Time-of-flight Neutron Diffraction as an Aid to Elucidating Enzyme Mechanisms: D-Xylose Isomerase

Jenny P. Glusker, Amy Katz, H. L. Carrell, Xinmin Li, B. Leif Hanson, Paul Langan, Benno P. Schoenborn, Gerard J. Bunick

Work supported by NIH grants CA-10925, CA-06927, GM-29818 and NASA NAG8-1826, the Office of Science, the Office of Biological and Environmental Research of the US Department of Energy.

FOX CHASE CANCER CENTER

Los Alamos

OAK RIDGE NATIONAL LABORATORY
U. S. DEPARTMENT OF ENERGY

UT-BATTELLE

What information can be obtained from neutron diffraction?

- **Location of hydrogen atom positions in proteins, nucleic acids and water molecules at modest resolution (~ 2 Å).**
- **Protonation states of active-site residues since these play critical roles in enzyme mechanisms.**
- **Information on labile and mobile hydrogen atoms since these indicate rigid or flexible interatomic interactions in the structure.**
- **The locations of hydrogen atoms in hydrogen bonds, particularly those connecting water with biological macromolecules or with other water molecules.**
- **Estimation of the local pH, for example in the active site.**
- **Multiple conformations of proton-containing groups may be detectable by neutron diffraction studies.**

What information is available from neutron- but not X-ray diffraction?

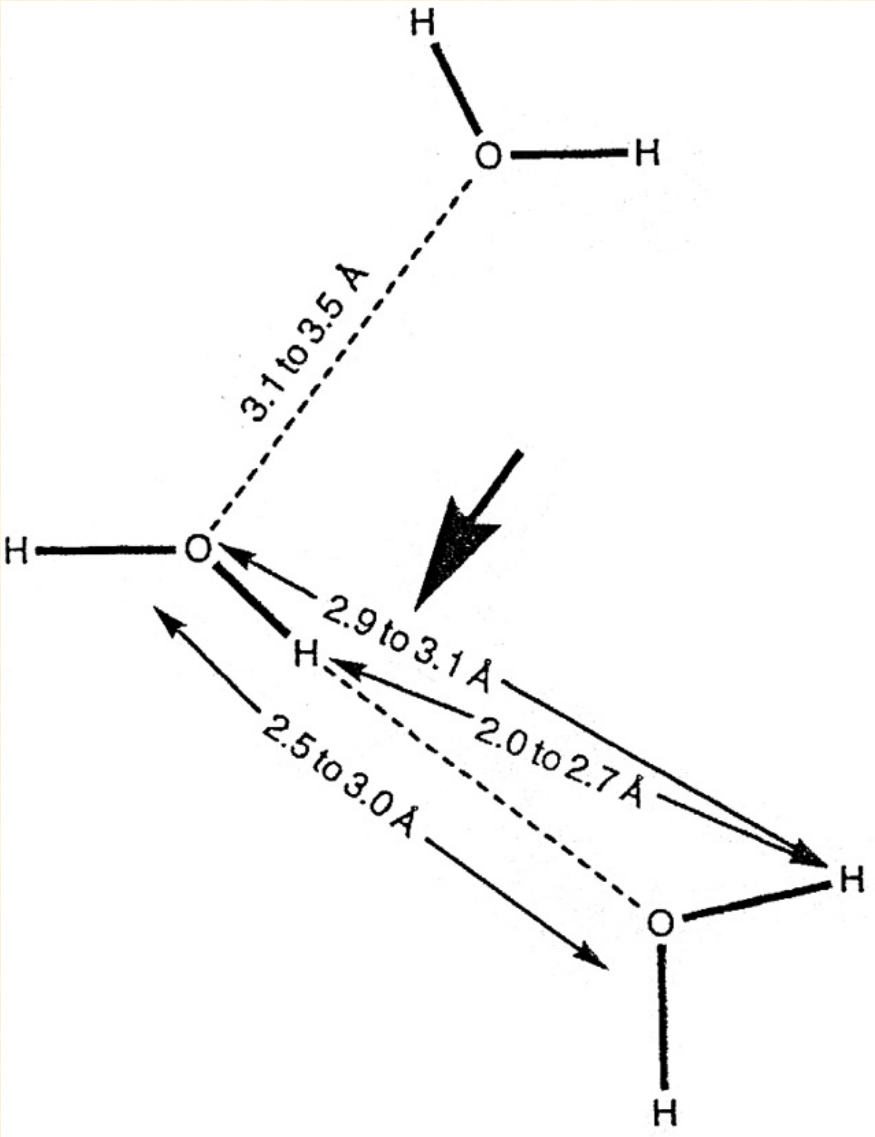
- **Water, hydrogen bonding networks (where are the H atoms?)**
- **Lysine, arginine, ammonium groups (location of H's on N?)**
- **Histidine ring nitrogens (is the histidine doubly, singly or not protonated?)**
- **Threonine, serine, tyrosine hydroxyl groups (how does the H of the OH group lie?)**
- **Cysteine thiol group (how does the H of the SH group lie, is the H there or is the thiol ionized?)**

Neutron versus X-ray scattering

•Element	neutron (fm)*	X ray (electrons)
•H	- 3.8	1
•D	6.5	1
•C	6.6	6
•N	9.4	7
•O	5.8	8
•Mg	5.3	12
•Ca	4.6	20
•Mn	- 3.6	25
•Fe	9.5	26
•Ni	10.0	28
•Zn	5.6	30

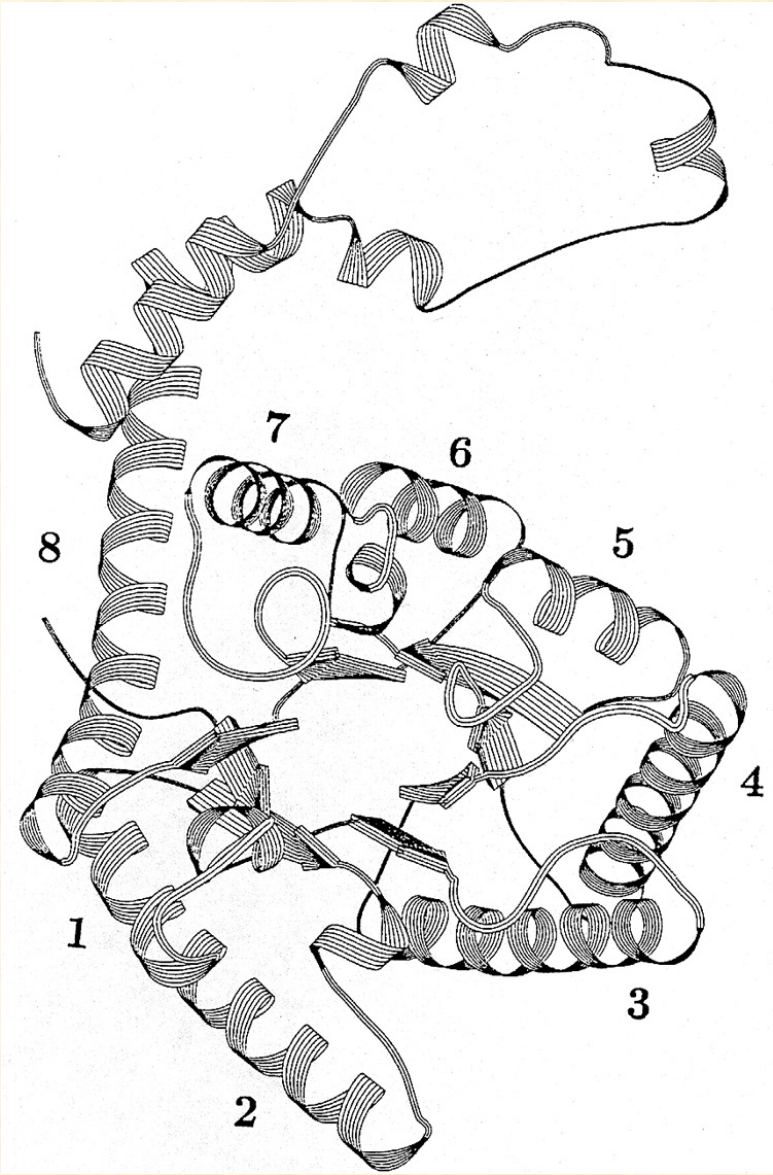
* fm = neutron scattering length in femtometers (10^{-15} m)

Interactions around water molecules from neutron diffraction studies



Savage and Finney, Nature 322, 717 (1986)

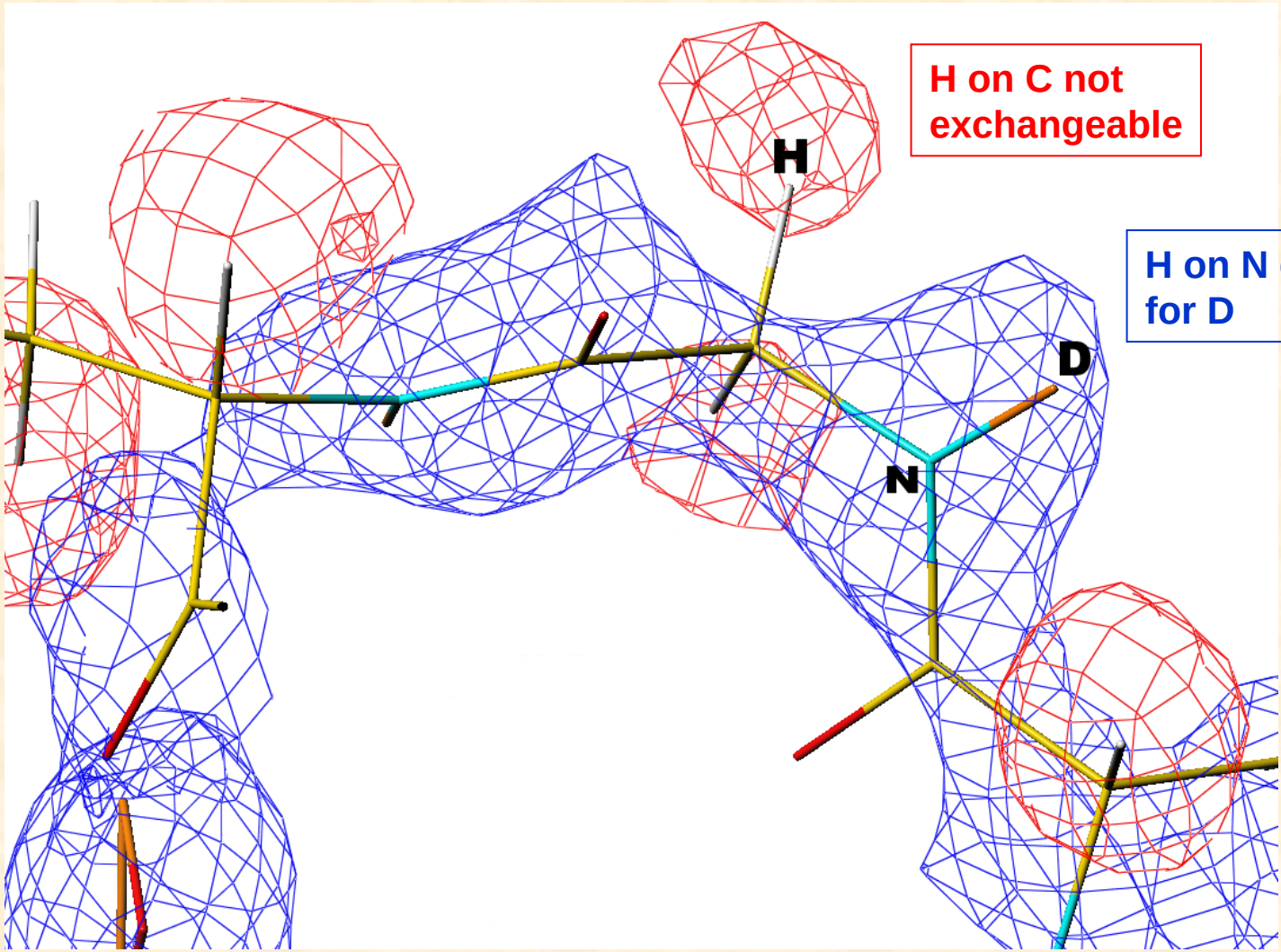
D-xylose isomerase, an eightfold (β/α) barrel



