CENTRE NATIONAL
DE LA RECHERCHE
SCIENTIFIQUE

Laboratoire
Léon Brillouin

LIFE SCIENCES

LABORATOIRE LÉON BRILLOUIN

A - Introduction

Neutron scattering in Life Sciences research emerged 15 years ago and is now playing an important role at the interface between biology, chemistry and physics. With a strong physical chemistry background obtained from studies on confined water, polymer solution and colloids, LLB was ready to tackle biological problems, especially the relationship between the structure and the dynamics of biological macromolecules in relation to the hydration water.

Protein is one of the basic components of food and makes all life possible. Amino acids are the building blocks of proteins. The structure and the specific behaviour of the thousands of proteins acting in living species is one of the major challenges of life science, and understanding of their role is of prime importance for medicine and pharmacology. All of the antibodies, enzymes and many of the hormones in the body are proteins. They are responsible for the transport of nutrients, oxygen and waste throughout the body. They also provide for the structure and contracting capability of muscles, and they provide collagen to connective tissues of the body and to skin, hair and nail tissues. Obviously, living species need proteins which are components of food and make all life possible.

Although X-ray with synchrotron radiation is a very powerful tool for structure determination of proteins, neutron scattering from crystallised proteins brings specific information mainly if a part of the protein is deuterated. A deuteration laboratory has been installed at ILL Grenoble and neutron protein crystallography measurements are mainly performed at ILL.

Research in Life Sciences at LLB focuses on two important aspects of the behaviour of proteins: their conformation in native and denatured states and their dynamics.

One of the challenges facing molecular biology is determining the rules that govern the acquisition by a nascent polypeptide chain of its three-dimensional and functional structure. Rapid progress in genome sequencing has made it all the more urgent to solve this problem. However, although protein folding is an extremely active field of research combining aspects of biology, chemistry, biochemistry, computer science, and physics, the detailed mechanisms of folding are not entirely clear.

All proteins fold in a defined and more or less compact conformation in times shorter than 100 seconds. If proteins randomly sought their lower energy conformation, the folding times would be larger than the universe lifetime. This is the Levinthal paradox. To solve this folding problem, various energy landscapes have been proposed. Studying protein unfolding and refolding is a good way to test all these hypotheses.

A complete understanding of protein folding requires the physical characterization of both native and denatured states and evaluation of the thermodynamic parameters of the system. This involves obtaining information concerning the structure and dynamics of proteins denatured under various conditions (temperature, pressure, chemical denaturant such as guanidinium chloride, pH).

Pressure has been used as a physicochemical perturbation to establish experimental conditions under which a different mechanism of aggregation might occur. The isolation of folding intermediates is crucial to understanding the protein misfolding and protein aggregation that are involved in many diseases (Alzheimer's and Parkinson's diseases, bovine spongiform encephalopathy). Moreover, transition between denatured states occurring under high pressure presents a great interest for the understanding of mechanisms involved into the amyloid diseases (due to beta-sheet formation).

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. Incoherent Quasielastic Neutron Scattering (IQENS) directly probes the internal dynamics of biomolecules on the picosecond time scale, providing information on diffusive motions and the geometry of the motions observed. These two components change significantly during denaturation. IQENS is a dynamic technique complementary to NMR and molecular dynamics simulation.

Beyond the native proteins, we also study the role of hydration water on protein dynamics, the influence of temperature and the effect of different constraints such as the confinement or the pressure, or stimuli (light) or environment (crowding) on either conformations or dynamics of proteins.

Protein denaturation and dynamics are studied by the LLB team, at the Orph_e reactor, by small angle and quasielastic neutron scattering, on the IN13 CRG instrument for which LLB contributes and also at ILL, Berlin, Julich and IPNS. The LLB is equipped with a confocal microscope and with the differential scanning calorimetry. Complementary information from UV visible absorbance spectroscopy, circular dichro_sm and fluorescence techniques is used.

B- Some studies performed at LLB

1 - HYDRATION WATER AND BULK WATER

• *Liquid-liquid transition in interfacial water and role of water in protein dynamics*
Hydration water plays a major role in the stability, dynamics and function of biological macromolecules. Water confined in various systems from model systems (porous hydrophilic Vycor glass) to proteins (lysozyme, C-phycocyanin (CPC)) has been studied. Nanosecond-time-scale measurements of dynamics of interfacial, non-crystalline water from hydrated Vycor have been done from 77 to 280 K. The experimental dynamic results 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. Moreover, we demonstrate that in hydrated lysozymes water dynamics is the driving force governing the slow, long range, protein internal motions that are relevant for protein-function. **[*C1*. J.M. Zanotti]**.

• *Influence of solvent (H_2O and D_2O) on dynamics of a hydrated C-phycocyanin protein*
The influence of the solvent (H_2O and D_2O) on the dynamics of a hydrated C-phycocyanin protein has been investigated. The evolution of the mean-square displacements as a function of temperature is different in H_2O and D_2O which means that the protein dynamic behaviour is different in H_2O and D_2O. Different dynamic transition temperatures are obtained for H_2O and D_2O which was confirmed by results of differential scanning calorimetry **[*C2*, S. Combet]**.

• *A direct determination of H-bond life time in bulk water*
Moreover, the high-Q performances of the spin-echo spectrometer MUSES, that measures the intermediate scattering function I(Q,t), offered the opportunity to study hydrogen-bond dynamics in bulk water by following the dynamics at a Q value where deuterium-deuterium pairs (D-D pairs) contribute significantly. This original procedure allowed a direct determination of H-bond life-time, the temperature dependence of which follows a classical Arrhenius law, while all the transport properties of water exhibit a non-Arrhenius temperature dependence **[*H1*, J. Teixeira]**.

2 - GLOBULAR PROTEINS IN NATIVE STATE

Several globular proteins have been studied so far by IQNS as D_2O-hydrated powders, including C-phycocyanin and parvalbumin. When describing protein dynamics, as biologists do, one has to consider a protein as a system designed for a specific biological function. The specificity of different parts of the protein has to be taken into account. This approach has been developed in the case of parvalbumin. Combined NMR and neutron scattering results suggested that peripheral water-protein interactions influence the protein dynamics in a global manner. We have determined that in the picosecond time range, the essential contribution comes from charged and polar side-chains residues at the protein surface. The formalism developed in the case of parvalbumin in hydrated powders, was subsequently extended to the case of small globular proteins (lysozyme, myoglobin, bovine pancreatic trypsin inhibitor (BPTI), calmodulin) in solution as well as to more complex systems as an enzyme, the aspartate transcarbamylase (ATCase) (J.-M. Zanotti et al, BBA, 2006).

• *Nanosecond dynamics of b-lactoglobulin (BLG) in a H_2O-protonated powder*
It must be noted that the neutron spin echo technique has been successfully used to probe the dynamics of protein. Our purpose was to learn about of the nanosecond dynamics of β-lactoglobulin (BLG) in a H_2O-protonated powder. The performances of the neutron spin-echo spectrometer MUSES allows one to get incoherent intermediate scattering functions (ISF) of hydrated BLG between 275 and 293 K for a Fourier time extending up to 1 nanosecond. From ISF, contributions from hydrogen atoms of surface water and of protein have been obtained. On one hand, the dynamics of the surface water follows a stretched expo-

nential function (the exponent is ~ 0.5), on the other hand, that of protein follows a single exponential function. This is in agreement with results from a photosynthetic C-phycocyanin (CPC) protein [*C3,* **K. Yoshida**] .

3 - UNFOLDED AND FOLDED STATES OF PROTEIN

3A - THERMAL DENATURATION, PRESSURE DENATURATION

Several studies have been carried out on thermal (between 20° C and 95° C) and/or pressure (between 1 bar and 7000 bar) denaturation of protein. The thermal and pressure denaturated states have been characterized on the basis of polymer theory.

• *Effects of temperature and pressure on bovine pancreatic trypsin inhibitor (BPTI) protein*

The structural investigation by small angle neutron scattering allowed us to observe an increase of the radius of gyration of the protein in solution at 95° C and a reduction of this radius under 6000 bar. Quasielastic neutron scattering allowed us to observe an opposite effect of temperature and pressure on translational diffusion coefficient and internal relaxation time of BPTI in solution. Increasing temperature induces a faster dynamics of these global and internal motions whereas increasing pressure induces a slowing down of these motions [*H2,* **M.-S. Appavou,** PhD thesis, 2005].

• *Effect of temperature on apo-calmodulin protein*

The conformations of apo-calmodulin protein have been studied as a function of temperature by SANS experiments. It appears that apo-calmodulin loses progressively its structure between 40° C and 80° C. At high temperature, apo-calmodulin adopts a "polymer-like" conformation (SANS spectrum follows a Debye law for QRg<3), with a radius of gyration of 32 Å. However, the high-Q exponent of 2.3 suggests the existence of residual secondary structures, also seen by circular dichro_sm. Indeed the 2.3 value is between polymer chain values (1.7 or 2) and the compact chain value. The effect of temperature on protein dynamics is under investigation as well as the effect of pressure on both protein forms (apo- and holo-calmodulin) [*C4,* **G. Gibrat**, PhD thesis].

• *Milk* β*-lactoglobulin aggregation under high pressure*

The SANS measurements show that at a pressure value around 150 MPa β-lactoglobulin is characterized by a swollen state. At pressure around 300 MPa the protein begins to form irreversible aggregates. This aggregation occurs between swollen dimeric units of the protein, which is very different from heat-induced aggregation where the resulting gel is formed between unfolded monomeric units. [*H3,* **C. Loupiac**].

3B - TRANSLOCATION

• *Protein refolding*

An original approach has been recently developed to studying protein refolding. It consists to performing *in vitro* translocation of an unfolded protein through nanochannel in lipid bilayer, synthetic nanoporous membranes, nanoporous track-etched PVDF membranes and aligned carbon nanotubes. Measurements of translocation events on single nanopore using fluorescence techniques usually associated with confocal microscopy (FRET or FCS) are now conceivable [*H4,* **D. Lairez**].

