s1.m4.p5 **Neutron protein crystallography is greatly enhanced using perdeuterated proteins.** Flora Meilleur[1,2], Dean A..A. Myles[2], 3, Michael Hartlein[1], [1]*Institut Laue Langevin, BP152, 38042 Grenoble (France),* [2]*EMBL, BP 181, 38042 Grenoble (France), 3ORNL, PB 2008, Oak Ridge TN 37831(USA). E-mail: meilleur@ill.fr*

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Protons and water molecules play critical roles in the enzymatic mechanism of many enzymes. Therefore, our understanding of enzymatic processes may greatly benefit from the localisation of hydrogen atoms in active site residues and water molecules [1]. Direct information on hydrogen positions can be obtained from ultra-high resolution X-ray crystallographic data or from neutron crystallographic data at more modest resolutions of 1.5 - 2.0 Å. Neutron crystallography is therefore a useful experimental tool to address specific problems where direct visualisation of hydrogen atoms is crucial. However, neutron protein crystallography raises many challenges. Unusually large crystals (> 1 mm^3) are required to compensate the weak flux of available neutron beams. Moreover, the large hydrogen incoherent scattering background significantly reduces the signal to noise. This problem can be overcome by deuterating the sample, either partially, by soaking crystals in deuterated mother liquor, or more fully, by preparing perdeuterated (*i.e.* fully deuterated) protein samples. Perdeuteration offers critical benefits in neutron diffraction experiments, principally in providing a 10-fold gain in signal/noise (enabling smaller crystals to be used) and also in enhancing the visibility of deuterium atoms and water positions in the resulting maps. We will present recent results from the LAue DIffractometer (ILL) on neutron protein structures determined from partially deuterated crystals. To overcome the difficulties arising from partial deuteration, efficient methods and protocols to clone, over-express and purify 100% D-labelled (perdeuterated) recombinant proteins in high yields from bacterial (*E. coli*) expression systems have been developed within the joint EMBL/ILL deuteration laboratory. The results obtained for cytochrome P450cam [2] are presented. We have assessed the integrity of the perdeuterated protein using X-ray crystallography [3] and FTIR [4] spectroscopy to ensure that the neutron structure of perdeuterated P450cam will be representative of the hydrogenated protein.

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s1.m4.p6 **Functional Relaxation determined by Protein Kinetics.** M Schmidt[a], A Krasselt[a], F Parak[a], K Nienhaus[b], U Nienhaus[b], V Srajer[c], R Pahl[c], [a]*Physikdepartment E17, TU München,* [b]*Abt. Biophysik, Univ. Ulm,* [c]*CARS, Univ. of Chicago. E-mail: marius@hexa.e17.physik.tu-muenchen.de*

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The function of proteins is related to their dynamics. By energizing a protein structure for example by flashing away CO from the heme in myoglobin new positions in the multi-dimensional potential energy space become accessible. Dynamic processes lead to the relaxation or adaptation of the protein structure into energy minima which were previously unreachable. In an ensemble of molecules, such as those in a crystal, the dynamics of a single molecule may become obscure. However, the dynamics of the ensemble, the kinetics, is usually measurable. Since only recently the kinetics can also be followed by time-resolved crystallography, if multiple data sets closely spaced in time are collected. The data must be analyzed in terms of chemical, kinetic mechanisms (1,2). The complexity of these mechanisms depends on the complexity of the energy landscape and the time ranges the molecules in the ensemble need to relax. We have collected 20 time-dependent crystallographic data sets after flashing away CO from the heme within the L29W mutant of myoglobin. The time points were roughly equidistant in logarithmic time from 1ns to 4s. From 1ns to 6 ms the CO is uniformly absent from the heme iron. The heme itself has almost completely relaxed to the deoxy structure even at the fastest times. No primary docking site of the CO can be identified. At about 100 ns the CO accumulates exponentially at the proximal XeI site and remains there until roughly 2 ms. However, rebinding to the heme-iron is one order of magnitude slower. Difference electron density values within the globin were globally analyzed by singular value decomposition. Only one significant singular value and a corresponding singular vector pair were found. Already at 1ns there is substantial relaxation. However, further relaxation was significantly non-exponential and the global relaxation is complete within a few hundred nano-seconds. This finding was confirmed by integrating the difference electron density values in selected segments of the globin. Final relaxation occurs in concert with the CO binding to the heme iron. Hence, in myoglobin complex mechanisms may account globally for the data on the initial, fast time scales and simple mechanism for time scales slower than about 100 ns.

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