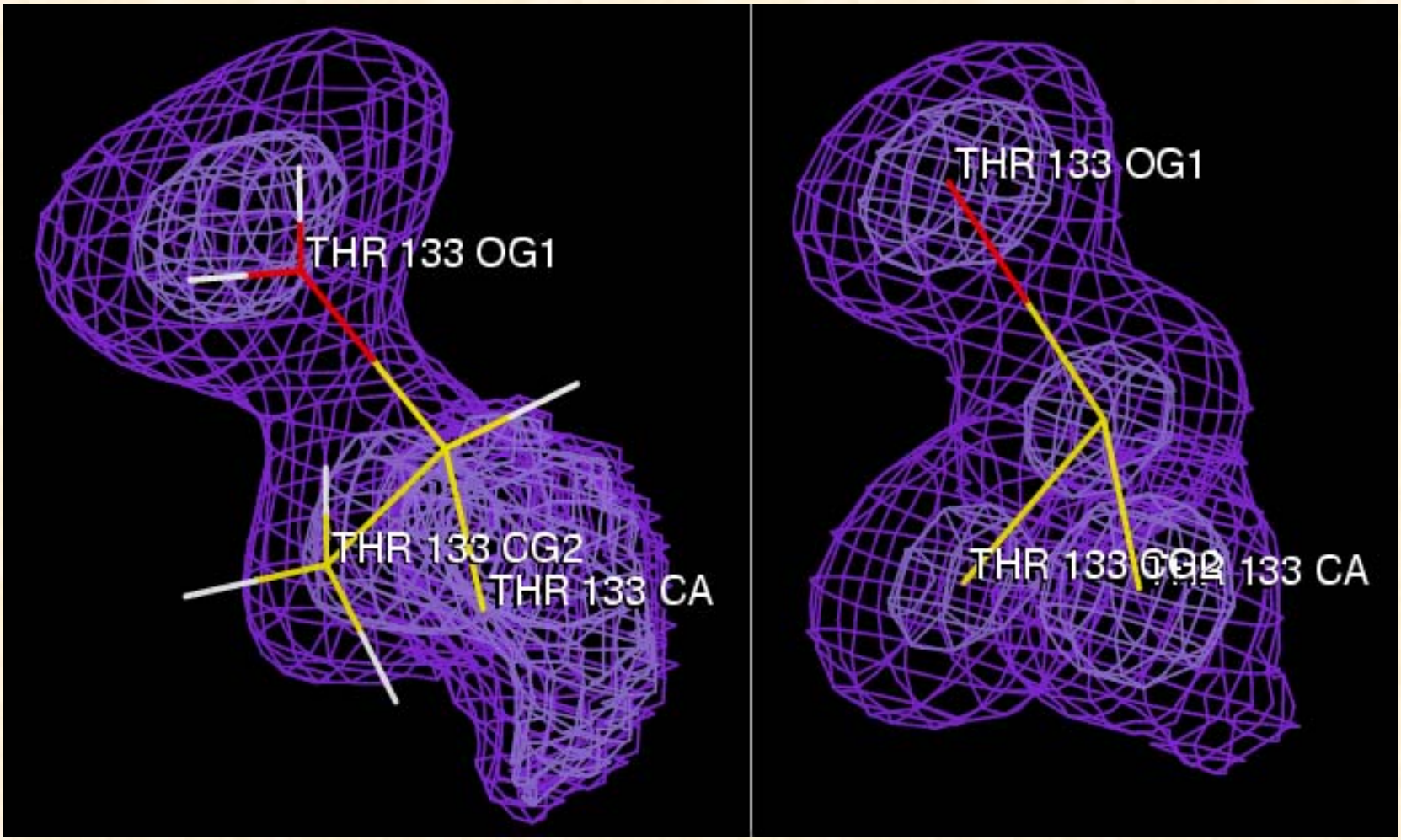
Carrell, Rubin, Hurley, Glusker
J. Biol. Chem. 259, 3230 (1984)

Interpreting neutron maps

2.0 Å neutron map, Blue positive, Red negative. (2 σ contour)

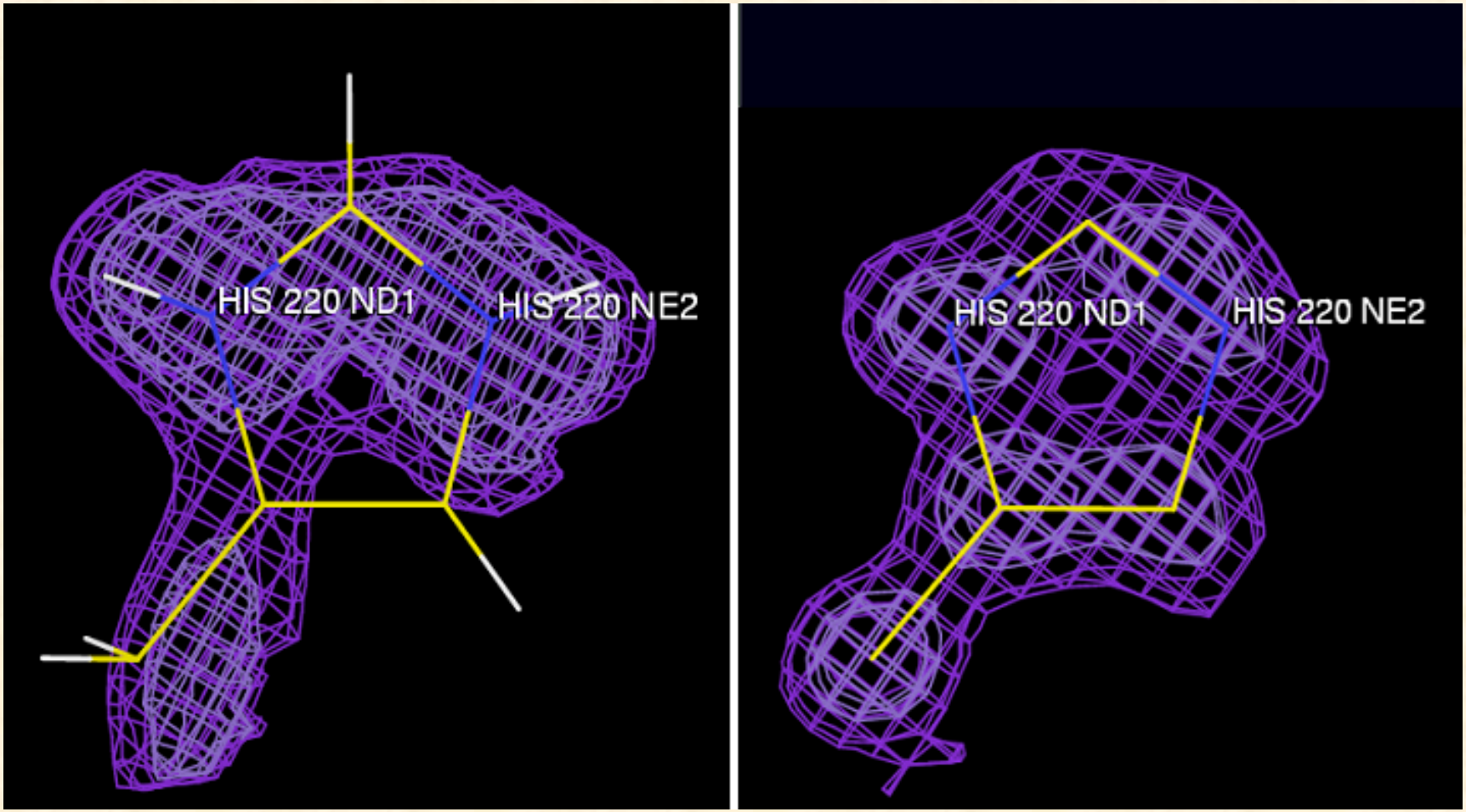


Neutron density shows multiple positions for OG1 proton whereas proton is unseen in electron density



Thr133 - neutron **Thr133 – X ray**

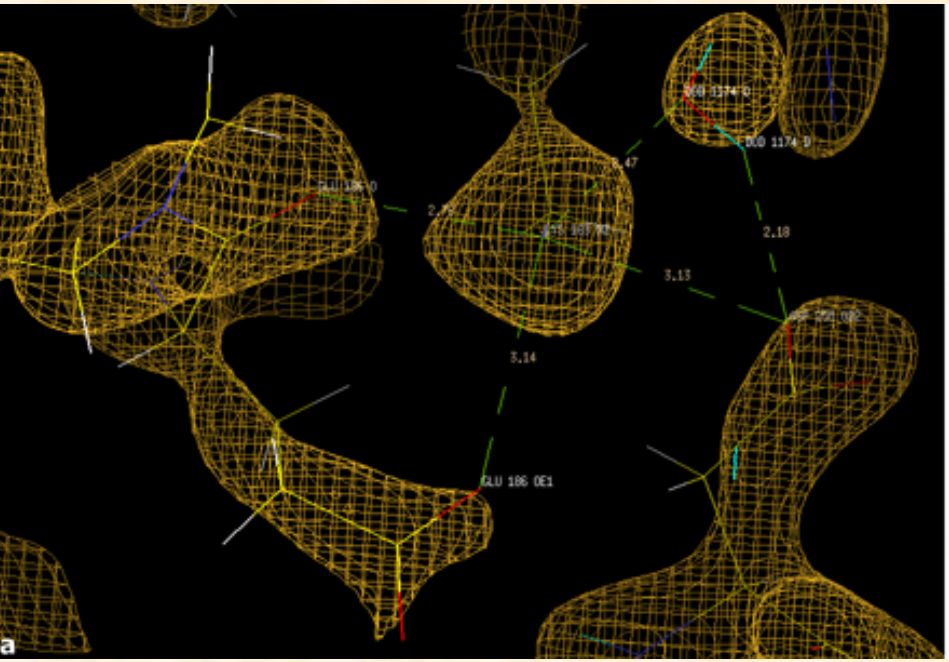
Doubly protonated histidine – neutron versus X ray

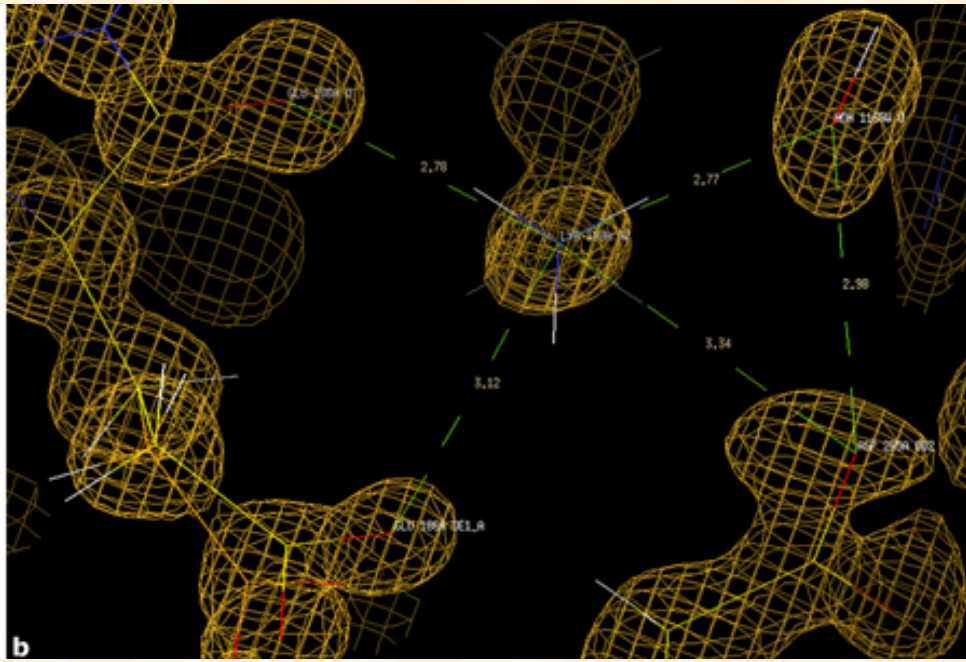


His220 - neutron

His220 – X ray

Comparison of neutron (1.8 Å) and electron density (0.94 Å)

