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Decoding the Role of the Global Proteome Dynamics for Cellular Thermal Stability

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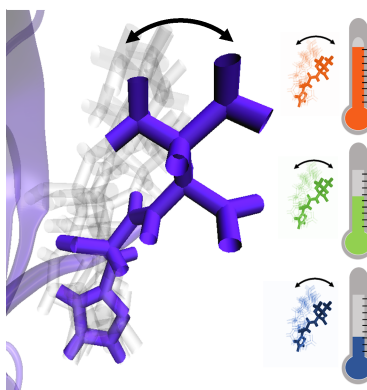
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Abstract

Molecular mechanisms underlying thermal response of cells remain elusive. Based on the recent result that the short-time diffusive dynamics of the *Escherichia coli* proteome is an excellent indicator of temperature-dependent bacterial metabolism and death, we used neutron scattering (NS) spectroscopy and molecular dynamics (MD) simulations to investigate the sub-nanosecond proteome mobility in psychro-, meso- and hyperthermophilic bacteria over a wide temperature range. The magnitude of thermal fluctuations, measured by atomic mean squared displacements, is similar among all studied bacteria at their respective thermal cell death. Global roto-translational motions turn out to be the main factor distinguishing the bacterial dynamical properties. We ascribe this behaviour to the difference in the average proteome net charge, which becomes less negative for increasing bacterial thermal stability. We propose that the chemical-physical properties of the cytoplasm and the resulting proteome's global dynamics are fine-tuned by evolution to uphold optimal thermal stability conditions.



Even small changes in environmental temperature impact the diversity of life on Earth, its evolution and adaptation. The outcomes are processes like biological migrations, extinctions, genetic divergence, and speciation of the living organisms.¹⁻³

At the cellular scale temperature concurs to control the kinetics of metabolic processes and the stability of the molecular machinery. A thorough comprehension of cellular thermal stability is crucial for modeling the impact of climate change on microbial growth,⁴ establishing theoretical boundaries for life in extreme environments,⁵ and optimizing thermal-based treatments for cancer.⁶ However, the molecular mechanisms that determine the cell's heat sensitivity, on the other hand, are largely unknown.

All biomolecular components in a cell are affected by heat, but the thermal sensitivity of proteins, which are the most abundant and least stable biomolecules,⁷ likely plays a significant role in determining temperature-dependent cellular activities.

Several hypotheses have been proposed to explain the degradation of the proteome in relation to the upper limit of the cellular thermal niche, known as the cell's death temperature (T_{CD}), and to quantify proteome thermal stability.⁸⁻¹⁰ Bacteria have been extensively studied as simplified cellular models, and the effect of temperature on cellular growth can be examined in bacteria adapted to different thermal domains, covering a wide temperature range from around 273.15 K (0°C) to 373.15 K (100°C). The death of bacteria has been first attributed to a proteome catastrophe, where most proteins unfold in a narrow temperature range near T_{CD} .^{9,11,12} However, successive experimental studies suggested that thermal adaptation results from the preferential stabilization of a subset of proteins that act as metabolic hubs and their unfolding triggers cell death.^{10,13} It has been also suggested that in cell ageing just a small fraction of the proteome is not in the folded state around the death midpoint.¹⁴

Very recently, we demonstrated that the nanosecond timescale dynamics of the *E. coli* proteome is an excellent indicator of temperature-dependent bacterial metabolism and death.¹⁵ We also showed that the cell death induced dynamical arrest of the proteome is caused by the unfolding of a small set of proteins, thus supporting a recent hub view.¹⁰ Building on

this finding, as well as previous research that reported the increasing macromolecular resilience of the bacteria thermal motions as their character shifts from psychrophilic (adapted to cold environments) to mesophilic (adapted to human like conditions) and then to hyperthermophilic (thriving in hot environments),^{16,17} we employed elastic incoherent neutron scattering (EINS) spectroscopy and molecular dynamics (MD) simulations to examine the sub-nanosecond dynamics of three bacteria, *Psychrobacter arcticus* (PA), *Escherichia coli* (EC), *Aquifex aeolicus* (AA), with varying thermal stability ($T_{\text{CD}}^{\text{PA}} \simeq 295$ K, $T_{\text{CD}}^{\text{EC}} \simeq 323$ K, and $T_{\text{CD}}^{\text{AA}} \simeq 368$ K) across a wide temperature range up to reaching cell death.

