CHEMICAL PHYSICS AND BIOLOGY

This very large chapter includes Soft Matter, and more precisely Polymers, and Biological Macromolecules. The latter part originates in previous studies of the structure and dynamics of water. It was realized some years ago that water has especially interesting properties when located in the vicinity of a hydrophilic or a hydrophobic surface. This led to a combined study of structural and dynamical properties of Proteins and of the water that is associated to them. It is interesting to note that a similar evolution occurred in Polymer Physics. There is a growing interest for water-soluble macromolecules, and/ or for copolymers with water-soluble sequences. This leads to conformations that are different from the structures that are usually considered. In some cases, the polymers are biological molecules that are studied in conditions very different from their normal working ones. Thus, there is a significant overlap between these apparently different topics. Among others, there is an interest in showing the strong similarity between the structure of a denaturated biological molecule and that of a synthetic polymer. Work in the second area focused on amorphous and mesomorphous Polymers in the bulk and at interfaces, on Gels, and on Polyelectrolytes. The first part was interested in the static and dynamic properties of soluble and membrane proteins and their associated water. It is indeed accepted that hydration water plays a central role in the stability and function of biological macromolecules. Therefore, it is important to look for the relation between structure, dynamics, and hydration water in proteins.
The neutron scattering experiments were performed essentially with the small angle spectrometers for the structures, by time-of-flight and spin-echo inelastic scattering techniques for the dynamics, and by reflectometry for interfaces.

1. BIOLOGY

1.1. Dynamics of proteins.

The dynamics of a photosynthetic protein, C-phycocyanin (CPC), was studied. This comprises the dynamics of the protein itself as well as of the water that is located at its immediate vicinity. Dynamical studies of the protein as a function of temperature and of the rate of hydration were made by time-of-flight technique, with the Mibemol spectrometer. They led to split the hydrogen atoms into motionless and mobile fractions. As temperature is varied, a dynamical transition is found at approximately 250 K ; it corresponds to the appearance of diffusive motions inside the protein. The radius of the diffusion sphere of the hydrogen atoms in the surface residues remains constant, at approximately 2 Å as temperature changes. But the fraction of motionless atoms decreases as temperature increases and reaches a limit that corresponds to the fraction that lies on the surface.

1.2. Dynamics of water.

A complementary study of the dynamics of the hydration water, in direct contact with CPC, was performed. In order to eliminate the contribution from bulk water, this was realized on powders at hydration rates such that the proteins are functional. Samples hydrated respectively with H_2O and D_2O were used for these experiments on Mibemol. The data may be interpreted with stretched exponentials, corresponding to a distribution of relaxation times for water. The average relaxation time varies as q^{-2}, where q is the momentum transfer. This leads to a diffusion constant equal to $1.5 \times 10^{-6} cm^2/s$, smaller than the 2×10^{-5} cm^2/s of bulk water. Thus, the hydrophylic surface has a slowing down effect on hydration water.
These results were also compared with those of a "model" system, made of supercooled water in a porous Vycor silica glass with large specific surface- 116 m^2/g. The latter is made of interconnected cylindrical pores of approximately 50 Å diameter, and is very hydrophilic. Two different hydration rates for Vycor were used. The quasi-elastic incoherent scattering experiments were made as a function of temperature. The data lead to a stretched exponential function, $\exp[-(t/\tau)^{\beta}]$. The relaxation time τ varies as q^{-2}. This leads to a diffusion coefficient equal to 1.1×10^{-5} cm^2/s, again smaller than that of bulk water. The exponent β is interpreted by a distribution of relaxation times corresponding to motions inside cages with a distribution of sizes. These structural and dynamical studies show that at room temperature, interfacial water behaves as supercooled water.
The influence of water was also specified by looking at changes in the internal dynamics of two globular proteins as one goes from a hydrated powder to a solution. This was realized on lysozyme and myoglobin. It was made on the time-of-flight spectrometer IN6 at ILL. Various hydration rates in D_2O were used. It was shown that surface residues progressively get a local diffusive motion as the protein is hydrated until there is a water monolayer.

