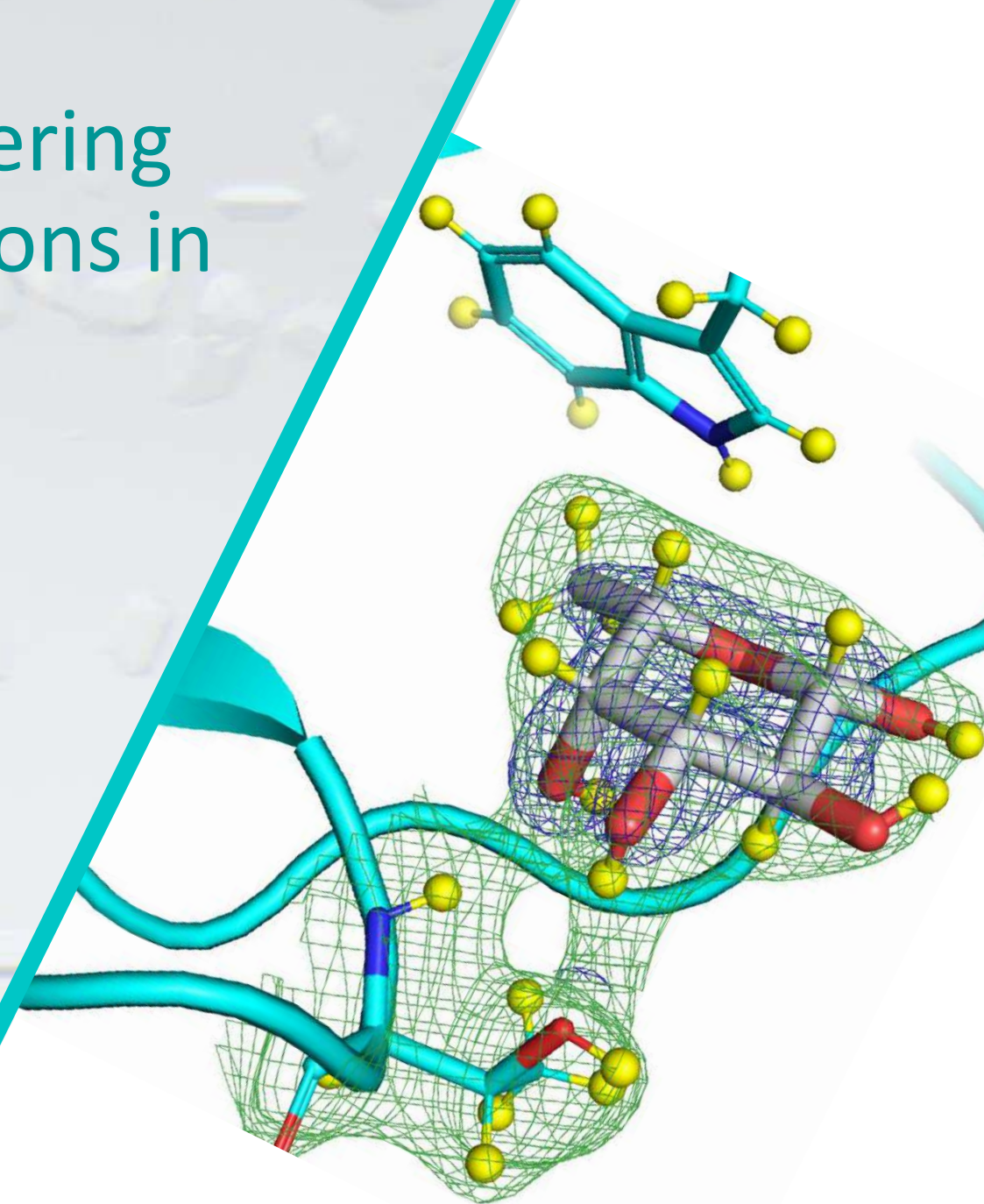


Neutron diffraction for deciphering protein-carbohydrate interactions in bacterial infection

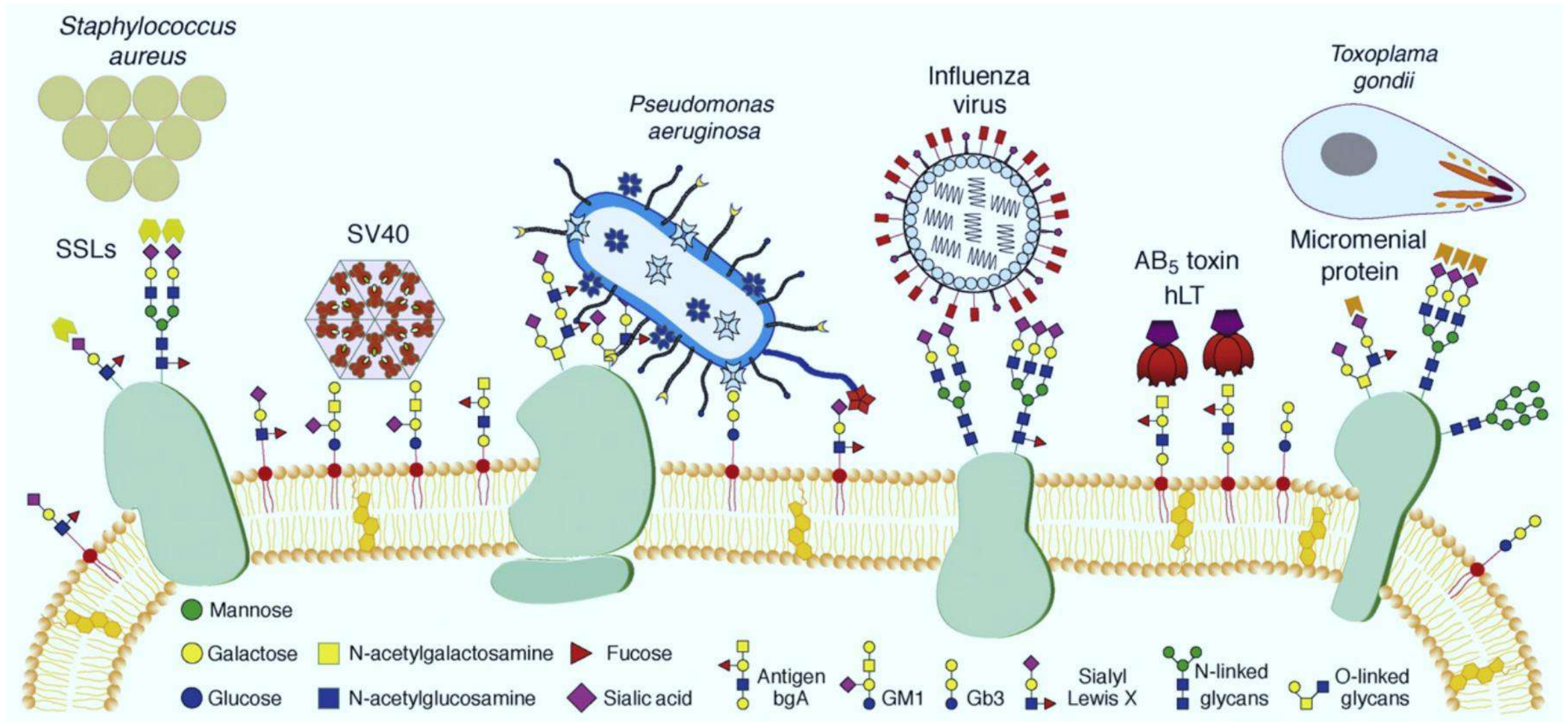
Lukáš Gajdoš

gajdosl@ill.fr

Glytunes 2023



Lectins from pathogenic organisms

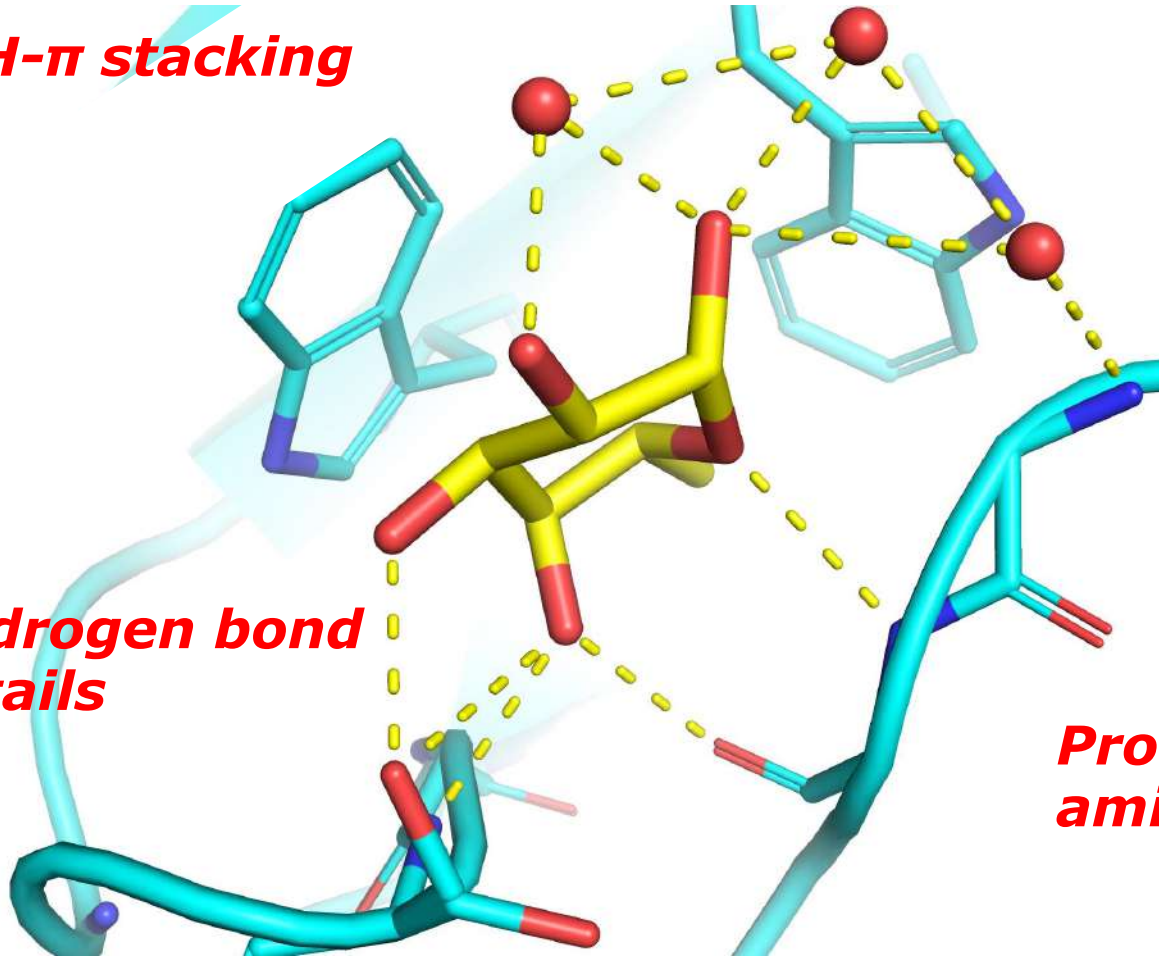


Protein-carbohydrate interactions

Fucose in PLL lectin

CH- π stacking

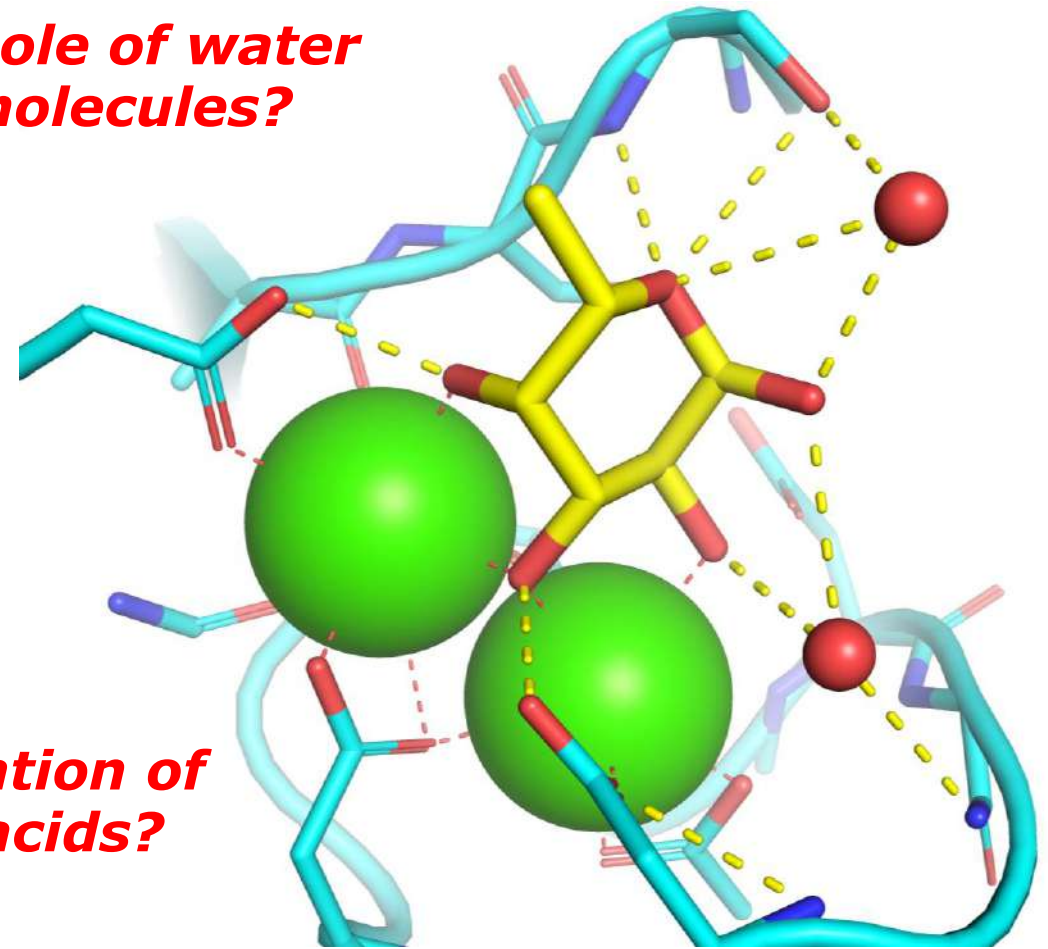
Hydrogen bond details



Fucose in LecB lectin

Role of water molecules?

