Molecular dynamics in cells: A neutron view

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This review is dedicated to Jeremy Smith in honor of his 60th birthday.

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Abstract

Experiments to characterize intracellular molecular dynamics *in vivo* are discussed following a description of the incoherent neutron scattering method. Work reviewed includes water diffusion in bacteria, archaea, red blood cells, brain cells and cancer cells, and the role of proteome molecular dynamics in adaptation to physiological temperature and pressure, and in response to low salt stress in an extremophile. A brief discussion of the potential links between neutron scattering results and MD simulations on in-cell dynamics concludes the review.

1. The beauty of incoherence

Seminar speakers at the Les Houches 1954 Summer School of Theoretical Physics included physicists prominent in neutron scattering theory and experiment; foremost amongst them were Enrico Fermi, Roy Glauber, Donald Hughes and Léon Van Hove. Van Hove brought with him his landmark paper introducing the analysis of neutron scattering in terms of $G(\mathrm{r},t)$, a pair distribution function in space *and* time (1). A manuscript of his presentation is kept in the Les Houches School library. As reviewed by Worcester et al (2), young scientists, who would later play a seminal role in the development of neutron scattering applications in magnetism, material science and biology, emphasized the importance of personal contacts they established at this school, in particular with Léon Van Hove.

Scattered neutron intensity by N atoms of scattering length b is expressed as a cross-section per unit solid angle and energy interval

$$\frac{d^2\sigma}{d\Omega d\omega} = \frac{k_1}{k_0} N b^2 S(\mathbf{Q},\omega) \qquad (1)$$

where k_1, k_0 are the magnitudes of the scattered and incident wave vectors (proportional to the incident and scattered neutron velocities), respectively. $S(\mathbf{Q},\omega)$ is the sample *dynamic structure factor*.

In the Van Hove analysis, the dynamic structure factor is the double Fourier transform of the space time distribution function, $G(\mathrm{r}, t)$

$$G(\mathbf{r},t) = N^{-1}\sum_{j.k}^{N} < \delta(\,\mathbf{r} - [\mathbf{r}_k(t) - \mathbf{r}_j(0)]) > \qquad (2)$$

The sum is over all atoms and the angular brackets refer to the thermal average. The distribution function counts the fraction of atoms that are separated by the vector **r** after a lapse of time *t*.

The Fourier transform operation is performed in two steps: first, r to Q to yield the intermediate scattering function, $I(\mathbf{Q},t)$, then *t* to ω to yield $S(\mathbf{Q},\omega)$

$$I(\mathbf{Q},t) = N^{-1}\sum_{j.k}^{N} < \exp[i\mathbf{Q}\cdot\mathbf{r}_k(t)]\exp\left[i\mathbf{Q}\cdot\mathbf{r}_j(0)\right] > \qquad (3)$$

$$S(\mathbf{Q},\omega) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} I(\mathbf{Q},t)\exp(-i\omega t)\,\mathrm{dt} \qquad (4)$$

Although the neutron-sample interaction is a quantum mechanical system and *G* is a quantum operator, under classical conditions, *G* takes on the real, positive value in equation (2). Van Hove further points out: "In the case of systems for which the symmetric or antisymmetric character of the wave function is of little importance, and which can thus be regarded as composed of distinguishable particles (Boltzmann statistics), the *G* function splits naturally into a part G_{self}, describing the correlation between positions of *one and the same* particle at different times (italics are mine), and a part G_{distinct} referring to pairs of distinct particles"(1).

A classical physics illustration of the progression from the *G* distribution (eq. 2) to the observed scattering (eq. 1) is given in figure 1. The neutron waves scattered by atom *j* at time zero and atom *k* at time *t* interfere to give the observed wave scattered by the *distinct* pair. For the *self*-case, the wave is scattered by the *same atom* ($j = k$) at time 0 and time *t*; *i.e.* at the end points of its *trajectory* in time *t*—interestingly, corresponding to trajectories calculated in MD simulations.

