Structural Glycoscience Workshop

IBS, Grenoble, 28-30th June 2016

Applications of neutron diffraction to glycoscience

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EMBL


Synchrotron X-rays:
• Allow observation of diffraction data *very quickly.* Good for systems that change rapidly
• Allow observation of diffraction data from small samples
Neutrons:
• Allow observation of hydrogen atoms and water - critical for biological systems
• Contrast variation
• Dynamics
• Allows exploitation of sample labelling (deuteration)

Partnership for Structural Biology (PSB)

NEUTRONS FOR SCIENCE

ESRF

EMBL GRENOBLE

ibs Institut de Biologie Structurale

UVHCI

PSB Platforms

Sample preparation

- RoBioMol for Molecular Biology and Protein Expression
- Eukaryotic Expression Facility
- Deuteration Laboratory (neutron scattering)
- Large crystal growth (neutron scattering)
- Labelling for NMR (13C & 15N)
- Cell free synthesis
- ESPRIT Construct Screening Platform
- High Throughput Crystallisation Platform

Characterisation

- Biophysical characterisation
- Mass Spectrometry and 1–D NMR
- Protein Sequencing
- MALLS
- SPR
- Cryobench
- AUC

Structure and Dynamics

- Synchrotron X-ray scattering
- Neutron crystallography
- SANS/SAXS
- High Field NMR
- Electron Microscopy
- Cell imaging

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High impact for user science

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- **Interdisciplinarity**: availability of all PSB platforms to ILL users
- **Complementarity and synergy**: SANS & NMR & deuteration & mass spectrometry & SAXS & EM; NMX & deuteration & XMX (crystallography) (& SAS)
- **Broadening engagement** of wider community in Life Sciences
- Wide range of **experience/expertise** amongst PSB partners – high mutual benefit


EMBL

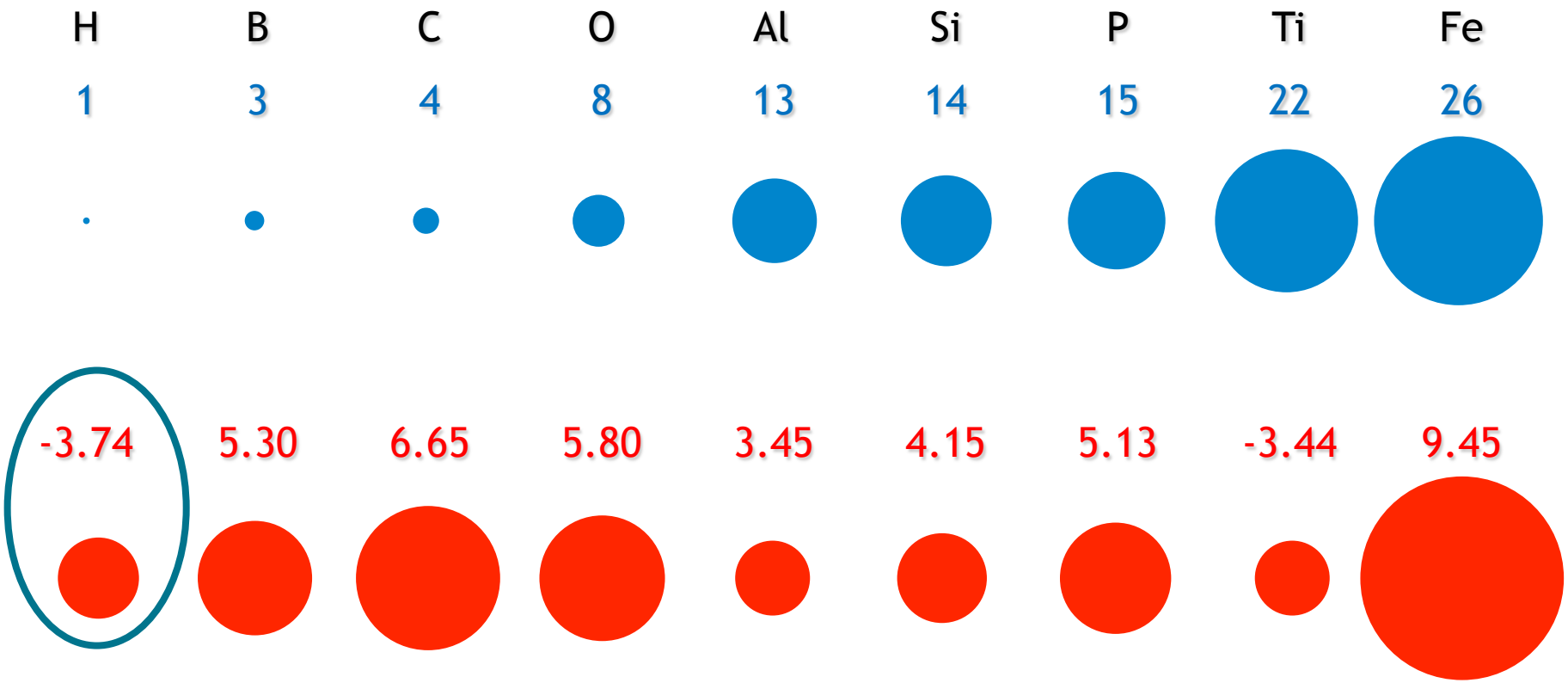
X-ray and neutron scattering

X-ray

Scattering proportional to Z

Neutron

Scattering not proportional to Z



X-ray and neutron scattering

X-ray

Scattering proportional to Z

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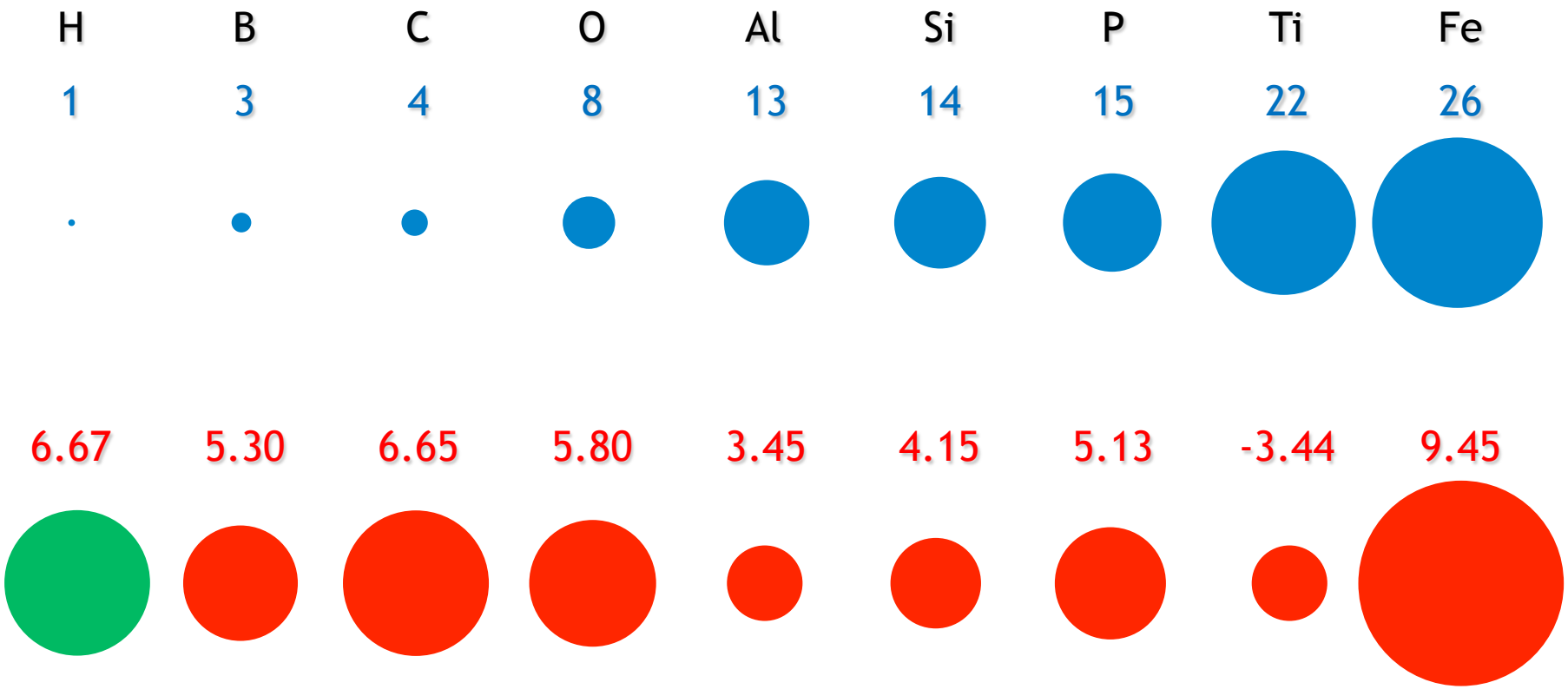


Molecules in crystals

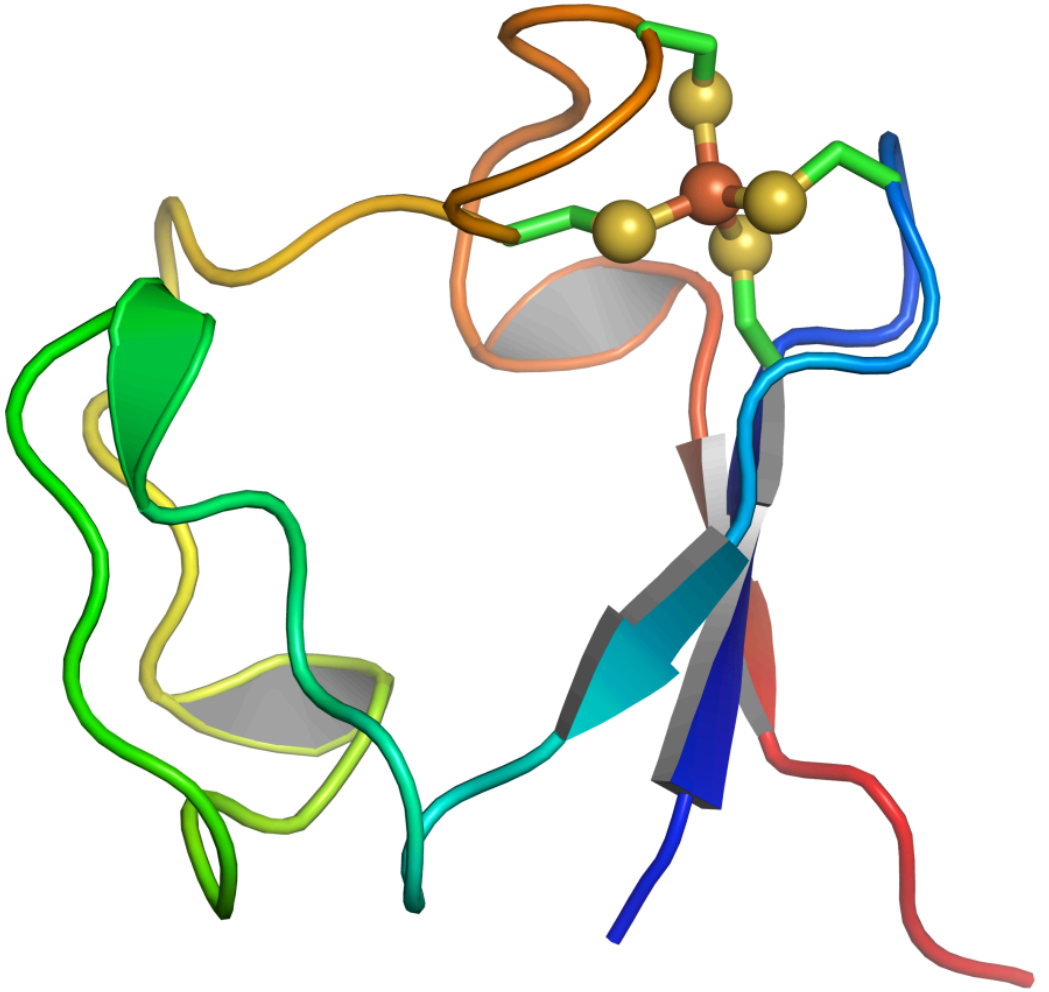
Rubredoxin from *Pyroccus furiosus*

Small iron-sulphur containing redox protein.