Further hydration increases the velocity of this diffusion, but does not involve more residues. Finally, the relaxation time in a solution is two times smaller than for a monolayer, with an amplitude of the motion three times larger.
Finally, the dynamics of hydrogen bonds was studied on a water/ dimethylsulfoxide (DMSO) mixture. The latter interacts strongly with water by forming H bonds. The mixture that was used (1DMSO/2H_2O) is eutectic, and is characterized by a very large decrease in the crystallization point, located at -70°C. Quasi-elastic neutron scattering experiments with deuterated DMSO allows the observation of the motion of the protons of water. They show that the dynamics is slower : the diffusion coefficient is 4 times smaller in this mixture than in pure water. A more surprising result is that the H-bond lifetime is found to be unchanged, whereas a longer time might be expected. These results indicate that hydrogen bonds are stronger than in pure water.

I.3. Structure- dynamics- function relationship

A collaboration with the Service de Biophysique des Protéines et des Membranes of CEA/Saclay was set up in order to study the relation between structure, dynamics and function of membrane proteins. Its aim is to understand completely the energetics of protein folding, and to establish a precise relationship between the structure and the functionality of these macromolecules. To this end, it is necessary to know the three dimensional structure of these proteins. It is also important to characterize with the best possible precision their internal motions. The most interesting time scales are between pico- and nanoseconds (10^{-12} to 10^{-9} s). They are accessible by inelastic neutron scattering. This work is done with membrane proteins involved in the first stages of photosynthetic processes in purple bacteria. Reaction Centers (RC) and light harvesting proteins (LH1 and LH2) were used. They form an interconnected protein network in the membrane, are available in large amount, and their structure is known.
Two examples are discussed in the highlights. The first one deals with time-of-flight experiments, giving access to motions with times in the picosecond range in the protein. The second one allows for a comparison of the collective motions in RC and LH2 by neutron spin-echo technique, for times about nanoseconds (10^{-9} s). An example of structure determination by Small Angle Neutron Scattering (SANS) for LH1 is also discussed. A combination of inelastic neutron scattering techniques and more classical methods in Biophysics such as absorption of light and vibrational spectroscopies makes it possible to study the relation between structure, dynamics, and functionality. This is a part of the PhD thesis of S. Dellerue.

I.4. Denaturated proteins.

A second kind of studies is intermediate between polymers and Biology. It involves denaturated proteins, and the possible similarities between their behavior and that of synthetic polymers. Denaturation was made either by heating or by using a strong denaturing agent, guanidinium chloride (GdmCl). Small-angle neutron studies of yeast phosphoglycerate kinase PGK denaturated by GdmCl show an excluded volume behavior, very similar to what was observed on neutral synthetic polymers. The second virial coefficient was measured, and is in agreement with the theoretical predictions for polymers. Similar results were obtained with beta-casein and neocarzinostatin (NCS) denaturated by GdmCl (PhD thesis of V. Receveur).
NCS denaturation by heating was studied in detail by a combination of SANS with other techniques. This protein has a structure made of a single domain, is very analogous to that of immunoglobulins, and also is present in other proteins. Three stages were observed during the denaturation. In the first one, the size remains almost constant. This is reminiscent of the "molten globule". Then, the radius increases until a reversible endothermic transition. The latter corresponds probably to the breaking of hydrogen bonds. In the last regime, the molecule reaches a final state where NCS behaves as an ideal chain, without any interactions. This corresponds to the so-called "theta" regime in polymer Physics.

2. POLYMERS.

Polymer studies are an excellence area of L.L.B. They dealt essentially with mesomorphous polymers at rest and under shear and elongation stresses, gels, and interfaces. Some experiments were also made on polyelectrolytes and on the dynamics of chains in the vicinity of the theta point.

2.1. Mesomorphous polymers.

The Liquid Crystalline Polymers (LCP) that were considered have comb-like structures, with a mesogenous part grafted on the main chain through a flexible spacer. An important effort on the synthesis allowed us to get monodisperse mass fractions. This made possible the determination by Small Angle Neutron Scattering (SANS) of the variation of the radius of gyration with molecular weight. At rest, it has the same variation as for a gaussian chain in the isotropic phase. In the nematic phase, it has an exponent intermediate between the gaussian and the random self-avoiding walk values. In the smectic phase, the chains are confined between the mesogenic layers. In the direction parallel to the layers, they have a gaussian character. In the transverse direction, the exponent implies a stretching of the polymers, therefore validating the assumption of jumps between successive layers.
Two kinds of deformations were studied.

