Experimental report

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Proposal: **8-04-763** **Council:** 4/2015

Title: Deep sea microbes under high pressure - a reference experiment with anew high pressure cell

Research area: Biology

This proposal is a new proposal

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Samples: T. Barophilus
T. Kodakarensis

Instrument	Requested days	Allocated days	From	To
IN13	7	7	30/11/2015	07/12/2015

Abstract:

The majority of the biosphere is a high pressure environment. Around 70% of the marine biosphere lies at depths below 1000 m, i.e. at pressures of 100 bars 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. Elastic incoherent neutron scattering studies, which provide access to information about molecular dynamics, constitute a very promising approach to decipher the structural adaptation in proteins living under high pressure. This approach has been used in the past to investigate the adaptation of biological systems to temperature and salinity and proved to be essential and complementary to structural studies. Therefore we want to investigate high pressure effects on cell dynamics using Thermococcales as models.

Report on IN13 experiments CRG 2231 and 8-04-763

Deep sea microbes under high pressure - a reference experiment with a new high pressure cell

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 [1]. 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 [1]. 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 2010 and 2011 [2, 3], first experiments using *E. coli* and thermococcal cells in a high pressure cylindrical sample holder, were performed on IN13. They were undertaken at ambient temperature as function of pressure between 0 and 1200 bar and the summed intensities compared to elastic scans as function of temperature of *E.coli* and *Kodakarensis* [3]. We clearly saw that both thermodynamic variables have inverse effects: pressure tends to increase the intensity, whereas temperature is decreasing it. *E. coli* showed a regular increase with pressure (corresponding to a decrease of the atomic mean square displacements, as the cell is losing flexibility under pressure), whereas *T. barophilus and T. kodakarensis* seemed to reach a plateau-like behaviour after a first increase [4].

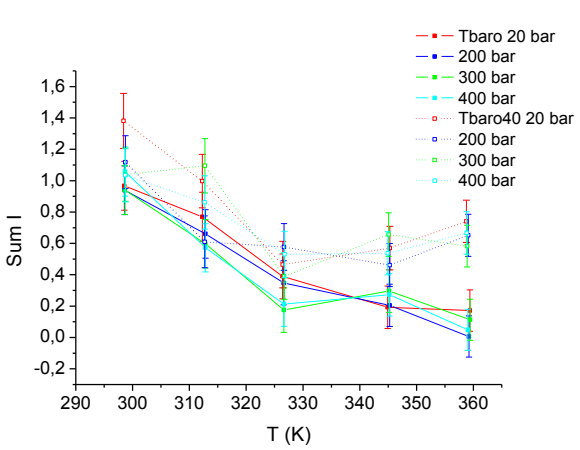
The main difficulty concerning the interpretation of these results arises from the fact that *T. kodakarensis* and *T. barophilus* originate from a high temperature environment. Both live close to hot vents at about 85 °C. However, the high-tensile aluminum alloy (7049-T6) used to build up the high pressure container so far [5] does not withstand temperatures higher than 50 °C, the material becomes then brittle and breaks. Therefore, the high pressure measurements were done at room temperature, which is far from the native conditions of these cells. In consequence, it was not clear how representative these results were with respect to the dynamics of the cells under growth permissive conditions.

