Published in final edited form as:
Chem Rev. 2016 July 13; 116(13): 7673–97. doi:10.1021/acs.chemrev.5b00664.

Water Determines the Structure and Dynamics of Proteins

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Abstract

Water is an essential participant in the stability, structure, dynamics, and function of proteins and other biomolecules. Thermodynamically, changes in the aqueous environment affect the stability of biomolecules. Structurally, water participates chemically in the catalytic function of proteins and nucleic acids and physically in the collapse of the protein chain during folding through hydrophobic collapse and mediates binding through the hydrogen bond in complex formation. Water is a partner that slaves the dynamics of proteins, and water interaction with proteins affect their dynamics. Here we provide a review of the experimental and computational advances over the past decade in understanding the role of water in the dynamics, structure, and function of proteins. We focus on the combination of X-ray and neutron crystallography, NMR, terahertz spectroscopy, mass spectroscopy, thermodynamics, and computer simulations to reveal how water assist proteins in their function. The recent advances in computer simulations and the enhanced sensitivity of experimental tools promise major advances in the understanding of protein dynamics, and water surely will be a protagonist.

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The authors declare no competing financial interest.

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1 Introduction

Water and its specific properties are fundamentally related to life and to the very peculiar properties of biological molecules like proteins and nucleic acids. Water properties have been studied in relation with the structure and dynamics of hydrogen bond network, the notions of hydrophobicity, crowding, and confinement of biomolecules. Biomolecular hydration is an active research area of great interest, in its theoretical and computational aspects and in a diversity of experimental methods. Biomolecular hydration is studied by X-ray and neutron scattering, solution and solid state NMR, and time-resolved techniques like ultrafast IR spectroscopy, electronic, and vibrational spectroscopy. Molecular simulations have become an integral part in the analysis and interpretation of experiments since experimental techniques cannot always provide a complete picture of protein hydration.

Hydration is a driving force for protein folding.[1] To perform their biological function, biomolecules must adopt a tertiary structure, known as the folded state. Globular proteins are water-soluble proteins that self-assemble into their folded state under appropriate conditions. This structure is the state in which the Gibbs free energy is lowest.[2] In some instances, to overcome kinetic traps, folding is catalyzed by other proteins, known as chaperones.[3,4] The stability and dynamics of proteins is closely linked to the properties of the aqueous solvent.[5–8] The stability of proteins, in vitro, depend on specific ions, ion concentration, small cosolvents known as osmolytes, sugars, etc., as well as thermodynamic variables such as temperature and pressure.[8,9] Hydrophobic interactions are known to be the driving force of protein folding.[1,10] The evolved sequence of a protein provides a minimally frustrated energy landscape on which the protein folds over a relatively short time scale, typically microseconds to milliseconds.[11,12] Hydrogen bond (H-bond) networks in the protein help stabilize the secondary and tertiary structures of the protein and help drive the protein to the folded state.[13,14] Hydrophobic interactions are responsible for the collapse of the protein chain in an aqueous environment.[15] Much of our understanding of protein folding comes from studies of model systems, peptides, and single domain fast folding proteins that fold in the submillisecond time scale.

In this review, we will focus on the behavior of proteins as a response to perturbations that change the structure and dynamics of water around biomolecules, and vice versa, on the changes in water dynamics induced by the protein surfaces. For a review on the role of water on biomolecular complexes, we refer the reader to the review by Levy and Onuchic.[8] We present our perspective on issues related to the structure and dynamics of water at a protein interface, as studied by NMR, X-ray and neutron scattering, and molecular simulations. Because of its novelty, a separate section is dedicated to the applications of terahertz spectroscopy. We will describe the hydration of proteins in the gas phase, which is relevant for the interpretation of mass spectroscopy experiments. We will also describe the effect of water on protein dynamics under various temperature and pressure conditions. Finally, we will describe the effect of hydrostatic pressure on protein stability.

2 Dynamics of Water

2.1 Dynamics of Water at a Protein Interface

The dynamics of water molecules at the interface with proteins have been the focus of extensive research for many years. The debate especially concerned the magnitude of the dynamic perturbation exerted by the protein surface on the solvent dynamics,[16–20] the extension of such a perturbation,[21] and the nature of its coupling.[22,23]

Time-dependent fluorescence Stokes Shift (TDFSS) experiments reveal the dynamics with which the environment of a chromophore relaxes following photoexcitation of the tryptophan.[19,24–26] In these experiments, the relaxation dynamics of small molecules in water is complete on a time scale of several picoseconds, while near proteins the dynamics can take tens of picoseconds or even longer. There have been two explanations for the molecular origin of the long time scale dynamics near proteins that have been advanced in the past decade. One model assumes that the electrostatic field of the protein renders the water molecules rotationally immobile, and hence to reorient, the water molecules need to exchange with bulk water molecules. This model interprets the slow component of TDFSS as originating from the intrinsic slow dynamics of water involving the exchange of water molecules between the bulk and hydration layer. On the other hand, Halle and Nilsson have interpreted the slow component in TDFSS experiments as originating from slow protein fluctuations.[16,17]

The origin of the slowdown observed at ambient condition for the rotational and translational motion of water is now understood. Systematic investigations using NMR have clearly assessed that water reorientation[16] and translation[27] are only a 3–5 times slower than in bulk. This was verified for many globular proteins. A similar mild slowdown was reported by computer simulations[28] for the average motion of water molecules in the hydration shell of globular proteins and showed to be quite independent of the water model used in the simulations. Both the geometrical disorder and the local energetics of the H-bond between water and protein sites (charged and polar groups in side chains and backbone) contribute to this average retardation and cause the heterogeneous character of water motion in the hydration shen.[27,29–33] Within the context of the interpretation of TDFSS experiments described earlier, the dynamical motions of side chains are a necessary criterion for observing slow components in the correlation functions. The most recent understanding of the process was recently granted by the application of the extended jump model introduce by Laage and Hynes for describing the H-bond reorientation in liquid water.[34,35] In this framework, the effect of the local geometrical and energetic disorders become two quantifiable free energy contributions to the barrier rate limiting the water reorientation via HB switch events, illustrated in Figure 1.[36] The anomalous character observed for water translation, transiently subdiffusive, is not yet associated with a clear molecular mechanism, but it is accepted that both the fractal-like dimension of the protein surface and the disordered distribution of H-bond-traps shape the mean square displacement of water when averaged over the entire hydration shell.[27,32,37]

The dynamics of water at the surface of proteins and small molecules used as model solutes reproducing the chemical features of amino acids was also investigated by a plethora of

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other methods, like neutron scattering,[38–41] optical Kerr effect,[42] extended depolarized light scattering (EDLS),[43] and terahertz[21] and two-dimensional (2D)-IR[44] spectroscopies. Because many of these techniques probe collective motions, the results obtained are not always easy to interpret, and generally systematic support from theory/modeling is required.[45,46] For nonlocal techniques, as for example EDLS, the collective relaxation processes must be carefully dissected:[45] for instance, the spatial extension of the perturbation depends on the quantity considered (density fluctuations vs total dipole fluctuations) and the effect of the size of the solute perturbing the process matters. This means that for collective motions, small peptides in solution cannot be used to infer behavior of water around a larger system as a protein.[43]

Quasielastic neutron scattering is a particularly well-suited experimental technique to probe water dynamics under different conditions. Energy resolved incoherent neutron scattering, especially in the elastic (EINS) and quasielastic (QENS) scattering modes, have been informing us of water dynamics under different conditions for decades. Because the method relies on incoherent scattering, samples need not be crystalline or even monodisperse and measurements can be performed on highly complex systems such as living cells or stacks of natural membranes. The incoherent neutron scattering cross section of hydrogen is more than an order of magnitude larger than that of other atomic nuclei usually found in biological material and their isotopes, including deuterium, and the development of in vivo specific deuterium-labeling methods permitted the focus on water dynamics in complex biological samples and its coupling with biological function and activity.

QENS allows access to the ranges of time and space scales for proteins, in particular global diffusive motions (translational and rotational modes) and internal diffusive motions. From inelastic neutron scattering, vibrational density of states of protein (VDOS) inform about the variation of internal modes in protein. The use of QENS to study the structure and dynamics of water at the surface of proteins and the dynamics of proteins is discussed in various sections in this review.

2.2 Water in the Interior of Globular Proteins

So far, we have focused on the dynamics of the majority of water molecules interfacing the external protein surface, but the protein interior can be hydrated as well.[47] The exchange dynamics of internal water generally occurs on the nanosecond time scale and longer. This exchange dynamics was first investigated by NMR experiments and correlated to slow rearrangement of the protein matrix.[47] The first detailed investigation at atomistic resolution have been carried out by computer simulations but limited to the nanosecond time scale.[48–50] Nowadays, thanks to technological development, it is possible to characterize this exchange process (e.g., individuate the path for the exchange and correlated protein motions) via computer simulation at much longer time scales and with an unprecedented resolution and richness of details, as shown in Figure 2.[51,52] Moreover, from the experimental perspective, the possibility to link proteins in a matrix of gel, hence reducing rotational tumbling, allowed NMR to dissect this in/out water exchange at a much finer temporal resolution.[53] Interior water molecules can be characterized as localized and fluctuating water molecules. Localized water molecules are identified by high resolution X-ray

crystallography and by water ^{17}O and ^{2}H nuclear magnetic relaxation dispersion (NMRD) measurements[47] and can have residence times on the millisecond time scale. Fluctuating water molecules cannot be easily detected as single water molecules but can be characterized by density or their relaxation properties. A computational study of a peptide-MHC complex show that fluctuating water molecules at the complex interface can have higher entropy than in bulk and can enhance the peptide binding affinity.[54] The nonpolar cavity in the protein interleulcin-l beta (IL-1β) has been reported to be filled by water on the basis of some experiments and simulations[55,56] and to be empty on the basis of others.[57] Yin et al.[58] studied the thermodynamics of filling the central nonpolar cavity of interleukin-1 beta by molecular dynamics simulation and found that water in the central nonpolar cavity is thermodynamically unstable. The favorable contribution of internal water to protein thermal stability has been recently pointed out[59,60] and complements previous investigations on the role of internal hydration to pressure induced unfolding discussed later in the review.

2.3 Coupling of Protein/Water Dynamics

How fast dynamics at the protein surface couples to the motion of the external exposed to solvent amino acids as well as to the protein interior and the escape dynamics of internal water relates to protein soft modes has been the object of intense research. Neutron scattering techniques, which are very sensitive to protons, are particularly suitable for these studies.[61] However, dielectric spectroscopy,[62] nuclear magnetic resonance (NMR),[63] and molecular dynamics simulation (MD) have led to significant results.[64–67]

In seminal studies of myoglobin over a wide temperature range by Doster et al.,[68] it was shown, by using Inelastic Neutron Scattering (INS), that at low temperatures (below 80 K) the protein behaves like a harmonic system and displays a linear increase in mean square displacement (MSD) with temperature. However, above 180 K, there is a dramatic increase in the MSD (Figure 3).[68] This increase in MSD was attributed to a dynamic transition [later labeled a protein dynamic transition (PTD)] arising from the excitation of nonvibrational motion, first attributed to torsional jumps between states and later attributed to methyl group rotations.[69] Earlier Mossbauer spectroscopy studies have shown similar behavior, but Mossbauer probes the protein (the heme iron) dynamics on a much slower time scale.[70,71] This sudden change of slope in the temperature dependence of mean-square displacement of hydrated proteins around 220 K has been extensively studied.[72] This effect is present in other proteins, although at different temperatures, and MD simulations reproduce the MSD temperature dependence.[73]

The interest in the protein's "dynamical transition" is due to the fact that this transition is supposed to be intimately connected to protein function and that this connection can be made for a wide variety of systems from small soluble globular proteins to membrane proteins. In the early steps of the transition, the role of the solvent surrounding the proteins has been recognized, since in the absence of hydration the 220 K dynamical transition vanishes. Moreover the transition temperature is controlled by the viscosity of the solvent, and the transition temperature is raised in the presence of cosolvents like sugars.[74,75] A clear connection among the role of solvent in controlling such thermal activation of protein vibrations has become more explicit by comparing the paralleling of the thermal dependence

of protein and water motions. A strong parallel evolution at 150 and 220 K between the mean-square displacements related to interfacial water rotational dynamics and to protons dynamics of a hydrated lysozyme protein[76] has been obtained (see Figure 4). This connection is made at the local scale (a few Angstroms) and on the time scale of nanoseconds. These observations provided evidence that interfacial water rotational dynamics are the real source of entropy driving protein dynamics. In the context of the previous findings, it has been possible to reach a final view of the protein-hydration water interaction[77] and how this interaction can drive the protein function: the protein external side-chains[78] short time motions, induced by fast water reorientational motion, propagate in a hierarchical way, along the protein structure from the residue side chains down to the protein core to induce the longer time scale motion of protein backbone necessary for its function.[79] The dynamical crossover experienced by water at 150 and 220 K are also detected on the protein dynamics, even though the time scales of the crossover can be different (longer times for protein than interfacial water). This was also observed by taking advantage of a deuterated protein, the maltose binding protein (MBP), and combining elastic incoherent neutron scattering with MD simulations.[73,80,81] The temperature-dependence of experimental and simulated dynamics of MBP and its hydration water, probed individually on the same sample, affirm the existence of a dynamical coupling between protein and solvent motion on the ps–ns time scale. These observations have been confirmed further using a different deuterated protein which allowed one to distinguish between protein dynamics and hydration water dynamics.[82] QENS and IR experiments on lysozyme powders suggest that the protein dynamical transition may have its origin in the liquid–liquid critical point in the protein hydration water.[83,84]

Nevertheless, the interpretation of the origin of the dynamic transition has been controversial.[86–91] Young et al.[87,88] have argued that the dynamical transition in Mossbauer experiments is caused by the incorrect separation of the spectrum into sharp and broad components. They argue that, given that proteins are not harmonic, the entire spectrum is inhomogeneous composed of sharp lines, with each sharp line comin,g from conformational substates in a complex, hierarchically ordered, free energy landscape. Frauenfelder and collaborators proposed a unified model of protein dynamics (UMPD) and showed that the Mossbauer and INS spectrum of myoglobin (Mb) can be explained above 180 K, without any fitting parameters, by the dielectric fluctuations in the hydration shell.[90] The UMPD can also explain DSC and INS in dehydrated samples without invoking dynamical transitions.[91] In this model, the striking change in dynamics near 180 K is a kinetic behavior that depends on the time scale probed by the experiment and not an equilibrium behavior. Fenimore et al. proposed that motions in proteins can be characterized into two classes: slaved and nonslaved.[89] Slaved processes are tightly coupled to the solvent, with the solvent being responsible for the activation enthalpy, and the protein and its hydration shell control the activation entropy. The dominant conformational motions are slaved by the hydration shell and the bulk solvent. The protein fluctuations are called α and β_h, following studies of the fluctuations of glass-forming liquids.[92] The alpha fluctuations are structural, and their rate constant is inversely proportional to the viscosity of the medium. The β_h fluctuations come from the hydration shell and influence the protein motions.[87,93] The β_h fluctuations proposed by Fenimore et al. are different than the typical β fluctuations in supercooled

liquids and glasses, which is a bulk solvent property. The microscopic origin of the β_h is not known but may be related to the behavior of interfacial fluids on the nanometer scale of proteins.[94] Molecular dynamics simulations and QENS on lysozyme-hydrated powders exhibit a logarithmic decay in the 10 ps to 1 ns time range, which is in the β-relaxation range of the protein.[95,96] Readers interested in the specific arguments related to this controversy are encouraged to read recent articles by Doster,[86] Young and Fenimore,[91] and Frauenfelder et al.[90]

2.4 Modeling Protein Hydration

We pause here to describe in detail the modeling of protein hydration by molecular simulations. In fact, molecular simulations play an important role in the understanding of the interactions of water with proteins. High-resolution experimental techniques, like X-ray crystallography, can determine the position and sometimes orientation of strongly bound water molecules. In most instances, water molecules in a crystal structure are placed only if they have two or more H-bonds with protein atoms, and highly fluctuating water molecules cannot be detected. Molecular simulations have been used to complement our understanding of hydration of biomolecules.

