Experimental Report

Proposal: **8-04-693** **Council:** 10/2012

Title: Deep sea microbes under high pressure

This proposal is a new proposal

Researh Area: Biology

Main proposer: **FRANZETTI Bruno**

Experimental Team: PETERS Judith

Local Contact: PETERS Judith

Samples: Thermococcus barophilus cells, Kodakarensis cells, Yayanosii cells, E.coli cells

Instrument	Req. Days	All. Days	From	To
IN13	6	6	14/05/2013	20/05/2013

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. The results suggested that adaptation occurs by selecting an appropriate resilience in order to maintain optimal macromolecular flexibility required for enzyme activity while modifying protein rigidity to prevent unfolding in extreme temperature conditions. 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 micro organic species adapted and non-adapted to high-pressure conditions (shallow and deep sea area). The present proposal is part of a funded four years scientific project (ANR Living Deep 2010-2014). The experiments proposed in this proposal are mandatory, together with the data collection already

Report on May 2013 IN13 experiment 8-04-693 and CRG 2033

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. 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.

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.

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.

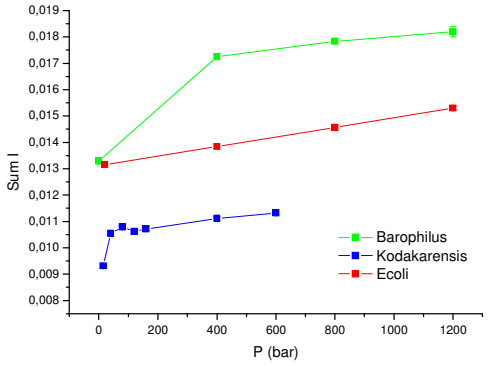

Figure 1: Summed intensities of elastic neutron scattering as function of pressure, data taken in 2012.

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 800 bar (figure 1). We wanted to test the reproducibility of the data taken in 2011(CRG report 1867) and 2012 (ILL report 8-04-665, figure 1) and to get more points in the transition region for *T. barophilus*.

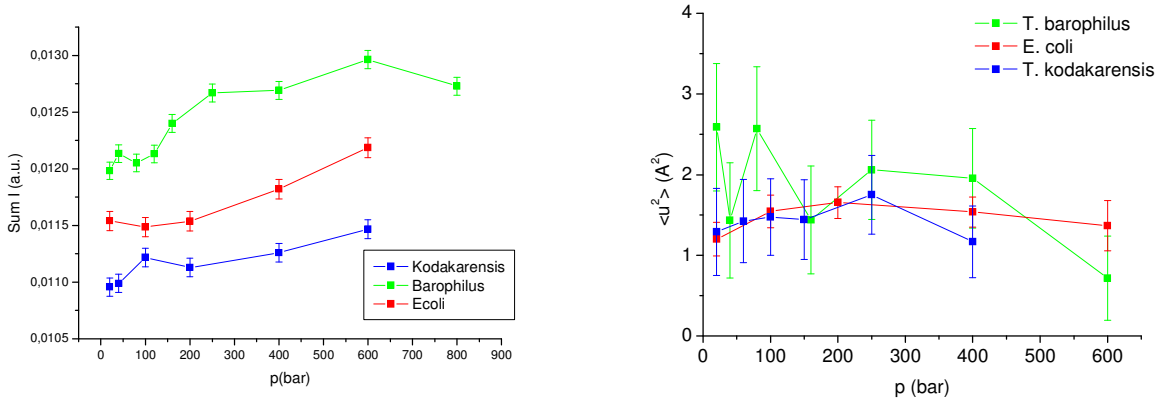

Figure 2: Summed intensities (left side) and mean square displacements (right side) extracted from elastic neutron scattering as function of pressure.

The absolute values of the summed intensities have no significance, but we could reproduce the trends found previously. The summed intensities of *E. coli* increased again with pressure and the jump in intensity at low pressure found for *T. kodakarensis* appeared again. We recorded much more points for *T. barophilus* now and we thus able to fix the transition at around 200 bar for this sample, which lives naturally in an environment of higher pressure.

We calculated furthermore the atomic mean square displacements $\langle u^2 \rangle$ for all three samples and found them slightly curved as function of pressure (see figure 2). In 2013, we did in addition quasi-elastic neutron scattering (QENS) measurements on IN5 and IN6 under high pressure (Peters et al., 2013). We develop actually a software program to analyse these data simultaneously for both instruments and resolutions to distinguish the part of confined and free water inside the cells. To complete this investigation we are asking now for beamtime on IN16B to perform QENS measurements which should be sensitive to translational motions.

References:

Tehei et al., (2004), EMBO Rep., 5:66-70.
Peters et al. (2013), ILL report LTP 8-4.