Intracellular molecular dynamics studied by neutron scattering

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Abstract. Incoherent neutron scattering experiments have produced important insights into intracellular molecular dynamics *in vivo*. Selected results highlight the role of water dynamics in cancer and brain cells, as well as cellular adaptation through the evolution of appropriate molecular dynamics, in order to respond to environmental challenges.

1 Introduction: dynamics, why?

Dynamics defines biological molecules through the forces that maintain their structures and allow appropriate functional motions. These forces have been studied by calorimetry, which revealed the thermodynamics of structural stability and importance of solvent interactions in the folding of a protein to achieve its active architecture [1]. They form the basis of force fields in molecular dynamics simulations [2].

The study of proteins from extremophiles, organisms that thrive under, for example, extreme temperature, pressure, or salinity, revealed the evolutionary adaptation of dynamics to maintain structure and activity under violent physical-chemical conditions. In Fig. 1, is shown the example of malate dehydrogenase, an enzyme of the Krebs cycle, from a halophile living in high salinity in the Dead Sea [3] and a hyperthermophile living at temperatures up to 95ºC, in oil fields [4].

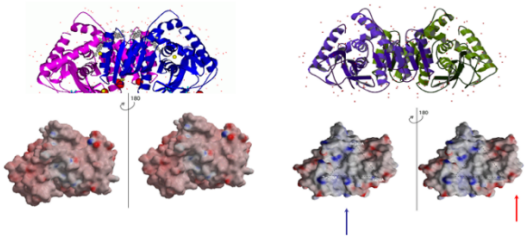

Fig. 1. Ribbon and surface structural representations of malate dehydrogenase from *Haloarcula marismortui* (on the Left) and *Archeoglobus fulgidus* (on the Right).

Note how, in spite of non-identical amino-acid composition, the two enzymes have maintained a similar tertiary structure throughout evolution. The adaptation to their respective physiological environments is clearly illustrated in the surface representation of the exploded monomers. The surface of the halophile enzyme is predominantly red, corresponding to an enrichment in negatively charged aspartic and glutamic acid residues, involved in stabilising the structure through solvent interactions with salt ions. In contrast, the surface of the hyperthermophile enzyme displays both positive (in blue) and negative charges (red), placed in such a way that in the formation of the dimer, by 180º rotation of one monomer over the other, the places indicated by red and blue arrows overlap for strong +/- ionic bonds to maintain stability at high temperature.

2 QENS and neutron incoherent elastic window scattering temperature scans

Quasi-elastic neutron scattering (QENS) is a classical approach to the study of molecular dynamics diffusive processes. The method has been applied successfully to measurements of intracellular molecular dynamics on the pico- to nanosecond time scale on back-scattering spectrometers at ILL, Juelich, ISIS and ORNL [5-11]. The Fourier transform of a diffusive process is a Lorentzian function, from which can be derived a diffusion coefficient and relaxation time. Cell contents being heterogeneous, the experimental data are the sum of scattering from several diffusive processes. A way to tackle the complexity is clearly explained in the pioneering works of Marques et al. on intracellular water dynamics in cancer cells [8, 10]. Three components were considered: (i) very slow motions from the largest organelles and cyto-skeleton and global motions of the macromolecules—represented by a Delta function (elastic line); (ii) slow diffusion of the intracellular water molecules (reorientations mediated by hydrogen bonds) for both the cytoplasmic and hydration water—defined by two Lorentzian functions; (iii) internal localized motions comprising conformational rearrangements from the biomolecules and lipid motions, such as the lateral diffusion of phospholipids and the movement of cholesterol within the membranes—ascribed to a broader Lorentzian.

The landmark 1989 paper by Doster et al. [12], introducing the neutron incoherent scattering elastic window temperature scan on IN13 [15] to study

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myoglobin, opened up new opportunities in experimental molecular dynamics in biology. A few years after, a similar shaped plot was obtained for bacteriorhodopsin in its natural membrane environment, suggesting it represented a general property of macromolecular dynamics [13]. The scan from [12] is shown in Fig. 2, together with data from a study of myoglobin in a trehalose glass [14].

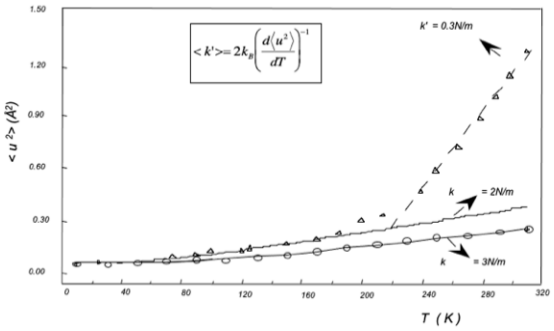

Fig. 2. Elastic window temperature scans on IN13 (8 μeV resolution) at the Institut Laue Langevin (ILL) [15] of myoglobin in a hydrated powder (triangles) from [12], and in a trehalose glass (circles) from [14].