The scattering length, *b,* of a given atom is also a function of the spin state of its nucleus. The cross-section of an assembly of nuclei with different spin states can be broken up into a

coherent term, including the average $b_{coh}^2 = < b^2 >$, which contributes to interference between waves from different particles and an incoherent term, representing waves scattered as if each nucleus were unaware of its surroundings.

$$b_{total}^2 S(\mathbf{Q}, \omega) = b_{coh}^2 S_{coh}(\mathbf{Q}, \omega) + b_{inc}^2 S_{inc}(\mathbf{Q}, \omega) \qquad (5)$$

where $b_{total}^2 = < b^2 >$, $b_{coh}^2 = < b >^2$ and $b_{inc}^2 = < b^2 > - < b >^2$

The coherent part contains information on G_{distinct} (the distribution of different particles). The incoherent part of the scattering informs on G_{self} (the trajectories of single particles moving in time).

The incoherent scattering, not containing structural information, is mostly considered as a nuisance background. This is especially true for samples containing natural abundance H atoms, whose incoherent cross section is more than an order of magnitude larger than for other natural abundance atoms and the deuterium isotope, D. D_2O/H_2O substitution and D/H labelling in SANS, membrane diffraction and crystallography are not only justified by contrast variation based on the difference between the coherent scattering lengths of H and D but also as a powerful way to reduce the background noise—the incoherent cross-section of D being about 40 times lower than that of H.

Immobile point particles scatter radiation isotropically. In the case of moving particles, however, scattered intensity displays an angular dependence, decreasing from a maximum in the direction of the incident beam. Information on single particle dynamics is obtained from the analysis of this angular dependence in energy resolved incoherent scattering. Neutron beams are flux limited, however, (severely so when compared to X-ray or electron beams) and the effect is small so that such experiments would not have been feasible were it not for the very large incoherent cross-section of H.

Incoherent neutron scattering (IncNS) experiments yield important information on the dynamics of biological systems, which are rich in homogeneously distributed H atoms, representative of global averaged molecular dynamics (3). H-bond diffusive dynamics is observed on the picosecond timescale, while, on longer timescales, hydrogen atoms reflect the conformational sampling motions of the groups to which they are bound (3). It is noteworthy that the information obtained is globally averaged, *i.e.* IncNS does not provide evidence on chemical site-specific motions. NMR or macromolecular crystallography, which do deliver high resolution site-specific data, are limited by constraints on sample preparation. Because IncNS focuses on single particle motions, however, there are no constraints on sample type, which can be crystalline or amorphous, liquid, in solution, a gel *etc*. A professor of mine taught that if you put carrot soup in the beam you will gather information on the *mean* motions of H atoms in carrot soup. This ability of IncNS to deliver average dynamics in extremely complex systems promised new perspectives in the study of large molecular machines and cells. But first it had to be shown that global average dynamics actually contained biologically relevant information. This was established by Tehei et al. (2004) (4) who revealed the role of specific global average information from a complex system can be broken down into component contributions either by specific deuteration (to lower the incoherent cross-section of the component to be masked, *e.g.* perdeuterated cells in H_2O to observe the water (5)), or by energy resolution of the scattered intensity as a time domain filter to separate components with different mobility (6, 7).

IncNS kept its promise. This review of molecular dynamics in highly complex biological systems is dedicated to young Jeremy Smith (whom I first met when he came to Grenoble for his PhD) in honor of his 60^{th} birthday. I am happy to take the opportunity to express my thanks and special appreciation for his contributions in unravelling the fascinating intricacies of neutron scattering and molecular dynamics theory.

2. Methods

2.1 *Elastic window temperature scan and QENS*

Elastic window temperature scans (ElTempSc) and quasi-elastic neutron scattering (QENS) inform on dynamics in a time-scale range from the pico- to the nano-second and length scale range from 0.1nm (Å) to tens of nanometers. They are the main methods used for the analysis of INS.