4 - CROWDED ENVIRONMENT

• *Influence of crowding on protein unfolding and stability*

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. It is interesting to measure the influence of crowding on protein unfolding and stability. This study is related to recent theoretical predictions of measurable influence of macromolecular crowding on unfolded protein state, with a consequence of stabilization of the folded state. Model systems where the crowding is performed by Ficoll (F70) and the unfolded protein by PEG are under investigation. Contrary to what is generally assumed, chemical interactions between cosolutes (Ficoll) cannot be neglected [*C5,* **S. Longeville**].

• *Phase transition of metastasic extracellular matrix*

An important topic is the understanding of the processes of the tumour dissemination and cell invasion that liquefy the extracellular matrix gel and lead to its degradation. Experimental and theoretical developments have been made with a model system and lead to the conclusion that the gel degradation kinetics is diffusion-limited [*C6,* **D. Lairez**].

5 - PHOTO EXCITATION

• *Dynamics of a photoexcited C-phycocyanin*
In order to get a better understanding of the relationship between the dynamics and function of proteins, we have chosen to study the dynamics of a photoexcited C-phycocyanin, a light-harvesting protein, by synchronising a laser beam with a neutron beam. The aim of this study is to investigate whether dissipation of excitation energy in PC leads to modifications of the protein internal dynamics on longer timescales and larger amplitudes than that of localized vibrations of the protein pigments. The MIBEMOL data acquisition system has been successfully modified to synchronize the laser excitation flashes with the neutron pulses at the position of the sample and get "double beam" relative measurements ("light" and "dark"). This "double beam" procedure is extremely novel and eliminates spurious effects that could occur in the sample during the experiment **[*C7,* S. Combet]**.

6 - FOOD INDUSTRY

• *Model systems of cryoconcentrated sucrose solutions*
Our collaboration with the food industry has been very productive (ENSBANA, Dijon and LLB). The objective of ENSBANA is to enhance the taste, texture or appearance of the food, to produce a product with a longer shelf-life or a healthier image, or to improve manufacturing. In the case of foods colloids, it is especially important to understand how the interfacial and aggregation behaviour of constituents (polysaccharides, proteins, pectins...) are affected by processing conditions or by molecular interactions with other constituents. The first results concern glass transitions of model systems of cryoconcentrated sucrose solutions and combine neutron scattering techniques and calorimetry measurements that are in full agreement: the first transition at -48°ΔC is to be correlated to a dynamic change of the sucrose molecule, whereas the other one seems to be linked to a change of water dynamic. The sharp evolution of <u_> seen at higher temperature (around -10°ΔC) is due to ice melting, which acts like the dilution of the liquid phase **[*C8,* D. Champion]**.

C - Prospects

Our projects concern the continuation of activities about interfacial water, the extension of studies on thermal and pressure denaturation to other proteins, the continuation of studies of protein translocation through different nanoporous media, the continuation of studies on crowded environment (entire cells, extracellular metastasic matrix) and the influence of confinement on protein denatured states, the observation of photo-induced dynamics in protein. The conformation of membrane protein such as water channel in lipid bilayers is now ready to be investigated because of the significant progress in sample preparation. Other objectives deal, on one hand, with the conformation of big biological assemblies, and, on the other hand, with the crystallographic structure of protein using neutron crystallography, which in both cases needs some specific deuteration.

• *Interfacial water dynamics*
As far as interfacial water dynamics is concerned we now need to pursue the two following routes: observing the individual dynamics at times longer than few nanoseconds (low resolution solid NMR experiment, SCM) and accessing the collective dynamic behaviour. In this respect, inelastic X-ray scattering (ESRF) is a promising technique.

• *Photo-induced dynamics in protein*
The studies of the photo-induced dynamics in protein will be extended to other proteins (rhodopsin) as well as to photosynthetic proteins (allophycocyanin, phycoerythrin).

• *The study of conformation and dynamics of membrane proteins*
Membrane proteins are very difficult to purify in large amounts while it is necessary to get concentrated samples for neutron scattering. The AQP1 water channel (membrane protein) has already been purified from human red blood cells in the laboratory, and preliminary experiments are in progress to insert the protein in supported lipid bilayers for neutron reflectivity measurements. New perspectives have now been opened up by the possibility of obtaining recombinant water channels (AQPZ and GlpF) from bacteria *E. coli* in the laboratory, which has just been equipped for bacteria culture (S. Combet).

• *Focussing on conformations of big biological assemblies and accessing protein crystallographic structure by neutron crystallography*
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 obtaining fully and specifically deuterated biological samples. We have already started activities in neutron protein crystallography. For this purpose, fully deuterated C-phycocyanin protein samples have been obtained in big amounts 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 N. Adir, Israel; F. Meilleur, ILL) as well as studies of internal slow collective motions in big protein samples using spin-echo techniques.

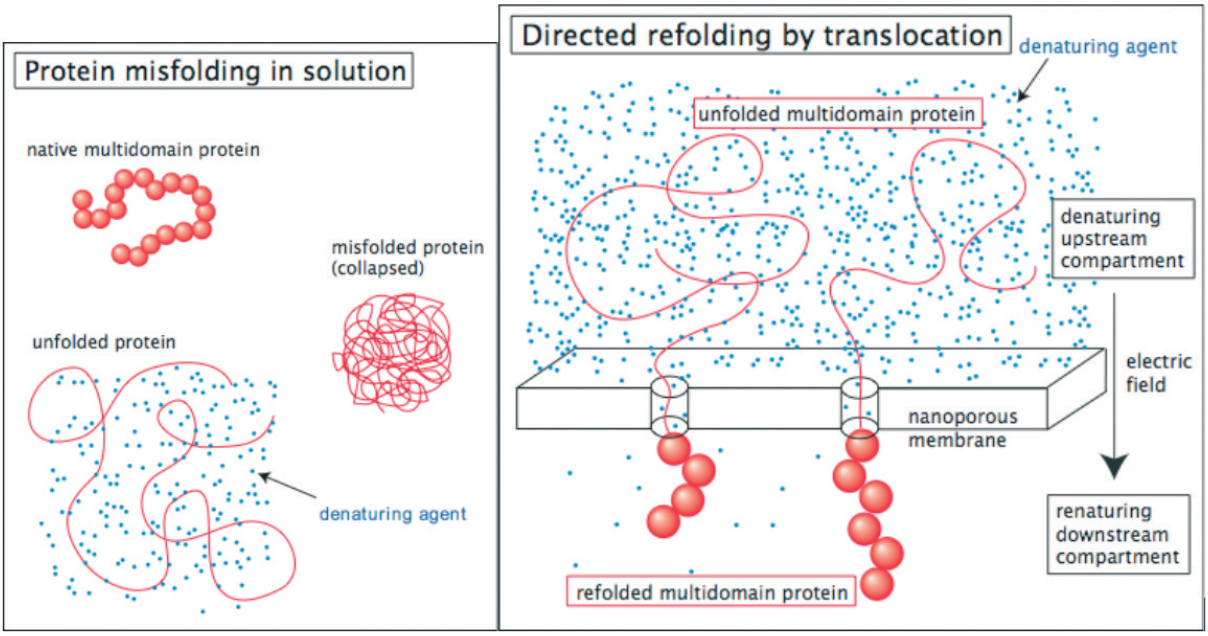
D - Collaborations

A new contract for the CRG IN13 (collaboration LLB, IBS (Grenoble) and INFM (Italy)) has been signed. The CRG IN13 resumed on January 2005.

On January 2003, the Department of Life Sciences of CNRS has been 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 most recent one concerns a School devoted to water in biological media (" l'Eau dans les milieux biologiques ", Roscoff, 25-29 October 2006).

One has to note the fruitful collaborations in Life Sciences, at the French and European level, with the following organisms: SBE (Service de Bio-Energétique), SPEC, CEA, Saclay, IBS and ILL, Grenoble; INRA, Nantes; University of Cergy-Pontoise; University of Porto, Portugal; Technical University of Munich; HMI at Berlin; Institut Curie (Orsay); Inserm, (Kremlin-Bicêtre hospital). At the level of CEA, a new group at the interface between chemistry, physics and biology has just been created. Collaborations involve also SPEC, LSI, University of Evry. A new collaboration in the field of food industry has become operative during the last two years with ENSBANA, Dijon. The long-time collaboration M. Desmadril (IBBMC, Orsay) is still active.

LIFE SCIENCES



H1. Hydrogen-bond dynamics in bulk water
S. Longeville, J. Teixeira

H2. Influence of temperature and pressure on structure and dynamics of a model protein belonging to the regulation of the enzymatic catalysis : the bovine pancreatic trypsin inhibitor : a neutron scattering study
M.-S. Appavou

H3. Milk proteins aggregation under high pressure studied by small angle neutron scattering
C. Loupiac, M. Bonetti, S. Pin, P. Calmettes

H4 Protein refolding and translocation: biology meets nanoscience
D. Lairez, J. Pelta, L. Auvray, O. Cuscito, M.-C. Clochard, M. Mayne-L'Hermite, G. Zalczer

[*C1*. J.M. Zanotti] Evidence that interfacial water is the driving force behind protein dynamics

[*C2*. S. Combet] Influence of hydration solvent on the dynamic transition of phycocyanin

[*C3*. K. Yoshida] Hydration water in dynamics of a hydrated beta-lactoglobulin

[*C4*. G. Gibrat] Thermal denaturation of apo-calmodulin.

[*C5*. S. Longeville] Influence of macromolecular crowding on protein folding and stability: a model for unfolded chain

[*C6*. D. Lairez] Phase transition of metastasic extracellular matrix: theory and experiment.

[*C7*. S. Combet] Dynamics of a photo-excited antenna protein

[*C8*. D. Champion] Glass transitions in cryoconcentrated sucrose solutions.