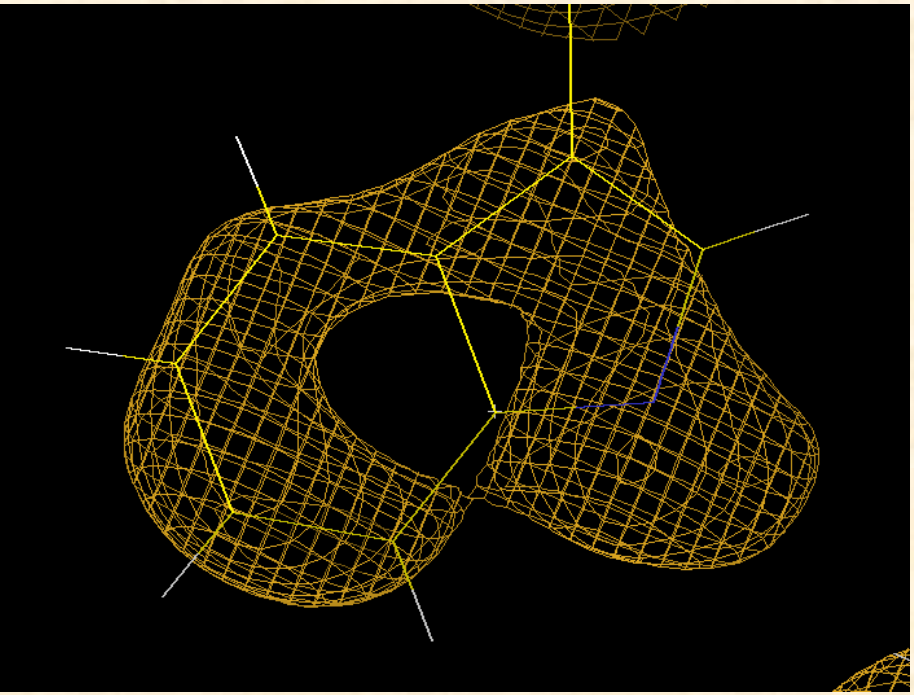




Lys183 - neutron

Lys183 – X ray

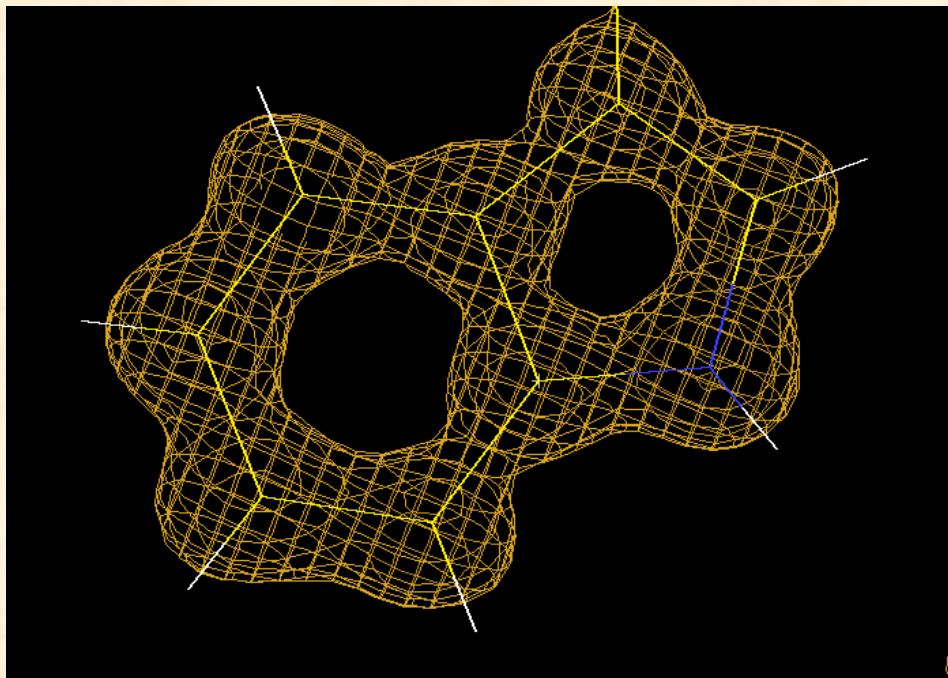
Comparison of neutron (1.8 Å) and electron density (0.94 Å)



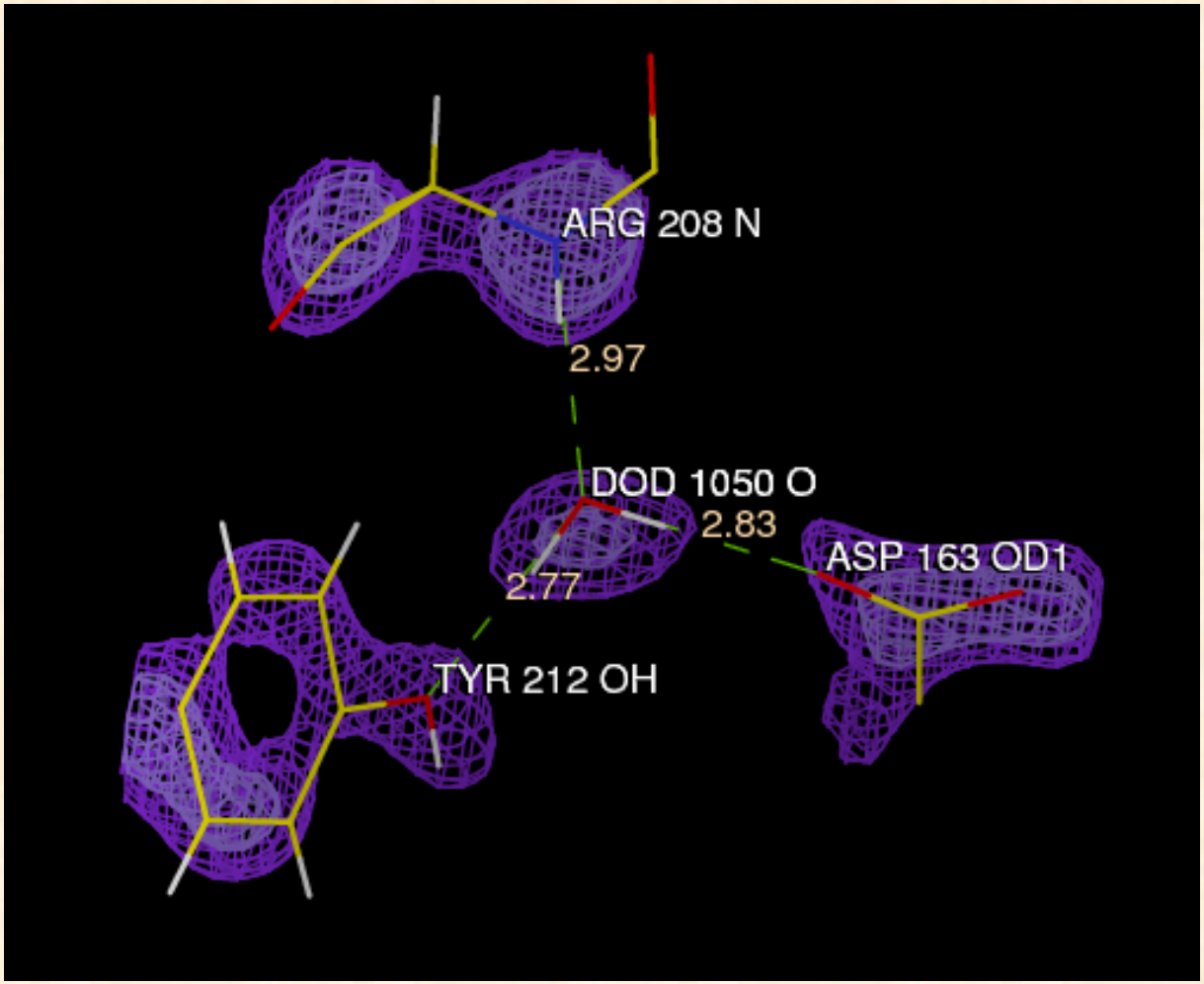
Trp137 - neutron

Note the H on the N in the neutron map.