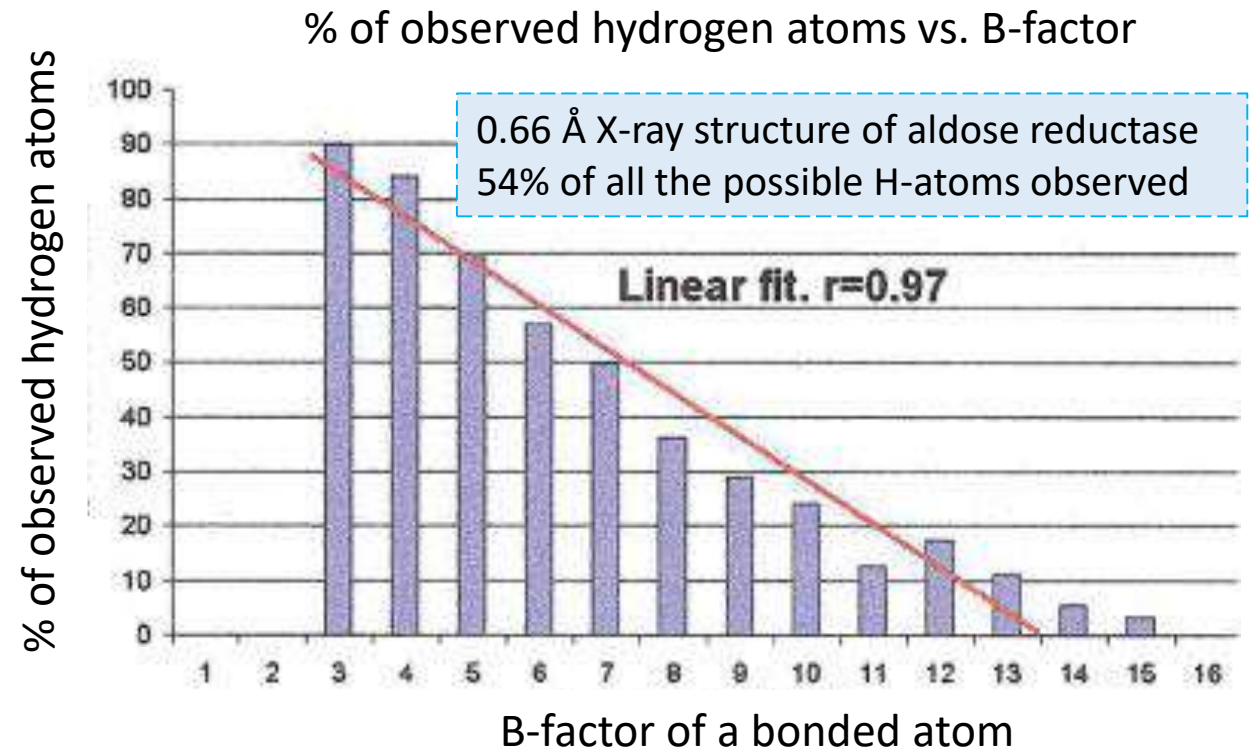
Protonation of amino acids?



Wanted : Location of hydrogen atoms

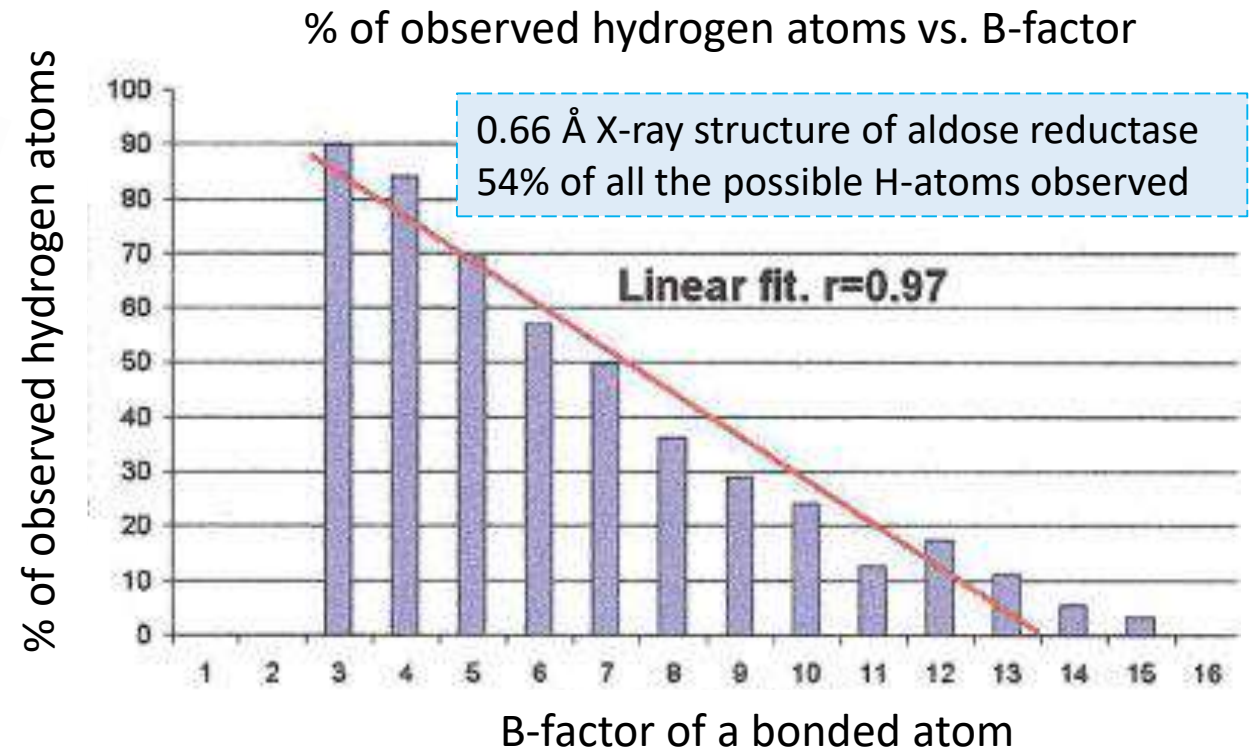
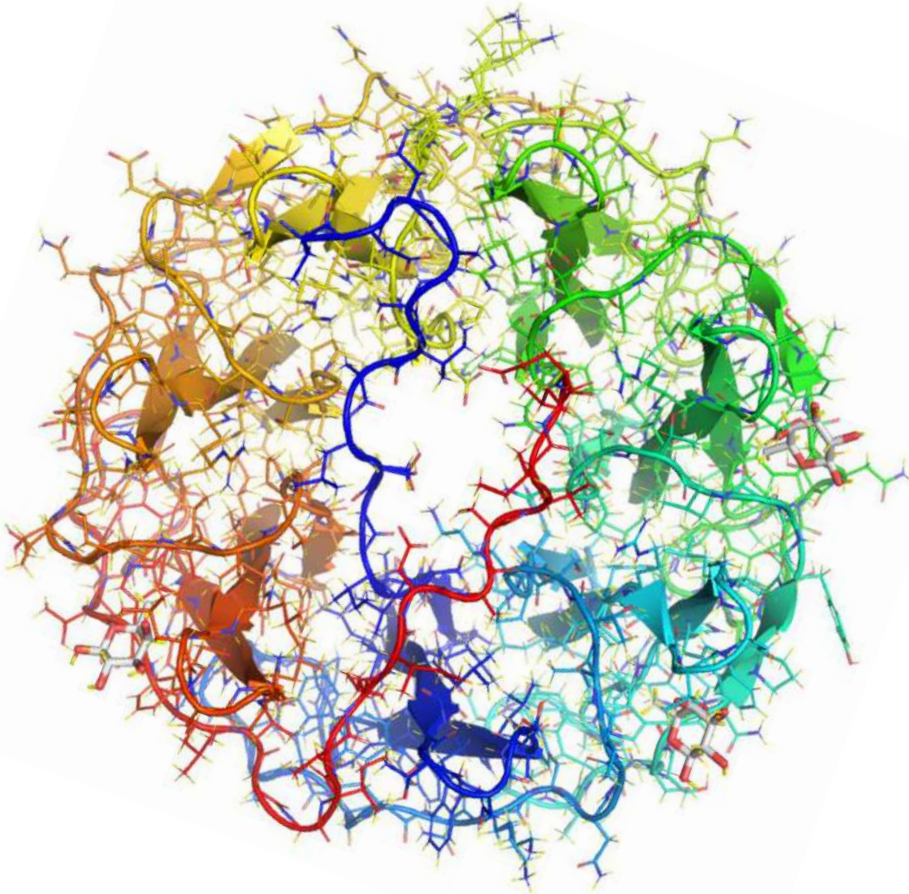
H atoms „invisible“ in X-ray structures

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



H atoms „invisible“ in X-ray structures

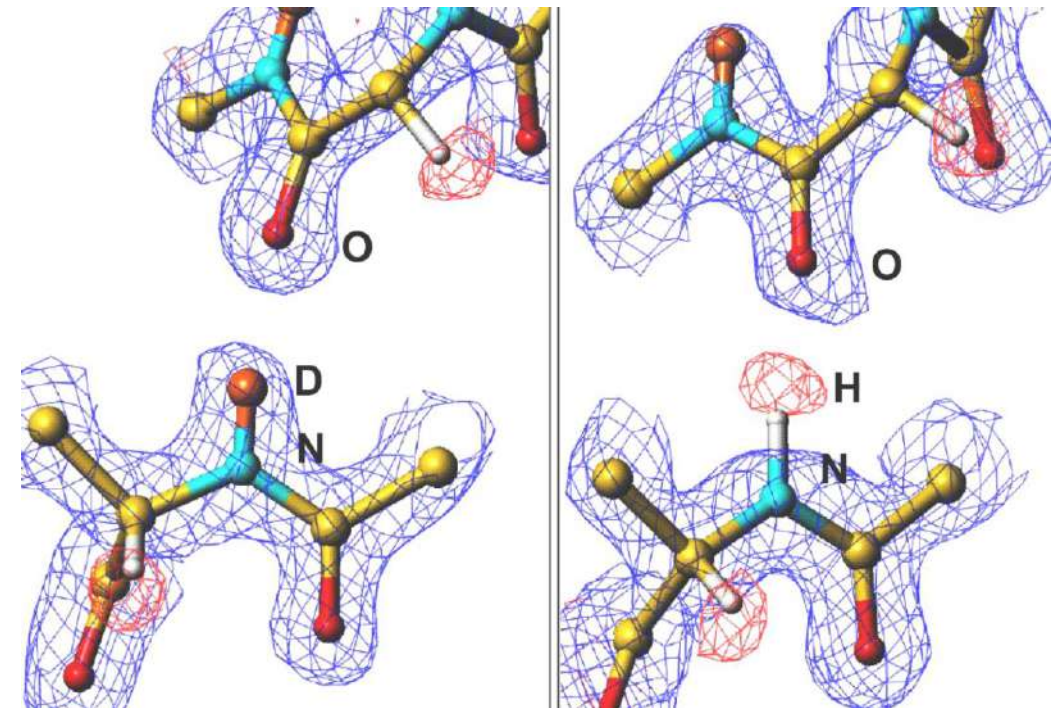
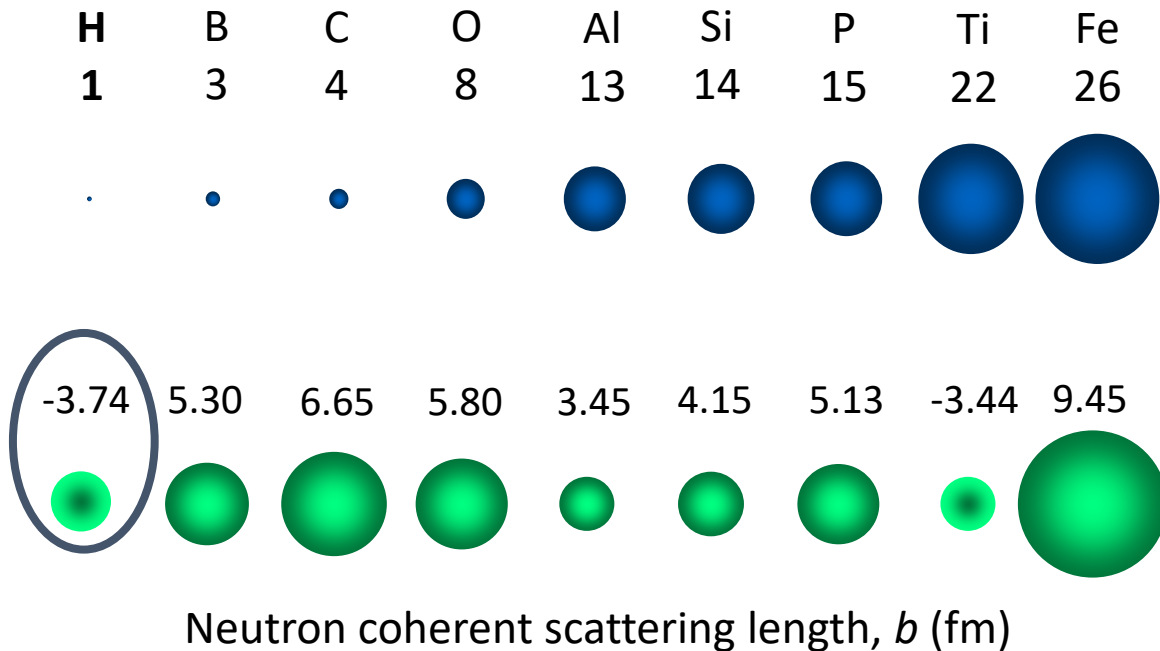
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Neutrons as a diffraction probe

