Experimental report

09/02/2019

Proposal: 8-04-839 **Council:** 4/2018

Title: Proteome dynamics as a proxy for cellular thermal stability

Research area: Biology

This proposal is a new proposal

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Samples: Psychrobacter arcticus bacterium
Escherichia coli

Instrument	Requested days	Allocated days	From	To
IN5	4	4	28/09/2018	02/10/2018
IN13	8	5	01/10/2018	06/10/2018

Abstract:

The biophysical principles at the basis of the adaptation of life to extreme environments are still elusive. Here we propose to investigate different bacteria with psychrophile to hyperthermophile character to establish the link between the sub-nanosecond timescale dynamics of their proteome and their thermal stability. We aim at providing a novel biophysical picture of the way fast local protein motions determine the thermal stability of cellular proteome and consequently the temperature range where microbial life can thrive. In this first phase of the project, we plan to measure the dynamics of psychrophilic and mesophilic bacterial proteome at the IN13 and IN5 spectrometers.

EXPERIMENT 8-04-839, 28/09/2018 — 2/10/2018

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PROTEOME DYNAMICS AS A PROXY FOR CELLULAR THERMAL STABILITY

— experimental report —

In order to establish a link between the sub-nanosecond timescale dynamics of the cellular proteome and the thermal stability of the cell, we studied the fast dynamics of two bacteria with different characteristic growth and death temperatures (CGT and CDT):

- *Escherichia Coli* (E. Coli) which is a mesophile with a CDT of about *320 K* [1]
- *Psychrobacter Arcticus* (P. A.) which is a psychrophile with a CDT of about *295 K* [2].

The samples, measured in light water solutions to rule out changes of thermal stability due to the effect of D2O, have been provided by the group of Dr. M.T. Giudici-Orticoni and Dr. M. Guiral from the Laboratoire de Bioénergétique et Ingénierie des Protéines (CNRS, Marseille — http://bip.cnrs-mrs.fr/).

The experiment was carried out at the IN13 and IN5 spectrometers. On IN13, the measurements were performed in the fixed elastic window mode, where the signal from the water is mostly outside the energy resolution. On IN5, the quasielastic signal was collected with an incident wavelength of *8 Å*, a comparable energy resolution as IN13 and an acceptable neutron flux. Moreover, since most of the biomolecules within the cells are proteins, the measured signals for such systems is mainly representative of the proteome dynamics [3].

The data were corrected for the sample holder contribution and normalized by vanadium standard. The transmission of each sample was measured and the correction for self-absorption using Paalman-Pings coefficients was performed using the LAMP software [4].

Thus, in order to obtain some preliminary information on the way the fast dynamics of these systems is related to the bacteria thermal stability, the hydrogen mean square displacement (MSD) was calculated in the Gaussian approximation (valid for small Q^2) from the IN13 data [5]:

$$ln\,[\, I_{el}\,(Q, E = 0 \pm \Delta E, T)\,] \approx const. - MSD \cdot Q^2 / 6 \qquad (1)$$

$$0.27\ Å^{-1} < Q < 1.47\ Å^{-1} \qquad (2)$$

where $ln\,[\,I_{el}(Q, E = 0 \pm \Delta E, T)\,]$ is the logarithm of the incoherent elastic intensity.

The results of this analysis are shown in the following graph for both the bacteria we measured in the present ILL beamtime (E. Coli and P. A.) and the ones we measured during the CRG beamtime (CRG 2537), namely: the thermophile *Thermus Thermophilus* (T. T.) and the hyperthermophile *Aquifex Aeolicus* (A. A.).

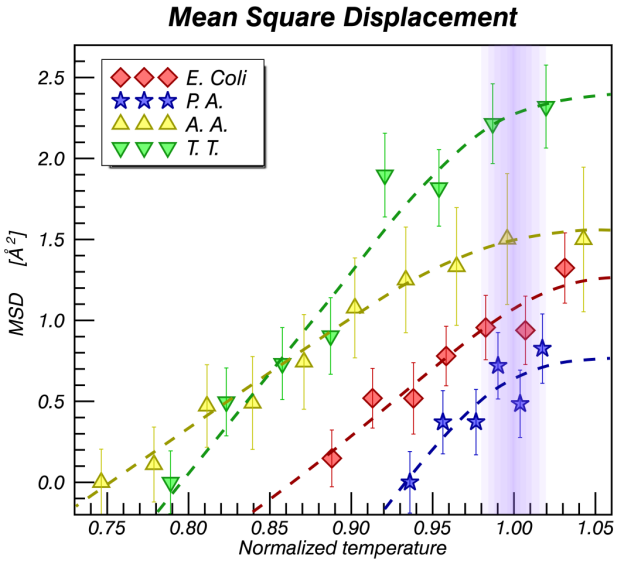

Fig. 1: MSD of each bacteria, in the Gaussian approximation (1). Temperatures were normalized to the CDT of the corresponding bacteria. Dashed lines are a guide to the eye.

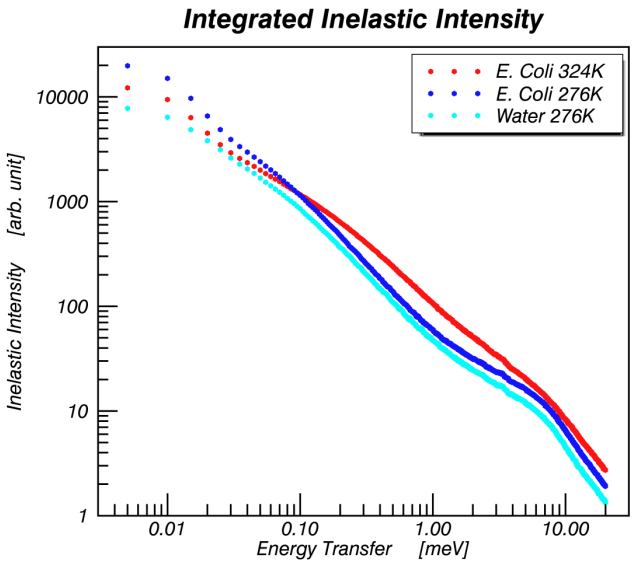

Fig. 2: The graph shows the inelastically scattered incoherent intensity integrated over all the scattering angles for the E. Coli bacteria and a sample of light water.

In **Fig. 1** it is shown that the MSD of the bacteria attains a plateau at around the CDT, thus suggesting a possible dynamical change. This result, if confirmed, will support the idea that there is a connection between the fast dynamics of the bacteria and their thermal stability.

In **Fig. 2** it is displayed that, for the E. Coli, there is a clear temperature dependence of the spectral features revealed with the IN5 measurements. It was also verified that this dependence on the temperature is also present, in a different form, for the P. A. bacteria. In order to better understand the dynamical mechanisms behind this trend, both the quasielastic and the inelastic features of these spectra will be analyzed through the implementation of an accurate data analysis that will include global large-scale and local confined motions.

REFERENCES

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[3] Bicout, D. J., & Field, M. J. (1996). *The Journal of Physical Chemistry*, *100*(7), 2489-2497.

[4] Richard et al. (1996). *J. Neutron Research* 4, 33-39. DOI: 10.1080/10238169608200065

[5] Peters et al. (2013). *The European Physical Journal E*, *36*(7), 78.