H1. HYDROGEN-BOND DYNAMICS IN BULK WATER

STÉPHANE LONGEVILLE, JOSÉ TEIXEIRA

Laboratoire Léon Brillouin, CEA-Saclay, 91191 Gif-sur-Yvette, France

Water is a simple molecule made up of three atoms in a V configuration displaying an electronic distribution almost perfectly spherical. However, the properties of the liquid are very complex and not totally understood despite a huge number of experimental and simulation studies. In a very general way, one may say that the so-called "anomalous" behaviour of water derives from hydrogen bonds (HB) which generate an anisotropic potential and strong although fragile inter-molecular forces.

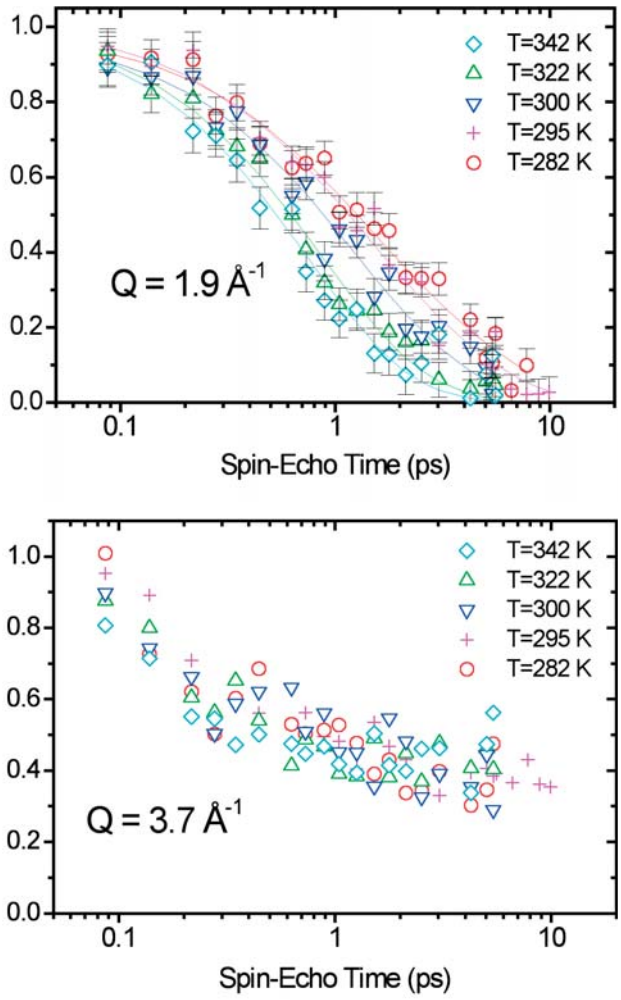
Historically, many experiments tried to catch and understand the topological and dynamic properties of HB and the way they may be related to the thermodynamic and transport properties of the liquid. Alternatively, the remarkable development of simulations of molecular dynamics made very popular "efficient potentials" to describing complex liquids such as water. For most of such potentials, the anisotropy of the potential is indirectly taken into account by the assumption of a molecular anisotropy fixed ad hoc in order to reconstitute, at the best and within classical concepts, the room temperature properties of liquid water. As a consequence, HB are poorly described because they are nothing more than a consequence of Coulombic forces between point charge molecules.

We have been among the experimentalists who, in the past, tried to identify the important role of hydrogen bonds, particularly studying the dynamics of supercooled water, i.e. at temperatures where they represent the determining factor. It is relatively difficult to isolate the dynamics of HB in a way that is, as much as possible, model independent. The performances of the spin-echo spectrometer MUSES, that measures the intermediate function I(Q,t), gave us the opportunity of using an original way to studying HB dynamics in a way that is almost independent of other contributions to the scattered intensity.

We took profit of the good knowledge of the partial structure factors of heavy water (D_2O). Looking into detail to the Q dependence of the three factors, one realizes that, by accident, at Q = 3.7 Å^{-1}, $S_{DD}(Q)$ is the only partial that contributes significantly to the scattered intensity. Consequently, the measurement of I(Q,t) at this value of the momentum transfer, yields a specific information about the dynamics of deuterium atoms directly implied in HB, and without any important contribution of the diffusion movements of the molecular centres of mass. In order to establish a convincing comparison, we measured as well I(Q,t) at Q = 1.9 Å^{-1}, i.e. at the vicinity of the structural peak in S(Q), where the scattered intensity is maximum and all the motions contribute to the signal.

The two main results are depicted on the figure. The two time dependences take place in very different time domains. As expected, the dynamics of the DD pairs is naturally faster than the molecular motions. But, the more important evidence concerns the temperature dependences, which are dramatically different. While at Q = 1.9 Å^{-1} we retrieve the well known non-Arrhenius temperature dependence of all the transport properties of water, at Q = 3.7 Å^{-1} the temperature dependence is much weaker and follows a classical Arrhenius law, demonstrating that, at the level of HB, there is no anomalous temperature dependence. This rather direct experimental determination of HB dynamics in liquid water reminds how important are all studies of bonds in water that can relate the two observed dynamics without calling for analytical, sometimes exotic models.

At this point, one may admit that the glass transition temperature of water (130 K) corresponds to the "freezing" of the motion of hydrogen atoms which remain extremely mobile even under 228 K, a virtual temperature that corresponds to numerical extrapolations of transport properties and that, actually, can be associated to the temperature of homogeneous nucleation of ice. In our view, this experimental result is a strong argument to say that temperatures obtained by extrapolations or from simulations reflect simply the increase of the number of HB with decreasing temperature and the formation of embryos



H2. INFLUENCE OF TEMPERATURE AND PRESSURE ON STRUCTURE AND DYNAMICS OF A MODEL PROTEIN BELONGING TO THE REGULATION OF THE ENZYMATIC CATALYSIS : THE BOVINE PANCREATIC TRYPSIN INHIBITOR : A NEUTRON SCATTERING STUDY

M.-S. APPAVOU

Laboratoire Léon Brillouin, UMR 12 CEA-CNRS, CEA Saclay, 91191 Gif-sur-Yvette. France.

Motions in proteins occur at different time scale from millisecond for enzymatic reaction to femtosecond for electronic transitions. Quasielastic Neutron scattering allows to probe picosecond to nanosecond time scale internal motions [1].Bovine Pancreatic Trypsin Inhibitor is a small protein belonging to the enzymatic catalysis. This protein is a model system because of its small amount of residues (58 amino acid residues) and low molecular weight value (6500 Da), these characteristics allowed molecular dynamic simulation studies [2]. It was also studied by several other techniques : BPTI has a very high stability since it cannot be denatured at temperature below 95°C as it have been shown by Raman spectroscopy [3] or at pressure below 14 kbar as shown by Fourier Transform Infrared spectroscopy [4,5]. This stability is due to the presence of three disulphide bridges and three salt bridges. We have studied the structure and the dynamics of native state and thermal [6] and pressure [7] denatured states of BPTI by neutron scattering technique. For our high pressure study, we used a hydrostatic pressure cell developed at the Laboratoire Léon Brillouin [8].

The structural investigation by small angle neutron scattering allowed us to observe an increase of the radius of gyration of the protein in solution at 95°C and a reduction of this radius under 6000 bar. (Figure 1)
The ellipsoidal shape of the molecule in the native state do not change between 22°C et 95°C but we have observed an increase of the volume of BPTI. Indeed, the shape of BPTI is modified from an ellipsoidal one to a spherical one at 3000 bar, while it is well represented by a micelle when applied pressure values reach 5000 and 6000 bar. (Figure 2)
Further experiments by infrared spectroscopy and by UV-visible spectroscopy as a function of temperature and pressure allowed us to confirm our results [6].
Quasielastic neutron scattering allowed us to observe an opposite effect of temperature and pressure on translational diffusion coefficient and internal relaxation time of BPTI in solution (Figure 3). Increasing temperature induces a faster dynamics of these global and internal motions whereas increasing pressure induces a slowing down of these motions.

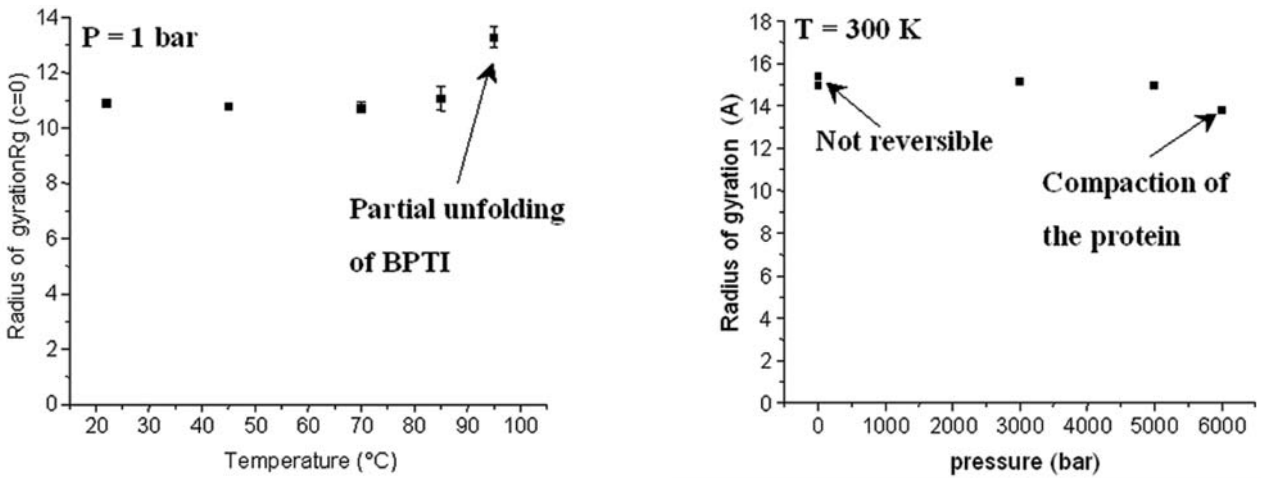


Figure 1 : Evolution of the radius of gyration of BPTI as a function of pressure (left) and as a function of temperature (right).

