Experimental Report

Proposal: **8-04-665** **Council:** 4/2012

Title: "Molecular adaptation of deep sea microbes"

This proposal is a new proposal

Researh Area: Biology

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Samples: Thermococcus barophilus and Kodakarensis cells, Pyrococcus furiosus and yayanosii cells, E.coli cells

Instrument	Req. Days	All. Days	From	To
IN13	9	3	30/11/2012	03/12/2012

Abstract:

In the past, neutron experiments performed on bacteria and purified proteins proved to be successful in quantifying the extent to which macromolecular dynamics is affected by adaptation to extreme temperatures (Tehei et al., 2004 and 2005). Dynamics fluctuations and force constants were found by recording elastic incoherent neutron scattering on IN13. High pressure conditions prevail in a vast part of the biosphere. The existence of a pressure adaptation at the molecular dynamics level is still not established. To address this question, we will explore the effects of hydrostatic pressure on the macromolecular dynamics of different microorganisms originated from surface and deep sea environments. The intact cells will be exposed to high pressure within the sample holder in the domain between 0 and 1200 bar. Lyzed cells samples will also be measured under high pressure. Indeed, intracellular crowding conditions are expected to contribute to the stabilization of macromolecular structure and might therefore hamper the detection of high-pressure effects on protein intracellular dynamics.

Report on November/December 2012 IN13 experiment 8-04-665 and CRG 1908

Molecular adaptation of deep sea microbes

Elastic incoherent neutron scattering experiments on IN13 have revealed important differences in the mean molecular dynamics between organisms adapted to high and low temperature habitats (Tehei et al., 2004). The mean flexibility and resilience of the macromolecules were obtained and gave valuable insights on how thermoadaptation in macromolecules is achieved. These experiments were performed on whole living cells thus allowing studying the dynamics of biomolecules in their cellular context. The signal obtained from elastic scattering experiments on whole cells is expected to be dominated by the contribution from the proteins (Tehei et al., 2004). In adaptation studies, measuring whole cell systems is advantageous compared to experiments on individual proteins as the adaptation mechanism of one specific protein can deviate significantly from the predominant mechanism. IN13 is the most appropriate instrument for such studies as it permits to see internal motions without mixing them with a signal from global diffusive motions or water motion.

In the context of an ANR research program (Living Deep. 2011-2014) we propose to study the dynamics of macromolecules in whole cells from organisms isolated at different depths in order to gain insights on high pressure adaptation on the protein dynamics level. The ANR consortium brings together microbiologists from Brest, Lyon and Grenoble and biophysicists from ILL. The results from such a study would also contribute to other ongoing research in biology and biophysics. First, it has been suggested that high pressure environments might have played an important role in the origin of life (Daniel et al. 2006, DiGiulio 2005). Studying the pressure adaptation in existing organisms will help to verify this hypothesis. Second, our understanding of the mechanisms and forces that control protein structure, function and dynamics cannot be complete without understanding the effects of the two fundamental thermodynamic variables, temperature and pressure (Kauzmann 1987, Royer 2005). Whereas the effects of temperature are relatively well understood, much less is known on the effects of pressure. Third, the effect of high pressure on proteins is of great interest in biotechnology, especially in food processing (Knorr et al. 2006, Kouassi et al 2007).

Methods:

The different types of surface and deep-sea cells were cultivated at Brest (UMR 6197 UBO-IFREMER-CNRS-laboratoire de microbiologie des environnements extrêmes). The deep sea environment team from Brest has a worldclass expertise in growing hyperthermophilic and piezophilic anaerobic cells. The freshly cultivated cells were transferred to Grenoble in special cartridges and loaded into the high pressure cell in excess of water under anaerobic conditions. Cell counts and viability tests were performed prior and after the experiments (on resuspended pellets). Because of the energy resolution on IN13, the data provide information only on the dynamics of hydrogen atoms associated to relatively local chemical groups such as the side chains of proteins. They are not sensitive to the longer range diffusive motions of water or small molecules. This allowed us to measure samples without exchanging H_2O to D_2O.

E. coli cells were measured first. However, the long term objective of the « living deep » consortium is to compare the molecular dynamics of cells originating from different high pressure environments. For this purpose we have chosen model cells that are phylogenetically related to reduce bias that can occur due to differences in the cytosol content (proteome composition, nucleic acids and compatible solutes contents). In addition to pressure, temperature may affect the dynamics of the systems. Therefore one needs to measure cells that live at similar physiological temperatures. The strains to be studied are: *Thermococcus kodakaraensis (*a piezosensitive strain isolated from a solfatara on the shore of Kodakara Island, Kagoshima, Japan) and *Thermococcus barophilus* (a piezophile strain isolated from a vent chimney, Mid Atlantic Ridge, 3550 m depth), *Pyrococcus horikoshii* (1400 m depth), *P. abyssi* (2200 m depth), *P. furiosus* (shallow vent) and *Pyrococcus yayanosii* (strain CH1 a first obligate piezophile, 4100 m depth). These organisms stop growing but remain alive when handled at ambient temperature and pressure. During the last year, the microbiology team from Brest has developed protocols to maintain a good viability rate of the different strains in the conditions that are used for neutron experiments on IN13.

