AXIS 3 Research: Soft Matter and Biophysics

Researchers (18): C. Alba-Simionesco (CNRS), V. Arluison (Univ. Paris VII), F. Audonnet (Univ. Paris XI), M.-C. Bellissent-Funel (CNRS Emeritus), F. Boué (CNRS), A. Brûlet (CNRS), G. Carrot (CEA), S. Combet-Jeancenel (CNRS), F. Cousin (CEA), G. Fadda (Univ. Paris XIII), J. Jestin (CNRS), D. Lairez (CEA), A. Lapp (CEA), L.T. Lee (CNRS), S. Longeville (CEA), N. Malikova (CNRS), N. Brodie-Linder (Univ. Cergy-Pontoise), J. Teixeira (CNRS Emeritus), J.-M. Zanotti (CEA).

Associated researchers (1): E. Buhler (Univ. Paris VII)

PhD students (11): D. Bhowmik (2008-), G. Chahine (2008-), C. Chevigny (2006-2009), A.-L. Fameau (2008-), F. Gal (2008-), N. Jouault (2006-2009), A.-S. Robbes (2008-), C. Said (2008-), C. Le Coeur (2007-2010), K. Lagrené (2005-2008), J. Jelassi (2007-2010)

Post-doc students (5): I. Colinet (2008-2009), C. de Rezende (2007-2008), C. Thibierge (2009-2010), A. Koutsioumpas (2009-), F. Muller (2009-)

Keywords

- SANS, reflectivity, inelastic scattering, polymer and particles chemical synthesis, rheology
- Complex fluids, polymers, surfactants, colloids, self-assembly, nanoparticles, grafting, nanocomposites, nanopores, multicomponents systems, selfassembly, gels and networks.
- Protein folding-unfolding, protein crowding, protein dynamics, proteins/polyelectrolyte, biocompatible polymers, biopolymers, water properties.
- Polymer dynamics, transport properties, diffusion, anomalous diffusion, slow dynamics, complex fluids dynamics, glass transition, confinement.

Scope

In the field of soft matter, many complex new systems are flourishing. The emergence of these newfound capabilities, many of which marry different technologies and components, and work at the nanometer scale (1-100 nm) has become a major current trend. At LLB, research has progressed in similar directions, though some of the historical specificities of the lab, such as polymer research, are kept as main components. With the advantages of neutron scattering in mind (i.e., labeling and contrast matching), we often marry the reciprocal space (SANS and SAXS) with other techniques, either in real space or at macroscopic scales. The following topics are presently developed: nanoparticles and hybrid systems; organic systems and self-organization; polymer dynamics; and electrostatic complexes. In the following review, we will consider multicomponent systems that involve polymers, while differentiating between: 1) systems where one of the components is intrinsically nanosized (e.g., nanoparticles or nanopores), and the relevant structure and dynamics can be kept at a comparable size, depending on its interactions with the polymer; and 2) systems where the architecture of the basic elements is bound to lead to self-organization at the nano- or micro-scale.
Research at the interface of physics and biology is based on three main topics. 1) Proteins in complex media viewed as model systems for living environments. Experiments here are concerned with macro- or supra-molecular scales and their analysis is strongly influenced by our background in polymer physics, statistical physics, and phase transition physics. 2)

The local dynamics of proteins and hydration water in relation to the dynamic transition of proteins and their enzymatic activity. Neutron scattering techniques, which are very sensitive to protons, are particularly suitable for these studies. 3) Water and its specific properties are fundamentally related to life and to the very peculiar properties of some biological molecules, such as proteins. Here, the properties of water are studied in relation tothe dynamics of hydrogen bond networks, and to the notions of hydrophobicity and confinement.
During the period from 2008 to 2010, 138 publications have been produced on these topics (51 in 2008, 36 in 2009, and 51 in 2010). The presented topics have also been the subject of 57 invited conferences. One *Habilitations à Diriger les Recherches* and 5 PhD have been defended.
The research in Soft Matter and Biophysics is also supported by a number of research contracts: 1 bilateral contract with Argentina, 7 national ANR contracts, 2 regional contracts within the RTRA and C'nano organizations, 2 industrial contracts.

Current research

Hybrid organic-inorganic systems: nanoparticles plus polymers, nanocomposites, and nanopores

These hybrid systems contain a combination of organic components (mainly polymers) and mineral components, and take the form of nanoparticles. Often such synthesis includes functionalization, or the grafting of polymers onto the surface of particles. This is done with various levels of partnership – from training, to external chemical synthesis in external laboratories, to in-house synthesis, for which our chemistry lab facilities are crucial.

Direct dispersion of nanoparticles for well-defined structures and related properties

Our aim was to relate the mechanical reinforcement of polymer melts that contain nanoparticles (NP), as a model for nanocomposites (which include the case of filled rubber, as in tires), to the structure of NP dispersion and chain conformation, at rest and under deformation. We synthesized model nanocomposites having dispersions of nanoparticles at the desired levels (i.e. non-aggregated or partially aggregated), in clusters of various compaction/fractalness, and with various degrees of connectivity. The first route to prepare these model nanocomposites was to disperse them directly by mixing them with the solution and evaporating the solvent, which generated homogeneously dispersed fractal aggregates. This method allowed inter-aggregate distances to be controlled, as determined by TEM and SAXS and SANS (N. Jouault, PhD, 2009)[1], with basically no global glass transition temperature (T_g) shift. A first striking result was the strong increase in elastic modulus at low silica concentrations ($\lesssim$ 5%), although aggregate distances between the NP were still in the non-connected range. If this result was due to the presence of " slowed down" regions around or between the particles, they would be expected to have sizes(between NP) greater than several tens of nm, which would be 10 times larger than the values proposed from fits of mechanical measurements, or than those extrapolated from high-temperature NMR analysis of different polymers.

A second striking result was obtained from deuterated chains under deformation: the behaviors of the chains and the matrix were similar[2]: the labeled chains inside the matrix deformed exactly as they did in the non-reinforced system (Fig. 3.1). Thus, there was no influence of slowing down, or of other interfacial or confinement phenomena.

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[1] *Well dispersed fractal aggregates as filler in polymer-silica nanocomposites: long range effects in rheology,*
N. Jouault, P. Vallat, F. Dalmas, S. Said, J. Jestin, F. Boué, MACROMOLECULES **42**:2031 (2009)

[2] *Direct SANS observation of stretched chain conformation in nanocomposites: more insight of polymer contributions in mechanical reinforcement,*
N. Jouault, F. Dalmas, S. Said, E. Di-Cola, R. Schweins, J. Jestin, F. Boué, PHYS. REV. E. **82**:031801 (2010);
Direct measurement of polymer chain conformation in well controlled model nanocomposites by combining SANS and SAXS
N. Jouault, F. Dalmas, S. Said, E. Di-Cola, R. Schweins, J. Jestin, F. Boué, , MACROMOLECULES, **43**, 9881-9891 (2010).

Organization

In essence, research in " soft matter and biophysics" is interdisciplinary. At LLB, three groups are involved in this domain (" soft matter" , " biology and disordered systems" , and " reflectivity"). Neutron scattering and a variety of complementary techniques are used to study the structures and dynamics of polymers, complex fluids, colloids, surfactants, and biologically relevant systems. In many cases, the contrast variation capabilities of neutron scattering, as well as its sensitivity to protons, make this technology a very powerful tool for these studies.
Hence, experimental studies are organized around the small angle neutron spectrometers PACE, PAXE and PAXY; the new very small angle spectrometer, TPA; the time-of-flight spectrometer, MIBEMOL; the neutron spin echo spectrometer, MUSES; and the neutron reflectometer, EROS. Many experiments are also conducted using lighter devices.
Additionally, these same groups are responsible for operating and upgrading their assigned spectrometers. They are also in charge of organizing the support for external visitors coming to perform experiments at the LLB. This organization allows the laboratory to operate the spectrometers efficiently by providing instruments that are constantly upgraded, and allows it to provide a high level of expertise in each respective technique, both of which greatly benefit all users of these instruments.

1. Small Angle Neutron Scattering (SANS) - to study large length-scale spatial correlations. LLB operates 5 SANS instruments: PAXY, PACE, PAXY, PAPYRUS (to be closed), and TPA (the new, very small angle scattering spectrometer).
2. Neutron reflectivity - for the study of solid/fluid and fluid/fluid interfaces. Two reflectometers are operated for these studies: EROS and PRISM.
3. Inelastic neutron scattering - to study dynamic properties. The Time-of-Flight spectrometer, MIBEMOL; as well as the Spin-Echo spectrometer (Zero Field), are used primarily for these studies.
4. Other equipment:

a. Rheometers, shearing and stretching devices, light scattering, opticam microscope, confocal microscope, voltage-clamp measurements.
b. Chemistry and physico-chemistry equipment, for synthesis (e.g., fume hoods) and characterization, UV and IR spectroscopy, DSC and TGA, and permeation-adsorption measurements.
c. Biology equipment: DNA analysis and production techniques (e.g., thermocyclers, gel migration, and gel imaging); recombinant protein preparation (microbiology equipment); sonicator; protein separation (FPLC) and characterization (SDS-PAGE, Western-blotting); UV and IR fluorescence spectroscopy, centrifugation, cold room, -80° C freezer.

Reinforcement at large deformation can therefore be decomposed into a chain contribution, which is independent from the filler fraction, and a filler contribution, which increases with Ø but is essentially constant with deformation. This suggests progressive rearrangement of particles when they hit, which is analogous to buckling.

More studies of direct polymer – inorganic mixtures, from a materials science view point, are shown in Axis 2. Examples discussed are Hevea Latex plus clay platelets[3]; latex[4]; and melting that is anisotropically reinforced by magnetic nanoparticles under field.