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

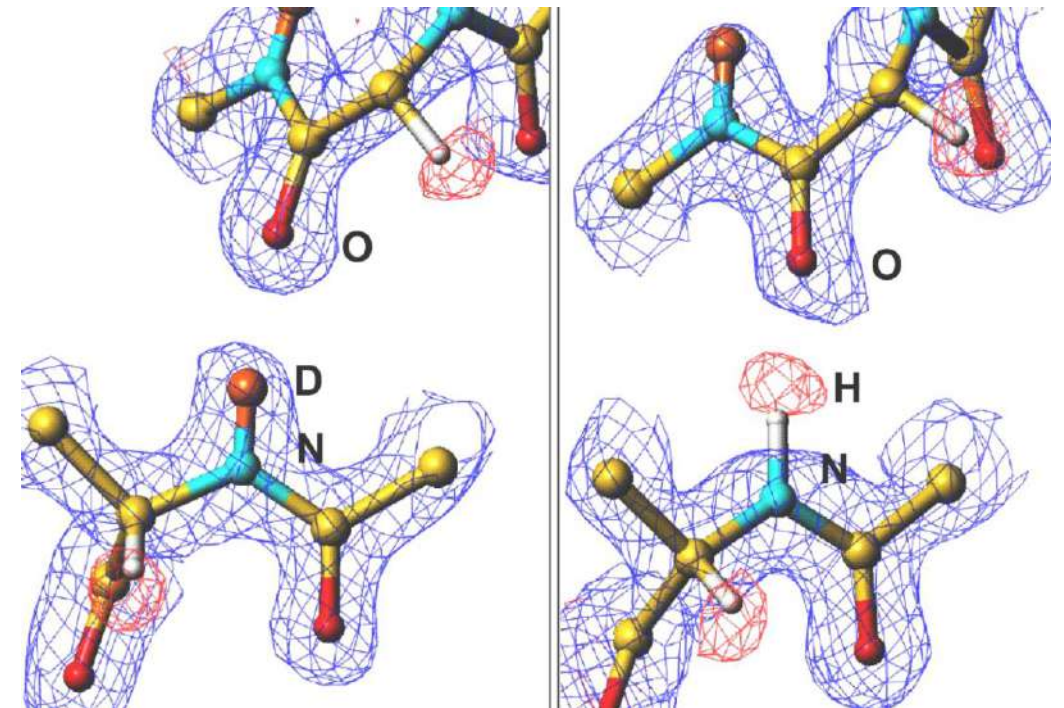
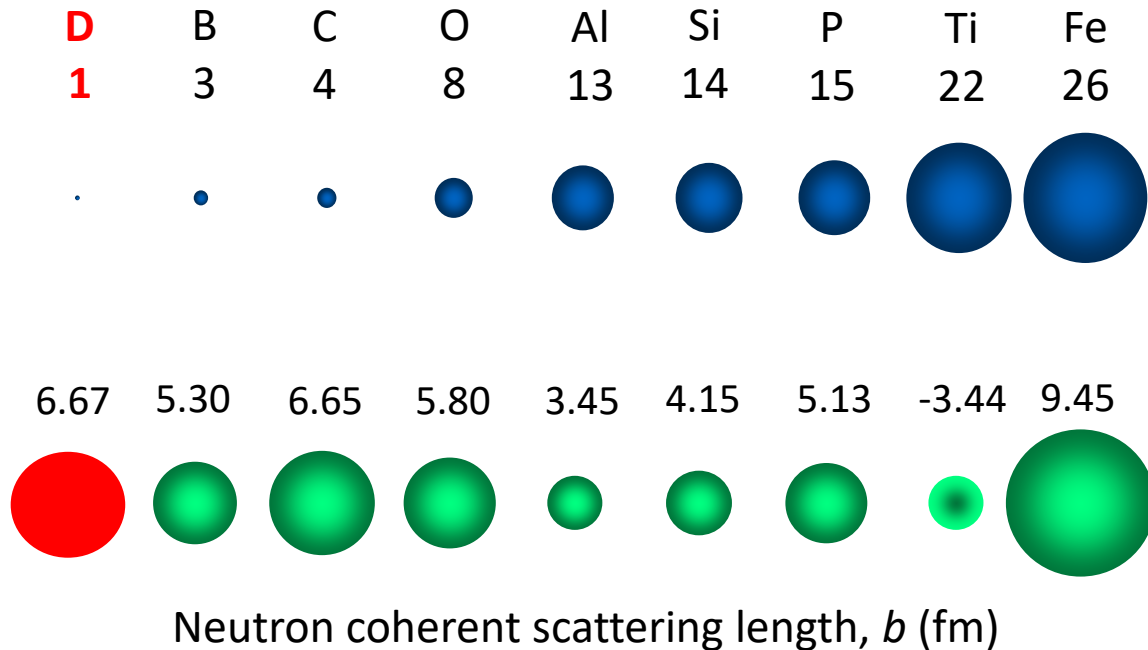
X-ray scattering proportional to Z



Neutrons as a diffraction probe

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X-ray scattering proportional to Z



Why neutron protein crystallography?

- **Advantages:**

- Visualisation of H/D atoms
- Nondestructive probe
- Ambient temperature

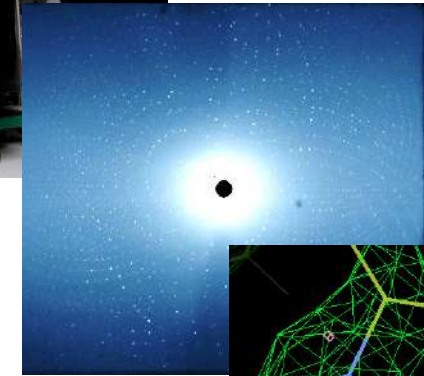
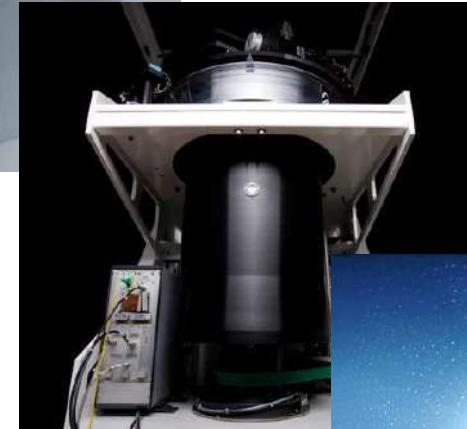
- **Limitations:**

- Low flux of neutrons (compared to X-rays)
- Large sample size (mm^3)
- Long data collections (days)

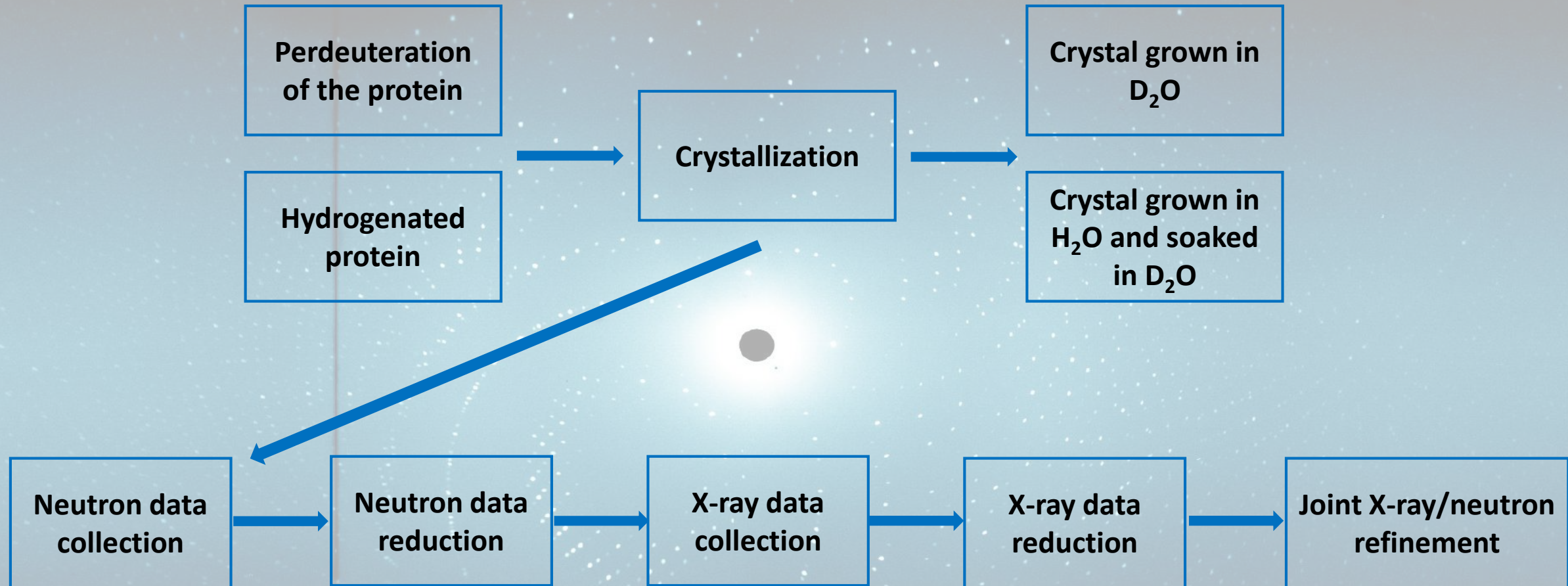
- Protein Data Bank:

- **174 899** X-ray crystal structures (May 2023)

- **212** neutron crystal structures (May 2023)