A work under shear showed the richness of the possible behaviors by a combination of large angle and SANS observations. The former gives the orientation of the director. The latter are made in the three planes defined by the velocity, velocity gradient, and neutral vectors. For a polymer having both a nematic and a smectic phase, a transition was found : in the high temperature nematic phase, the director is parallel to the velocity. For low temperatures, it orders along the neutral axis. This is coupled to a change in the orientation of the chains: for high temperatures, they are parallel to the mesogens, while at lower temperatures, they are normal to the director. This transition may be interpreted by assuming a coupling between smectic fluctuations and shear.
A work under uniaxial extension also combined SANS, X-rays, and rheological measurements. Introducing the parameter " duration of relaxation after stopping the deformation" allowed for a study of the dynamics of a chain for various values of the diffraction vector q. In order to extract the effect of the comb architecture from that of the nematic interactions, two isomers were prepared in similar thermal conditions. The first one was in the isotropic phase. The second one was in the nematic phase. The experiments in the isotropic phase show that the chain is as flexible as the corresponding amorphous polymer. But both the dynamics and the rheology are non classical: the relaxation time and the viscosity have molecular weight dependences that are more important than for linear polystyrene for instance. Under uniaxial applied strain, the rate of deformation of a single chain is constant, but smaller than for the macroscopic sample. This might be explained by assuming the existence of clusters. The latter are related to the existence of temporary junctions between the teeth of the combs. In the nematic phase, the deformation is also pseudo-affine. But it relaxes as a stretched exponential : the nematic interactions slow down the local relaxation of the chains (PhD thesis of V. Fourmaux-Demange).

2.2. Gels.

The work on gels dealt with their structure, in the various cases discussed below, as well as other phenomena occurring in these structures, because of their interfacial and confining properties. Therefore the study of liquid mixtures in Vycor is included in this part.
Studies concerning the structures concerned complexes made by the association of polymers, and model systems describing composite materials made of polymer and mineral charges.

Associative gels

The former gels were made in water by hydrogen bonding between polyacids and polybases. They may then be controlled by changing the pH for instance. Comparing the structures of free and of complexed chains in similar conditions allowed us to show by contrast variation that complexation is stoichiometric, and that complexed chains are tightly bound together and have compact structures.
Gels may also form, as for instance in methyl-cellulose, by associating chains with hydrophylic and hydrophobic sequences. Agregation in this case was considered in the dilute regime, where gelation is not possible, and where phase separation may eventually occur and be studied. This was done as a function of temperature by SANS and by elastic and quasi-elastic light scattering. The results indicate that there is a wide distribution of masses that decreases as a power law with increasing masses. However the aggregates are not self-similar because growth and local densification occur together.

Composites

Model composite materials are made with two types of spheres, respectively made of silica and of nanolatex. The degree of aggregation is also controlled by pH. The composites were studied by SANS and by rheological measurements for various sizes and stretching. The results are currently being interpreted with a computer simulation.

Confining effects

Two applications were considered. Both show the confining effects in gels. They show its influence on a first and a second order transition. SANS allows us to follow the processes in a confining medium because it is easy to eliminate its contribution to the scattering.
The confining effect of gels in crystalline nucleation of a protein - lysozyme- was studied. It may lead either to a promoting or to an inhibiting effect.
Agarose gel is in the first class. It was shown by SANS that the gel is an inert medium for the protein, and that aggregates with sizes larger than 500 Angström are present, as in solutions. But as one lowers temperature, their number becomes larger in a gel.
Silica gels belong to the second class. It was shown that a fraction of the molecules adsorb on the inner surface of the gel. It was possible to determine by SANS the effective fractal dimension of this adsorbed layer. When the solution is under saturated, the dimension is that of the gel. By increasing oversaturation, the dimension increases and goes to a limiting value on the order of 3.4. Because of this adsorption, the concentration in the solution decreases, and this may explain the decrease of the nucleation rate. Crystal growth seems to be related to desorption.

