Experimental report

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Title: Diffusive Dynamics of the proteomeas a Proxy of Cell Death for psychrophilic and hyperthermophilic bacteria

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Samples: Aquifex aeolicus
psychrobacter arcticus

Instrument	Requested days	Allocated days	From	To
IN16B	5	4	17/11/2023	21/11/2023

Abstract:

The biophysical principles at the basis of the adaptation of life to extreme environments are still elusive. Very recently we combined Neutron Scattering (NS) spectroscopy and Molecular Dynamics (MD) simulations to show that the global motions of the ¿E. coli¿ proteome are intimately related to the cell¿s metabolism and thermal stability in the temperature range where microbial life can thrive. We aim now at applying the same approach to two bacteria with a radically different character, i.e. the psychrophilic ¿P. Articus¿ (PA) and the hyper-thermophilic ¿A. Aeolicus¿ (AA). Therefore, we propose to study the ns timescale global diffusive dynamics of the proteome of PA and AA in their temperature niche. By comparing previous results on ¿E. coli¿ with those on PA and AA, we will provide a possibly general picture on the relationship between ns timecsale proteome dynamics, thermal stability and metabolism.

Exp. Report 8-04-960

Diffusive Dynamics of the proteome as a Proxy of Cell Death for psychrophilic and hyperthermophilic bacteria

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Temperature changes have profound and manifold effects on cells. Even small changes in environmental temperature can cause biological migrations, extinctions, genetic divergence, and speciation [1-3]. Modelling the influence of climate change on microbial organism growth [4], establishing theoretical limitations for life in severe conditions [5], and optimizing thermal-based cancer treatments [6] all require a thorough knowledge of cell thermal stability. The molecular mechanisms that determine the cell's heat sensitivity, on the other hand, are largely unknown.
Since proteins are both the least stable and most common biomolecules in cells, their thermal stability and that of their proteome must be tightly linked. Indeed, quite recently it has been proposed that the cell's death takes place in coincidence with a denaturation catastrophe of its proteome [7]. On these grounds, it is key to investigate the role played by the protein dynamics in determining the cell's death. Our group has already shown that there is an intimate relationship between the thermal stability and the fast dynamics of proteins and both are modulated by their molecular environments [8]. It is also known that the fast dynamics of the proteome of bacteria with a different nature, from psychrophilic to hyper-thermophilic character, is related to their different thermal stability [9]. Very recently, by using Neutron Scattering (NS) spectroscopy and multi-scale molecular dynamics (MD) simulations, we have shown that the diffusive dynamics of the E. coli proteome is an excellent proxy for temperature-dependent bacterial metabolism and death [10].

Aim of the proposals
The aim of the present proposal was to understand how the proteome fast (nanosecond) dynamics of the two bacteria *Psychrophilic Arcticus* (PA) and *Aquifex Aeolicus* (AA) is related to their specific thermal stability, also in comparison with our previous results for *E. coli* (EC). The existence of critical dynamic regimes related to the bacterial thermal stability provides novel insights about the microscopic picture of life adaptation to environments at extreme temperatures. Particularly we reveal the fraction of unfolded proteome at the cell death temperature (T_{CD}) and the relationship between ns dynamics and bacterial metabolism.

Experiments on IN16B
All bacteria samples were freshly prepared for the experiment by Dr. M.T. Giudici-Orticoni and Dr. M. Guiral from the Laboratoire de Bioénergétique et Ingénierie des Protéines (CNRS, Marseille). The signal of the bacteria was measured in a light water solution, in order to rule out changes of thermal stability due to the effect of D_2O. Since most of the biomolecules within the bacteria are proteins, the signal is mainly representative of the proteome dynamics. The IN16B spectrometer has already proven to be very effective to investigate the global mobility of proteins in the ns timescale in crowded conditions [4,5]. Therefore, we performed QENS on

the IN16B spectrometer (Institut Laue Langevin (ILL), Grenoble). We investigated three bacteria with varying thermal stabilities: the psychrophilic *Psychrobacter arcticus* ($T_{CD} \simeq 295$ K), the mesophilic *Escherichia coli* ($T_{CD} \simeq 323$ K), and the hyperthermophilic *Aquifex aeolicus* ($T_{CD} \simeq 368$ K). For that we scanned from 276 K to 324 K in 6 K increments for PA, from 276 to 350 K in 8 K steps for EC and from 290 K to 390 K in 10 K steps for AA.

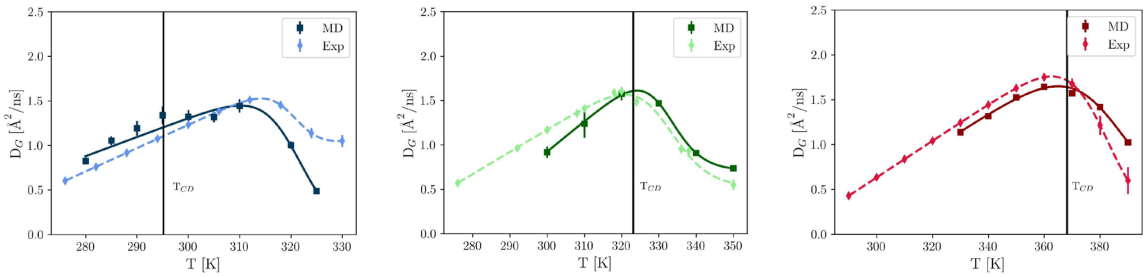

Figure 1: For *P. arcticus* (left), *E. coli* (middle) and *A. aeolicus* (right) global dynamics from QENS experiments (light color) and global dynamics obtained from a combination of folded and unfolded global diffusion coefficients and by an optimal combination of concentration levels to minimize the least squares difference of MD and QENS diffusion (dark color).

In Figure 1, we show the global diffusion coefficient D_G extracted from the experiment following the same approach as described in [10]. As previously found for EC, the diffusion coefficient presented a sharp drop around the cell death temperature for AA, but a surprising behavior for the psychrophilic bacterium PA. Here the diffusion coefficient is still increasing up to 20 K above T_{CD}. We compared our experimental results to coarse-grained and atomic MD simulations [11], mimicking the drop in dynamics by combining the folded and unfolded proteome within the cells. This permits in addition to extract information on the ratio of unfolded protein at the cell death temperature. We completed our experimental studies with DSC measurements of the same systems at the PSCM facility. The reason for the deviant behavior of the psychrophilic bacterium is still debated.

References

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