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Uncovering the Dynamics of Confined Water Using Neutron Scattering: Perspectives

Heloisa N. Bordallo[1]* *and Gerald R. Kneller*[2]

[1]Niels Bohr Institute, University of Copenhagen, Copenhagen, Denmark, [2]Centre de Biophysique Moléculaire CNRS and University of Orléans, Orléans, France

The main characteristic of liquid water is the formation of dynamic hydrogen bond networks that occur over a broad range of time scales from tens of femtoseconds to picoseconds and are responsible for water's unique properties. However, in many important processes water does not exist in its bulk form, but in confined nanometer scale environments. The investigation of this confined water dynamics is challenging since the intermediate strength of the hydrogen bonds makes it possible to alter the structure and dynamics of this constrained water. Even if no single experimental technique can give a full picture of such intricate dynamics, it is well established that quasielastic neutron scattering (QENS) is a powerful tool to study the modification of hydrogen bonds in confinement in various materials. This is possible because *neutrons tell us where the atoms are and what they are doing*, can detect hydrogen, are penetrative and non-destructive. Furthermore, QENS is the only spectroscopic technique that provides information on the dynamics and atomic-motion amplitudes over a predetermined length scale. However scientific value of these data is hardly exploited and never to its full potential. This perspective highlights how new developments on instrumentation and data analysis will lead to appreciable progress in our understanding of the dynamics of complex systems, ranging from biological organisms to cloud formation.

Keywords: water, complex systems, neutron scattering, dynamics, modelling

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***Correspondence:**
Heloisa N. Bordallo
bordallo@nbi.ku.dk

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INTRODUCTION

Everything that happens in a complex system can be fully rationalized by a description at the level of matter in motion–atoms and molecules moving, exerting forces, interacting, and exchanging energy with each other [1, 2] From a simplistic point of view, we can argue that structure-enabled interactions derive from the arrangement of the component molecules, while information on how these interactions change in space and time relates to function. In real organisms the function is enabled by multiscale atomic motions. This is indeed characteristic of many systems where water dynamics is a key component of function [2, 3]. An excellent experimental tool for describing such complex water interactions on multiple length and time scales is quasielastic neutron scattering (QENS). Here, QENS is an ideal tool due to the neutron's natural scattering sensitivity [4–6] and scattering length density contrast between H and D [7]. QENS further allows to study motions in the time region of 10^{-9} to 10^{-13} s, while monitoring such motions in space from 0.1 to 10 nm [8, 9]. This is vital since in complex systems competing short- and long-range interactions result in frustration, and this complex coupling between different variables leads to a non-

Markovian, scale-invariant multiple scale relaxation process [10]. In QENS experiments these variables are the positions of the H-atoms in time. Additionally, the neutron's natural scattering contrast between hydrogen (H) and deuterium (H2, D), both in magnitude and phase, provides for an extraordinary method for highlighting (or hiding) H-containing components in complex systems [5], making neutron scattering especially successful for studies of structural characteristics [11] and dynamics of confined water [3, 4, 6–8, 12–14]. Furthermore, QENS is the only spectroscopic technique that provides information on the dynamics and atomic-motion amplitudes over a predetermined length scale [15–17].

QENS unique ability to access information on the geometry of the motion and its utmost importance for complex soft systems where long-range order is mostly absent will become even more distinct with the increased neutron flux coupled with the development of new instrumentation available on cutting-edge neutron sources [18–20]. New neutron spectrometers will allow simultaneous access to broad regions of time and space and, consequently a better description of complex water interactions. Analysis, interpretation, and modeling of QENS data is however complex, and this is a significant barrier to generating scientific innovation from the experiments performed. While QENS spectra contain the desired information to understand the dynamics in complex soft systems where long-range order is mostly absent [21], our current methods of analysis fail to completely capture the full dynamic nature arising from the multiscale atomic motions [22]. This hinders a more generalized understanding of the mechanisms behind their organizing nature. On one hand, the complex molecular mechanism describing the water dynamics probed by QENS is poorly captured in molecular dynamics (MD) models. The challenge here is the lack of accuracy of the interatomic potentials, hampering real predictive modelling of atomic-scale dynamics in time [23]. While on the other, the complexity of such process makes the analysis, interpretation, and modeling of QENS data difficult [3, 6, 16]. Usually in QENS analysis, empirical models for special atomic motion types are used to extract diffusion coefficients and mean-square displacements [24, 25]. This approach works well when dealing with simple processes. In complex systems, such as water dynamics in a constrained local environment, however, one is faced with an incompletely described problem, needing probabilistic methods to choose between likely models. An alternative, and very promising, approach is to compute the QENS spectrum theoretical parameters to identify physical models to analyze QENS data [14, 15, 26], and thus provide the needed experimental verification by using an energy landscape-based method [12, 27]. In this new framework the QENS spectrum is described by its form, and the parameters related to the structure of the energy landscape; a concept in which the (free) energy of the system is represented by a convex envelope with many local minima and a distribution of barrier heights localized in a restricted domain of phase space.