Computational modeling of proteins has become a large field of science over the last 30 years. Whereas the first studies treated only peptides and small proteins on picosecond time scales,[97,98] recent simulations study all atom models of a mature HIV-1 viral capsid.[99] Millisecond time scale simulations are now performed to study protein folding,[100] protein dynamics,[101] and ligand binding to receptor proteins[102–106] with unprecedented accuracy.[107] The size and complexity of biological systems makes it only feasible to treat them with force field approaches (i.e., empirical classical potentials). A number of such force fields exist, each with specific strengths. Due to historical reasons, protein force fields were typically developed in conjunction with a specific water model, for instance, GROMOS[108] with SPC,[109] CHARMM[110] and AMBER[111] with TIP3P,[112] and OPLS/AA[113] with TIP4P,[112] and modifications of these models.[114,115] Although a plethora of water models for molecular simulation exist,[116] only the simple ones have gained popularity for biomolecular simulation. This does not mean that the reproduction of experimental data by the models is equally good. The dynamic and dielectric properties of TIP3P are far from the experimental values, see for instance,[117] while the thermodynamic properties are regarded as very good.[117] For the SPC/E model,[118] the observations are rather the reverse. The relative merits of the different force field/water model combinations are difficult to establish when studying proteins, although some differences can be noted.[119,120] Due to the difficulty of evaluating the suitability of force field/water model combinations for studying protein hydration, a defacto “cease-fire” has come into being, where the choice of a particular force field/water model combination is not questioned in the community, although some papers present validation calculations with additional models. We note that it is possible to test force fields in detail using model systems like amino-acid analogs in water,[121] organic molecules in water,[122] or even pure organic liquids,[123] but this falls outside the scope of this review. There is some hope that force fields may make a leap in accuracy and predictive power in the near future though. The recent introduction of complete models for studying biomolecular hydration including polarization effects, like Amoeba[124] and CHARMM[125]

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each with corresponding water models,[126,127] makes it possible and necessary to explicitly compare the performance of these models to older force fields. If these new models indeed prove to yield significantly improved reproduction of experimental properties, as has been speculated for a long time, older force fields may at last move out of fashion. For practical reasons, the CHARMM polarizable force field[125] is interesting because the force field is implemented in both the CHARMM software[128] and the NAMD package,[129] while efforts to incorporate it into GROMACS[130] are underway, meaning it will be available to a large community of users. Computer time requirements are obviously higher for these more complex force field model estimates, depending on the level of approximations applied, between a factor of 2 and 10 increase in computer time.

3 Terahertz Studies of Biomolecular Hydration

Due to the recent fast progress in the fields of optics and electronics, terahertz (THz) spectroscopy has received much research interest due to its wide use in scientific fields.[131] THz spectroscopy can detect and control properties of matter with electromagnetic fields that are in the frequency range between a few hundred gigahertz and several terahertz (i.e., between microwave and infrared in the electromagnetic spectrum). Unlike X-ray, THz radiation is intrinsically safe, nondestructive, and noninvasive. It creates a powerful spectroscopic and imaging technique for characterizing molecular structures such as those in biomolecular hydration. Specifically, THz spectroscopy can probe large amplitude motions and the hydration of proteins,[132] in particular the long-range and fast dynamics of collective hydrogen bond networks, which are averaged out in many static or dynamic scattering experiments. Nowadays, water films extending several tens of micrometers can be analyzed by THz spectroscopy[133] as well as fully hydrated biological solutes like carbohydrates, amino acids, model peptides, nucleic acids, lipids, osmolytes, and proteins.[134]

Thanks to the availability of THz laser sources, the THz spectrum of pure water could be recorded in ambient conditions decades ago.[135] Two pronounced bands at approximately 200 and 650 cm^{-1} were found and assigned to hydrogen bond stretching vibrations and librations, respectively. Later, with the interest in understanding protein dynamics and its relation to protein conformational change and biomolecular function, the groups of Markelz, Heilweil[136] and Jepsen[137] observed a broad absorption of THz radiation for lyophilized DNA, bovine serum albumin and collagen, displaying a large number of low-frequency collective modes of the biomolecules and confirmed by normal mode calculations. The Grischkowsky group used a parallel-plate waveguide approach and cooling to investigate the polycrystalline film of amino acids with reduced broadening.[138] Jepsen and co-workers reported THz frequency absorption characteristics of polycrystalline saccharide stereoisomers in various states.[139] Due to the fact that water has a high absorption coefficient, all the above-mentioned measurements are based on samples in the form of dry pellets, crystals, or lowly hydrated thin films. Therefore, the interpretation of the measurements as an attempt to relate the dynamics of the biomolecules to their functions should be very cautious, since the degree of hydration deeply affects biological fUnctions.[140,141]

The most obvious restriction for exploring the dynamics of biomolecules in bulk-like aqueous solution by THz spectroscopy is the lack of high power THz sources.[142] Another reason is that water in the THz region shows a wide broadening and largely overlaps or couples to the low-frequency modes of biomolecules, making it quite difficult to decouple various motions of the biomolecules and the solvent water. The complex combinations of the vibrational modes that contribute to the THz spectra of aqueous solutions hence need much joint effort from both experiment and simulation for better interpretation of the spectra with molecular insights.

Recently, Havenith and collaborators have built up a p-Ge difference spectrometer, which uses a pulsed p-Ge laser with an average output power of 2 W and a duty cycle of 5% in the frequency range between 2.4 and 2.7 THz, to carry out high-precision THz absorption measurements under controlled humidity and temperatures.[143] In most solutions, the THz absorption (between 1 and 3 THz) of the solvent due to the intermolecular vibrations of the hydrogen bond water network by far exceeds that of the solute, meaning that the solute exhibits a “THz defect” with respect to the solvent. This implies that a linear decrease of the THz absorption with increasing solute concentration is expected. However, for disaccharides in solution, a “THz excess” and an onset of nonlinearity at specific concentrations have been found between 2.4 and 2.7 THz. These could be modeled when taking into account that the water molecules in a dynamical hydration shell show a distinct and larger absorbance than that of bulk water. For homogeneous solutions, this can be described very well by means of a three-component model, which takes the solute, the bulk water, and the hydration water into account. The THz spectroscopy thus allows a direct investigation on the dynamical hydration shell and the determination of its size (i.e., how far the influence of the biomolecules on the solvent can reach).[144]

In studies on sugars that have homogeneous surface such as lactose, trehalose, and glucose, Havenith and collaborators fit the concentration-dependent THz absorbance to a three-component model to obtain the size and absorption coefficient of the dynamical hydration shell of the carbohydrates.[145] As a result, the hydration shell of the monosaccharide glucose was found to extend to approximately 4 Å from the carbohydrate surface and around 6–7 Å for the disaccharides lactose and trehalose. This could be correlated to the hydrogen bonding between the carbohydrates and the water.

Later, their investigation was extended to proteins such as λ-repressor, antifreeze protein, ubiquitin, and human serum albumin.[146] In these studies, it was possible to determine the dynamical hydration shells around the proteins go beyond 15 Å from the protein surface, corresponding to at least five shells of hydration water or a volume of approximately 7500 $Å^3$. By examining vibrational density of states and hydrogen bond dynamics of the hydration water, accompanying molecular dynamics simulation attributes the increased THz absorption to a significant retardation of dynamical processes on the picosecond time scale, including hydrogen bond breaking and reforming, rotational relaxation, and translational diffusion.[147] Therefore, THz spectroscopy has proven to be a sensitive tool to probe the collective hydrogen bond network vibrations of water without the use of artificial probes or chromophores. However, a more quantitative description of the absorption certainly requires considerations of the heterogeneity of the protein-water interface, a concentration-dependent

coupling of internal vibrational modes of the protein and the surrounding hydrogen bond network, and the effects of protein aggregation or oligomerization in solution.

A recent advancement in biomolecular hydration is the application of multiple time-resolved spectroscopic techni-ques.[148] By merging transient fluorescence kinetics, stopped-flow X-ray absorption spectroscopy (XAS), and kinetic absorption spectroscopy (KITA), it was possible to follow kinetically biochemical reactions at the active site and the changes in the THz response of the surrounding hydration water in real-time. This had been considered a challenge especially when monitoring fast changes of the aqueous environment upon biochemical processes such as enzyme catalysis.[149] Ultrafast fluorescence spectroscopy and diffraction techniques such as neutron scattering can access the regime but limit their focus on the static interactions between water and protein side chains.[149]

The first biochemical reaction examined by this technique is the catalysis of a matrix metalloproteinase (MMP). MMP is a zinc-dependent endopeptidase for collagen degrading proteins with enzymatic activity.[150] For the experimental setup (Figure 5), a stopped-flow mixer with the enzyme and a fluorogenic substrate were placed in two reservoirs in the focused THz beam of a THz time-domain spectrometer in transmission configuration. A rapid mixing was then initiated for the enzymatic reaction followed by the XAS for the active site structural kinetics and by KITA measurement for the collective water network dynamics in the enzyme–substrate mixture. It was observed that in the first few nanoseconds after the mixing, there is an unspecific binding of the substrate at the enzyme surface before a specific binding at the active site. By using the XAS to monitor the change of the charge state of the metal ion of the enzyme, the formation of the Michaelis enzyme– substrate complex occurred with a change in the coupled protein-hydration dynamics. In this work, the authors then proposed that the collective water network dynamics and enzymatic proteolysis are also coupled and tested the hypothesis by varying the substrate.

The analysis on three types of hydration water in different locations (i.e., near the active site, near the separated enzyme and substrate, and near the substrate in the Michaelis complex) displays a gradient of water dynamics near the active site of the metalloprotease MT1-MMP and water network dynamics during the Michaelis complex formation. The combined experiments and molecular simulation studies led to a model in which there is a sharp gradient of water dynamics near the active site prior to the binding. Right after the mixing, the substrate binds to the metalloprotease but not the active site. The active site gradient of hydrogen bond exchange dynamics supports the substrate association toward the catalytic metal ion via charge-induced water retardation near the active site. Then, upon Michaelis complex formation, the hydration dynamics of the substrate is further retarded and a mild gradient of water dynamics is established near the active site. Altogether, a prebuilt gradient of fast-to-slow coupled protein–water motions known as a “hydration funnel” toward the active site was detected before the formation of the complex, suggesting solvent-mediated induced-fit as a possible nature of enzyme catalysis.

The past decade has witnessed the extensive success of THz spectroscopy in biomolecular hydration research. Due to its inherent sensitivity to water hydrogen bonding, it has become an indispensible tool for direct observation of fast and coupled biomolecular water network

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such as that surrounding sugars and proteins. The ultrafast view by THz spectroscopy has brought a more comprehensive understanding of biomolecular hydration. Water, rather than a bystander in the interactions with biomolecules, actively participates in various biomolecular dynamics and functions. It can perturb the dynamics of biomolecules by both direct short-range interaction in the first hydration shell and indirect long-range interaction up to several hydration shells via its unique hydrogen bond network. The two interactions are coupled dynamically, leading to a water-mediated induced-fit between the substrate and the enzyme in the enzyme catalysis, for instance. The future of THz spectroscopy in biomolecular hydration research will surely expand our horizon on the hydration to complex environment such as under extremes, at interfaces, and under confinement and crowding.

4 Relationship between Water-Structure and Protein Function

4.1 H-Bond Networking at Biological Surfaces

Following the seminal work by Careri et al.,[140,141] great interest has been placed in characterizing the water–water hydrogen bond network enveloping proteins and finding correlations with their stabilities and functions. High-resolution crystallographic structures provide visual evidence of a well-defined organization of water at the surface of proteins. Of course, this localization/ structuring is generally very specific and could be sensitive to the low temperature of the experimental setup. Combining results from crystallography, neutron scattering, and simulations, it was proposed that this layer is generally 10% more dense than bulk water;[151,152] however, the solvent packing depends on the nature of the local surface[49] and extended hydrophobic patches induce large density fluctuation.[153,154]

In a recent survey,[155] the distribution of the size of the water oligomeric clusters observed in high-resolution structures (1.5 A) of more than 1500 proteins was reported. Trigons and pentagons are the larger populations found. The impact of these local organizations on function and stability is unsolved. For example, the persistence at ambient temperature of organized closed clusters at the protein surface could give contributions to the specific heat of unfolding of the protein by reducing the effect caused by the solvent ordering upon solvation of the hydrophobic groups of the protein core.[156]

Early attempts to characterize the spatial correlation of water–protein interfaces were done by using neutron diffraction as functions of temperature and hydration level.[157] Figure 6 shows data obtained for the protein C-phycocyanin.[157]

The correlation distance of 3.5 Å measured in these diffraction experiments compared well with computer simulations work on polypeptides and proteins[97,158] and has been interpreted as resulting from some increase in the clustering of water molecules. For the highest hydrated sample ($h = 0.365$), a definite peak appears at 3.5 Å. This is the average distance between the center of mass of a water molecule in the first hydration layer and amino-acid residues on the surface of the protein. In the case of the lowest hydrated sample ($h = 0.175$), the perturbation to the structure of protein due to water of hydration is not detectable. Some similarity between the behavior of water close to C-phycocyanin protein and close to hydrophilic model systems can be stressed. In fact, for low hydrated protein samples, no crystallization of water is detectable,[159] while for more than one monolayer coverage there

is appearance of the hexagonal crystalline ice; moreover, the peak at 3.5 Å is also detected. At 77 K, the structure of protein hydration water looks similar to that of water as a monolayer at surface of a hydrophilic porous glass of Vycor. There is no appearance of Bragg peaks characteristic of hexagonal ice.[157] In the case of Vycor, the structure of interfacial water at 77 K is characteristic of that of low density amorphous ice.[85] It seems that a transition between a low density liquid and a liquid with a higher density occurs for water at the protein surface, as it has been obtained for a water monolayer at the Vycor surface.[85] However, it should be noted that at the highest hydration level, water nucleates into hexagonal ice at low temperature; this is in contrast to hydrated Vycor where water nucleates into cubic ice.[160]

The presence of highly structured interfacial water was correlated to specific functional states of proteins. As an example, we mention here the case of the snow flea antifreeze protein (sfAFP), where the crystallographic structure comes along with an ordered layer of water mimicking the template of the ice structure (Pdbcode 2pne).[161] A recently published crystallographic structure of the antifreeze protein Maxi[162] also surprised the community because of the structure of crystallographic water in which the interior of the four helices bundle is filled by an unconventional amount of ice-like water well-connected to the external surface. This finding challenges the common understanding of protein folding associated with the formation of a dry hydrophobic core. Other meaningful examples are represented by water networking in protein channels.[163]

Percolation theory combined with atomistic simulations proved the persistence/extension of the water–water H-Bond network at the surface of globular proteins[163] and its correlations with the chemical composition of the underlying surfaces.[164] In fact, by removing pinning sites (site accepting or donating H-Bond) at the protein surface, water tends to assume the well-known inverted structure typically observed at a flat nanoscale hydrophobic surface[32] and therefore loses in-shell connectivities and percolation is broken. For low-hydrated systems, the thermal stability of the network seems correlated to the thermophilic character of the underlying protein, while in dilute solution, the stability of the H-bond network around small peptides was probed to be quite insensitive to the chemistry of the peptide and is mainly controlled by the backbone exposed surface.[165]