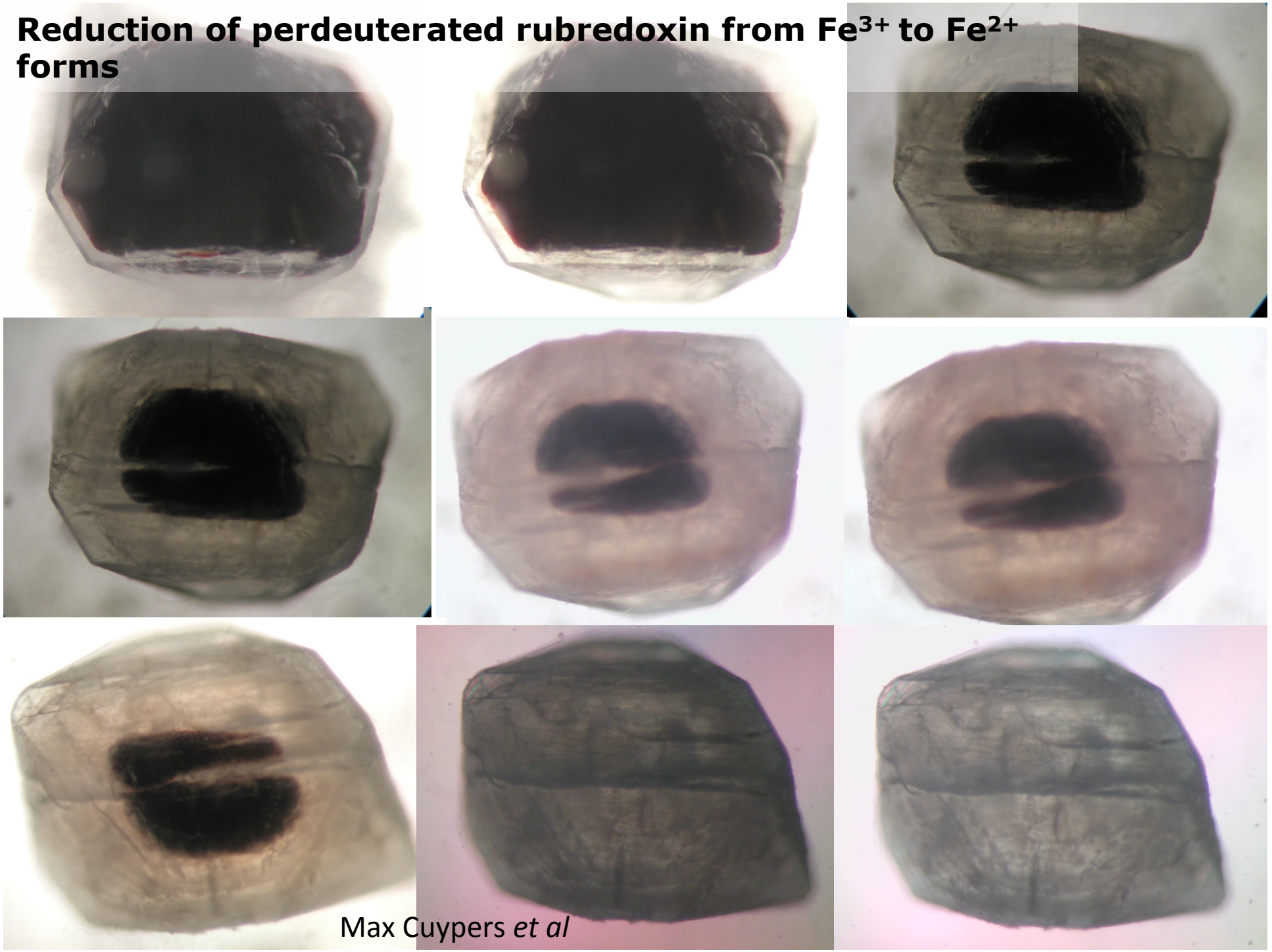
Biological function not well understood

Remarkable properties of thermostability

Important model system for an understanding of electron transfer processes associated with redox activity.



Reduction of perdeuterated rubredoxin from Fe^{3+} to Fe^{2+} forms



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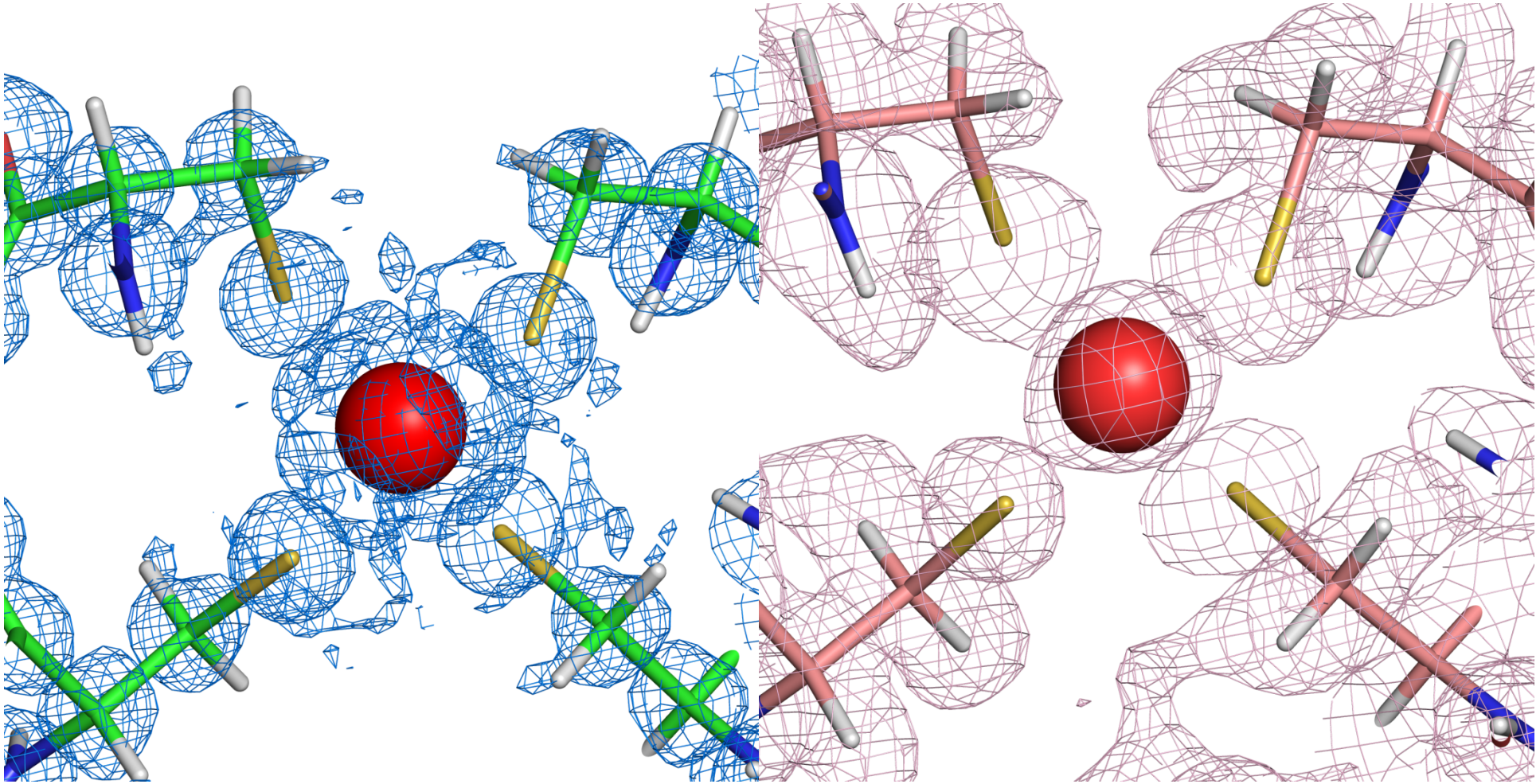
Perdeuterated rubredoxin Fe^{3+} form



X-ray structure

neutron structure

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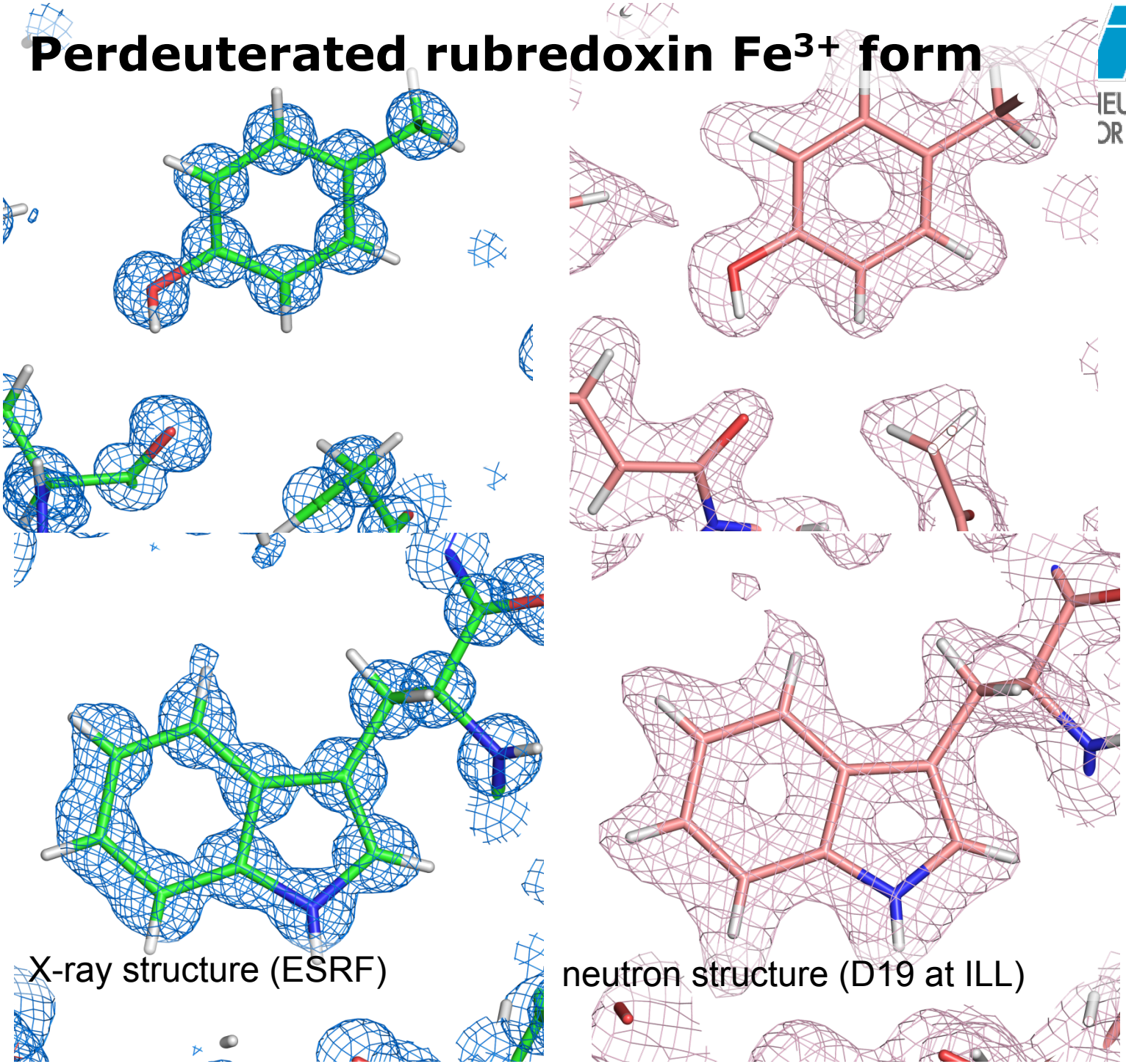
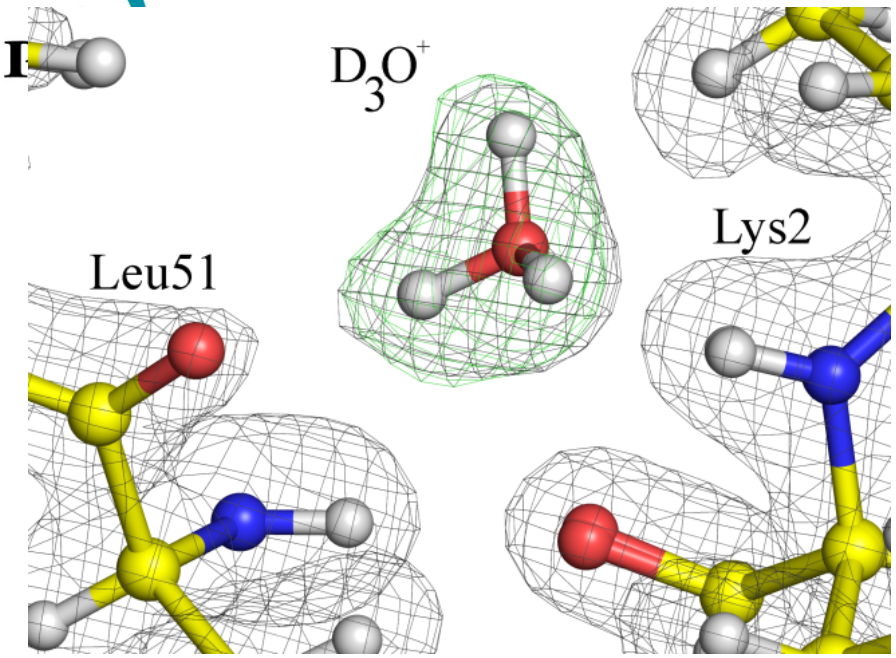
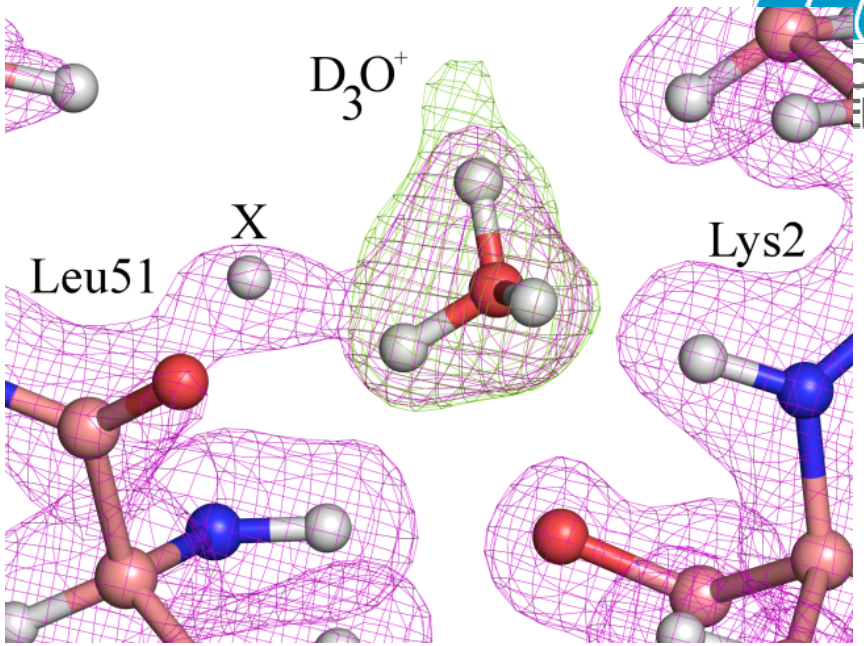
Perdeuterated rubredoxin Fe^{3+} form