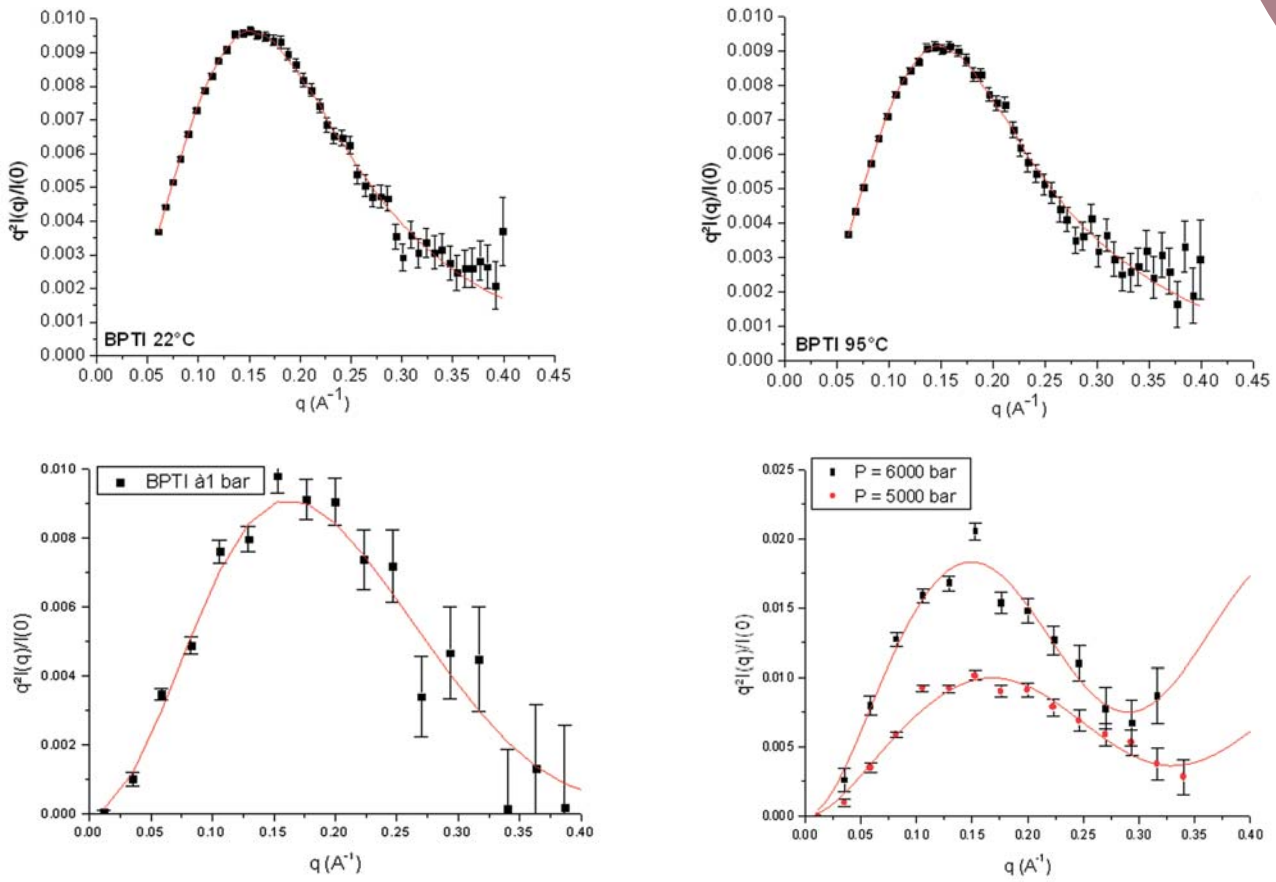


Figure 2 : Kratky plot of SANS spectrum for BPTI at ambient and high temperature (top) and at atmospheric pressure and high pressure (bottom).

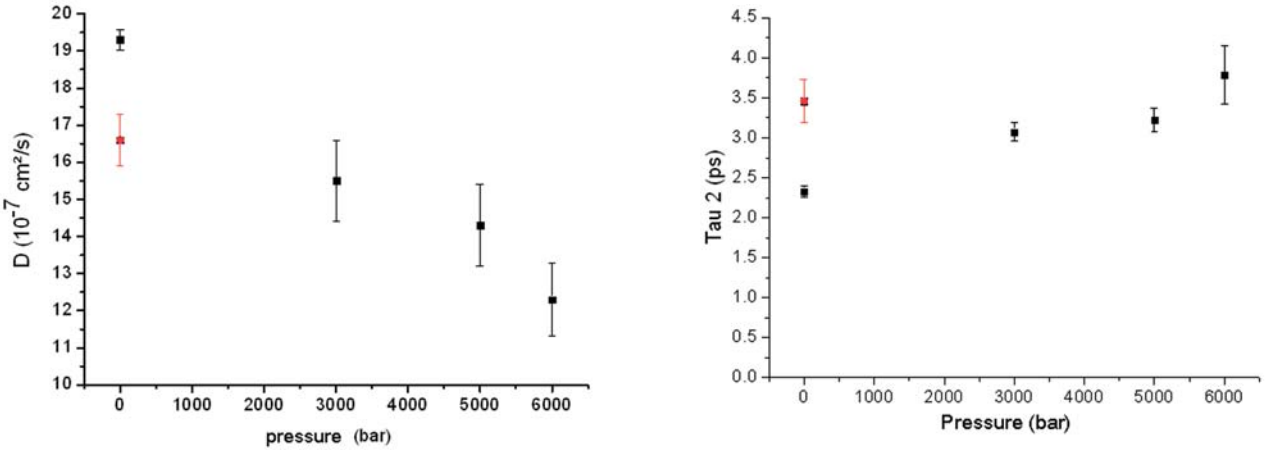


Figure 3 : Effect of pressure on global (left) and internal motions (right)

[1] McCammon J. A. & Harvey S. C., *Dynamics of proteins and nucleic acids*, Cambridge, University Press, 1987.
[2] Hayward JA., Finney JL., Daniel R.M., et Smith JC., *Biophys. J.*, **85**, 2003, pp 679-685.
[3] Carmona P, Molina M, Rodriguez-Casado A, *Eur Biophys J*, **32**, 2003, pp137 –143
[4] Goosens K, Smeller L, Frank J, Heremans K, *Eur. J. Biochem.*, **236**, 1996 , pp 254-262,
[5] Takeda N, Nakano K, Kato M, Taniguchi Y, *Biospectroscopy*, **4**, 1998, pp 209-216
[6] Appavou M.-S., Gibrat G., Bellissent-Funel M.-C., *BBA.*, in preparation
[7] Appavou M.-S., Gibrat G., Bellissent-Funel M.-C., *BBA.*,**1764**(3), 2006, pp 414-423
[8] Appavou M.-S., Gibrat G., Bellissent-Funel M.-C., Plazanet M, Pieper J, Buchsteiner A, and Annighöfer B, *J. Phys.: Condens. Matter* **17**, 2005, S3093-S3099.

H3. MILK PROTEINS AGGREGATION UNDER HIGH PRESSURE STUDIED BY SMALL ANGLE NEUTRON SCATTERING

C. LOUPIAC[1], M. BONETTI[2], S. PIN[3], P. CALMETTES[4]

[1]-Equipe d'Ingénierie Moléculaire et Sensorielle des Aliments et des Produits de Santé, ENSBANA, Dijon
[2]- Service de Physique de l'Etat Condensé, CEA, Saclay
[3]- Service de Chimie Moléculaire, URA 331 CNRS, CEA, Saclay
[4]- Laboratoire Léon Brillouin, UMR 12 CNRS, CEA, Saclay

The food scientist is commonly confronted with the challenge of modifying the formulation of a food product. The objective may be to enhance the taste, texture or appearance of the food, to obtain a product with longer shelf-life or healthier image, or to improve manufacturing efficiency by incorporating cheaper ingredient or adopting a new processing technology. The speed with which these objectives can be accomplished depends on the level of fundamental understanding that exists on the key physico-chemical factors affecting products properties. In the case of foods colloids, it is especially important to understand how the interfacial and aggregation behaviour of polymer constituents (polysaccharides, proteins, pectins...) are affected by processing conditions (heat, drying, freezing, shear forces), or by molecular interactions with other constituents (fat, hydrocolloids, aroma, water...). One of our goal is to improve insights into such factors by taking advantage of polymer science concepts and neutron scattering technique applications to such systems, to the systematic study of model food systems [1].

In foodstuffs, proteins are very often used for their functional properties. Most of the time their abilities to act as emulsifiant, gelation or foaming agents, are related to their structure. Processing foods under high pressure often results at the molecular level in structural changes of the protein [2]. Experimental and theoretical approaches indicate that one of the underlying mechanism of pressure unfolding is the penetration of water into the protein, several intermediate states of the protein have been shown to exist, with their properties depending on the experimental conditions [3]. The isolation of folding intermediates is crucial to understand protein misfolding and protein aggregation. β-lactoglobulin (BLG) is the main protein constituent of the milk whey from ruminant. This protein is an important functional protein in foods, as it is the major component of many dairy gel and emulsions. A basic challenge of this study was to better understand the mechanism of pressure unfolding, dissociation and aggregation of BLG. We used pressure as a physicochemical perturbation to establish experimental conditions under which a different mechanism of aggregation might occur. From the small-angle neutron scattering (SANS) measurements the overall conformation of the β-lactoglobulin was studied at pH 7 on the dimeric form of the protein in a pressure range going from 50 to 300 MPa. These measurements were done "on-line" by gradually increasing the pressure. We can determine whether the dissociation of the dimeric units occurs and if the aggregation mechanism involves the monomeric form of the protein. To determine the pressure effects on the protein interactions and the variation of the value of the actual radius of gyration, the SANS measurements were performed at different protein concentrations.