How the water network is capable of hydrating the rough protein surface impacts the local dielectric response of the protein/water interface[166] and has a potential effect on protein–protein binding[167] as well as the stability of key local interactions like the salt-bridges.[168] It is also argued that the thin hydration layer plays a key lubricant action when proteins are segregated in organic solvents. This skin ensures the protein stability and flexibility by saturating the H-bond propensity of polar/charged groups and avoids their collapse and intramolecular rigidification. Generally, when a small amount of water is introduced in enzymes/organic solvent solutions, the activity of the enzyme is significantly increased[169] and a clear correlation among the mobility of water and the enzymatic activity of subtilisin Carlsberg in organic solvent was recently reported.[170]

4.2 Proton Wires

In 1978, Nagle and Morowitz[171] introduced the notion of hydrogen-bonded chains that would form through a series of sequential hydrogen bonds involving the hydroxyl side chain group of several serine amino acids. These hydrogen-bonded wires could serve as conduits along which proton transfer occurred. Numerous experimental and theoretical studies later on indeed confirmed that fairly long-lived water-wires formed in a plethora of biological systems particularly membrane proteins and allowed for correlated or concerted protonhopping events in which several protons hop over many water molecules simultaneously leading to enhanced mobility.[172–175]

Water and proton wires may not be a unique feature of water in confined environments. Taking the view of water as a percolating 3D hydrogen bond network yields the presence of closed rings or loops, the segments along which lead to wirelike structures. In the presence of topological defects such as the proton or hydroxide ion, concerted proton hopping can also occur.[176] Rather than being able to form more linearshaped and long-lived topological structures like in proteins, the proton wires in water will be a lot more coiled up and instead much shorter lived. Understanding both the structural and dynamical properties of these networks will likely play an important role in attracting and funneling protons to the active site such as in proteins like the green fluorescence protein.[177,178]

Besides their role in conducting protons, the fluctuations and evolution of hydrogen bond networks and wires has been shown to play an important role in the binding of protein interfaces. Extensive molecular dynamics simulations of the interaction of two hydrophilic proteins have shown that the interfacial water between the proteins acts as an adhesive-directed network, resulting in a dielectric shielding which results in highly directed electrostatic interactions between the hydrophilic groups of the protein.[168] The role of collective hydrogen bond networks has also been observed recently in the folding of antifreeze proteins. In particular, this protein is an alanine rich polypeptide forming helical bundles that are stabilized by clathrate-like pentagonal hydrogen bond net-works.[162,179]

One of the most fundamental processes in protein biophysics is the exchange of the amide backbone hydrogen atoms with the surrounding solvent. The time scales of amide exchange is a very useful probe into the flexibility of the protein. Despite its ubiquitous occurrence, the molecular mechanism of amide backbone hydrogen exchange is still not completely understood, although it is believed that the process is catalyzed by the close proximity of a hydroxide ion near the amide backbone. Using long classical molecular dynamics simulations, Persson et al.[180] have proposed a mechanism whereby a hydroxide ion approaches the N–H bond through a water wire. They suggest, based on the interpretation of their classical simulations, that the number of water molecules and orientation of waters within the wire plays an important role in ensuring successful amide proton exchange.

4.3 Protein Hydration in the Gas Phase

An interesting way to study protein hydration is in the gas phase, where hydration in principle can be studied “one molecule at a time”. Without bulk water around, the activation barrier to hydrogen bond breaking becomes higher, like it is in a hydrophobic environment,

[181] and water is kinetically trapped on the surface. A prerequisite for such experiments is that proteins are introduced into the gas-phase in a gentle maner, typically using electrospray ionization (ESI)[182,183] and that the flying time in vacuum is relatively short. Seeing that the ESI process is difficult to study recent research has focused on model systems, including water/methanol mixture and polymers.[183]

Computer simulations of proteins under electrospray conditions have shown that proteins undergo a collapse to a compact state, in which the number of intra molecular hydrogen bonds increases.[184,185] Unless the protein is activated (e.g., in a hot gas chamber) or if it is sprayed from a low pH solution, it will typically remain in a folded state close to the native one.[186] Complex arrangements of proteins in micelles have been reported from mass spectrometry studies as well[187] and corresponding theoretical papers.[188,189] The general observation from these studies is that the interaction energy is minimized under a limited time until the protein is kinetically trapped. In complex arrangements, water plays a crucial role to “lubricate” interactions between proteins and lipids.[187,188] Evaporation of water from droplets in the gas phase removes energy,[190,191] and this happens in cases where there are proteins in the droplet as well; simulations of the process[185] suggest that temperatures drop by several tens of K within a few nanoseconds due to evaporation (see Figure 7). In the same work, hydration of proteins in the gas phase was shown to be reproducible; that is, in triplicate MD simulations water consistently bound to hydrophilic groups on protein and to other water molecules.

5 Pressure and Temperature Unfolding of Proteins

Under appropriate conditions, proteins adopt a folded state. As temperature is increased, the entropy of the system increases, and at some temperature, the entropy (solvent and protein configurational entropy) dominates over the enthalpic stabilization, leading to the unfolding of proteins. Temperature unfolding of proteins is well-characterized. For reviews, we refer to the work of Makhatadze and Privalov.[192] The changes in heat capacity can be modeled using simple models that calculate the changes in polar and nonpolar surface areas between the folded and unfolded states.[193,194] The Gibbs free energy change upon unfolding of a protein can be described by the Gibbs–Helmholtz equation. This equation describes the free energy as a concave curve that crosses zero at two temperatures. The high temperature crossing corresponds to temperature unfolding, and the lower temperature corresponds to cold denaturation.[195] For most proteins, the cold-denaturation temperature is below the water freezing point, but the transition can be explored using cosolvents. There are also examples of cold denaturation under physiological conditions.[196,197] The cold denatured state can be observed under liquid water conditions (including supercooled water) by adding cosolvents or changing hydrostatic pressure.[198–206] The structure of the cold denatured state of a protein dimer has been characterized by NMR studies.[207] Hydrogen exchange experiments on phosophofructokinase-2 shows that cold denaturation primarily occurs by solvent penetration into the hydrophobic core of proteins, probably in a sequence-independent manner.[208]

The effects of pressure on organic systems have been known from qualitative experiments since the 19th century.[209] The first example of pressure-driven denaturation of a protein was

described by Bridgman in 1914.[210] Bridgman described how a protein is unfolded by increasing the hydrostatic pressure of the solution (typically to values over 300 MPa). He also described that the ease of unfolding increases slightly when the temperature is reduced (i.e., it cold denatures). At ambient conditions, in case of globular soluble proteins, the globule is relatively loosely packed with and a hydrophobic core. By applying pressure, the system will be driven to a lower volume state, which includes the elimination of filling of cavities. From pressure driven unfolding of proteins, one comes to the following conclusions: (i) the variation of volume of the protein ΔV is negative because water molecules get into the protein while the hydrophobic groups do not come into the water. Although matter is always compressible, the electrostriction of charged and polar side chains, hydrophobic hydration, hydrogen bonds stabilization, and the elimination of packing defects are considered to be the main cause of the volume change.[211] (ii) The protein unfolds as a result of water penetration.[212] (iii) The protein becomes more solvated by water molecules.

There are several reasons why one may want to measure the effect of pressure on a wide variety of thermodynamic systems. Perhaps the most important argument is that one can separate the effects of volume and thermal energy changes, which appear simultaneously in temperature experiments.[2] Moreover, high pressure can induce unfolding of protein in a different way from thermal denaturation.[214] The pressure studies have considerably increased in the past decade.[211,215–219]

The effects of pressure on hemeproteins have been the subject of numerous investigations.[220,221] The crucial role of water on pressure unfolding has been seen in myoglobin at pressures up to 300 MPa, where the reorganization of the secondary structure and of the active site occurs. It has been shown that the partial specific volume of Mb decreases by 5.4% between atmospheric pressure and 300 MPa, while the isothermal compressibility remains almost constant.[222] Moreover, it was found that the compactness of the protein was not altered and that the interactions between the molecules were always strongly repulsive. The β-lactoglobulin (βLG) protein has mainly a β-sheet secondary structure and a large central hydrophobic cavity. Under certain experimental conditions and at a pressure around 300 MPa, the protein begins to form irreversible aggregates.[223] This aggregation occurs between dimeric units of the protein. It looks as if under pressure the water penetration was hindered. A recent work on β-lactoglobulin[224] is in favor (at pD close to 2.3) of βLG dissociation and modification of protein–protein interaction with an unfolding process at pressures higher than 160 MPa. βLG is considered as a sensitive model system to study denaturation, oligomerization, and nucleation of proteins under destabilizing conditions, which can lead to a series of related diseases (due to beta-sheet formation).[225]

5.1 Pressure-Assisted Cold Denaturation

The study of proteins at low or subzero temperatures is based on the unusual behavior of water under pressure, which allows for the study of protein denaturation without ice crystal formation. An extensive study of pressure-assisted cold denaturation on β-lactoglobulin has been done by combining fourth derivative UV spectroscopy under pressure (300 MPa) with gel permeation chromatography and DSC measurements.[226] It has been shown that at a

given pH, low temperature-pressurization is very useful to minimize structural changes and therefore losses in protein functionality.

Thermodynamically, the pressure denaturation and pressure-assisted cold denaturation observed by Bridgman can be described by a simple parabolic P-T stability diagram, first described by Hawley. Figure 8 shows a P–T stability diagram obtained from molecular dynamics simulations on a small protein.[228] Notice that at low temperature and high pressure the protein can unfold upon cooling. This diagram shows isentropic and isochoric lines on which the entropy and volume changes of the transition are zero. For P–T states below the $\Delta V = 0$ curve, the volume of the unfolded state is higher than the volume of the folded state, while above this curve the volume of the folded state is higher. We must emphasize that here volumes and entropy changes refer to the overall changes on the system, not just the protein. This suggests that for the region above, $\Delta V = 0$ (i.e., $\Delta V = V_{\text{unfolded}} - V_{\text{folded}} < 0$), as required by Le Chattelier's principle, the unfolded state should be preferred at higher pressures. Equally interesting are the P–T regions separated by the $\Delta S = 0$ curve. Below this curve, the protein solution has larger entropy in the unfolded state over the folded state, as is typically described in textbooks. However, above the $\Delta S = 0$ curve, the folded state will have higher entropy than the unfolded state, and, as the system is driven toward the more ordered state as temperature is lowered, the protein will adopt the (cold denatured) unfolded state. This behavior has been observed experimentally for model proteins and has been described by molecular dynamics simulations.[228–232]

The P–T diagram of the Trp-cage for an alpha helical peptide (AK16) and for a β-hairpin forming peptide known as the GB1 peptide calculated by Hatch et al.[232] are shown in Figure 9. The diagram for the Trp-cage shows pressure denaturation at low pressures as well as high pressures. However, the GB1 β-hairpin only shows high-pressure denaturation. Hatch et al. suggest that the behavior of the Trp-cage and GB1 β-hairpin under negative pressure is related to the position of the center of the elliptical stability diagram relative to the spinoidal for the bulk liquid described by Tip3P water.

5.2 Cavities in Folded Proteins

The structure of the temperature-unfolded state of a protein is modeled as a random coil with little ordered content of secondary structure. However, the pressure-denatured state (at room temperature) of a protein is much more compact than the random coil and preserves much of the protein's secondary structure.[214,233] The compactness of the pressure-unfolded state of a protein can be explained in terms of the interaction of hydrophobic groups in water as a function of pressure. A computational study of the potential of mean force of two hard spheres in water as a function of pressure by Hummer et al. showed that as pressure is increased the solvent separated minimum becomes stabilized relative to the contact minimum.[212] This led the authors to suggest a model in which the pressure denatured states of a protein consists of an ensemble in which the hydrophobic core of the protein is disrupted by water molecules that are stabilized between hydrophobic groups in the protein. To test the hypothesis that water will penetrate the hydrophobic core of a protein at high pressures, Collins et al. studied a mutant of T4 lysozyme (L99A), which opens a 160 A^3 cavity in the protein by X-ray crystallography at various pressures.[234,235] It was found that

at 0.1 MPa (1 atm), the cavity was empty, but at pressures of 100 MPa, they observed water density corresponding to a population of half a water molecule in the cavity. At higher pressures, 200 MPa, they found that four molecules occupy the cavity. Therefore, these experiments demonstrated that modest pressure can change the hydration of protein cavities, and this change occurs within a narrow range of pressures, suggesting a cooperative effect. At 200 MPa, T4 lysozyme remains folded.

The susceptibility of T4 lysozyme L99A mutant to pressure is suppressed when the protein hydrophobic cavity is occupied by a benzene molecule.[236] Merski and co-workers determined the structure of eight alkyl benzenes bound to the L99A mutant of T4 lysozyme and pointed out that low-energy excited states of the protein can be able to accommodate increasingly larger ligands.[237] Lerch and co-workers found that under high pressure, there is a small fraction of the population of the protein in which protein side chains fill the hydrophobic cavity.[238] The balance between volume changes and water penetration is fragile. For example, the effect of pressure on T4 lysozyme L99A mutant is suppressed when the protein is encapsulated in reverse miscelles. Nucci and collaborators found that under these confinement conditions, the hydrophobic cavity is preferentially filled with water (as found in crystal structures) rather than driving the system to the unfolded state, which is unfavorable under the nanoscale volume of the reverse micelle.[236] Maeno et al. combined high-pressure NMR, MD simulations, and 3D RISM calculations to conclude that the water containing cavity in the L99A mutant of T4 lysozyme are the source of conformational fluctuations.[239]

Roche et al. have shown that protein cavities are the main source of volume changes upon pressure denaturation of proteins.[211,215–217,240] Solvation affects are also present but are not the dominant contributor to volume changes. MD dynamics simulations on a small protein have been used to characterize the changes in solvation between folded and unfolded states.[241] It has been shown that most of the solvation changes occur around hydrophobic groups, while polar groups do not exhibit significant changes in water density with pressure. The small changes in water density around polar and charged groups occur since the density of water around these groups is already higher at low pressures due to electrostriction.

Molecular dynamics studies of the pressure denaturation of α-helices showed that pressure does not destabilize α-helices,[232,242] yet experimental studies have shown that pressure stabilizes α-helices since they form compact, low-volume conformations.[243–245] This is consistent with the observation of high α-helical content in the unfolded state of proteins at high pressures and room temperature.[214,246]

The kinetics of proteins is also affected by pressure. Brun et al. studied the folding kinetics of mutants of SNase by fluorescence spectroscopy and determined the structural and physical character of the transition state ensemble of the protein.[219] They found a large activation volume for folding, showing that the major effect of pressure is to decrease the folding rate. When the protein occupies the transition state ensemble (TSE), the core of the protein is dehydrated. However, mutants that introduced ionizable groups into the protein core show an increase in the unfolding rate due to a large negative activation volume for

unfolding. This suggests that the effect of pressure on proteins depends on the protein, since a single mutation can result in large variations in the activation volume of proteins.