For a system with isotropic motions, the dynamic structure factor at zero energy exchange, (ElTempSc intensity) can be approximated by a Gaussian (6, 7)

$$S_{el}(Q, \omega = 0 \pm \Delta E) \approx S_0 \exp(-\frac{1}{6} < u^2 > Q^2) \qquad (6)$$

where ΔE is instrumental energy resolution related to the time window and $<u^2>$ (the square of G_{self}) is the average time-dependent atomic mean square displacement (MSD) in the limit defined by ΔE (8) (9).

The Q range of validity for the approximation is good to $<u^2>\ Q^2 \approx 1$ but can extend further depending on the geometry of the motion (10). The $<u^2>$ is obtained for each temperature from the slope of the semi-logarithmic plot of the incoherent scattering function through

$$\langle u^2 \rangle \approx -6 \frac{\mathrm{d} \ln S_{el}(Q, \omega = 0 \pm \Delta E)}{\mathrm{d} Q^2} \qquad (7)$$

An effective average force constant for sample dynamics $< k >$ can be calculated from the slope of $<u^2>$ as a function of temperature by applying a quasi-harmonic approximation (11) (12):

$$< k >= \frac{0.00276}{\mathrm{d}<u^2>/\mathrm{d}T} \qquad (8)$$

In equation (3), the numerical constant is for $< k >$ expressed in Newton per meter when $< u^2 >$ is given in Ångstrom squared and T is the temperature in Kelvin.

QENS contains information on the time constants of different dynamics processes. QENS is centered on zero energy transfer and appears as a broadening of the wings of the elastic peak. As in dynamic light scattering, QENS arises from diffusing particles, with the energy transfer width of the curve inversely proportional to a relaxation time τ.

In the case of linear diffusion obeying Fick's law, the intermediate scattering function calculated from the correlation function, G_{self}, is written:

$$I(Q,t) = \exp(-DQ^2t) \qquad (9)$$

where D is the diffusion coefficient and $DQ^2 = 1/\tau$. The Fourier transform of an exponential decay function is a Lorentzian function

$$S(Q,\omega) = \frac{1}{\pi}\frac{DQ^2}{(DQ^2)^2+\omega^2} \qquad (10)$$

The Lorentzian dynamic structure factor describes a curve of full width at half maximum, Γ, equal to $2DQ^2$ in frequency units.

If the diffusion model is appropriate, plotting experimental $\Gamma(Q)$ versus Q^2 yields a straight line at low Q of slope related to the translational diffusion coefficient. In practice, however, a simple diffusion model is more likely to be valid at high Q values, *i.e.* for small

displacements for which it is less likely the diffusion process will be hampered by interactions with the atomic environment. In practice, more involved models than simple diffusion (such as the *jump diffusion* model) need to be used in the analysis of QENS to inform on water dynamics in the bulk and different biological environments as well as on the slower diffusive motions of macromolecule bound H atoms (reviewed in (13)).

2.2 *Samples in the papers reviewed*

Bacterial, archaeal and cancer cells were always freshly cultured or propagated and prepared before loading into the neutron aluminum sample containers. Human venous blood was drawn from healthy adults into tubes containing heparin to prevent coagulation, just before loading. Brain tissue was extracted from fresh post-mortem bovine brains obtained from the slaughterhouse.

3. Review of neutron results on in-cell dynamics

3.1 *Water, water, everywhere*

3.1.1 *The water dance*

Water in the liquid state takes part in a mad dance, neighboring molecules exchanging partners through hydrogen bonds on a picosecond beat (experimental and MD simulations reviewed by John Finney in reference (14)). Its highly dynamic disordered structure turns water into a vital entropy sink for biochemical reactions in aqueous solution, the hydrophobic effect, which plays determining roles in the folding of the polypeptides into active proteins and self-assembly of cellular molecular machines and membranes, as well as for the hydration effects that trigger functional dynamics in macromolecules. Since most of these interactions occur inside cells, it would seem likely that intracellular water would share the dynamic properties of bulk water. Nevertheless, the properties of water, the matrix of Life

itself, have been the object of wild metaphysical speculations, even within the 'hard' science community. Intracellular water dynamics did not escape controversy (15) until clarified by IncNS (16) (17) (18) and NMR (19) (discussed in a PCCP perspectives article (5)).