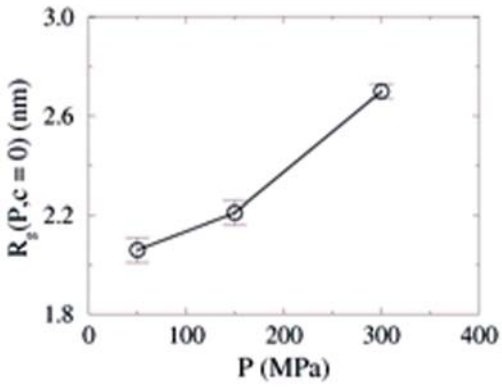


Figure 1. Value of the actual radius of gyration, Rg (c = 0) as function of pressure, P. Measurements as been made on PACE spectrometer. The range of the wave-number was between 0.07 and 0.74 nm^{-1}. A sapphire–anvil cell specially designed to perform SANS measurements has been used (M. Bonetti, SPEC, CEA, Saclay)

One of the questions addressed in this work was to know whether or not the aggregation process induced by applying pressure involves the dimeric unit or monomeric unit of the protein. This has been answered by the analysis of the evolution of the radius of gyration as a function of applied pressure (Figure 1). Our analysis shows that no dissociation of the dimmer occurs in the 50-150 MPa pressure range as our measured radius of gyration (Rg = 2.20 nm) is far away from the monomeric form (Rg = 2.06 nm). Increasing pressure up to 150 MPa leads to a swollen state of the protein that gives rise to an increase of the radius of gyration by about 7 %.

The measurements show an aggregation process occurring above 150 MPa, irreversible aggregates are formed at pressure around 300 MPa. This aggregation occurs between swollen dimeric units of the protein, which is very different that for heat-induced gel that occurs between unfolded monomeric units. Different parameters could lead to this swollen state of the protein after applying pressure: hindrance of water inside the protein matrix and/or change in the hydrogen bonds network and/or breaking down the electrostatic bonds and some of the protein hydrophobic interactions. Within this pressure range, the observation of the second virial coefficient (A_2) indicates that the interaction between macromolecules weakens although it remains repulsive (Figure 2).

It can be stated that a pressure value around 150 MPa leads to a swollen state of β-lactoglobulin and that at pressure around 300 MPa the protein begins to form irreversible aggregates. In the future it will be interesting to see the repercussion of this aggregation between dimeric units on the gels properties (rheological and neutron scattering studies).

Biochimica and Biophysica Acta, 1764, 2006, p211-216

[1] Gimel J-C., Durand D., Nicolai T., *Macromolecules*, 1994, **27**, 583-589.
[2] Silva J.L., Foguel D., Royer C.A., *Trends Biochem. Sci.*, 2001, **26** (10), 612-618
[3]Loupiac C., Bonetti M., Pin S., Calmettes P., *Eur. J. Biochem.*, 2002, **269**, 4731-4737

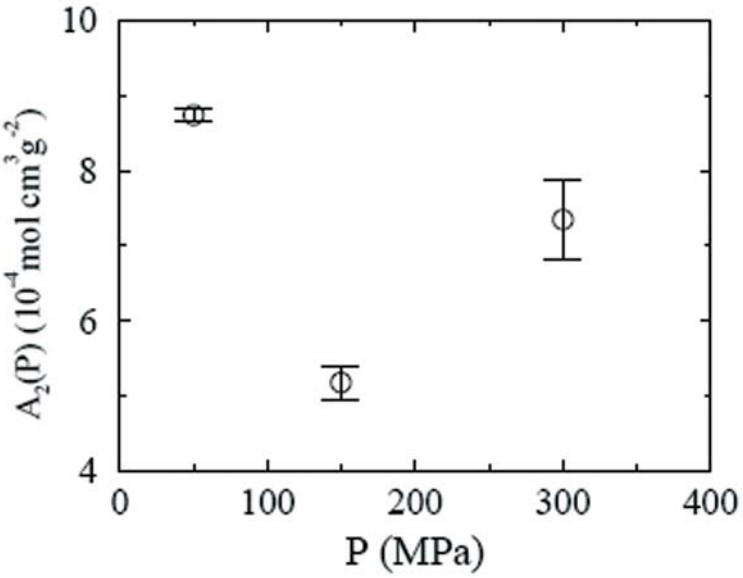


Figure 2. Second virial coefficient as function of pressure

H4. PROTEIN REFOLDING AND TRANSLOCATION: BIOLOGY MEETS NANOSCIENCE

D. LAIREZ[1], J. PELTA[2], L. AUVRAY[3], O. CUSCITO[4], M.-C. CLOCHARD[4], M. MAYNE-L'HERMITE[5], G. ZALCZER[6]

[1] Laboratoire Léon Brillouin, CEA-CNRS UMR-12, CEA-Saclay, 91191 Gif-sur-Yvette cedex
[2] Groupe microenvironnements et comportements cellulaires, Université de Cergy-Pontoise, 95302 Cergy-Pontoise
[3] Laboratoire Matériaux Polymères aux Interfaces, CNRS-UMR 7581, Université d'Evry, 91025 Evry
[4] LSI, Ecole Polytechnique, CEA-DSM, CNRS, 91128 Palaiseau cedex
[5] Laboratoire Francis Perrin, URA-2453, DSM/DRECAM/SPAM, CEA-Saclay, 91191 Gif-sur-Yvette cedex
[6] Service de Physique de l'Etat Condensé, CEA-Saclay, 91191 Gif-sur-Yvette cedex

The understanding of protein folding is a central problem in present post-genomic biology. On one hand, protein misfolding is involved in many diseases: Alzheimer's and Parkinson's diseases, bovine spongiform encephalopathy... On the other hand, in many cases recombinant protein synthesis comes up against the formation of inclusion bodies. These inclusion bodies are solubilized using a denaturing agent such as urea or guanidinium chloride. Then *in vitro* refolding difficulties are often encountered, more particularly for high molecular weight and multidomain proteins. Current view of protein folding involves a minimum of free energy pathway through the conformational energy landscape. To progress in the understanding of protein folding, this paradigm needs to be overcome. Actually *in vivo*, the nascent protein folding mechanism, as well as unfolding-translocation-refolding cycles observed in many cases, suggests that sequential refolding is a key feature. Sequential refolding means that one extremity of the peptide chain begins to refold without the knowledge of the remaining peptide chain sequence. This is the key point we try to mimic *in vitro*.

Our approach consists in studying protein refolding by performing *in vitro* translocation (see Fig. 1), developing techniques to measure and control translocation time and developing nanoporous media adapted to this application. To this end, different strategies are investigated.

Protein translocation through single protein nanochannel in lipid bilayer is studied. Here, nanochanel is α-Hemolysin from Staphylococcus Aureus that has been already used for DNA translocation. This single molecule experiment allows patch-clamp technique to be used for measurement of translocation events (frequency of events, duration of a single event, see Fig. 1).

Ref. 1 reports the first experiment concerned with *in vitro* translocation of an unfolded protein, Maltose Binding Protein, that has the ability to be unfolded at low concentration of denaturing agent ([Gdm-HCl ~1 M) leaving intact the proteic nanochannel. This result demonstrates translocation feasibility in the case of unfolded protein and opens up to new means of investigation for unfolding-refolding mechanism.

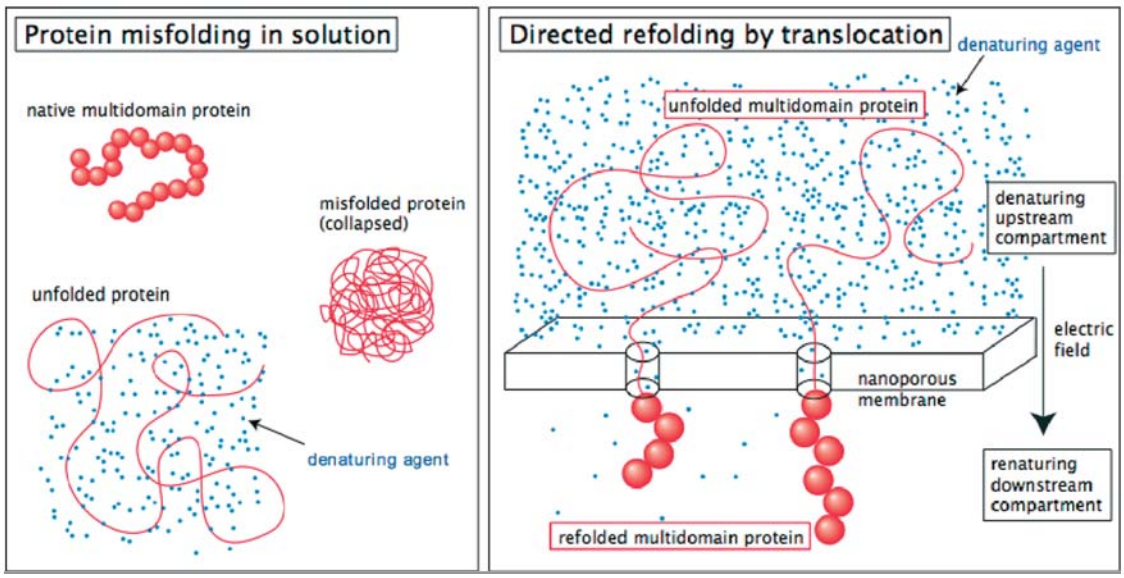


Figure 1. Protein refolding directed by in vitro translocation. An adequate translocation velocity should favour a correct refolding of multidomain proteins.

