5 - LIFE SCIENCES

This chapter reports on the research activities on Life Sciences. It collects results obtained during the past two years by LLB scientists as well as scientists collaborating with LLB. Some results have been selected to appear as "Highlights" at the end of the chapter.

Our research activities in the Life Sciences are to characterize the movements and dynamic structural equilibria of proteins, including fast internal motions, domain motions and global unfolding and refolding transitions, to understand how the primary sequence of the protein controls these dynamic properties and to relate the specific structural dynamics to the specific functional properties of the proteins. Neutrons produced by present research-oriented sources typically have wavelengths on the order of angstroms, and energies of tens of wavenumbers, and hence are well-suited for probing the structures and motions of molecules. Neutrons scatter from nuclei, both coherently and incoherently. Coherent scattering provides information on structure and collective dynamics, and incoherent scattering on single particle motion. The large difference in scattering cross-sections between hydrogen and deuterium provides a powerful method for accentuating (or masking) the scattering from particular parts of a system by selective deuteration. Neutron scattering has been demonstrated to be a powerful tool for characterizing the structures and dynamics of biological molecules. We present recent results of applications of a wide variety of neutron scattering measurements (small-angle scattering for structure; quasi-elastic scattering, spin echo for dynamics). Theoretical molecular models for interpreting the data are presented in the chapter dedicated to Theory. The thesis work of V. Hamon is an example of collaboration between experimentalists and theoreticians (see Highlight in chapter Theory).

The research activities have progressed along the lines described previously:

- studies of conformation of proteins in solution (native and unfolded states)
- relationship between structure, dynamics and function of biological macromolecules and role of hydration water in this relationship with orientation to complex systems

Role of hydration water

Hydration water plays a major role in the stability, dynamics and function of biological macromolecules. The knowledge of structure and dynamics of hydration water is needed to get a better understanding of the role of water in biology. Water in various systems from model systems to complex ones like DNA and entire cells has been studied.

Water as a monolayer at the hydrophilic surface of a porous Vycor glass

Nanosecond-time-scale measurements of local rotational and translational dynamics of interfacial, non-crystalline, water from 77 to 280 K have been done. These experimental dynamic results meet calorimetric and diffraction data to show that after exhibiting a glass transition at 165 K, interfacial water experiences a first order liquid-liquid transition at 240 K from a low density to a high density liquid. This is the first direct evidence of the existence of a liquid-liquid transition involving water
(see Highlight). Collaboration J.-M. Zanotti, M.-C. Bellissent-Funel (LLB), S.H. Chen (MIT)

Water in assemblies of identical small peptides

A chain of five alanins has been hydrated, at various levels of hydration, providing with 1 to 25 water molecules. At high level of hydration, the H-bond lifetime is slightly longer than that observed in bulk water. Higher the hydration and higher the diffusion that tends asymptotically to the self-diffusion in bulk water. As obtained in previous works, the residence time is almost constant with a value similar to that bulk water at a temperature 30 K lower.
(see Highlight**).** Collaboration D. Russo (postdoctorant, Berkeley), P. Baglioni (Florence), J. Teixeira (LLB)

Water in ds-DNA and ss-DNA

Analysis of quasielastic neutron scattering data of hydrated samples of ds-DNA and ss-DNA indicates that in the ds-DNA sample one can distinguish two types of protons—those belonging to water molecules strongly attached to the ds-DNA surface and another fraction belonging to water that has an isotropic diffusion. For

ss-DNA, on the other hand, no indication was found of motionally restricted or confined water. Further, the fraction of protons strongly attached to the ds-DNA surface corresponds to 0.16 g H_2O/g ds-DNA, which equals the amount of water that is released by ds-DNA upon thermal denaturation, by differential scanning calorimetry. These results represent, thus, a completely independent measurement of water characteristics and behavior in ds- and ss-DNA at critical hydration values, and therefore substantiate the previous suggestions/conclusions of the results obtained by calorimetry.
(see Highlight). Collaboration M. Bastos, V. Castro, G. Mrevlishvili (Porto), J. Teixeira (LLB)