Our results indicate that the extent of proteome thermal fluctuations, as measured by atomic mean squared fluctuations, is quite similar across all the studied bacteria at their respective thermal cell death. MD simulations suggest that the global movements of the proteome are the primary factor that distinguishes the dynamic properties of the bacteria with different thermostability, and not the proteins' internal motions.

Mobility of Proteomes Reaches a Common Value at the Bacterial Thermal Death. The quantity $-\ln S(Q, E \sim 0)$ against Q^2 extracted from the NS experiments is shown in Fig. 1, demonstrating that the Gaussian approximation provides an excellent description of the measured intensity in the Q^2 range up to 2.2 \AA^{-2} . It is noteworthy that the scattering signal is predominantly caused by the high incoherent scattering cross section of hydrogen atoms. This signal mainly reflects the self-diffusive dynamics of the average protein, or proteome, in the bacterial cytoplasm,^{18,19} which represents the most abundant dry weight cellular composition (see SI for details). As seen in Fig. 2, the calculated mean square displacements $\langle u^2 \rangle$ for all the bacteria show a linear temperature dependence, with a clear trend, where $\langle u^2 \rangle_{\text{PA}}$ is larger than $\langle u^2 \rangle_{\text{EC}}$ that is in turn larger than $\langle u^2 \rangle_{\text{AA}}$ in the whole investigated temperature range. Quite interestingly, the proteome mobility is inversely proportional to the bacterial thermal stability and the $\langle u^2 \rangle$ s attain a very similar value in the range close to the T_{CD} of each bacterium (see also in Fig. 2 the range of values marked in gray).

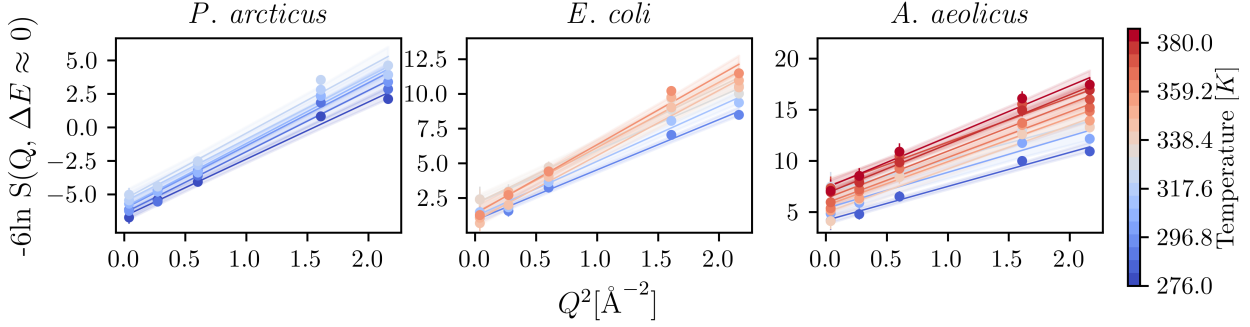


Figure 1: Fit of $-6 \ln S(Q, E \sim 0)$ as a function of Q^2 , where $S(Q, E \sim 0)$ represents the Elastic Incoherent Neutron Scattering (EINS) intensity measured for *P. arcticus* (left panel), *E. coli* (middle panel), *A. aeolicus* (right panel).

As already observed for proteins global diffusivity in *E. coli*,¹⁵ this result suggests that there exists a critical dynamics of the proteome linked to the bacterial thermal death. The existence of a critical threshold for the amplitude of thermal fluctuations has been also proposed in the case of simple model systems such as proteins in powder environment undergoing melting.^{20,21} In these studies it was suggested that biomolecules exhibit an analogous behaviour as solid-state systems obeying the Lindemann criterion, i.e. their structure becomes unstable when the extent of the atomic mean square displacements exceeds a certain fraction of the typical interatomic distance. The $\langle u^2 \rangle$ values here reported correspond to sub-nanosecond timescale motions, therefore we expect that they include contributions from both global roto-translation and local intra-protein dynamics.^{22,23} Actually, both these contributions may explain the observed linear trend. The translational cytoplasmic protein mobility, which provides most part of the global dynamics,^{15,18,19} can be described by Brownian diffusion²⁴ and, if the Stokes-Einstein equation holds, the former should contribute with a term $\sim 6D_{av}^t \tau_R = K_B T / \eta_{app} a_{av}$, where D_{av}^t , τ_R , η_{app} and a_{av} are the short-time average protein translational diffusion coefficient, the accessible experimental time, the apparent cytoplasmic viscosity (also accounting for inter-protein interactions) and the average protein hydrodynamic radius, respectively. On the other hand, also the protein internal dynamics in the anharmonic regime, a region in which the molecular system's energy landscape deviates from its simple harmonic behaviour, can be reasonably well described with a linear tem-