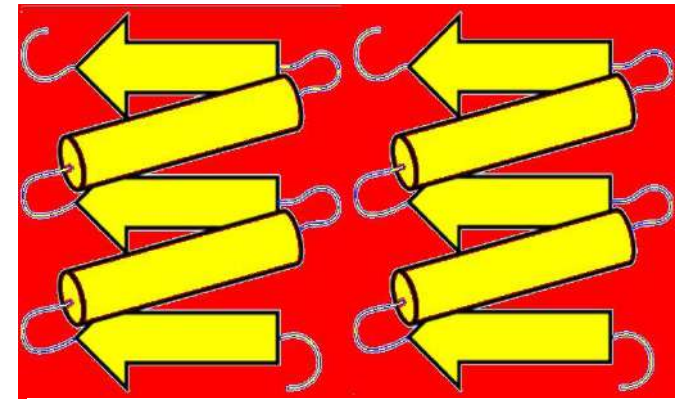


Neutron protein crystallography flow chart

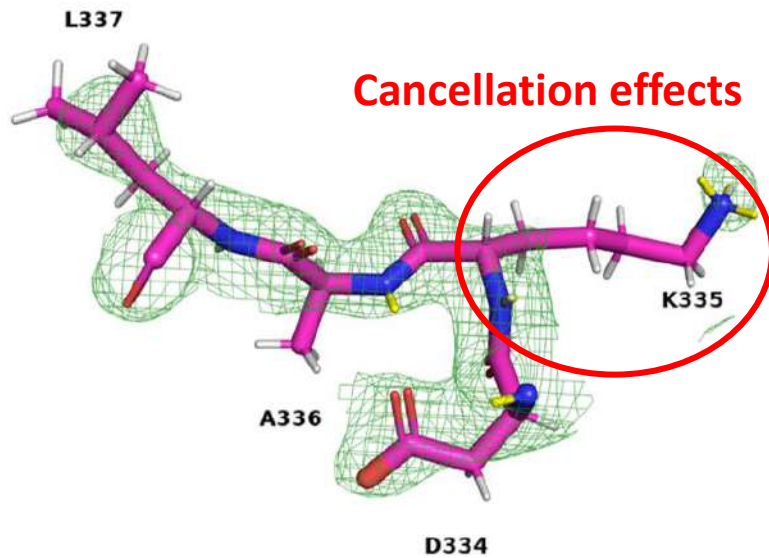


Need of perdeuteration

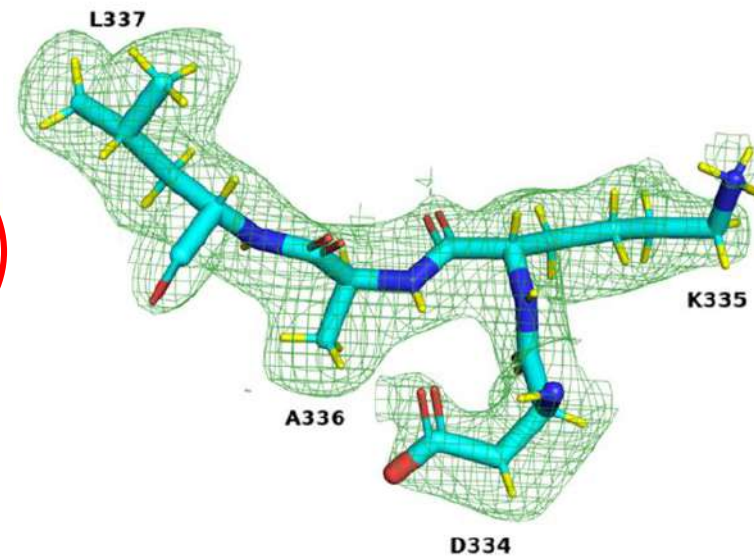
- Full replacement of all **hydrogen (H)** atoms by **deuterium (D)** atoms
- **Reduce** the large **incoherent** scattering of H (~ 40 times larger than for D)
- **Reduces the background** and **increases the signal-noise ratio**
- Clearer visualization of neutron scattering density maps
- **Cancellation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



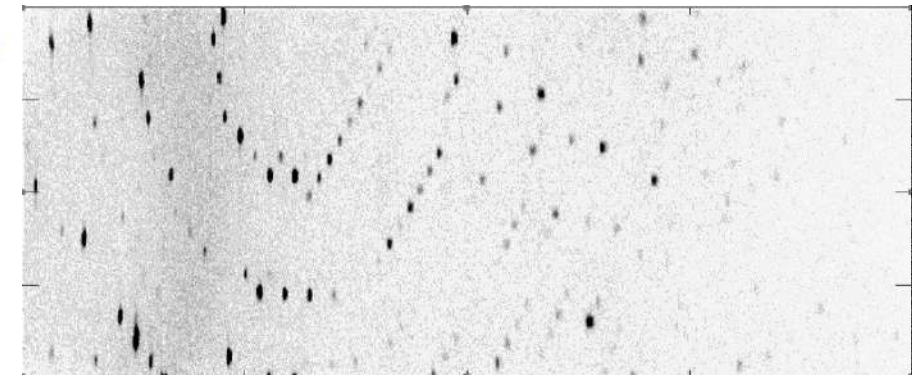
Perdeuterated protein, D_2O solvent



H/D-exchanged protein



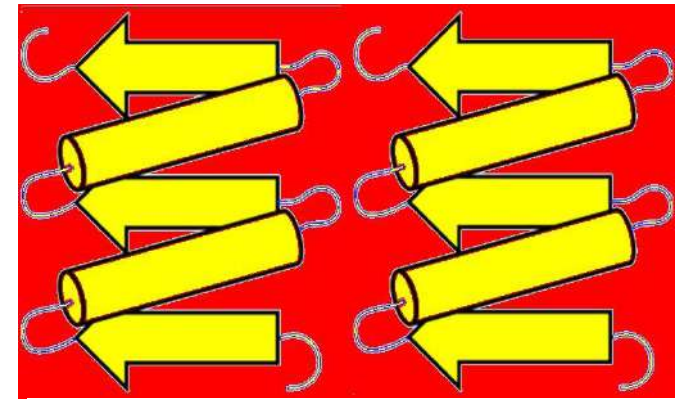
Perdeuterated protein



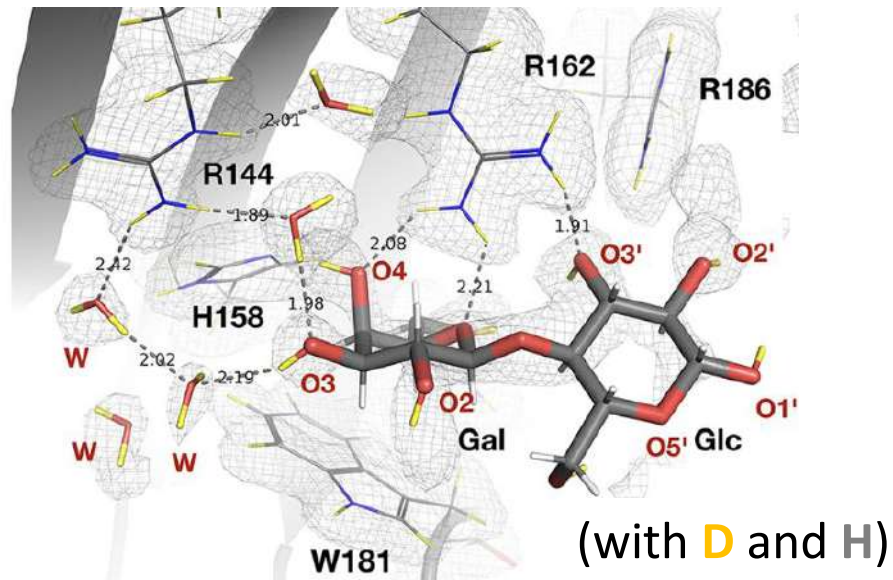
Courtesy of prof. Trevor Forsyth

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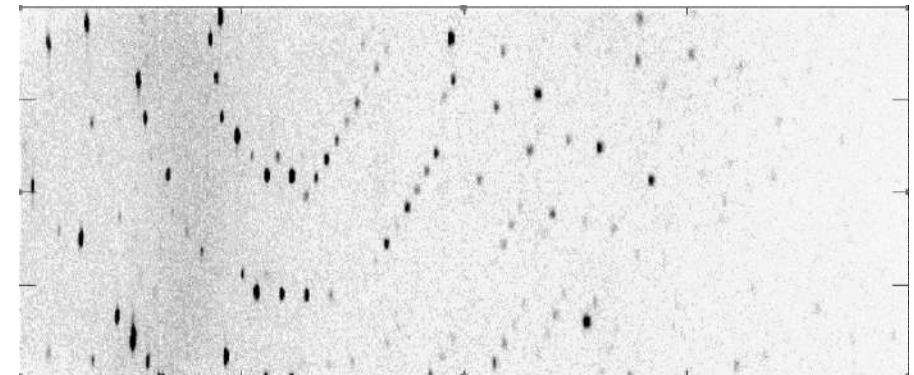


Perdeuterated protein, D_2O solvent



D-Galectin-3C/lactose complex

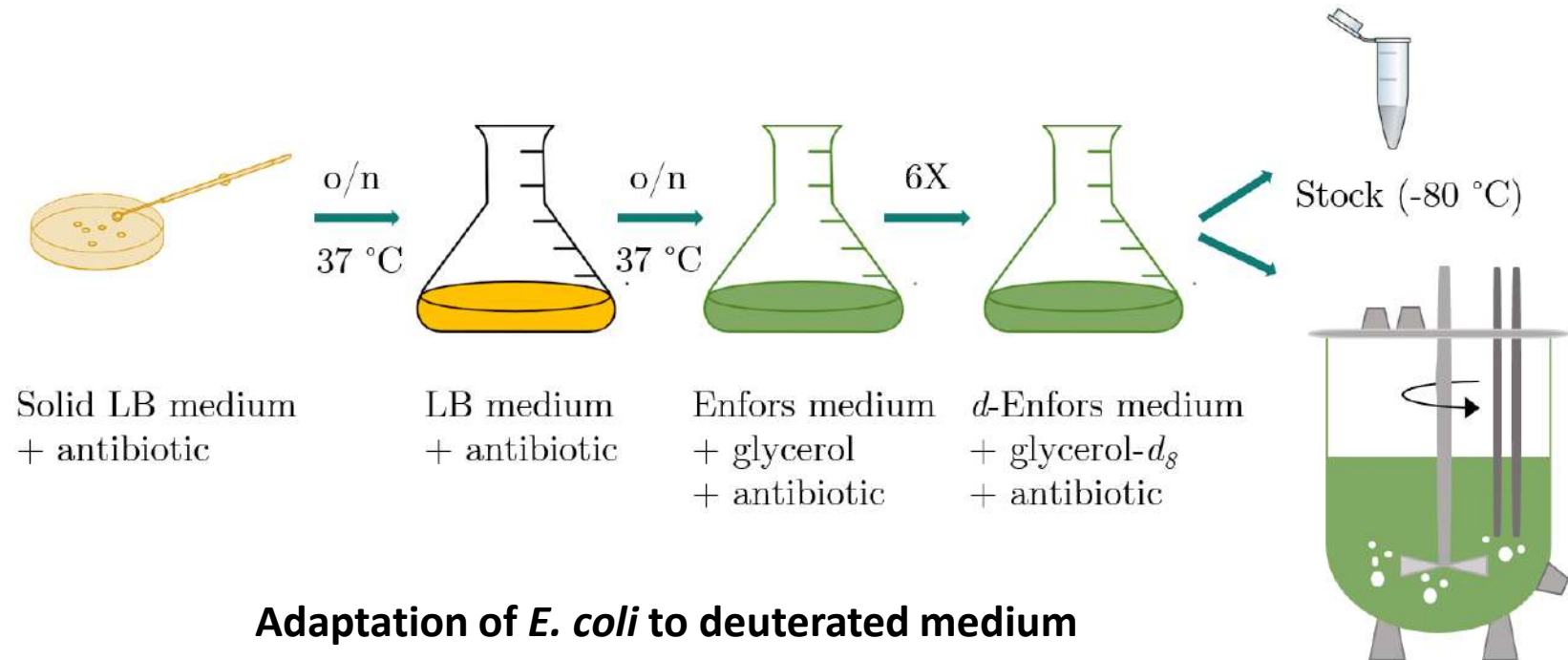
Manzoni F. et al., *J. Med. Chem.*, 2018



Courtesy of prof. Trevor Forsyth

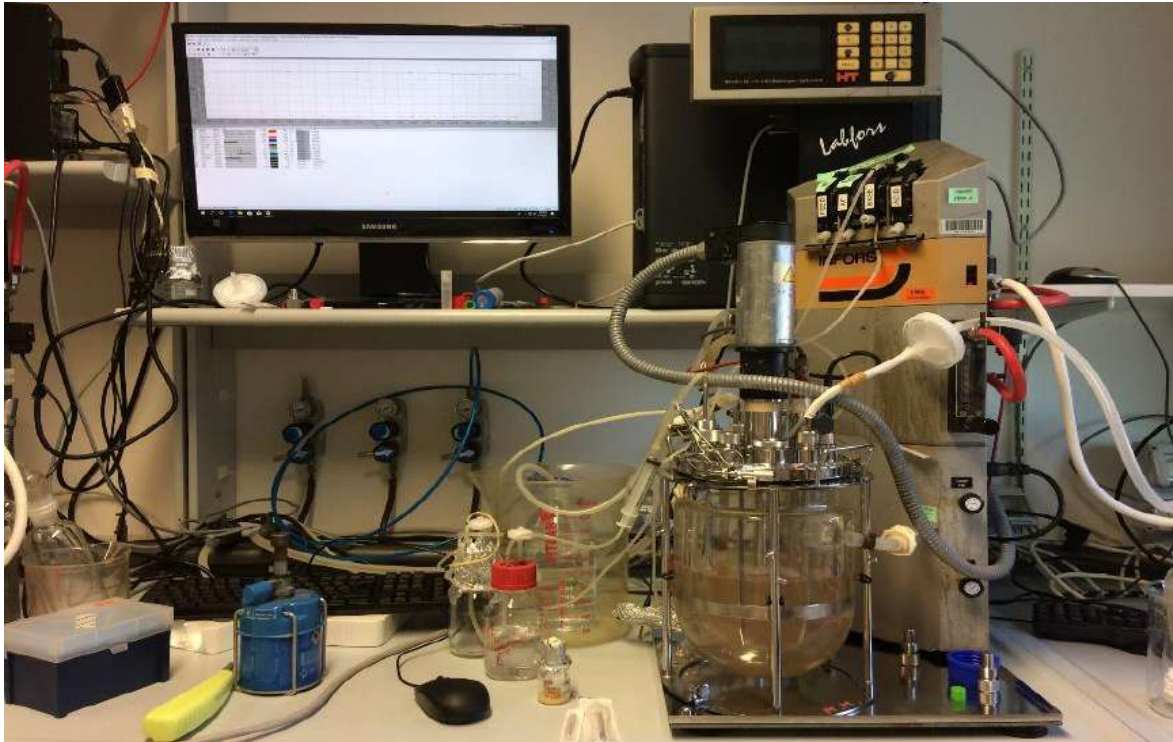
How to obtain perdeuterated biomolecules?