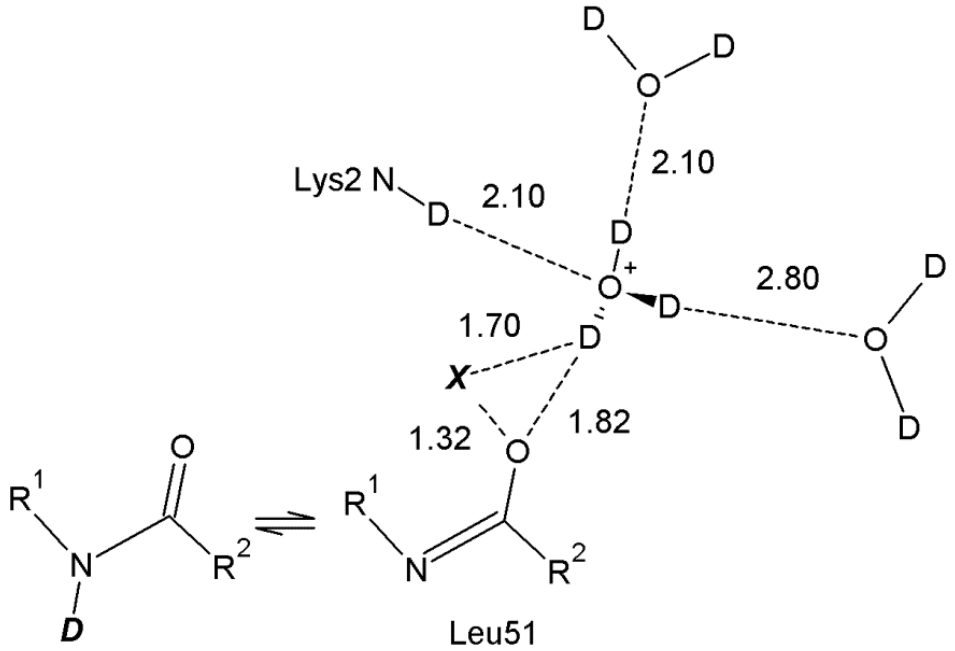
X-ray structure (ESRF)

neutron structure (D19 at ILL)





Observation of hydronium ions and of tautomeric shifts following the change from the oxidised form to the reduced form



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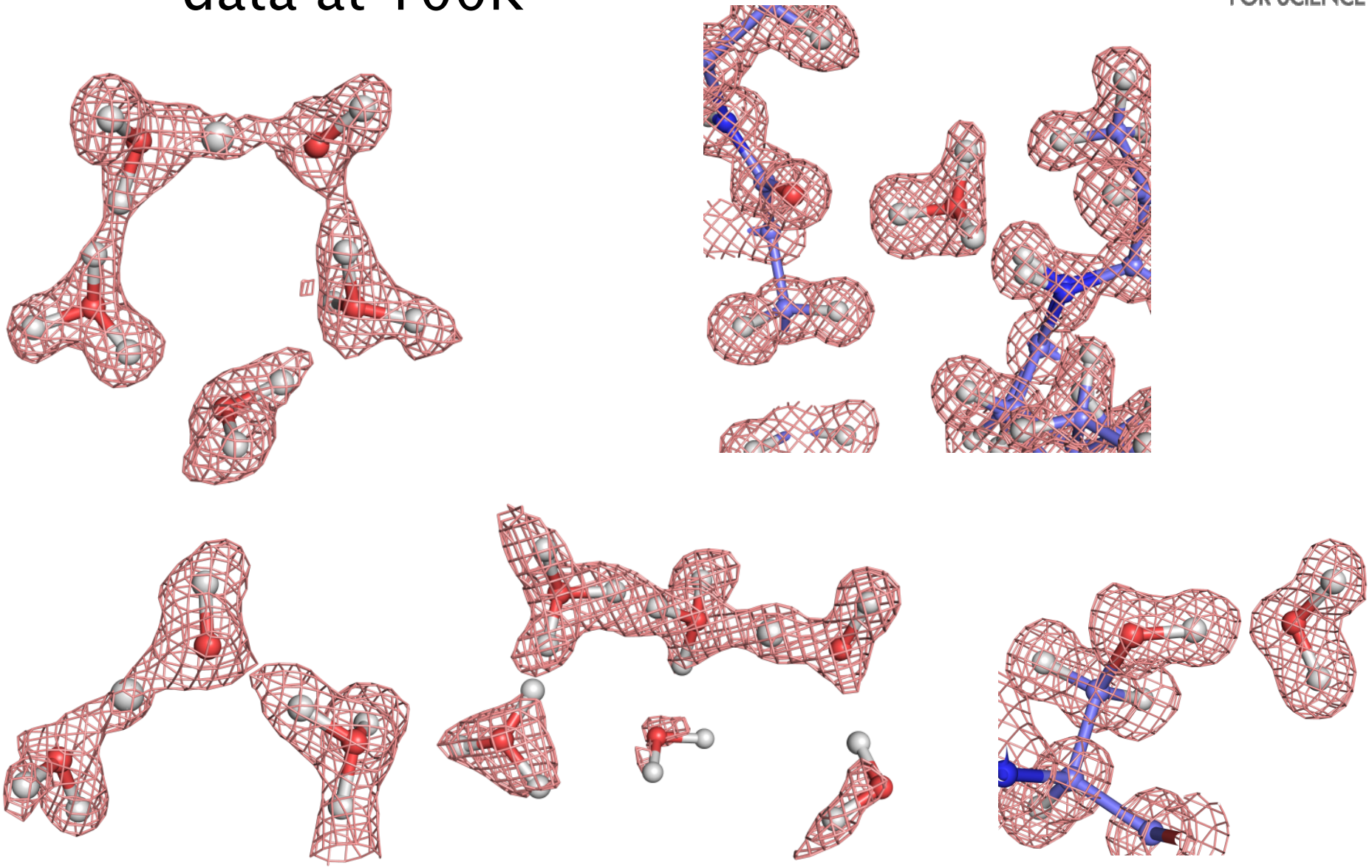
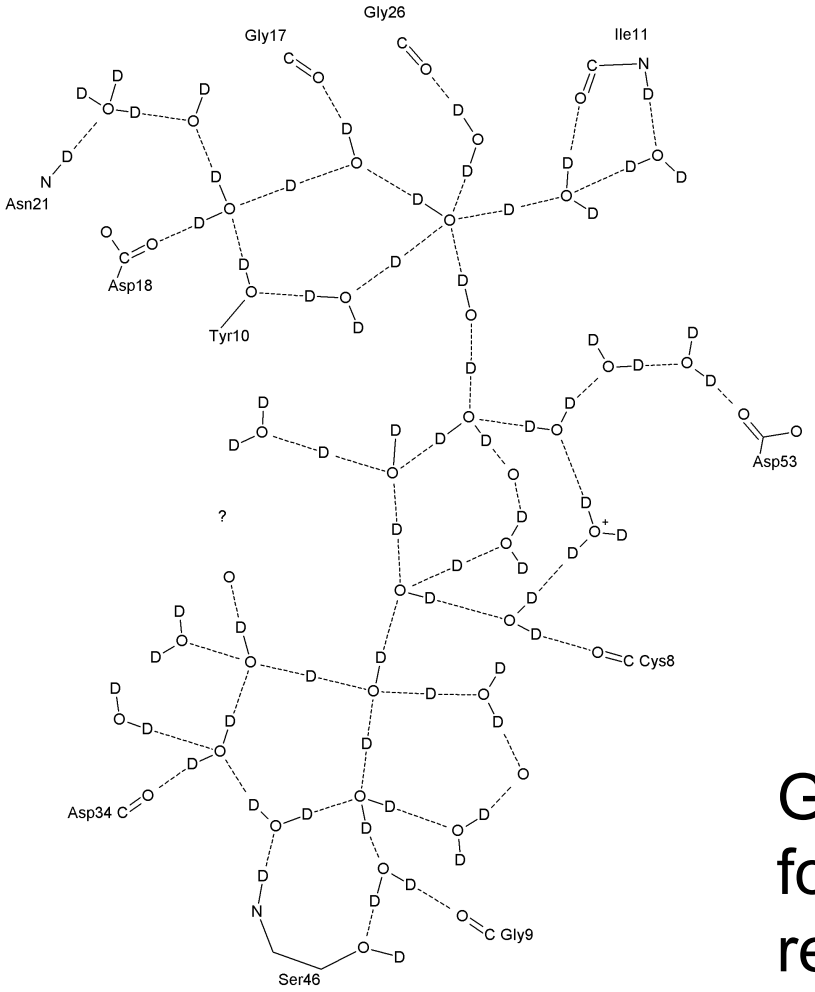
Ultra-high (0.88A) resolution neutron data at 100K

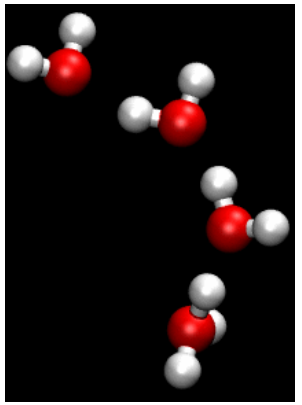


Extensive solvent ordering



- 5 and 6 water rings
- Intermediate O---D---O
- Distance O-O= **2,58-2,87 Å**
- Normal O-D dist. is 0,98 Å
- Zundel $H_5O_2^+$'s O-O dist 2.32 -2.52 Å

Grotthuss mechanism for proton transfer in a redox protein?



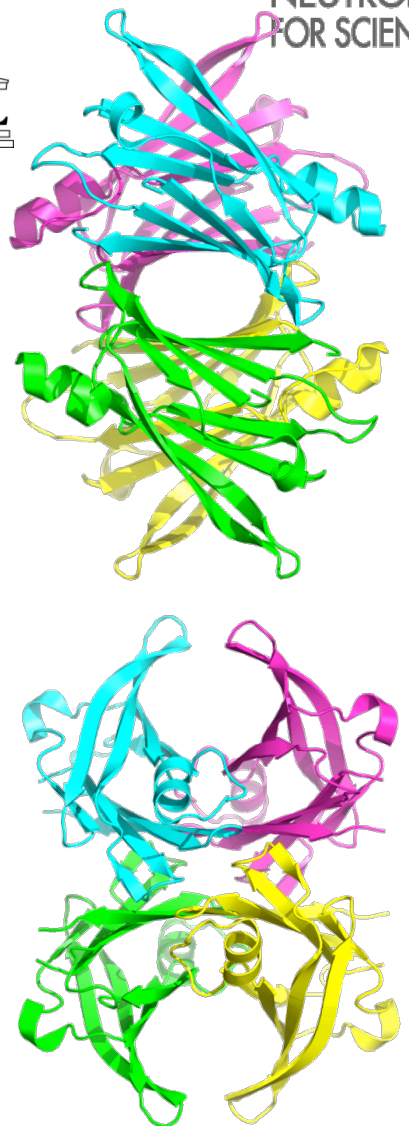
Transthyretin amyloidosis – insights from neutron and X-ray crystallography

- Human transthyretin (TTR) is a 55 kDa homotetramer that transports thyroxine and retinol binding protein in the blood and cerebrospinal fluid.

- Wild type TTR is inherently amyloidogenic and frequently leads to senile systemic amyloidosis (SSA).