In 2010 and 2011 (Franzetti et al., 2010, 2011), pilot experiments using *E. coli* and thermococcal cells were performed on IN13. The reports 8-04-612 and CRG 1790 contain reference measurements as function of temperature, the reports 8-04-562, CRG 1692 and CRG 1867 summarize first results as function of high pressure. Temperature and pressure have inverse effects, i.e. the summed intensities (which are inversely proportional to the square root of the mean square displacements and thus to the flexibility of the sample) are decreasing as function of temperature, but increasing in dependence of high pressure, translating the loose of flexibility under such condition.

In the present experiment we measured three cell types: *E.coli*, as a mesophilic control; *T. kodakaraensis* and *T. barophilus* as hyperthermophilic cells. Elastic experiments were performed at ambient temperature as function of pressure between 0 and 1200 bar (figure 1). We wanted to test the reproducibility of the data taken in 2011 (CRG report 1867, figure 1 ride sight) and to get more points in the transition region for *T. Kodakarensis*.

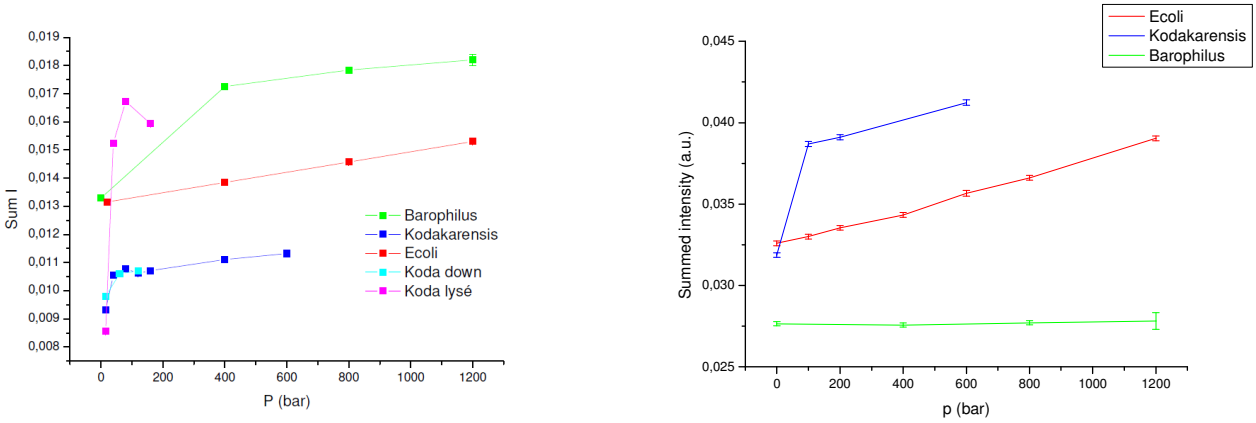

Figure 1: Summed intensities of elastic neutron scattering as function of pressure, data taken in 2012: left, and in 2011: right.

The summed intensities of *E. coli* increased again linearly with pressure. *T. kodakarensis* presented a jump in intensity and we were able to fix more precisely now around 80 bar. These results could thus be fully validated. In contrary *T. barophilus* exhibited now a similar behaviour to *T. kodakarensis*, certainly due to a little dysfunction of the pressure cell during the experiment in 2011. Thus we plan to repeat the measurement of this sample once more to better characterize the dependence of *T. barophilus* upon pressure in the domain between 0 and 400 bar.

We had an additional sample of lyzed cells from *T. kodakarensis*. The idea was to investigate the effect of confined water on the dynamics and of the forces in a very restricted environment. Indeed, when the cells are lyzed, the crowding disappears, but all components from the cytosol are conserved within the sample. The lyzed sample exhibits a curve similar to the intact cells, but the jump in intensity around 80 bar is much higher. Partly, it can be due to different masses of the samples, but it is very likely that the destroyed cell structure is much more flexible and shows thus a much higher response against pressure.

Further data analysis is still under progress and other experiments are scheduled on IN13 and IN5 in 2013. IN5 will give access for a first time to QENS data of such samples under high pressure and help to disentangle the part of confined and free water inside the cells.

References:

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