perature dependence, whose slope is inversely proportional to the molecular conformational rigidity.²⁵

The slope of the $\langle u^2 \rangle$ values was extracted to obtain the resilience of each bacterium, which is defined²⁵ as $k = 0.00276/(d\langle u^2 \rangle/dT)$. For PA a value of 0.1109 ± 0.022 N/m was obtained, for EC the resilience is equal to 0.0930 ± 0.012 N/m, and for AA a resilience value of 0.1690 ± 0.017 N/m was retrieved. As also reported in the past,¹⁶ there is an increase in resilience in passing from mesophile to hyperthermophile. Also for PA and EC, which attain very similar values, an increase within the error-bar can be observed. While the values may not align precisely with those in Ref. 16, this discrepancy could be attributed to variations in the experimental setup. However, it is noteworthy that the ratio between the resilience of the hyperthermophile and the mesophile remains related, where $k_{AA}/k_{EC} = 1.8$ was found similar to 1.5 in Ref. 16.

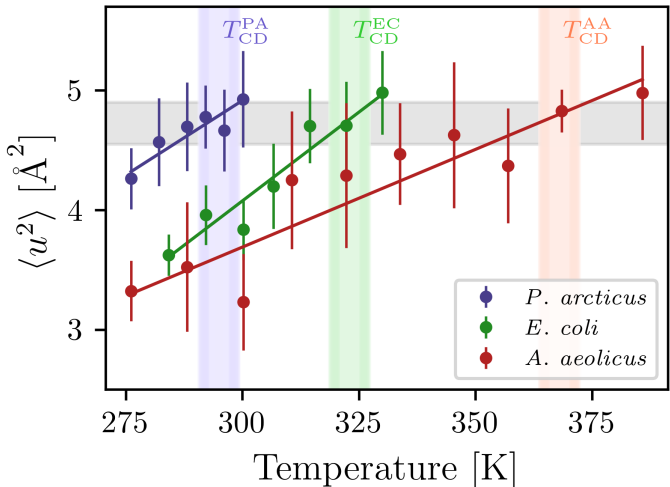


Figure 2: Mean square displacements extracted from the EINS fits using the Gaussian approximation for PA (blue), EC (green), AA (red). The mean square displacements were fitted with a linear function. The vertical color ranges in lightblue, lightgreen, and lightred represent the cell death temperature range for the respective organisms. The gray zone indicates the common range of values at the different cell death temperatures.

Thermal Stability of Bacteria Dictates Protein Global Motions. The question arises as to whether the specific trend in the bacterial mean squared displacements comes

from a difference in the proteome global diffusive roto-translational dynamics or the internal dynamics of the average protein of the three bacteria or both. To determine the specific role of the global and internal dynamics of the average protein of each kind of bacteria we performed MD simulations on three representative systems modeling the bacterial cytoplasm (see Fig. 3.A and supplementary material). In Fig. 3.B we see that the $\langle u^2 \rangle$ s from computations confirm the trend from NS experiments, with the proteome mobility decreasing at the same temperature on passing from psychrophile to mesophile and then to hyperthermophile. For PA and EC the $\langle u^2 \rangle$ s from MD simulations have also values consistent with the experimental ones, while in the case of AA the numerical estimates are quite larger than expected. By inspecting the individual proteins in simulations at the same temperatures we further confirmed the difference in the mean square amplitudes for each protein type validating the experimental results, see Fig. S5 in the supplementary material.