- Point mutations in the genome often result in an early disease onset due to de-stabilisation of the tetramer, rendering the protein prone to dissociation and aggregation. Eg FAP, FAC.

- Interest in factors that impart stability to the TTR tetramer



TTR Structure

Melina Haupt *et al*

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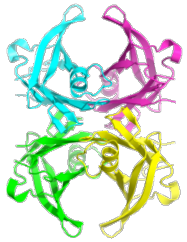

tetramer

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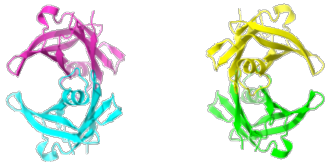

dimer

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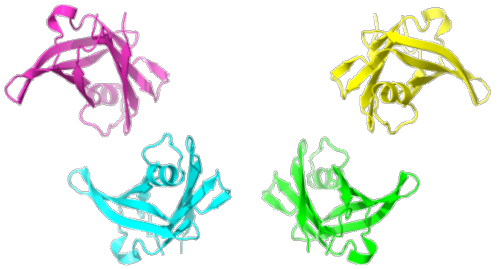

monomer

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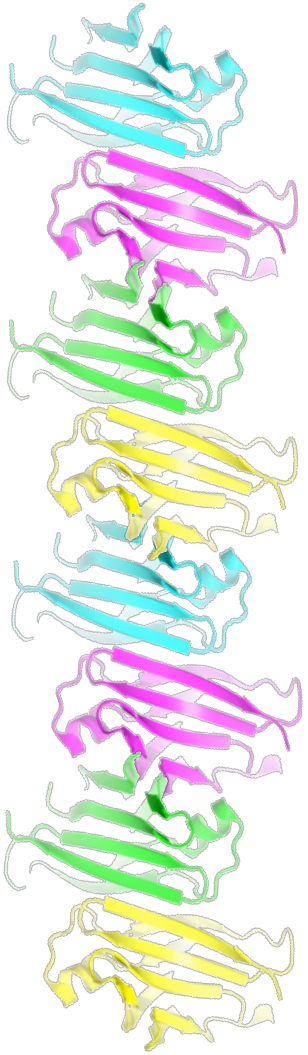

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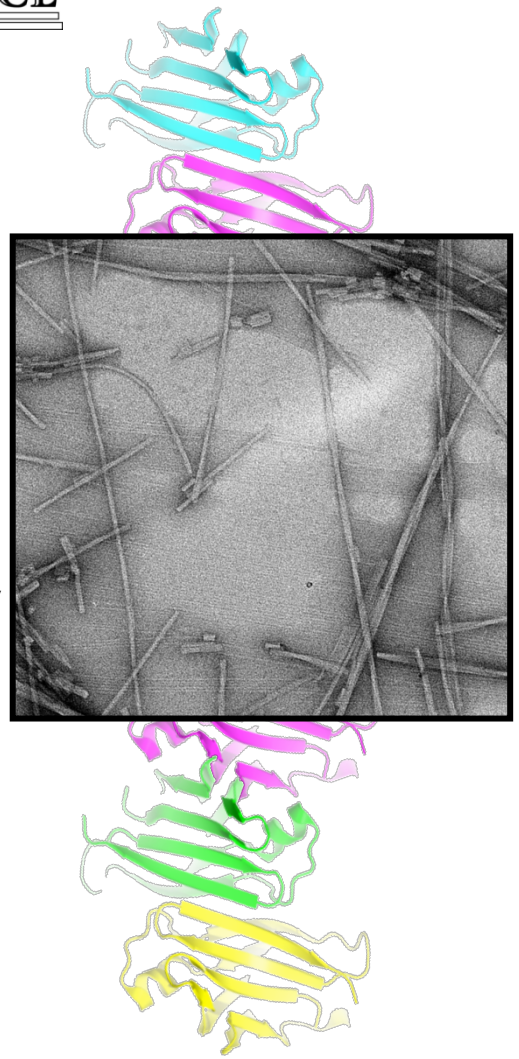

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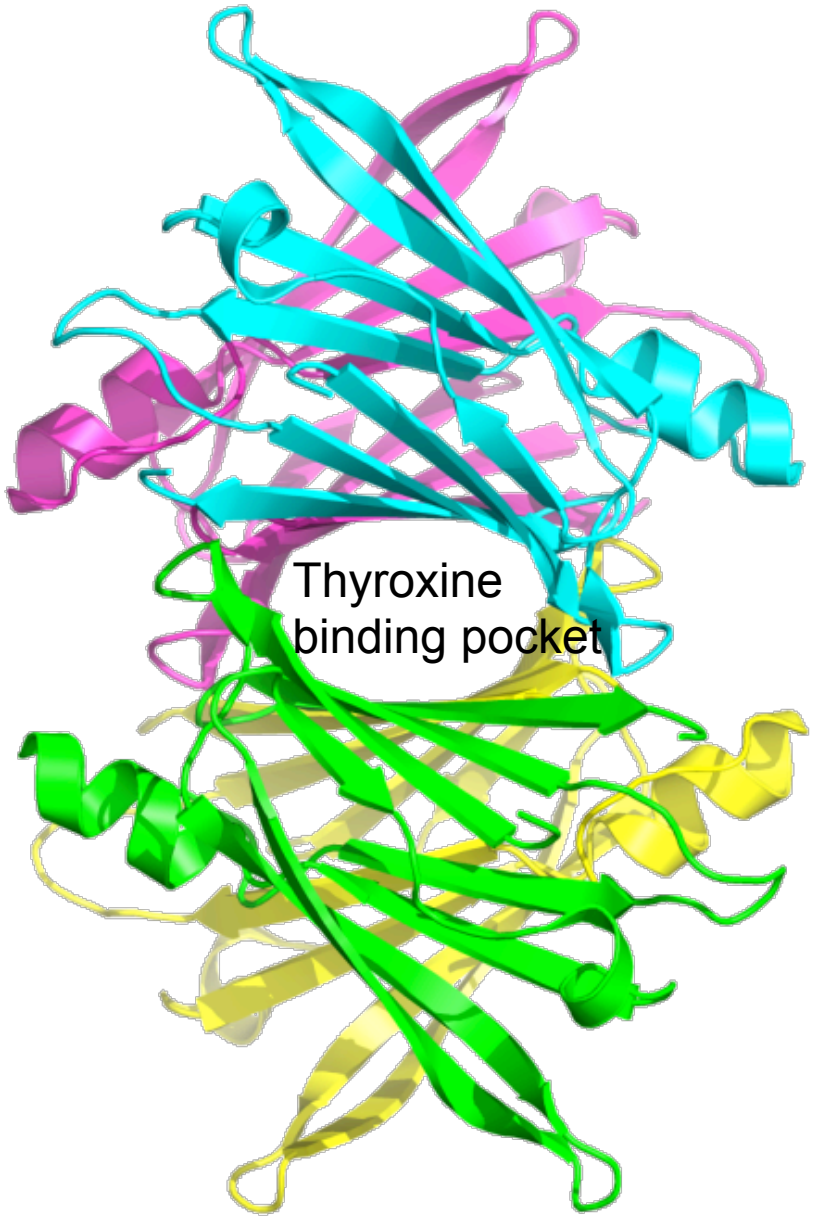
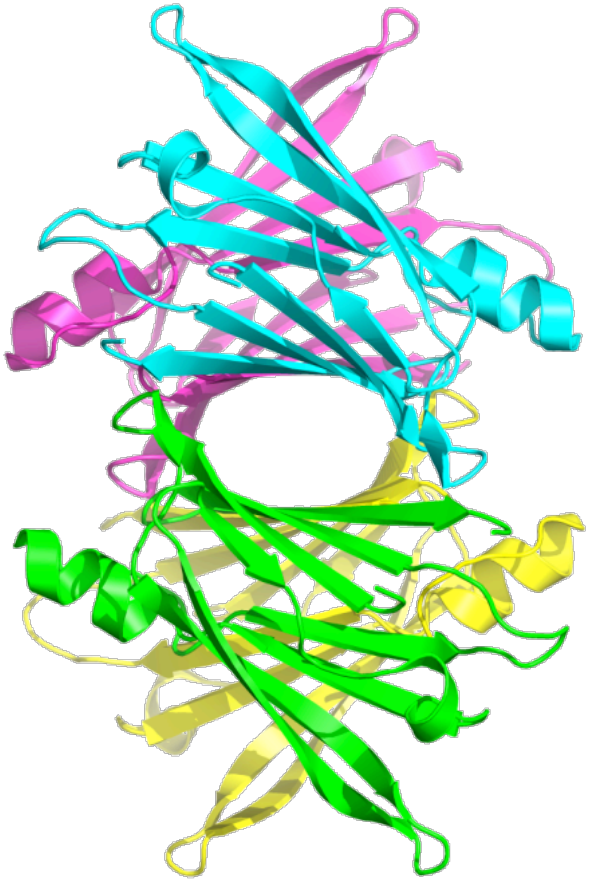
TTR: significance of monomer, dimer, and tetramer structure

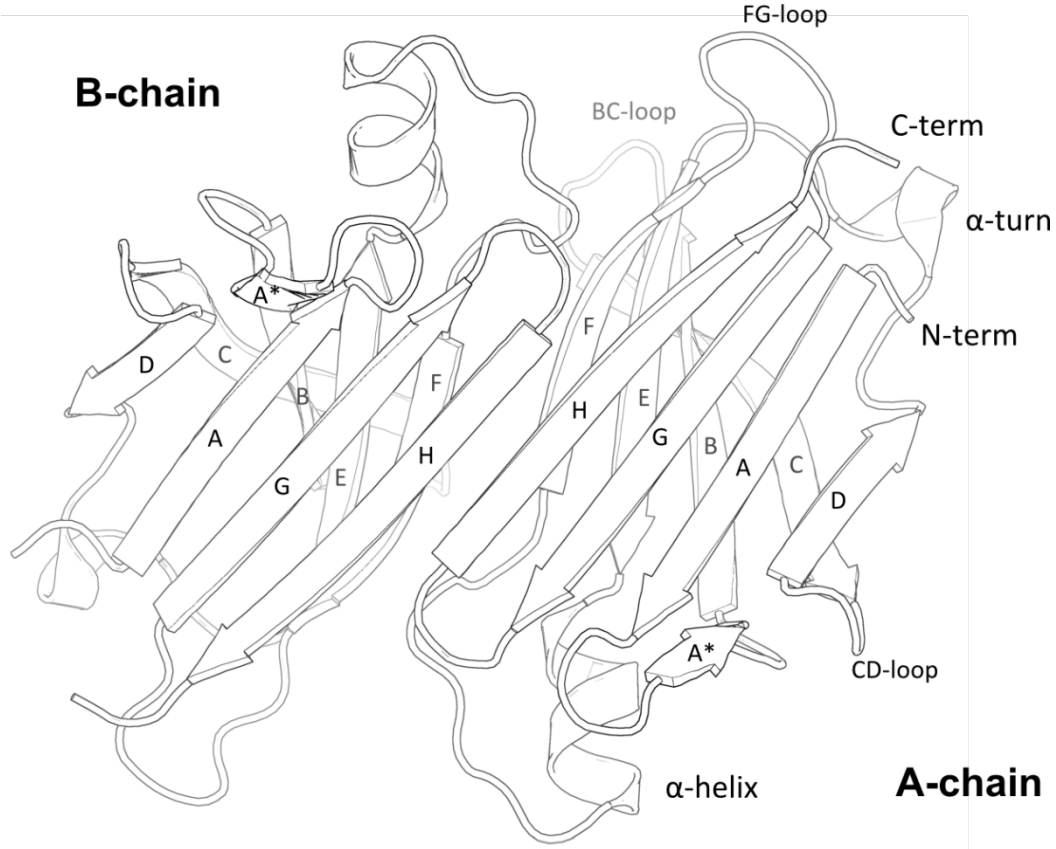


Primary dimer

Secondary dimer

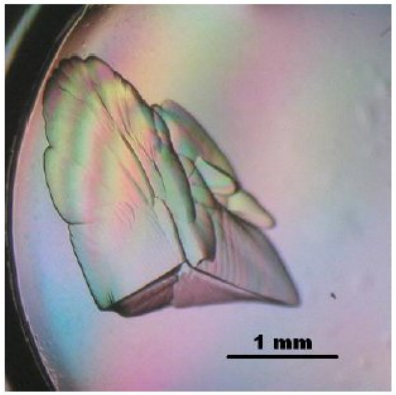
TTR primary dimer

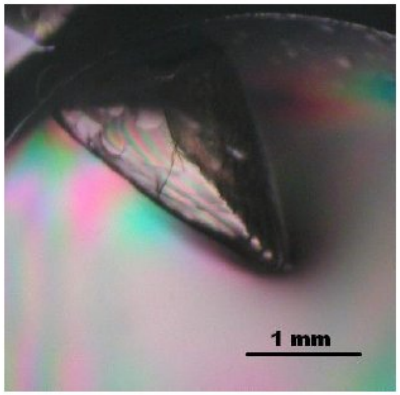


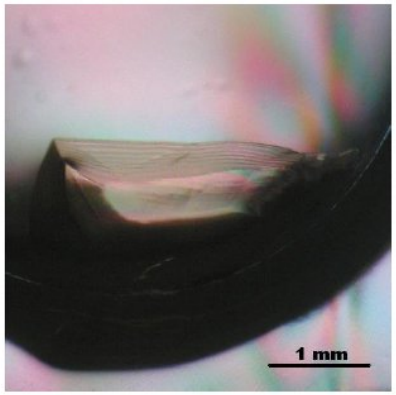


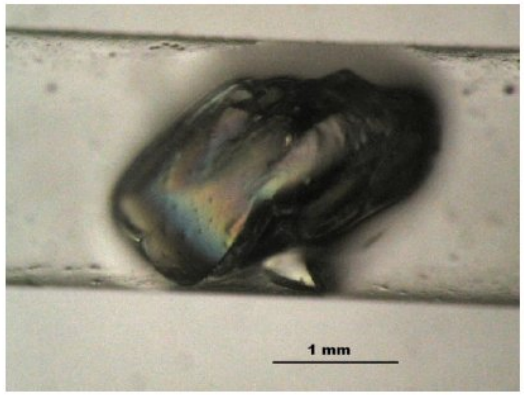
The primary dimer. Schematic representation of the primary dimer. The dimer-dimer interface is oriented towards the observer