The scans are plots of the mean square displacement (msd), $\langle u^2 \rangle$, calculated from the elastic scattering (see [12-14]), as a function of absolute temperature. The hydrated powder msd values follow a straight line until a break at about T = 200 K, where the structure *softens* and the msd climb steeply with temperature. For myoglobin trapped in a trehalose glass, the initial straight line subsists until just below 320 K, the highest temperature measured. A quasi-harmonic interpretation of the scans has been proposed by Zaccai [16]. The linear dependence of the msd at low temperature, extrapolating to zero at $T = 0$, is indicative of a harmonic regime, described by the equation in the inset in Fig. 2, where k' is a force constant. The quasi-harmonic analysis applies the harmonic equation to the portion of the plot above the break at ~200 K, with $\langle k' \rangle$, now, a mean effective force constant. As shown in Fig. 3, $\langle k' \rangle$ can be related to free energy configurations (such as the *double-well*) that have been used by authors to interpret elastic window temperature scans [12, 17, 18].

In qualitative terms, the msd informs on the *flexibility* of the macromolecular structure and the mean effective force constant informs on its *resilience* [16-18].

The $\langle k' \rangle$ values derived from the different portions of the scans are shown in Fig. 2. Interestingly, they are of the same order as values in our macroscopic world, like the stiffness of a new tennis ball, ~1 N/m, or that of a soft rubber ball, ~0.1 N/m.

3 The length-time window and incoherent scattering from H_2O

The energy resolution and Q range of a neutron spectrometer delimit the length-time window seen by elastic incoherent neutron scattering [29]. The IN13 Italian-French back-scattering spectrometer at ILL is specifically dedicated to biology [15]. In particular, the 8 μeV resolution and large Q range delimit a window of ~ 1 Å / 100 ps. Neutron scattering is strongly dominated by the incoherent cross-section of H atoms. On the one hand, atoms bound to macromolecular groups, in proteins, for example, will move well inside the window. The square of the diffusion length of H_2O at 20 °C, on the other hand, is 25 Å^2, well outside the IN13 window. An elastic window scan on IN13, therefore, would filter out most of the incoherent scattering from bulk water. This was an important realisation. Formerly, neutron experiments on biological molecules were performed in D_2O, to avoid the signal from being swamped by the incoherent scattering of H_2O. There is an isotope effect, however, and macromolecular dynamics is significantly different in H_2O and D_2O as measured, for example, in the halophilic malate dehydrogenase [19]. The IN13 length/time window permitted to extend the studies of biological macromolecules to solutions in H_2O—most relevant to physiological conditions.

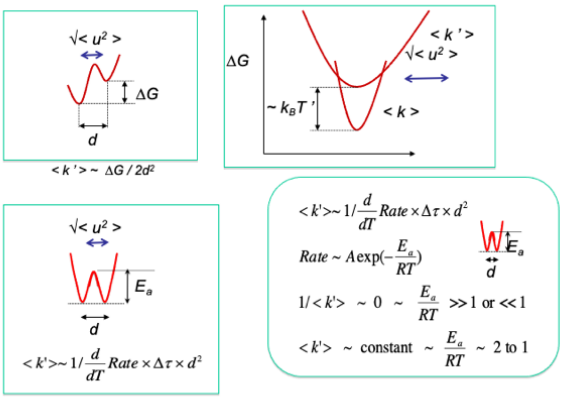

Fig. 3. The two-well model (top left) is from [12], the top right one is from [17]. The model on the bottom, in which two wells of the same free energy are separated by an activation energy barrier, would be appropriate, for example, to methyl group rotations

4 Live cell samples

The elastic window neutron scattering method is not site-specific but provides mean values over the motions of all H atoms in the sample. From this point of view a protein is already a complex entity in which the mean is over H atoms bound to different groups moving in different environments, as well as in strongly associated water molecules. It was obviously interesting to go one step further and to examine live cells. But would the measured mean values (msd, $\langle k' \rangle$) contain useful information for biology?

In an exploratory experiment, neutron scattering from live cells of bacteria adapted to life at different temperatures was measured on IN13 [20]. In order to avoid freezing or over-heating the samples, the temperature range of the scans was adjusted to between a few degrees C to below 40 °C. The msd *versus* T plots for four bacterial species are shown in Fig. 4.

The plot for the psychrophile was particularly encouraging that the scans gave meaningful information. It revealed a break in the line above 20 °C from a softening of the macromolecular structures due

to denaturation at this *high* temperature from the bacterium's view-point!

