# The role of water in the protein activity: lysozyme from the glass to the unfolded state.

### *(Nuclear Magnetic Resonance and Neutron Spectroscopy studies from the protein dynamical crossover to the irreversible unfolding)*

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The effect of water on proteins is studied in a very large temperature range from 180 to 370 K. By using in a comparative way the Nuclear Magnetic Resonance and the Neutron scattering we explore this protein system at different hydration level h (h=0.3, 0.37, 0.42 and 0.61). The hydration level h=0.3 is equivalent to a single monolayer of water around the globular protein. Our interest is focused to study the water role in the protein dynamical transition (glass transition or the transition from a harmonic solid like behavior to an anharmonic and liquid like motion) and the irreversible unfolding. We demonstrate also by considering neutron scattering experiments that the protein dynamical transition belongs to the universal class of dynamical crossover characterizing supercooled liquids and materials.

The thermal evolution of the spectral features allows identifying that the dynamical crossover observed for water coincides with that of the protein dynamical transition. We stress that we are able to demonstrate at a molecular level the interaction of water with the protein peptides and how via the HB it drives the protein activity. In addition on considering water thermodynamics we identify a special temperature T* that marks the crossover of water, by increasing T, from the state of a complex anomalous liquid to that of a simple conventional one. Furthermore, the combination of Scattering and NMR data allows us to clarify the role of T* in the protein properties, in particular T* is the limit of the protein native state.

At the same time we are able to clarify at microscopic level the underlying mechanisms that govern the reversibility of the folding-unfolding and irreversible denaturation processes of the protein. New NMR observations at the temperature above and below the protein irreversible unfolding ($T_D$) show that folding-unfolding process takes place as a function of the temperature; we observe that T acts as a control parameter of the measured nuclear magnetization M(T). Whereas far from this singular temperature, in the protein native state, the M(T) behavior is Arrhenius, approaching $T_D$ (in a large T-interval) the system changes dramatically it energetic configurations by means a power law behavior. Hence, by following the thermal behavior of different protein-peptide metabolites we are able to explore the funneled energy landscape. On these bases, by taking advantage of the polymer physics we propose this complex process (protein folding/unfolding) as a sort of sol-gel transition driven by water as the cross-linker between different protein peptides, an with $T_D$ as the percolation threshold temperature.