If the translational dynamics is subtracted, the $\langle u^2 \rangle$ s of the three bacteria decrease significantly. In the temperature range of 320 K to 350 K, where both $\langle u^2 \rangle_{EC}$ and $\langle u^2 \rangle_{AA}$ were calculated, they become very similar. When the rotational contribution is also removed, the $\langle u^2 \rangle$ s decrease even further and appear to follow a trend common to all the bacteria. This behaviour strongly suggests that the global roto-translational dynamics, but not the local internal protein dynamics, is correlated to the specific thermal stability of the investigated bacteria. Our results support the early intuition by Creuhet and coworkers²⁶ and their revisiting of EINS data²⁷ on Malate/Lactate mesophilic/thermophilic homologues.

As mentioned above, the computed $\langle u^2 \rangle$ s for the hyperthermophilic proteins are larger than the experimental measurements. The explanation for this disagreement is possibly linked to features exhibited by bacteria thriving at high temperatures that are not accounted for in the MD simulations, such as a relatively high cellular cytoplasmic viscosity that would allow thermophiles and hyperthermophiles to mitigate the thermal deterioration of NADH (nicotinammina adenina dinucleotide).²⁸ This is reminiscent of the "viscoadaptation" response to temperature increases that in budding yeast triggers compensatory changes that can main-