Water in entire cells

Although water (which constitutes around 80% of a cell) plays a crucial role in nearly all biochemical processes, the properties of water in the cytoplasm are still under much discussion. Former NMR experiments had suggested a very different cytosolic environment in *Haloarcula marismortui* (*Hm*) and *Halobacterium salinarum* (*Hs*). Both are extreme halophiles, but hydration interactions appeared to be much stronger in *Hm* than in *Hs*.
Deuterated *Haloarcula marismortui* cells were cultivated, and whole cell pellets were prepared to measure cellular water dynamics. Quasielastic measurements were performed on Muses for a Q value of 1.7 $Å^{-1}$ and a significant difference can be observed between the water in the cells and the buffer (a high concentration of salts in D_2O). By fitting the curves with a stretched exponential, one gets for intracellular water a characteristic diffusion time which is a factor ten longer than for bulk water.
(see Highlight). Collaboration K. Wood, B. Franzetti, G. Zaccai (Grenoble), D. Oesterhelt (Martinsried), M. Ginzburg and B.-Z. Ginzburg (Jerusalem), S. Longeville(LLB), M.-C. Bellissent-Funel (LLB)

Dynamics and function of biological systems

The dynamical transition temperature

The physical origin of the dynamical transition still remains a matter of debate. Several models to analyze the temperature dependence of the mean square displacement $\langle u^2 \rangle$ have been proposed. Recently, in a series of recent papers, the hypothesis was developed that the strong increase in the $\langle u^2 \rangle$ around 200 K could at least partially arise from an increase in the low energy part of the density of states. Studies are under way to try to discriminate between mode softening and anharmonic contributions in the dynamical transition.
S. Longeville, J.-M. Zanotti (LLB)

Effects of co-solvents on β-lactoglobulin dynamics

Quasi-elastic and elastic neutron scattering preliminary measurements performed on Mibemol spectrometer, have shown that the β-lactoglobulin (β-LG) is a very rigid protein (picosecond time scale). Indeed, the evolutions of the $\langle \mu^2 \rangle$ as a function of temperature for the dry and wet protein (0.7g D_2O/g protein) are exactly the same. No dynamical transition was observed on the hydrated β-LG around 220 K as usually observed on other globular proteins.
Collaboration C. Loupiac (ENSBANA,Dijon), J.-M. Zanotti (LLB)

Dynamics of Aquaporin-1 (AQP1)

Aquaporin-1 (AQP1) is a small membrane protein that functions as a selective water channel in different cell types, including red blood cells. Its structure is now known at 2.2 Å and molecular dynamics simulations suggest that water dipole rotation during passage in the pore of the channel is essential for water selectivity and proton impermeability. Our aim is to investigate AQP1 dynamics *in vitro*, using neutron scattering and contrast matching strategy with hydrogen-deuterium substitution. A first series of experiments by neutron scattering has been carried out on AQP1 *via* elastic scans on MIBEMOL spectrometer (LLB). The high hydrophobicity of AQP1 allowed its partial purification with native membranes from human red blood cells, before freeze-drying. Mean square displacements (MSDs) of the hydrogen population were determined for D_2O-hydrated membrane powder (0.38 g D_2O/g dry sample), compared to controlled dehydrated sample. The extracted MSDs in D_2O-hydrated sample reveal an increase beginning at ~ 230 K called the "dynamical transition", comparable to other membrane proteins like bacteriorhodopsin but with a smoother transition. For a better comparison with dynamic behaviour of other membrane proteins, sample preparation is under improvement. AQP1 is purified in detergent by chromatography to avoid contamination from red blood cell lipids, before reconstitution in deuterated liposomes to match the membranes. Quasi-elastic and inelastic neutron scattering measurements in the intermediate temperature range will also be carried out to provide more information on the nature of the movements occurring in this regime.
S. Combet-Jeancenel, J.-M. Zanotti and M.-C.Bellissent-Funel (LLB)