[3] *Nanoclay natural latex composites: structure and reinforcement under high deformation,* L.T.Lee, C. Rezende, T. Doi, F. Boué, POLYMER **51**:3644 (2010)

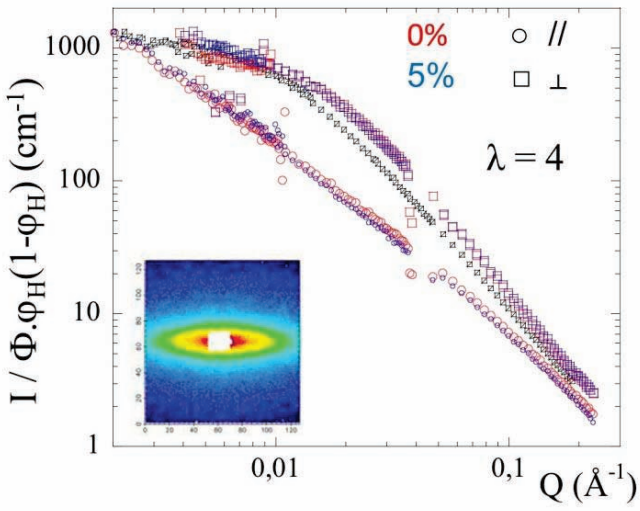

Figure 3.1. *Comparison of chain deformation between unfilled polymer (red) and nanocomposites (blue): Scattering signal (log-log) of deformed chains in pure polymer and filled with 5%v/v (blue curves) in directions // and ⊥ to stretching for elongation ratios λ=4.*

Single polymer chain dynamics under nanometric confinement

Confinement is thought to deeply modify the dynamic properties of polymers. Recent NMR relaxometry results have suggested that the confinement of a polymer melt in a nanoscopic isotropic porous matrix leads to chain dynamics that are dramatically different from their dynamics in bulk, i.e., the reptation tube diameter under confinement would be only a few angstroms; or one order of magnitude smaller than in the bulk. This would actually correspond to a situation where the chain experiences reptation alongside its own physical contour so that this phenomenon has been called the *corset effect*.

We have challenged the existence of this *corset effect* in a neutron spin-echo (NSE) experiment. We demonstrated how NSE, combined with contrast matching and zero average contrast (ZAC), allowed us to simultaneously (i) match the intense porous detrimental elastic small angle neutron scattering contribution to the total intermediate scattering function $I_{Chain}(Q, t)$, and (ii) measure the Q dependence of the dynamic modes of a single chain under confinement[5].

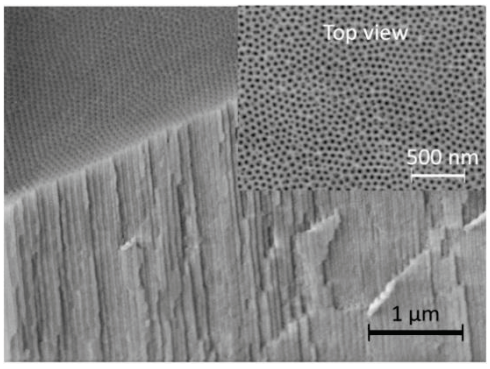

Figure 3.2. *Scanning electron microscope image of an Anodic Aluminium Oxide membrane[6]. A highly oriented array of cylindrical pores (radius 9 nm) goes from the top down to the bulk of the membrane.*

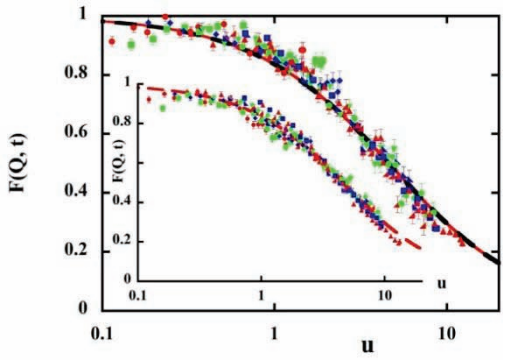

Figure 3.3. *$F(Q,t) = I_{Chain}(Q, t) / I_{Chain}(Q, t=0) - P(Q) / (1 - P(Q))$ versus reduced time Rouse parameter $u=Q^2\sqrt{(W\sigma^4 t)}$, for the confined and bulk polymer (inset). P(Q) is the form factor of the reptation tube. The tenfold reduction of the reptation tube diameter predicted by the "corset effect" is not observed.*

Our measurements obtained on Poly(Ethylene Oxide) (PEO) chains in Anodic Alumium Oxide membrane (AAO) (K. Lagrené, PhD 2008) are in contradiction with a tenfold reduction of the reptation tube diameter predicted by the " corset effect" [5] (Figs. 3.2 & 3.3). The method

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[4] *Anisotropic reinforcement of nanocomposites tuned by magnetic orientation of the filler network,*
J. Jestin, F. Cousin, I. Dubois, C. Ménager, J. Oberdisse, R. Schweins, F. Boué, ADV. MAT. **20**:2533 (2008)

[5] *Large-scale dynamics of a single polymer chain under severe confinement,*
K. Lagrené, J.-M. Zanotti, M. Daoud, B. Farago, P. Judeinstein, Phys. Rev. E **81**:060801 (2010)

developed has a general relevance when probing the large scale dynamics of a system of large molecular mass under confinement. The use of the host porous material as an electrolyte for lithium batteries has been extended to a patent[6].

Templating nanoparticle surface assembly by polymer dewetting patterns

Here, we used specific interactions between a polymer and nanoparticles to foster self-organization on a substrate. For functional applications, the assembly process must be directed in order to obtain open and complex arrays. Different approaches for this include the use of external electric field force, chemically pre-patterned substrates, and biomolecular templates. Our alternative approach was to use the dewetted morphologies of a polymer solution to template nanoparticle organization. An aqueous solution of non-ionic polymer will dewet a substrate to form different morphologies, which depend on the physicochemical properties at work and the drying conditions. We used elongated dewet patterns to template nanowires / nanoyarns of nanoparticles via a two-stage mechanism: nucleation of an ordered phase by lateral capillary attractions, which confined the nanoparticles in the dewet pattern; and convective transport of the particles towards the ordered region in order to grow the ordered nanoparticle array. The efficiency of this process was diminished, however, due to the presence of particle-substrate attraction, which hinders convective transport such that the formed nanostructures coexist with a non-organized phase. Neutron reflectivity showed that such nanoparticle attachment could be suppressed by adding an anionic surfactant (Fig. 3.4, left). These results, in conjunction with well-controlled drying conditions, enabled us to direct the formation of nanoyarn arrays that were longer than several tens of microns[7] (Fig. 3.4, right).

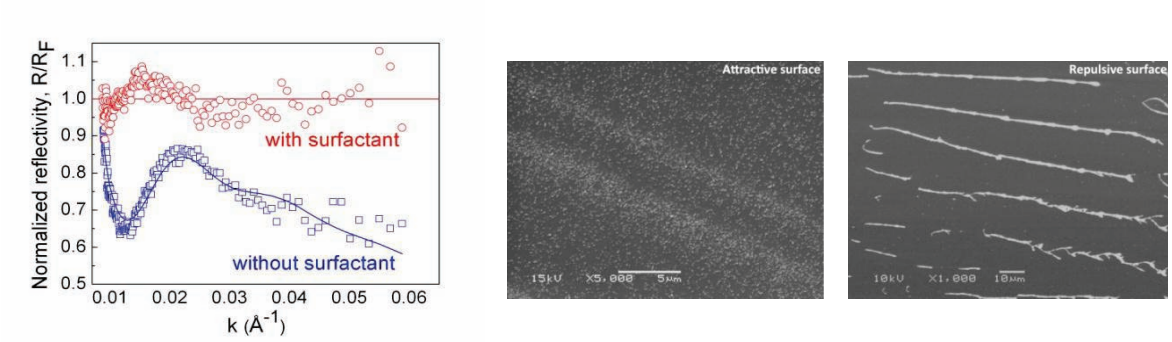

Figure 3.4. *Normalized neutron reflectivity showing strong adsorption of polymer-coated silica nanoparticles at a model hydrophobic interface (water-air). Addition of a charged surfactant transforms the attractive surface to a repulsive one, resulting in displacement of the nanoparticles from the surface (R/RF = 1). Similar suppression of nanoparticle adhesion on a solid substrate promotes long range organization of nanoyarns templated by dewetting morphology (SEM images).*

Grafted nanoparticles

Following the pioneering work on nanosilica[8], the chemistry of grafting has become an important theme in the laboratory. It is now largely developed along new chemical syntheses for each different application. From the thesis work of J. Vinas (PhD, 2008), routes for synthesizing silica and its direct subsequent grafting to hydrosoluble polymers, including a new initiator[9], have been developed in the CROPS group of Marseille and studied at LLB.

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[6] *Membrane minérale électrolytes pour dispositifs électrochimiques,*
J.-M. Zanotti, K. Lagrené, BREVET n°FR 10/56178

[7] *Liquid-templating for nanoparticle organization into complex patterns,*
C.A. Rezende, L.T. Lee, F. Galembeck, LANGMUIR **23**:2824 (2007)
Silica nanoparticles at interfaces modulated by amphiphilic polymer and surfactant,
C.A. Rezende, L.T. Lee, Galembeck F., LANGMUIR **24**:7346 (2008);
Surface mechanical properties of thin polymer films investigated by AFM in pulsed force mode,
C.A. Rezende, L.T.Lee, Galembeck F., LANGMUIR **25**:9938 (2009)

[8] *Surface–atom transfer radical polymerization from silica nanoparticles with controlled colloidal stability,*
A. El Harrak, G. Carrot, J. Oberdisse, C. Eychenne-Baron, F. Boué, MACROMOLECULES **37**:6376 (2004)

[9] *SG1-based alkoxyamine bearing a N-succinimidyl ester: A versatile tool for advanced polymer synthesis,*
SG1 and BLOCBUILDER® technology: a versatile toolbox for the elaboration of complex macromolecular architectures,
J. Vinas, N. Chagneux, D. Gigmes, T. Trimaille, A. Favier, D. Bertin, Polymer, **49**:3639 (2008) ;

Currently, gold nanoparticles are being grafted by C. Said (PhD) to thermosensitive polymer permitting the distance between particles and the optical properties (plasmon resonance) to be controlled (see " Projects"). In between these efforts, two different grafting approaches were developed, which are detailed in the next section.

Inclusion of grafted nanoparticles in polymer for mechanical properties

Model nanocomposites for reinforcement studies can also take the form of grafted nanoparticles in polymer. This second route of nanocomposite preparation focuses on using the chemical interactions between the matrix and fillers to influence reinforcement. Nanosilica-controlled grafting was performed through Nitroxyde Mediated Polymerization (C. Chevigny, PhD 2009, in collaboration with D. Gigmes & D. Bertin, Univ. Marseille-Aix), using a MAMA-SG1 (BlocBuilder™) -derived alkoxyamine bearing a N-succinimidyl (NHS) ester group, which allowed grafting to silica. This new grafting technique produces well- dispersed single nanoparticles in organic solvent, which are characterized as having an individual core with a polymer corona[10,11]. We are currently extending this method to grafting from the surface of magnetic (maghemite) nanoparticles[12] (PhD of A.-S. Robbes).