In this Perspective article, we briefly describe why and how the MIRACLES time-of-flight backscattering spectrometer [19, 28] being built at the European Spallation Source in Lund [18] will increase our understanding of water dynamics in complex systems. We will also discuss on how the vast scientific value of these data can be better exploited by using a new methodology in which the QENS spectrum theoretical parameters are computed and used to identify physical models to analyze QENS data [15, 16, 26] In this short Perspective, we do not attempt to lengthily review the field of QENS (and neutrons in general), we refer the interested reader to a very stimulating book where a general introduction into the production and properties of neutrons is followed by a series of papers describing the neutron scattering techniques used to study biological and biologically relevant systems [29].

WATER DYNAMICS IN BIOSYSTEMS AND THE MIRACLES BACKSCATTERING INSTRUMENT AT THE EUROPEAN SPALLATION SOURCE

As mentioned in the Introduction, QENS is unique in its ability to provide temporal and spatial information on molecular motions in the same measurement. Detailed microscopic characterization of diffusive motions in systems with nm length scale confinement have been carried out successfully for some time [5]. Furthermore, the ability to access information on the geometry of the motion is of utmost importance in systems where long-range order is mostly absent, such as in soft matter, including polymers, biomaterials, and macromolecules in living systems. The new generation spallation source and reactor backscattering spectrometers have already opened new doors in recent years for high-resolution neutron spectroscopy. For instance, in life sciences it is now possible to unravel the complex dynamics of such water-dependent complex morphologies using neutron spectroscopy [30]. Nevertheless, structural information from QENS experiments on complex systems depends on the observation-time $(\Delta t)_H$, which is defined by the spectrometer setting and its resolution function [31]. As a consequence, data from experiments carried out on a single time scale defined by $(\Delta t)_H$, i.e. implying a single Fourier-time window, are usually incomplete. This implies that even if current QENS methods allow for a range of observation time covering four orders of magnitude (10^{-9}–10^{-13} s), a combination of several different spectrometers is normally required to obtain a full understanding of the relevant dynamics [17, 32, 33]. However, the extraordinary flexibility of the time-of-flight backscattering spectrometer MIRACLES [19, 28] being built at ESS will bring a paradigm shift to QENS studies. The variable energy resolution of MIRACLES will allow to better focus on either the water dynamics (well covered at 10 μeV energy resolution) [17] or on proteins/membranes interactions (well covered at 2 μeV energy resolution) [34]. Additionally, the high flux offered by MIRACLES will provide for data of high statistical accuracy to be collected faster, thus avoiding biological degradation. Finally, the wider scattering vector (directly related to the geometry of the motion) at higher-energy resolutions together with the large energy transfer range will make it

