Neutron protein crystallography with LADI: recent results, technical advances and perspectives

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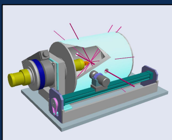
NEUTRONS FOR SCIENCE

Neutron Protein Crystallography

Neutron protein crystallography allows the direct visualisation of hydrogen/deuterium atom positions in biological structures even at the typical resolutions of most protein structure determinations (1.5 – 2.0 Å).

		H	D	C	N	O	Mg
X-Ray	f(q=0) (e-)	1	1	6	7	8	12
Neutron	bcoh (fm)	-3.74	+6.67	+6.65	+9.36	+5.81	+5.37
	Sinc (barns)	80.27	2.05	0	0.49	0	0

LADI



Detector Cylinder covered with image plates
Neutron image plate Gd_2O_3 doped BaF(Br.I):Eu^{2+}
Configuration cylinder camera
Radius 159.19 mm; **Length** 400 mm
Active area 800 ´ 400 mm²; 4 400´200 mm²-NIPs
Angle subtended 144° in T, 52° in v
Pixel size 200 x 200 μm²

Protein	Unit cell volume v_0 (Å³)	Crystal volume V (mm³)	Resolution (Å)	Reference
Lysozyme	228.999	6.00	2.0	Niimura *et al.*, 1997
Lysozyme	22.771	2.00	1.7	Bon *et al.*, 1999
Cubic ConA	479.534	15.00	2.4	Habash *et al.*, 2000
Endothiapepsin	158.139	3.50	2.0	Coates *et al.*, 2001
Trp repressor	93.420	1.12	2.1	Daniels *et al.*, 2003
Sacch. free ConA	236.469	1.6/5.6	2.5	Blakeley et *al*, 2004
Rubredoxin	53.300	1.40	1.7	Blakeley *et al.*
Lysozyme	22.771	4.00	1.6	Meilleur *et al.*
Xylose Isomerase	463.424	4.00	2.2	Meilleur *et al.*
Aldose Reductase	161.535	0.15	2.2	Podjarny *et al.*
DHFR	518.136	0.30	2.2	Bennett *et al.*
Urate Oxidase	407.036	1.8	2.1	Bonnete *et al.*

15 K (red) — Perdeuterated (ILL/EMBL Deuteration Laboratory) (green)

Perdeuteration: Aldose reductase

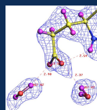
I. Hazemann, A. Mitschler, A. Podjarny (IGBMC); M.-T. Dauvergne (EMBL); D. Myles (ORNL)

Human-AR belongs to the aldo-keto reductase family and is implicated in diabetic complications.

The large hydrogen incoherent scattering background significantly reduces the signal-to-noise ratio. This problem can be fully overcome by deuterating the sample by preparing completely (per)deuterated proteins. Neutron diffraction data have been collected to 2.2 Å resolution from a small (0.15mm³) crystal of perdeuterated human Aldose Reductase (h-AR) in order to help determine the protonation state of the activer site residues.

	MW (kDa)	Crystal	Space Group Unit Cell param.	Resolution (Å)
hAR	36	Perdeut. 0.15 mm³	$P2_1$ a = 50.1Å, b = 67.3 Å, c = 48.0 Å, β = 92.5°	2.2

Fully deuterated crystal of h-AR(D) used for data collection (1.0 x 0.67 x 0.23mm³)



Perdeuteration also helps the interpretation of the neutron map, preventing map cancellation.

Residue Glu 53 + 2 water molecules.

Large unit cell systems: XI

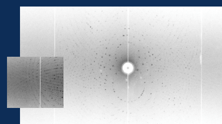
E. Snell (Hauptman-Woodward Medical Research Institute); R. Judge (Abbott Laboratories); D. Myles (ORNL)

DHFR

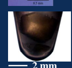
B. Bennett, C. Dealwis (University of Tennessee); D. Myles (ORNL)

Dihydrofolate reductases (DHFRs) are conserved across species from *Archaea* to the higher mammals and are critical for multiple metabolic pathways, including pyrimidine and amino acid biosynthesis as well as other processes involving one-carbon transfer reactions.