One advantage to using these particles is that the various states of dispersion for the particles in the polymer matrix, from lumps to individually dispersed particles, can be controlled by varying the ratio (R) of the grafted chain mass over matrix chain mass, as shown by SAXS and TEM[13]. This is shown in Figure 3.5 below, which also shows an apparent correlation with the thickness of the corona. The latter could be extrapolated from SAXS analysis in all dispersion states, and was subsequently confirmed by direct measurement in the dispersed state using a combination of SAXS and SANS[14]. Corona thickness decreases with respect to its value in solvent, and also decreases when passing into the aggregated state. The same corona form factor measurement could be achieved in the deformed state, so that we were able to follow the effect of the stress on such coronae. Under stretching, corona deformation was found to be comparable to the deformation of the matrix. We also found that particle arrangement in response to deformation is dominated by the local inter-particle interaction. Finally, these mechanical measurements enlighten our understanding of the non-grafted particle dispersion described above.

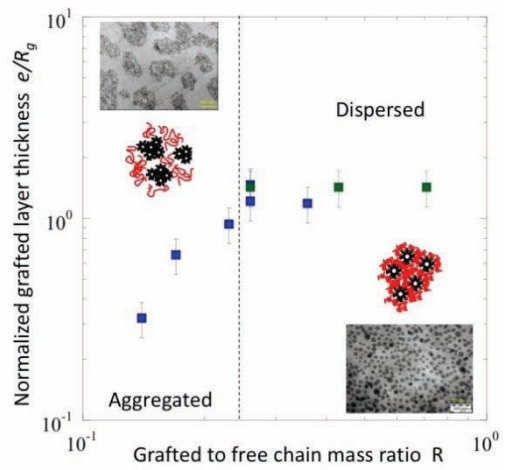

Figure 3.5. *Corona thickness versus grafted/matrix mass ratio in model nanoparticles*

D. Gigmes, J. Vinas, N. Chagneux, C. Lefay, T. N. T. Phan, T. Trimaille, P. E. Dufils, Y. Guillaneuf, G. Carrot, F. Boué, D. Bertin, ACS Symposium Series, Advances in Controlled/Living Radical Polymerization", Krzysztof Matyjaszewski Ed., 2009.

[10] *Polystyrene grafting from silica nanoparticles via nitroxide-mediated-polymerization (NMP): synthesis and SANS analysis with contrast variation method,*
C. Chevigny, D. Gigmes, D. Bertin, J. Jestin, F. Boué, SOFT MATTER **5**:3741 (2009)

[11] *Controlled grafting of polystyrene on silica nanoparticles using NMP: a new route without free initiator to tune the grafted chain length,*
C. Chevigny, D. Gigmes, D. Bertin, R. Schweins, J. Jestin, F. Boué, POLYMER CHEMISTRY, Communication, **2**, 567-571 (2011).

[12] *Homogeneous dispersion of magnetic nanoparticles aggregates in a PS nanocomposite: highly reproducible hierarchical structure tuned by the nanoparticles' size,*
A.-S. Robbes, J. Jestin, F. Meneau, F. Dalmas, O. Sandre, J. Perez, F. Boué, F. Cousin, MACROMOLECULES **43**:5785 (2010)

[13] *Polymer-grafted-nanoparticles nanocomposites: dispersion, grafted chain conformation and rheological behavior,*
C. Chevigny, F. Dalmas, E. Di Cola, D. Gigmes, D. Bertin, F. Boué, J. Jestin, MACROMOLECULES **44**; 122–133 (2011).

[14] *"Wet-to-dry" conformational transition of polymer layers grafted to nanoparticles in nanocomposites,*
C. Chevigny, J. Jestin, D. Gigmes, R. Schweins, E. Di Cola, F. Dalmas, D. Bertin, F. Boué, MACROMOLECULES, **43**:4833 (2010)

Nanocomposites for biology

Another development of our pioneering work in grafting of polymers onto nanosilica via controlled polymerization (surface-ATRP)[8, 15], is now to functionalize platinum nanoparticles (Pt-NPs[16]).

Enzyme/polymer-grafted platinum nanoparticles: towards construction of glucose probes. The thesis work of F. Gal (PhD defense, Nov 2010), in collaboration with H. Perez (IRAMIS/SPAM), showed that polymer-grafted-PtNPs could be chemically modified at different stages to form bio-hybrid objects. The first stage involves surface-ATRP, followed by an hydrolysis step to form a poly(methacrylic acid) corona. Finally, the latter is activated in order to bind enzyme (Fig. 3.6)[17]. Using contrast matching data from the Pt core, and the signal from polymer corona, we determined: that the particles were well dispersed (no aggregation); the size and number of grafted chains per object; and the size of the object (Fig. 3.6). Extrapolation at zero concentration yielded the total molecular weight of all chains[16,17]. Then, to study the electrochemical properties of protein/polymer-grafted-PtNPs, it was necessary to transfer these nano-objects onto electrodes, using the Langmuir-Blodgett technique for depositing homogeneous films onto a gold surface. Controls of homogeneity, thickness, and roughness, were attested by neutron reflectivity and TEM.

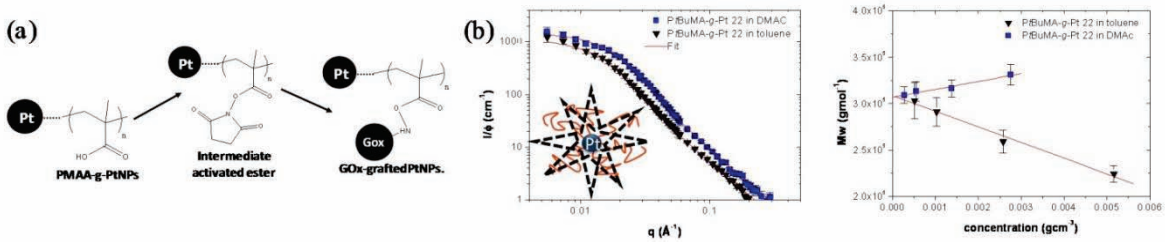

Figure 3.6. *(Left) Synthesis of the glucose oxidase (Gox)-poly(methacrylic acid)-grafted-PtNPs: Activation of the PMAA-g-PtNPs with N-hydroxy-succinimide (NHS) and subsequent binding of the glucose oxidase. (middle) Relative scattered intensity I/ϕ in cm^{-1} as a function of scattering wave vector in $Å^{-1}$ of PtBuMA-g-Pt 22 in 100% d-toluene and DMAc (95.7% d, 4.3% h).(Right) Molecular weight of the scattering objects as a function of the concentration.*

Grafting biocompatible polymers onto nanoparticles via click chemistry. The main limitations of the " grafting from" approach (described above) are that our polymerization route is restricted to the use of only vinylic monomers, and that the length of the grafted-from polymer chains is difficult to determine. Due to these limitations, there was great interest in developing a " grafting to" approach for grafting various well-defined preformed polymers, such as the biocompatible polymers PEG and PCL, directly onto PtNPs for their use in biosensor and targeted drug delivery applications. In this scope, the recently developed concept of click chemistry was particularly attractive[18]. The most reliable example of this is copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) that exclusively yields 1,4-disubstituted 1,2,3-triazole linkages. This reaction is highly efficient, tolerant to a wide range of functional groups, and uses mild conditions in protic or aprotic solvents. Our novel " grafting to" approach for the functionalization of PtNPs uses complementary combinations of CuAAC and polymer chemistry[19,20]. For example, tailor-made PtNPs having bromide

[15] *Polymer grafting from 10-nm individual particles: proving control by neutron scattering,*
G. Carrot, A. El Harrak, J. Oberdisse, J. Jestin, F. Boué, SOFT MATTER **2**:1043 (2006)
[16] *Polymer-grafted-platinum nanoparticles: from three-dimensional small angle neutron scattering study to tunable two-dimensional array formation,*
Carrot G., Gal F., Cremona C., Vinas J., Perez H., LANGMUIR **25**:471 (2009)
[17] *Protein/polymer-grafted platinum nanoparticles: towards the construction of glucose probes,*
F. Gal, V. Noel, H. Perez, G., POLYMER PREP. **51**:216 (2010)
[18] V. Rostovtsev et al., ANGEW. CHEM. INT. ED. **41**: 2596 (2002)
[19] *Smart combination of "grafting from" and "grafting to" for the design of 2D and 3D hybrid architecture,*
G. Carrot, S. Al-Akhrass, I. Colinet, F. Gal, D. Damiron, E. Drockenmuller, POLYMER PREP. **51**: 220 (2010)
[20] *Efficient approaches for the surface modification of platinum nanoparticles via click chemistry,*
E. Drockenmuller, I. Colinet, D. Damiron, F. Gal, H. Perez, G. Carrot, MACROMOLECULES (2010) (DOI: 10.1021/ma102188d).

functionalities at their surface (Br-PtNPs) were converted to azido-functionalized PtNPs (N3-PtNPs) by substituting the bromide atoms for sodium azide (NaN_3) groups. In parallel, ω-alkyne PCL and PEG were respectively obtained by esterification of PCL with pentynoic acid, and by alkylation of monomethoxy-PEG with propargyl bromide. After the CuAAC "grafting to" process, we obtained relatively high grafting densities (0.7 to 1.75 chains/nm^2 as determined by thermogravimetric analysis of the organic content). SANS measurements showed that the radius of gyration (R_g) of the polymer corona increased slightly (5.9 to 7.7 nm) as the molecular weight of the polymer precursors was increased. Moreover, the intensity at low-q values is consistent with the complete grafting of all chains and a non-aggregated state of the hybrid PtNPs. Thus, the CuAAC "grafting to" method successfully functionalized PtNPs with biocompatible polymers. The same strategy is being applied to functionalize silicon nanoparticles with PEG (with N. Herlin, IRAMIS/SPAM). These silicon-based objects are potentially available for use as biomarkers, and have the added advantage being photoluminescent.

Supramolecular polymer organization

Supramolecular polymers are an increasingly important class of polymers, in which designed intermolecular interactions allow the polymer's properties to be specifically tailored. Numerous studies have focused on the structures and properties of these molecules.

