# Experimental report

24/05/2022

**Proposal:** 8-04-922 **Council:** 4/2021

**Title:** Global proteome dynamics as a proxy for cellular thermal stability.

**Research area:** Biology

**This proposal is a new proposal**


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**Experimental team:** Daniele DI BARI

**Local contacts:** Tilo SEYDEL


**Samples:** Escherichia coli bacteria

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| IN16B | 1 | 1 | 30/06/2021 | 01/07/2021 |


## Abstract:

he biophysical principles at the basis of the adaptation of life to extreme environments are still elusive. Here we propose to study the reversibility of the ns timescale global diffusive contribution to the E. Coli proteome average dynamics after the cell thermal death. We aim at providing a novel biophysical picture of the way fast global protein motions are related to the thermal stability of the very cellular proteome and consequently the temperature range where microbial life can thrive. The results from the spectrometer IN16B will be combined with mesoscale molecular dynamics simulations.

Exp. Report 8-04-879 and 8-04-922

**Global proteome dynamics as a proxy for cellular thermal stability**

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Life on Earth exhibits an amazing adaptive capacity. One of the most striking evidence of adaptation to extremely adverse environments is the presence of microbial life in a vast temperature range from below 0 °C in glacial waters to above 100 °C in deep ocean hot vents. The mechanisms that preserve bacteria from decline under these extreme conditions are still elusive. Since proteins are both the least stable and most common biomolecule, the thermal stability of cells and their proteome must be tightly linked. Indeed, quite recently it had been proposed that the cell's death takes place in coincidence with the denaturation catastrophe of its proteome [1]. Neutron scattering (NS) spectroscopy has already shown that the internal fast dynamics of proteins in the pico- to nanosecond time scale well reflects the different thermal stability of the proteome of bacteria with a different nature, ranging from psychrophilic to hyperthermophilic character [2, 3]. This study was done in a range close to room temperature extrapolating the mean square displacement values to high temperature, but a detailed picture of the trend of the proteome dynamics in the range close to cell's death is still missing.

**Aim of the proposals**

First we wanted to understand the relationship between the fast (nanosecond dynamics) of biological systems *in vivo*, namely of the well-studied bacterium *Escherichia coli*, with their specific thermal stability. The existence of critical dynamic regimes related to the bacterial thermal stability can provide novel insights about the microscopic picture of life adaptation to environments at extreme temperatures.

In a second time, we proposed to study the reversibility of the ns timescale global diffusive contribution to the E. Coli proteome average dynamics after the cell thermal death. This allows to directly compare the dynamic features of native *E. Coli* proteome with those of partially unfolded *E. Coli*. Such information is crucial to better understand the dynamical changes of the bacterial proteome that are connected to the thermal death of the cell.

**Experiments on IN16B**

The *E. coli* samples were freshly prepared for each experiment and provided 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 investigated the average diffusion of the proteomes of the mesophile *E. coli* bacteria on the ns timescales, where hydrodynamic interactions dominate over negligible protein collisions. *E. coli* (with a cell death temperature (CDT) = 322 K) was measured from 276 K to 360 K, and then at decreasing temperatures from 360K down to 300K.