3.1.2 *IncNS and NMR studies of intracellular water in prokaryotes and red blood cells*

NMR and IncNS are complementary. In the prokaryote intracellular water experiments, the spin relaxation rate of ^{2}H or ^{17}O nuclei in isotopically labelled water revealed water dynamics on a range of time scales from the pico to nanosecond range (overlapping with IncNS) to the microsecond range. The spin relaxation method is sensitive to water molecule rotational diffusion in the bulk or when confined by interactions with solutes and surfaces. It is not sensitive to linear diffusion.

Physicists comfortable with Laws and generalizations tend towards the belief that results from a favorite model system are generally applicable. At first, the case of intracellular water was apprehended with the expectation that its behavior would be the same in all cells. But if there were a fundamental law of biology, it would be the law of diversity. A variety of biophysical experiments from the early 1970s, by Ben Zion and Margaret Ginzburg on *Haloarcula marismortui* (Hmm) an extreme halophile from the Dead Sea, proposed the existence of an intracellular water component, specific to this organism (they informally named it 'funny water'), with the ability to maintain strongly bound K^+ ions within the halophilic cell in absence of metabolism (reviewed in (20)).

To explore intracellular water dynamics in Hmm, QENS experiments were performed on perdeuterated cultures pelleted in either H_2O or D_2O molar saline buffer (16). Similar

experiments on perdeuterated *E. coli* grown and prepared in the same way were carried out for comparison. Rotational and translation diffusion coefficients for intracellular water were measured on the ten picosecond and nanosecond ranges, respectively. On the shorter time, translational and rotational diffusion parameters were found with values close to those measured for bulk water in high salt. On the nanosecond time-scale, however, a very slow water component was detected in Hmm, and suggested to correspond to Ginzburg funny water. The water H nuclei of this component displayed a residence time two orders of magnitude larger than for bulk water and a translational diffusion coefficient two orders of magnitude slower. Surprisingly, the rotational diffusion parameters of the slow component were only slightly slower than for bulk water. By comparing the scattering signals from the two time scales, Tehei et al. (16) published that the funny water component made up 76% of the total intracellular water. This value is probably overestimated, the calculation depending on a comparison between data collected on different spectrometers with different energy resolutions (corresponding to the two timescales). No funny water was found in *E. coli* measured under identical conditions.

Spin relaxation NMR is sensitive to water rotational diffusion (but not translational diffusion) over the time of bulk water movements to 'immobile' water trapped inside macromolecular structures. Persson and Halle, using spin relaxation NMR, found similar water dynamics in *E. coli* and Hmm (15). Samples were identical to the ones used in QENS except that only the water and not cellular components were isotope labelled in the NMR study. Approximately 85% of cell water in both organisms displayed bulk-like rotational dynamics. The remaining approximately 15% was found to be retarded in its rotation rate and was assumed to interact directly with biomolecular surfaces. The relaxation data on the microsecond time scale (for essentially immobile water) showed that about 0.1% of cell water exchanges from buried hydration sites hydration in solutions and crystals.

NMR could not confirm or exclude the existence of funny water in Hmm since (from the QENS data) its rotational diffusion is only slightly slowed down with respect to other intracellular water. The highly slowed down translational diffusion coefficient found by QENS suggests the existence of a volume of restricted diffusion within the cell perhaps associated with the sequestering of K^+ ions (as originally speculated by the Ginzburgs (20)) by carboxylic groups in structures similar to the ones observed in the potassium channel protein (5).

Water diffusion in *E. coli* and red blood cells (RBC) was found to be similar by both NMR (15) and QENS (17) (18). In these cells, 90% of the water displays bulk dynamics with its essential biological properties as an entropy sink, while the remaining 10% exhibits the confined dynamics of macromolecular hydration shells (21). As perdeuterated RBC cannot be prepared, QENS measurements were on natural abundance RBC in H_2O buffer and in D_2O with the water contribution obtained by careful subtraction. It is to be noted, however, that RBC are not typical eukaryotes. They are highly specialized cells that do not contain organelles, a nucleus, DNA or RNA. They are bags hemoglobin molecules, which make up 92% of the dry weight. The confined water measured in RBC corresponds satisfactorily to a monolayer of hydration around each hemoglobin molecule.