Dynamic supramolecular polymers

Dynamic supramolecular polymers consist of reversibly associated monomers that connect to each other via reversible covalent bonds or non-covalent bonds (e.g., hydrogen bonds). Due to their dynamic nature, the structure, length, and composition of these polymers are amenable to evolving in response to varied polymerization conditions (e.g., temperature, pH variation, monomer concentration, shear stress, presence of a molecular target, etc.). Due to these constitutional changes, they can be envisaged as smart materials[21,22,23,24,25,26,27]. By altering the reversibility of the monomer connections in response to physical and/or chemical effectors[21,24], the team of Prof. J.-M. Lehn at ISIS-Strasbourg has developed novel polymer features which ultimately affect their structural ordering and dynamic nature. In addition, the group of Prof. N. Giuseppone at ICS-Strasbourg has developed responsive combinatorial mesophases from libraries of dynamic block copolymers (Dynablocks)[25,27]. These types of studies are opening the way to biologically inspired polymer structuring on the nano- and micro-scales.

Block copolymers for vesicle-like tunable structures

Polymer vesicles that can be obtained from hydrophilic/hydrophobic diblock copolymers have attracted considerable attention due to an array of beneficial properties (e.g., toughness, stability, tailorable size, and membrane properties[28]), which make them attractive candidates for a number of important applications, including encapsulation, drug delivery, nanoreactors, and templates for micro- or nano-structured materials. Many applications require the ability to control the release of substances encapsulated in the interior compartment and/or in the hydrophobic core of membrane. To address this goal, we developed polymersomes, in which specific external stimuli are able to destabilize the vesicle structure. Diblock copolymer vesicles, studied in collaboration with Dr. M.H. Li (Institut Curie, Paris), are intrinsically responsive to external physical stimuli, such as UV irradiation or a magnetic field. Indeed, the hydrophobic

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[21] *Reversible constitutional switching between macrocycles and polymers induced by shape change in a dynamic covalent system,* S. Ulrich, E. Buhler, J. M. Lehn, NEW J. CHEM. **33**: 271 (2009)

[22] *Room temperature dynamic polymers based on Diels-Alder chemistry,* P. Reutenauer, E. Buhler, P.J. Boul, S. J. Candau, J. M. Lehn, CHEM. EUR. J. **15:**1893 (2009)

[23] *Glycodynamers: dynamic polymers bearing oligosaccharides residues - generation, structure, physicochemical, component exchange, and lectin binding properties,* Y. Ruff, E. Buhler, S. J. Candau, E. Kesselman, Y. Talmon, J. M. Lehn, J. AM. CHEM. SOC. **132**:2573 (2010)

[24] *Cooperative bottom-up generation of rigid-rod nanostructures through dynamic polymer chemistry,* J. F. Folmer-Andersen, E. Buhler, S. J. Candau, M. Schmutz, J. M. Lehn, POLYM. INT., published online (2010)

[25] *Dynamic combinatorial evolution within self-Replicating supramolecular assemblies,* R. Nguyen, L. Allouche, E. Buhler, N. Giuseppone, ANGEW. CHEM. INT. ED., **48**:1093 (2009)

[26] *Dynablocks: structural modulation of responsive combinatorial self-assemblies at mesoscale,* R. Nguyen, E. Buhler, N. Giuseppone, MACROMOLECULES **42**:5913 (2009)

[27] *The hierarchical self-assembly of charge nano-carriers: a highly cooperative process promoted by visible light,* E. Moulin, F. Niess, M. Maaloum, E. Buhler, I. Nyrkova, N. Giuseppone, ANGEWANDTE **49**:6974 (2010)

[28] *pH and temperature responsive polymeric micelles and polymersomes by self-assembly of poly[2-(dimethylamino)ethyl methacrylate]-b-[poly(glutamic acid)] double hydrophilic block copolymers,* W. Agut, A. Brûlet, C. Schatz, D. Taton, S. Lecommandoux, LANGMUIR **26**:10546 (2010)

group is an azobenzene-containing liquid crystal (LC) polymer. Upon *in situ* application of a magnetic field and elevated temperature, huge increases in the thickness of the vesicle membrane (Fig. 3.7) caused by partial dehydration of the hydrophilic bloc and mixing with LC polymer have been measured[29].

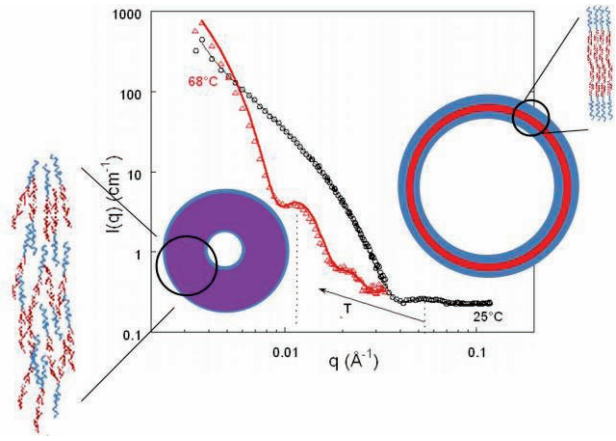

Figure 3.7. *Temperature variation of SANS by vesicular structures obtained with LC block-polyethylene glycol "polymersomes".*

With the group of S. Lecommandoux (LCPO-Bordeaux), we studied new hybrid vesicles made from biodegradable amphiphilic block copolymers and magnetic nanoparticles (NP). SANS experiments performed in different contrast conditions showed that high loading of magnetic NP inside the vesicle membrane could be prepared.[30] These magnetic vesicles have properties suitable for biomedical applications, including: being guided by an external magnetic field gradient; producing local hyperthermia under application of an oscillating magnetic field; and contrast enhancement in Magnetic Resonance Imaging with a nanomolar detection limit.

Multiple steps in the organization of alpha-CD/PEO based polyrotaxanes

Polyrotaxanes (PR) comprise another dynamic supramolecular system, which consists of a stable supramacromolecular pearl necklace assembly of macrocycles (here α-cyclodextrins) on a template polymer chain (here PEO). In a first stage of synthesis, N α-CDs (N depending on affinity with PEO) are threaded along PEO chains in aqueous solution, resulting in so-called pseudo-polyrotaxane (PPR). In the second stage, capping both ends with bulky groups prevents decomplexation, and leads to the polyrotaxane. If N is high, this allows further functionalization of the PRs, which may include crosslinking to form supramolecular " sliding" gels (S-Gels). Neutron scattering experiments have been performed to describe the kinetics of self-organization of pseudo-polyrotaxanes in water[31]. PPRs are obtained by quenching an α CD/PEO mixture in water from 70°C down to T < 30°C, and is possible due to favorable interactions between the α-CD cavities and the PEO chains. At 30°C, the kinetics of formation of the physical gel are slow enough to distinguish:

- In the first step, α-CDs thread onto PEO chains, forming PPR molecules for which water is a bad solvent, thus, these molecules rapidly aggregate to form threaded α-CD based nanorods. At a higher length scale, the nanorods associate in a Gaussian way and form precipitated domains.
- After 20 min, the system remains liquid, and reorganizes only after more than 150 min, when the precipitated domains compact and form a physical gel.

At intermediate temperatures, the structure formed is a multiblock copolymer[32,33] with alternating rigid blocks of with alternating rigid blocks of st$_{rod}$) of ≈7 nm, and flexible naked

[29] *Structural changes induced by temperature variation and magnetic filed in liquid crystal polymer vesicle,* S. Hocine, A. Brûlet, L. Jia, J. Yang, A. Di Cicco, L. Bouteiller, M.H. Li, submitted to SOFT MATTER

[30] *Dorubixin loaded magnetic polymersomes: a multifiunctional nanocarrier for theranostics* Ch. Sanson, O. Diou, E. Ibarboure, A. Brûlet, S. Miraux, O. Sandre, S. Lecommandoux, submitted to J. AM.CHEM. SOC.

[31] *Formation and Self-Organization Kinetics of alpha-CD/PEO-Based Pseudo-Polyrotaxanes in Water. A Specific Behavior at 30°C,* C. Travelet, G. Schlatter, P. Hébraud, C. Brochon, A. Lapp, G. Hadziioannou, LANGMUIR **25**:8723 (2009)

[32] *Multiblock copolymer behaviour of alpha-CD/PEO-based polyrotaxanes: towards nano-cylinder self-organization of alpha-CDs,* C. Travelet, G. Schlatter, P. Hébraud, C. Brochon, A. Lapp, D. Anokin, D. Ivanov, C. Gaillard, G. Hadziioannou, SOFT MATTER **4**:1855 (2008)

PEO segments. Hence, on cooling to room temperature, the naked PEO segments crystallize, but the αCD rod-like tubes aggregate to form regular nano-bundles whose size is limited to the nanometric scale because the naked PEO moieties act as a compatibilizer (Fig. 3.8). In this way, the nanometric scale and the gel' s transparency are preserved.

Finally, a new class of polymer networks with sliding crosslink points can be obtained, based on the crosslinking of some αcyclodextrins of the polyrotaxane precursors: the crosslink points created are not fixed but can slide along the chain of polyrotaxane).