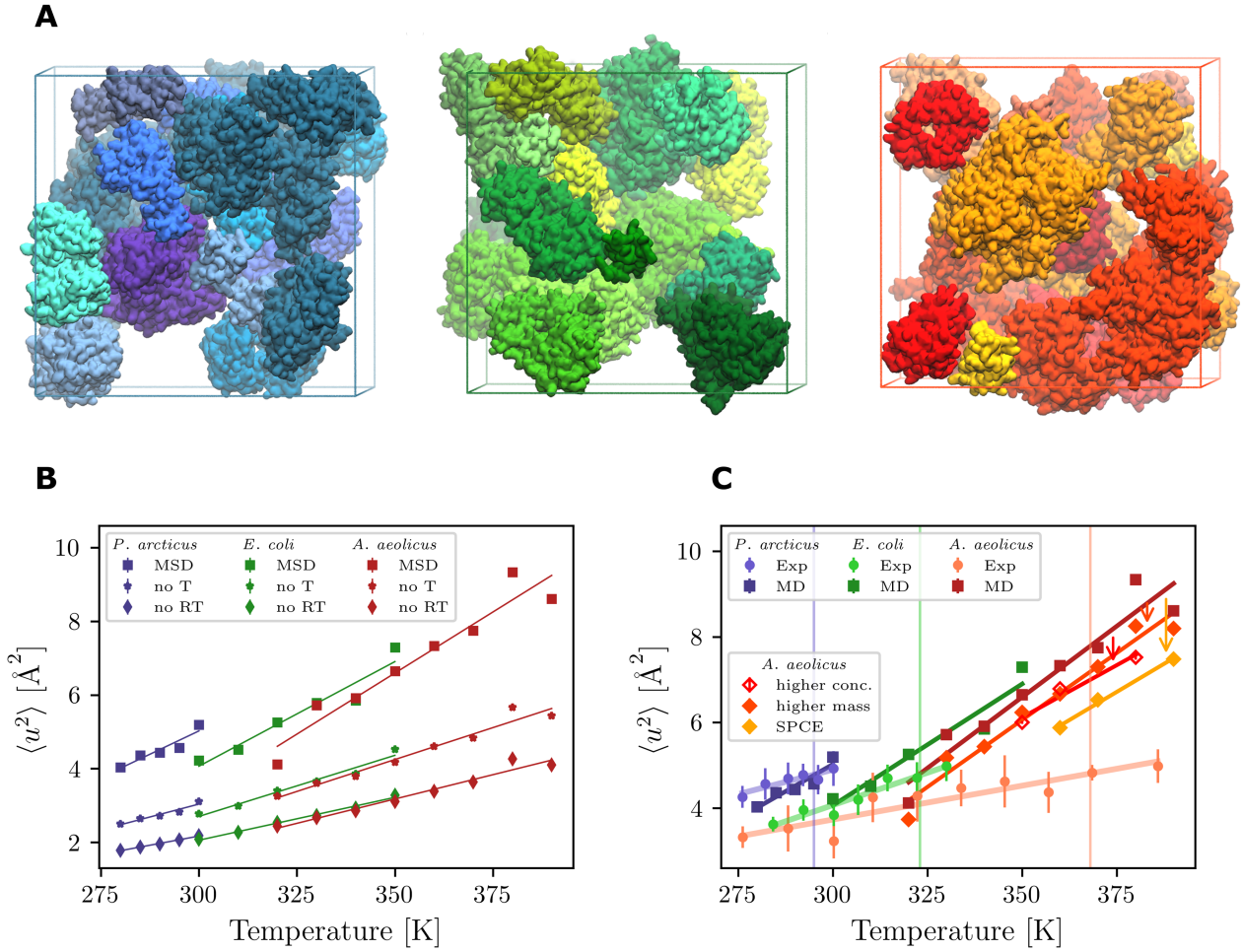


Figure 3: Mean square displacements of the three bacteria PA, EC, AA from Molecular dynamics and Neutron Scattering. (A) Representations of the three systems modeling the cytoplasm of the psychrophile, PA (left), the mesophile EC (middle), the hyperthermophile, AA (right). (B) Mean square displacements calculated from molecular dynamics simulations of the protein systems of PA (blue), EC (green), AA (darkred). The $\langle u^2 \rangle$ s including all motions are represented as squares. The $\langle u^2 \rangle$ s after removal of translational motions are shown as stars. The $\langle u^2 \rangle$ s without roto-translations are noted as diamonds. (C) Comparison between experimental (circles) and molecular dynamics (squares and diamonds) mean square displacements of the proteins in PA (blue), EC (green), AA (darkred). The colored vertical lines represent the respective cell death temperature. The MD simulations of the three built systems (squares) showed a good agreement with the experiments in the case of PA and EC, while the system of AA had exceedingly high $\langle u^2 \rangle$ values. Three further scenarios have been analysed showing a decrease in molecular dynamics $\langle u^2 \rangle$ values for AA: (i) a system with a higher concentration of proteins (red), (ii) considering only $\langle u^2 \rangle$ s of proteins with a higher mass, (iii) a system using SPC/E water model instead of TIP3P.

tain invariant diffusion across a 293.15 K temperature range.²⁹ To dig further, we computed the viscosity of the protein solutions at 320 K by integrating the correlation function of the stress-tensors components,³⁰

$$\eta_{ij} = \frac{V}{k_B T} \int_0^\infty \langle P_{ij}(0) P_{ij}(\tau) \rangle d\tau$$

where V is the volume of the box, k_B is the Boltzmann constant, T the temperature of the system, and P_{ij} the stress tensor component (see supplementary material for further details). The results indicate an increase in the viscosity passing from 14.14 ± 2.28 mPa·s for PA to 17.45 ± 3.24 mPa·s for EC¹⁵ and further to 21.40 ± 3.99 mPa·s for AA. As evidently shown by the Einstein relation, the viscosity of the system is tidily linked to the dynamics of the proteins. Our findings suggest that an adaptation of both, the dynamics and the viscosity, to the different temperature ranges takes place in the proteomes of the different organisms. We then consider three possible conditions that would alter the local environment of the hyperthermophilic cytoplasm so to impose a better match with our experimental results. We first run simulations of the system, but at a higher crowding condition of around 340 g/L. As shown in Fig. 3.B, upon increasing concentration the $\langle u^2 \rangle$ s decrease. We then computed the $\langle u^2 \rangle$ s from the original setup, but considering only proteins with larger masses, see supplemental information for details. Also in this case the $\langle u^2 \rangle$ decrease. Finally we have run a protein system with a different model of water, namely the SPC/E model that exhibits slower diffusivity and higher viscosity with respect to TIP3P.³¹ Again, the mean square displacements decrease when compared to the original set up. Besides force field quality, all these tests, and the experimental measurements, point to the fact that the modelling of the hyperthermophilic cytoplasm must be more sophisticated, in terms of proteins, and metabolites, composition and concentration so to correctly reproduce the effect of the environmental viscosity as temperature increases up to the cell death.

Less Negative Protein Charge Reduces Global Proteome Mobility. The set of

proteins included in the MD simulations exhibits a predominantly negative total net charge, and this charge becomes on the average less and less negative when passing from the PA to the EC and further to the AA, as illustrated in Fig. 4.A. A similar trend is observed for the surface net charge (see Fig. 4.B). Therefore, we propose that the increasing net (surface) charge may be at the origin of the decrease of the proteome mean squared displacements on passing from the psychrophilic to mesophilic and hyperthermophilic bacteria we investigated.