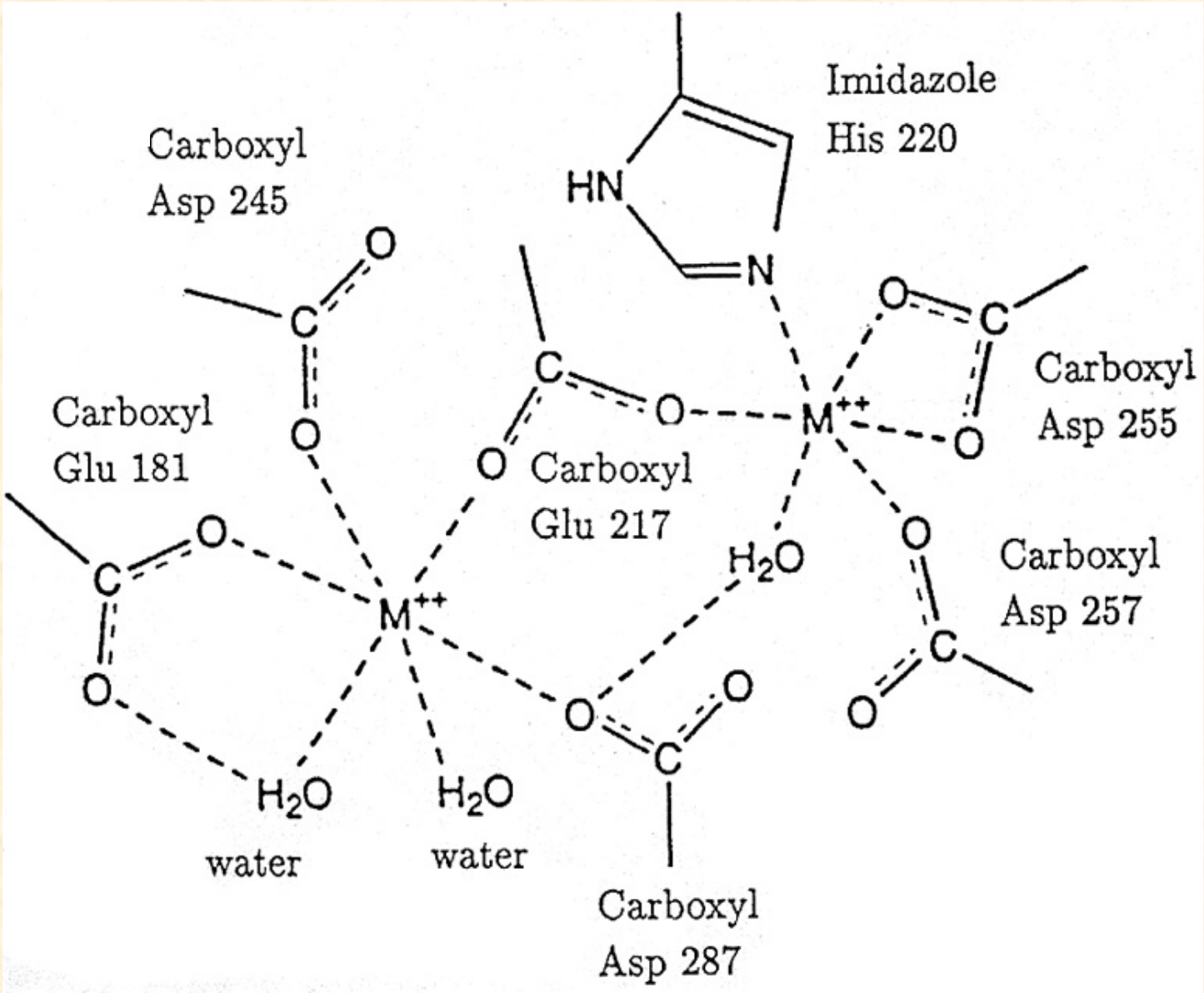
Trp137 – X ray

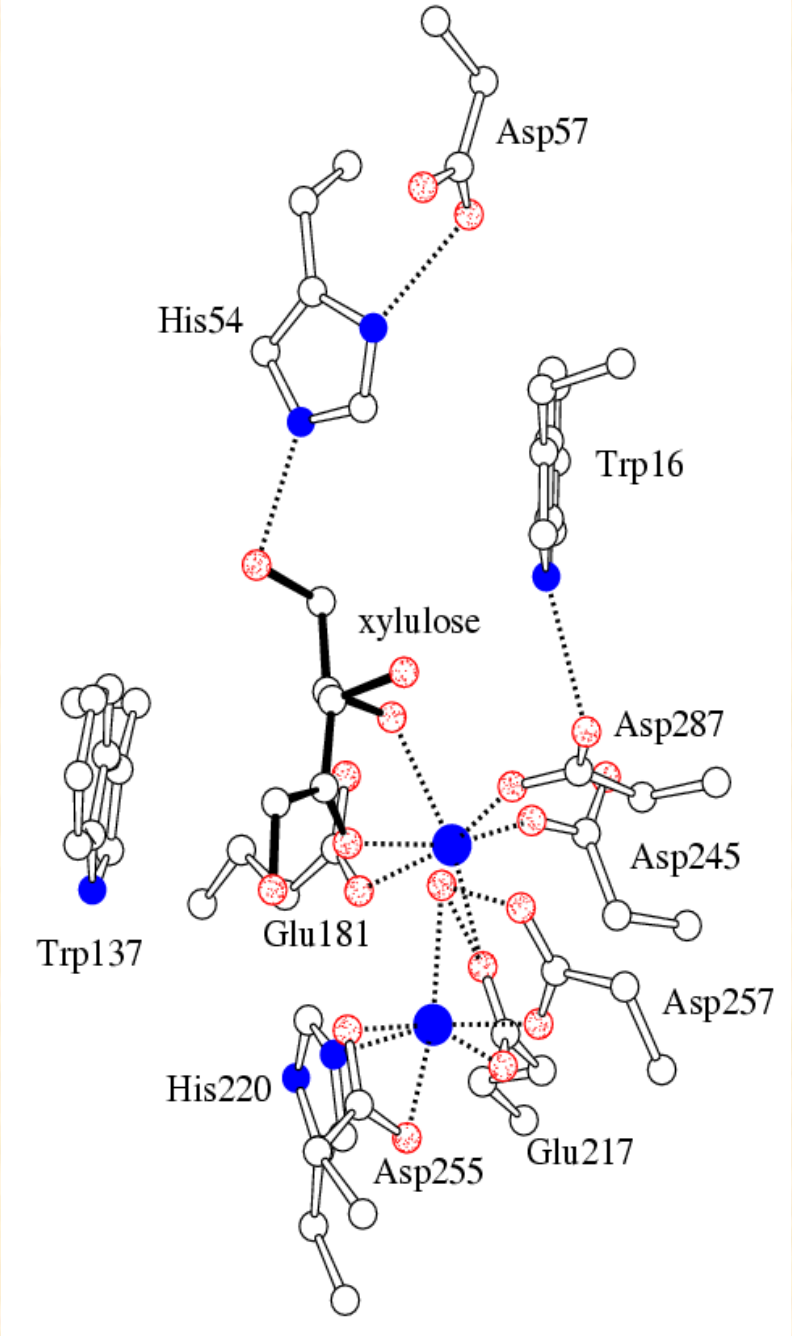


Neutron example of a water molecule originally reported (X ray) to be a metal ion