- **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})



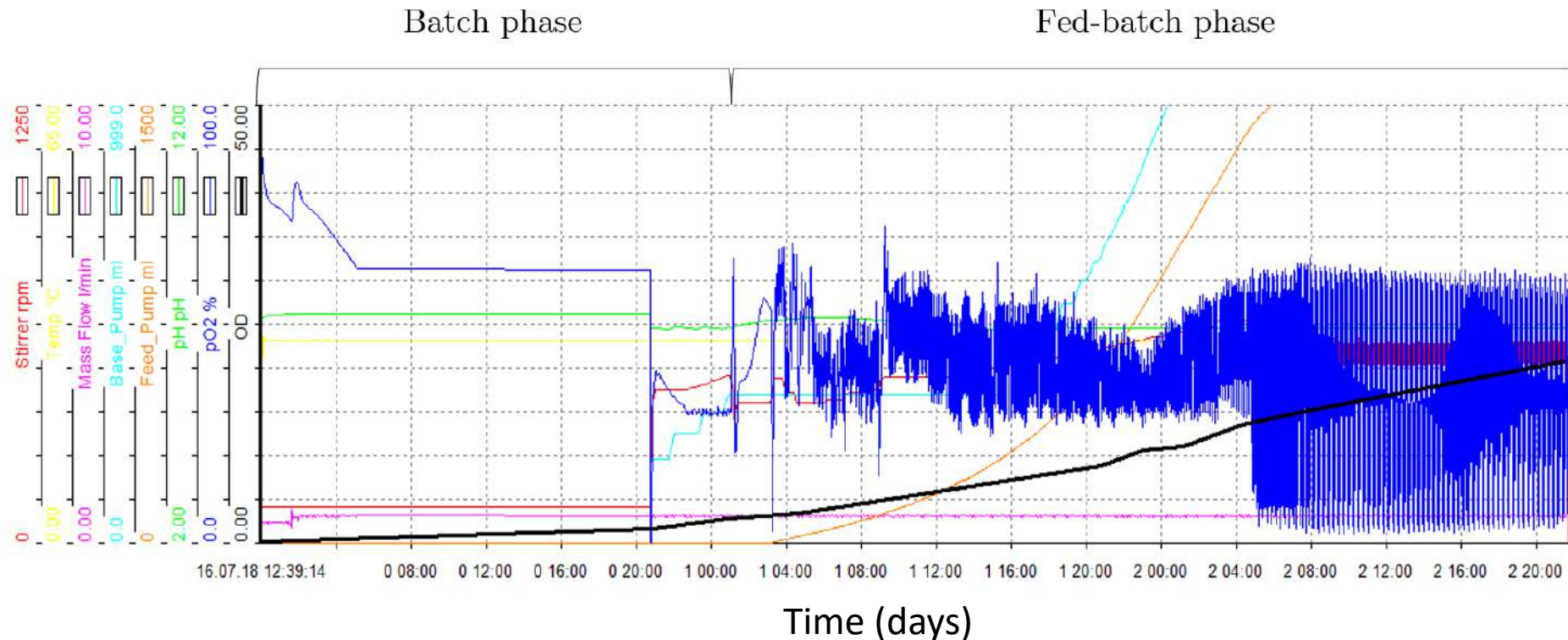
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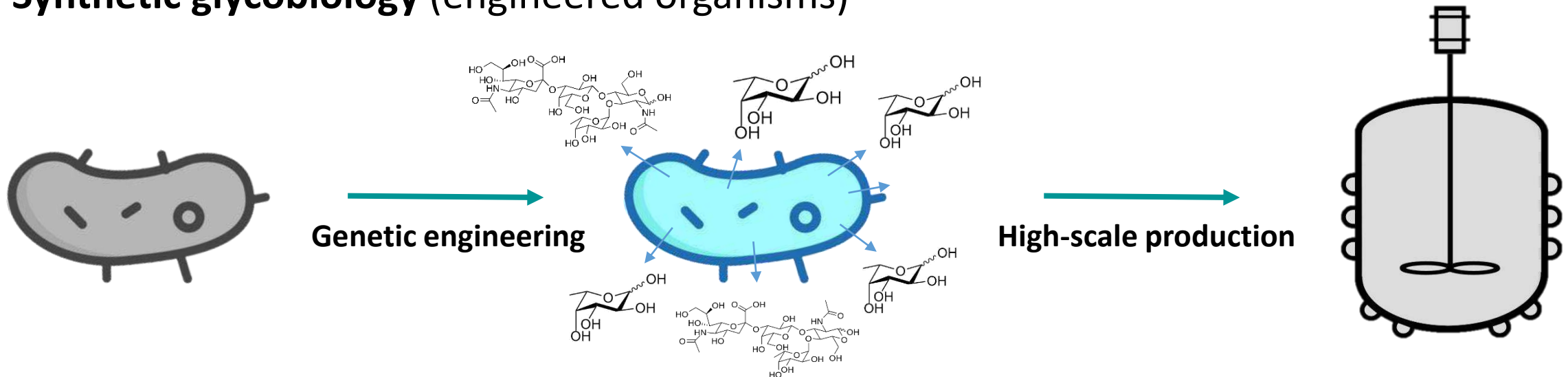
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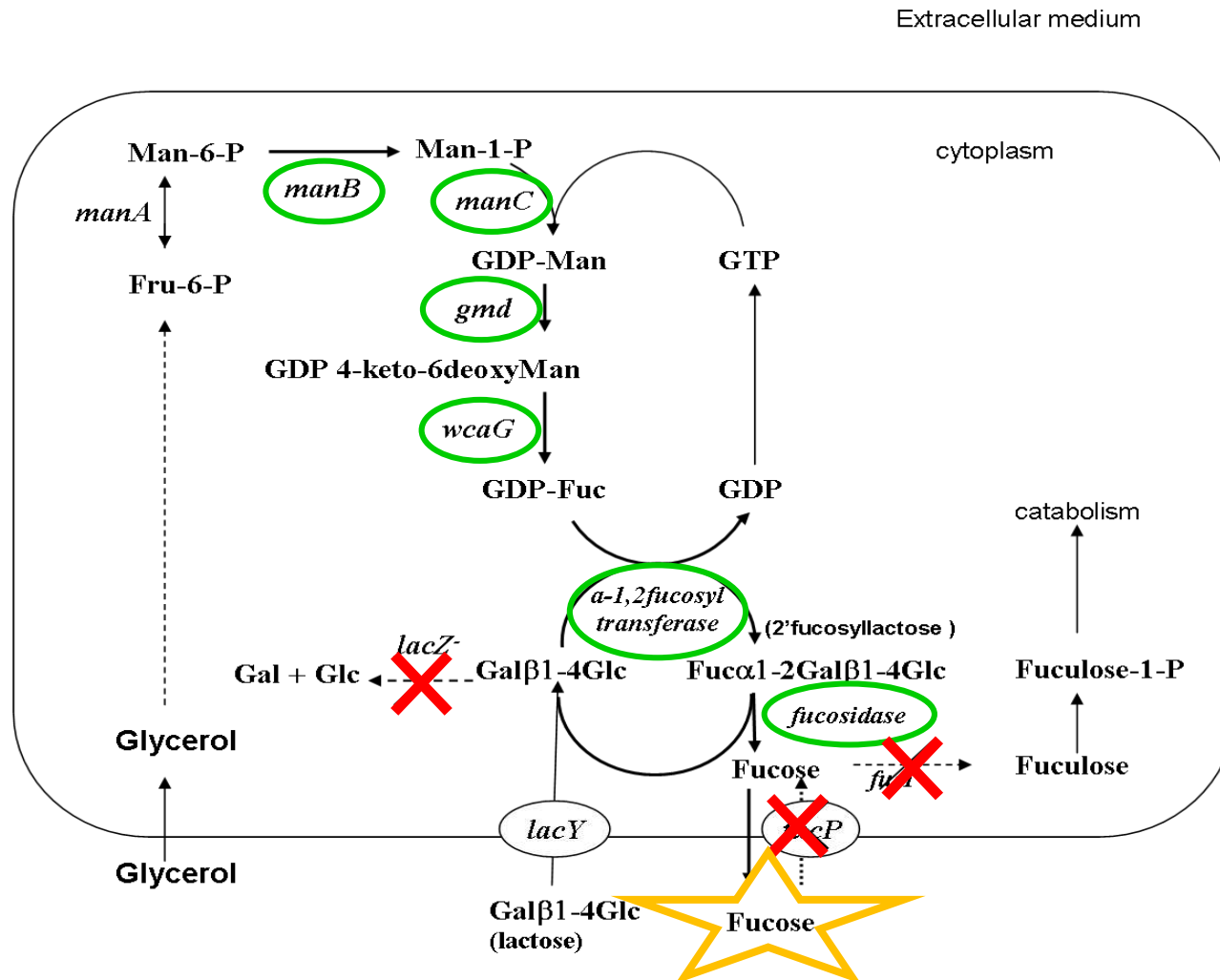
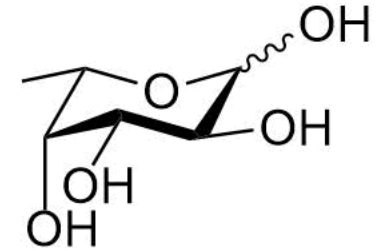
Production of perdeuterated carbohydrates

- Glucose-d₁₂ from alga grown in D₂O, hydrolysis of cellulose
- Direct deuteration on Raney nickel catalyst
- Chemical synthesis from deuterated precursors
- Chemoenzymatic
- **Synthetic glycobiology** (engineered organisms)



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*

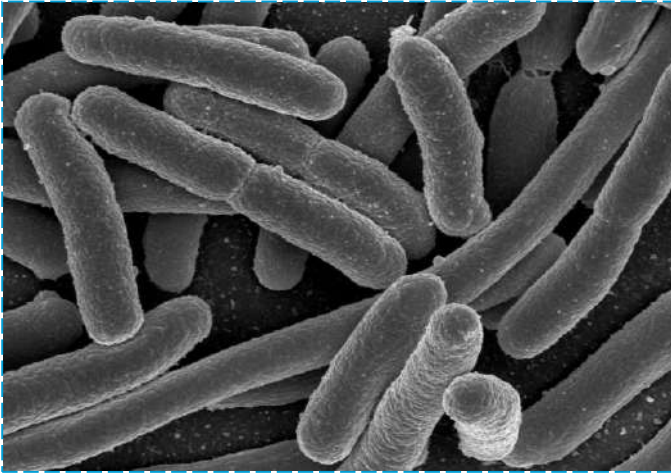


Knocked-out genes

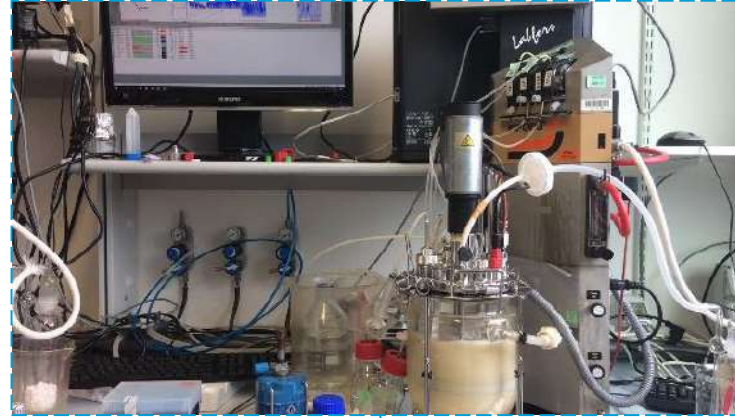
- lacZ*: β-galactosidase
- fucI*: fucose isomerase
- fucP*: fucose permease