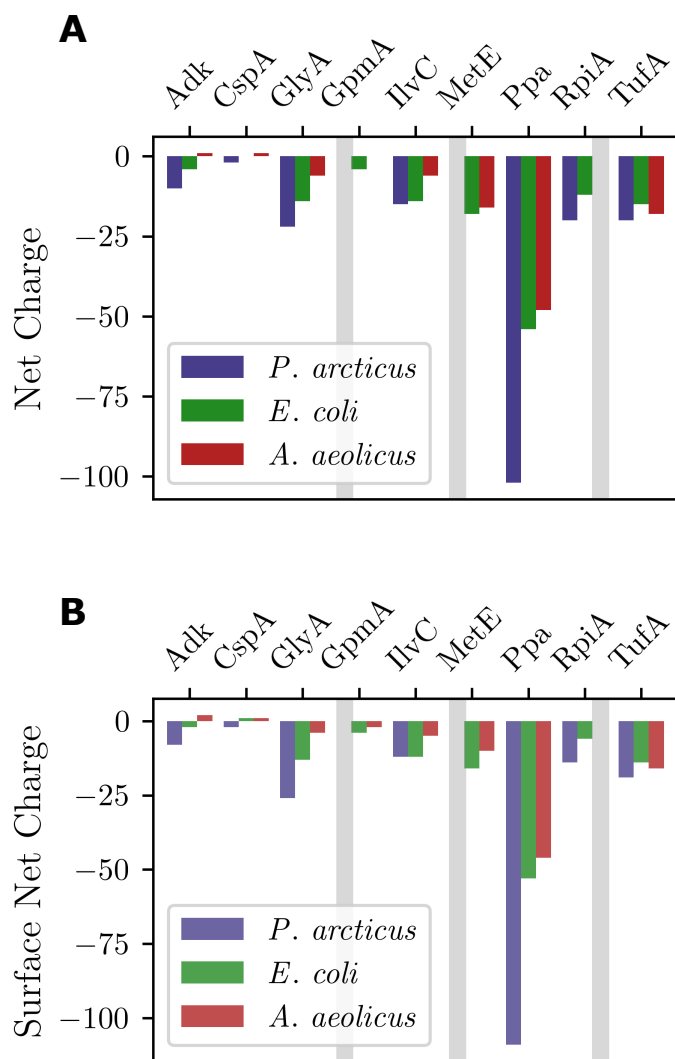


Figure 4: Analysis of the proteins' net charge in the system of PA (blue), EC (green), AA (red). The gray lines display the missing protein type in the respective system. (A) Net charge of all the residues in the proteins in the three systems. (B) Net charge computed on the residues lying on the surface of the proteins in the systems.

In fact, recent research has shown that protein rotational diffusion in cells is less affected by transient binding to other proteins or macromolecules when the protein has a larger net negative charge.³²⁻³⁴ The net (surface) charge present in the bacterial proteome would generate a repulsive force that effectively balances out the attractive dispersion force potentially promoting uncontrolled aggregation.³⁵ Furthermore, it has been discovered that the diffusion coefficient in *E. coli* is influenced by both the net charge and the distribution of the charge across the protein.³⁶ In particular, proteins with a positive net charge have been observed to diffuse up to hundred times slower than those with a negative charge.³⁶ The normalized short-time translational diffusion coefficient predicted by colloid theory for charged spheres, which has been already used to explain the trend of crowded protein solutions,¹⁸ can be approximated as $D^t/D_0^t = 1 - 15\Phi/8x$,³⁷ where D_0^t is the dilute limit translational diffusion coefficient, Φ is the protein volume fraction and $x = \sigma^{EHS}/\sigma$, with σ^{EHS} and σ the effective and the hydrodynamic diameter of the charged sphere. Within this scheme, and supposing that D_0^t and σ of the average protein do not vary significantly in the investigated bacteria, the trend of the $\langle u^2 \rangle$ suggests that (given that the protein volume fraction Φ is maintained over the different systems), σ^{EHS} decreases on passing from PA to EC and then to AA. This is consistent with larger effective diameters for more negatively charged proteins. We have verified that the inter-proteins contacts – an indirect quantification of the effective pairing between proteins in the crowded solution and that inversely correlates to σ_{EHS} – increases passing from PA to EC and AA.