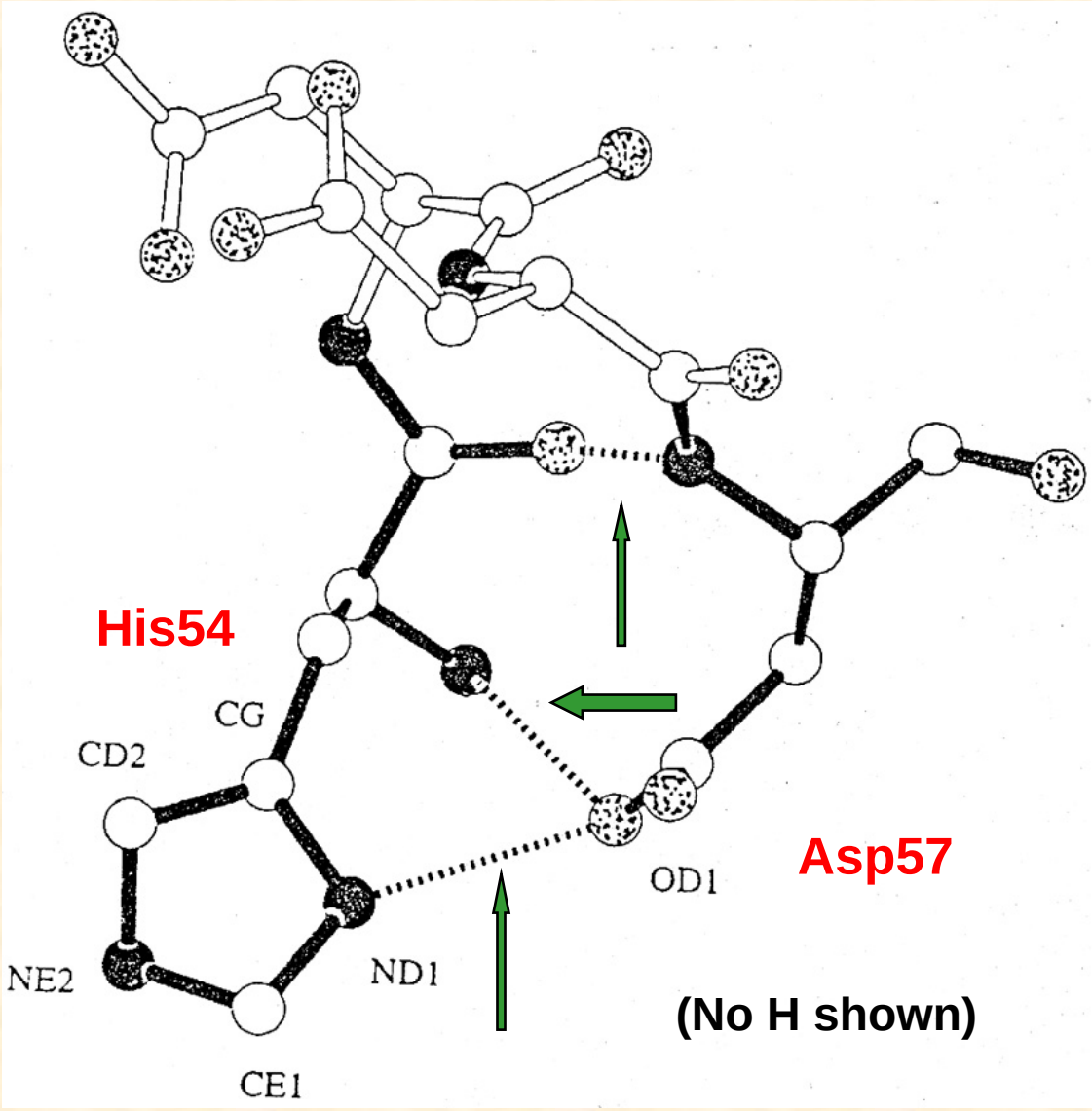
The active site of D-xylose isomerase



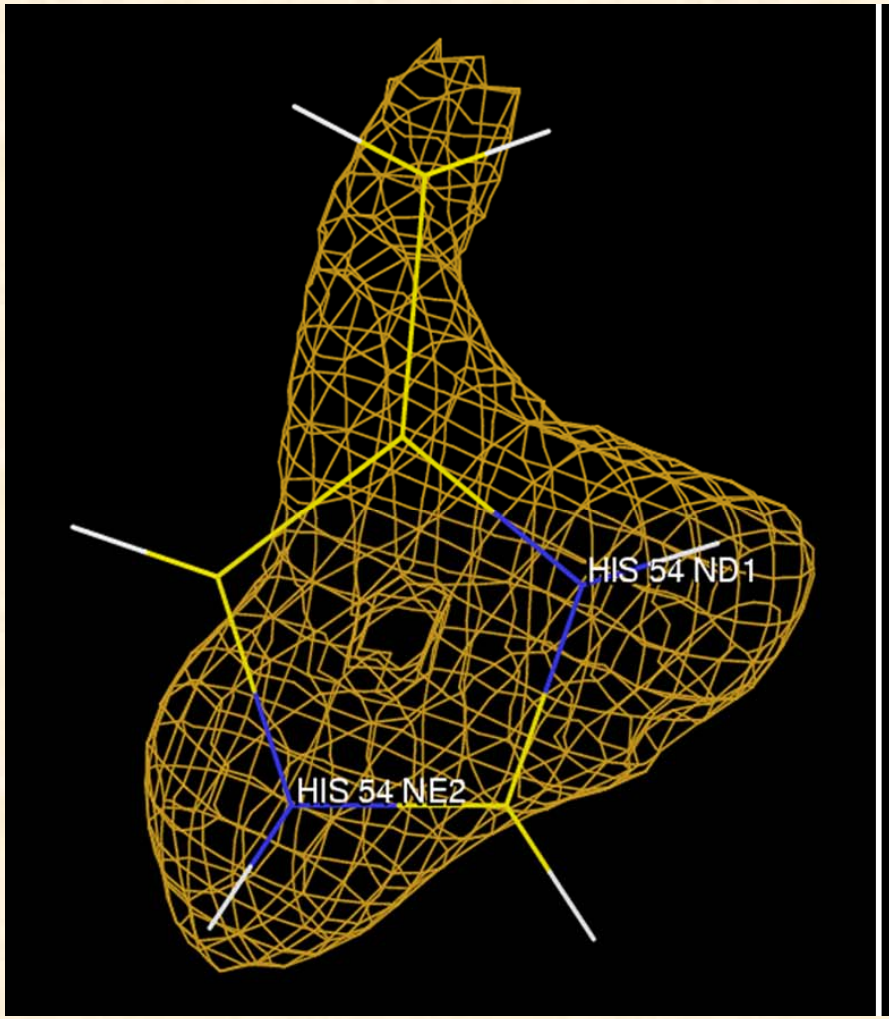


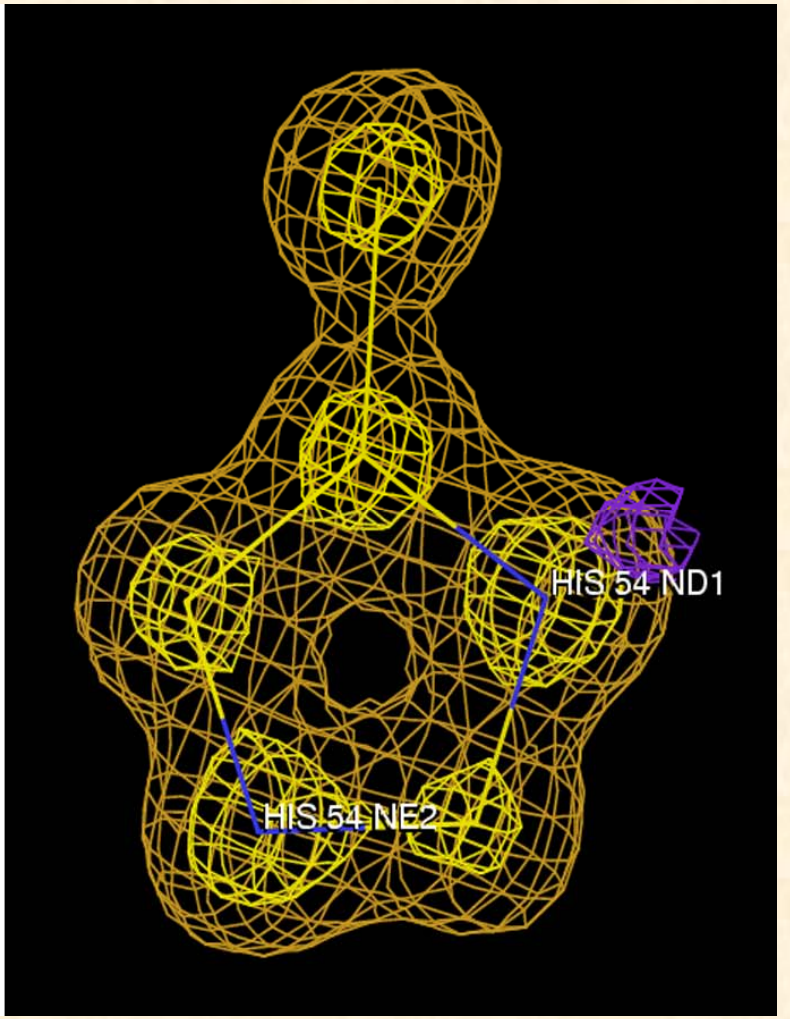
Active site of D-xylose isomerase showing bound xylulose (solid bonds), two histidines and two tryptophanes defining the substrate channel. The two large filled circles are the metal ions (Mg^{++}, Mn^{++}, or Co^{++}).

Interactions between His54 and Asp57 in the active site of D-xylose isomerase



Comparison of neutron (1.8 Å) and electron density (0.94 Å)





His54 - neutron

His54 – X ray

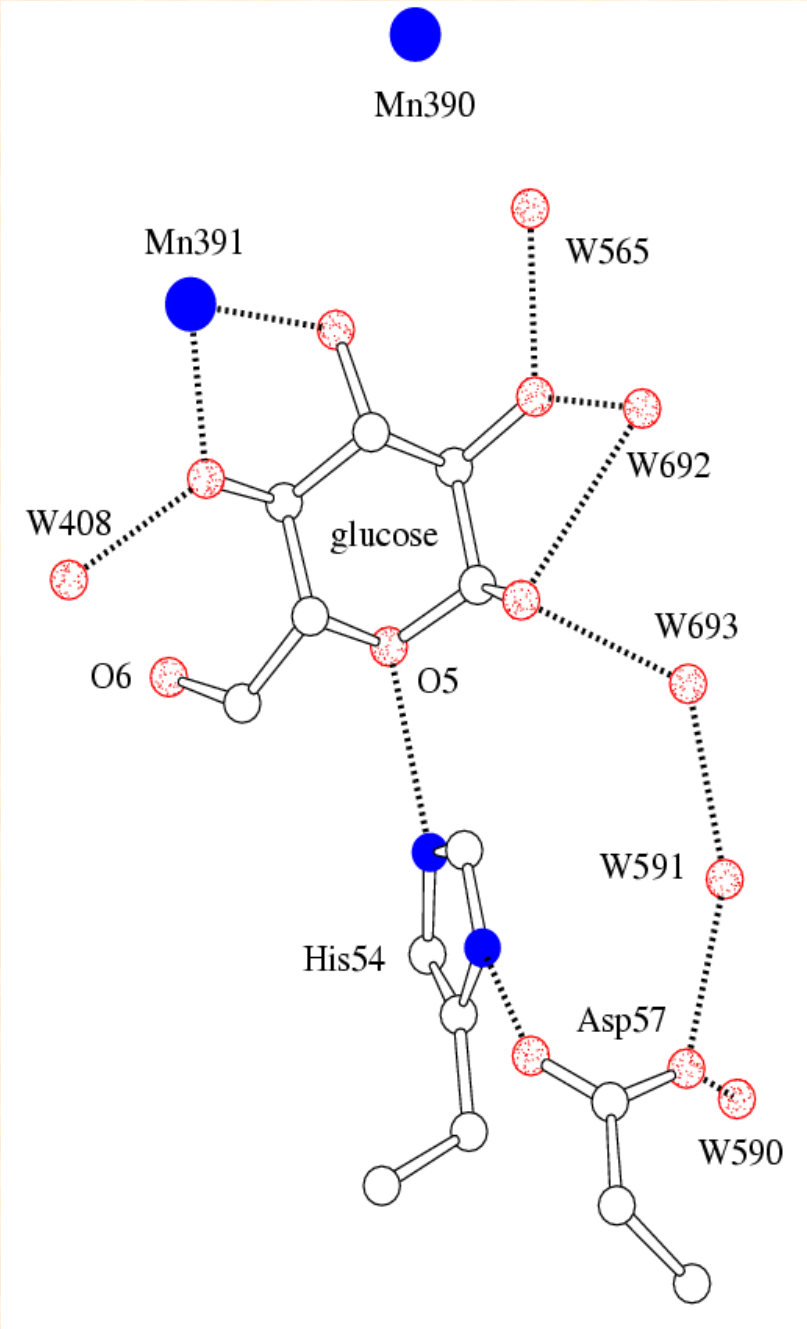
Note the H on NE2 in the neutron map

Singly protonated (proton located on either ND1 or NE2)

His	ND1 to	%D	NE2 to	%D	ND1 B	NE2 B	ND1 e.d.	NE2 e.d.
	neutron				X ray			
49	Pro-7 O	37	-	-	15.8	14.4	2 σ	none
71	W1204 (D2)	0	W1281 (O)	46	18.9	19.8	none	none
96	Val-98 N	0	W1210 (O)	32	10.8	13.3	none	1.5 σ

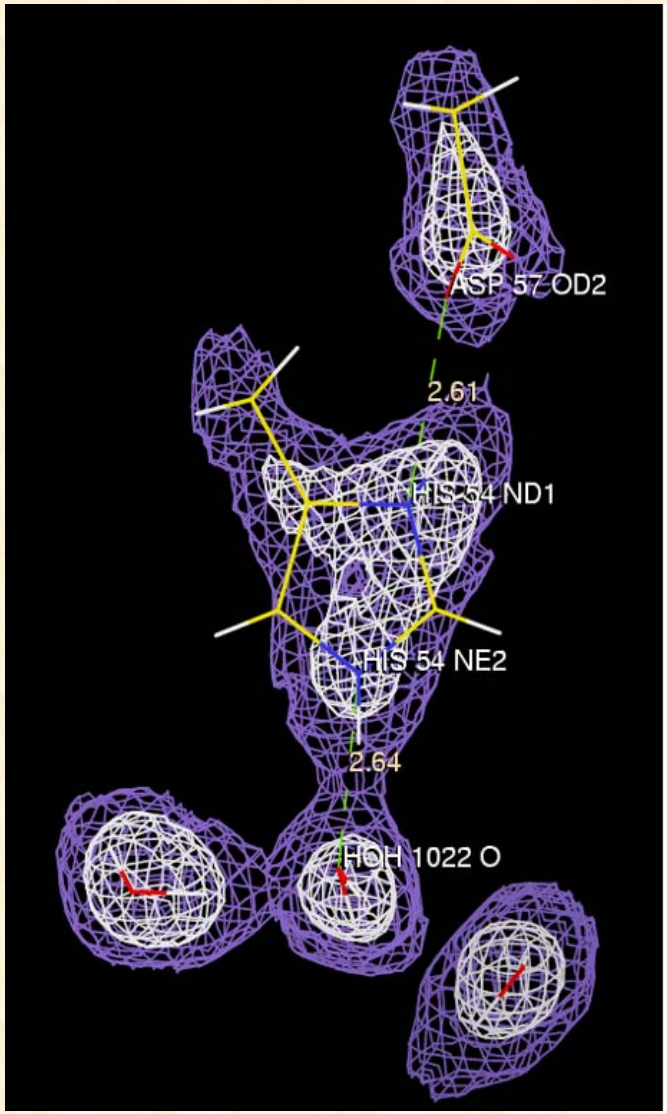
Doubly protonated (proton located on both ND1 and NE2)

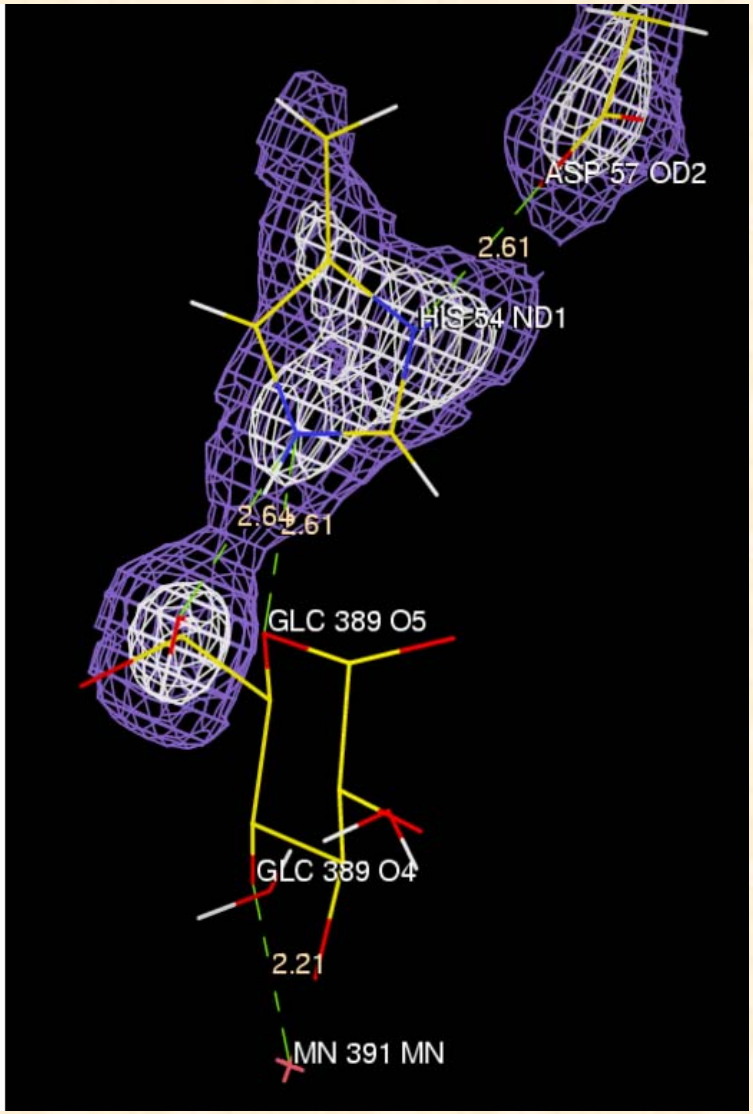
His	ND1 to	%D	NE2 to	%D	ND1 B	NE2 B	ND1 e.d.	NE2 e.d.
	neutron				X ray			
54	Asp-57	67	W1022	50	10.6	10.7	2 σ	none
198	Thr-195 DG1	54	W1023	52	8.4	9.2	2 σ	2 σ
220	Pro-182 O	64	metal	57	14.4	17.7	none	none
230	W1065	67	W1214	87	8.9	9.5	2 σ	none
243	Asn-215 OD1	91	W1026	32	13.2	14.1	2 σ	none
285	Asp-245	100	Thr-52 DG1	34	10.3	10.4	2 σ	none
382	W1109	69	Asp-323	49	10.6	10.7	none	none



Surroundings of glucose in a complex with D-xylose isomerase (Carrell, Hoier, Glusker. Acta Cryst. D 50:113-123, 1994). Note the interconnecting water molecules.