Influence of concentration on the diffusion process of myoglobin and haemoglobin

The interior of cells is often filled with a very wide variety of "objects" with respect to the size and shape. Proteins are present *in-vivo* in a very crowded environment, with sometimes volume fraction up to Φ~0.3. This environment can affect some physical, chemical or biological properties of the macromolecules. As a particular aspect, diffusion mechanism in highly concentrated protein solutions is of high interest, with the aim to address the question of transport properties and the possible diffusion assisted or limited biochemical reactions. Combined SANS and NSE analyses of protein solution have been used to analyze the effect of non-specific protein-protein interactions on myoglobin diffusion. Direct protein-protein interactions are modeled using DLVO potential. The diffusion mechanisms can be measured up to volume fractions of Φ~0.4. From the volume fraction dependence of $D^s_s(\Phi)/D_0$ we have extracted i: the magnitude of the short range hydrodynamic interaction which are related to the local viscosity, ii: computed the protein friction, iii: shown that theories for hydrodynamic interaction failed for describing the $H(Q\rightarrow\infty)$ but give a qualitatively correct description of the wave vector dependence. When compared to the results of $D^I_s(\Phi)/D_0$ our results show that hydrodynamic interactions are the leading interactions that drive the macromolecular mobility even over long distances. These results are the first attempt to measure the wave vector dependent solvent mediated interactions in protein solutions.

Collaboration S. Longeville (LLB), W. Doster (Munich)

Protein folding

A complete understanding of protein folding requires the physical characterization of both native and denaturated states and the evaluation of the thermodynamic parameters of the system. This involves obtaining information concerning the structure and dynamics of proteins denatured under various conditions (temperature, pH, chemicals). The application of hydrostatic pressure to a protein solution also provides a controlled manner to alter these physical properties. Thus, characterisation of the denatured states of proteins is important for a complete understanding of the factors stabilising their folded conformation. Small-angle scattering, of either neutrons or X-rays, is a very powerful tool giving structural information at low and medium resolution. Complementary information from circular dichroïsm, fluorescence and differential scanning calorimetry is used.

Effects of high pressure on β-lactoglobulin structure

Studies of high pressure on the structure of this milk protein are interesting for the food industry as a way to make gel with new properties and texture. The effect of high pressure (50 MPa to 450 MPa) on the structure of the protein has been obtained from measurements of the radius of gyration and analysis of the initial forward scattering intensity by SANS on PACE spectrometer at LLB. At pressures lower than 200 MPa, the change of radius of gyration is about 0.15 nm. According to other studies with only pH change, where the β-LG goes from dimer to monomer, one expects that the dissociation should at least be linked to a change of 0.7 nm of the radius of gyration. So one can say that the protein unfolds under this range of pressure but the dimer does not dissociate. Beyond 200 MPA aggregation occurs between partially unfolded dimers and the gelation process is initiated

Collaboration C. Loupiac (ENSBANA, Dijon), M. Bonetti (SPEC, CEA), S. Pin (Laboratoire Claude Fréjacques, CEA), P. Calmettes (LLB)

Effects of high pressure on BPTI (bovine pancreatic trypsin inhibitor) structure and dynamics (Part of Thesis work of M.-S. Appavou)

BPTI is a small protein, formed by 58 amino acids (6,5 kDa). The structure is well known, and contains both alpha-helix and beta-sheet patterns. Its heat-induced irreversible transition temperature occurs between 80 and 90°C.

Under pressure, a first structural transition has been observed between 3 and 6 kbar. The global shape of the protein studied by SANS starts to exhibit changes at 3 kbar, and the secondary structure studied by FTIR changes from 5kbar. This first transition concerns the breaking of α-helices, turned into unordered structures. A second structural transition occurs at about 14 kbar and is related to the change of unordered structures, accompanied by the formation of into β-sheets. This second structural transition has only been studied by FTIR. Such a transition presents, moreover, a great interest for the understanding of mechanisms involved into the amyloid diseases (due to beta-sheet formation).

In the same context as described above, the protein has been investigated by QENS (IN5,ILL; NEAT, HMI) to characterise the evolution of the dynamics during the heat and pressure induced denaturation. Changes have been observed around 3 kbar, and a clear transition occurs between 5 and 6 kbar. Data are analysed

with theoretical laws developed for polymeric systems, from which geometry of motions and distribution of relaxation times of various parts of the protein are obtained. One foresees to carry out experiments at higher pressures.
With SANS (NEAT, HMI), we get some structural information, enabling us to link structure and dynamics over the whole denaturation process. A new high-pressure cell (up to 14 kbar), suitable for SANS measurements and developed at LLB, will be used, This high-pressure range has not yet been investigated by SANS.
Collaboration M.S. Appavou, G. Gibrat, B. Annighofer, M.-C. Bellissent-Funel (LLB), M. Plazanet (ILL), A. Buchsteiner, J. Pieper (HMI, Berlin)