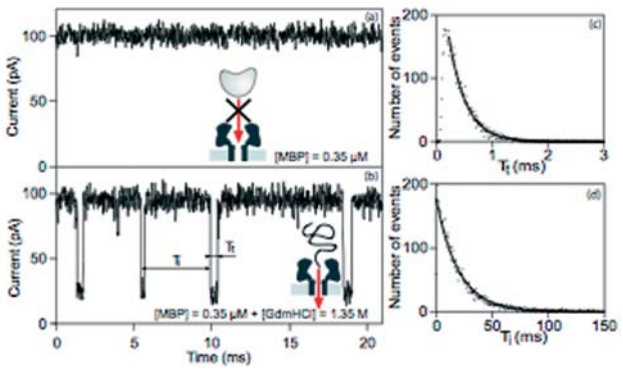


Figure 2 (from Ref.1). Current traces through a-Hemolysin (at100mV) in presence of Maltose Binding Protein (MBP) at 0.35µM. Left: a) with denaturing agent, native protein cannot passes through the nanochannel. Measured current is constant (100 pA). b) with denaturing agent ([GdmHCl=1.35M), MBP is unfolded the current trace decreases down to 20 pA when a molecule is in the pore. Right: c) Distribution of translocation times. d) Distribution of time intervals between two events.

Protein nanochannels technique knows limitations: fragility to osmotic gradient that should be necessary to direct the refolding of translocated proteins; low frequency of translocation events (single pore) leading to production of small quantities... For these reasons, synthetic nanoporous membranes have to be preferred and specially designed for this application. Two ways are currently investigated.

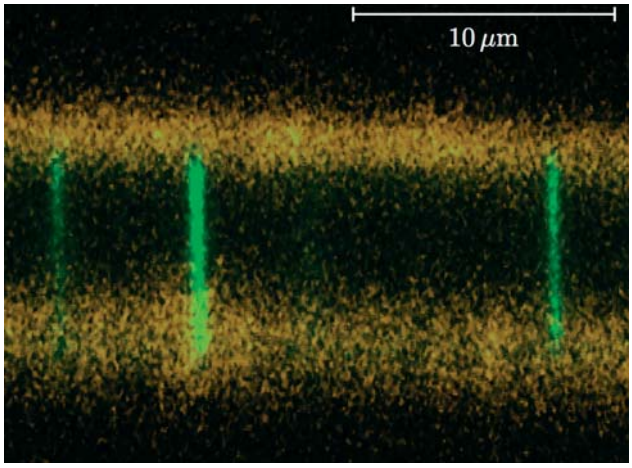


Figure 3 (from Ref.2). PVDF membrane obtained by heavy ion irradiation and ion-track etching. Radicals that persist in nanopores after etching allows us a selective radiografting of poly(acrylic acid). Then a selective pore labelling with fluorophore is possible. The image the *xz*-plan cross-section of the membrane obtained by confocal laser scanning microscopy.

Nanoporous track-etched PVDF membranes are obtained by heavy ion irradiation of 9 µm thick PVDF films and track-etching. Nanopores have a nice straight cylindrical shape that has been evidenced by Small Angle Neutron Scattering. Recently, radiographting of poly(acrylic-acid) has been selectively driven at the surface of pore-walls. This chemical modification allows membrane pores to be selectively labelled with fluorescence molecules and then to be imaged by confocal laser scanning microscopy (see Fig. 3 and ref. 2). With so prepared membranes, measurement of translocation events on single nanopore using fluorescence techniques usually associated with confocal microscopy (such as Fluorescence Resonance Energy Transfer, or Fluorescence Correlation Spectroscopy) are now conceivable.

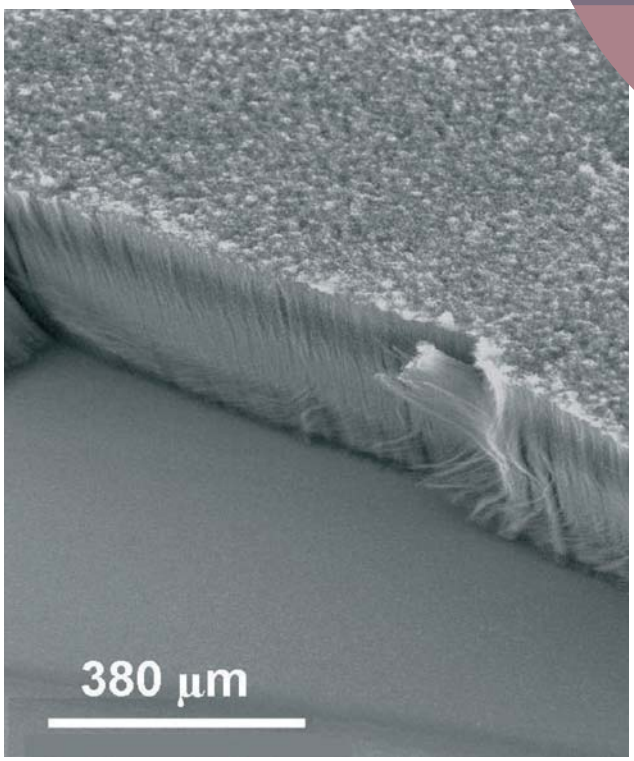


Figure 4 (from Ref.3). Scanning Electron Microscopy image of aligned carbon nanotubes (a-CNT) synthesized on silicon substrates by aerosol-assisted catalytic chemical vapour deposition from toluene/ferrocene aerosol. The a-CNT obtained are multiwalled (] M. Pinault *et al.*, Nano Lett., 5, 12, 2394-2398 (2005).

Aligned carbon nanotubes (nanotubes carpet see Fig. 4) are suitable to elaborate polymer-based composites in which the nanotube fillers exhibit a unidirectional orientation. After impregnation with polystyrene or epoxy-resin, a thinning procedure with a polishing device is performed to adjust the thickness of the composite and to open nanotube ends. Nanoporous membranes of 100-200 µm tihckness are so elaborated with the hollow central channels of nanotubes as nanopores (Ref. 3). With respect to our application, the permeation characteristics of these membranes are very interesting with a monodisperse internal diameter of the order of 7 nm (that can be adjusted) and an optimal pores density of the order of 10^{10} tubes/cm^2.

[1] G. Oukhaled, J. Mathé, A.-L. Biance, L. Bacri, J.-M. Betton, D. Lairez, J. Pelta, L. Auvray. "Unfolding of proteins and long transient conformations detected by single nanopore recording". Submitted in Phys. Rev. Lett.

[2] O. Cuscito, M.-C. Clochard, S. Esnouf, N. Betz, D. Lairez. "Nanoporous PVDF membranes with selectively functionalized pores". Submitted for publication in Nuclear Instr. Meth. Phys. Res. B

[3] S. Barrau, M. Mayne-l'Hermite, D. Lairez, C. Reynaud. "Control of aligned carbon nanotubes synthesis parameters for elaboration of nanoporous membranes". Submitted for publication in Chem. Phys. Lett.

[C1. J.M. Zanotti] Evidence that interfacial water is the driving force behind protein dynamics

The atomic scale behaviour of water as a monolayer on a porous silica glass is the result of a subtle coupling of local rotational and long range translational dynamics. We have been able to discriminate between these two contributions to shows that interfacial water experiences a glass transition at 165 K and a liquid-liquid transition at 240 K from a low-density to a high density-liquid. This unusual behaviour, compared to the bulk, is due to a strong weakening of the hydrogen-bond strength when water molecule lay in a 2D situation.

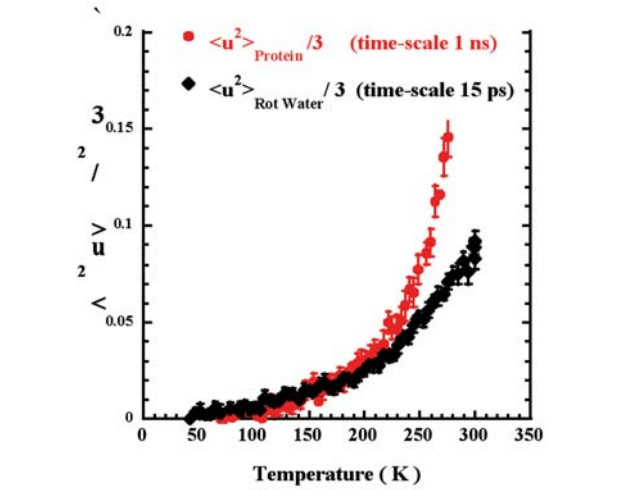


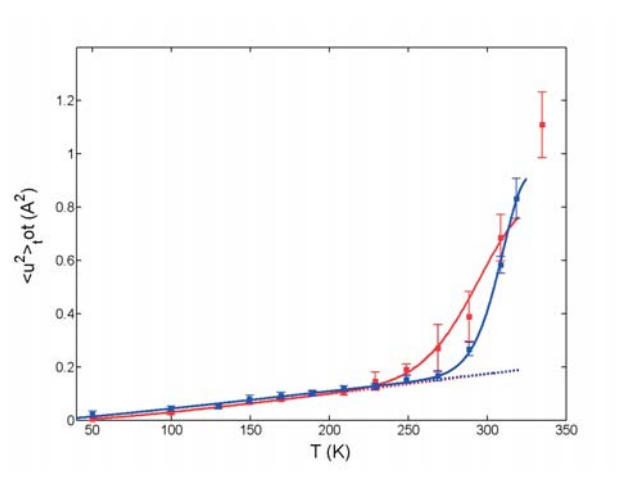
Figure 1. The atomic scale behaviour of water is the result of a subtle coupling of local rotational and long range translational dynamics. Here we show the temperature dependence of (i) $\langle u^2 \rangle_{\text{Rot Water}}$ the short time scale (15 ps, i.e. 80 μeV) rotational mean-square displacements of interfacial water and (ii) $\langle u^2 \rangle_{\text{Protein}}$ the hydrogen atom mean-square displacement of lysozyme hydrated with a monolayer of D_2O. We observe a strong correlation between the local reorientational transition in interfacial water at 220K and the onset of the long time (1 ns i.e. 1 μeV) large amplitude over-damped motions responsible for the $\langle u^2 \rangle_{\text{Protein}}$ to increase above 220K. The observed correlation suggests that water dynamics is the driving force governing the protein-function-relevant slow, long range, protein internal motion.