The role of hydration on protein folding has been studied by molecular dynamics simulation. Umbrella sampling,[247–249] REMD,[250] coarse-grained models,[251] and knowledge-based (structure prediction) models[252] have been used to study the hydration of the protein along the folding progress, as represented by structure-based order parameters. These calculations showed that, in addition to inducing the hydrophobic collapse of the chain, water guides the folding process by forming folding intermediates with hydrated cores. The hydrophobic cores are then dehydrated in the final steps of folding.[251] Predictions obtained for SH3 using a modified minimalist model[251] that used solvent-mediated hydrophobic interactions[253] were confirmed experimentally.[254] The presence of a hydrated hydrophobic core in unfolded states is consistent with pressure having an effect on the folding rate of proteins.[43]

5.3 Characterization of the Unfolded State of Proteins

The influence of the secondary structure on thermal and chemical denatured states has been investigated for different proteins in aqueous solutions: phosphoglycerate kinase (PGK),[255] β-casein,[256] neocarzinostatine (NCS),[257] bovine pancreatic trypsin inhibitor (BPTI),[258] apo-calmodulin,[259] and β-lactoglobulin (βLG).[225] Recently, the heat-denatured states of apo-calmodulin that is a small, mainly a, soluble protein have been studied. Conformational differences between native and thermal denatured states have been detected by using SANS and other biophysical techniques. The results, shown in Figure 10, show that secondary and tertiary structures of apo-calmodulin evolve in a synchronous way, indicating the absence in the unfolding pathway of molten-globule state sufficiently stable to affect transition curves. From SANS experiments, at 85 °C, apo-calmodulin adopts a polymer chain conformation with some residual local structures. After cooling down, apo-calmodulin recovers a compact state, with a secondary structure close to the native one but with a higher radius of gyration and a different tyrosine environment. In fact, on a time scale of a few minutes, heat denaturation of apo-calmodulin is partially reversible, but on a time scale of hours (for SANS experiments), the long exposure to heat lead to nonreversibility due to some chemical perturbation of the protein. Mass spectrometry measurements have permitted evidence of dehydration and deamidation of heated apo-calmodulin.[259]

5.4 Influence of Pressure on the Low-Frequency Dynamics of Lysozyme

The influence of pressure on the low-frequency part (less than 10 meV = 80 cm^{-1}) of the vibrational density of states $g(\omega)$ of lysozyme and water has been investigated by a complementary inelastic neutron scattering (INS) and molecular dynamics simulation study.[260] The experimental and simulated $g(\omega)$ for bulk water and lysozyme are in good agreement. To shed light on the effect of the pressure on the translational and rotational motions of hydration water, the translational and rotational $g(\omega)$ for bulk and hydration water of lysozyme have been computed. Figure 11 shows $g_{\text{translational}}(\omega)$ and $g_{\text{rotational}}(\omega)$ for bulk and lysozyme hydration water at 0.1 MPa (1 atm) and at 600 MPa. The two bands of $g_{\text{translational}}$ close to 6 and 20 meV, called I and II, have been ascribed to the O–O–O bending and O–O stretching motions of hydrogen-bonded water molecules, respectively. The positions of the two bands for lysozyme hydration water are found at frequencies higher

than those of the $g(\omega)$of bulk water by about 1 and 2 meV, respectively. These shifts clearly reflect the stiffening of water motions induced by the protein surface and show that the translational motions of hydration water are hindered compared with those of bulk water. As observed for the $g_{\text{translational}}(\omega)$, pressure induces a broadening and a high frequency shift of the $g_{\text{rotational}}(\omega)$, which suggests a stiffening of the librational motions of water in line with the stiffening of the bending and the stretching motions of hydrogen-bonded water molecules. This effect is also higher for hydration water than for bulk water.

The study also indicates that the structural, dynamical, and vibrational properties of the hydration water of lysozyme are less sensitive to pressure than those of bulk water, thereby evidencing the strong influence of the protein surface on hydration water.

6 Water in (BIO)Molecular Crowded Environments

The structure and dynamics of water under spatial confinement exhibit important deviation from bulk behavior with consequent impact on elementary processes occurring in the aqueous medium. These include among others ion and molecular transport and chemical reactivity. Here below, we focus on confinement involving biomolecules, a special class of soft matter, but it is worth mentioning that interesting issues relate to the behavior of confined water in condensed matter, see refs 261 and 262 and refs therein.

Experiments mainly based on infrared spectroscopy have been extensively used to characterize the water pool in reverse micelles that can be considered a prototype of molecular nanoreactors and confiners. By controlling the nature of the surfactants forming the micelle and the size of the water pool, it was proposed that the main factor affecting the relaxation process of water is a global perturbation due to the reduced space and the local specific interactions among the solvent and the polar heads have a secondary effect.[263,264] Computer simulations have been used to describe the dynamics and structure of the internal pool at molecular level. It was shown that the simplistic spherical model generally assumed to derive the water to surfactant ratio in the experiment does not hold,[265,266] and moreover, the key role of specific interactions at the interface involving H-bond between water and the polar or ionic surfactant head groups in perturbing locally both reorientation and diffusion of water was pointed out.[264,267] Reverse micelles have found a strategic application for investigating the slowdown of water at the interface of proteins too. Hydration dynamics of protein encapsulated in the interior of a reverse micelle is systematically retarded with respect to the dilute solution. This kinetic shift enables NMR technique to access (via probing the nuclear and rotating-frame Overhauser effects) and characterize the motion and location of slow reorienting molecules.[268,269] However, it is yet unclear whether the extract information can be readily transposed to the hydration of a protein in a dilute condition, thus assuming that the confinement acts only as a global scaling of the solvent kinetics or instead specific enclosures of water at the interface between the protein and the micellar interface make visible strong residence sites at the protein surface otherwise quickly washed by interfacial water.[270]

A more complex example of biological confinement is represented by the cytoplasmic space where proteins live. Understanding this realistic environment and the cascade of effects on

the protein functionality is a great challenge for modern biophysics and molecular biology. Moreover, the perturbation of water under confinement could contribute to the specificities of biological activity in vivo. In this spirit, NMR experiments were conducted to monitor the dynamical perturbation of water reorientation in the cytoplasm.[271] Two different cellular models were selected, the mesophilic bacterium *E. coli* and the halophilic archeon *Haloarcula marismortui.* For the 15% of the total water in the cell that is perturbed by contact with the macromolecules, a retardation factor of 15 with respect to bulk is measured. Subsequent experiments were performed on model of bacterial spores.[272,273] These systems can recover metabolic functionality from very long dormant states. This highly resistant "hibernation" is supposed to be induced by a glassy-state of the internal components causing dynamic arrest, see refs 272 and 273 and refs therein. NMR was able to detect a meaningful retardation of internal water, a slowdown with respect to bulk of a factor 30, but the magnitude of this dynamical perturbation cannot induce the freezing of molecular mobility in the interior of the cell that is instead required to explain the associated dormant functionality of proteins. When moving from dilute solution to crowded states, the increasing perturbation of water reorientation with respect to bulk (from a factor 5 up to a factor 30) emerges as a natural consequence of the decreased level of hydration, from 7.5 h (h is the hydration level in units of g H_2O [g dry protein)$^{-1}$] for a protein in water solution down to 0.6 h for the spores, which correlates to an increase of the amount of water confined between macromolecules and less and less exposed to a reservoir of bulky solvent that could ease the mobility via H-bond exchanges.[274] The interprotein length scale for the transition from a dilute solution to a strong slowdown by an order of magnitude with respect to bulk and associated with a more collective process of the water reorganization was estimated to be in the range of 40–50 Å by 2D infrared spectroscopy for lysozyme crowded solutions.[275]

The results from NMR partially conflict with analysis of neutron scattering experiments. In fact, while reporting mild slowdown for water dynamics in the *E. coli* cell in agreement with NMR, a very strong retardation was deduced when the analysis is performed on the halophilic archeon Haloarcula marismortui (Hmm).[276,277] To the best of our knowledge, this contradiction is not resolved. The strong model dependence of the interpretation of neutron scattering experiments is invoked from one side,[272,273] while others explain the discrepancy by stressing that NMR and NS access separately different motions (rotation vs translation) that could decouple in extreme environments.[276,277]

Computer simulations of water in crowded protein solutions have recently appeared in literature. The results are qualitatively in line with experiments. A drastic slowdown is reported when the occupied volume fraction is larger than 30%.[278] The effect of this slowdown is also suggested to act back on the relaxation dynamics of biomolecules themselves. In fact, how solvent-mediated interactions influence the mobility of proteins in cells and similar crowded conditions is essential to understand many functional processes in vivo. Experiments reported that diffusion of biomolecules is about 1 order of magnitude smaller in cell than in a dilute solution.[279,280] This slowdown is monitored at both short (nanosecond) and long (microsecond) time scales. In silico modeling of the cellular environment suggested that this reduced mobility is not just an effect of excluded volume but is essentially caused by solvent-mediated interactions. Skolnick and co-workers[281] clearly demonstrated that only by including hydrodynamics interactions in water-free

coarse-grained simulations of proteins (rigid spheres) in a celllike environment the slowdown with respect to the dilute solution is as large as in experiments. The challenge to simulate realistically the cellular environment has been undertaken recently by several groups (sees ref 282 and refs therein). For example McGuffee and Elcock were the first to present a massive simulation of a portion of the cytoplasm of *E. coli*.[283] Unfortunately because of the size of the system, the essential hydrodynamic interactions were not included. A novel promising multiscale algorithm based on the Lattice Boltzmann methodology has been recently implemented to deal with this problem; as proof of concept, the method was delivered to simulate a system of huge size (about 18000 proteins) at quasiatomistic resolution and including hydrodynamics. This allowed effectively for the investigation of the role of solvent-mediated interactions on protein mobility.[284]

7 Water in Membrane Channels

Water mobility adjacent to proteinaceous surfaces is commonly assessed by spectroscopic means. For example, neutron scattering studies found the surface water molecules on fully deuterated GFP molecules less mobile than bulk water molecules. Measuring the water flux through proteinaceous pores represents an alternative way of obtaining the mobility of interfacial water molecules. The most interesting insight comes from studies of pores that are too narrow to let two water molecules overtake each other. A variety of biological important membrane channels belong to this class: water channel proteins (aquaporins, AQPs), excitatory sodium and potassium channels and their bacterial ancestors (KcsA and KvAP), and some antibiotics like gramicidin-A channels.

The extreme narrowness of the channels has profound implications on hydrodynamics: we may not expect a nonslip boundary layer of water molecules that adheres to the channel wall, since there would be no flow if the nonslip boundary layer were to exist. The flow velocity cannot increase from the channel wall toward the channel center as it does in wider channels (i.e., there is no way to observe a parabolic streaming profile across a single water molecule that is in contact with the channel wall along its perimeter, shown in Figure 12). Channel length and diameter cannot play the role of determinants for fluid flow as they do in macroscopic hydrodynamics (Hagen–Poiseuille's law) because the unitary water permeability, p_f, of single-file channels varies by several orders of magnitude, even though (i) the length of these channels can only vary within the limits of membrane thickness and (ii) its diameter is roughly fixed to that of one water molecule.[285]

Single-file flow through channels are diffusional in nature.[286] Thus, it is governed by the interactions between the water molecules and the channel wall.[287] Variations in channel geometry may additionally contribute to the variability in p_f [288] but only to a minor extent.

p_f is Linked to the Water Diffusion Coefficient within the Pore

p_f is defined by the volume of water that a narrow channel conducts per unit of time in response to an osmotic pressure difference:

$$\Phi_w = -p_f \Delta c_s \quad (1)$$

where Φ_w and Δc_s are the osmotic water flux (in mole per second) and the difference in impermeant solute concentration in the two solutions at both channel ends (in mole per unit volume). With the assumption that the water densities inside and outside of the channel are equal to each other, Φ_w can also be expressed as the product of the average linear drift velocity, v, and water concentration, c_w:[286]

$$\Phi_w = -Ac_w v = -Ac_w \frac{F}{\gamma} \quad (2)$$

where A, F, and γ are the channel cross section, the force acting on an individual water molecule, and the molecular friction coefficient, respectively. Eq 2 can be rewritten since $F = A\Pi = A\Delta c_s N_A k_b T$, where $A = v_w/z$:

$$\Phi_w = -\frac{v_w}{z} c_w \frac{v_w}{z} \Delta c_s \frac{N_A k_b T}{\gamma} = -\left[\frac{v_w}{z}\right]^2 \frac{1}{N_A v_w} \Delta c_s N_A D_w \quad (3)$$

where D_w, N_A, and z and are the diffusion constant of the water molecules within the channel, Avogadro's number, and the distance between water molecules, respectively. Eqs 1 and 3 combine to give[289]

$$D_w = \frac{z^2 p_f}{v_w} \quad (4)$$

Eq 4 corresponds well with $p_f = v_w k_0$ [290,291] or $p_f = 1/2\Phi_0 v_w$,[292] where k_0 and Φ_0 are defined as the rate by which all single-file water molecules shift by the separation distance between two neighboring water molecules or the intrinsic flux, respectively.

7.1 Mobility of Single-File Water Molecules as Judged from p_f Values

To visualize single-file water diffusion, molecular dynamics simulations have been employed. Bulk water models[293,294] are commonly used since the calculated p_f values do not deviate much from experimental ones; however, it is not really clear to which extent these models reflect the physical and chemical properties of confined water.[295] For example, p_f of the human red cell water channel protein, aquaporin 1 (AQP1), varied in silico between 0.7 and 1×10^{-13} cm^3 s^{-1}.[293,294] Part of these data are in reasonable agreement with recent experiments in which p_f was found to be 5×10^{-13} cm^3 s^{-1}.[287] Thus, they confirm the observation that confinement of water to subnanometer films does not grossly alter its fluidity.[296]

In accordance with eq 4, the p_f of 5×10^{-13} cm^3 s^{-1} indicates that water molecules retain bulk water mobility. Water rushes through the slightly wider bacterial glycerol facilitator GlpF even faster as indicated by its p_f of 19×10^{-13} cm^3 s^{-1}.[287] That is, within the shorter single-file region of GlpF, water molecules attain a mobility that is slightly above that of bulk water molecules.

7.2 Energetics of Single-File Water Transport

When water enters the pore, it is partially dehydrated because every molecule may retain no more than two of the four hydrogen bonds that it forms with neighboring water molecules. Usually only a fraction of the lost energy can be recovered through interactions with pore-lining residues. In the extreme case of water conduction through the hydrophobic channel of a carbon nanotube, there is no replacement for the lost hydrogen bonds. The carbon atoms of the nanotube only allow for van der Waals interactions, which recover no more than 4 kcal mol^{-1} of the 10 kcal mol^{-1} entrance penalty in silico.[262,297]

The energetics of water passage through gramicidin-like peptidic pores is different. Hydrogen bonds may form between the single-file waters and the backbone of amino acids lining the pore.[298] Although the backbone carbonyls may act as surrogates for the waters of hydration, the rate at which water can transform its hydration environment from that of the channel exterior to that of the interior was identified as the major source of the resistance to water permeation.[298] Molecular dynamics simulations of water transport through the pore of a D,L-polyalanine helix revealed an access barrier of 3.6 $k_B T$ (or 2.1 kcal mol^{-1}). Since the calculated energy for movements between binding sites within the pore was on the order of $k_B T$,[289] the access barrier appeared to dominate the whole transport process. It has however remained unclear why the smaller transport barrier did not translate into a higher p_f of the peptidic channels: p_f was more than an order of magnitude smaller than that calculated for carbon nanotubes.[289,297] Systematically varying the polarity of the D,L-polyalanine helix with the gramicidin A fold did not solve the conundrum: the access penalty was always larger than the barrier for permeating the pore.[299] Transient channel occlusions by lipid head-groups[300] explained part of the phenomenon: gramicidin acetylation increased p_f by sterically preventing pore occlusion.[301]

Yet, even without pore occlusion, water permeation through the peptidic pores remained slower than through carbon nanotubes. The experimentally found dependence of p_f on channel length[302] provided the first clue for a better understanding of the permeation process. p_f rapidly decreases with increasing channel length because the number of backbone carbonyls which may form hydrogen bonds with the single-file waters increases. Similarly, an increase in the total number of hydrogen bonds (N_h) that any of the single-file water molecules may form with pore-lining residues while traversing aquaporins decreases their p_f.[287] In order to obtain that result, the accuracy of p_f measurements was improved by (i) minimizing unstirred layer effects due protein reconstitution into vesicles that were not larger than 120 nm, (ii) precisely counting the number of reconstituted proteins per proteoliposome using both fluorescence correlation spectroscopy and high-speed atomic force microscopy, and (iii) by a new adaptation of the Rayleigh-Gans-Debye equation to follow the kinetics of vesicle shrinkage from the intensity of scattered light. The bacterial potassium channel KcsA, which may also conduct water[290,303] falls into the same logarithmic dependency between N_h and p_f (Figure 13).