Vycor glass was used for its confining properties to study by SANS a critical mixture of alcane and perfluoroalcane. The temperatures that were considered were both above and below the critical temperature of the mixture. The latter has the interesting property that both components have very similar wetting properties. The results seem to indicate that the latter do not play a central role. The observed signal is interpreted as the sum of an Ornstein- Zernicke contribution, and another one related to the interactions between Vycor, a superficial layer rich in one of the components and a core rich in the other one. This allowed the observation of critical fluctuations the size of which may be significantly larger than the pore size.

2.3. Polymer solutions.

Dynamics at the Theta point.

A detailed study of the dynamics of a linear chain in the vicinity of the theta temperature was made by Spin Echo. It shows that the relevant length in the semi-dilute regime is the diameter of the tube rather than the screening length. It was also shown that the local viscosity η_{loc} depends on the concentration C of the solution :
$\eta_{loc} = \eta_0 (1+BC)$, where η_0 is the solvent viscosity, and B is a constant that vanishes when the solvent becomes good.

Shearing of polymer solutions.

This is the subject of a thesis made in collaboration with ILL (PhD thesis of I. Morfin). It analysed by SANS the concentration fluctuations induced by shear, and connected them with small-angle light scattering and rheological measurements. Two regimes are found. In the first one, the fluctuations are enhanced by shear. Then, fluctuations diverge and there is a phase separation into two regions respectively rich and poor in polymers. This transition occurs either by increasing the mechanical perturbation or by lowering temperature. The latter brings the mixture at rest closer to the coexistence curve.

Polyelectrolytes.

Studies of the form factor of polyelectrolytes were extended to Sodium sulfonate polystyrene in the presence of multivalent ions, Ca^{2+} and La^{3+}. For high concentrations, trivalent ions lead to a phase separation that is attributed to interchain linking. More precisely, it is observed on the form factor that this leads to the individual collapse of the chains. The effect of Calcium ions seems to be similar, and this is more surprising because there is no phase separation in this case. But there is probably a local thickening of the chains.
We chose to select a work that is external to our group in this area. This was realized by a team of Institut Charles Sadron (Strasbourg) both at LLB and ILL and deals with polyelectrolyte star-shaped polymers (see highlights). Two peaks are observed in the scattered intensity. The first one varies as $C^{-1/3}$, and corresponds to the average distance between centers of the stars. The second one corresponds, in semi-dilute solutions, to the average screening length of the solution. In the dilute regime, it becomes the average on a star of the screening length, that decreases as one goes from the periphery of the star towards its center.

2.4. Interfaces.

Proteins and polymers at interfaces.

The work on polymers at interfaces also considered macromolecules with biological origin, namely lipase and beta casein. The first one is an enzyme with an activity that increases significantly at the oil/ water interface. Neutron reflectivity experiments show that the enzyme is adsorbed, and that the superficial layer has the size of the molecule. In the presence of an anionic surfactant, SDS, the layer does not change, thus indicating that there are few interactions between species with same charge. In the presence of a cationic surfactant, TTAB, adsorption increases. In the vicinity of the bulk precipitation point, there appears a thick layer. This seems to imply the possible formation of complexes at the surface. For both cases, when the surfactant concentration reaches the critical micellar concentration (CMC), a small amount only of the enzyme is detected at the surface. The adsorbed layer becomes dilute, and its width becomes on the order of five times that of lipase alone. A possible interpretation is in terms of unfolding of lipase.
The adsorption of a thermosensitive polymer, poly(N-isopropylacrylamide) (PNIPAM), in the presence of SDS has also been studied at the air/ water interface (PhD thesis of B. Jean). The adsorbed layer density increases with temperature, an effect due to a decrease in the solvent quality. In the presence of SDS, adsorption of PNIPAM is modified above the critical aggregation concentration (CAC). The polymer is then progressively displaced from the surface at all temperatures studied. The concentration profiles suggest the presence of micellar- surfactant complexes in the adsorbed layer (see highlights).

Beta casein is a naturally unfolded protein, which adsorbs at the air/water interface. A simplified model was studied. Assuming a symmetrical structure of hydrophilic and hydrophobic sequences, it was possible to get a phase diagram in a temperature concentration plane. The various measurable quantities as well as the structure of the protein were determined in all the regimes of this diagram.

Polymers at the surface of vesicles.