3.1.3 *Water in cancer cells*

In a study published on-line just as I began writing this review, Martins et al. combined thermal analysis and neutron scattering to compare water mobility in untreated and treated breast cancer cells (22). First, the cells were probed by differential scanning calorimetry as reservoirs of bulk-like and confined water populations. Interaction with the anti-tumor drug, paclitaxel, significantly increased the confined population as shown by the reduced melting enthalpy and lower specific heat measured at room temperature. Inelastic neutron scattering

spectra (probing collective motions by coherent scattering) were measured for treated and untreated cells. The spectrum of the confined water component in each case was determined by subtraction of the bulk water spectrum. The increased amount of confined water found by calorimetry in treated cells was confirmed by the amplification of collective atomic motions in this population. Finally, QENS analysis on the nanosecond timescale led the authors to hypothesize that, in the particular cell line, intracellular bulk-like water diffusion increased in response to the action of paclitaxel. A theoretical model analysis of the QENS suggested that the intra-cellular water in the treated cells displayed translational diffusional mobility on the probed time and length-scales different than that of the untreated cells whose behavior deviated considerably from expected bulk water jump diffusion. The authors concluded that their first descriptive findings support the hypothesis that cellular water dynamics plays an active role in the cell cycle (paclitaxel 'freezes' the cells in mitosis) and cellular response to external stimuli and should be investigated further in healthy and cancer cell lines and for distinct chemotherapy drugs.

3.1.4 *Water in brain*

The principles of Magnetic Resonance Imaging (MRI) as applied to brain scans are described for non-specialists in reference (23). Blood brain activation can be mapped directly through slowing down of water diffusion in activated cells and diffusion MRI (*d*MRI) of the brain has been used effectively to diagnose ischemia and brain tumors in stroke patients. The method is sensitive to linear diffusion on the ten-millisecond time scale in which water molecules diffuse over micron distances corresponding to several cellular elements. Other than the fact that water diffusion on this scale is not Gaussian, the mechanisms and interactions are not sufficiently characterized to be modelled in MD simulations. In an article, currently in press, Natali et al. (24) reported combined *d*MRI and pioneering neutron scattering experiments on

bovine brain tissue. Neutron diffraction scans to examine internal structures were dominated by the diffraction peaks from myelin lamellar sheaths of the nerve cell membranes. *d*MRI and QENS both revealed the co-existence of two water populations of different dynamical behavior. On the μm/ms of *d*MRI the dynamical heterogeneity was traced to cellular structures forming obstacles to the diffusing water. On the Å/ps length/time scale of QENS, the water translational dynamics appeared as essentially 'jump-like' with a heterogeneity due to water interactions with cell membranes. The combination of *d*MRI and QENS provided quantitative results on diffusing water components in brain on the full length/time range from the micrometer/ms down to the atomic scale—highly relevant data to improve the MD simulation and understanding of intra and extracellular water diffusion in the brain with evident benefits for applications in medical imaging and diagnostics.

3.2 *Intracellular macromolecular dynamics in prokaryotes*

3.2.1 M*acromolecular dynamics in mesophile, psychrophile and thermophile Bacteria*

Neutron scattering experiments reported by Tehei et al. measured intracellular molecular dynamics (dominated by the proteome) in bacteria adapted to different physiological temperatures (4). MSD and $< k >$ values (see Methods) were extracted from ElTempSc between 280K and 315K. The main result was that intramolecular forces ($< k >$) had been selected to maintain similar structural flexibility (MSD) at the respective physiological temperatures Psychrophile bacteria (adapted to 4ºC) displayed weaker forces to maintain appropriate *fluctuations* at the low temperature while thermophiles (adapted to ~70ºC) presented stronger forces to maintain *stability* with appropriate motions at the high temperature. Mesophilic *E. coli* (37ºC gut temperature) attained similar molecular motions with intermediate force values. Tehei and collaborators concluded that adaptation occurs *via* evolutionary selection of dynamics. What does this mean? Systems biology is the study of

pathways from extracellular signals to the control of gene expression. Actors in the pathways are macromolecules, themselves adapted to function under physiological conditions. The statement of Tehei et al. that evolution selects dynamics, therefore, simply states the obvious: that gene products have to be stabilized by forces that permit

functional structures *and* motions under physiological conditions. Evolution, therefore, 'has to' select these forces correctly.