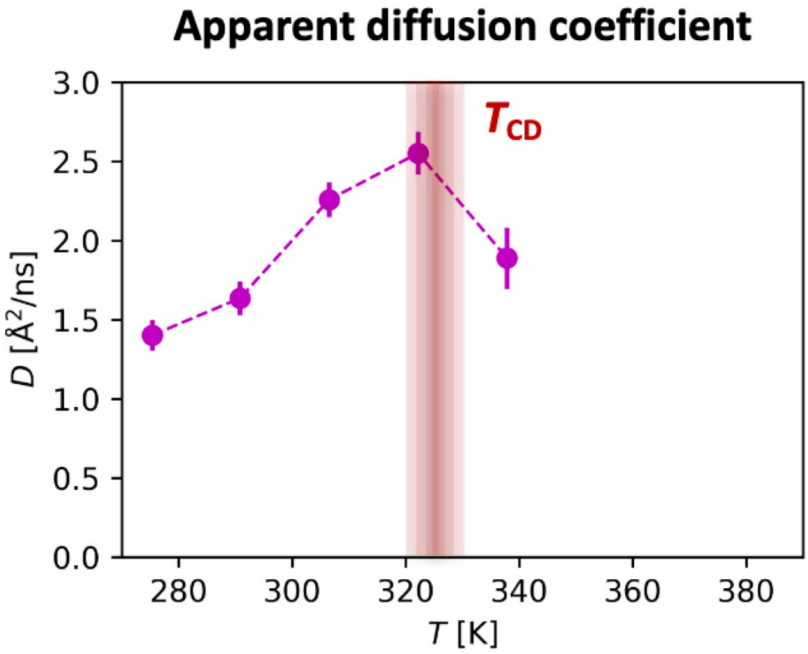


**Figure 1:** Average apparent diffusion coefficient D of the E. Coli proteins at increasing temperature.

In Figure 1, we show the average apparent diffusion coefficient D extracted from our first experiment in 2019 from *E. Coli* proteome at increasing temperatures obtained from the simultaneous fit of the QENS signal assuming a jump-model diffusion for the global dynamics of the proteins [6]. As shown in the graph, we found a clear reduction of D after CDT, and we verified this result by testing different fitting procedures (simpler fits with less assumptions, variation of the parameters, etc.). A similar drop in D was observed previously for homogeneous crowded protein solutions above their thermal denaturation temperature [7]. Therefore, our results reinforce the connection between the proteome unfolding and the thermal death of the cell.

During the second experiment in 2021, we checked the (non)-reversibility of molecular dynamics, when increasing the temperature beyond CDT and decreasing it again. Figure 2 shows the extracted mean square displacements along with fits according to a model suggested by O. Matsarskaia et al. [8]. The denaturation temperature $T_d$ found in this way is very similar to our CDT. It describes partial unfolding of the protein, where aggregation processes set in and lead to a non-reversibility of the process when lowering the temperature again.

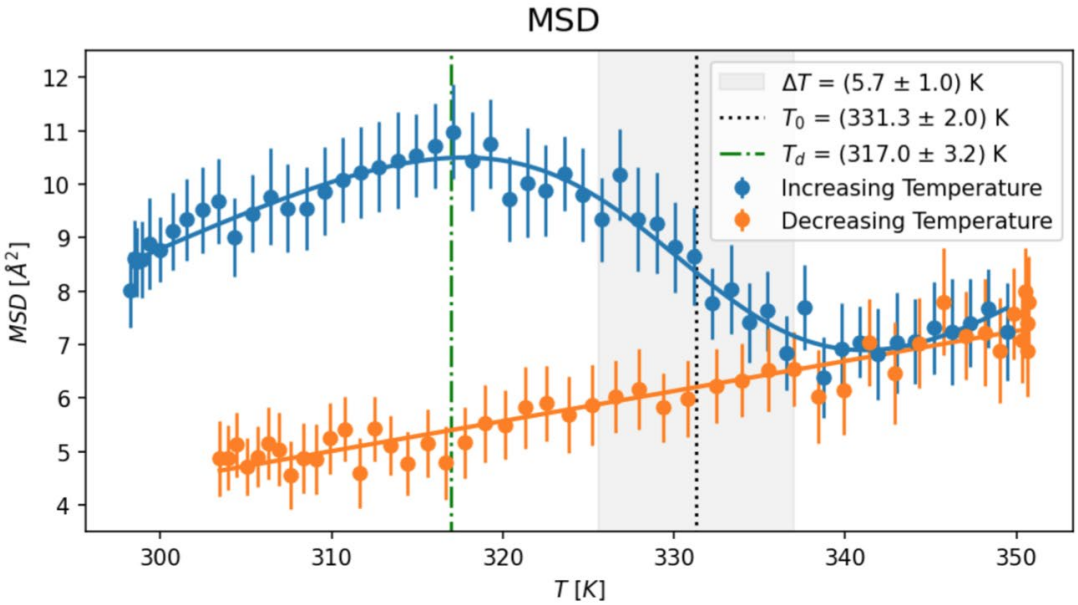


**Figure 2:** Mean square displacements extracted from elastic data taken on IN16B when increasing and decreasing the temperature.

**References:**

[1] Dill K et al. (2011). PNAS 108, 17876
[2] Tehei M et al. (2004). EMBO Reports 5, 66

[3] Katava M. et al. (2017) PNAS 114: 9361–9366

[4] Roosen-Runge F. et al. PNAS (2011) 108, 11815–11820

[5] Grimaldo M. et al (2019) J. Phys. Chem. Lett. 10, 1709-1715.

[6] Jasnin M. et al. (2008) Biophys. J. 95 857–864

[7] Grimaldo M. et al. (2015) EPJ Web Conf. 83

[8] O. Matsarskaia et al. (2020) PCCP 22, 18507