It is not entirely clear as to why the dependency should be logarithmic. Assuming that the breaking and forming of a hydrogen bond occurs at a constant rate would rather lead to a linear dependence. Strictly speaking, this is only true if there were a lone water molecule in the pore that may have formed just one bond at a time. If, however, a whole column of water

molecules was moving in a concerted fashion, all of the singlefile waters would have had to break their hydrogen bonds with pore-lining residues at the same time. Even if such an event was to happen, neighboring water binding sites may be situated very close to each other, so that there is only slight progress (if any) after bond reformation. For example, yeast aquaporin-1 offers four water positions in the selectivity filter that are too closely spaced to be simultaneously occupied.[304] Thus, it seems to be the joint multiplicity of all the binding options of all singlewater molecules taken together that result in the logarithmic dependence of p_f on N_H, since every one of them is capable of simultaneously forming four hydrogen bonds.

7.3 Channel Geometry

Apart from the gating peculiarities of individual water channels, the opening angle of the pore is also predicted to affect p_f. The calculated p_f was lowest for channels with cylinder geometry and highest for channels with an opening angle that matched that of the hourglass shape of aquaporins.[288] The single-file movement inside the central part of the AQP was not captured in these calculations at all, since a continuum description was used. That is, the single-file movement was assumed to occur free of any resistance.

Experimental support for this oversimplified view has yet to be obtained. Figure 13 compares channels with cylindrical geometry (peptidic pores: gramicidin, minigramicidin, and midigramicidin), hourglass symmetry (aquaporin-1, GlpF, and AqpZ), and channels (KcsA) with a cylindrical entrance on one side and a vestibule on the other. They all fit into the logarithmic dependence of p_f on N_H, suggesting that the main resistance has to be attributed to the narrowest region (constriction site) and not to the much wider entrance regions. Nevertheless, the shorter cylindrical peptidic pores (minigramicidin) are likely to have a smaller hydrophobic thickness than the surrounding lipid so that an hourglass-shaped lipidic entrance may have formed, which could be responsible for the increment in p_f above the regression line (Figure 13). That is, pore geometry may well act to modulate the p_f value which is chiefly determined by N_H.

7.4 Hydrophobic Gating

There are many cases in which the X-ray structures of supposedly closed conformations of ion channels and receptors do not show a fully occluded constriction site. Although the constriction site is wide enough to accommodate the fully hydrated ion, the space remains empty. Exclusion of ions from the closed conformation is hypothesized to be achieved by hydrophobic gating.[306] Molecular dynamics simulations visualize the process as liquid vapor oscillations. That is, water cannot permanently occupy the narrow constriction zone due to its increased hydro-phobicity.[307] Examples have been provided by molecular dynamics simulations of the small mechanosensitive channel MscS[308] and the acetylcholine receptor.[307] Upon activation, pore widening gradually increases the phases of capillary condensation until the pore is filled with water molecules for longer periods. At that stage even ions can enter it. Interestingly, liquid vapor oscillations do not preclude water conductance: during burst-like periods of water conductance, the water diffusion coefficient increases up to 5-fold.[297,307]

An alternative explanation for hydrophobic gating is that corresponding X-ray structures capture an intermediate channel conformation that differs from the truly closed state. For example, the narrowest site of the acetylcholine receptor was found to be too constricting for a sodium or potassium ion to pass through while retaining its first hydration shell.[309] Since substitutes for the water of hydration are missing at the constriction site, the ion cannot readily lose part of its hydration shell.

8 Conclusions

We have provided a review of the recent literature describing the dynamics of water when coupled to proteins and how the dynamics of proteins is affected by the solvent dynamics. Given the extent of the field, we did not cover all the aspects related to biomolecular dynamics and hydration. However, we presented a perspective of the use of X-ray and neutron scattering and diffraction, NMR, terahertz spectroscopy, and molecular simulations. This is a growing field in which the sensitivity of the molecular probes is improving and the MD simulations are starting to provide insights in the time scale covered by the experiments with improved accuracy. We expect that the understanding of the role of water in protein stability, dynamics, and function will grow over the next decade.

Acknowledgments

This work was supported by the National Science Foundation in the USA (MCB-1050966 to AEG) and the Austrian Science Fund (FWF, Grant P23679 to P.P.). F.S. acknowledges funding from the European Research Council (ERC) under the European Community's Seventh Framework Programme (FP7/2007–2013), Grant 258748. A.E.G. acknowledges support from Los Alamos National Laboratory LDRD funds. This review was initiated during the NORDITA (Nordic Institute for Theoretical Physics) scientific program "Water - the Most Anomalous Liquid". Additional financial support for this program was provided by the Royal Swedish Academy of Sciences through its Nobel Institutes for Physics and Chemistry, by the Swedish Research Council, and by the Department of Physics at Stockholm University.

Biographies

Marie-Claire Bellissent-Funel received her degree in Physics from the University of Paris and her Thesis of Doctor ès Sciences Physiques from the University of Grenoble (France) in 1977. She taught at the University of Grenoble before joining the Centre National de la Recherche Scientifique (CNRS). Presently, she holds the position of a Director of Research Emeritus at the Laboratoire Léon Brillouin where she was in charge of the research group centered on biology and disorderd systems. She has been elected a Fellow of the Institute of Physics (UK) and granted the title of Chartered Physicist. From the International Academy of Lutèce, she received in 2006 the "Grand Prix" of Sciences for her novel work on water in confined media. Her current research interests are water related and use different experimental (neutron and x-ray scattering, biophysical techniques) and modelling approaches. They include the structural and dynamic properties of bulk water in various conditions of temperature and pressure, the properties of water in confinement, the role of water in the stability and function of biological macromolecules, and the study of thermal and pressure denatured states of proteins.

Ali Hassanali was born in Tanzania and grew up in Kenya where he attended high school. He then moved to the US where he obtained his undergraduate and Master's degree at

Purdue University. He then moved to OSU (The Ohio State University) for his Ph.D. in Biophysics working with Sherwin Singerand and Dongping Zhong. After a three year postdoc with Michele Parrinello at ETHZ & University of Lugano, he moved to the International Center for Theoretical Physics (ICTP) in Trieste, Italy, where he is currently a Junior Research Scientist in the Condensed Matter and Statistical Physics and Quantitative Life Sciences sections. His research interests are focused in understanding the mechanisms by which fundamental processes like water autoionization & proton transfer occur in bulk water as well as water near interfaces.

Martina Havenith studied physics and mathematics at the university in Bonn, Germany. During her graduate and postdoc time she worked in Bonn and at UC Berkeley and Radboud University in Nijmegen studying the high-resolution spectra of molecular cluster in the FIR and IR range. She received a Heisenberg grant of the German Science Foundation. Since 1998, she has held a chair in physical chemistry at the Ruhr University Bochum. She is a member of the Leopoldina, the German Academy of Sciences. In 2014, she received the Miller Professorship of UC Berkeley. Her research is focused on studying solvation of small solutes and biomolecules on a molecular level using laser spectroscopic techniques. She is the director of the Center of the Molecular Spectroscopy and Simulation of Solvent Driven Processes in Bochum.

Richard Henchman graduated from the University of Sydney with a B.Sc. Honours degree in 1996, where he modelled diffusion in glassy polymers and examined the thermodynamics and crystallization of supercooled water. He obtained his Ph.D. from the University of Southampton in 2000 for studying the selectivity of molecular binding using free energy methods. During his postdoctoral research at the University of California, San Diego, from 2000–2004, he studied biomolecular hydration, binding, and function, and discovered how to calculate the entropy of a liquid by combining cell theory and computer simulation. He then moved to the University of Manchester as Lecturer and Senior Lecturer where he generalizes his liquid-state entropy theory to complex molecular systems.

Peter Pohl obtained his diploma in Biophysics at the Piragov Institute Moscow in 1989 and his M.D. at the Martin Luther University Halle (Saale) in 1994. After having completed his habilitation in 2001, he joined the Leibniz Institute of Molecular Pharmacology in Berlin as Heisenberg fellow of the Deutsche Forschungsgemeinschaft. He then became a guest professor at the Institute of Biology of the Humboldt University Berlin in 2002–2003 and was appointed full Professor of Biophysics in 2004 at the Physics Department of the Johannes Kepler University Linz. His research focuses on membrane transport of water by aquaporins, ion channels, and cotransporters; proton migration along membranes; protein translocation through membranes; and the coupling of membrane leaflets.

Fabio Sterpone is currently a researcher at the CNRS, France. He graduated from the University of Paris UPMC (biophysics) and then occupied several postdoctoral positions in which he dealt with quantum classical simulations of materials and the effect of solvent on biomolecular structure and dynamics. Presently, he is mainly interested in the study of protein stability and aggregation in extreme environments by applying and developing multiscale simulation methodologies.

David van der Spoel studied engineering physics in Groningen, The Netherlands, where he proceeded to obtain a Ph.D. in computational chemistry with Herman Berendsen. During his Ph.D. work, he was part of the team that developed the GROMACS software suite for molecular simulations, a project in which he is still involved. After his Ph.D., he moved to the university of Uppsala, Sweden, where he has been a professor of Computational Molecular Biophysics since 2008. His research focuses on force field and methods development for molecular simulations and applications of simulations ranging from molecular liquids to large biomolecular systems like viruses.

Yao Xu earned his B.Sc. in Applied Chemistry from Beijing University of Chemical Technology in P. R. China in 2006. He joined the doctoral program in Chemical Physics at the University of Nevada, Reno, Nevada, where he obtained his Ph.D. under the supervision of Prof. David M. Leitner on the topic of solvation dynamics at the interface of proteins in 2013. Now he is a postdoctoral researcher with Prof. Martina Havenith at Ruhr University Bochum, Bochum, Germany. He is interested in connecting solvation dynamics to biomolecular functions by molecular simulation and laser spectroscopy.

Angel E. Garcia earned his B.Sc. in Physics from the University of Puerto Rico and a Ph.D. in Theoretical Physics from Cornell University. During 1987–1989, he was a postdoctoral fellow at Los Alamos National Laboratory. From 1989–2005, he was a staff member at the Theoretical Biology and Biophysics Group at Los Alamos. From 2005–2015, he was a chaired Professor of Physics at Rensselaer Polytechnic Institute. Since 2015, he has been the Director of the Center for Nonlinear Studies (CNLS) at Los Alamos National Laboratory. He is a fellow of the American Physical Society and the Biophysical Society. His research interests are in protein folding and dynamics, membrane proteins, and nucleic acids.

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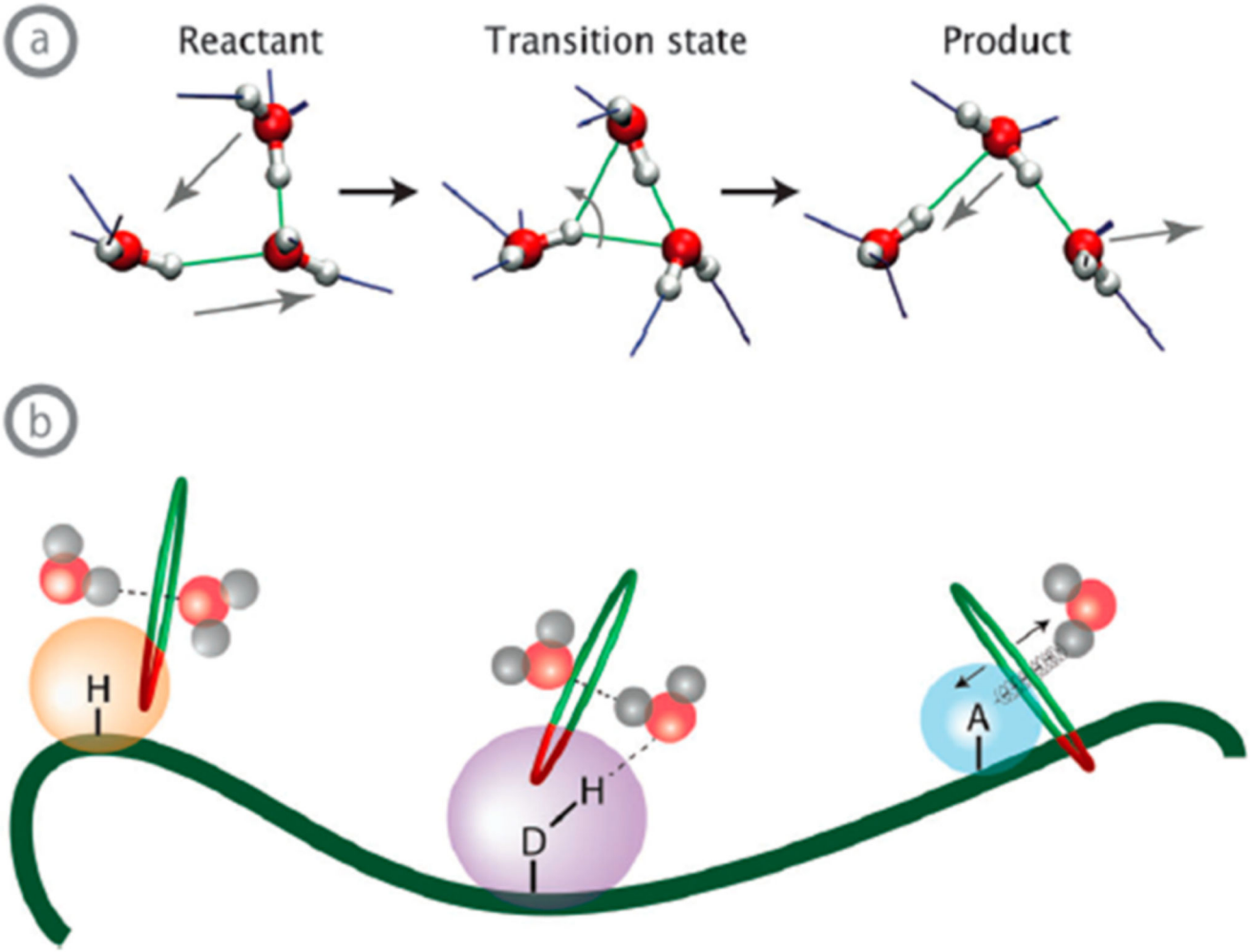

Figure 1.

(a) Molecular jump mechanism. (b) Schematic figure with a protein interface and the three types of sites, respectively hydrophobic, H-bond donor, and H-bond acceptor, together with a pictorial representation of the types of perturbation they induce on water dynamics (excluded volume and H-bond strength factors). This figure is reproduced with permission from ref 36. Copyright 2013 Royal Society of Chemistry.

