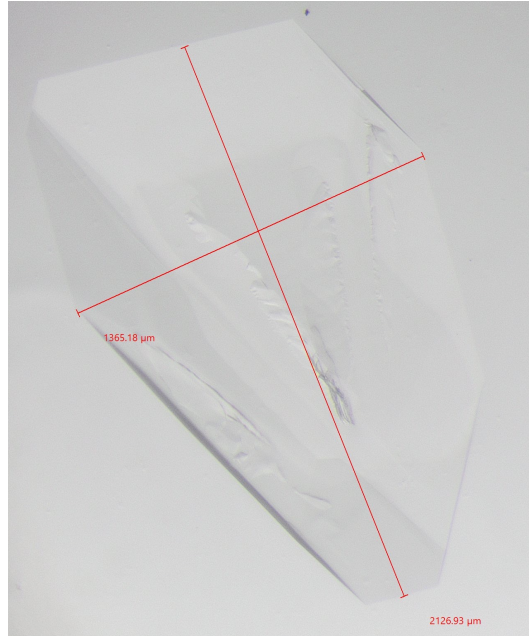


Globotriaosylceramide (Gb3) structure

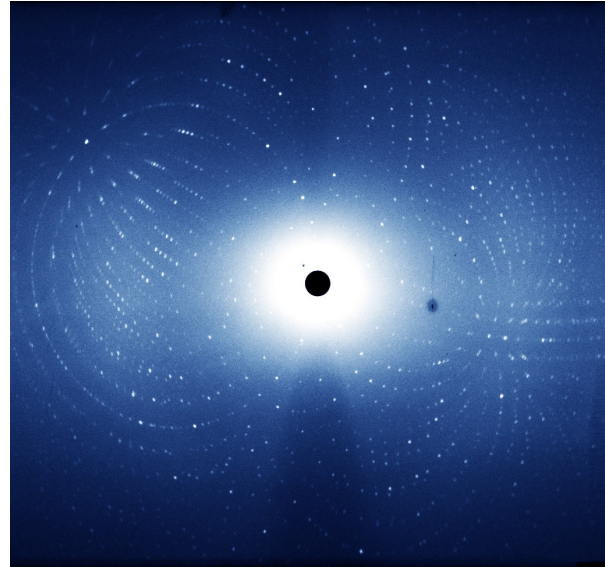
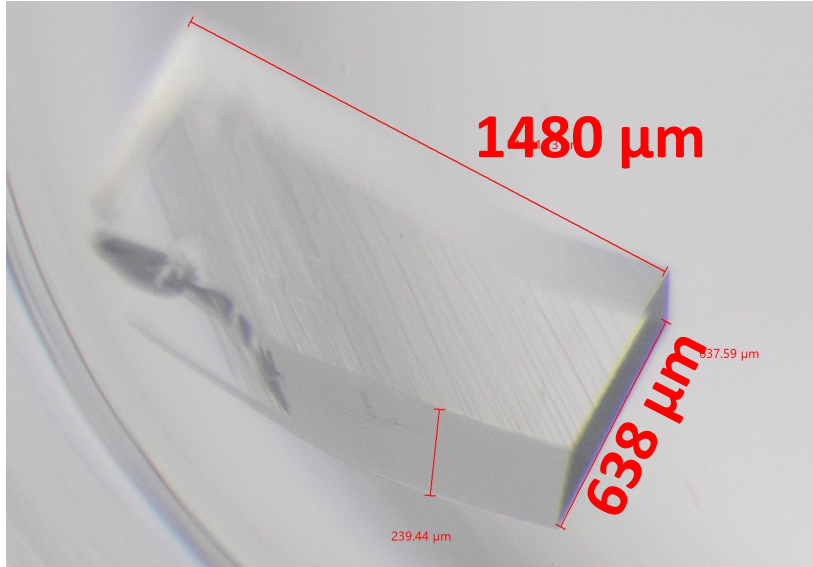