3.2.2 *Water isotope effects on macromolecular dynamics in E. coli*

Heavy/light water exchange is widely used for contrast variation in small angle neutron scattering (23), solvent isotope effects on structures at low resolution being negligible. Because of the difference between H-bond and D-bond dynamics, however, macromolecular dynamics in H_2O and D_2O is expected to be different. Jasnin et al. (25) applied elastic incoherent neutron scattering temperature scans (ElTempSc, see Methods above) to investigate solvent isotope effects on macromolecular dynamics *in vivo* in *E.coli*. Measurements were performed on bacteria samples in H_2O and D_2O, respectively, between 280K and 315K. MSD (flexibility) and mean force constant $< k >$ (resilience) values were extracted from the ElTempSc intensities and interpreted. They inform on the mean dynamics of all macromolecules inside the cell, dominated in practice by the internal motions of the protein fraction. Resilience and flexibility were both found to be smaller in D_2O when compared to H_2O. The hydrophobic effect is known to be stronger in D_2O, favoring the burial of non-polar surfaces as well as Van der Waals' packing in macromolecule cores and leading to the observed smaller MSD. The lower resilience suggested a larger entropy content in D_2O due to increased sampling of macromolecular conformational sub-states.

3.3 *Intracellular macromolecular dynamics in Archaea*

3.3.1 *Dynamics response to stress in extreme halophiles*

Halobacterium salinarum (Hs), an extreme halophilic archaeon has an absolute requirement for multi-molar salt and thrive in salt lakes and saline ponds. In order to counterbalance extracellular osmotic pressure due to multi-molar NaCl, Hs accumulates molar concentrations of KCl in the cytosol. Its metabolism, therefore, is specifically adapted to function in high salt—a damaging environmental condition for non-adapted cells, through effects on macromolecular dynamics, protein unfolding and aggregation, screening of ionic contacts in protein-nucleic acid interactions *etc*. Hs, however, despite being an obligate halophile, displays a stress survival response when environmental conditions become temporarily unfavorable through variations in environmental salt concentration or temperature (*e.g.* following intensive rainfall or exceptional hot weather). Stress in Hs can be followed by a microbiological response that includes increased expression and accumulation of the thermosome complex, a chaperone that slows down protein unfolding and prevents aggregation.

The Hs intracellular molecular dynamics reaction to high temperature (26) and low salt (27) was measured by combining microbiological and neutron scattering experiments. MSD and resilience, $< k >$, values from ElTempSc were revealed to be a good indicator of protein functionality and cell viability. The lower resilience, $< k >$, values observed in stressed cell intracellular macromolecular dynamics when compared to unstressed cells, reflected weakened intramolecular forces leading to a softening of the structures. In spite of the crowded cytosol and induction of protein quality control systems like the thermosome, the molecular dynamics of a large fraction of the proteome was strongly perturbed under thermal or low salt stress. Curiously, Hs cells recovered easily when replaced in optimal growth conditions.

In a paper, recently submitted to *Scientific Reports*, Vauclare, Natali et al. explored in more detail the reactivation capacity of Hs sub-populations after incubation in low salt media. Respiratory oxygen consumption of stressed cells was measured and cell viability was assessed by Live/Dead staining and flow cytometry to follow multiple cellular parameters including cell membrane integrity. Again, IncNS experiments represented a good indicator of protein functionality. The results showed that the recovery of Hs sub-populations exposed to severe low salt conditions was correlated with a rapid retrieval of functional intracellular molecular dynamics.