Neutron Data Collection on the Quasi-Laue diffractometer









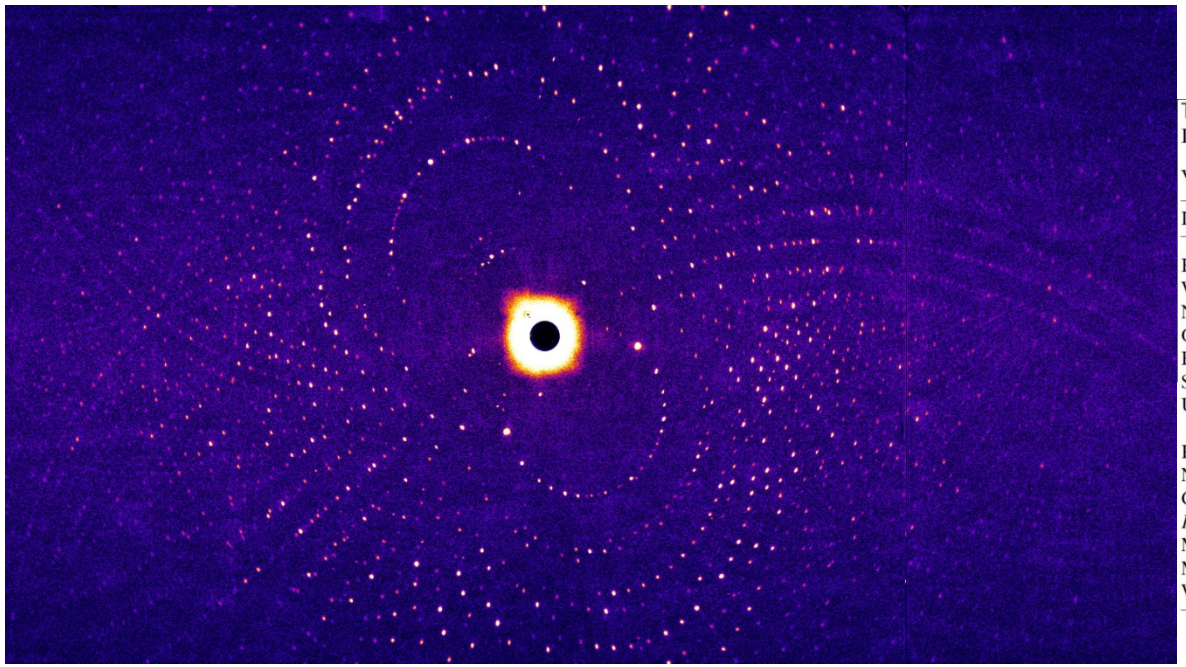


Table 1
Data-collection and structure-solution statistics.

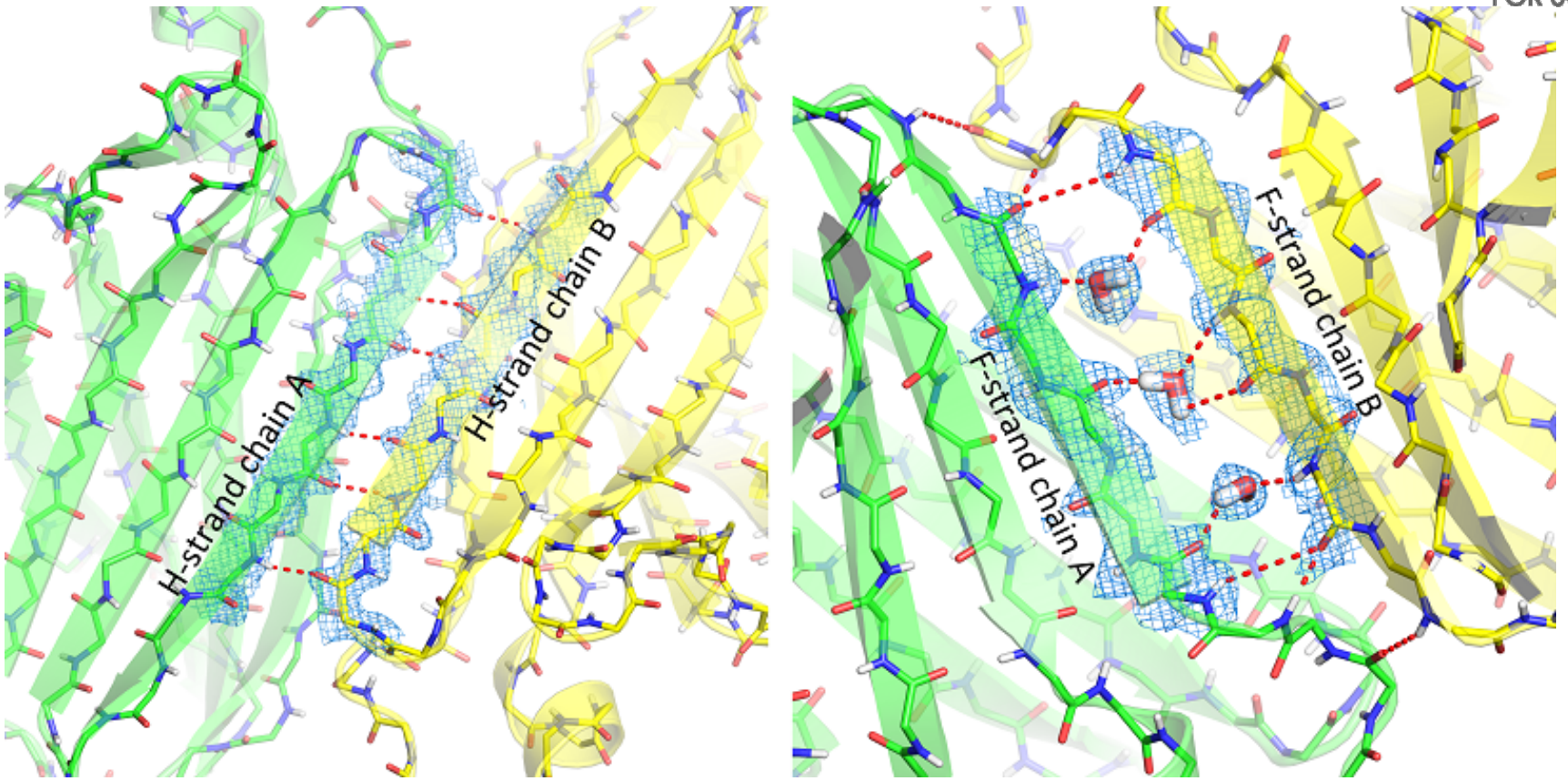
Values in parentheses are for the outer shell.

Data set	Neutron	X-ray
Beamline	LADI-III, ILL	ID23-1, ESRF
Wavelength (Å)	3.24–4.18	0.9791
No. of images	23	180
Oscillation angle (°)	7 [step angle]	0.5
Exposure time per image (s)	18470 [5.13 h per image]	0.1 [beam attenuated to 0.74%]
Space group	$P2_12_12$	$P2_12_12$
Unit-cell parameters (Å)	a = 43.68, b = 86.26, c = 65.72	a = 43.68, b = 86.26, c = 65.72
Resolution range (Å)	43.68–2.00 (2.11–2.00)	52.28–1.85 (1.95–1.85)
No. of unique reflections	13480 (1524)	21718 (3060)
Completeness (%)	78.3 (62.1)	99.4 (97.9)
R_{merge}	0.182 (0.337)	0.093 (0.420)
Multiplicity	5.1 (4.0)	3.53 (3.49)
Mean $I/\sigma(I)$	7.3 (3.4)	7.49 (2.67)
Wilson B factor (Å^2)		19.8

Melina Haupt *et al*

X-ray data: R_{work} = 0.1737 and R_{free} = 0.2083
neutron data: R_{work} = 0.1998 and R_{free} = 0.2579