The well-known protein dynamical transition is clearly visible at about 220 K (Fig.1) and is strongly correlated, to the onset of short time-scale reorientational fluctuations that initiate structural rearrangements within the transient H-bond network of interfacial water surrounding the protein. This result seems to be the first experimental evidence supporting a possible mechanism controlling protein dynamics. Within the framework of this model, the protein external side-chain short time motions, induced by fast water reorientational motion ($\langle u^2 \rangle_{\text{Rot water}}$ Fig.1), propagate in a hierarchical way, along the protein structure from the residue side chains down to the protein core to induce the longer timescale protein backbone motion necessary for its function.

[Collaboration : J.M. Zanotti, M.C. Bellissent-Funel (LLB), Chen (MIT) and Kolesnikov (ANL/IPNS), J. Phys.: Condens. Matter 18 S2299–S2304 (2006)].

[C2. S. Combet] Influence of hydration solvent on the dynamic transition of phycocyanin

Phycocyanin (PC) is a light-harvesting protein present in the antenna of cyanobacteria, where it is involved in the first steps of photosynthesis. This protein, which can be fully deuteriated, has been used as a model to study hydration water dynamics at protein surface by neutron scattering. The aim of the present study was to compare the influence of hydration solvent (H_2O and D_2O) on the dynamics of PC by elastic neutron scattering. Samples of hydrogenated PC powder have been hydrated in H_2O (0.4 g/g PC) or D_2O (0.46 g/g PC) to obtain one similar monolayer of water molecules at the protein surface. Neutron elastic scattering spectra have been analysed by the double well-model. Evolution of the mean square displacements, as well as of associated thermodynamics values, was significantly different along the entire temperature range (20-320 K) between PC hydrated in H_2O and PC hydrated in D_2O. Dynamic transition temperatures between harmonic and anharmonic modes were, respectively, 220 ± 10 K and 270 ± 20 K for PC in H_2O and PC in D_2O. Differential microcalorimetry measurements confirmed these data with different slopes and vitreous transition temperatures between PC hydrated in H_2O (220 K) and PC hydrated in D_2O (235 K).



Mean square displacements of PC hydrated with 0.4 g H_2O/g PC (red) and PC hydrated with 0.46 g D_2O/g PC (blue).

[Collaboration: S. Combet, G. Gibrat, M.-C. Bellissent-Funel, LLB; M. Tehei, ILL].

[C3. K. Yoshida] Hydration water in dynamics of a hydrated beta-lactoglobulin

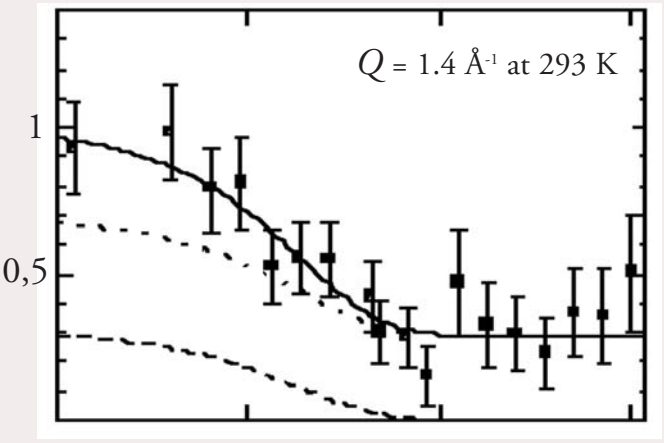


Figure 1. Typical intermediate scattering functions I(Q, t) / I(Q, 0) at Q = 1.4 Å^{-1} at 293 K. The solid line indicates the fitting results by the KWW equation. The upper and lower dashed lines are contributions from the protein and the surface water, respectively

It is well-known that water plays an important role in protein folding and function of proteins. Although computer simulation is a powerful tool to investigate the dynamics of protein and hydrated water, its result depends on the interatomic potential used in the calculation. Therefore, it is important to verify simulation results by comparing the calculated intermediate scattering function I(Q,t) with that obtained from experiment. In the present study, incoherent spin echo signals of a hydrated β-lactoglobulin protein were measured at 275 and 293 K. In the measured protein, ~69 % of water exists on the surface of the protein and the rest remains as the bulk. The intermediate scattering functions were divided into two contributions from surface water and protein, respectively, as shown in Figure 1. On one hand, the dynamics of the surface water follows a Kohlrausch-Williams-Watt (KWW) stretched exponential function (the exponent is ~0.5), on the other hand, that of the protein follows a single exponential. The behavior of elastic incoherent structure factor (EISF) as a function of Q shows the feature of the confined diffusion. The present results are consistent with our previous results of hydrated C-phycocyanin combining elastic and quasielastic neutron scattering and by molecular dynamics simulation. Moreover, the behavior of surface water is similar to that of water confined in hydrophilic porous materials. We can stress that water confined in hydrophilic porous materials is an adequate model to investigate water in biomolecules.

[Collaboration : K. Yoshida, T. Yamaguchi, Fukuoka Univ. , Japan; M.-C. Bellissent-Funel, S. Longeville, LLB]

[C4. G. Gibrat] Thermal denaturation of apo-calmodulin

Calmodulin is a small (16.7 kDa) calci-protein (a protein that can fix calcium ions) that is well adapted to neutron scattering experiments. Indeed, it allows reaching concentrations of about 100 g/L in physiological-like conditions (pH 7.5 and [KCl] ~ 100mM) without any aggregation. Moreover calmodulin is made of two N- and C-terminal domains, with a 70% sequence homology, linked by a central _-helix. Despite the high sequence homology, these two domains show significantly different stabilities (about 10°C difference in thermal denaturation temperatures). It is so an interesting system to study the sequence-folding relationship.

From fluorescence, circular dichroïsm and UV absorption spectroscopy experiments, it is quite clear that for apo-calmodulin (calmodulin without calcium) thermal denaturation occurs at least in two steps, corresponding to the successive unfolding of the two N- and C-terminal domains (respectively Tm=63°C and Tm=51°C). Holo-calmodulin (calmodulin with calcium) is stable up to 100°C. From SANS measurements, it appears that apo-calmodulin looses progressively its structure between 40°C and 80°C. At high temperature, apo-calmodulin adopts a "polymer-like" conformation (SANS spectrum follows a Debye law for QRg<3), with a radius of gyration of 32Å. However, the high Q exponent of 2.3 suggests the existence of residual secondary structures, also seen by circular dichroïsm. Indeed the 2.3 value is between polymer chain values (1.7 or 2) and the compact chain value (4).

[Collaboration :G. Gibrat, LLB; G. Hui Bon Hoa, Inserm U473; Y. Blouquit, Inserm U759/Institut Curie-Orsay; C. Craescu, Inserm U759/Institut Curie-Orsay; M-C. Bellissent-Funel, LLB]

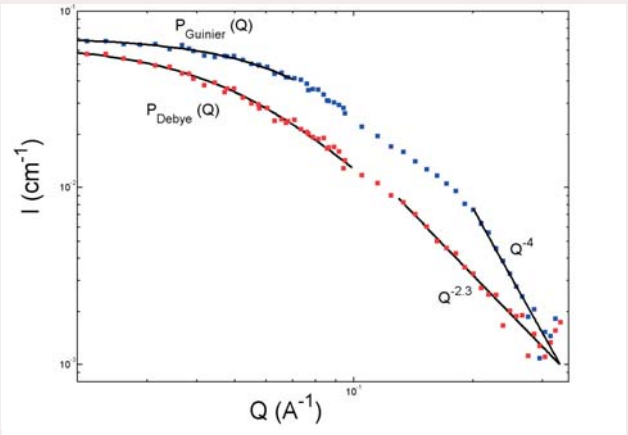


Figure 1. SANS spectra of native apo-calmodulin (in blue) and of calmodulin at 80°C (in red)

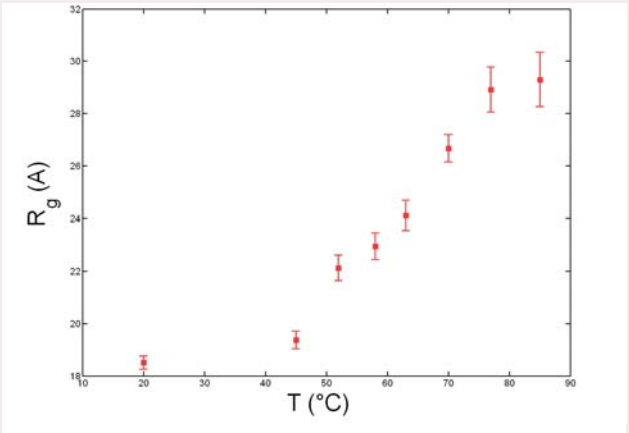


Figure 2. Radius of gyration of apo-calmodulin at 5g/L as a function of temperature

[C5. S. Longeville] Influence of macromolecular crowding on protein folding and stability: a model for unfolded chain

The cytoplasm, the interior of cells, is filled with a very high quantity of objects with respect to shape and size. In most of the cases, each species is present at rather low concentration but the overall occupied volume fraction can reach Φ~0.3-0.4. The term "crowding" is generally used to describe this environment rather than high concentration which appears less appropriate in this context.

Under crowding environment protein-protein interactions play a fundamental role because the distances between molecules are of the order of few tens of Å. The crowding environment can affect some physical, chemical or biological properties of biological macromolecules [1][2]. The structure and the reactivity can be very strongly modified as a function of inert crowding agents. A particularly interesting aspect concerns the effect of crowding on protein folding and stability. Usually protein folding is studied *in-vitro* at very low concentration. Under such conditions small globular single chain proteins can unfold and refold quite rapidly depending mainly on the nature of the solvent.

The aim of our project is to search for the possible differences between the process of protein folding/unfolding studied *in-vitro* where the protein are very diluted and surrounded by solvent only and the mechanism *in-vivo* where proteins are in very crowded environment.