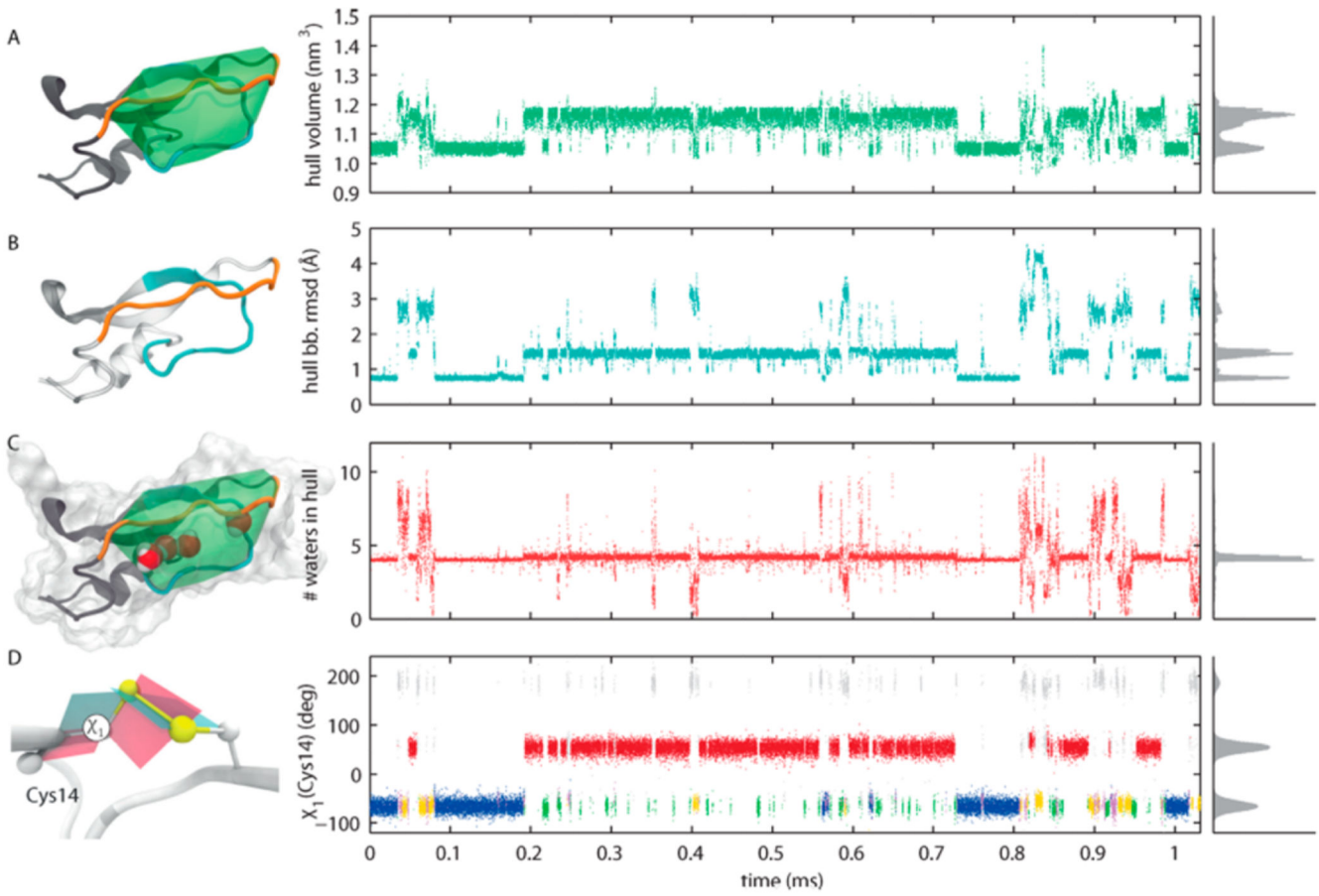

Figure 2.

Fluctuations in the interloop region during 1 ms. (A) Volume of the convex hull spanning the four internal hydration sites. (B) Backbone RMSD, relative to crystal structure 5pti, for the residues defining the hull. (C) Number of water molecules inside the hull. (D) Dihedral angle $\chi 1$(C14), with disulfide isomeric states color-coded: M1 (blue), M2 (magenta), M3 (yellow), mC14 (red), mC38 (green), and other (gray). The time series values plotted here are either averaged over consecutive 25 ns windows (A–C) or sampled with 25 ns resolution (D). The full-trajectory distributions are projected on the right-hand axis. This figure is reprinted from ref 51. Copyright 2013 American Chemical Society.

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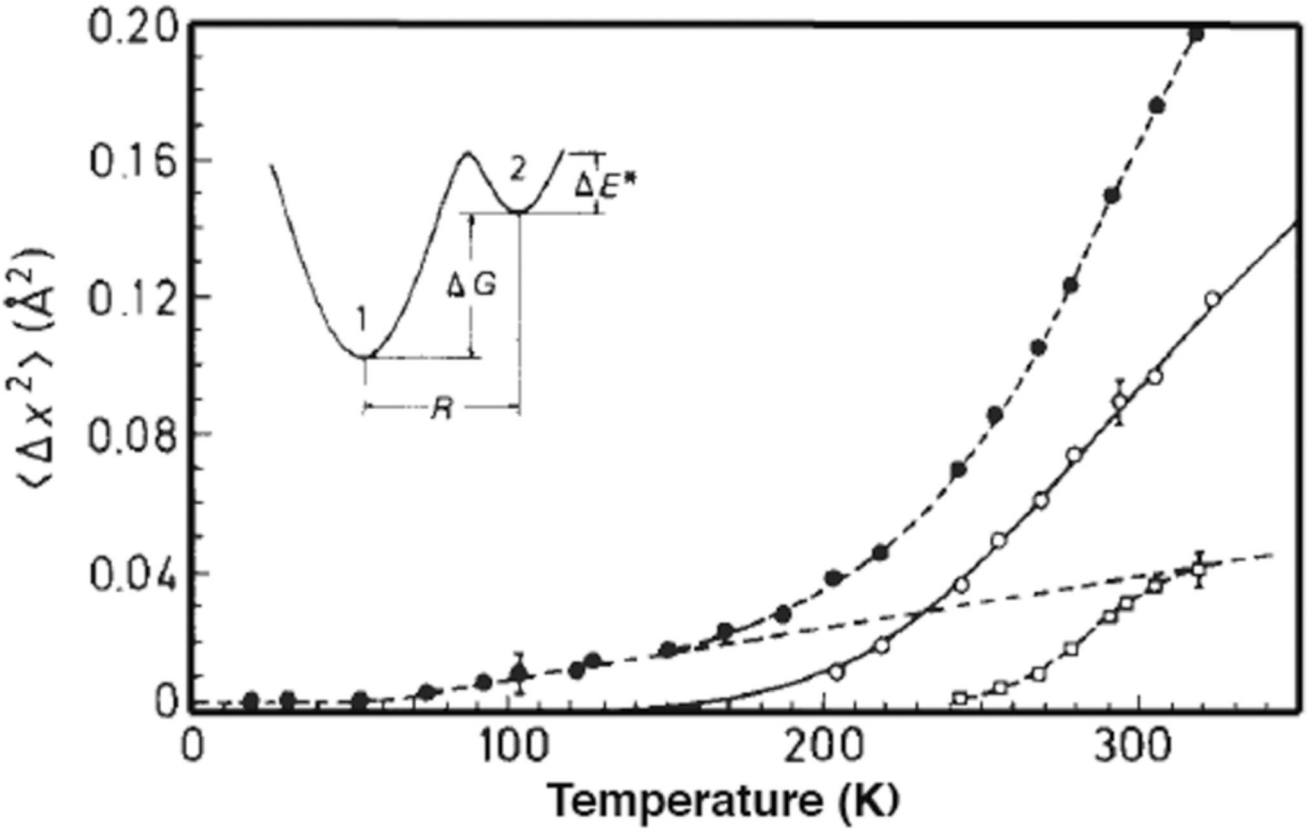

Figure 3.

Mean square displacements in dry and wet myoglobin.[68] The sharp increase in mean square displacements at around 200 K for the hydrated sample is absent in the dehydrated sample. Reproduced with permission from ref 68. Copyright 1989 Macmillan Publishers Ltd., Nature.

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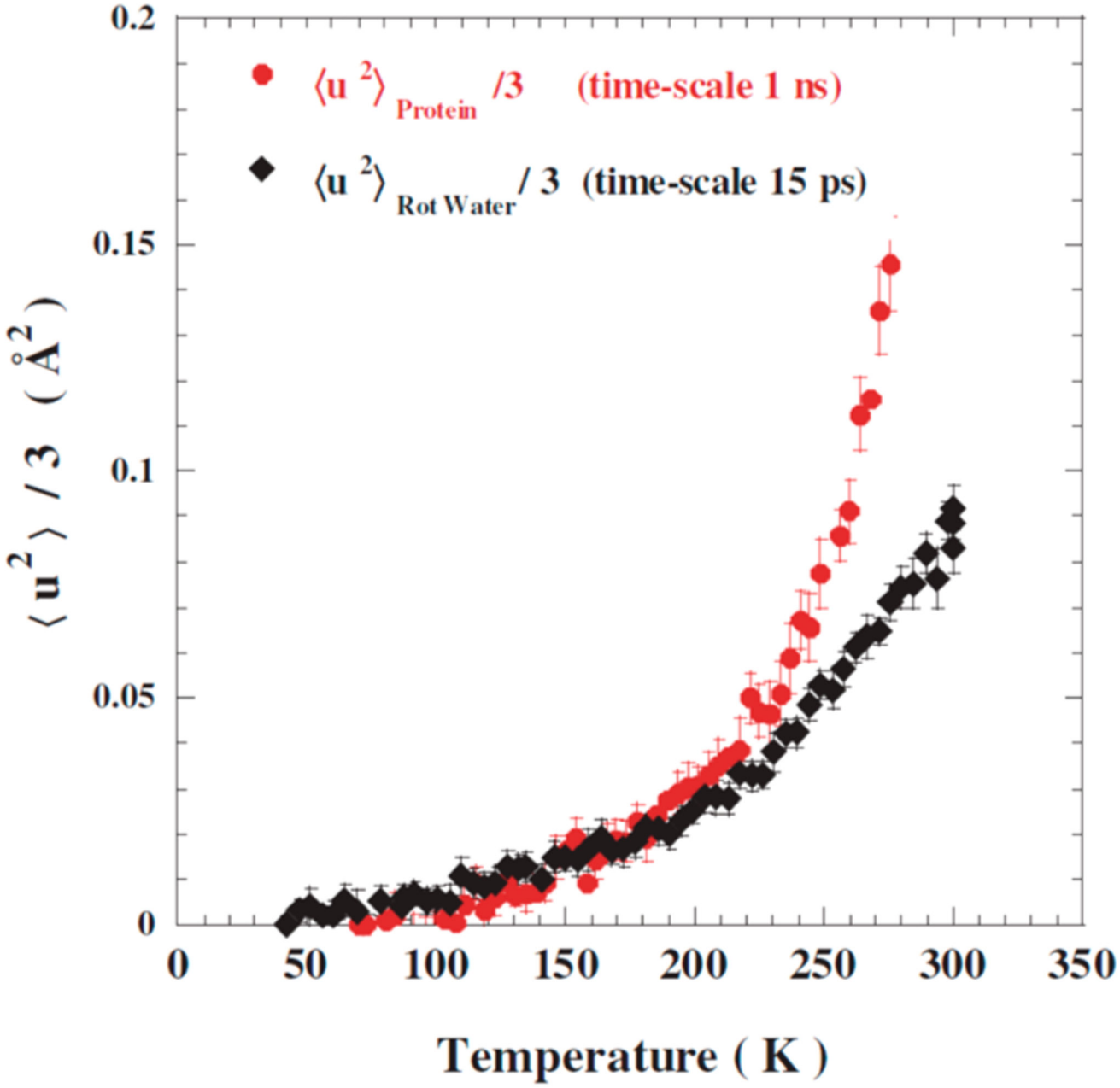

Figure 4.

Temperature dependence if the hydrogen atom mean-square displacement of lysozyme hydrated with a monolayer of D2O. Zanotti et al.[85] observed a strong correlation between the local reorientational transition observed in water at 220 K, $\langle u^2_{\text{rot water}} \rangle$, and the onset of the long time (1 ns) large amplitude overdamped motions responsible for the $\langle u^2_{\text{protein}} \rangle$ to increase above 220 K. The correlation between protein dynamical crossovers at 150 and 220 K and interfacial water rotational dynamics (no correlation with translational dynamics): it was suggested that the sole hydration water rotational dynamics trigger the protein dynamics. This figure is reproduced with permission from ref 85. Copyright 2007 Springer.

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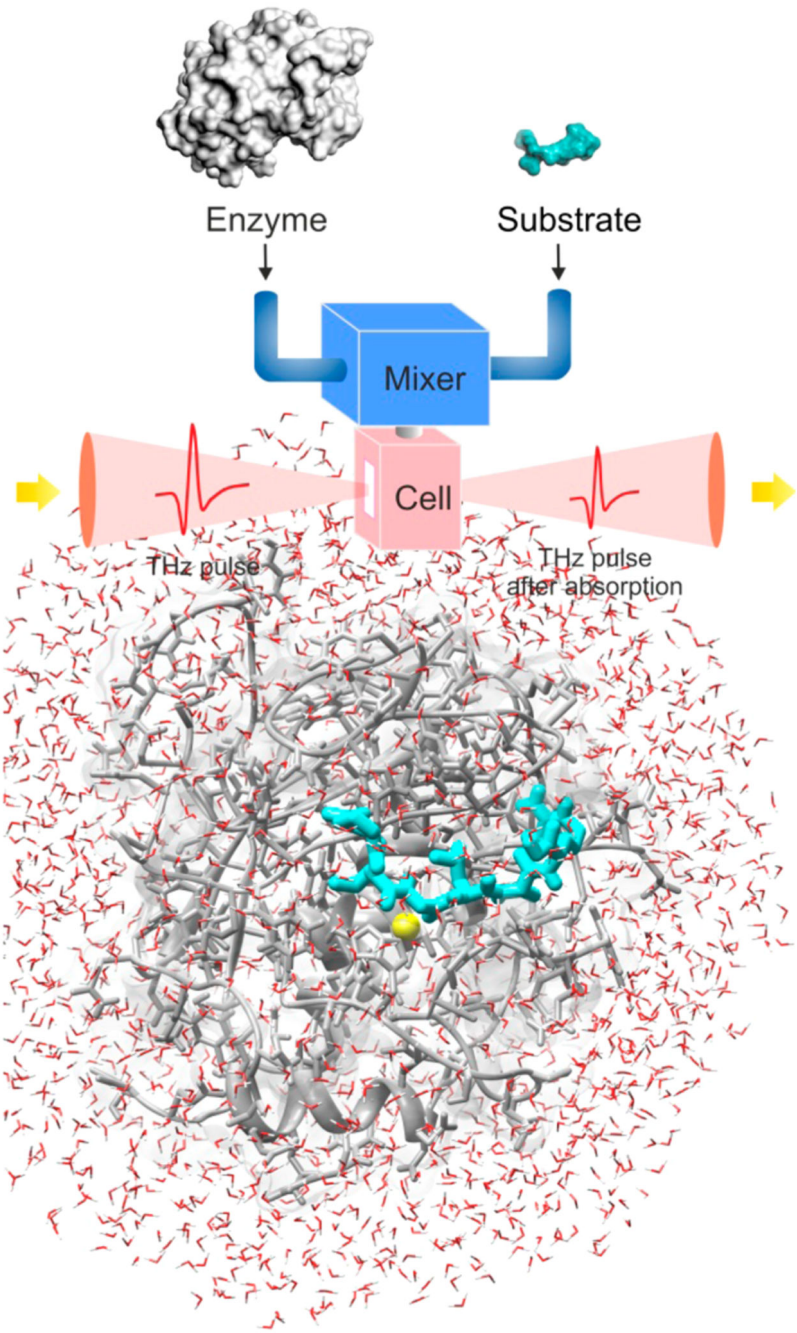

Figure 5.
A schematic view of the kinetic absorption spectroscopy setup.

