Experimental report

21/04/2020

Proposal: CRG-2664 **Council:** 4/2019

Title: Proteome dynamics as a proxy for cellular thermal stability. Aquifex Aelicus Hyperthermophile bacteria.

Research area:

This proposal is a new proposal

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Samples: Aquifex aeolicus

Instrument	Requested days	Allocated days	From	To
IN13	4	4	06/09/2019	10/09/2019

Abstract:

EXPERIMENT CRG 2664, 1/9/2019 – 8/9/2019

PROTEOME DYNAMICS AS A PROXY FOR CELLULAR THERMAL STABILITY

— experimental report —

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This experiment is part of a more general study where, combining neutron scattering and multi-scale molecular dynamics, we aim at providing a novel biophysical picture of the way the proteome dynamic and thermodynamic properties are linked to, and possibly define, the cellular thermal stability. In particular, this experiment is the continuation of the experiment CRG 2537 and it was performed to verify the obtained results for the two bacteria that, living at high temperature, require more measurements.

We measured the elastic incoherent scattered intensities of bacteria with different characteristic growth and death temperatures (T_{CG} and T_{CD}):

- *Thermus Thermophilus* (T.T.) which is a thermophile with a T_{CD} of about *350 K* [1],
- *Aquifex Aeolicus* (A.A.) which is an hyperthermophile with a T_{CD} of about *370 K* [2].

The samples 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. The bacteria were suspended in light water in order to rule out changes of thermal stability due to the effect of D_2O. The measurements were performed at the IN13 spectrometer in the fixed elastic window mode, where the signal from H_2O is mostly outside the energy resolution. Therefore, since most of the biomolecules within the cells are proteins, the signal for such systems is mainly representative of the proteome dynamics [3]. The data were corrected for the sample holder contribution and normalized by the lowest temperature in order to enhance the changes of the scattered intensity due to variation of the sample temperature[1]. The transmission of each sample was measured and the correction for self-absorption using Paalman-Pings coefficients was performed using the LAMP software [4].

Then, to obtain 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) [5]:

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

$$0.5\ \text{Å}^{-1} < Q < 1.7\ \text{Å}^{-1} \qquad (2)$$

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

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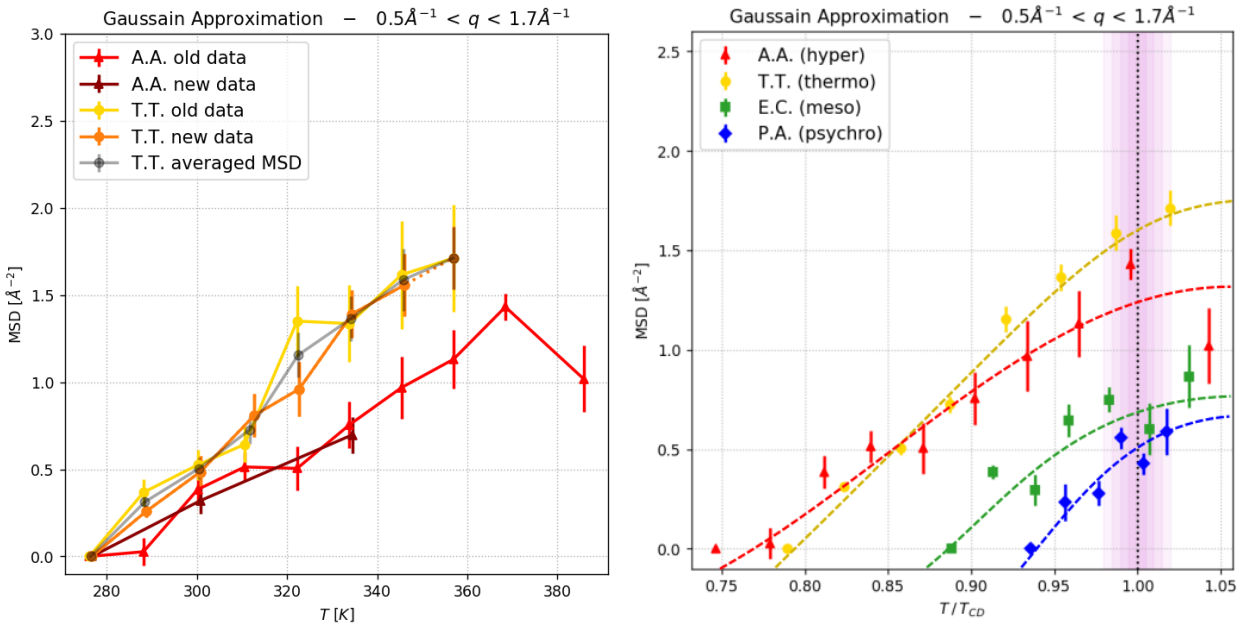
[1] Due to the complexity of the studied systems, the simple Gaussian approximation does not work very well, especially for the data normalized to the Vanadium. Therefore, in order to have first insights into the dynamics of the systems and to emphasize the changes of the MSD due to the changes in temperature, we decide to try with the normalization to the lowest temperature (276K). The drawback of this procedure, of course, is that it can yield only qualitative results.

In the **Fig. 1a** we show the MSD for both the bacteria that we have measured here and we compared these results with the ones of the CRG 2537 experiment. The MSD obtained from the new data confirm the trend observed in the previous experiment.



(a) **(b)**

Fig. 1: The graph on the left, **Fig. 1a**, shows the comparison between the MSD of the two bacteria derived from current experiment (new data) and the ones obtained from the CRG 2537 experiment (old data). On the right, **Fig. 1b** shows the MSD vs. T / T_{CD} for all the studied bacteria — dashed lines were added as a guide to the eye.

In **Fig. 1b** are reported the MSD vs. T/T_{CD} of all the bacteria that we are studying: hyper-thermophile A. A., the thermophile T. T., the mesophile *Escherichia Coli* (E.C.) and the psychrophile *Psychrobacter Arcticus* (P.A.) — E.C. and P.A. were measured during the ILL beamtime (Experiment 8-04-839).

Quite interestingly, it seems that there is a change in the slope of the MSD around the T_{CD}. This suggests that, at about T_{CD}, the bacterial proteomes attain a special dynamical regime.

To better understand the dynamical mechanisms behind this trend, and to have a quantitative measure of the MSD, we are now implementing a more accurate data analysis using different models for the calculation of the MSD to take into account the dynamical heterogeneity of the systems [6] and remove properly the contribution of the water.

REFERENCES

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