Expression of the perdeuterated LecA lectin (D-LecA) followed by SDS-PAGE

Crystallization of LecA lectin

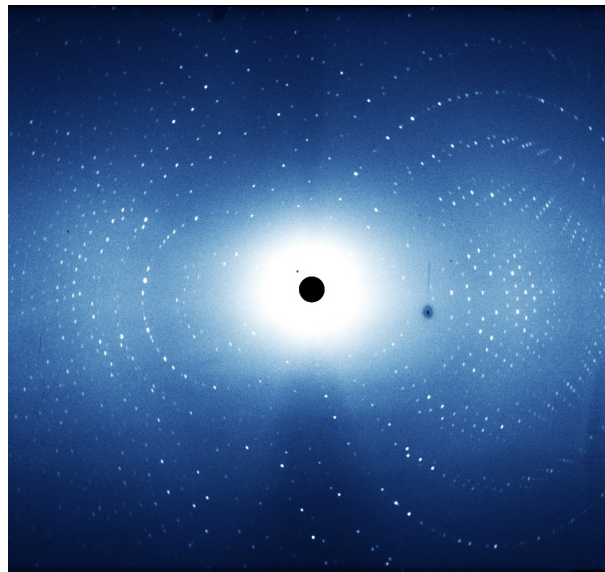


Neutron diffraction data collections from LecA



D-LecA/d-galactose

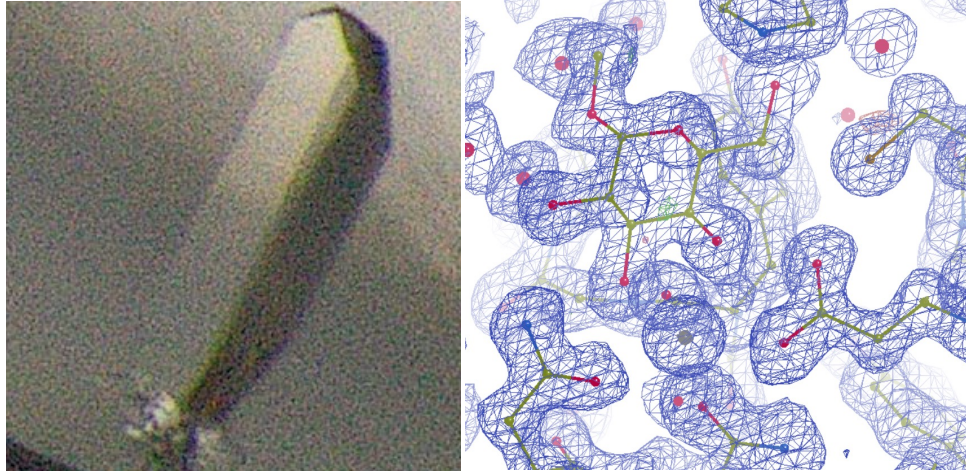
LADI
P2₁
18h exposures
1.9 Å resolution



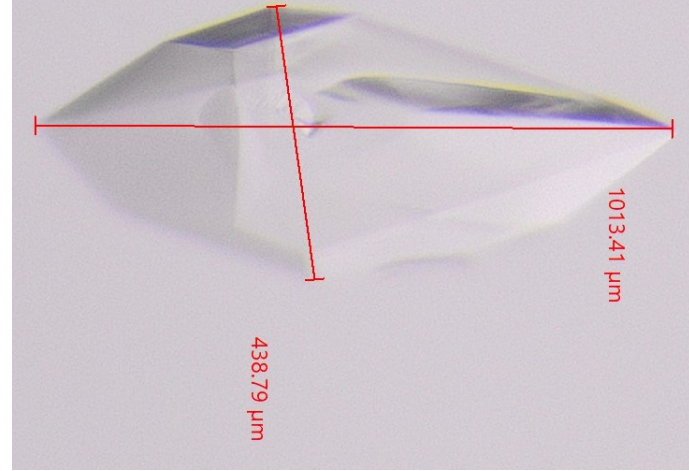
D-LecA/disaccharide

DALI
I222
18h exposures
2Å resolution

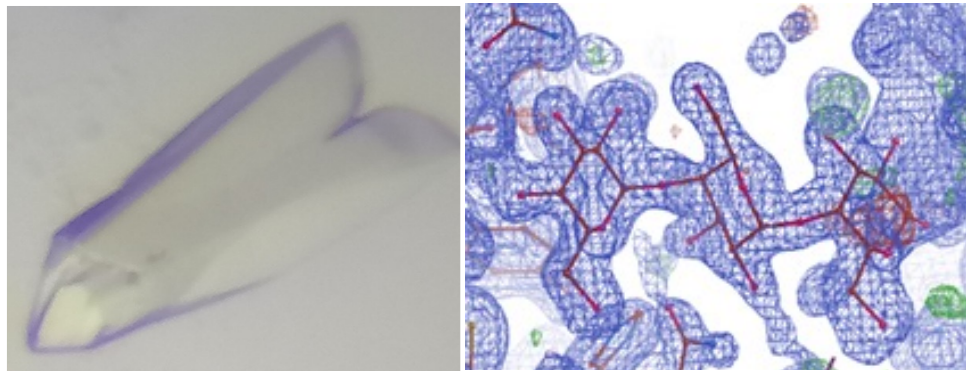
X-ray diffraction data from LecA crystals



D-LecA/methyl- α -galactoside



D-LecA/disaccharide (galabiose?)