Environment of His54

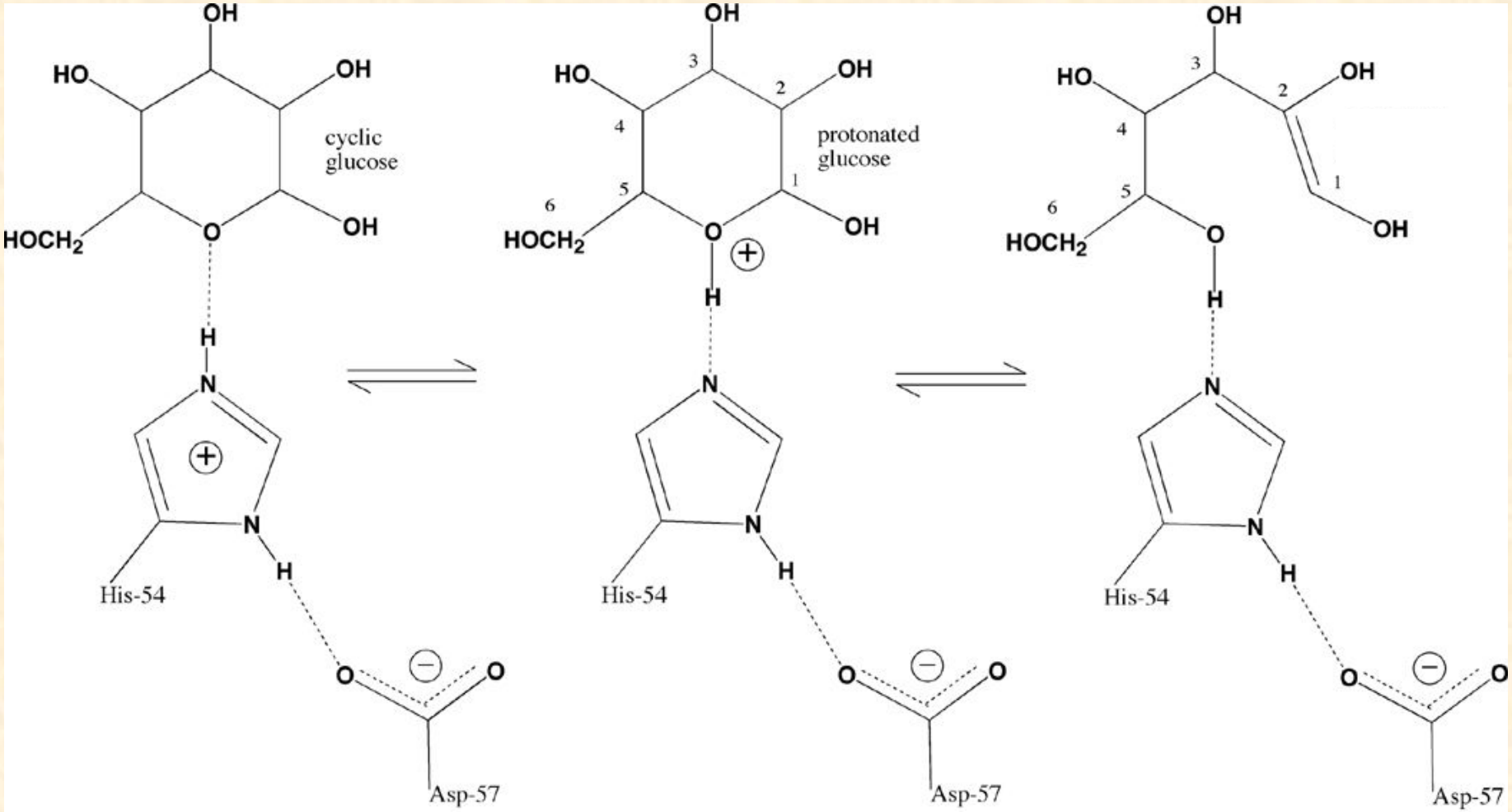


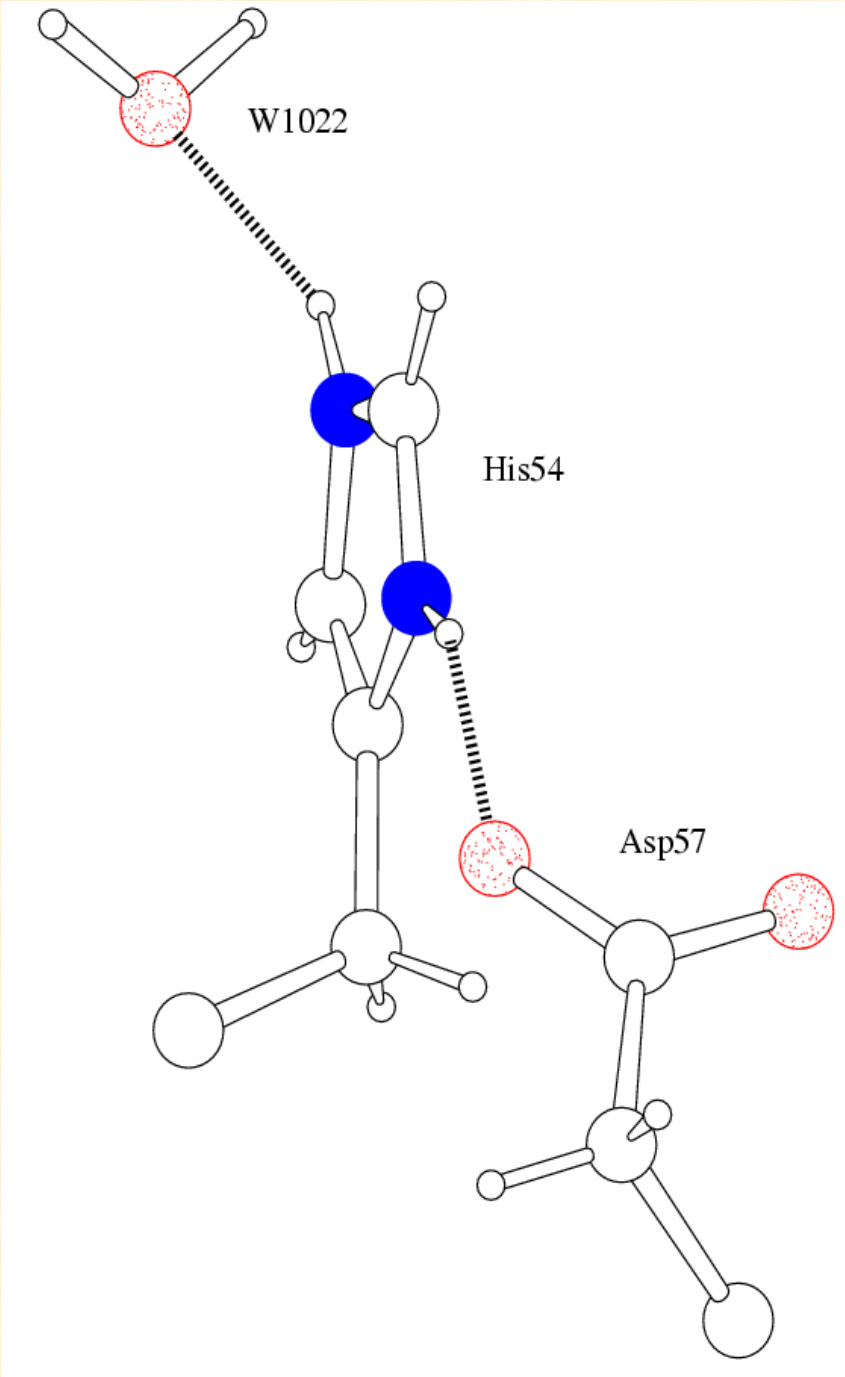


Water structure around His54
In neutron map

Glucose from an X-ray study added to show possible mechanism of protonation

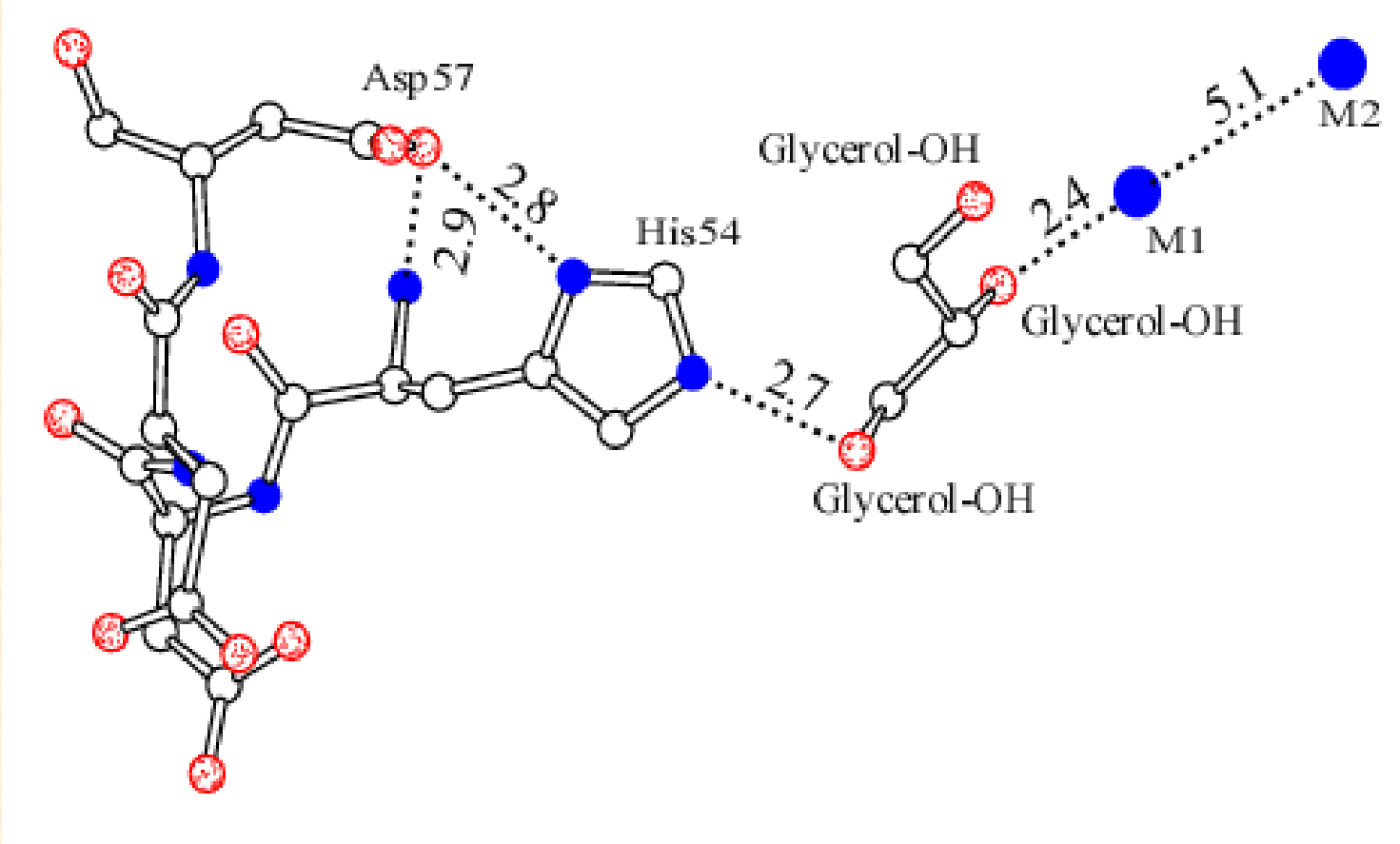
His54 and the ring-opening mechanism



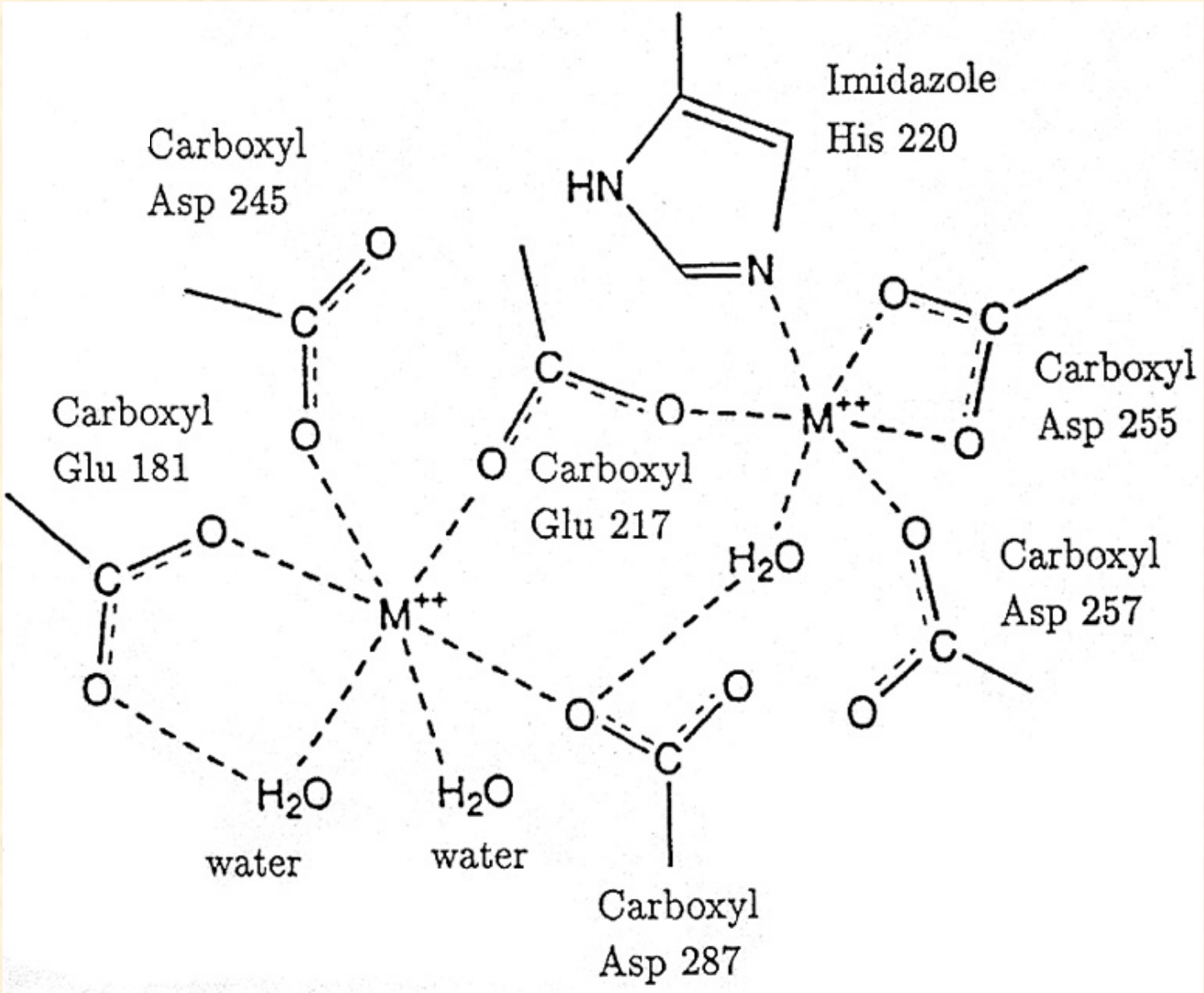


The serine-protease motif in D-xylose isomerase.

Serine-protease catalytic triad found in D-xylose isomerase