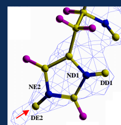
D-xylose ketol-isomerase (XI) is an enzyme that catalyses the reversible isomerisation between aldose and ketose. Xylose isomerase catalyses the first reaction in the catabolism of D-xylose, but is also able to convert D-glucose to D-fructose.



Typical neutron quasi-Laue diffraction pattern for Xylose isomerase.

		MW (kDa)	Crystal	Space Group Unit Cell param.	Resolution (Å)
	DHFR	18	D_2O-grown 0.3 mm³	$P6_1$ a = b = 90.9 Å, c = 72.4 Å	2.2
	XI	40	D_2O-grown 5 mm³	I222 a = 92.8 Å, b = 98.4 Å, c = 101.5 Å	2.0

2 mm



Neutron analysis of XI allowed direct observation of His53 NE2 protonation state. This result validates the mechanism suggested by an ultra high-resolution X-ray analysis (Fenn et al., 2004).

LADI-III

- *routinely study protein crystals of volume <1mm³*
- *reduce the exposure time*
- *study larger systems*

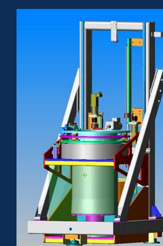
Phase I

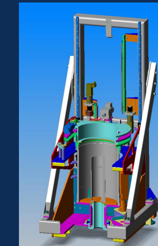
- Diffractometer upgrading

Image plates and readout system inside the drum (measurements showed a *3-fold gain* in neutron detection compared to LADI)

Delivery: May 2006

- H142 guide refit (gain in flux: 30%)





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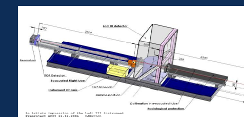
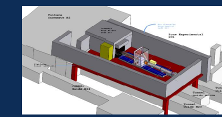
Beam height adjustable from 630 to 1400 mm
Diameter 400 mm; Length 450 mm
Active area 1200 × 450 mm²; 1.5 800×450 mm²-NIPs
Angle subtended 172° in T, 49° in v

Phase II

- Focussing optic design to concentrate the beam at the sample (1-4 fold)

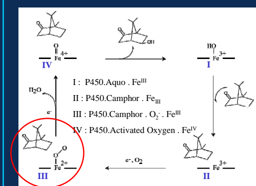
- Instrument relocation to a higher intensity beam (2-4 fold)



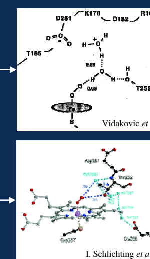


Perdeuteration / Intermediate freeze-trapping: Cytochrome P450cam

Enzymatic mechanism question:

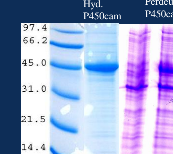


?

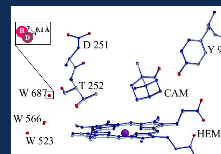


Production of perdeuterated P450cam:

- Fermentation in bioreactors using perdeuterated minimal media.
- High Cell Density Cultures.
- Purification protocol of (per)deuterated P450cam similar to hydrogenated condition.
- Deuteration level: 98% (MS)



Perdeuterated structure validation: X-ray analysis of perdeuterated P450cam



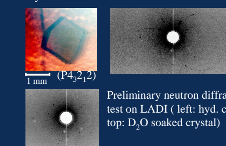
H-bond (Cam-Tyr96):
Hyd. P450cam: 2.67 Å
Perd. P450cam: 2.75 Å

•Conformation of the key residues AsP251 and Thr252 conserved.

•Water molecule structure conserved.

Growth of large crystal:

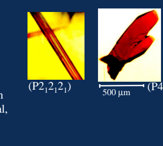
Hyd. P450cam:



(P4₃2₁2) 1 mm

Preliminary neutron diffraction test on LADI (left: hyd. crystal, top: D_2O soaked crystal)

Perdeuterated P450cam:



($P2_12_12_1$) 500 µm ($P4_32_12$)

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LADI : F. Dauvergne (EMBL-Grenoble); D. Myles (ORNL); Clive Wilkinson.

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