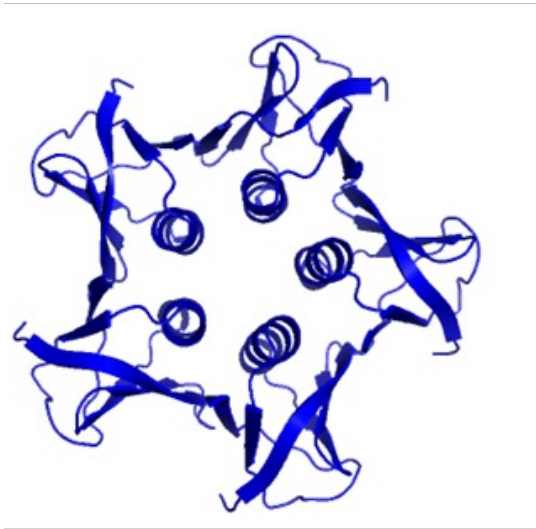
LecA/galatriose



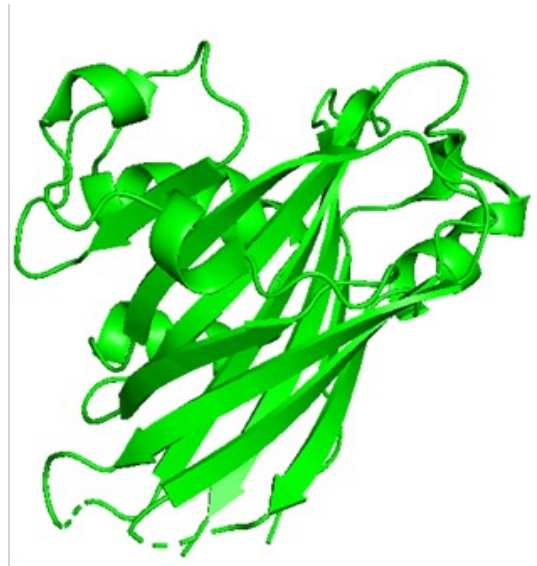
D-LecA/d-galactose

Future plans

- PhD thesis of Theodore Arnaud
- New oligosaccharides producing strains (trisaccharides, tetrasaccharides) and their perdeuteration
- New protein targets such as **Shiga-like toxin 1 or 2** from enterohemorrhagic *E. coli* and **Factor H-binding protein (Fhb)** from *Streptococcus suis* that both bind to Gb3 cell receptors



Stx-1B



Fhb

Acknowledgements



Theodore Arnaud
Matthew Blakeley
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