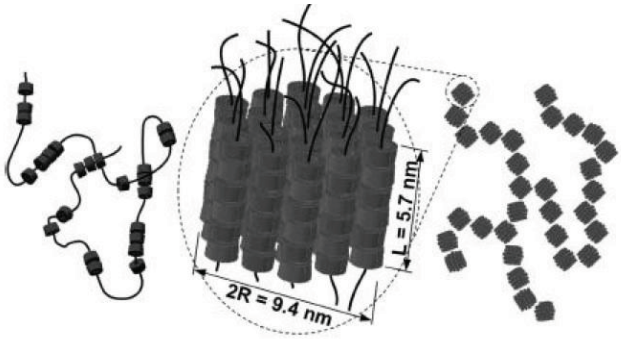

Figure 3.8. *Schematic representation of a PPR molecule showing αCD rod-like tubes and naked PEO segments; a threaded αCD based nanorod (at a higher length scale); and the nanorod associated in a Gaussian way.*

Asphaltene aggregates: oil-water emulsions and deposition mechanisms

Transportation of heavy crude oil is facilitated by using emulsions, which abate the oil' s viscosity to that of water. Knowing the fine structure of these oil-water interfaces explains their macroscopic stability. With the Institut Français du Pétrole, we developed a protocol for a SANS study of oil macro-emulsions stabilized by asphaltenes. Contrast matching gives access to the composition and the quantity of the interface: it is made of a monolayer of asphaltene aggregates, the thickness of which directly correlates to the size (volume) of the aggregates. Emulsion stability was found to improve when the interactions between aggregates inside the film are strengthened[34]. During transport or production of crude oil, reduced pressure will induce flocculation of asphaltene aggregates, causing adsorption or deposition of aggregate multilayers, which may lead to plugging. To investigate this problem, we used neutron reflectivity to study asphaltene adsorption on various model surfaces. Under static conditions, the aggregates adsorb in a dense monolayer having a thickness close to the size of the aggregates in solution (10 nm) [35]. Following addition of a bad solvent, there is a transition towards an adsorption profile that consists of multiple layers. Under *in situ* shear, we evidenced a critical shear rate, above which multilayer deposition saturates. Thus, it is possible to control local deposition by adjusting the flow rate inside the pipes[36]. We also used SANS to study asphaltene deposition mechanisms inside porous media[37], and used very dilute solutions to accurately characterize the aggregates using SAXS and SANS[38, 39].

Electrostatic-based complexes: the role of the polyion

Electrostatics is a driving force that is frequently used to create complex systems from two components having opposite electrostatic charges. Initially, we used polystyrene sulfonate (PSS), which has been the subject of detailed studies, in either full or partially sulfonated

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[33] *Temperature-Dependent Structure of α-CD/PEO-Based Polyrotaxanes in Concentrated Solution in DMSO: Kinetics and Multiblock Copolymer Behavior,*
C. Travelet, P. Hébraud, C. Perry, C. Brochon, G. Hadziioannou, A. Lapp, G Schlatter, MACROMOLECULES **43**:1915 (2010)

[34] *A Small Angle Neutron Scattering study of crude oil emulsions. Structure of the oil-water interfaces,*
G. Alvarez, J. Jestin, J.-F. Argillier, D. Langevin, LANGMUIR **25**:3985 (2009)

[35] *Asphaltene adsorption mechanisms at the local scale probed by neutron reflectivity: transition from mono to multilayer growth above flocculation threshold,*
N. Jouault, Y. Corvis, F. Cousin, J. Jestin, L. Barré, LANGMUIR **25**:3991 (2009)

[36] *Relation between solution and interfacial properties of asphaltene aggregates,*
S. Simon, J. Jestin, T. Palermo, L. Barré, ENER. FUELS, **23**:306 (2009)

[37] *Asphaltene multilayer growth in porous medium probed by SANS,*
J. Gummel, Y. Corvis, J. Jestin, J. M'Hamdi, L. Barré, EUR. PHYS. J. Special Topics, **168**:171 (2009)

[38] *Relation between nanoscale structure of asphaltene aggregates and their macroscopic solution properties,*
L. Barré, J. Jestin, A. Morisset, T. Palermo, S. Simon, OIL & GAS SCIENCE AND TECHNOLOGY, **64**:617 (2009)

[39] *Insight into asphaltene nano-aggregate structure inferred by small angle neutron and X-ray scattering,*
J. Eyssautier, P. Levitz, D. Espinat, J. Jestin, J. Gummel, I. Grillo, L. Barré, (submitted to J. AM.CHEM. SOC.)

versions[40] that were complexed with lysozyme, a small globular protein. PSS can be advantageously deuterated for different types of contrast experiments, we were able to characterize the threshold between a gel state and a globular structure. Additionally, we defined the counterions released from the globule[41], the contents in both components, the relation between the size and the screening length[42], the polyion chain conformation[43], and the possible reorganization of these globules[44]. In order to understand the role of the polyion in such complexes, we investigated the effect of increasing the rigidity of the backbone. This led us to use semi-rigid polysaccharides (biopolymers), which have a wide range of applications since such complexes are frequently used in the field of natural materials (food, pharmacy, biology). Unfortunately, deuterated versions of these polymers are not available, but due to the similarities between polysaccharides complexed with lysozyme or pectin[45] and PSS-lysozyme complexes, we have been able to profit from the knowledge obtained from deuterated PSS complexes. Surprisingly, for lysozyme complexed with hyaluronan, a very regular biopolymer, the scattering signals indicate a new structure, namely very well-defined rodlike complexes (Morfin[46] ; see Fig. 3.9). From the point of view of soft matter, data obtained from the latter system suggested the possible existence of " single complexes" leading to phase separation, which is one of the favored hypothesis for " complex coacervation" (see Axis 3: Projects).

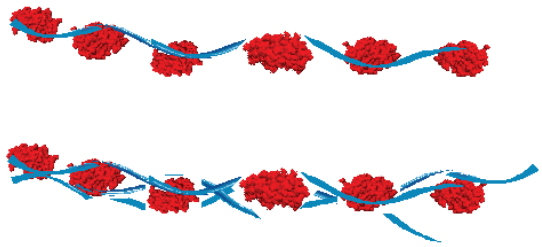

***Figure 3.9**. Elementary rodlike complex between lysozyme and Hyaluronan chain, single (up) or multiple (down).*

Protein interactions with DNA[47]

In this area, we have established patterns of interaction between DNA and Hfq, a bacterial protein involved in nucleic acid structuring and whose mechanism remains unclear. We examined the conformation of DNA bound to Hfq by combining vibrational spectroscopy and neutron scattering. Our analysis revealed that Hfq, which preferentially interacts with deoxyadenosine-rich regions (A-tracts), induces partial opening of double stranded dA-dT sequences, which is accompanied by a conformational change in the sugars of the dA strand. We observed that this conformational change correlated with dehydration of the DNA when it is complexed with the protein. By taking into account Hfq' s preferential binding to A-tracts, which are commonly found in transcriptional promoters, the biological implications of Hfq binding to DNA are of primary importance. Thus, we are currently trying to analyze the geometry and stoichiometry of complexes of Hfq and DNA, using templates of defined sizes. These results are combined with atomic force microscopy and electron microscopy in order to scan the complexes at different scales (Å to µm).

[40] *Hydrophobic polyelectrolytes in better polar solvent. Structure and chain conformation as seen by SAXS and SANS,*
W. Essafi, M.N. Spiteri, C. E. Williams, F. Boué, MACROMOLECULES **42**:9568 (2009)
[41] *Counterions release from electrostatic complexes of polyelectrolytes and proteins of opposite charge: a direct measurement,*
J. Gummel, F. Cousin, F. Boué, JACS (communication) **129**:5806 (2007)
[42] *Finite size and inner structure controlled by electrostatic screening in globular complexes of proteins and polyelectrolytes,*
J. Gummel, F. Boué, D. Clemens, F. Cousin, SOFT MATTER **4**:1653 (2008)
[43] *Structure transition in PSS/lysozyme complexes: a chain-conformation-driven process, as directly seen by small angle neutron scattering*
J. Gummel, F. Cousin, F. Boué, MACROMOLECULES **41**; 2898 (2008)
[44] *Multiscale reorganization of electrostatic complexes of PolyStyreneSulfonate and lysozyme,*
F. Cousin, J. Gummel, D. Clemens, I. Grillo, F. Boué, LANGMUIR **26**:7078 (2010)
[45] *Spatial structure and composition of polysaccharide-protein complexes from small angle neutron scattering*
I. Schmidt, F. Cousin, C. Huchon, F. Boué, M. A.V. Axelos, BIOMACROMOLECULES **10**:1346 (2009)
[46] *Rodlike Complexes of a Polyelectrolyte (Hyaluronan) and a Protein (Lysozyme) observed by SANS,*
I. Morfin, E. Buhler, F. Cousin, I Grillo, F. Boué. ASAP Biomacromolecules DOI: **10**.1021/bm100861g
[47] *Conformational transition of DNA bound to Hfq probed by infrared spectroscopy,*
F. Geinguenaud, V. Calandrini, J. Teixeira, C. Mayer, J. Liquier, C. Lavelle, V. Arluison, PHYS. CHEM CHEM PHYSICS (2010, to appear)

Proteins in complex media

The effects temperature and high hydrostatic pressure on proteins

Studying the structure and dynamics of the native and denatured states of a protein may shed some light on the mechanism of amino acid sequence folding that results in a functional 3D conformation. Currently, physical parameters, such as temperature and pressure are used for bioconservation and sterilization in food industries and can present different folding pathways for protein. In contrast to thermal sterilization, which can affect the color and taste of food due to the breaking of covalent bonds, sterilization by pressure is gentler and more economic because it can be applied at temperatures below 100°C where only low energy bonds are broken. Proteins require internal flexibility to perform their functions, and include particularly fast conformational fluctuations that occur at the picosecond and nanosecond time scales, and at 1 to 20 Å spatial extension. Quasielastic neutron scattering allows us to access these scales of time and space. Small Angle Neutron Scattering (SANS) experiments have been performed in order to investigate the evolution of the radius of gyration; intermolecular interactions; and the shape of proteins at different states, including in solution in a native conformation, in thermally denatured states at temperatures up to 95°C, and in pressure denaturated states at pressures up to 6000 bar. A basic challenge has been to better understand the mechanism of thermal- and pressure-induced unfolding of bovine pancreatic trypsin inhibitor (BPTI)[48,49] and calmodulin[50]. Another protein of interest is beta-lactaglobulin (BLG), which is a sensitive model system for studying denaturation, oligomerization, and nucleation of proteins under destabilizing conditions, which resemble processes known to occur in a number of disease states that involve beta-sheet formation.

As an extension of the previous studies, we are now interested in studying protein denaturation under pressure in relation to cold denaturation (ANR BIOSTAB). The overall goal of the BIOSTAB project is to carry out a research program on the stabilization of biological materials having therapeutic interest. An additional goal is to understand the effects of protectants on biomolecules subjected to different extreme conditions (low temperature, pH, dehydration), making it possible to justify the use of these co-solvents to obtain better stability during the storage of highly active biomolecules. The proteins used for these investigations are lysozyme and lactate dehydrogenase (LDH).

For these studies, small-angle scattering (neutrons or X-rays) has been combined with circular dichroïsm, fluorescence, and differential scanning calorimetry. Inelastic neutron scattering is combined with Raman spectroscopy and MD simulations.