3.3.2 *Deep ocean Archaea*

A large part of the biosphere lies below 1000 m in the deep ocean, at hydrostatic pressures of 100 bars (10 MPa) or higher. The genetic and structural bases of adaptation in deep ocean organisms, however, are still unknown. The thermodynamic response of a system to high hydrostatic pressure (HHP) is governed by *Le Chatelier's principle* ('When any system at equilibrium for a long period of time is subjected to change in concentration, temperature, volume, or pressure, the system changes to a new equilibrium that partly counteracts the applied change') as expressed in the Van't Hoff kinetic equation

$$\frac{\partial}{\partial p}\ln k = \frac{-V_a}{RT}$$

where k is a rate constant and V_a is the activation volume. In other words, a system under pressure will tend to reduce its volume. HHP induces a complex response in proteins because volume reducing interactions can be structure destabilizing or stabilizing. HHP provoked

electrostriction and reorganization of water molecules, for example, are destabilizing while reduced van der Waals atomic separations and H-bond lengths are stabilizing. The pressure-temperature phase diagram for protein stabilization is reentrant with the thermal and pressure limits of the native state defined by an ellipse. In general, HHP up to 2 kbar causes dissociation of oligomeric proteins and large complexes; from 3 to 8 kbar, HHP will unfold proteins (see special issue of *Biochimica et Biophysica Acta* edited by Balny, Masson, and Heremans (28)).

HHP neutron scattering studies have been published for lipid multilayer vesicle dynamics (29) and protein dynamics (*e.g.* on lysozyme (30) and hemoglobin (31)) as well as MD simulations of proteins under pressure (*e.g.* a nanosecond simulation of lysozyme (32)). As in the cases of adaptation to temperature or salinity discussed above, however, it is of interest to measure the cellular molecular dynamics response to HHP of the total proteome, since individual proteins might display specific different reactions.

Peters et al. (33) and Martinez et al. (34) explored HHP effects on intracellular molecular dynamics in *Thermococcales.* They examined the hyperthermophile *T. kodakarensis*, which thrives at 85ºC and atmospheric pressure, the barophilic hyperthermophile *T. barophilus*, which grows optimally in the deep ocean at 85ºC and 40 MPa pressure, and, in reference (33), *E. coli* as a mesophilic control. Experiments were difficult to realize because samples had to be handled under anaerobic conditions and because of poor signal-to-noise ratios from the pressure cell.

In reference (33), MSDs extracted from the IncNS in the 100 ps time window as a function of pressure (up to 1.2 kbar) displayed reproducible trends in spite of large error bars. Similar MSD versus pressure negative slopes observed for pressure sensitive *T. kodakarensis* and *E.coli* indicated a tightening of mean proteome molecular flexibility under HHP as expected

from *Le Chatelier's Principle*. In contrast, HHP had a limited impact on the MSD of barophilic *T. barophilus*. The HHP cell could not withstand high temperatures, however, and it was not possible to measure MSDs close to 85ºC in order to assess the effect of pressure under physiological conditions.

Martinez et al. (34) analyzed QENS in the 1 to 100 ps time window from *T. kodakarensis* and *T. barophilus* in D_2O, at atmospheric pressure (0.1 MPa) and 40 MPa, respectively. A high temperature pressure cell was not available and experiments were conducted at room temperature. QENS intensity was fitted with five components, the elastic peak (defined by instrumental resolution), three Lorentzians and a background. The diffusion rates extracted from the Lorentzians were attributed, respectively, to bulk water (~ $2.3x10^{-5}cm^2s^{-1}$), hydration water (< $2.3x10^{-5}cm^2s^{-1}$) and slower proteome and membrane component diffusional motions ($10^{-5}cm^2s^{-1}$ to $10^{-7}cm^2s^{-1}$). Water in the samples having been exchanged with D_2O, the authors assumed that the QENS water signals were dominated by intracellular H_2O that had not exchanged fully because of low cell membrane permeability. The results on pressure dependence complemented the earlier MSD results published in reference (33). Intracellular bulk water dynamics behaved similarly in both organisms and was affected by pressure as expected for free water. Compared to *T. kodakarensis* the diffusion coefficient of hydration water was found to be reduced in *T. barophilus* and the fast proteome and membrane dynamics enhanced at HHP. The authors suggested the enhanced macromolecular dynamics and 'slow' hydration water at HHP in the piezophile are adaptive strategies. They interpreted the more energetic dynamics as higher flexibility, based on the previous work (33) that showed MSD values that did not decrease at HHP as would have been expected from *Le Chatelier's Principle*. Faster dynamics at HHP have been observed by QENS also for an oligomeric protein from the hyperthermophilic deep sea microbe, *Thermococcus thioreducens* (35).

