Powder diffraction is an important and often unique crystallographic tool to determine the structures of polycrystalline materials in powder crystallography, for measurement of residual strain in bulk materials, in in-situ measurements for the exploration of phase diagrams with temperature, pressure, the kinetics of chemical reactions, following the changes in the crystalline components in electrochemical cells with charging and discharging, etc. One of the major general strengths of powder diffraction is the ability to carry out measurements under a wide range of conditions.

At the High Resolution Powder Diffraction beamline ID31 at the ESRF, ancillary equipment is available for experiments in a wide range of temperatures, from room temperature down to 2.5 K using either a cold nitrogen gas blower or a liquid-helium-cooled cryostat; or at high temperature, up to 900 °C, using a hot air blower. Higher temperature can be reached using a parabolic mirror furnace, using halogen lamps; in this case the sample has to be contained in platinum capillary and it can be heated to ~1500 °C. An in-situ gas-handling system has been necessary to allow volatile compounds to be condensed in a capillary mounted on the axis of the diffractometer for standard powder X-ray diffraction studies. This gas-handling cell has also been employed in in-situ experiment that required fluxing gas through the sample during data collection, at modest pressures (up to 20 bar).

More recently, systematic pH- and temperature variations of crystallization conditions of relatively small protein were monitored using high-resolution powder diffraction data. Structural modifications of polycrystalline hen egg-white lysozymes (HEWL), precipitated in the pH range between 6.56 and 3.33, at 4 °C and at room temperature (for a total of 48 samples) were revealed from X-ray powder diffraction data. The experiment was also possible thanks to the automation in data collection achieved by employing robotic equipment for sample change.

In this presentation, a general overview of the diverse research activities carried out at the High Resolution Powder Diffraction beamline ID31 at the ESRF, in Grenoble (France), will be given.
X-ray and neutron crystallography are highly complementary techniques. While the non-hydrogen atom positions of biomacromolecules are accurately determined by X-ray crystallography, information on the hydrogen atom positions is only possible when ultra-high resolution data (better than 1 Å) can be collected. Neutron crystallography, on the other hand, can often reveal critically important hydrogen’s even at medium resolutions of 1.5 – 2.5 Å. Thus neutron crystallography can provide key insight into hydrogen bond networks, water orientations and proton shuttles involved for instance in enzyme catalytic mechanisms.

Whereas development of third generation synchrotron sources has allowed X-ray protein structures to be solved from crystals of a few microns in their smallest dimensions, a major hurdle to neutron protein crystallography is that unusually voluminous crystals (~ 1mm³) are required to compensate for the weak flux of available neutron sources [1]. However if the protein is fully perdeuterated (all hydrogen replaced by deuterium) the required crystal volume is considerably reduced as perdeuteration largely avoids the large incoherent scattering background of hydrogen, and thus can provide a tenfold gain in the signal/noise ratio of diffraction images [2]. Obtaining large deuterated crystals suitable for neutron analysis involves performing systematic studies of the solubility and protein-protein interactions in deuterated crystallization conditions, as H-D exchange alters the physico-chemical properties of protein solutions and affects the crystallization process in a significant way [3].

We will discuss recent technological advances achieved at the ILL/EMBL Deuteration Laboratory in Grenoble in preparing D-labelled biological material, and in growing large D-labelled crystals suitable for neutron diffraction. Finally we will present one of the recent neutron protein structures determined with the Laue diffractometer (LADI) at the ILL focusing on the complex of recombinant urate oxidase enzyme with 8-azaxanthin [4].