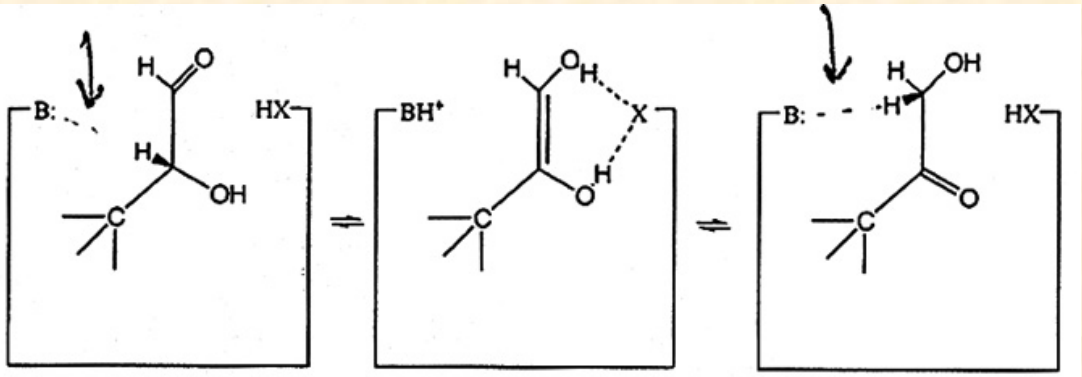


The active site of D-xylose isomerase

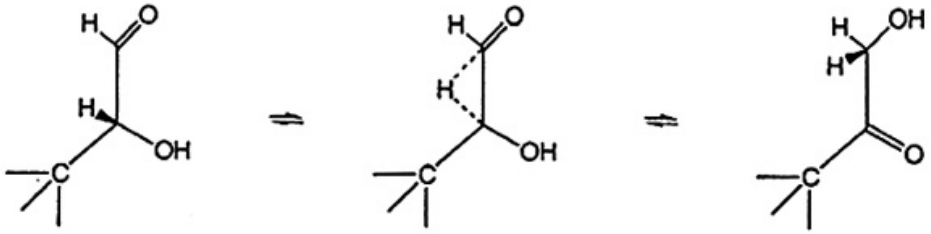


Proposed mechanisms for D-xylose isomerase

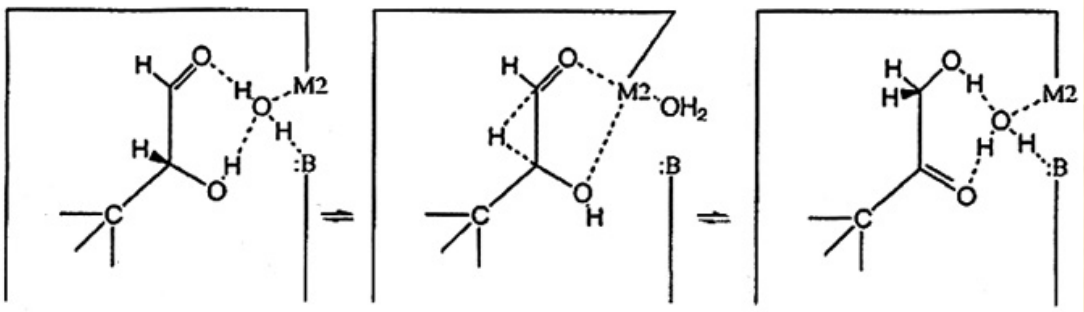
cis-ene diol



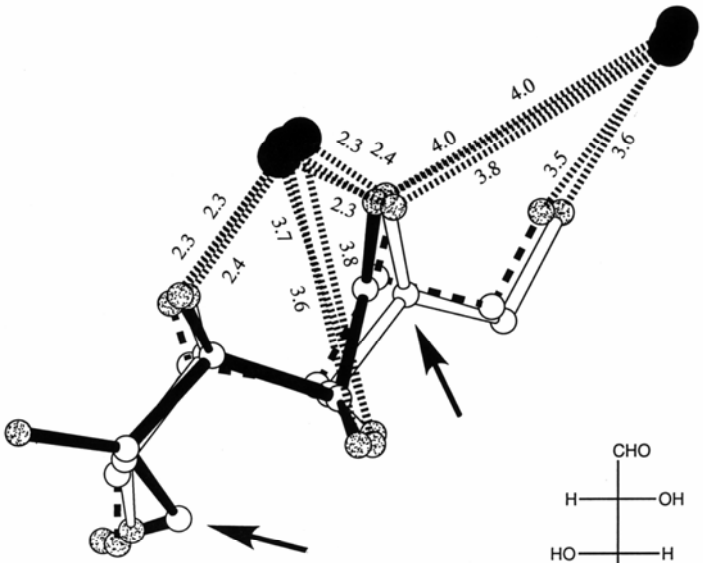
hydride shift



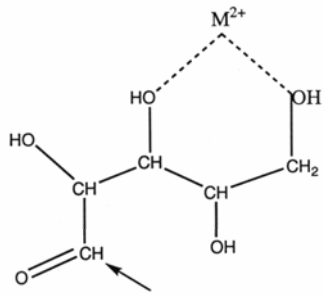
metal-assisted hydride shift



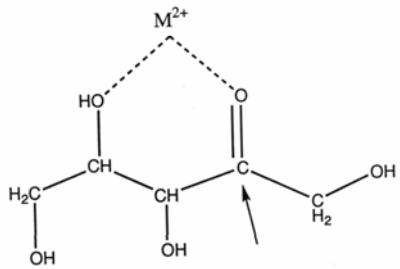
Modes of ligand binding to D-xylose isomerase