possible to fully explore restricted diffusion and fitting the free-like water contribution. The technical origin of these striking capabilities rests on the instrument design that optimizes the use of the long source pulse provided by the ESS. It is thus foreseen that MIRACLES will engender unprecedented opportunities for neutron scattering in areas of science not yet fully explored by QENS, such as life sciences, biomaterials, and climate change [29, 35, 36]. This will make MIRACLES to not only do what can be done today much faster, but more importantly, provide for appreciable progress in our understanding of the dynamics of complex systems, ranging from polymers to biological organisms. Questions might remain in how one can disentangle the convoluted dynamics of the hydrogen atoms in living organisms even if we can separate their dynamics by varying the observation time. Certainly, such studies are facilitated with neutron scattering supported by deuteration [7]. This is however a very challenging subject, which has trigged a fascinating study by Okuda et al [37] to which we refer the reader. It is therefore expected that new neutron instrumentation will generate further developments in this area as well. Lastly, the time and space domain covered by Molecular Dynamics (MD) simulations is ideally matched to that offered by MIRACLES. This property facilitates a symbiotic relationship between QENS and MD, in which MD can be used to understand QENS, and conversely QENS can be used to improve and confirm the potentials used in MD. This area of research has grown considerably in the last years, and will continue to do so [38, 39].

Case 1: Short Time Dynamics of Water in Living Systems

Molecular crowding and complexity in and around cells can in principle produce marked slowing of diffusion as well as anomalous and complex diffusive behaviors, such as strongly size-dependent diffusion [40, 41]. Indeed, it is widely assumed that the time scale of diffusion dynamics governs many important biological processes. Thus, information about mobile and immobile fractions of labeled molecules and their diffusion properties are essential for processes such as nuclear organization and signaling in cell division, differentiation, and migration. In pharmaceutical research, it would permit the improvement of drug delivery systems relying on the slow release or on the control of docking [42–44]. Complex dynamics of protein solutions as a function of the ionic strength and protein surface charge patterns is usually studied using light scattering [45]. However, light scattering can only access the global collective diffusion of proteins in solution and cannot detect the internal motions of the proteins or the self-dynamics. Incoherent neutron scattering, on the other hand can provide unique and unambiguous access to the self-diffusion coefficient, but the investigations of the diffusion dynamics of living systems using neutron spectrometers has been severely hindered until recently because of the limited flux provided by the instruments. However, the advent of such research has just begun due to the development of the third-generation neutron backscattering instruments [4, 30, 46–48], but continued growth will depend on the highest-flux and flexibility provided by the backscattering spectrometer MIRACLES. Additionally, neutron scattering offers the unique capability of performing experiments in various extreme environments, such as humidity [49], pressure [50], electrical stimuli [51], pump probe [52].

Case 2: Dynamics of Biomolecules in Non-aqueous Environments

Biomolecules embedded in non-aqueous environments may exhibit novel exploitable properties, such as altered functionality and increased stability. For example, enzymatic selectivity—including substrate-, stereo-, regio- and chemoselectivity—can be markedly affected and sometimes even inverted by the solvent [53]. On the other hand, sugars and more generally polyols show an outstanding ability in preserving structure and functionality of biomolecules, which has been largely exploited in food, pharmaceutical and biotechnology sciences to optimize protein lyophilization and long-term storage of pharmaceuticals [54]. Fast protein dynamics has been shown to play a key role in determining and tuning these modified functional and stability properties [55]. Currently this type of study is restricted to an approached called elastic window scan [34], while the analysis of the quasielastic signal is very often neglected because of the poor statistics achievable in these cases [56]. The high flux available on MIRACLES, together with its variable energy resolution, will provide efficient simultaneous measurements of both elastic and quasielastic responses in this important time window, thus offering vital information on the coupling between protein and solvent dynamics, on a timescale often left unexplored, yet crucial for the delicate balance between biological functionality and stability.

Case 3: Climate Change and Ice Nucleation

Clouds affect climate, but changes in the climate, in turn, affect the clouds. This relationship implies that clouds modulate Earth's radiation and water balances. Retention of water may increase the freezing probability of water and can in turn serve as seeds for secondary nucleation of ice crystals that potentially grow into cirrus clouds, which can modify the radiative balance and change the climate. Dust, usually a primary catalyst, plays a large role in what takes place in clouds as it supports ice formation [57]. An improvement of our understanding of ice nucleation in clouds is therefore crucial to understanding the effects of global warming. In addition, pollen, bacteria, and spores of fungi can also induce ice nucleation [58]. Since microorganisms, which inhabit plant surfaces, are able to initiate the ice formation, this process can result in frost injury to frost-sensitive plants, which does, of course, have a rather negative effect on agricultural crop yields. Knowledge of water structure and water adsorption behavior on these catalyzing particles is therefore essential for any investigation of this process of ice nucleation. A determination of the dynamics and the structure of condensed water on the surface of chemical and biological ice nucleators would form the basis for sub-sequent parameterization of cloud physics models. However, until now, this type of information is scarce [59, 60]. In the future, the time scale covered by MIRACLES will allow observing relaxation processes related to the coupling of the

