# Experimental Report

01/07/2015

**Proposal:** **LTP-8-4** **Council:** 10/2011

**Title:** High pressure studies on biological systems

**This proposal is a new proposal**

**Researh Area:**

**Main proposer:** **PETERS Judith**

**Experimental Team:**
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GROBELNY Sebastian
LEME Mathieu

**Local Contact:**
PETERS Judith
DEME Bruno
FRICK Bernhard
SEYDEL Tilo

**Samples:**
Lipid membranes (DMPC, DOPC)
Natural membranes from Thermococcus barophilus
Acetylcholinesterase
1,2-dimyristoyl-snglycero-3-phoshatidylcholine (DMPC)
Hen Egg white Lysozyme (from Sigma)
Polar Lipids
Lysozyme (C H N O S)/D2O
Kodakarensis cells
Yayanosii cells
E.coli cells
Egg yolk Very low density lipoprotein
Beta-Lactoglobulin (Sigma)
PSII membrane stacks

| Instrument | Req. Days | All. Days | From | To |
|---|---|---|---|---|
| D16 | 6 | 8 | 05/11/2012 | 08/11/2012 |
| | | | 22/07/2013 | 25/07/2013 |
| IN13 | 24 | 24 | 11/06/2012 | 19/06/2012 |
| | | | 12/07/2013 | 19/07/2013 |
| | | | 03/11/2014 | 11/11/2014 |
| IN6 | 9 | 9 | 13/03/2013 | 19/03/2013 |
| | | | 09/10/2014 | 12/10/2014 |
| IN16 | 8 | 6 | 24/09/2012 | 28/09/2012 |
| | | | 01/07/2013 | 08/07/2013 |
| IN5 | 9 | 9 | 25/06/2012 | 28/06/2012 |
| | | | 08/03/2013 | 11/03/2013 |
| | | | 17/11/2014 | 20/11/2014 |
| IN16B | 3 | 3 | 10/11/2014 | 13/11/2014 |

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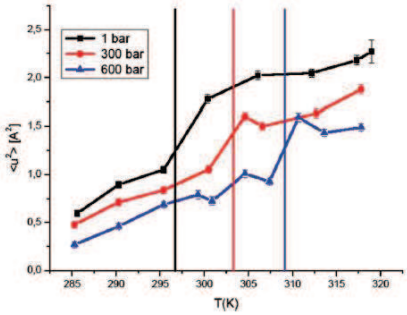
**Abstract:**

The effects of hydrostatic pressureon biological samples are poorly explored. The temperature influences the thermal energy as well as other thermodynamical parameters including the volume and the effects are difficult to separate. Pressure is a thermodynamical variable which has so far not been used extensively to probe biological samples. It provides, in particular, a well adapted tool for the study of protein folding and dynamic energy landscapes and makes it possible to characterise thermodynamically complex biological systems. It can open access to intermediate states, which cannot be reached by temperature variation. The lack of research using pressure is due to technical challenges and experimental difficulties, especially in combination with neutron scattering. Thus we will further develop the high pressure equipment present at the ILL and apply it for milestone experiments.

**LTP 8-4 report: High pressure equipment for samples in solution at the ILL**

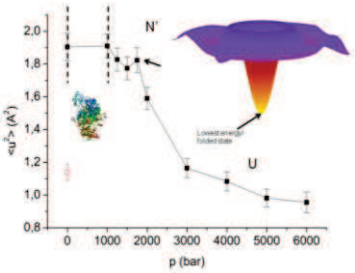
J. Peters

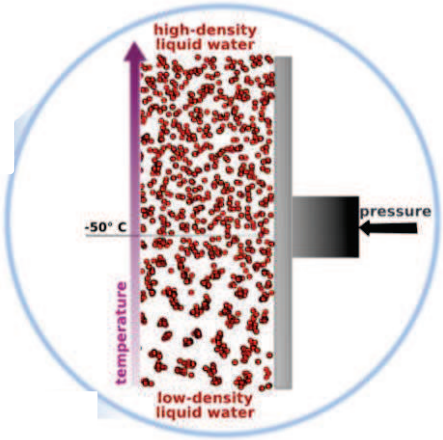
In 2011, a long-term proposal (LTP 8-4) was granted to Judith Peters and many co-workers with the aim to develop high pressure equipment and especially high pressure cells for structural and dynamical studies on biological samples in solution. The project resulted in several new cells, designed and tested by the Services for Advanced Neutron Environment (SANE), a specific funding by the CNRS (Défi Instrumentation aux Limites 2014) has been obtained for that, several experiments were run on the instruments IN5, IN6, IN13, IN16, IN16B and D16 and the necessary infrastructure was installed (high pressure module on NOMAD and a cable to permit the remote control of the pressure controller). Different types of high pressure cells cover the range of 0.1 – 700 MPa (0.001 – 7 kbar) and some of them permit to combine high pressure with high temperature up to 100 °C. They have a cylindrical shape corresponding to a sample volume of about 1 ml [1]. Moreover, new collaborations were started, among others with R. Prassl et al. from the Medical University Graz, A. Cupane et al. from the University Palermo and R. Winter et al. from the Technical University Dortmund, which took advantage from this specific equipment. Two projects got funding from the ANR: Living Deep, which deals with the question of adaptation to a high pressure environment of microorganisms from the deep sea, and LDLPRESS, which permits to investigate the behavior of natural nanoparticles, lipoproteins, under high pressure conditions (studies still under progress).




