Experimental report

10/05/2017

Proposal: 8-04-783 **Council:** 4/2016

Title: Probing the dynamics of Thermococcus barophilus under high temperature and high pressure

Research area: Biology

This proposal is a continuation of 8-04-763

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Samples: Thermococcus barophilus

Instrument	Requested days	Allocated days	From	To
IN5	4	4	24/11/2016	28/11/2016

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. Therefore we want to investigate high pressure effects on cell dynamics using Thermococcales as models. Former experiments on two of the species showed the influence pressure has on these microbes, but to date only measurements at 300 K could be done, as the high pressure cell did not withstand high temperature. As these organisms life close to hot vents we developed a new cell and want to repeate now a QENS measurement at native temperature conditions (360 K) to be compared to the former results. For that we would imperatively need IN5 to stay in the same instrumental conditions.

Report on IN5 experiment 8-04-783, 24 – 28/11/2016

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Probing the dynamics of *Thermococcus barophilus* under high temperature and high pressure

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. We wanted to investigate high pressure effects on cell dynamics using Thermococcales as models, in particular *Thermococcus barophilus*, the first true piezophile (an organism which thrives at high pressure) hyperthermophile described in 1999 [1]. Recently, we did a first experiment on IN5 at ambient temperature and high pressure [2], as no high pressure cell withstanding pressure and high temperature at the same time was available. Meanwhile a new high pressure cell going up to 100 °C was developed by SANE and tested, so that we wanted to investigate our sample in native conditions, too. The organism lives close to hot vents at 85 °C, therefore we wanted to compare QENS measurements at ambient temperature (22 °C) and native temperature conditions (87 °C), low pressure (30bar) to native pressure conditions (400 bar).

QENS measurements permit to discriminate between different kind of diffusive motions and various populations as bulk water, interfacial water and Hydrogen atoms belonging to the sample cells. To shed light on the effects of pressure adaptation, we investigated *T. barophilus* samples grown under native pressure and ambient pressure conditions, as the first one seems much more stable against extreme conditions, according to a test experiment done on IN13 [3].

We did the experiment on IN5, to get advantage of its higher flux, at a resolution similar to that of IN13 (about 10 µeV) and at a lower resolution (about 50 µeV). We measured *T. barophilus* at 22 and 87 °C, as function of pressure (at 30, 200, 300 and 400 bar) and at two different energy resolutions (corresponding to the incident wavelength of 5Å and 10Å). We also measured the buffer at low and high temperature and 30 and 400 bar for both resolutions, the empty cell at low and high temperature for both resolutions and the vanadium at both resolutions to do the analysis.

Preliminary results using a sum of Lorentzians to fit the different motional populations show the expected different diffusive motions (figure 1), but a more careful analysis to study the different contributions will be done.

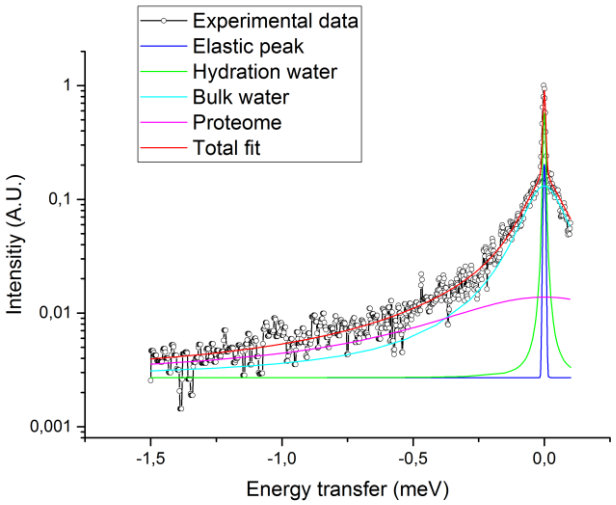

Figure 2. Experimental data and fit for T.barophilus grown under native pressure, measured at 400 bar and 22°C.

[1] Marteinsson VT, Birrien JL, Reysenbach AL, Vernet M, Marie D, Gambacorta A, Messner P, Sleytr UB, Prieur D (1999) *Thermococcus barophilus* sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. Int J Syst Bacteriol 49, 351-359.
[2] Martinez N, Michoud G, Cario A, Ollivier J, Franzetti B, Jebbar M, Oger P, Peters J (2016) High protein flexibility and reduced hydration water dynamics are key pressure adaptive strategies in prokaryotes. Sci Rep 6:32816.
[3] Peters et al. (2015), ILL exp. Report CRG 2231 and 8-04-763.