Crowded environments

In vivo, the cytoplasm and many extracellular compartments are filled with very high quantities of macromolecules that occupy a total volume fraction in the range of 30-40%. As a consequence, interactions between components are significantly enhanced due to their close proximities, which are on the order of 1 nm. The term “crowding” is generally used to describe these environments which affect the thermodynamic equilibria and dynamic properties of proteins with respect to those of proteins in dilute solutions. Neutron scattering techniques are invaluable methods for studying these effects, because they probe the characteristic length and time scales for proteins within the typical ranges of size and intermolecular distance. Secondly, thanks to the scattering length density difference between hydrogen isotopes and contrast variation methods, it is possible to observe the signal of macromolecules at low concentration in the presence of very high concentrations of other components.

Understanding the equilibrium between the different possible conformations of a macromolecule is a fundamental concept in biology. If conformational changes of a molecule

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[48] *Influence of pressure on structure and dynamics of bovine pancreatic trypsin inhibitor (BPTI): Small angle and quasi-elastic neutron scattering studies,*
M.-S. Appavou, G. Gibrat, M.-C. Bellissent-Funel, BBA **1764**:414 (2006).

[49] *Temperature dependence on structure and dynamics of Bovine Pancreatic Trypsin Inhibitor (BPTI): A neutron scattering study,*
M.-S. Appavou, G. Gibrat, M.-C. Bellissent-Funel , BBA-PROTEINS & PROTEOMICS **1794**:1398 (2009)

[50] *Biophysical studies of thermal denaturation of calmodulin protein: Dynamics of native and unfilded states,*
G. Gibrat, Y. Blouquit, C. T. Craescu, M.-C. Bellissent-Funel, BIOPHYS. J. **95**:5247 (2008).

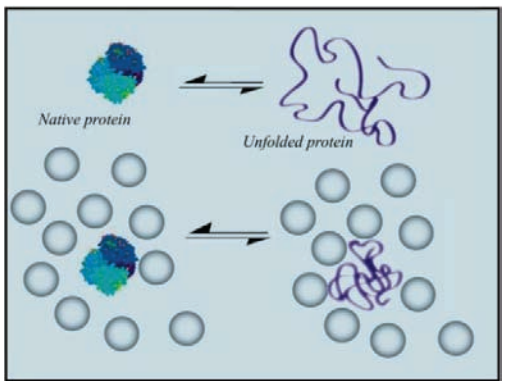

Figure 3.10. *The physical principle of protein stabilization due to macromolecular crowding. The excluded volume tends to destabilize the more extended (unfolded) conformation with respect to more compact ones including the native state. This effect shifts the N⇔U equilibrium to favor the native state.*

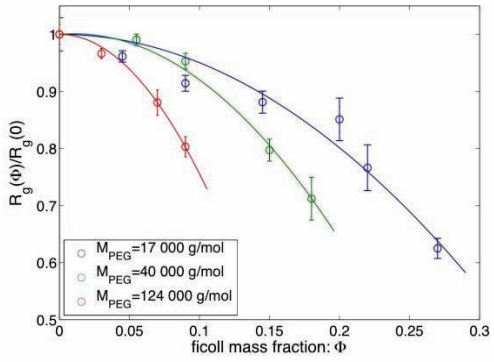

Figure 3.11. *Evolution of the radii of gyration of Gaussian chains due to the presence of Ficoll (crowding agent), obtained for polymer chains of different molecular weights.*

alter its effective volume, then its structural equilibrium inside the cell is shifted with respect to this equilibrium in dilute solution. Protein folding-unfolding falls into this category. It was predicted[51] that inert co-solutes stabilize the native state (N) of proteins against unfolding mainly by destabilizing the unfolded state (U). Osmotic pressure induced by the co-solutes diminishes the volume of a protein' s unfolded state, shifting its N⇔U equilibrium towards the native state. We undertook a detailed SANS experiment to quantify the effects of inert co-solutes on the structure of a " model" unfolded chain. We first observed the compaction of a gaussian chain due to the presence of inert cosolutes. Using contrast methods in SANS experiments, the contribution of the crowding agent was matched and the conformation of a linear polymer chain was studied as a function of the crowder concentration. It was shown that when the free volume is reduced by the crowder (300 mg/ml of cosolutes), the polymer chain is compressed and its radius of gyration is reduced by 30%[52]. To compare our results with the predictions of excluded volume theory, we studied the effect of varying the size ratio between the random coils and the crowder[53]. We then studied the temperature dependence of protein unfolding in a presence of macromolecular crowder. Preliminary results show that protein stability correlated to compression of the unfolded state.

In some cases, macromolecule diffusion is the key parameter that controls the kinetics and/or the mechanism of a biological process. Thus, a protein' s mobility in crowded surroundings plays a major role in its activity. A typical example of this concept occurs in the oxygen transport system of vertebrate organisms, in which transport is mediated by oxygen-binding proteins. Neutron Spin-Echo spectrometry is a powerful technique for studying the reduced mobility of oxygen-binding proteins in crowded or concentrated solutions because it is able to probe protein motions over appropriate intermolecular distances. We studied the details of myoglobin diffusion in solution when protein concentration was varied. It was thus possible to compare these experimental results to theoretical models developed for colloidal solutions[54]. We showed that, by rescaling the hydrodynamic volume that accounts for the hydration shell, the theories are mostly applicable up to substantial volume fraction[55]. It was also possible to measure the diffusion of hemoglobin *in vivo*, inside red blood cells (RBC),

[51] A. P. Minton, BIOPHYSICAL J., 88, 971 (2005)
[52] *Compression of random coils due to macromolecular crowding,*
C. Le Coeur, B. Demée, S. Longeville, PHYS. REV. E. **79**: 031910 (2009)
[53] *Compression of random coils due to macromolecular crowding: Scaling effects,*
C. Le Coeur, J. Teixeira, P. Busch, S. Longeville, PHYS. REV. E. 81:061914 (2010)
[54] *Myoglobin in crowded solutions: structure and diffusion,*
S. Longeville, W. Doster, G. Kali, CHEM. PHYS. **292**: 413 (2003)
[55] *Microscopic protein diffusion at high concentration by neutron spin-echo spectroscopy,*
C. Le Cœur, S. Longeville, CHEM. PHYS. **345**: 298 (2008)

which is not directly possible using other experimental approaches[56]. For a long time now, hemoglobin diffusion has been recognized as facilitating the rate of oxygen uptake by RBC in the lungs. Using a simple model, we have shown that the hemoglobin concentration observed in human RBC (330 mg/ml) corresponds to an optimum for oxygen transport, if we account for: 1) the trivial increase in transport capability that occurs with increasing hemoglobin concentration; 2) the reduced mobility of hemoglobin caused by increased protein-protein interactions; and 3) the limited time that RBC spend near the alveolar sac in the lungs to capture oxygen.

Another example of a crowded environment is the extra cellular matrix (ECM). The ECM resembles a gel that consists mainly of proteins, such as collagen, and serves to embed internal organs. It plays a central role in cancer metastasis, as both cellular invasion and tumor vascularization imply that cells must pass across this solid barrier. Cells are able to migrate through the ECM by producing proteinases. As a result, these enzymes are potential targets in cancer therapy, however, they have not been well-studied with respect to the phase transition (from a gel to a liquid state) they are able to catalyze. Cell motion responds to physical properties of the ECM at the cellular length scale, which is three orders of magnitude larger than that of typical macromolecules. These properties are governed by the physical mechanism of the phase transition (i.e., its universality class). We pointed out that this mechanism is inherently controlled by the diffusion of enzymes within the gel[57] that introduce correlations in broken bond loci. From kinetics measurements on the gelatin/thermolysin system, and by varying solvent viscosity as well as gel and enzyme concentrations, we proposed scaling relations and reduced variables that account for the experimental results, and demonstrated that the random walk of enzymes in this system is self-attracting, leading to a continuum percolation model for gel degradation[58]. We intend to pursue this pioneering work in the framework of two collaborations (with Univ. Cergy-Pontoise and INRA Reims).

Cells are organized in closed compartments that are usually surrounded by lipid bilayer membranes. This organization requires communication between compartments, i.e., the passage of nucleic aids or proteins across the membranes that separate the compartments. In many cases, this " translocation" process is achieved via proteins or peptides that self-organize within the lipid bilayer to form nanochannels having diameters that are typically between 2 nm and 5 nm. The ability to perfectly control translocation in *in vitro* studies has given rise to increasing research activity.

Many small peptides, either natural or synthetic, are known for their ability to make holes in cell membranes and for their ability to transfect DNA[59] efficiently, which allows them to have potential applications in gene therapy. Using voltage-clamp measurements, we focused[60] our attention on ionic current fluctuations observed at the onset of pore formation. The dynamics in this regime might provide clues to the trigger of pore opening. Indeed, the time autocorrelation function of the ionic current exhibited a slow power law decrease, from which no average lifetime could be computed. This is reminiscent of many-body problems near a jamming transition, such as the one occurring in Random Sequential Adsorption (RSA), rather than single particles dynamics. We have shown that four different peptides exhibit this generic feature. The exact mechanism of the membrane damage they produce is still being debated, but at least one point is established: prior to pore formation, peptides adsorb in parallel onto the membrane surface up to a high surface density at which pores begin to be observed. Our results tend to indicate that the dynamics of pore formation is controlled by fluctuations in the peptide concentration at the crowded surface. Also, they give a new insight into how these pores could be viewed as quenched or jammed structures.