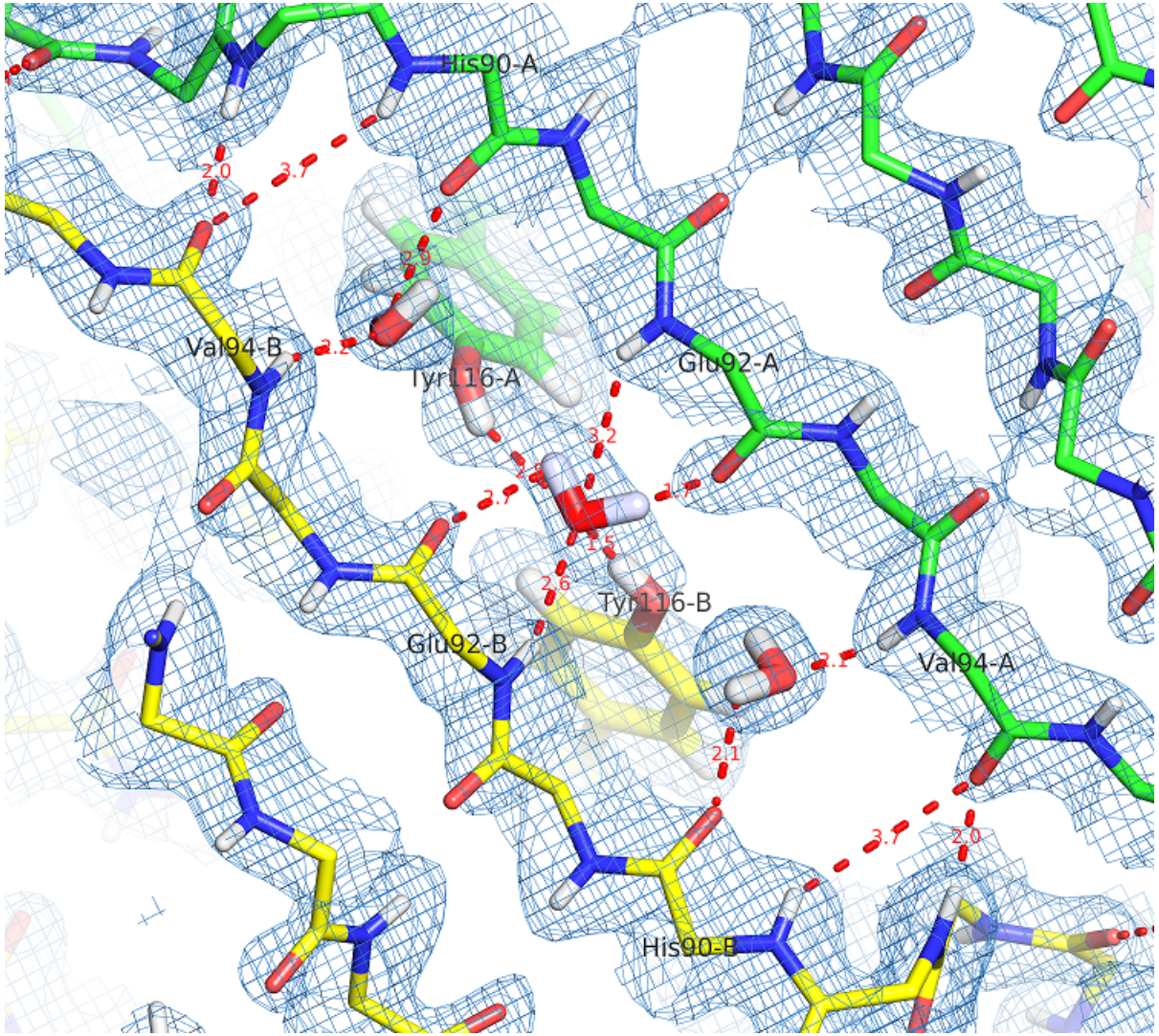
Primary dimer interface



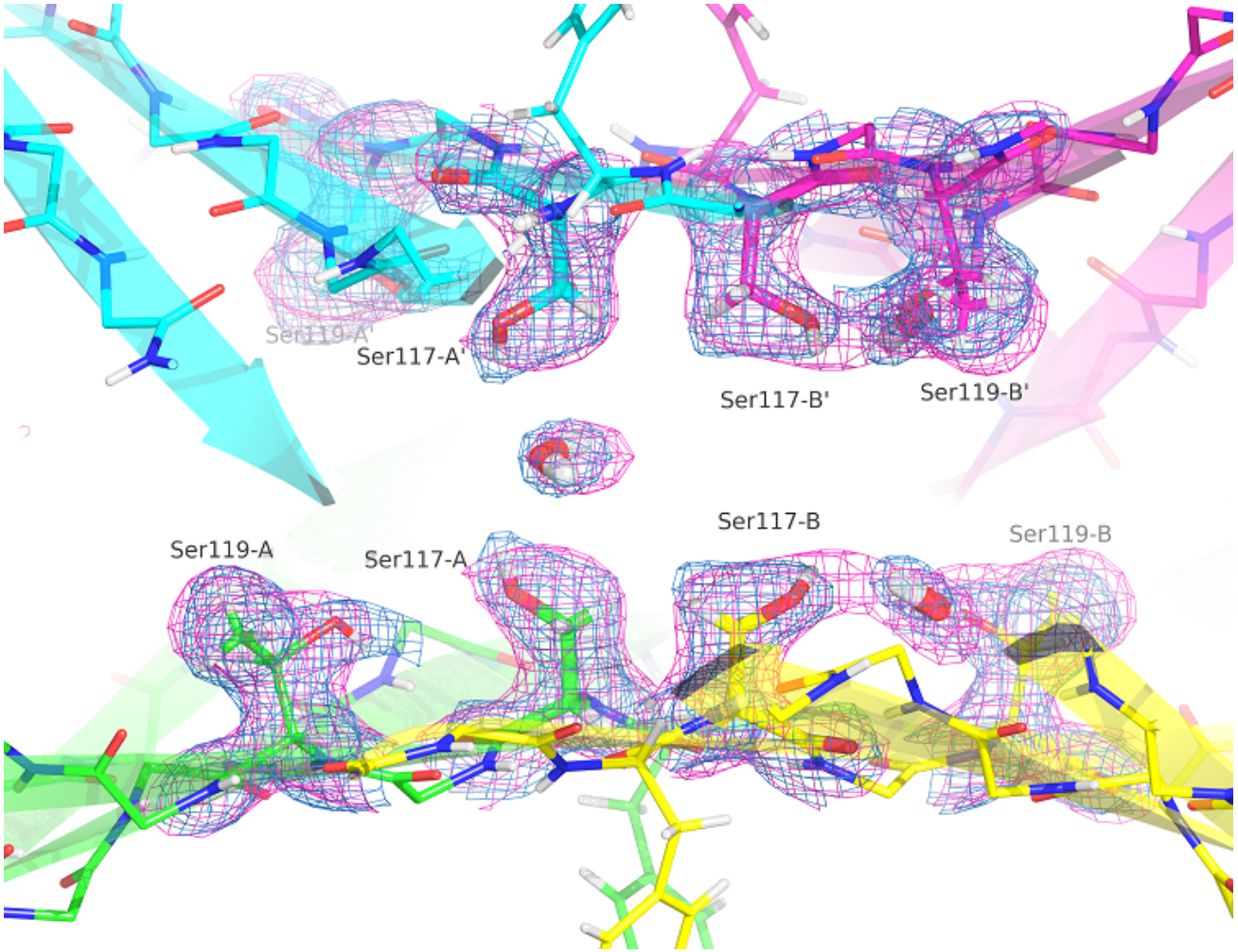
View from binding channel

View from exterior solvent

Left panel shows the continuous beta-sheet formed by the contact of the H-strands from subunit A (green) and subunit B (yellow) hosting the binding channel. The right panel shows the somewhat looser assembly on the solvent-exposed side of the beta-sandwich. Three water molecules are wedged between strands F and F', preventing a smooth circular sequel of the CBEF-sheet.

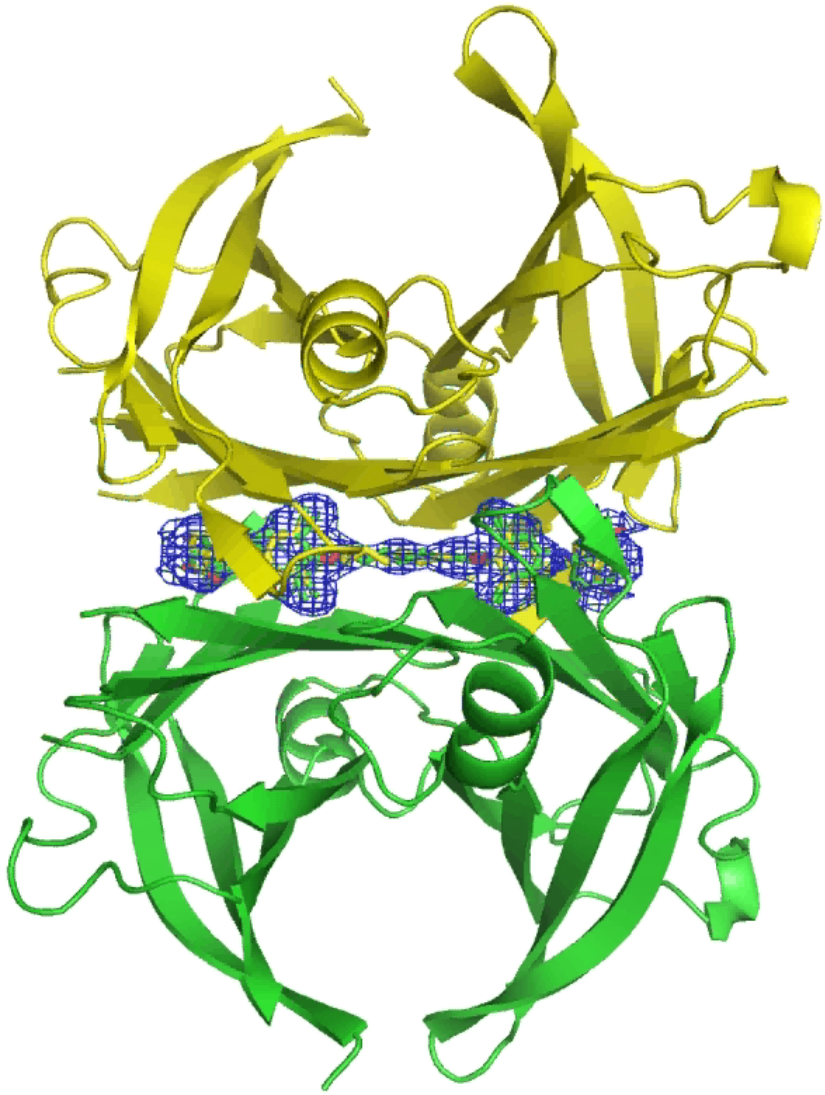


Hydrogen bond networks in the region of the symmetry axis in the primary dimer as revealed by the $2F_o$-F_c neutron diffraction map. The central water molecule is trapped between Tyr116 h-hydroxyl groups and Glu92 backbone CO and NH hydrogen bonds. The water molecule establishes a very close hydrogen bond with Tyr116 in the B-chain, whereas the h-hydroxyl group of the A chain tyrosine points backwards away to avoid steric hindrance.



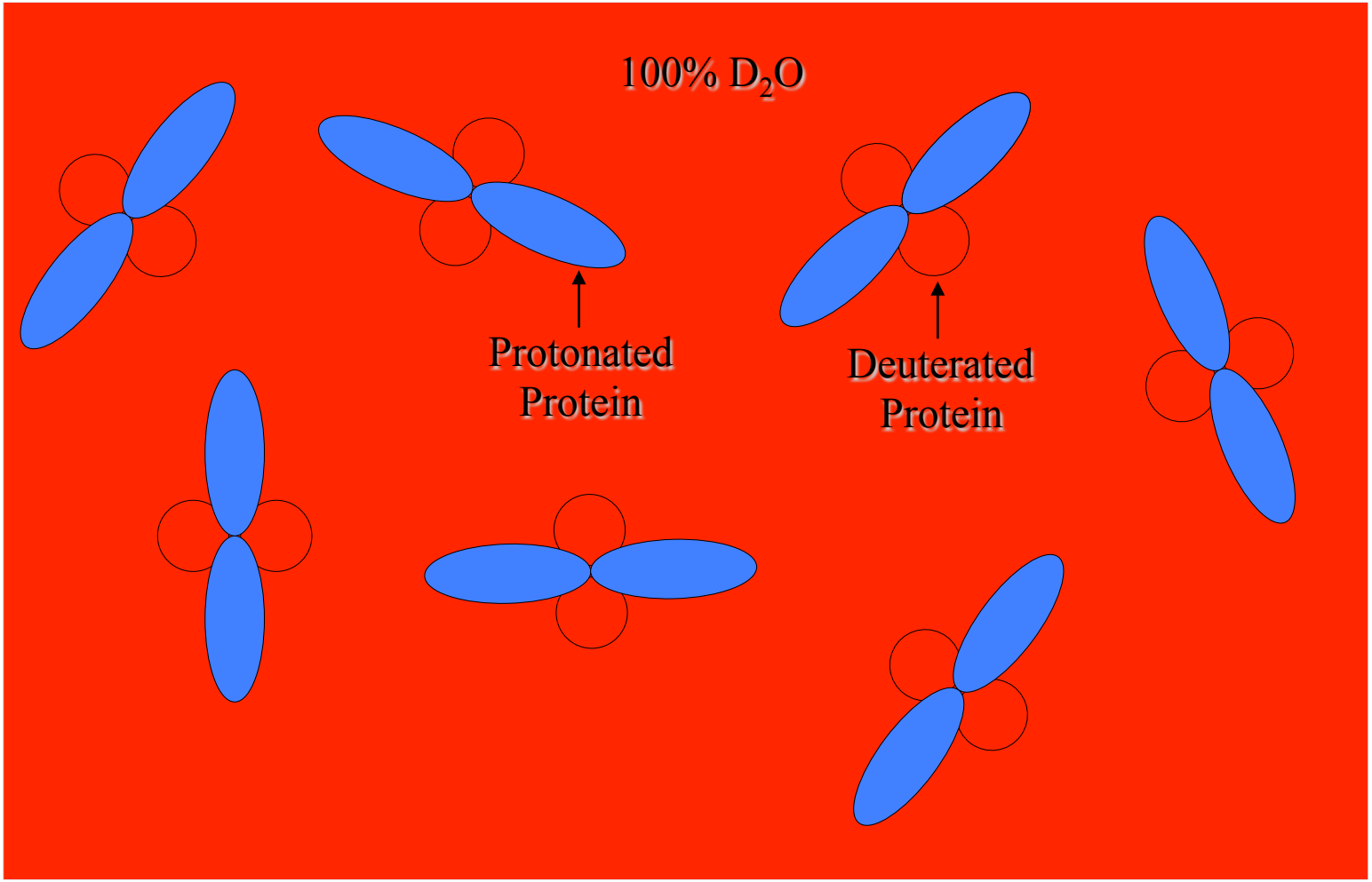
The highly symmetrical centre of the homotetramer. The sidechains of the four Ser117 residues form a barrier between the two hormone binding sites. The $2F_o$-F_c neutron difference map (s = 1.2) shows clearly the different orientation of the g-deuterium atoms of serine residues in chain A and B and the presence of only one water molecule.