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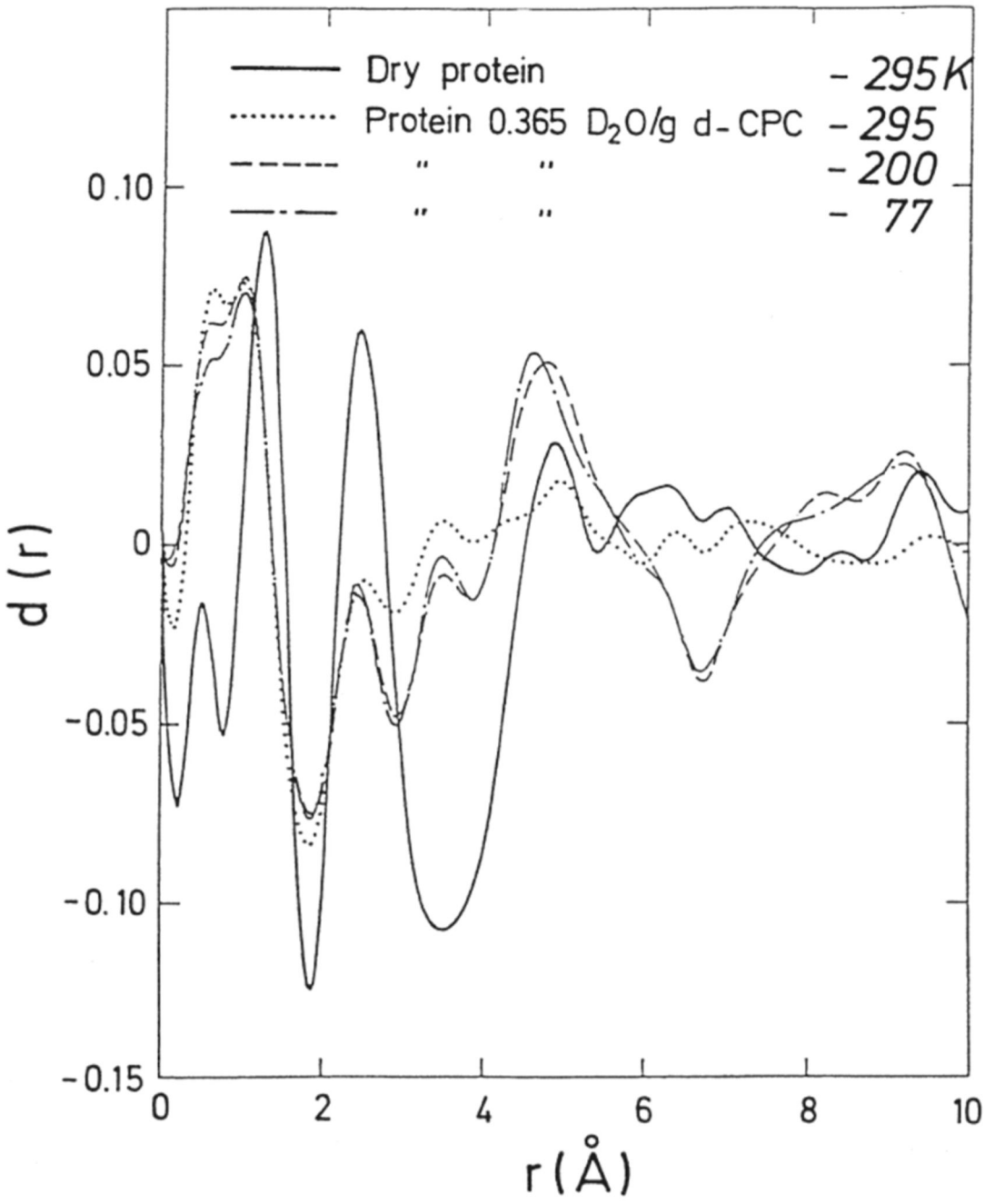

Figure 6.

Pair correlation function d(r) for a dry deuterated C-phycocyanin(d-CPC) protein at 295 K and for a D_2O-hydrated (h = 0.365) d-CPC protein at different temperatures. This figure is reproduced with permission from ref 157. Copyright 1993 Elsevier.

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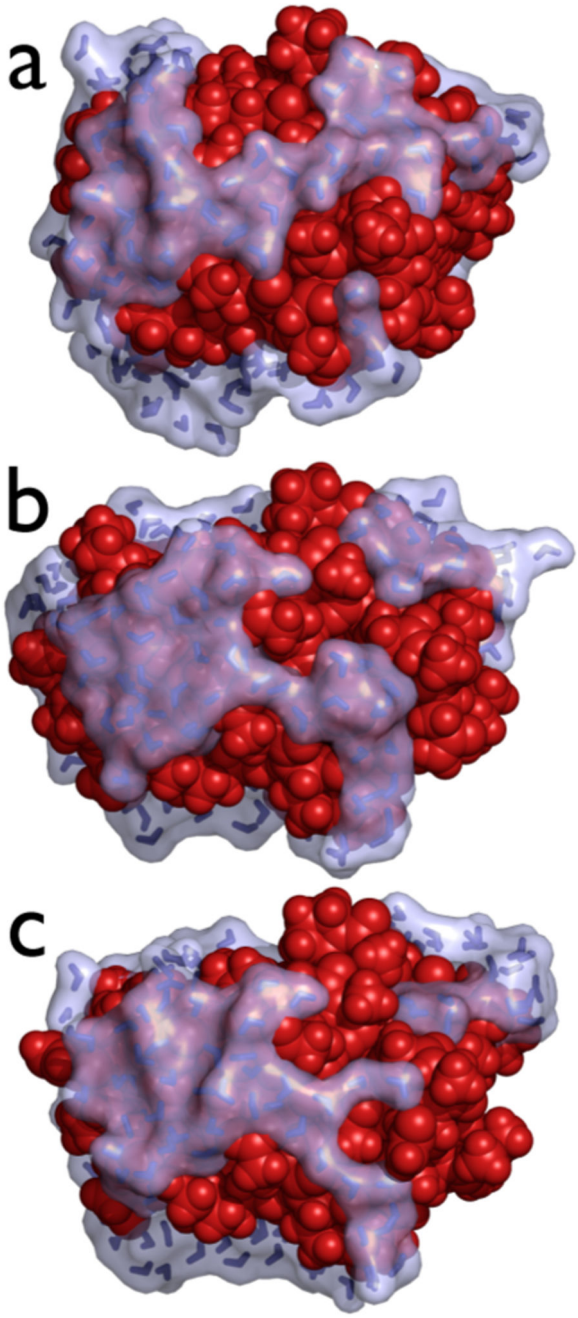

Figure 7.
Structures resulting from three replicate simulations of the C-terminal fragment of L7/L12 ribosomal protein showing that solvation (blue, transparent) of the protein is quite reproducible. Figure adapted with permission from ref 185. Copyright 2009 Royal Society of Chemistry.

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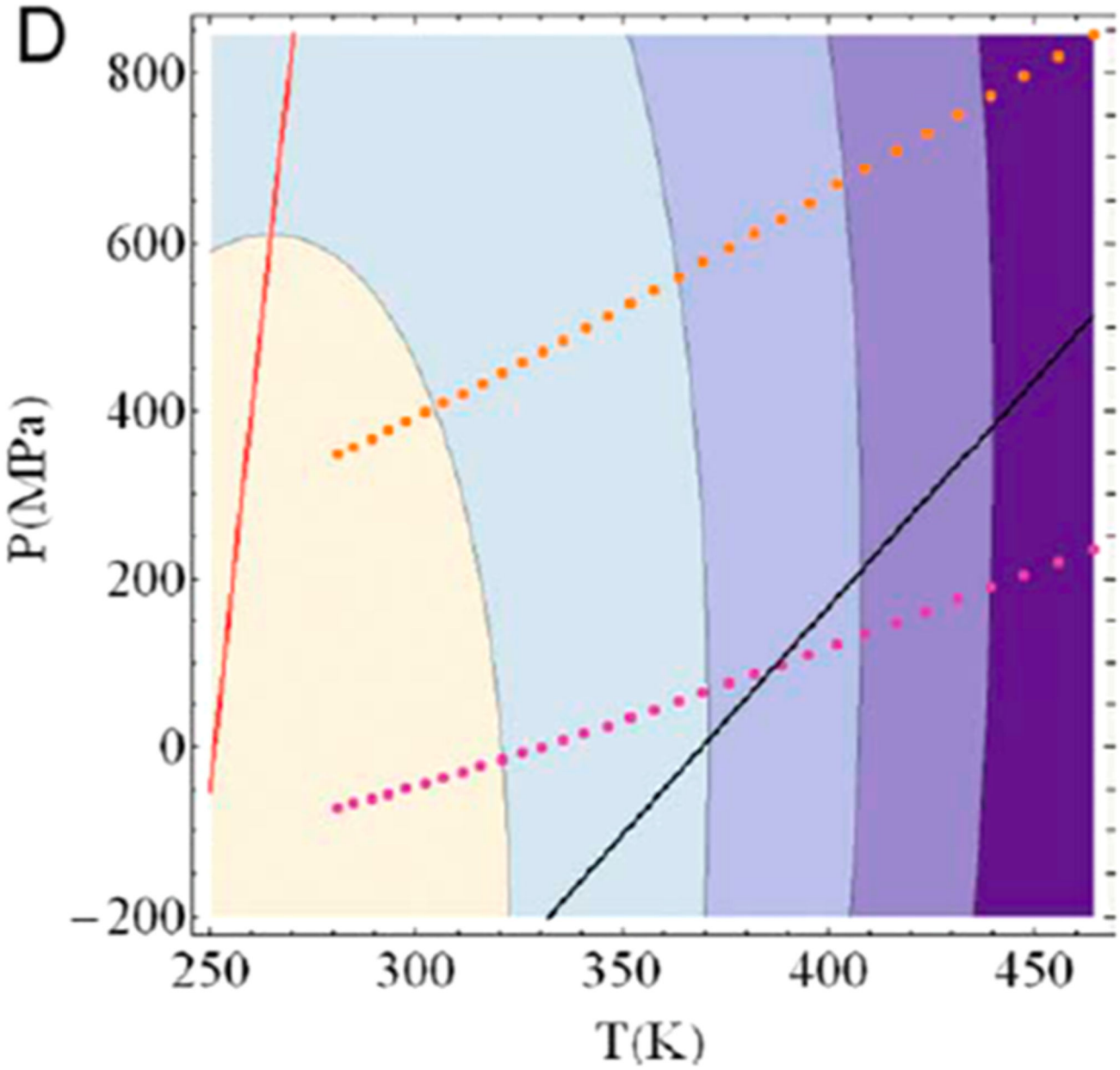

Figure 8.

Calculated *P*–*T* stability diagram for the Trp-cage miniprotein shows an elliptical shape that predicts pressure and cold denaturation at high pressures. The $\Delta G = 0$ curve is the innermost contour. Other contours show $\Delta G = -15$, −10, and 15 kJ mol^{-1}. The dotted lines show the thermodynamic states sampled in two independent REMD simulations. The black line shows the $\Delta V = 0$ isochore; the solid red line shows the $\Delta S = 0$ isentrope. This figure is reproduced with permission from ref 228. Copyright 2010 Wiley-Liss, Inc.

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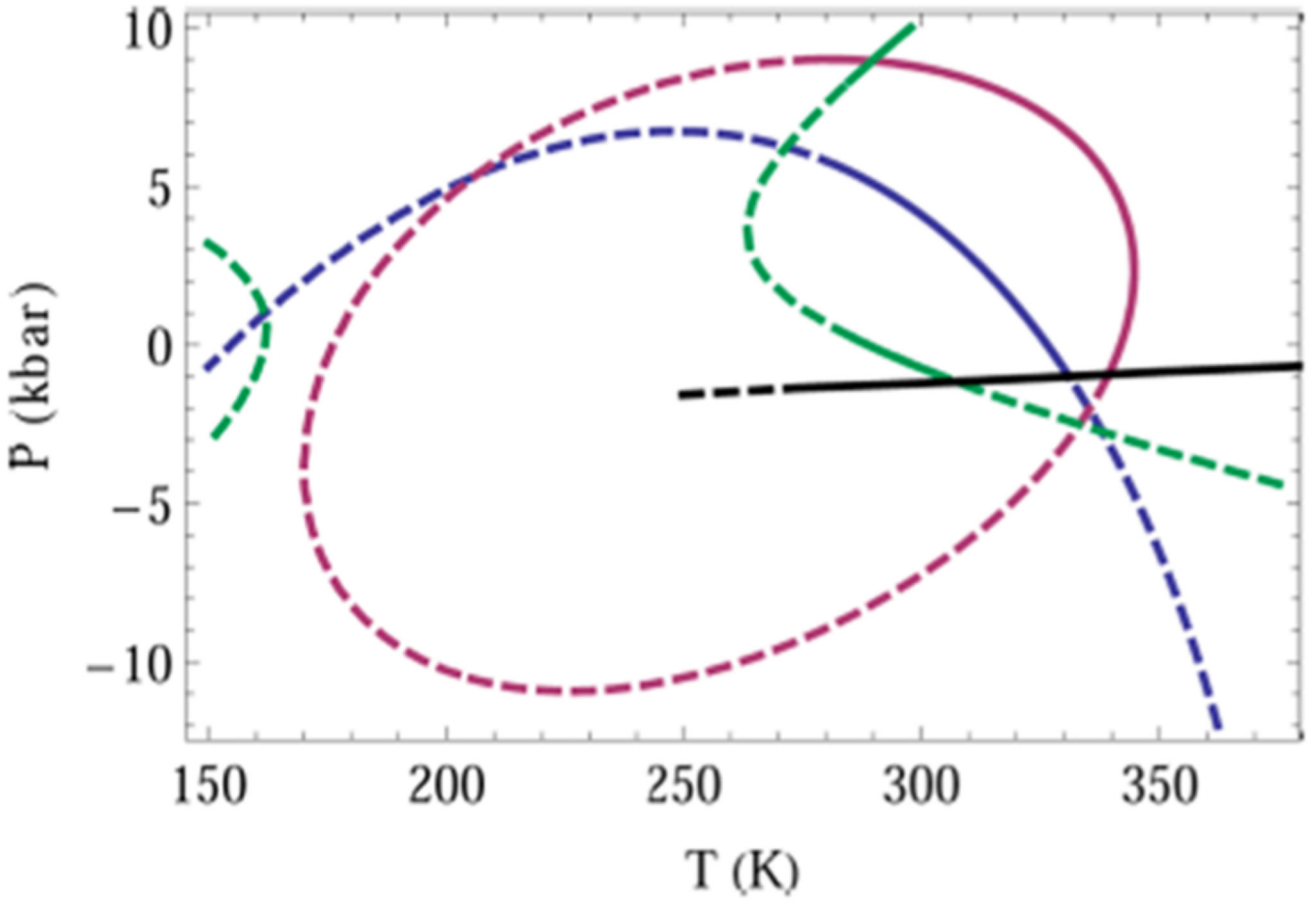

Figure 9.

Calculated *P*–*T* diagram for a small protein and peptides. The colored lines are the loci of the 0.5 fraction folded for the Trp-cage (magenta), 0.5 fraction folded for the GB1 β-hairpin (blue), and 0.25 fraction folded for the AK16 (green) α helix. Dashed lines are in the vicinity of the simulation data. The back line is a numerical estimate of the spinoidal of the bulk solution. The figure and caption are reprinted from ref 232. Copyright 2014 American Chemical Society.