Therefore, our findings support the notion that the net (surface) charge of the typical protein exerts a significant influence on the overall roto-translational diffusion within the cytoplasm, which are the main dynamic components that are specifically related to the different thermal stabilities of the bacteria. A recent study has shown that the protein surface-charge density is a key evolutionary determinant, with optimal values varying across organisms with different intracellular conditions.³⁸ This finding allows for the comprehensive functional classification of organisms across all kingdoms of life. Based on these results, our

study substantiate the idea that the global diffusive dynamics of the proteome are subject to evolutionary control and fine-tuned across different organisms to maintain optimal metabolic function at proper thermal stability conditions. The high value of the net negative charge of the psychrophilic ensures cytoplasm fluidity even at low temperature, while when the net charge decreases, as in hyperthermophilic species, a higher viscous environment damps proteins' mobility, and fluidity is recovered only at high temperature. The corresponding state principle early invoked by Somero³⁹ to describe the stability-function trade-off in extremophilic enzymes extends here to the whole physical properties of the cytoplasm and proteome diffusivity up to the cell death where all organisms show a common value of thermal fluctuations, anticipating the dynamical arrest caused by unfolding.^{15,21}

Conclusion. In this work we combined EINS experiments and MD simulations to investigate the sub-nanosecond dynamics of the proteome from bacteria adapted to different thermal environments. We were able to record their dynamics in a wide range of temperatures up to the cell death. The experimental measurements confirm two facts previously reported in the literature for other systems but in a restricted temperature regime^{16,17} : i) in the common range of temperatures around ambient condition the sub-nanosecond motion of the proteins is hierarchically correlated with the thermal character of the organism, with hyperthermophilic bacteria exhibiting the smallest amplitude of motion and psychrophilic bacteria exhibiting the largest; ii) when looking at the temperature response, the hyperthermophile shows a strong resilient character. Moreover, and very interesting, our measurements show that at their respective cell death temperatures the three organisms have comparable sub-nanosecond dynamics. Thus, the so-called corresponding-state picture relating protein dynamics/flexibility to optimal working conditions,^{39,40} extends to the cell death regime. By deploying atomistic MD simulations, and simulating models of the three bacteria cytoplasm, we clearly show that the different dynamics of the proteome is caused by the global rotational motion of the macromolecules, and not by the internal fluctuations of proteins. In fact, when the global motion is removed, the internal dynamics of the proteins from the

different organisms collapse on each other. We therefore indicate, for the first time, that in cell-like conditions, the average protein flexibility is identical for homologous proteins.

Our results support the early intuition by Crehuet and coworkers:²⁶ organisms adapted to different thermal conditions tend to have a comparable cytoplasmic environment (viscosity, protein diffusivity) at their respective working-to-death thermal niche. The different global motion relates to the different net charge contents of the homologous proteins in the three proteome models. A strong negative charge content on protein surfaces ensures high fluidity to the psychrophilic cytoplasm even at low temperature. On the contrary, the very peculiar small amplitude motion (low diffusion contribution) and the high resilience to thermal increase of the hyperthermophilic bacteria, suggest that for this organism the cytoplasmic composition and concentration are specifically adapted to extremely high temperatures. The biochemical/physical origin of such a viscous environment is a newly opened question in the field of life adaptation to extreme conditions. In future work, the global motion at a longer time-scale of extremophiles will be investigated to confirm experimentally our *in silico* analysis, and verify how thermal adaptation of the bacteria fine tunes the hub picture.¹⁰ The present results reinforce the view that the physical properties of the proteome are tightly related to the cellular stability.

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Supporting Information Available

The Supporting Information is available free of charge at ACS website. It contains additional details on neutron scattering experiments, molecular dynamics simulations, data analysis, and supplementary figures and tables.

Author contributions

This study is part of the Ph.D. project of BC, carried out under the joint supervision of JP, AP and FS. J.P., F.S. and A.P. designed research; M.G. and M.T.G.O. contributed samples; B.C., D.D.B., S.T. performed experiments and simulations; B.C. and D.D.B. analyzed data; FS, JP and AP jointly supervised the data analysis. B.C., C.P., J.P., F.S. and A.P. wrote the paper.

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