Applications

- Structure of proteins including hydrogen atoms at medium to high resolution

Selected examples

**Water molecules in lysozyme**

Neutron crystallography is the method of choice in determining water structure in protein crystals. When crystals are soaked in D₂O the water of crystallisation becomes deuterated and thanks to the large coherent scattering length of deuterium all three atoms of the water molecule become visible. This is in contrast to the case of X-rays where only the central oxygen atom is observed due to the very weak scattering power of hydrogen. Neutrons, therefore, allow us to locate not only the centre of mass of the water molecule but also its orientation which is all-important in defining its hydrogen bonding. The figure shows the characteristic “boomerang” shape of a water molecule in the crystal structure of lysozyme as determined on LADI.

Reference:


**Protonation in the active site of an enzyme**

The mode of action of many enzymes depends on whether particular amino acid side chains in the active site are protonated or not. Neutron diffraction studies on crystals of the aspartic protease endothiapepsin, soaked in D₂O, have enabled the full geometry of the active site including hydrogen (replaced by deuterium) atoms to be elucidated leading to an understanding of the enzymic mechanism. Below is a 2.1Å neutron density map near the active site of Endothiapepsin/H261 transition state analogue, showing protonation at the outer carboxyl oxygen of Asp 215.

Reference:

The Laue diffractometer LADI is mainly used for single-crystal studies of small protein systems at medium or high resolution in order to locate individual hydrogen atoms of special interest, water structures or other small molecules that can be marked with deuterium to be particularly visible.

**Large-scale structure diffractometers**

**Instrument description**

The instrument is mainly used for single-crystal studies of small protein systems at medium or high resolution in order to locate individual hydrogen atoms of special interest, water structures or other small molecules that can be marked with deuterium to be particularly visible. The size of the unit cell that can be studied is clearly dependant on the size of the crystal, and beyond around 300,000 Å^3 crystals of several cubic mm are required. To optimise the density of the peaks on the detector surface and the peak-to-background ratio typically a bandwidth of 20% is used, but other band-width filters can be made available.

The detector, employing neutron-sensitive image plates, is capable of high spatial resolution, has good homogeneity, a large dynamic range, extended linearity and no dead-time, and subdents very large angles at the specimen. The neutron-sensitive plates are based on the same storage-phosphor (BaFBr doped with Eu^{2+} ions) which is used for X-ray image plates, but with GdO_{2} added. This enables the Gd nuclei to act as neutron scintillators by creating a cascade of X-rays and conversion electrons. The cylindrical neutron image-plate detector is shown below.


The sample crystal is mounted on a goniometer head on the cylinder axis, and can be rotated around this axis. The neutron beam, which enters and leaves via opposed holes in the cylinder, produces Bragg reflections and other, more general scattering patterns, which pass through the aluminium wall and are recorded on the image plates mounted on the outside cylinder surface. The detector, which has a radius of 159.2 mm and a length of 400 mm is read off in a phonographic mode with the reading head tracking slowly horizontally while the cylinder rotates at high speed. The readout time is three minutes, giving an image of the pattern recorded on the plates comprising 4000 x 2000 square pixels 200 μm on edge. The sample axis is horizontal and the opening for the sample holder has only a diameter of 10 cm, so this detector would generally be unsuitable for physics/chemistry type work, where bulky environment-control units - often with vertical geometry - are used.

(Such experiments may be carried out on the VIVADI instrument - see page 46).

**Instrument Data**

**Guide hall n°1, cold guide H142**

<table>
<thead>
<tr>
<th>monochromator</th>
<th>Laue white beam</th>
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<tbody>
<tr>
<td>quasi-Laue (δλ/λ&lt;30%)</td>
<td>Ti/N multilayer band-pass filter</td>
</tr>
<tr>
<td>standard filters (λ=3.5 Å)</td>
<td>δλ/λ 8% or 20%</td>
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**collimation**

| pinholes | 0.5 to 4 mm |

**sample**

| flux at specimen (λ= 3.5 Å; δλ/λ= 20%) | 3 x 10^10 cm^2 s^{-1} |

**detector**

| cylinder covered with image plates | GdO_{2}doped BaF(Br,I):Eu^{2+} |
| configuration | cylinder camera |
| radius | 159 mm |
| length | 400 mm |
| active area | 800 x 400 mm^2 |
| angle subtended | 144° in φ, 52° in ψ |
| pixel size | 200 x 200 μm^2 |

**sample environment**

displex cryostat going to approx. 12 K.

web: www.ill.fr/YellowBook/LADI/