Production, purification and characterization of L-fucose-d₁₂

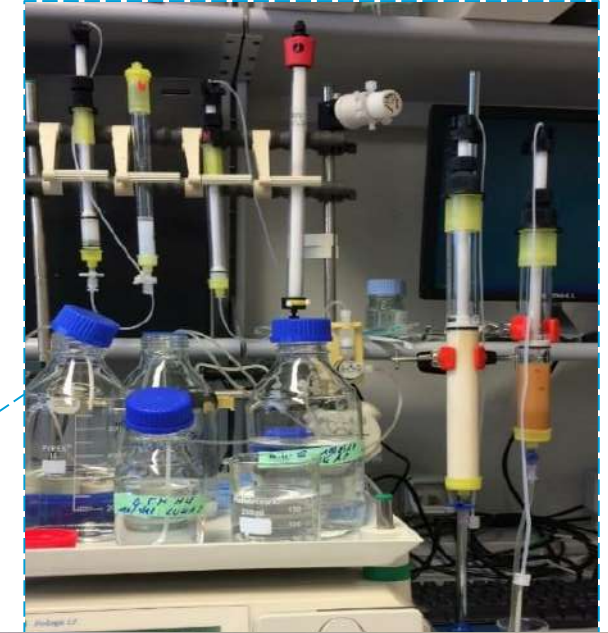
Adaptation to D₂O and glycerol-d₈



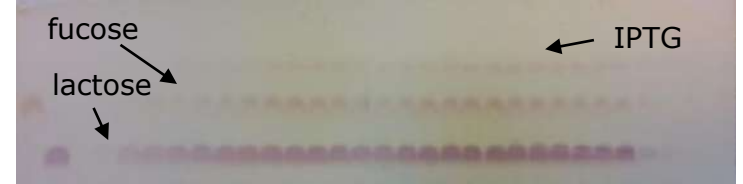
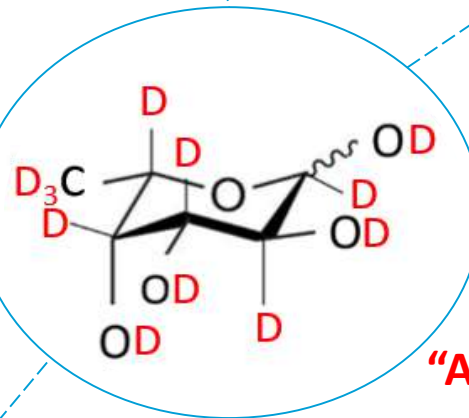
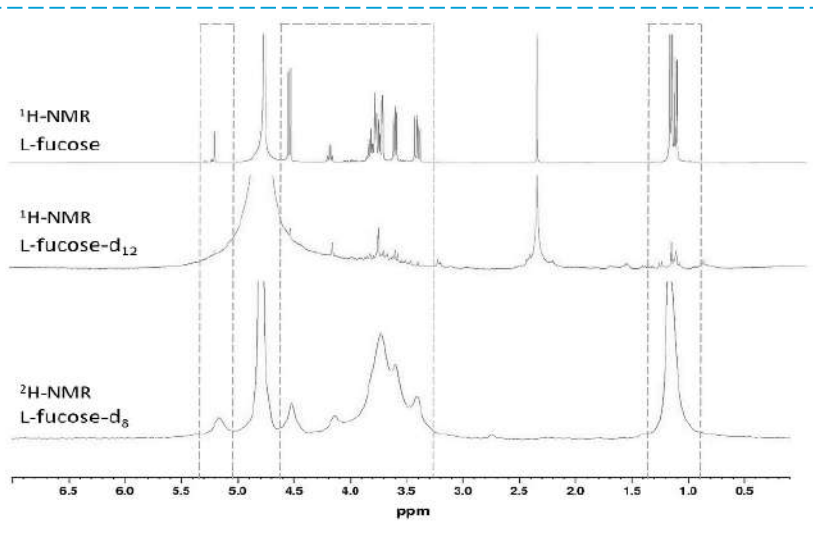
Batch production



Purification



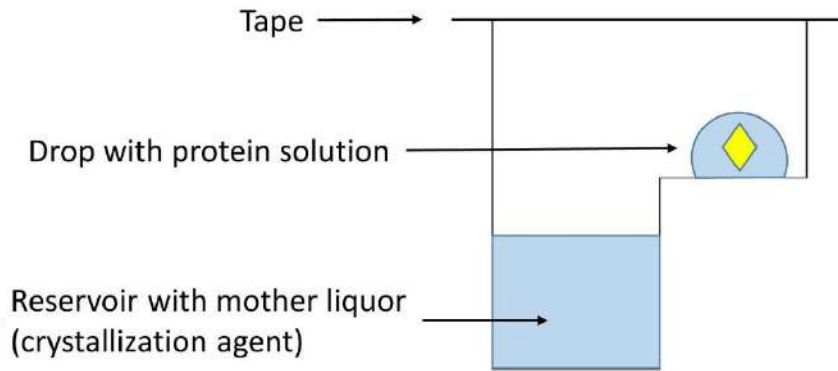
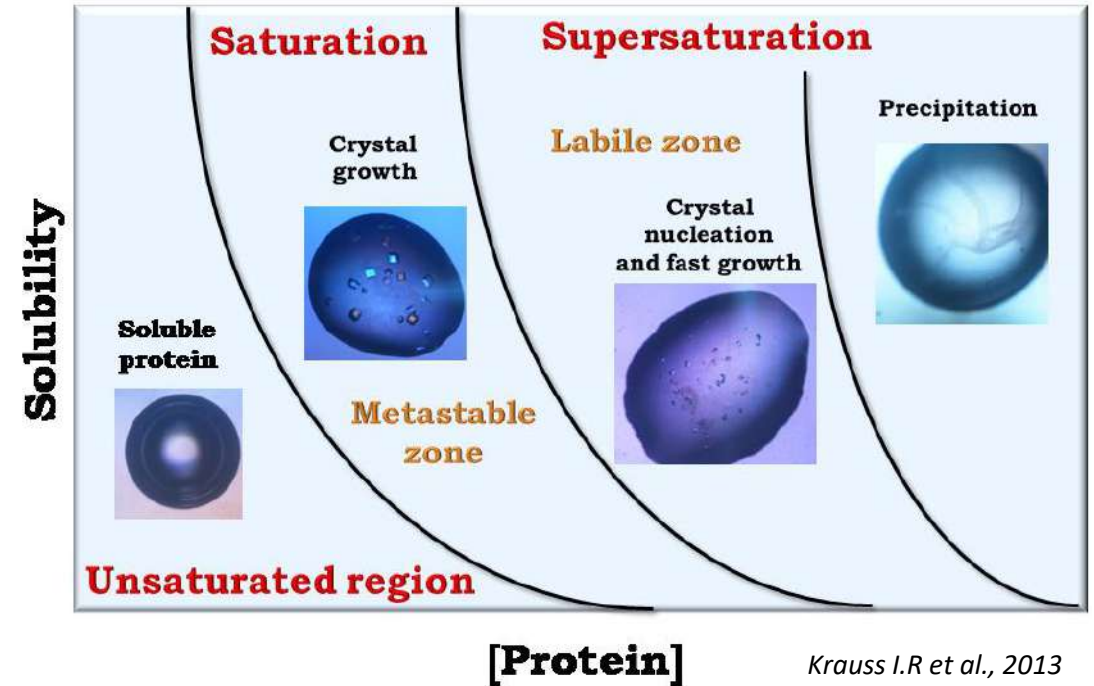
Characterization (Mass spec, NMR)



“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”

Large crystal growth

- To **compensate** for the **lower fluxes** of neutrons
- Typically **0.1-1 mm³** crystals needed
- **Optimization** of known conditions
- **Vapour-diffusion**, counter diffusion, seeding, **feeding**, microgravity..

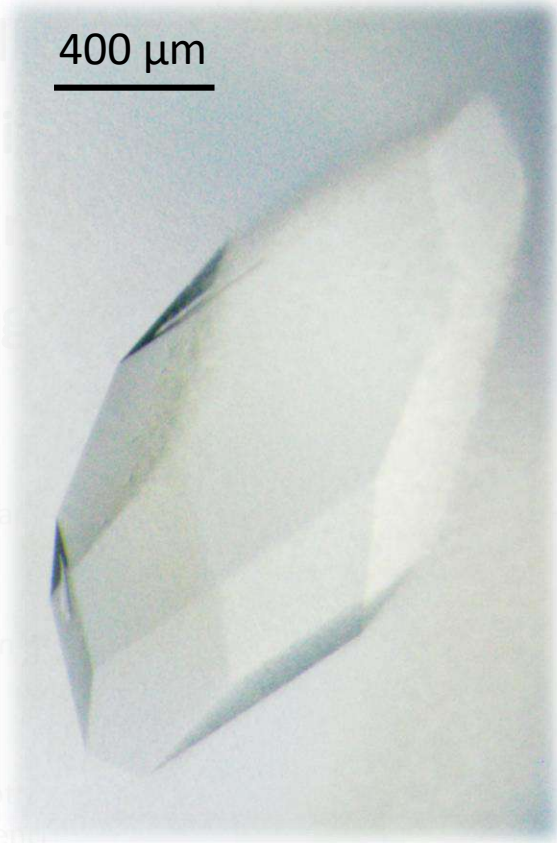


Sitting-drop vapour-diffusion method

Feeding over a period of several months

Large crystal growth

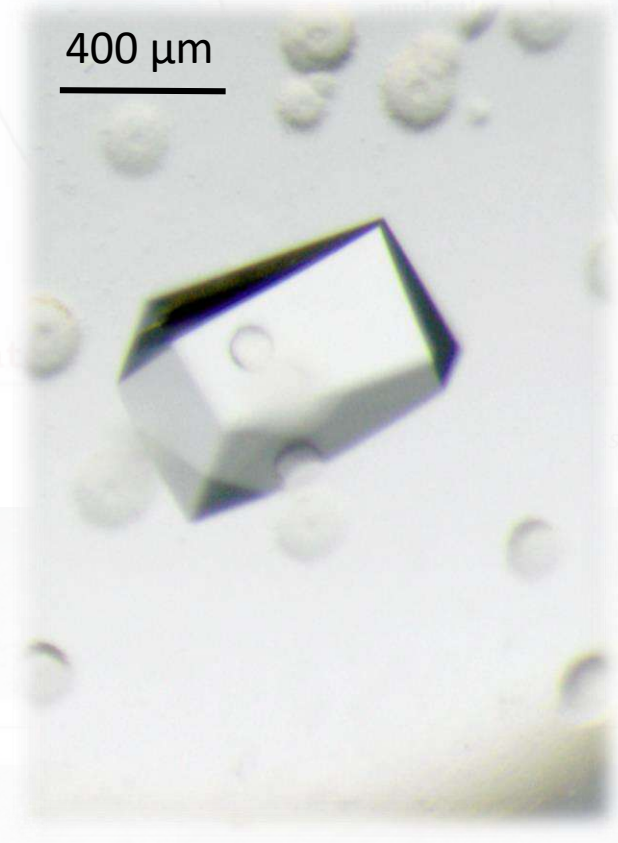
- To compensate for the lower fluxes of neutrons
- Typical needs
- Optimized conditions
- Vapour diffusion
- feeding