We tested first a reference system, multilamellar vesicles from the lipids DMPC, under high pressure on IN13, as their behavior as function of pressure was well known. Especially, the main phase transition temperature is shifted to higher temperature under pressure. Such findings could be verified, structurally by measuring the corresponding d-spacings on D16, and dynamically by extracting mean square displacements on IN13 [2].

As examples for further investigations on IN13 (in collaboration with F. Nachon, M. Trovaslet and P. Masson), let us mention the investigation of a molten globule state (a metastable state intermediate between the folded and the unfolded state) of the enzyme human acetylcholinesterase at around 170 MPa, where molecular dynamics are clearly enhanced [3].

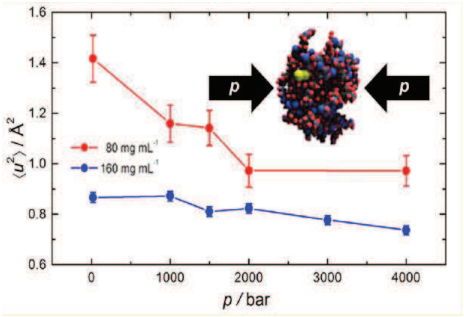


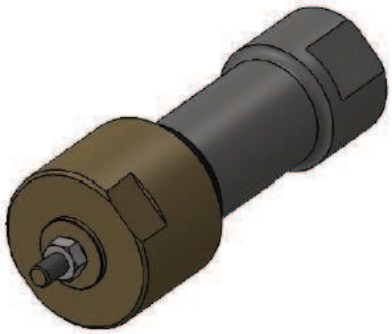

Together with A. Cupane and co-workers we investigated supercooled water confined in the pores of a three-dimensional disordered $SiO_2$ xerogel as function of temperature and pressure. Besides a glass transition evidenced by DSC at about 170 K, a first-order-like endothermic transition occurring at about 230 K that, in view of the neutron scattering results, could be attributed to a liquid-liquid crossover. Our results gave experimental evidence for the presence of a crossover occurring at about 230 K (at ambient pressure) from a

liquid phase predominant at 210 K to another liquid phase predominant at 250 K; therefore, they were fully consistent with the liquid-liquid transition hypothesis [4].

Recently, we studied lysozyme under high pressure conditions for two different protein concentrations, mimicking at higher concentration the crowded cellular environment. We found out that protein structural and interaction parameters as well as the dynamical properties of the protein are affected by pressure in a nonlinear way [5].




Finally, it is a matter of fact that the majority of the biosphere is a high pressure environment. Around 70% of the marine biosphere lies at depths below 1000m, i.e. at pressures of 0.1 MPa or higher. To survive in these environments, deep-biosphere organisms have adapted to life at high pressure. *In vitro* studies showed that the activity of certain proteins originating from deep-sea organisms is less affected by high pressure than that of enzymes from surface organisms. However, the genetic and structural bases for this increased pressure resistance are still unknown. Within the framework of a dedicated ANR project we therefore used elastic incoherent neutron scattering to shed light on adaptation mechanisms of microbes from the deep sea [6]. However, our observations would need further investigations, since the experiments could not be performed at native temperatures, ca. 85 °C, for *T. kodakarensis* and *T. barophilus*, as the high pressure cell was not withstanding high temperatures. Therefore, we raised funds from the CNRS for the development of a new high pressure cell permitting both extreme conditions. It will be tested in May 2015 on IN13. The high pressure equipment is available at the ILL and the sample cells can be borrowed from J. Peters (peters@ill.fr).



[1] J. Peters et al., High Press. Res. 32 (2012), 97 – 102.
[2] M. Trapp et al., Phys. Chem. Chem. Phys. 15 (2013) 20951.
[3] J. Marion et al., Phys. Chem. Chem. Phys. 17 (2015), 3157 - 3163.
[4] A. Cupane et al., Phys. Rev. Lett. 113 (2014), 215701.
[5] M. Erlkamp et al., J. Phys. Chem. B 119 (2015), 4842 - 4848.
[6] J. Peters et al., Z. Phys. Chem. 228 (2014), 1121-1133.