Theoretically the problem was studied by the introduction of the concept of excluded volume [3]. In a recent paper [4], Minton uses a statistical thermodynamic model to address the question. He predicted that inert cosolutes stabilize the native state of proteins against unfolding mainly by destabilizing the unfolded state and that the dimension of the unfolded state decreases with increasing the concentration of solute in a measurable way.

In a first series of experiment we have measured by SANS the effect of a classically used inert co-solute F70 on the conformation of a deuterated polymer (PEG). We choose a solvent mixture of D_20 and H_20 at the matching point of the F70 in order to observe only the polymer in good solvent, assumed to be a model for an unfolded chain [5].

On the contrary to what is generally assumed chemical interactions can not be neglected leading to partial segregation of the two components but we show that the polymer density is increase when adding F70.

[Collaboration: S. Longeville, LLB, B. Demé ILL]

[1] R. J. Ellis, Trends in Biochem. sciences **26** (2001) 597-604. [2] A. P. Minton, The J. of Biol. Chem. **276** (2001) 10577-10580. [3] Zhou Y. and C. K. Hall, Biopolymers **38** (1996) 273-284. [4] A. P. Minton, Biophysical J. **78** (2000) 101-109. [5] P. Calmettes et al, Biophysical Chemistry **53** (1994) 105-114

[C6. D. Lairez] Phase transition of metastasic extracellular matrix: theory and experiment

The extracellular matrix is a gel made of various macromolecules that isolates organs. In tumour dissemination, invasive cells liquefy the extracellular matrix gel by producing proteolytic enzymes. We study the physical aspects of their actual role in cell invasion: proteinases by hydrolyzing peptide bonds between gel crosslinks, catalyze a phase transition from a gel and solid state to a liquid [1]. A key feature has to be considered: *in vivo*, the enzyme concentration range is so small that enzymes must diffuse within the gel to significantly damage it. Enzyme diffusion introduces space correlations and then controls the gel degradation mechanism [2] and its universality class [3].

Recently [4], a model system consisting in gelatin (denatured collagen) and thermolysin as proteinase was studied at different gel volume fraction, ϕ_{gel}, and enzyme concentration, *[E]*, and varying the solvent viscosity η_r. The degradation time t_c varies as:

$$t_c \mu \eta_r \times \phi_{gel}^{2.50\pm0.05} \times [E]^{-1.46\pm0.07}$$

This result provides clear evidence, which was missing until now, that the gel degradation kinetics is diffusion-limited. We propose a scaling argument and reduced variables for anomalous enzyme diffusion that fully account for experiments. Plotting $[E]\xi_0^3$ as a function of t_c/τ_0 with ξ_0 the mesh size of the gel network and τ_0 the diffusion time of enzyme over this length, allows us to obtain a single master curve independent of ϕ_{gel}. This scaling argument is consistent with self-attracting memory effect on enzyme random walk, i.e. enzyme has some facilities for going back in previously visited area (already damaged gel) rather than for exploring new area (intact gel).

This self-attracting random walk leads to a "Swiss Cheese" model for gel degradation that belongs to the continuum percolation class.

[Collaboration: D. Lairez, LLB, G. Zalczer and J.-P. Carton SPEC/DRECAM/DSM, J. Pelta, Université de Cergy-Pontoise]

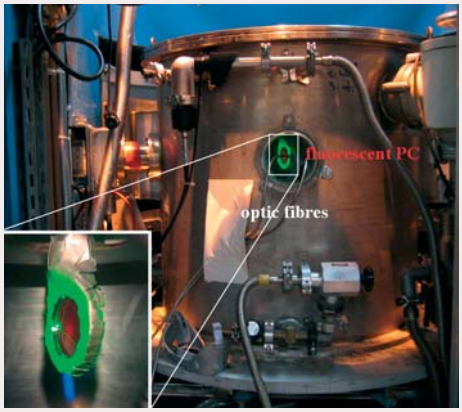
[1] H. Berry, J. Pelta, D. Lairez, and V. Larreta-Garde. Biochem. Biophys. Arch., 1524:110–117, 2000. [2] G. C. Fadda, D. Lairez, B. Arrio, J. P. Carton, and V. Larreta-Garde. Biophys. J., 85:2808–2817, 2003. [3] T. Abete, A. de Candia, D. Lairez, and A. Coniglio. Phys. Rev. Lett., 93:228301, 2004. [4] D. Lairez, J. P. Carton, G. Zalczer, and J. Pelta submitted for publication in Phys. Rev. Lett., 2006.

[C7. S. Combet] Dynamics of a photo-excited antenna protein

Phycocyanin (PC), a blue protein present in the light-harvesting system of cyanobacteria, plays a key role in the first steps of photosynthesis. For the isolated PC, one part of captured light energy is dissipated *via* emission of fluorescence and the other part is dissipated by fast and localized dynamics of the pigment-protein complex. The aim of this project is to investigate whether dissipation of excitation energy in PC leads to modifications of the protein internal dynamics on longer timescales and larger amplitudes than that of localized vibrations of the pigments.

We measured photo-induced dynamics of PC on MIBEMOL time-of-flight spectrometer (LLB) with a pulsed Nd:YAG laser (λ = 532 nm, 5 ns pulses) at different energy and frequency values (20 mJ and 14 Hz max.) to illuminate *via* optic fibres both sides of the sample (130 g/L of hydrogenated PC solubilized in 20 mM Na_2DPO_4). MIBEMOL data acquisition system has been successfully modified to synchronize the laser excitation flashes with the neutron pulses at sample position and get "double beam" relative measurements ("light" and "dark"). This "double beam" procedure is extremely novel and eliminates spurious effects that could occur in the sample during the experiment. We used an aluminium sample holder surrounding sapphire glasses, which exhibit a much lower neutron scattering than quartz glasses. In a preliminary experiment, a difference of 3% between PC illuminated and PC in the dark has been observed in the maximum of elastic scattering peak, with the "low energy" mode of the laser (pulses ~ 110 µs similar to the duration of the neutron pulses ~ 87 µs). Further experiments will take place very soon to improve this device (laser wavelength closer to the maximum absorption (620 nm) of PC, integrating sphere to illuminate the sample inside uniformly) and also to get reasonable statistics.

[Collaboration: S. Combet, J.-M. Zanotti, M.-C. Bellissent-Funel, LLB; J. Pieper, TU-Berlin].



Side view of the sample environment on MIBEMOL time-of-flight spectrometer during illumination experiments on PC. Insert: fluorescence of the PC (red color) in the sample holder with the green laser beam reflected on the cadmium mask.

[C8. D. Champion] Glass transitions in cryoconcentrated sucrose solutions.

Since it drives the rate of diffusion limited bio-chemical reactions, the glass transition temperature is a key parameter for frozen food conservation. DSC signal of a maximally cryo-concentrated sucrose solution (50% sucrose mass fraction), considered here as a model system, shows a two steps baseline shift (Figure 1a).

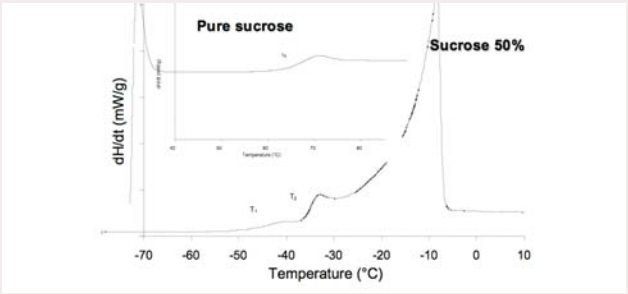
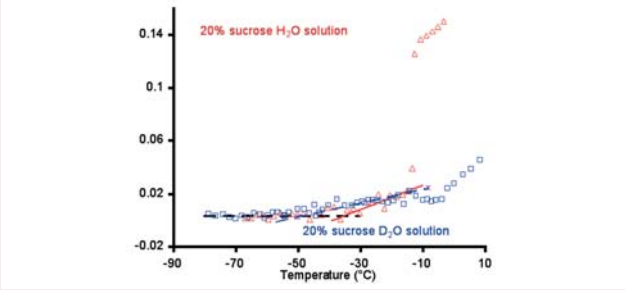


Figure 1 a): Comparison of the DSC results between a pure glassy sucrose and the cryoconcentrated sucrose solution.



b): Temperature dependence of the mean square displacements for 20% sucrose solution in H_2O and D_2O.

To identify the onset temperature of water mobility in a 20% sucrose solution, H/D isotopic labelling has been used to directly probe molecular motions of water (H_2O+sucrose) then sucrose (D_2O+sucrose). The mean square displacement ($\langle u^2 \rangle$) as measured on Mibémol shows a small slope change at -37°C and -48°C in 20% sucrose/H_2O and sucrose /D_2O mixtures respectively. These results are in fully agreement with DSC observations: the first transition at -48°C is to be correlated to a dynamic change of the sucrose molecule whereas the other one seems to be linked to a change of water dynamic. The sharp evolution of $\langle u^2 \rangle$ seen at higher temperature (around -10°C) is due to ice melting, which acts like the dilution of the liquid phase. In order to study if the water dynamical change around -35°C affects the ice structure during its formation, diffraction studies were also carried out with MIBEMOL. The Bragg peak intensity were analysed as a function temperature during cooling. The crystallization of heavy water started at temperatures around -7°C. This temperature is around the same as the temperature of $\langle u^2 \rangle$ evolution variation (Figure 1b). The intensity of both Q=1.61 $Å^{-1}$ and Q=1.71 $Å^{-1}$ peaks were followed during temperature sweep. The observed change in the evolution of the Q=1.71 $Å^{-1}$ peak intensity at the temperature around T_2 on DSC (Figure 1a) may be the consequence of cubic ice formation. Indeed, confined water beyond the interfacial region created by the high cryo-concentration of sucrose may crystallize into a distorted form of cubic ice in contrast to bulk water which crystallizes into ordinary hexagonal ice.

[Collaboration : Champion D., Loupiac C., Simatos D., ENSBANA, Dijon and Zanotti JM (LLB]