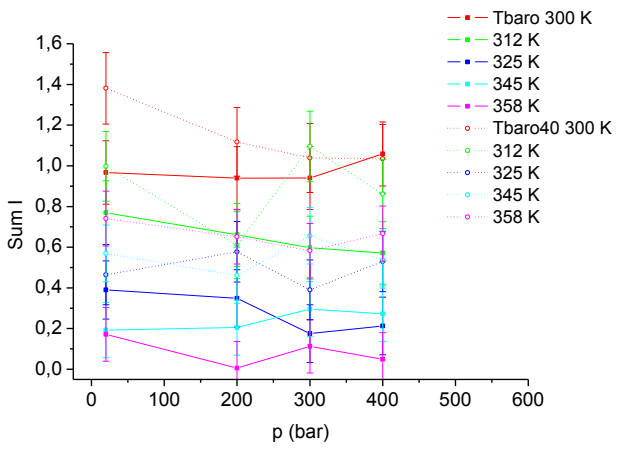
We recently got a financing from the CNRS (Défi Instrumentation aux limites) to develop a new cell which should withstand high pressure and high temperatures (up to 100°C) simultaneously. Such a cell was designed by the SANE group of the ILL using TiZr and Al for the different parts and was ready for a test experiment with real samples. The two experiments were undertaken to study cells of *T. barophilus* in solution within pressures of 1 – 400 bar and temperatures of 300 – 360 K. The first CRG experiment of 3 days (6. – 9.11.2015) failed as the HP stick was not connected to the heater, therefore the HP transmitting liquid was frozen and only temperature was transmitted. For the second experiment 8-04-763 of 7 days (30.11. – 7.12.2015) we had no such problems, but the controller "Louise" was not available and we had to change pressure manually. Two samples were investigated: *T. barophilus* grown at 85 °C and ambient pressure (Tbaro) and *T. barophilus* grown at 85°C but at 400 bar (Tbaro40).

The new high pressure cell withstands well pressure and temperature, but absorbs neutrons a lot (transmission of the empty cell is about 63 %, only !). Therefore the signal-to-noise ratio was poor and requested complicated correction calculations.

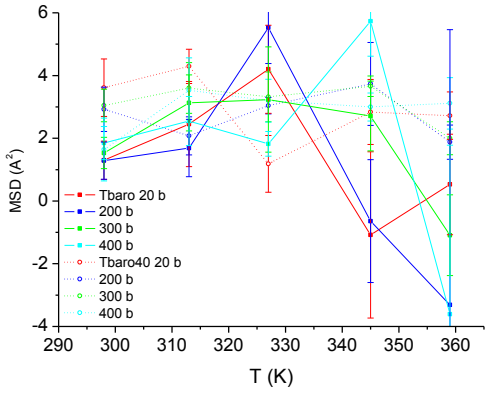
We extracted intensities summed over the Q-range (0.19 – 1.27 A^{-1}) (see Figures) and MSD, as function of T (left column) and as function of p (right column):

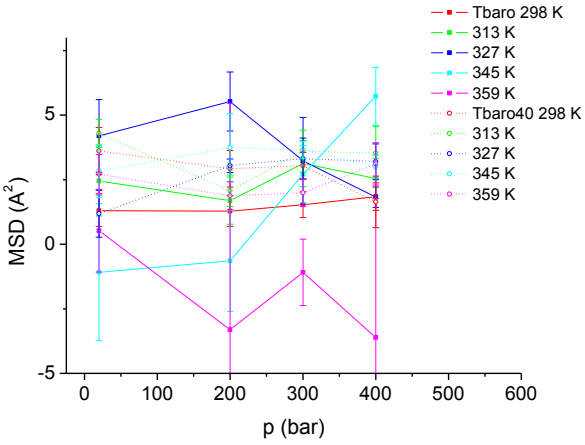
T barophilus:





T barophilus grown at 400 bar*:*





Although the data quality is not very good due to the high absorption, we tested successfully the reliable functioning of the high pressure cell. We remark that the extracted MSD (curves not shown) fluctuated much less for the second sample (*T. barophilus* grown under native conditions) indicating a higher stability of these microbes against pressure and temperature and therefore a better adaptation to extreme conditions. We would like to repeat now the experiment on the same kind of samples on IN5 to get advantage of the higher flux on this instrument and to investigate QENS studies at a resolution similar to that of IN13.

References:

[1] Tehei et al., (2004), EMBO Rep., 5:66-70.
[2] Franzetti et al. (2010), ILL exp. Report 8-04-562 and CRG 1692
[3] Franzetti et al. (2011), ILL exp. Report CRG 1790 and 8-04-612
[4] Peters et al. (2014), Z. f. Phys. Chemie, **228** (2014), 1121-1133.
[5] Peters et al. (2012), High Pressure Research (2012) **32**, 97 – 102.