Complex systems

Fibronectin matrix assembly by endothelial cells as a function of fibronectin conformations.
Fibronectin is a high molecular weight multidomain glycoprotein witch is distributed in a soluble form in plasma and most body fluid. Fibronectin is also found in a polymerized form as part of the extracellular matrix (ECM) of various connective tissues. Recently, one has obtained and caracterised in terms of size, volume fraction and shape by angle neutron scattering experiments three different fibronectin conformations: a flexible string of 56 globules, a globular shape, at large scale with a low protein density inside the globule (Gaussian local structure), a still open conformation, at large scale with a very low density. In the absence of endogeneous fibronectin synthesis, the cells are able to form fribrillar matrix with the different exogenous fibronectins. The extended and the globular conformations are assembled into extensive fibrillar network, where as the string of beads conformation is assembled into different short fibrils (figure). The cell adherence and the spreading are increased at the same level independently of the exogenous fibronectin conformation. These data show that the cell capability to form a fibronectin network with exogenous protein is principally due to the fibronectin conformation. These results are consistent with the hypothesis that cells stretch mechanically fibronectin during fibrillogenesis.
Collaboration B. Thiébot, M.-F. Breton, (Cergy-Pontoise), J. Pelta (Cergy-Pontoise and LLB), D. Lairez (LLB)

Structure of proteins-polyelectrolyte complexes
Small Angle Neutron Scattering (SANS) has been used to reveal the inner structure of complexes that can be formed when proteins (here lysozyme) and polyelectrolyte of opposite charges are mixed.
In a first time, we mix lysozyme protein with a fully deuterated synthetic polyelectrolyte (PSSNa) to reveal both the structure of lysozyme and PSS chains within the complexes for different PSS chain length and lysoszyme/PSS chains ratios. We identify three main complexes types that are in accordance with the macroscopical mechanical properties. For large lysozyme/PSS ratios we get: (i) dense aggregates with short PSS chains and (ii) a PSS network cross-linked by lysozyme for long chains. (iii) for low lysozyme/PSS ratio, lysozyme is unfolded and behaves as an excluded volume chain. We get a costructure formed by the two chainlike objects.
In a second time, in collaboration with an external team (INRA), we study a system of biological interest formed by lysozyme and pectin. Though matching contrast is not possible here, we reveal the structure of complexes on the basis of the study of the first part. A data treatment enables us to get the inner volume fraction of complexes and to link it with the electrostatic interactions.
(see Highlight). Collaboration F. Cousin, J. Gummel, C. Huchon, F. Boué (LLB), I. Schmidt, M. Axelos (INRA, Nantes)

Conclusion
In the post genomic area, one is aware that proteomics will be central to the functional genomics efforts. In the field of proteomics, neutrons can be decisive to solving conformations of big biological assemblies. For this purpose, efforts must be devoted to get fully and specifically deuterated biological samples. Fully deuterated C-phycocyanin protein samples have been obtained in big amount from cultures of cyanobacteria in D_2O (Stage of DESS of A. Ould-Ouali, in collaboration with A. Boussac and D. Kourilovsky, SBE, DSV, Saclay). Location of protons and water molecules in a deuterated crystal of C-phycocyanin is now possible (collaboration with ILL) as well as studies of internal slow collective motions in big protein samples using spin-echo techniques.

A new contract for the CRG IN13 is in preparation. This involves the collaboration between LLB, IBS (Grenoble) and INFM (Italy). The CRG IN13 must restart on January 2005.
On January 2003, the Department of Life Sciences of CNRS has renewed (for four years) the GDR-1862 entitled 'Fonction et Dynamique des Macromolécules Biologiques' (Director: M.-C. Bellissent-Funel, Co-Director: J. Parello). In the frame of the GDR successful research activities have been undertaken. The last ones concerned "Journées Thématiques: *Molécules de l'Adhérence Cellulaire: Forces et Dynamique*, (Institut Curie and ENS, Paris, January 2004).
One has to notice the fruitful collaborations in Life Sciences, at the French and European level, with the following organisms: SBE (Service de Bio-Energétique), CEA, Saclay, IBS and ILL, Grenoble; INRA, Nantes; University of Cergy-Pontoise; University of Porto, Portugal; Technical University of Munich, HMI, Berlin.