Ionic current recording through single nanopores gives rise to hopes of using this technique for the study of biological macromolecules. Notable applications include DNA sequencing and

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[56] *Microscopic diffusion and hydrodynamic interactions of hemoglobin in red blood cells,*
W. Doster, S. Longeville, BIOPHYS. J. **93**: 1360 (2007)

[57] *catalyzed gel proteolysis: an anomalous diffusion-controlled mechanism.,*
G. C. Fadda, D. Lairez, B. Arrio, J.-P. Carton, and V. Larreta-Garde, Enzyme BIOPHYS. J. **85**:2808 (2003);
Percolation model for enzyme gel degradation.,
T. Abete, A. de Candia, D. Lairez, and A. Coniglio, PHYS. REV. LETT. **93**:228301 (2004)

[58] *Scaling and continuum percolation model for enzyme-catalyzed gel degradation,*
D. Lairez, J. P. Carton, G. Zalczer, and J. Pelta, PHYS. REV. LETT. **98**:228302 (2007)

[59] M. Martin and K. Rice, Peptide-guided gene delivery, AAPS Journal **9**:E18 (2007)

[60] *Fluctuations of ionic current through lipid bilayer at the onset of peptide attacks and pore formation.,*
G.C. Fadda, D. Lairez, and G. Zalczer, PHYS. REV. LETT. **103**:180601 (2009)

protein folding-unfolding studies at a single molecule level[61]. Initially concerned with biological nanopores, recent reports have considered artificial nanopores because of their versatility. Nanopore sensing of macromolecules is based on the idea that individual chain translocation causes a resistive pulse in the ionic conductance of the channel and, more generally, current fluctuations. Analysis of these fluctuations is expected to be a signature of the solute. While quite promising, progress in this domain has been impeded by a low frequency (1/f) noise of the current spectrum that is observed even for nanopores filled with solvent alone. We studied single artificial nanopores (obtained by chemical etching of heavy ion track on irradiated polymer film) with the goals of understanding and reducing this noise, which will be crucial to making the most of translocation studies[62]. We found that the relative noise amplitude is virtually independent of concentration and pH for KCl solutions, but varies strongly for ionic liquids. We have shown that whether the transport of charge carriers is either strongly facilitated (low noise and higher conductivity than in the bulk) or jammed depends on the ionic liquid. These results show that the origin of the 1/f noise cannot be ascribed to fluctuations in the properties of the pore, but rather to a cooperative effect on ion motion in a confined geometry.

Local dynamics of proteins

The sudden change at 220 K in the slope of the temperature dependence of the mean-square displacement for hydrated proteins has been extensively studied (for a review see ref. [63] and references therein). The reason for such focus on this so-called dynamical transition is twofold. First, this transition is intimately connected to protein function. Second, and this is even more interesting, this connection can be made for a wide variety of systems, from small soluble globular proteins[64] to membrane proteins. In the early steps, the role of the solvent surrounding the proteins[65] was recognized, i.e., in the absence of hydration, the 220 K dynamical transition vanishes. Also, the transition temperature is controlled by the viscosity of the solvent, i.e., the transition temperature increases in the presence of co-solvents, such as sugar[66,67]. A consensus has now emerged for the idea of a deep interplay between protein motion, and therefore function, and the surrounding solvent[68].

Dynamic processes at play in water physics are now well understood, at least in bulk water. In bulk, a water molecule is hydrogen bonded, on average, to slightly less than four neighboring molecules. If, due to thermal energy, a hydrogen bond O---H-O deviates from linearity by an angle larger than 25°, the bond breaks. When several H-bonds engaged by a molecule are simultaneously broken, the molecule is free to experience a rotational diffusive motion until several hydrogen bonds are formed again leading to the formation of a transient localization "site". It is a key point to note that within this mechanism, long range translational dynamics can only occur if rotational dynamics is present. In other words, translation is driven by rotations. Therefore, while the notion of "water dynamics" appears to be a convenient shortcut, it is actually rather vague and imprecise. Gaining real insight into the physics of water and hydration-related phenomena requires us to be able to distinguish between rotational and translational contributions.

In interfacial water, when dealing with just a monolayer of water molecules, the mechanism above is still at play even when the average number of H-bonds per molecule is

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reduced to three[69]. We have been able[70] to discriminate between the rotational and translational contributions of water molecules in such a monolayer situation at a hydrophilic surface (Vycor glass). The originality of this work was not only to discriminate between the translational and rotational water components, but also to perform this decomposition over a wide range of temperature, from 70 to 280 K. We have recently extended these results to the very active field of biophysics, which is devoted to understanding how the function of a bio-molecule can be shaped by the structural and dynamic properties of the surrounding solvent.

We have shown that interfacial water at the surface of Vycor (a hydrophilic, but chemically and dynamically inert material) experiences different dynamical crossovers. As far as rotational motion of water is concerned, transitions are detected at 150 and 220 K. At 150 K, the H-Bond becomes softer[71] . But no change in the H-Bond strength has been detected at 220 K. The 220 K dynamical crossover could then be associated to a large-scale structural change in H-Bond connectivity. We have shown[72] a strong parallel evolution at 150 and 220 K between the mean-square displacements related to (i) interfacial water rotational dynamics, and (ii) proton dynamics of a hydrated protein. This connection is made at the local scale (few Angstroms) and in the nanosecond timescale. We interpret these observations as evidence that the rotational dynamics of interfacial water is the real source of entropy that drives protein dynamics.

All together, we reach this final view of the protein-hydration water interaction[73] and how this interaction can drive protein function: the short time motions of a protein' s external side-chains[74], induced by fast, water-reorientational motion, propagate in a hierarchical way along the protein structure, from the residue side chains down to the protein core to induce the longer the timescale motion of the protein backbone that is necessary for its function. The dynamical crossovers experienced by water at 150 and 220 K are also detected on the protein dynamics, even though the timescales of the crossover can be different (longer times for protein than interfacial water).

Finally, it should be noted that LLB has started an ambitious program to routinely couple, online, a neutron experiment with other experimental techniques - not to simply make two different measurements simultaneously, since the final result is often disappointing - but in the sense of a pump-probe approach. A key point is that the excitation must be triggered by the neutron spectrometer. The performances of the next generation of neutron spectrometers (Fa#, MultiMuse) give indeed the possibility to envision time-resolved inelastic experiments following an external stimulus (laser, electric field..). To gain expertise in this new way of using neutron scattering, we have developed such an experiment, in the field of biology. The sample was a light harvesting protein under excitation with a laser[75]. The goal was to detect a possible correlation between the light harvesting process and specific dynamical modes of the protein (Figure. 3.12).

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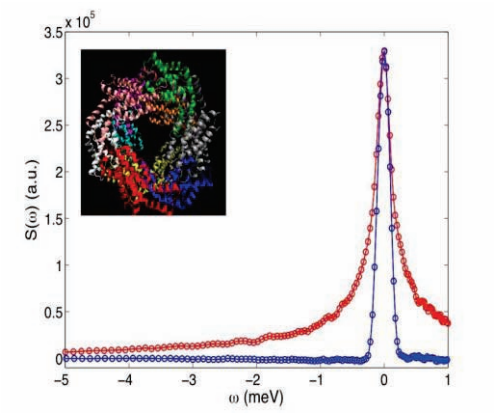

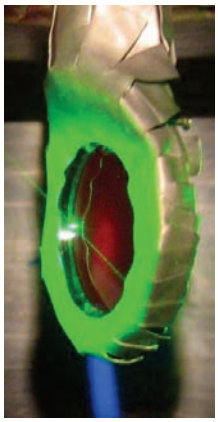

Figure 3.12. *Measurement (left) (Mibémol spectrometer) of the internal dynamics of the light harvesting protein C-phycocyanin (hexamer, inset) under excitation by a green laser (right).*

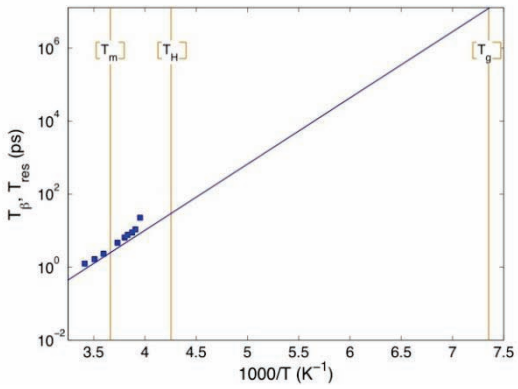

Figure 3.13. *Arrhenius plot of dynamic processes in supercooled water. Squares are residence times, T_{res}, obtained from neutron scattering experiments at temperatures above –20°C[78]. The solid blue line represents β processes, in this case, the dynamics of hydrogen bond dynamics, which follows Arrhenius temperature dependence. Above –20°C this characteristic time, T_β, is obtained from the same experimental results. T_m, T_H and T_g are the temperatures of melting, homogeneous nucleation and glass transition, respectively.*

Water properties

Water and its specific properties are fundamentally related to life and to the very peculiar properties of biological molecules, such as proteins (cf. previous topic). Here, water properties are studied in relation to the dynamics of the hydrogen bond network, as well as the notions of hydrophobicity and confinement.

The properties of bulk liquid water in the very large temperature domain extending from the lowest accessible metastable states (approximately –25°C) to the glass transition (–138°C), are always the object of speculation, such as those based on extrapolations of simulations, or on the hazardous interpretation of the properties of confined water. Efforts to explain the polyamorphism of water; in particular, the large difference in density between the low and high density forms of amorphous ice, have led to the reappearance of mixture models[76] despite the large amount of experimental evidence against their validity. In order to clarify this controversy, we performed a careful analysis and interpretation of small angle X-ray scattering experiments, and showed that the small angle scattering intensity of water at

[76] C. Huang et al PNAS **106**: 15214 (2009)

low temperature is completely explained by enhanced density fluctuation, which ruled out the existence of clusters[77].

In our view, the main unresolved problem of metastable bulk water derives from the poor knowledge of intermolecular directional hydrogen bonds. While definitions of an intact or broken bond, and its lifetime, are somewhat arbitrary, in our experiments we separate two types of dynamics in bulk water: that of the bonds and that of the molecules. The latter depends dramatically on the number of intact bonds, which increases with decreasing temperature. Its temperature dependence is non-Arrhenius with an apparent divergence at the homogeneous nucleation temperature, while the hydrogen bond dynamics depicts a simple Arrhenius temperature dependence[78].

While the coordinance of ions in aqueous solutions is well established, studies of the water structure in highly concentrated solutions are scarce and can be achieved only by a combination of scattering techniques, taking advantage of isotopic substitutions, and computer simulations. Actually, the structure of water in NaCl solutions, namely the hydrogen bond-related partials HH and OH, is not significantly modified by of the presence of ions. Instead, as expected, the number of intermolecular hydrogen bonds decreases with increasing salt concentration[79], explaining the smaller thermodynamic anomalies as compared to bulk water.

Among the puzzling phenomena observed with pure water, the " water bridge" is one of the most spectacular. A bridge of water is formed and remains stable over long times between two glass beakers filled with water and submitted to a differential electrical voltage of 15 kV[80]. Although the understanding of this phenomenon is more likely to be in the macroscopic properties of liquid water, a diffraction study showed that air microbubbles are likely present in the bridge accompanying the steady flow established between the beakers[81].