water hydrogen-bond to the dust-surface groups [31], while Raman scattering can be used to map the vibrational spectroscopic features arising from the hydration shells and the catalyst [61]. Thus a combination of *in-situ* Raman spectroscopy and imaging with systematic neutron studies performed on MIRACLES, can provide answers to the following questions: How important is the catalyst concentration for ice nucleator? Is aggregation of dust particles important? Can we distinguish relevant changes in the hydration shell before freezing?

EXPECTED IMPACT OF NEW MODELLING APPROACHES TO PROPERLY DESCRIBE WATER-MEDIATED INTERACTIONS OBSERVED BY QUASIELASTIC NEUTRON SCATTERING

Implementation of quantum interpretation of QENS describing the emergent properties of complex systems are urgently needed for a better description of the physical and chemical processes involved in complex systems. Development of "minimalistic" models to explain multiscale relaxation and anomalous diffusion, accounting for the non-Markovian properties of the dynamical heterogeneity of the water molecules constrained in a hydration environment is a promising idea [12, 15, 16, 26]. The concept is to capture the form of QENS spectra with very few parameters and to relate these parameters to the form of the multi-minima "energy landscape" which is explored by the scattering atoms [12]. Here the dynamics of a complex system is described by transitions between different states instead of using the traditional method of trying to fit a QENS spectrum by models for specific atomic motions. The main point of this proposed approach is that it accounts with very few, physically interpretable parameters for the multiscale dynamics in complex systems. The key word here is the "self-similarity" of the observed QENS spectra, i.e., their form invariance under a change of the time/frequency scale. Further development of this new physical interpretation of QENS experiments requires, in a first step, replacing the traditional least squares fitting method for these minimal multiscale models that consider the self-similarity, the most important physical property of the relaxation and diffusion processes in complex systems. The second step is to explore the full information contained on the experimental elastic incoherent structure factor (EISF). The insight resulting from Ref. [15] that the EISF probes effectively the size of the wave function of the hydrogen atoms and not the amplitude of their motions provides a radically new interpretation of elastic neutron scattering, which is central for a quantum mechanical understanding of energy landscapes. It gives also a new physical interpretation for the complex quantum version of the classical van Hove correlation functions, where we consider simply classical trajectories to explain the diffusion process. This methodology will enable to capture the dynamic nature of complex systems and understanding the dynamics of hydration (interfacial) water. It is our view that a proper description of water-mediated interactions will impact many areas of research, ranging from the understanding of cloud formation [50] and long-term stability of protein therapeutics [62, 63] to improved food processing [64] and conservation of museum artifacts [65].

OUTLOOK

Chemical and physical properties are governed by quantum mechanics and to view these complex interactions, we are constructing a multi-billion large-neutron source in Lund [18]. ESS will provide unprecedented access to quantitative structural and functional information from the atomic scale to the relevant mesoscales where real-world functionality often emerges in materials and complex biological systems. Neutron spectroscopy allows for direct measurements of space- and time-dependent information, which in principle can be compared directly with theoretical calculations. More specifically, QENS data gives insight in particularly crowded environments in the nanometer-nanosecond window, and biological applications are particularly important in this context [4, 5, 16, 40, 41]. To exploit QENS' full potential and allowing for instance to link QENS studies of protein dynamics with spectroscopic or kinetic experiments on biologically relevant time scales, such as dielectric and fluorescence correlation spectroscopy [66], harvesting information contained in QENS spectra needs a radical change in paradigm [12, 15, 16, 26]. We consider that theoretical and molecular dynamics modelling, and consequently development of new models that provide direct insight into physical properties of bio-materials and -molecules, must be rigorously developed and implemented in the analysis of the experimental data.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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