Finally, polymers were grafted on vesicles in order to study their stability and their sizes as a function of the chains and of temperature. The radius increases as one heats the system, without changing noticeably the polydispersity. The characteristic time for growth decreases as membrane concentration increases. This implies a fusion of the latter. The temperature dependence may be explained by the existence of a potential barrier related to the existence of grafted chains. Addition of macromolecules leads to the formation of micelles, and to the destruction of vesicles. It seems that large masses have smaller perturbation effects on membranes. This might correspond to an effect predicted by Lipowsky, where isolated grafted chains create a tension effect in the membrane.

Polymer-polymer interfaces.

Finally, we studied the interface between a polymer melt, where the chains are free, and a network, where they are interconnected (PhD thesis of G. Bacri). The characteristics of the network (mesh size) and of the melt (length of the chains) are controlling the width of the interface. The aim of this work is to study the extent to which a modification of the width of the interface changes its mechanical properties, and more precisely its resistance to fracture (see highlights).

3. PERSPECTIVES.

In Biology, we intend to continue the following soaring activities :

i)-Denaturated states of proteins.

We will compare denaturation in various conditions:
1) by a chemical agent, namely guanidinium chloride,
2) by heating up or cooling down,
3) by pressure, which has hardly been studied.
Both the structure and the dynamics of the native and unfolded states will be studied by neutron and X-ray scattering techniques. Circular dichroism, static and time-resolved fluorescence, Differential Scanning Calorimetry and Fourier Transform Infra-Red techniques will be associated with the previous ones.
Kinetic studies will also be made in the presence of chemical denaturating agents, in order to characterise the intermediate states during denaturation and renaturation of a protein such as NCS, that we already studied.

ii)-Structure-dynamics- function relationship

This will be considered for soluble as well as for membrane proteins. The aim is the identification of those motions that are important for the function of the protein. We will use quasi-elastic and inelastic scattering of neutrons with selectively deuterated biological samples. This is a promising method for the identification of the motions in the pico- to nano-second time scale. NMR, Infra-Red (IR) spectroscopy and Raman will be associated. These perspectives depend crucially on the obtention of selectively deuterated biological samples. This is becoming easier with the growing interest of the biologists for the dynamics- function relation, that might explain some not so well understood properties of their systems.
For soluble proteins, we will take advantage of the possible studies of coupled effects hydration water/dynamics by coherent inelastic X-ray scattering.
The systems that will be studied are soluble photosynthetic proteins (C-phycocyanin) and membrane proteins, reaction centers and light harvesting proteins, hyperthermophilic and barophilic proteins (transcarbamylase aspartate), parvalbumin, and hemoglobin.

iii)-Molecular dynamics simulations.

Computer simulations by molecular dynamics will be continued, in collaboration with A. Petrescu (Romania) and J. Smith (Heidelberg). A project about the slow dynamics in the nanosecond scale within the Physics and Chemistry of Living Objects Programme (CNRS) is being set up in collaboration with G. Kneller (Orléans).
We will always work in physiological conditions, in close collaboration with various teams of biologists.

In the field of polymers, the following activities will be developed :
Collaboration with ICS (Strasbourg) for the study of the dynamics of grafted fullerenes, with various geometries (stars, dumb-bells, ..). The dynamical structure factors will be measured by Spin Echo technique. They exhibit a minimum for a scattering vector that corresponds approximatively to the cross-over between the global translation and the internal modes. This a new topic.

Study of reinforced polymers. This is in a growth stage. It aims at mimicking reinforcement of polymers by solid mineral charges, and at studying model composite systems.
Adhesion between two polymer layers is studied by coupling mechanical measurements of the adhesion energy and interfacial profiles by neutron reflectivity and ion beam measurements. With the latter techniques, one may follow the penetration of the chains from one layer into the other one. The relation with the resistance of the interface should allow us to determine whether the interfacial chains play the role of connectors between both layers.

The experimental and theoretical studies of multiblock copolymers at interfaces will be continued. These will be made by local and outside LLB experimentalists for the studies about beta-casein. There should also be studies on synthetic copolymers manufactured by Elf-Atochem.
Studies of polymers grafted on vesicles will also be continued, in order to understand the consequences of grafting both on the structure of the polymers and of the vesicles.