TTR structure and ligand binding

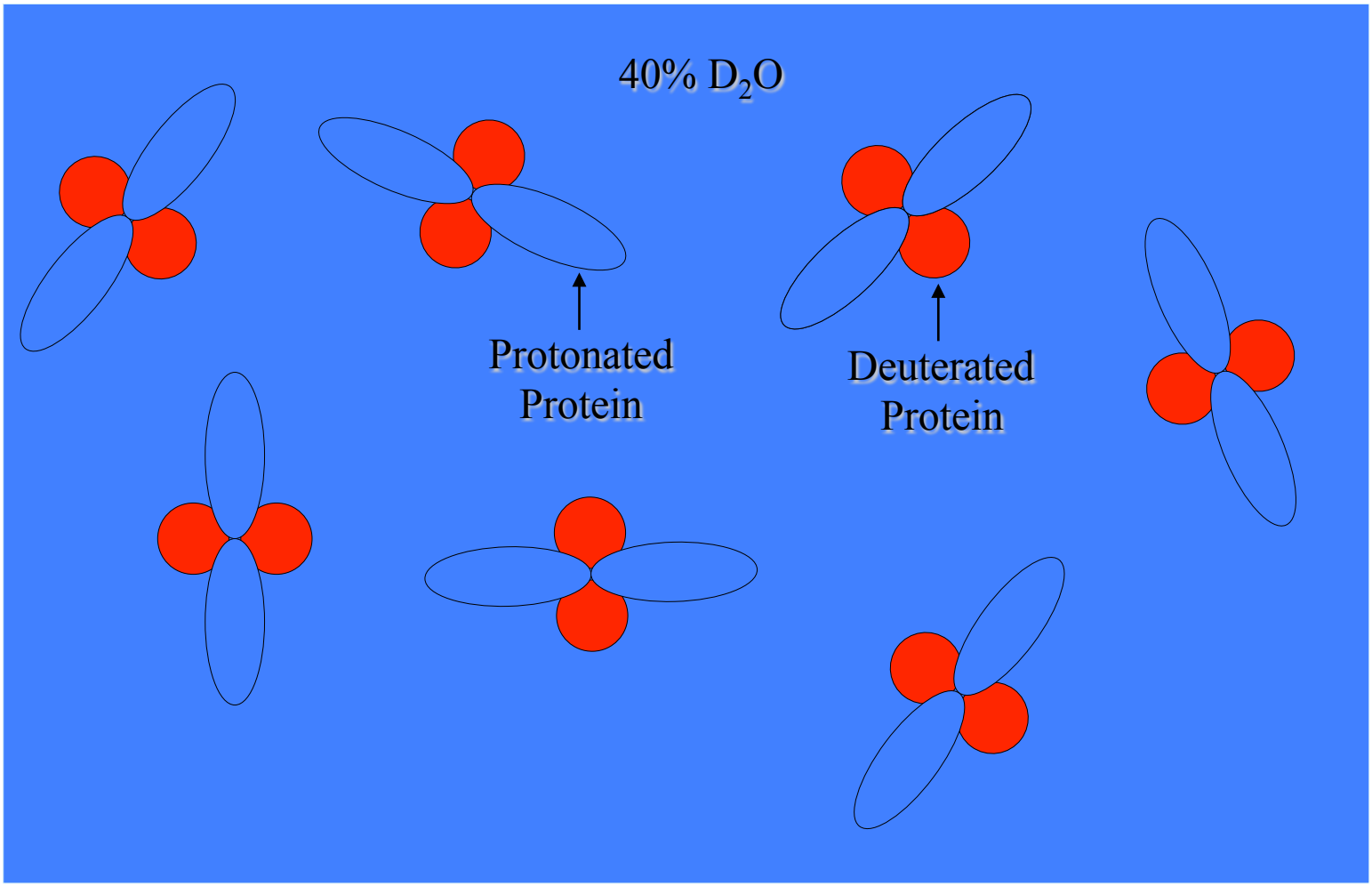


Molecules in solution

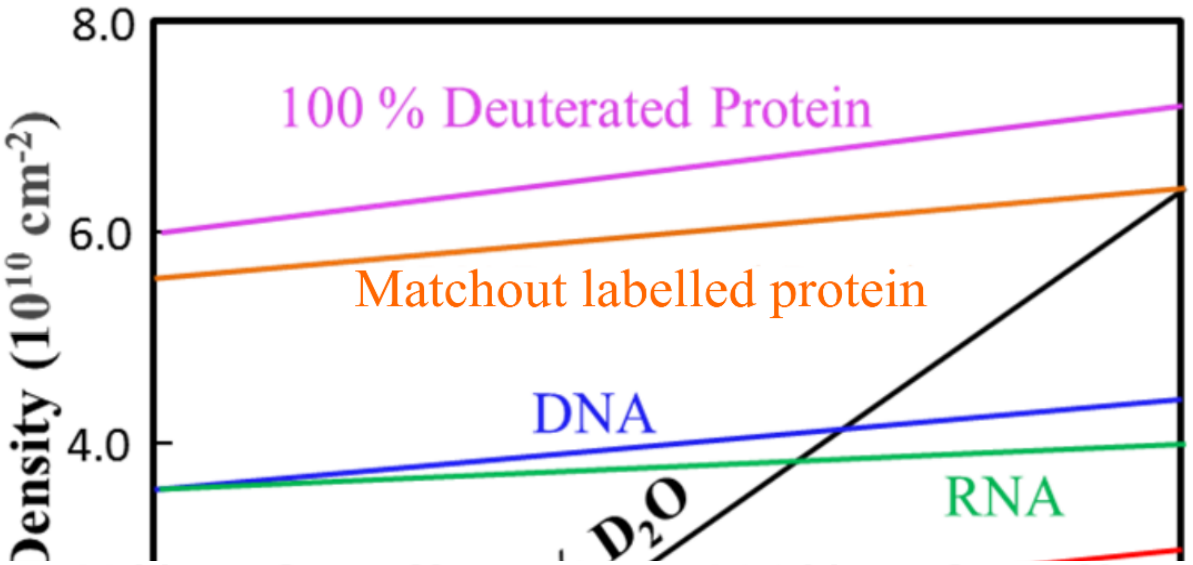
Contrast Variation



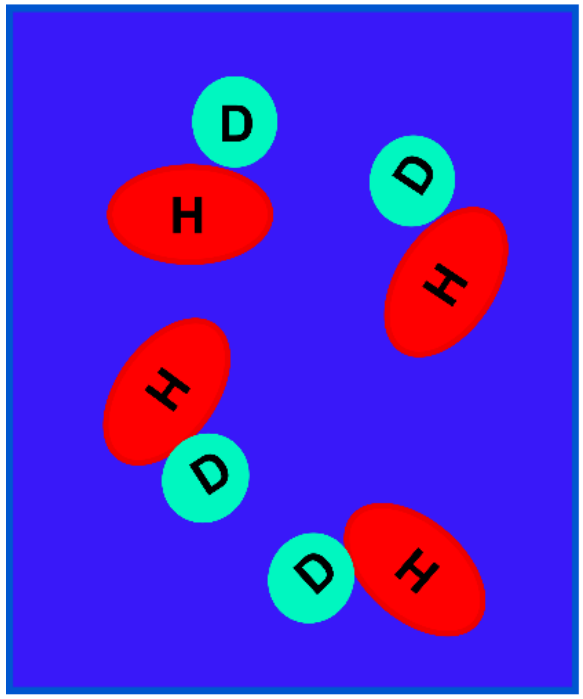
Contrast Variation



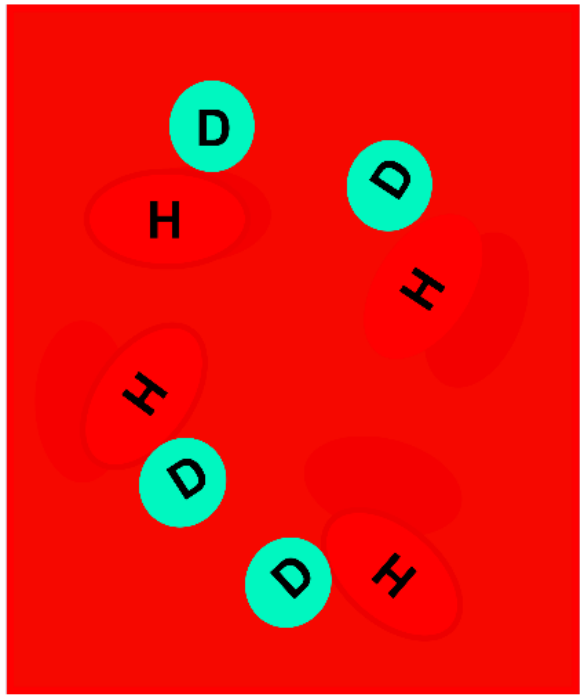
Matchout labelling for SANS



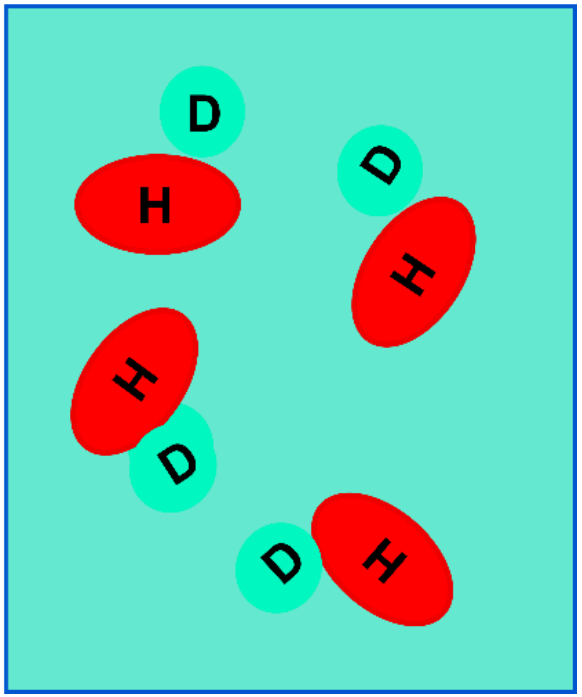
0% D_2O buffer



40% D_2O buffer

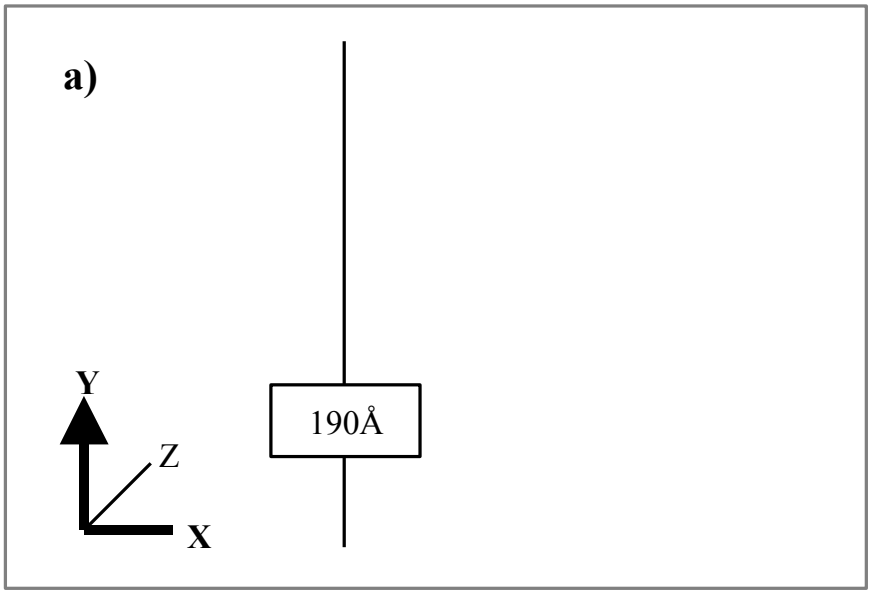


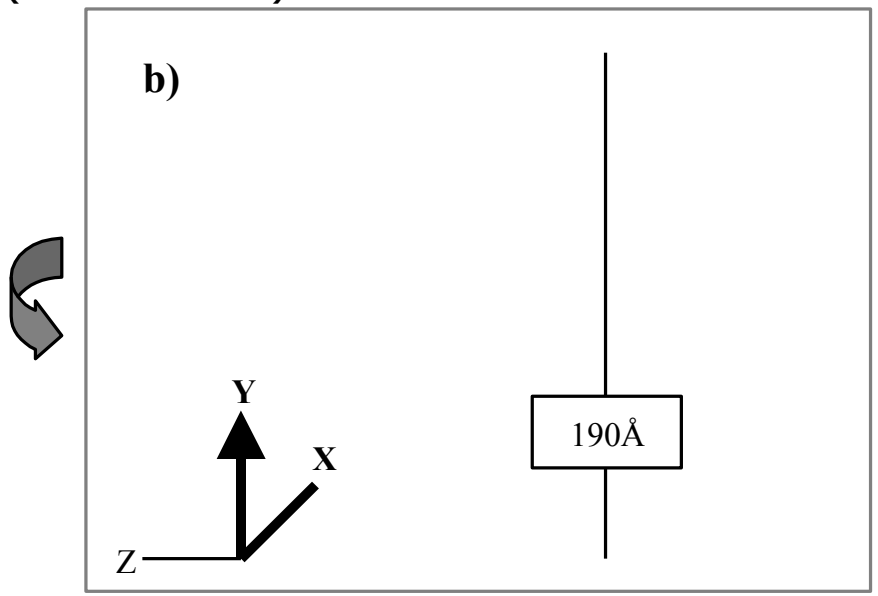
100% D_2O buffer

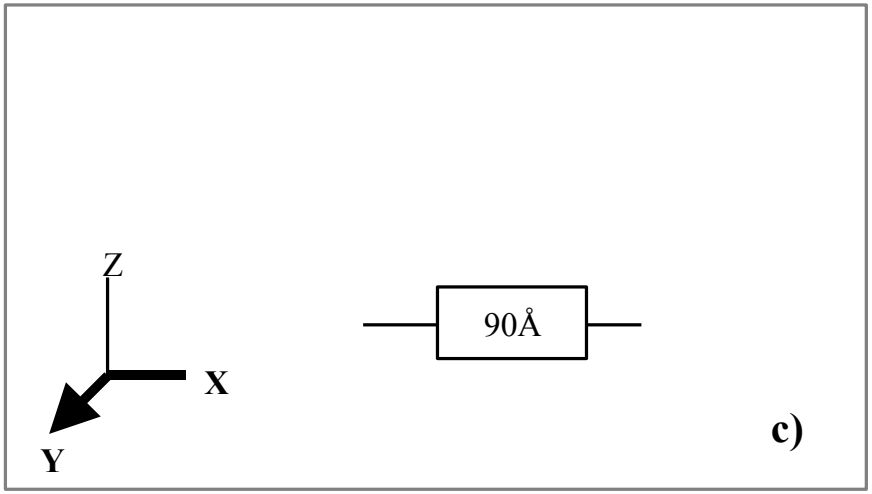


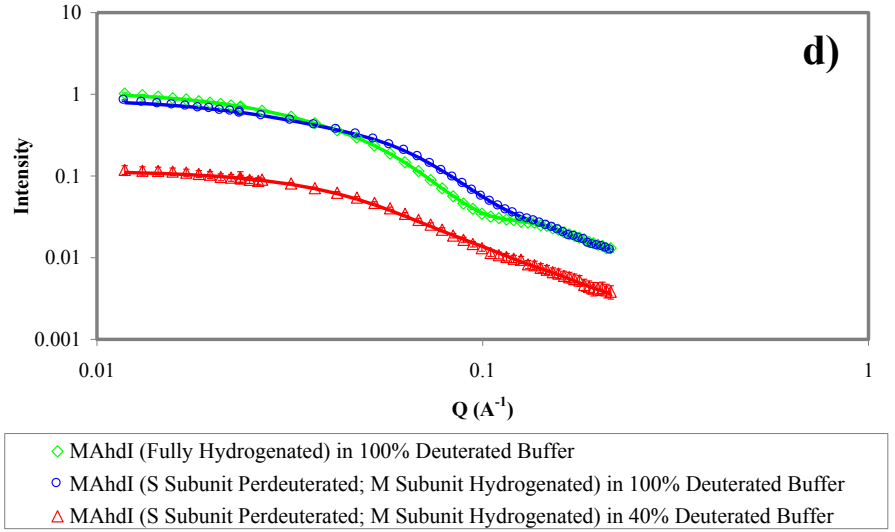
Small-angle neutron scattering studies of a restriction-modification system

Callow *et al*. (*J. Mol. Biol.*)



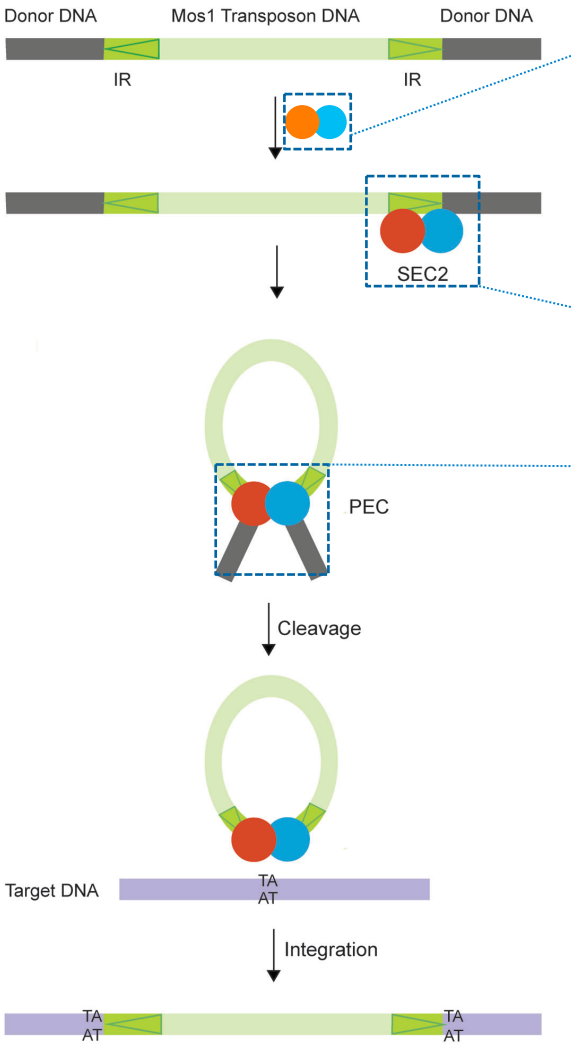




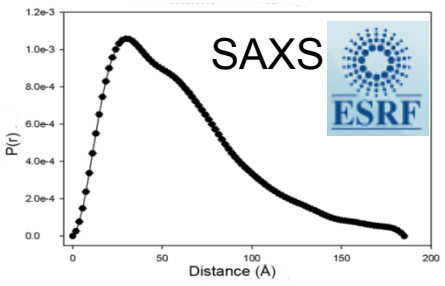


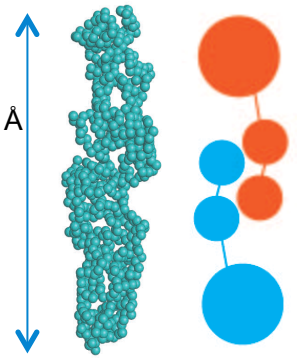
Mechanism of DNA Transposition – using selective deuteration, SANS and SAXS

Cuypers, Callow, Forsyth, Richardson, *Nucleic Acids Res*. **41(3),** 2020–2033 (2013)
Richardson *et al.* *Cell* **138(6)**, 1096-1108 (2009).

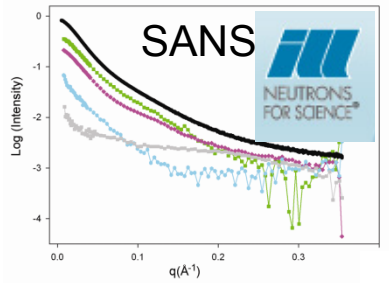


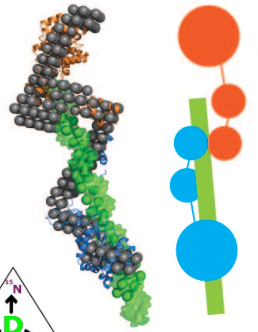
Mos1 Transposase is an elongated, N-terminal dimer in absence of DNA

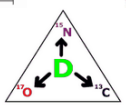


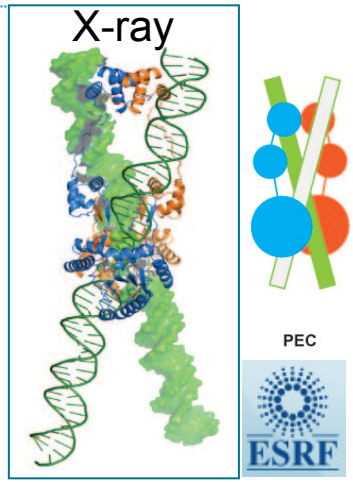


SEC2 - elongated transposase dimer, DNA bound to one monomer.









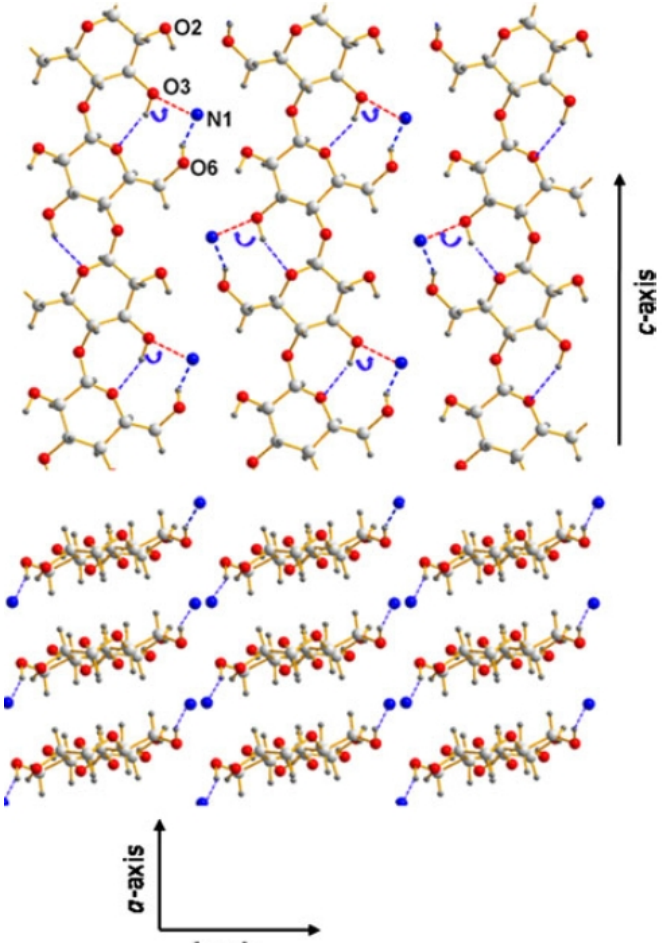
Mos1 PEC has compact crossed architecture

wellcome trust

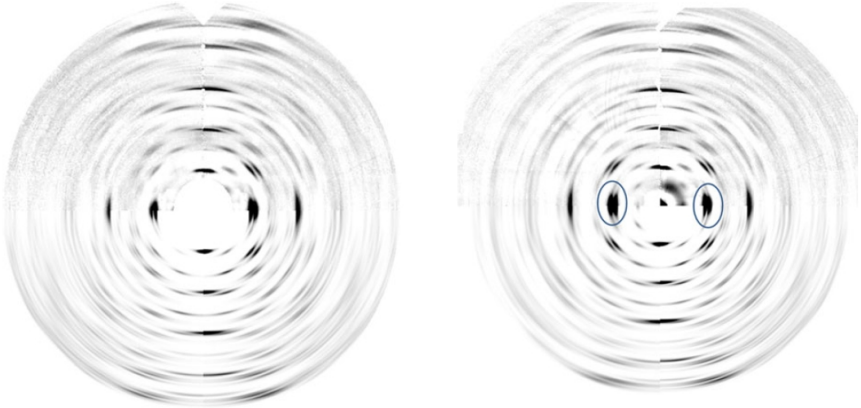
Partially ordered systems

Cellulose – conversion from cellulose I to cellulose III by ammonia treatment

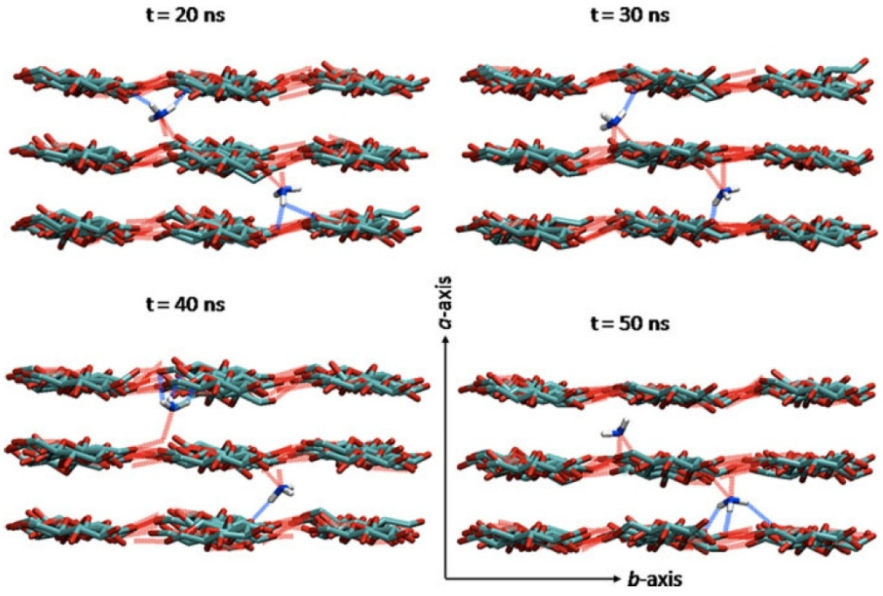