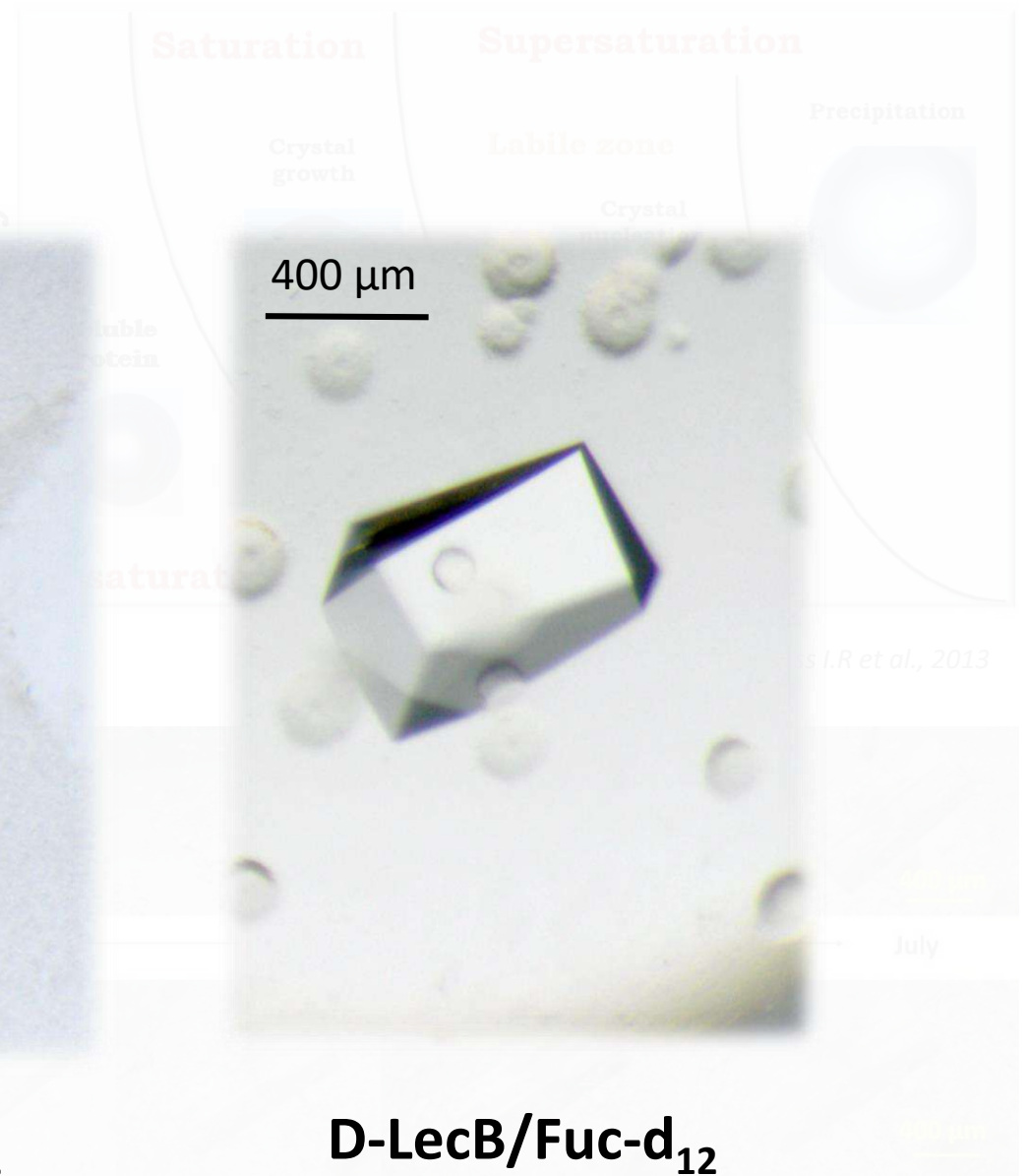
PLL lectin



D-PLL/Fuc-d₁₂



D-LecB/Fuc-d₁₂



Sitting-drop vapour-diffusion method

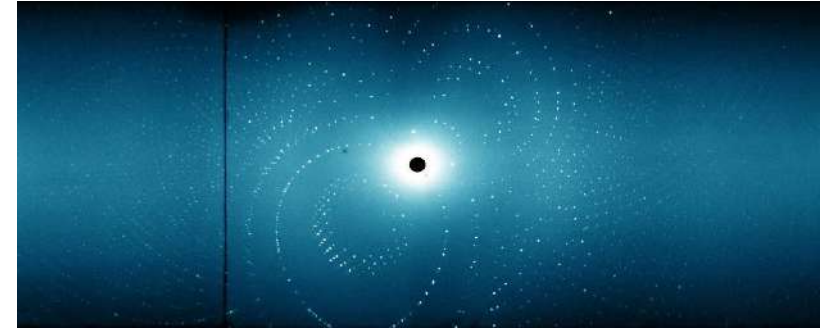
Laue diffractometer LADI-III at ILL

- Free neutrons produced by **nuclear fission**
- **Moderated** to decrease their energy from MeV to **meV** range



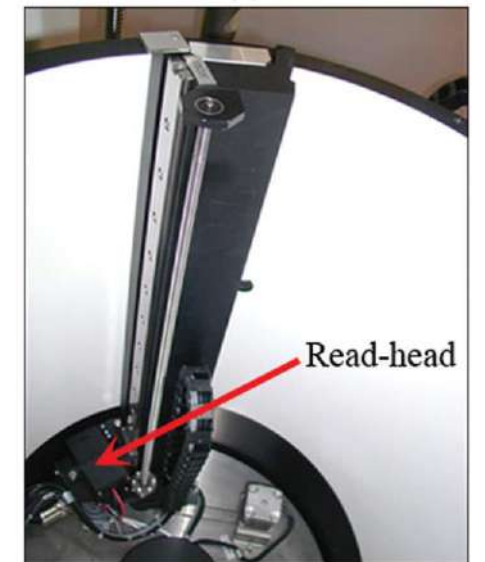
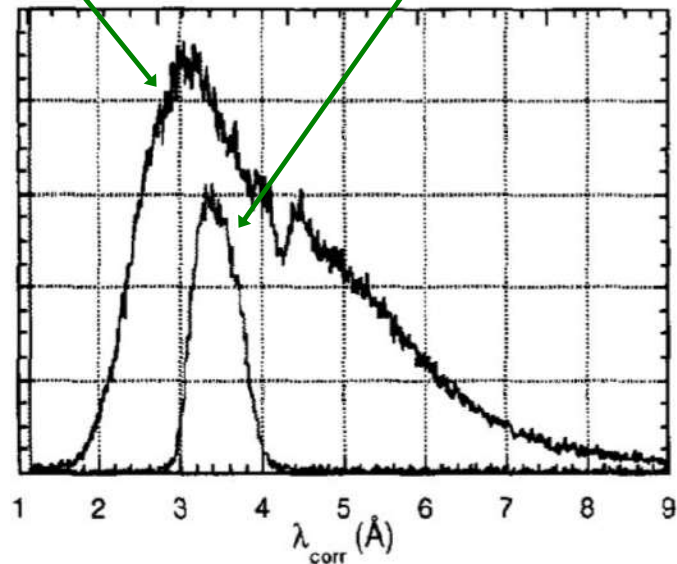
Laue diffractometer LADI-III at ILL

- Quasi-Laue diffraction method (pink beam of neutrons)
- Large cylindrical neutron-sensitive image plate detector
- Data collection at room temperature or cryo
- Crystal mounted in a quartz capillary



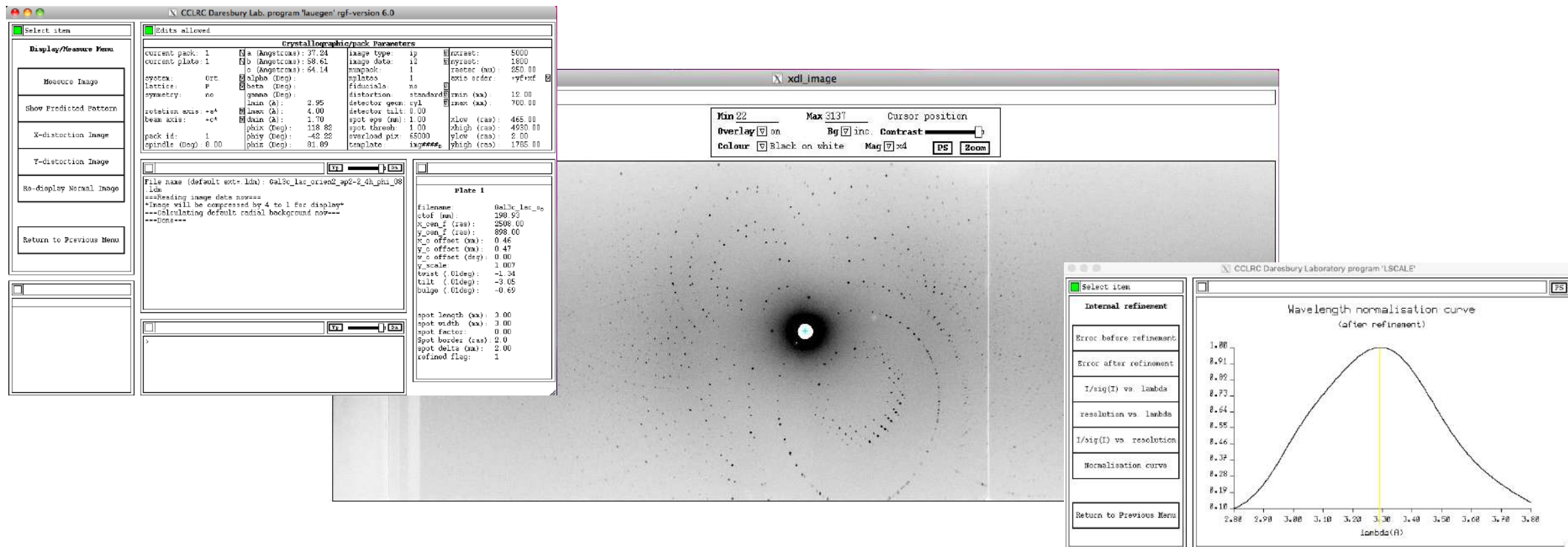
Laue (white) beam

Quasi-Laue (pink) beam



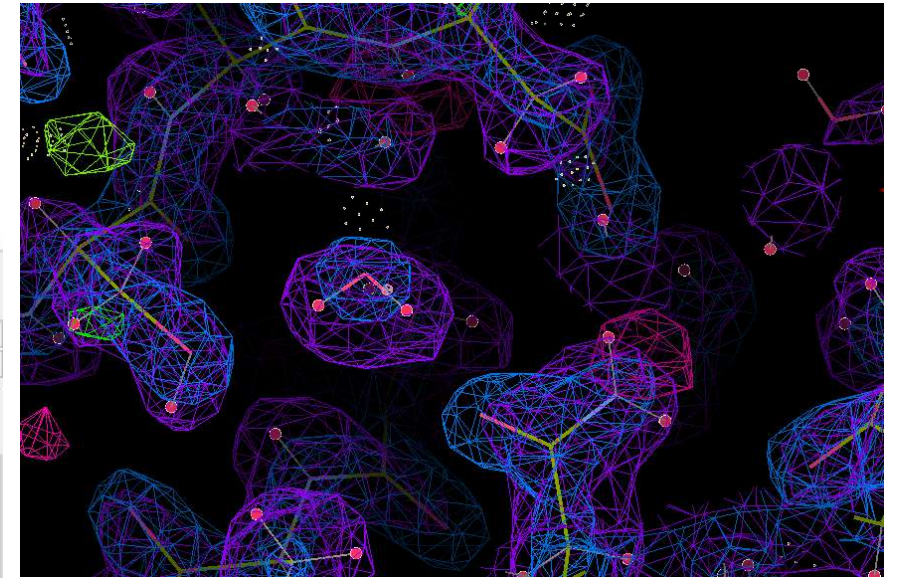
Neutron data reduction

- Quasi-Laue diffraction data indexed and integrated ($h, k, l, I, \text{sig}(I), \lambda$) using **LAUEGEN**. Intensities are λ -normalized using **LSCALE**.
- Data then processed with standard X-ray software from **CCP4** (<https://www.ccp4.ac.uk/>).



Joint X-ray/neutron structure refinement

- Increases the **data-to-parameters** ratio
- Uses **X-ray data** to better define the atoms that are subjected to cancellation effects in neutron diffraction
- Using *phenix.refine* within the PHENIX suite
- **H/D** atoms added and **refined individually**



File path | Format | Data type

C:\Users\gajdos\Phenix\JointXN_DLecB_Dfuc_LG50B1\Refine_13\JointXN_D...	ccp4_mtz	X-ray data, X-ray R-free, Neutron data...
C:\Users\gajdos\Phenix\JointXN_DLecB_Dfuc_LG50B1\FUL_restraints.ligan...	CIF	Restraints (CIF)
C:\Users\gajdos\Phenix\JointXN_DLecB_Dfuc_LG50B1\FUC.ligands.cif	CIF	Restraints (CIF)
C:\Users\gajdos\Phenix\JointXN_DLecB_Dfuc_LG50B1\JointXN_DLecB_Dfu...	PDB	Input model

Space group: P 1 2 1 1 | Unit cell: 52.8999 73.87 55.0033 90 94.58 90

X-ray data and experimental phases

Data labels: F-obs-xray,SIGF-obs-xr | R-free label: R-free-flags-xray | Test flag value: 1

High resolution: | Low resolution: | Phase labels: ---

Wavelength: | Options...

Neutron data

Data labels: F-obs-neutron,SIGF-obs | R-free label: R-free-flags-neutron | Test flag value: 1

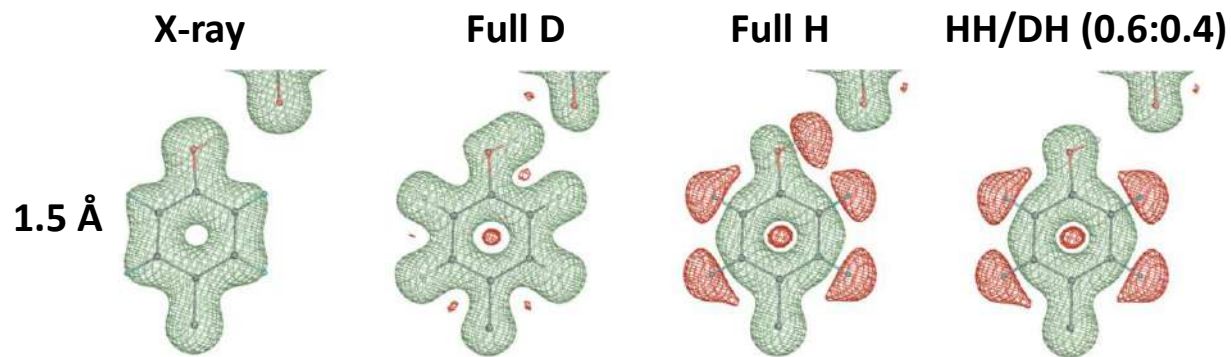
High resolution: | Low resolution: | Options...