Beyond such fundamental problems of the physics of bulk water, the effects of confinement are also important, and make up the largest part of the present research. Generally speaking, the behavior of fluids confined within nanometric pores (the size of a few molecular diameters) differs significantly from that of the bulk fluid. In the case of liquid water, as pointed out above, confined water shows a rich variety of behaviors depending on essentially all the properties of the substrate. Size, roughness of the interface, wetting, hydrophobicity, and the presence of charges are some of the parameters that any detailed study must consider. Some materials are prototypes, either because of their homogeneity (e.g. porous silica or carbon nanotubes) or because of the importance of their applications (e.g. clays or cement pastes).

A comprehensive view is emerging from experiments where the confinement is due to silica nanotubes, which have a well-defined diameter and surfaces that can be grafted in order to monitor their hydrophobicity. Differential Scanning Calorimetry allows the phase diagram of confined water to be established as a function of the pore diameter and the nature of the interface. The dynamics of the liquid form persists at temperatures that decrease with the size of the pore and with the hydrophobicity of the interface, reaching 206 K in the extreme conditions. For diameters below 2.3 nm, only amorphous ice is formed[82]. These results raise important questions about the relation between structure and dynamics, the extension of the interfacial effects, the validity of thermodynamic laws for mesoscopic systems, and the significance of the surface area/volume ratio.

Globally, the behavior of water under hydrophilic confinement is better understood despite a large variety of situations related to the arrangement and dynamics of hydrogen bonds, while the behavior of water confined under hydrophobic conditions remains at the center of fundamental and applied research. As briefly mentioned above, its roles in biological systems, protein folding, hydration, energizing technologies, nanofluidic devices, are indeed crucial. Intrusion of water inside hydrophobic porous materials is naturally difficult. We intend to profit from recent progress in the synthesis of nanomaterials[83], surface treatments, and high pressure experiments, to develop the study of water confined in hydrophobic nanopores in order to evaluate a thermodynamic limit for the melting/crystallization of water.

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Clays are an example of situations where the lamellar periodicity of water layers and alumino-silicates generates a complex array of quasi two-dimensional water slabs that can be studied only through complementary information coming from experiments and atomic-scale computer simulations[84]. Incoherent quasi-elastic neutron scattering has been used to probe the water motion in these systems[85]. Unconventional data analysis is necessary to highlight the signal of low-dimensional water diffusion in clays which, in the majority of cases, exist in a powder form and cannot be easily oriented macroscopically[86]. Clay-confined water molecules form the first hydration shells of ions in these systems. The ionic diffusion coefficients are difficult to obtain experimentally, and thus parallel atomic-level simulations are exploited to provide this kind of information[87]. Information on ion and water motion is of crucial importance in light of the role of clays in soil and their numerous applications (catalysis, radioactive waste-disposal, etc.).

The isotopic effect, i.e., the different affinity of H_2O or D_2O to forming bonds with a substrate, is another complex effect observed under confinement. We studied the kinetics of cement-water mixtures during their evolution towards the gel phase. Due to specific chemical reactions that take place in different time scales, the behaviors of mixtures prepared with H_2O or D_2O are completely different from each other. The cement paste exhibits well-known temporal oscillations. These oscillations are in phase with the formation of hydration products only in the case of H_2O [88], while they are asynchronous when hydration takes place with D_2O. This observed isotopic effect results from small differences in water diffusion on the rough interface of the cement due to the increased stability of hydrogen bonds in the case of D_2O. Small angle scattering experiments measure the fractal dimension of the interface in both cases, which can be correlated with the kinetics of the sol-gel process.

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Scientific Output

Scientific collaborations

The main partner organisms in France and abroad are listed in the two Tables hereafter:

Organization	Total
[INST LAUE LANGEVIN GRENOBLE -...	25
[INRA - FRANCE]	11
[UNIV PARIS 6 - FRANCE]	11
[UNIV PARIS 11 - FRANCE]	9
[ESPCI PARIS - FRANCE]	6
[UNIV AIX MARSEILLE - FRANCE]	5
[UNIV MONTPELLIER - FRANCE]	5
[UNIV PARIS 7 - FRANCE]	5
[UNIV BORDEAUX - FRANCE]	4
[UNIV STRASBOURG - FRANCE]	4
[INST FRANCAIS PETR - FRANCE]	4
[UNIV ORLEANS - FRANCE]	4
[UNIV RENNES - FRANCE]	3
[UNIV LYON - FRANCE]	2
[UNIV PAU - FRANCE]	2
[UNIV TOULOUSE - FRANCE]	2
[ENS PARIS - FRANCE]	2
[INSERM - FRANCE]	2
[UNIV GRENOBLE - FRANCE]	2
[UNIV REIMS - FRANCE]	2

Organization	Total
[JULICH - GERMANY]	6
[UNIV BERLIN - GERMANY]	6
[RUTHERFORD APPLETON LAB - UK]	4
[UNIV ROME - ITALY]	4
[UNIV TUNIS - TUNISIA]	4
[UNIV COMENIUS - SLOVAKIA]	4
[NIST - USA]	3
[UNIV MESSINA - ITALY]	3
[UNIV REGENSBURG GERMANY]	3
[CANADIAN NEUTRON BEAM - CANAD...	3
[DESY - GERMANY]	3
[HELMHOLTZ ZENTRUM BERLIN - GERMANY...	3
[INST OF ATHENS -GREECE]	3
[UNIV BAYREUTH - GERMANY]	3
[UNIV MUNICH - GERMANY]	3
[UNIV PATRAS - GREECE]	3
[UNIV AMSTERDAM - NETHERLANDS]	2
[UNIV CALIF BERKELEY USA]	2
[INST BUCHAREST - ROMANIA]	2
[UNIV BRUSSEL - BELGIUM]	2
[UNIV BUDAPEST - HUNGARY]	2
[UNIV RUTGERS - USA]	2

Scientific contracts

Our research activities are supported by a number of different research contracts:

- ANR-PNANO BIOSELF «Auto-assemblages de nanogels et nanocomposites bioinspirés », coordonné par L. Heux, CERMAV (MAcromolecules Végétales), Grenoble(F. Boué, F. Cousin, L.-T. Lee)
- ANR-PNANO 2006-2010: projet LISSIL " Développement d' une nouvelle famille d' électrolytes solides nanohybrides. Dynamique moléculaire en milieu liquide ionique nanoconfiné" , coordonné par J. le Bideau, Univ. Nantes
- ANR " MULTICLICK" (E. Drokenmuller, G. Carrot, F. Cousin)
- ANR-PCV 2007-2011: projet BIOSTAB " Optimisation de la stabilité de matériaux biologiques pour de nouvelles stratégies thérapeutiques" , coordonné par A. Hedoux, Univ. Lille 1.
- ANR-programme blanc international 2009-2012: projet TEMPLDISCO " Template confinement effects on discotic liquid-crystals" , coordonné par D. Morineau, Univ. Rennes.
- ANR-programme blanc 2008-2011: projet TRANSFOLDPROT, Dynamics and mechanics of protein transport, interaction and folding through different protein channels at the single molecule level, coordonné par J. Pelta, Univ. Evry.
- ANR-jeune chercheur 2009-2012: project DYPOLYPO, Modelling the dynamic properties of polyelectrolytes in charged porous media, coordinated by M. Jardat (University Paris VI)

- RELAXAN: RTRA, A Langmuir trough common to neutron and X-Ray reflectivity technique, F. Cousin, Ph. Fontaine.
- PA 20 building: RTRA + C' nano (A. Brûlet)
- CIFRE for PhD in partnership with MICHELIN Reinforced rubbers, (J. Jestin, F. Boué).
- OSEO-programme GENESIS 2008-2011, " Nanomatériaux" , coordonné par ARKEMA.
- Programme bilateral Ecos Sud A09B02 2010-2013: " Etude interdisciplinaire et multi-échelle de l' interaction entre la couche-S des bactéries lactiques et les proteines de la membrane externe des bactéries Gram(-)" , Univ. Paris Diderot (V. Arluison, E. Fort, C.mayer, C. Ricolleau)/Univ. de La plata - Argentine (A. Gomez Zavaglia, P. de Urraza)

Other

PhD theses defended, and in preparation, during the period 2008-2010

- J. Vinas, " Matériaux hybrides polymères-particules de silice: synthèse et caractérisation" , 2005-2008.
- K. Lagrené, " Etude dynamique de polymères sous confinement quasi-uniaxial", 2007-2010.
- C. Chevigny, " Nanocomposites polymères-particules greffées: de la synthèse à l' étude des propriétés macroscopiques" , 2006-2009.
- N. Jouault, (LLB-Univ. Bretagne Sud). " Nanocomposites Silice/Polymère. Structure des charges, Renforcement mécanique, Conformation des chaî nes, Evolution sous deformation" , 2006-2009.
- C. Le Cœur, " Influence de l'encombrement cytoplasmique sur la stabilité et la diffusion des protéines" , 2007-2010.
- C. Mohamed-Said (2008-) " Structural and optical properties of PNIPAM-coated gold nanoparticles" .
- A.-S. Robbes (2008-) (LLB-SOLEIL) " Nanocomposites magnétiques : contrôle de la dispersion par greffage et orientation des charges sous champ externe" .
- F. Gal " Nanoparticules de platine greffées polymère" , 2007-2010.
- A.-L. Fameau (2008-) (LLB-INRA Nantes) " Vers de nouveaux détergents : assemblages d' acides gras hydroxylés du volume aux interfaces, impact de la structure sur les propriétés moussantes et émulsifiantes" .
- L. Shi (2009-) (LLB-Univ Paris VII) " Assemblages électrostatiques macromoléculaires et supramoléculaires" .
- D. Bhowmik (2008-) (LLB - Paris VI) " Dynamics in complex fluids"

Post-docs working during the period 2008-2010

- Colinet (2008-2009) " ANR MULTICLICK"
- F. Muller (2009-2010) l' ANR BIOSELF " Elaboration de nouveaux biomatériaux à base de cellulose"
- C. Rezende (funded by DRI-CEA 05/2007-04/2008) nanoparticles: bulk and surface properties. Nanocomposites latex and clay: from structure to mechanical properties.

Habilitation à Diriger les Recherches defended during the period 2008-2010

- S. Longeville (Univ. Paris VI), 2009