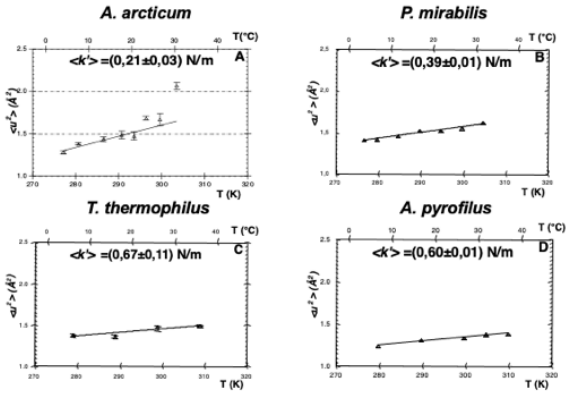

Fig. 4. From [20]. Elastic window temperature scans of *Aquaspirillum arcticum* (a psychrophile, adapted to 4 ºC in glacial waters), *Proteus mirabilis* (a mesophile adapted to body temperature), *Thermus thermophilus* (a thermophile, adapted to 65 ºC) and a hyperthermophile, *Aquifex pyrofilus* (85 ºC).

Cell content calculations proposed that the signal was dominated by the protein fraction in the cell (the proteome). The increasing macromolecular resilience with physiological temperature, suggested the dominance of the enthalpic term in the mean free energy of protein stabilisation, *i.e.*, stronger internal bonds maintained the folded structures to higher temperature. The question then arises why is psychrophile macromolecular resilience low, when a mesophile resilience, for example, would surely maintain structural stability above freezing? In order to achieve functional activity a macromolecule not only needs to be stable but also to have the appropriate *flexibility*. Both resilience and msd have to be adapted to the temperature. At psychrophile temperatures a mesophile resilience would lead to low msd values, incompatible with activity, and similarly for the mesophile if the resilience were as high as that of the thermophiles.

5 Live cells

The elastic window neutron QENS and elastic window neutron scattering experiments on live cells (partially reviewed in [21]) include studies on water mobility in Dead Sea archaea, in which a slow component was discovered, hypothetically associated with ion-binding structures [5], in *E. coli*, indicating about 90% of cytoplasmic water behaves similarly to bulk water, 10% being associated in macromolecular hydration shells [6], in *Shewanella oneidensis* at ambient and high pressure [7], in cancer [8, 10, 11, 15] and brain cells [23], as well as on intracellular macromolecular dynamics in red-blood cells and archaea (see below).

In their extensive study of intracellular water as a drug target in cancer cells [8, 10] and in the transition to malignancy [11], Marques et al. observed an increased intracellular plasticity. The combined results corroborated the key role of intracellular water dynamics in cell proliferation and differentiation. With respect to drug action, perturbation of the water's dynamical profile led to inhibition of cell growth and death. In the case of normal-to-malignant transformation, results suggested that water dynamics were closely associated with the onset of carcinogenesis and invasiveness.

Intracellular water dynamics in breast cancer cells, untreated and treated with the drug paclitaxel, was compared on TOSCA at ISIS, UK [22]. Paclitaxel is known to 'freeze' the cell-line in mitosis and, in this work also, the data supported the hypothesis that water mobility plays an important role in the cell cycle and response to chemotherapy drugs.

Diffusion Magnetic Resonance Imagery (dMRI) is used effectively to diagnose ischemic strokes and brain tumours by measuring water mobility in the brain. A combined dMRI/QENS quantified water dynamics in brain cells from the μm/ms scale of dMRI down to the atomic Å/ps scale of QENS [23]—important data for the understanding of water diffusion in the brain in order to improve MD simulations and assist medical diagnostics.

Comparative experiments on red blood cells of different animals revealed an adaptation of proteome molecular dynamics to body temperature. Remarkable because, in contrast to the psychrophile, mesophile and thermophile bacteria, the temperature range is small (~10 K) [24].

Studies of halophilic archaea showed how proteome molecular dynamics responded to temperature [25] and salt stress [26] in the organisms. The elastic window temperature scans turned out to be good indicators of protein functionality and cell viability.

Neutron scattering experiments at high hydrostatic pressure (HHP) on deep ocean archaea are particularly challenging. First results from elastic window scattering revealed molecular dynamics response mechanisms to HHP of hydration water and the proteome [27].

It is appropriate to conclude with a molecular dynamics study of *Mycoplasma genitalium* [28], which opens the way towards whole-cell simulations to link with experiment. QENS and elastic window neutron scattering data are already here to contribute when the times comes!

This work was supported by the French National Research Agency in the framework of the *Investissements d'Avenir* program (ANR-15-IDEX-02) through the funding of the "Origin of Life" project of the Univ. Grenoble-Alpes, and AlloSpace, ANR-21-CE44-0034-01). IBS acknowledges integration into the Interdisciplinary Research Institute of Grenoble (IRIG, CEA).

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