4. Can the neutron view link up with current in-cell MD simulations and NMR?

The rich interface between neutron scattering experiments and MD simulations has been developed mainly by Jeremy Smith and his collaborators (3). And, as shown by a recent neutron study of comparative molecular dynamics in 30S and 50S ribosomal subunits, there is good hope that the neutron/MD approach will be soon be extended to treat very large molecular machines (36). When I googled 'MD simulations of the cytosol' the first hit was a YouTube video posted by Riken in Japan, a five minute colorful, attractive representation of molecular interactions in a bacterial cell (37). The video is based on an atomistic calculation by Yu et al. of molecular interactions in the cytosol of *Mycoplasma genitalium* made on the K supercomputer of the Riken Advanced Institute for Computational Science (38). The bacterium was chosen because of its relative simplicity. The authors studied a model of the cytoplasm with 103 million atoms in a cubic box (100 nm side) and two subsections of the box with 12 million atoms in each. Unrestrained MD simulations were carried out for 20 ns, 140 ns, and 60 ns, respectively. The simulation highlighted ribosomes and GroEL chaperones as large molecular machines, proteins, RNAs, metabolites, ions and water molecules. The comparison with experimental work was made mainly with in-cytoplasm quenched H/D exchange NMR studies of protein stability (39) and weak quinary interactions (40).

The results of the Yu et al. simulation (37) focused on diffusion and intermolecular interactions in the crowded environment: (i) protein-protein interactions tended to destabilize native protein structures and contributed to significantly reduced macromolecular diffusion in the crowded conditions; (ii) metabolite interactions tended to induce more compact states due to electrostatic screening; (iii) metabolites exhibited significant two-dimensional surface diffusion and altered protein-ligand binding that may lead to a reduction of their *in vivo* effective concentration; (iv) evidence of weak quinary associations formed by metabolic

enzymes, attributed to solvation and entropic effects; (v) lowered water diffusion rates for cell water compared to free water, the calculation, however, did not distinguish between hydration water and bulk water.

Conclusions on in-cell protein structures were obtained by comparison with crystal structures using radii of gyration and root mean square displacements of atomic positions. It would have been of interest to extract MSD of atomic fluctuations on the time scale of the simulation to confront with neutron scattering data on proteins in other crowded conditions (*e.g.* in red blood cells (41)) compared with values from dilute solution.

The complementarity between IncNS and spin relaxation NMR to study intracellular water was discussed above in section 3.1.2. Now as the incoherent neutron scattering technique moves towards the examination of specific cellular components using deuterium labeling, the development of links between IncNS and H/D exchange NMR to study intracellular macromolecular dynamics holds great promise.

5. Conclusion

The *M. genitalium* simulation is an important step towards *in silico* whole-cell models to connect with molecular level experiments in cell biology. Hopefully, progress in computing power and methods will one day permit the comparison of functional molecular dynamics in different cell types. When this happens, neutron scattering results will already be there waiting to help validate the simulations!

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Figure 1

A neutron wave is scattered by atom *j* at position $\mathbf{r}_j(0)$ at time zero and atom *k* at position $\mathbf{r}_k(t)$ at time *t.* In the case of incoherent scattering *j* and *k* represent the same atom at different positions in time. In the scattering diagram, **k** and **Q** are wave-vectors and ω is wave energy.