Project: JointXN_DLecB_Dfuc_LG50B1

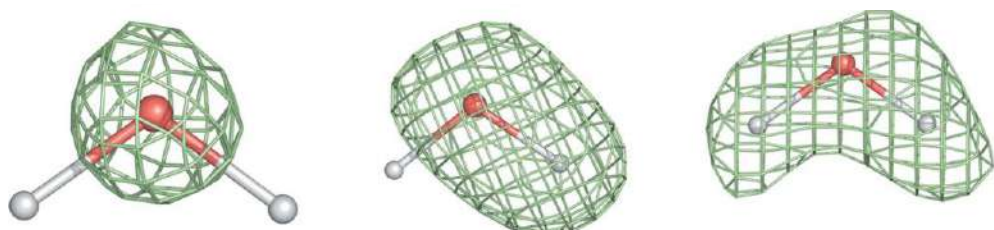
Before and after refinement: $2F_o - F_c$ electron density
 $2F_o - F_c$ neutron scattering length density

	Initial (X-ray)	Final (X-ray)	Initial (neutron)	Final (neutron)
R-work	0.1047	0.1042	0.3126	0.1914
R-free	0.1422	0.1419	0.3487	0.2461
Bonds	0.010	0.010	None	None
Angles	1.280	1.269	None	None

Neutron scattering length density maps

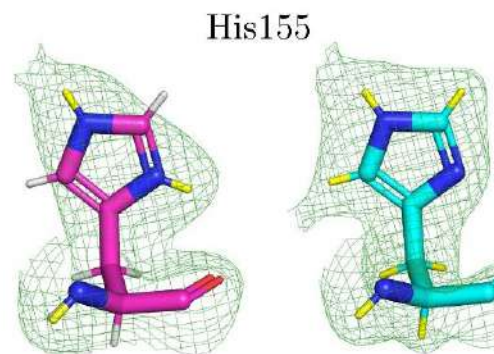
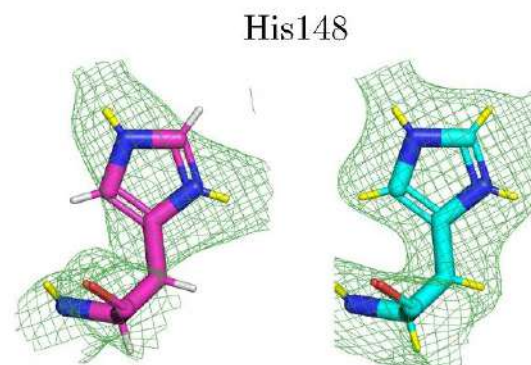


Orientation of water molecules



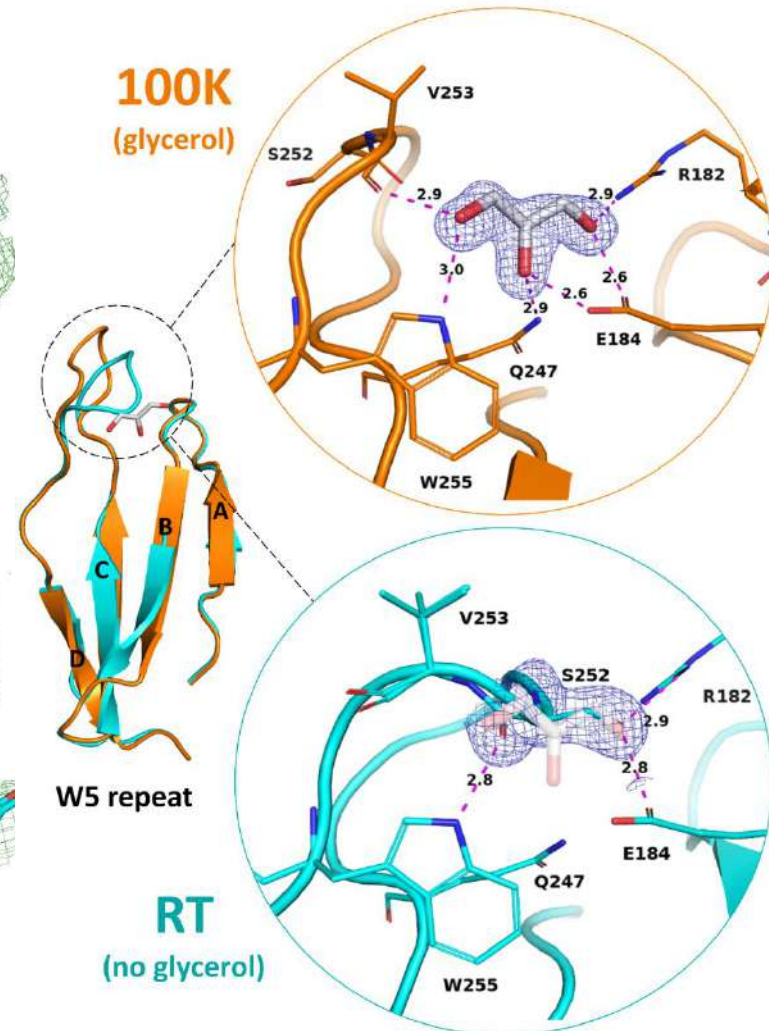
Neutron $2F_{\text{obs}} - DF_{\text{model}}$

Afonine P.V. et al., 2010



H/D

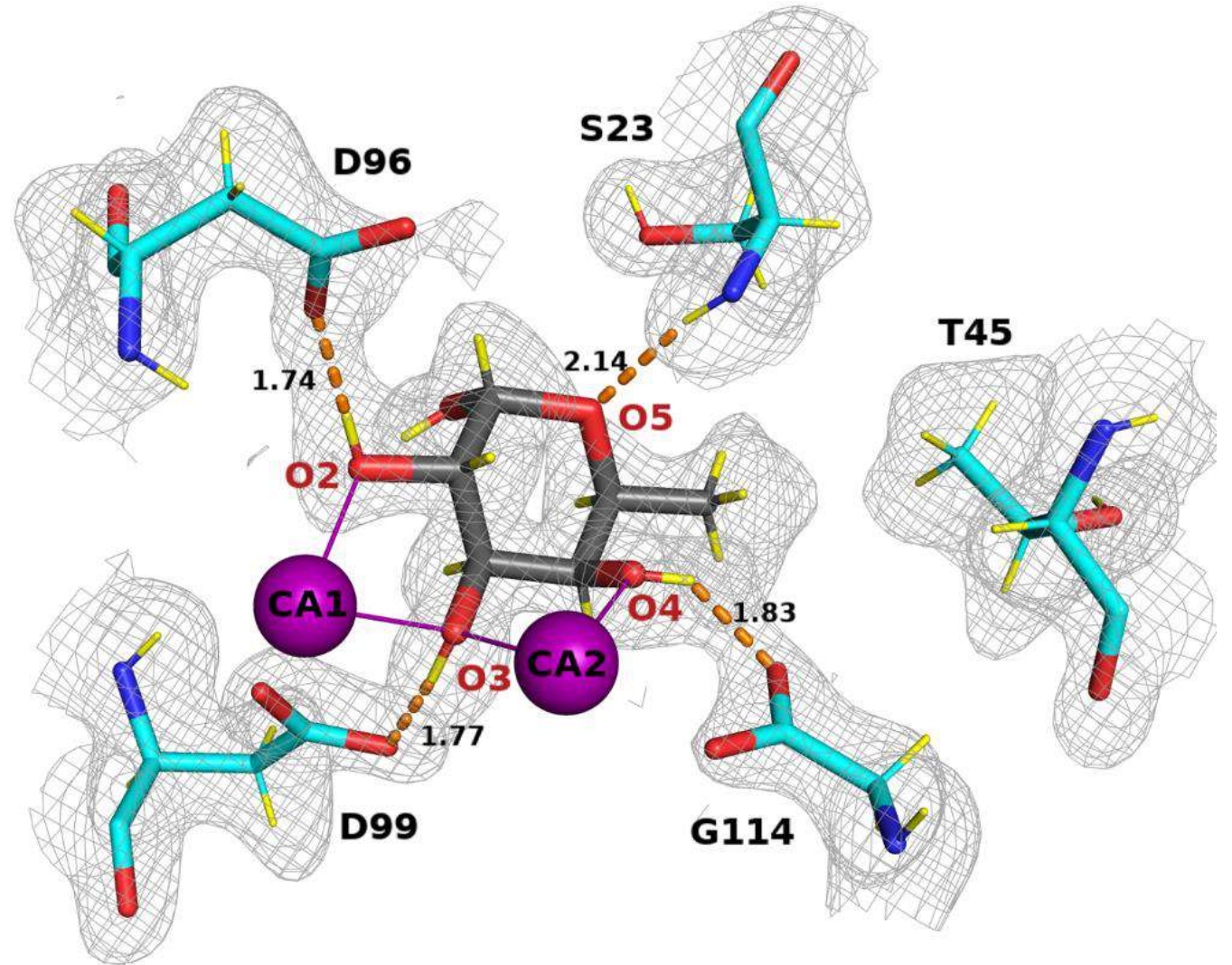
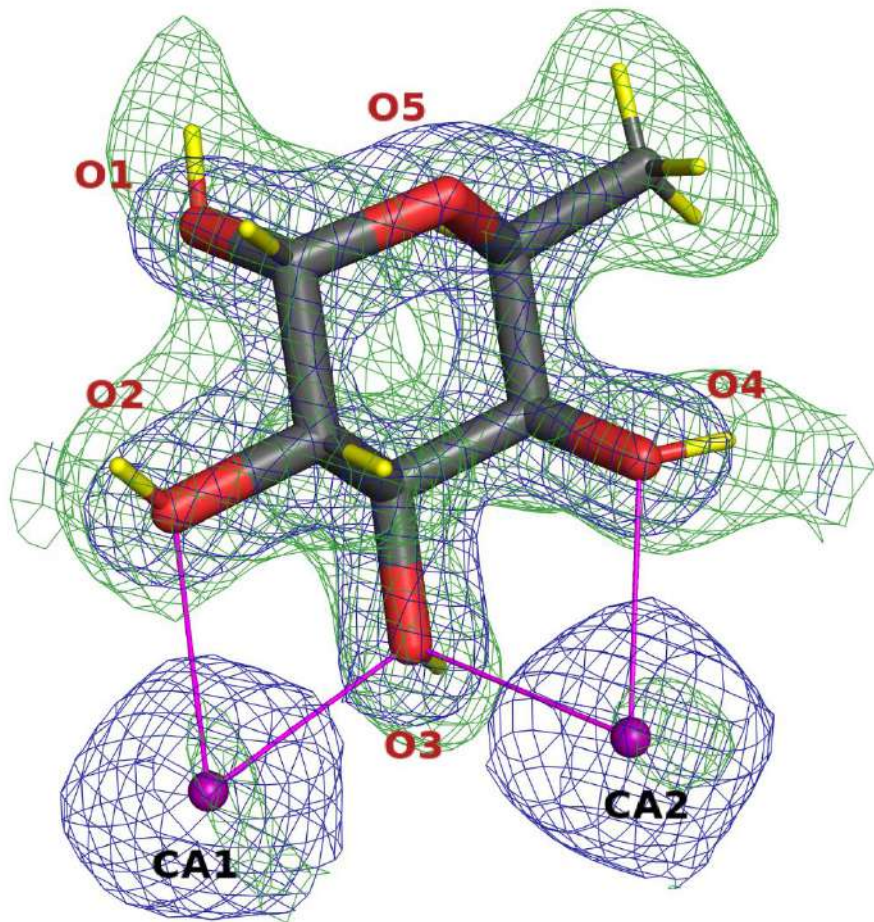
Full D



Gajdos L. et al., 2021

Perdeuterated fucose in the LecB binding site

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



Summary

- Neutron crystallography is a **complementary** technique to X-ray crystallography
- Provides **experimental determination** of hydrogen atom positions
- **Unambiguous** determination of protonation states, water orientations, ligand docking, H-bonding networks, proton transfer
- **Perdeuteration** is advantageous (smaller crystals needed and reduced data collection times)
- Crystals with volumes of **0.1-1 mm³**
- **Room-temperature** data collection (closer to physiological conditions)
- **Joint XN refinement improves** the model quality

Acknowledgements

Juliette Devos

Trevor Forsyth
Michael Haertlein
Life Sciences group

Anne Imberty

Annabelle Varrot
Molecular and
structural
glycobiology group



Matthew Blakeley