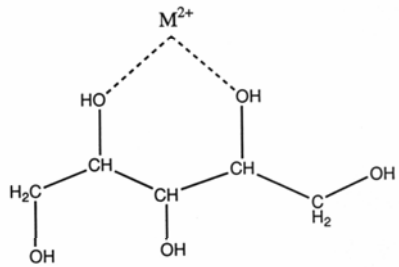
xylose



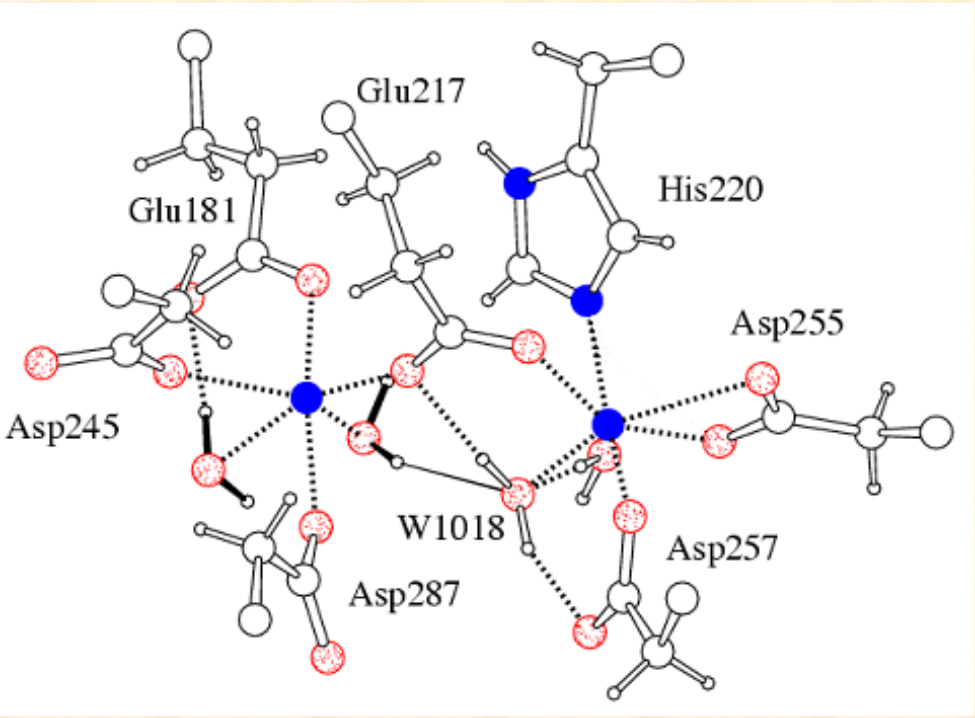
xylose



xylulose

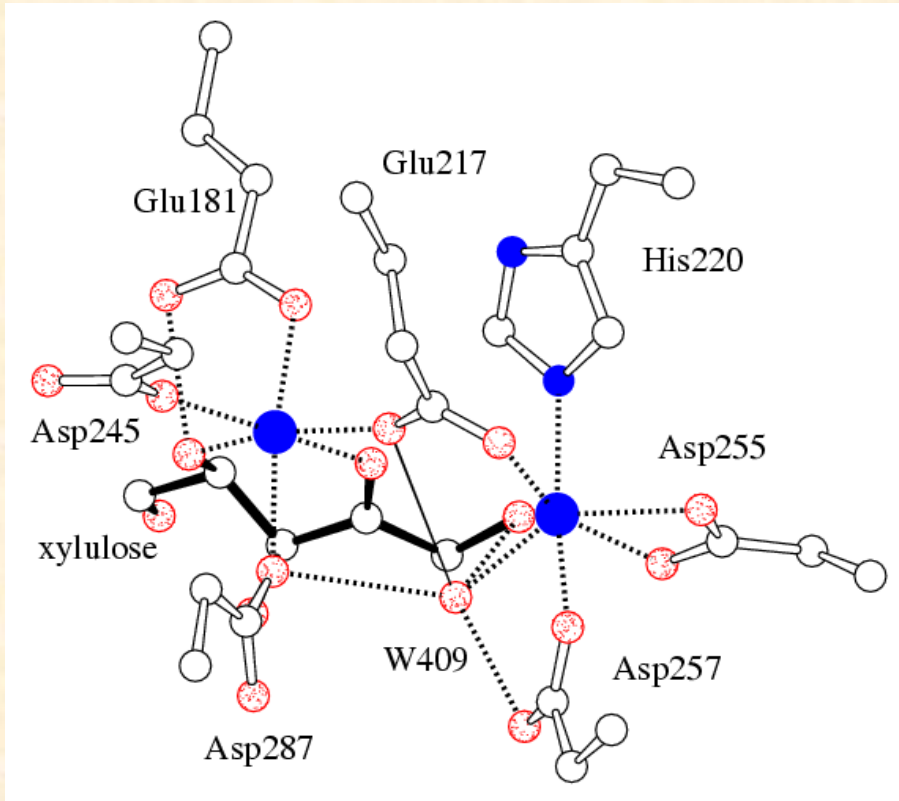


xylitol



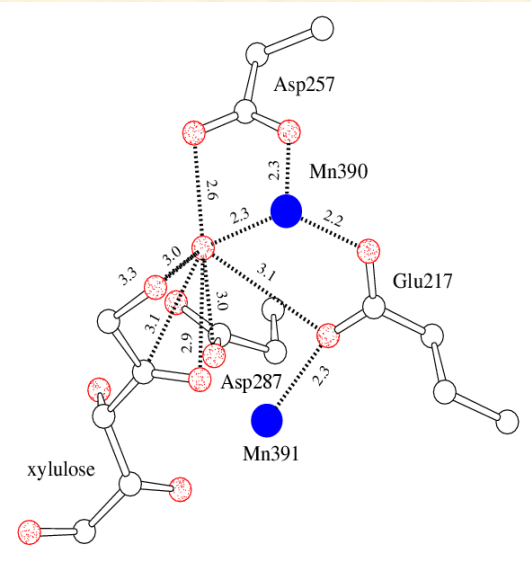
Above: Active site showing bound water

Below: Active site showing product xylulose replacing bound water

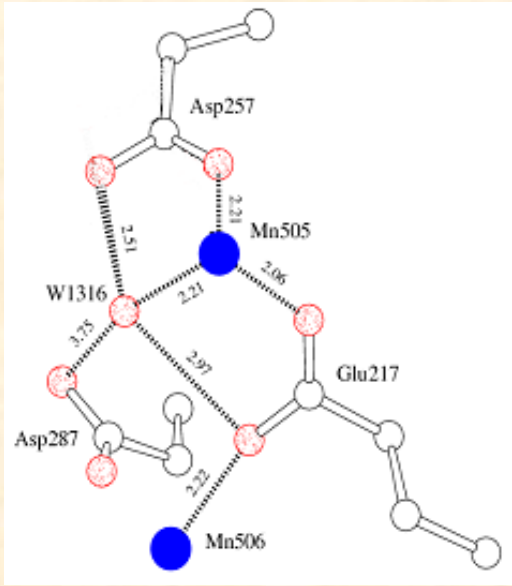


The power of the combination of X-ray diffraction and neutron diffraction

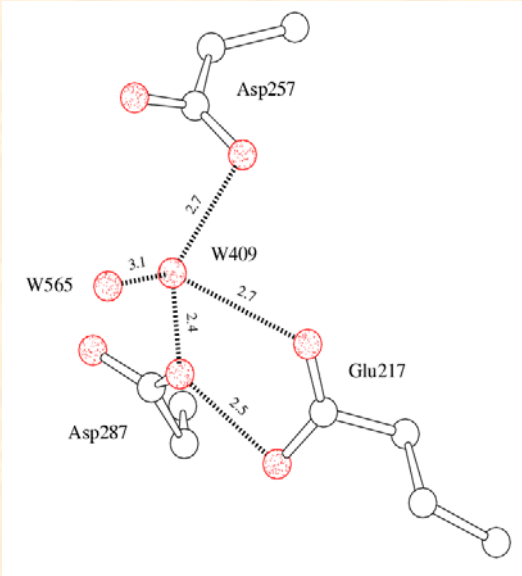
Environment of proposed catalytic water



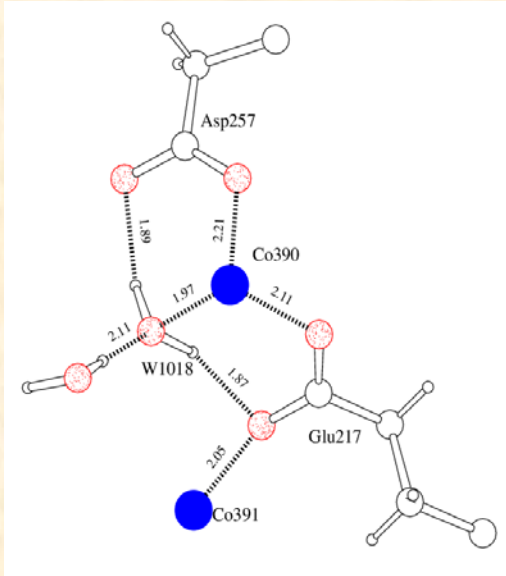
X ray structure 1XII, xylulose



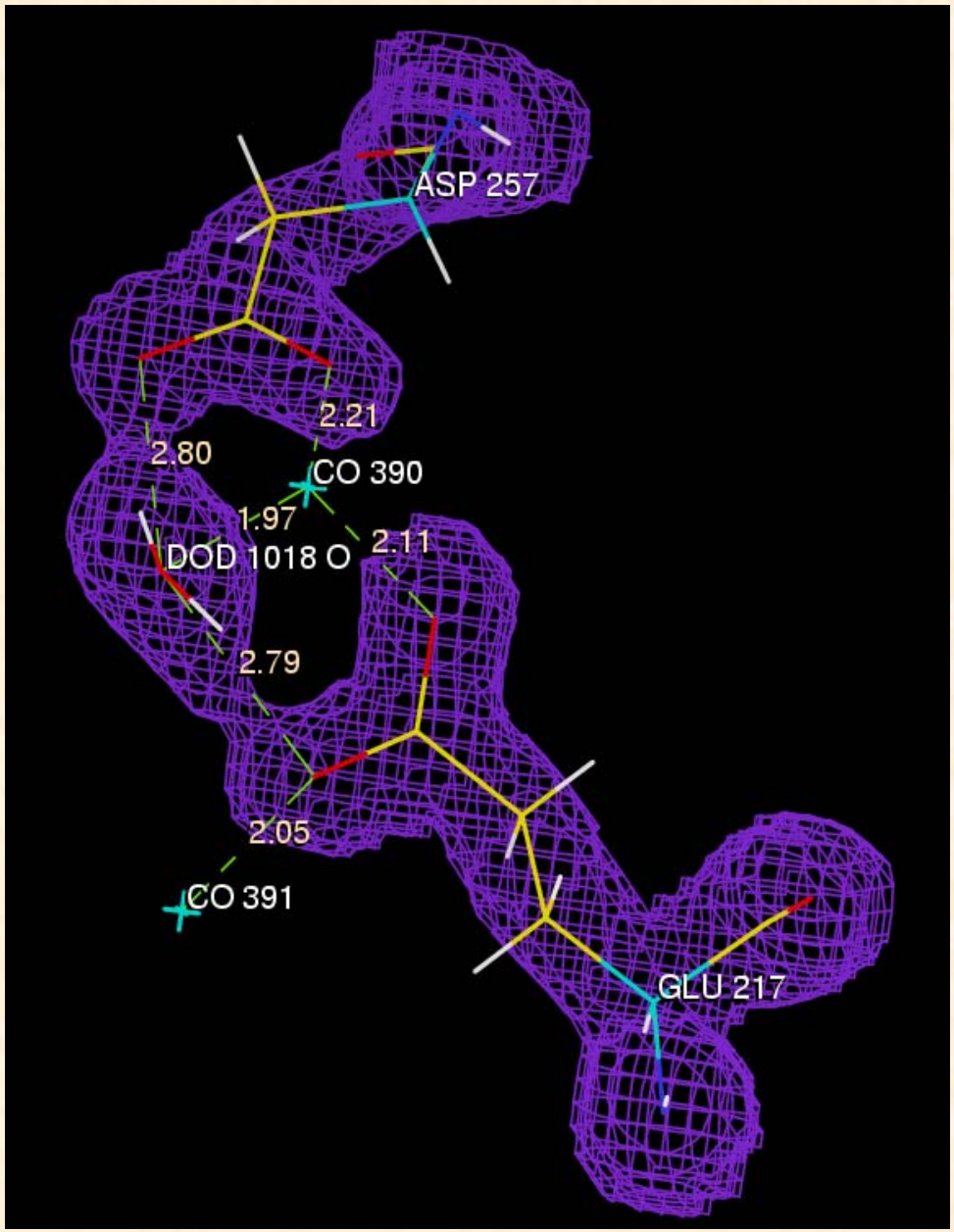
X ray structure, 0.94 Å



X ray structure, apoenzyme



Neutron structure, 1.8 Å



Metal ion-carboxylate-water motifs in D-xylose isomerase. Note how two motifs are shown by neutron diffraction to tie up the two protons on the metal ion-bound water molecule.

The information not previously known that we found using neutron diffraction

- **Location of protons on metal-bound waters**
- **The protonation state of His54**
- **The type of hydrogen bonding between water and Asp287**
- **The locations of protons on Lys183**
- **The protonation state of His220**

The ultra-high resolution X-ray study did not provide clear evidence on proton locations. Neutron diffraction did!