Ammonia treatment used to improve textile properties and also to being explored as a pretreatment for lignocellulosic biomass to improve conversion into sugars for the production of biofuels



Wada *et al*, *Cellulose*



Cellulose neutron fibre diffraction data from ILL's D19 diffractometer



Movement of ammonia molecules and the dynamic formation and breaking of H bonds. H bonds donated by the ammonia molecule are shown in blue, other H bonds in red. Only two ammonia molecules are shown for clarity

Deuteration Laboratory (D-LAB) access

INSTITUT Max von LAUE - Paul LANGEVIN

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http://www.ill.eu

PROPOSAL FOR USE OF THE DEUTERATION FACILITY (D-LAB)

(Please read the attached guidelines before submitting the completed proposal form to the above address)

Experiment title:	*Proposal number* *(to be completed by ILL)*
Proposer *(to whom correspondence will be addressed)* Full name and address:	Phone: Fax: Email: New neutron user? ☐ Yes ☐ No New ILL user? ☐ Yes ☐ No
Co-proposers ***mark with an asterisk*** *the main proposer in each laboratory)* Full name and address *(if different from above)*:	Phone/fax/email:

- Simple & rapid electronic peer review access, analogous to beam time applications
- Available to all ILL member state users regardless of facility where neutrons will be used. (users expected to cover cost of consumables used in biosynthesis if expt is not at ILL or if grant funding has been awarded for the work)
- Users can either take delivery of deuterated cell paste and purify at home, or purify in D-LAB.