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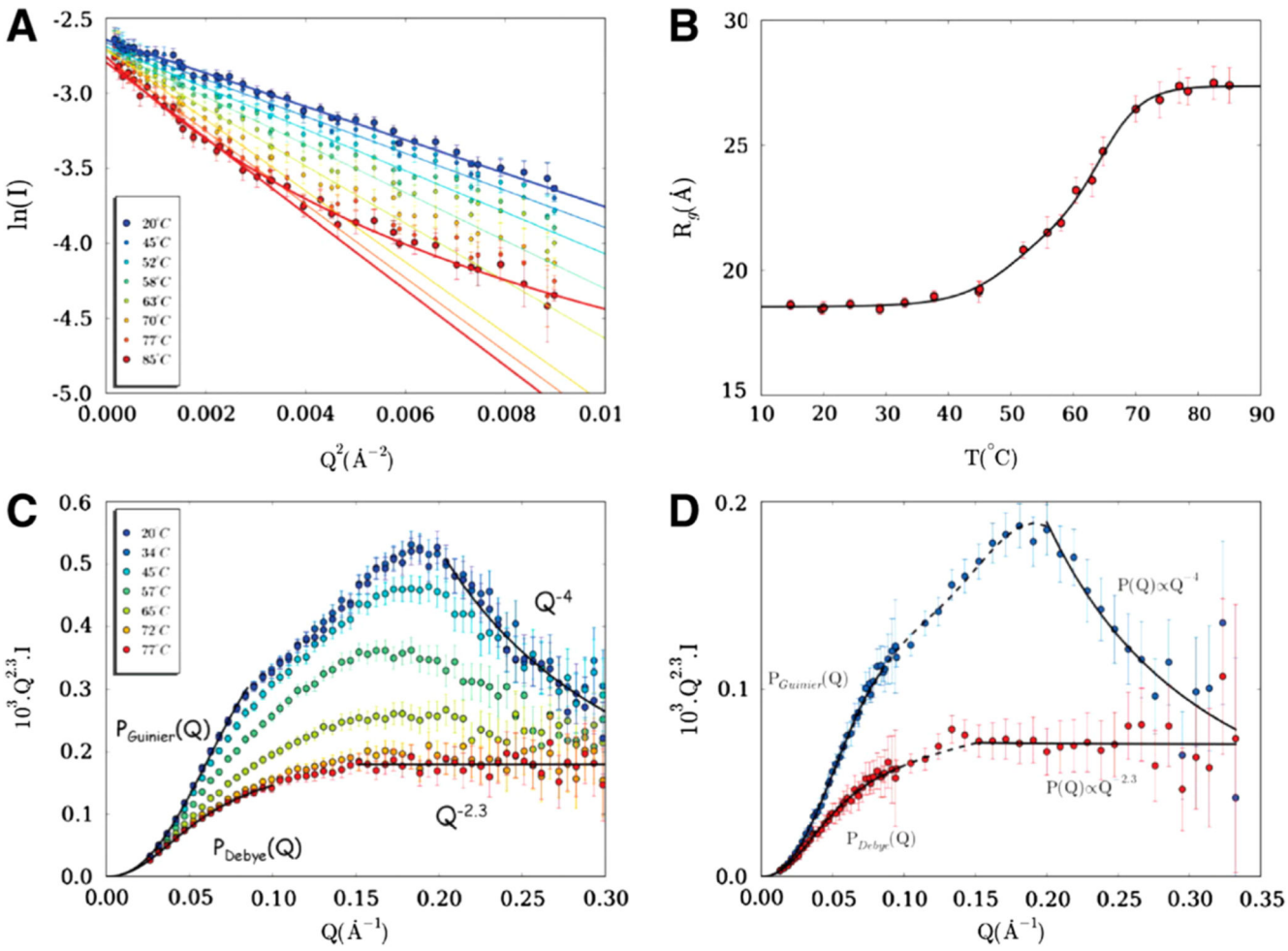

Figure 10.

(A) Guinier representation [ln I = f(Q2)] of SANS spectra of apo-calmodulin at 5 g/L for several temperatures, between 20 °C (in blue) and 85 °C (in red). Straight full lines are fits using Guinier laws leading to radius of gyration. The red curved full line is a fit of SANS spectra of apo-calmodulin at 85 °C, using the Debye law. (B) Radius of gyration, R_g, of apo-calmodulin in D_2O at 5 g/L as a function of temperature. In black is the fit with a three-state transition model, with the same thermodynamic parameters as for circular dichroism. (C) Adapted Kratky [Q2.3.I(Q) = f(Q)] representation of SANS spectra at 18 g/L of apo-calmodulin at several temperatures between 20 °C (in blue) and 77 °C (in red). (D) Adapted Kratky [Q2.3. I(Q) = f(Q)] representation of SANS spectra at 5 g/L of apo calmodulin at 20 °C (in blue) and 85 °C (in red). The samples are solutions of apo-calmodulin in a 50 mM D2O Tris buffer, pD = 7.6 with 80 mM KCl and 500 μM EDTA. The figure and caption are reproduced with permission from ref 259. Copyright 2012 Elsevier B.V.

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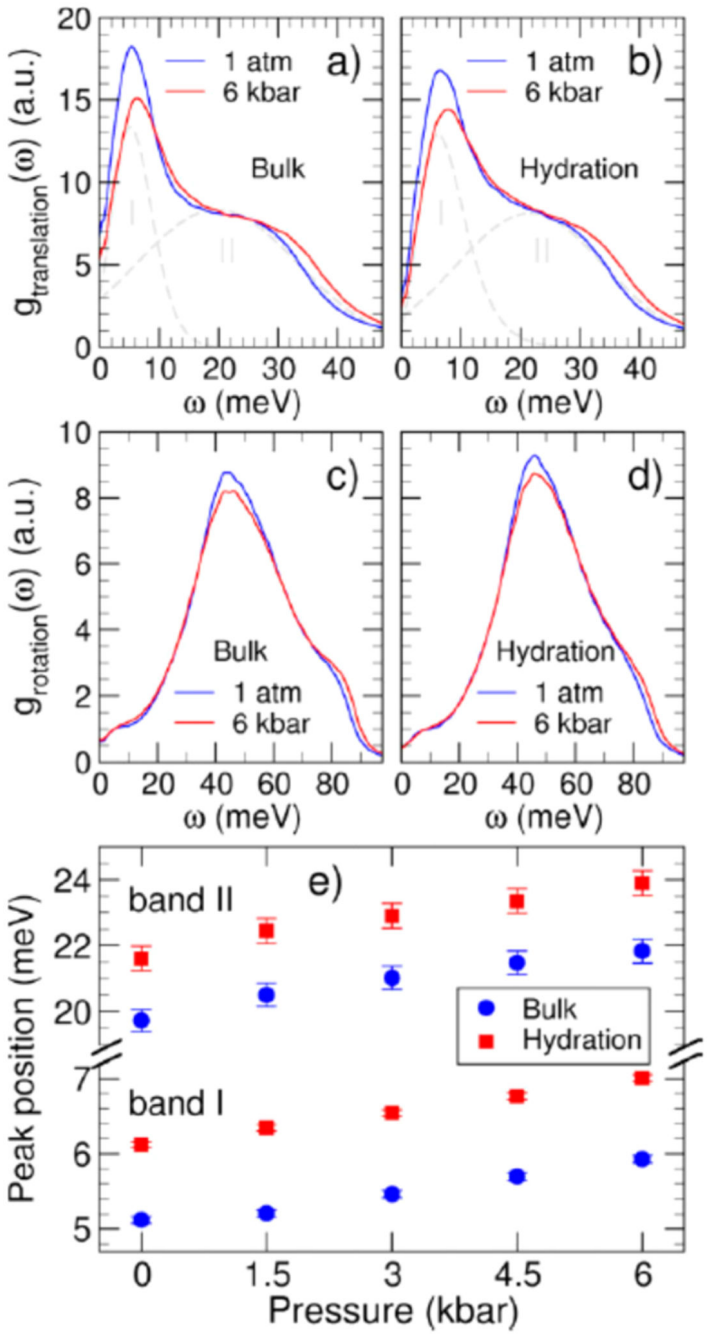

Figure 11.

(Top) Translational density of states of (a) bulk and (b) lysozyme hydration water determined by MD simulations at 0.1 MPa (1 atm) and 600 MPa. (Middle) Rotational density of states of (c) bulk and (d) lysozyme hydration water determined by MD simulations at 0.1 and 600 MPa. The curves have been determined by averaging the $g(x)$ of bulk and hydration water over 10 and 25 10 ps trajectories, respectively. The translational $g(x)$ of bulk and hydration water have been fitted using a log-normal and Gaussian function that represents the O_O_O bending and O_O stretching bands, respectively, denoted as I and

II. The two bands determined for the $g(x)$ of bulk and hydration water at 1 atm are shown as dashed gray lines in (a) and (b), respectively. The pressure dependence of the positions of these bands is shown in (e).[260] This figure is reproduced with permission from ref 260. Copyright 2013 Wiley Periodicals, Inc.

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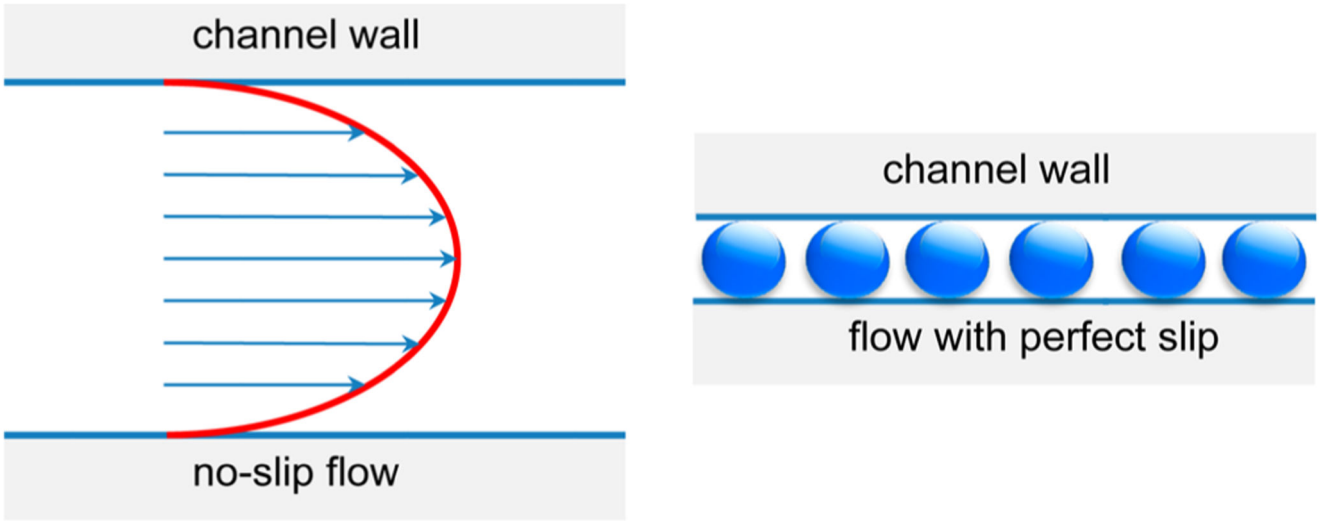

Figure 12.
Macroscopic hydrodynamics (Hagen–Poiseuille's flow) versus single-file flow (not drawn to scale). The water molecules in contact with the walls of a macroscopic tube are thought to be immobile (nonslip condition), whereas the water molecules within nanoscopic channels may be as mobile as in bulk water.

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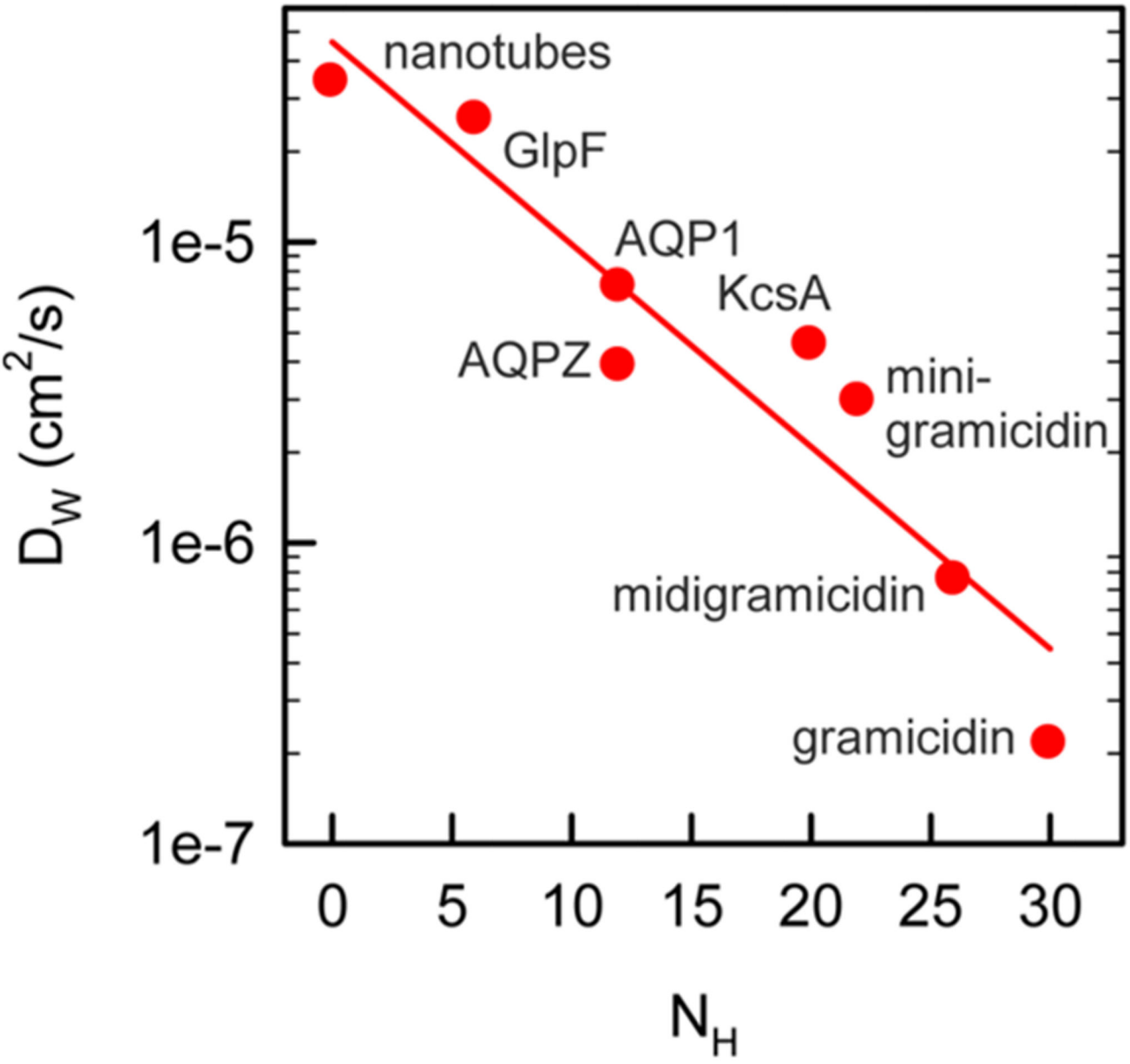

Figure 13.

Total number N_H of hydrogen bonds that single-file water molecules may form with pore-lining residues while traversing the channel governs water mobility. The latter is expressed in terms of the water diffusion coefficient D_w in the pore. The data for the peptidic channels gramicidin, midigramicidin, and minigramicidin are from refs 302 and 305 for the potassium channel KcsA from Hoomann et al., 2013 (with corrections as outlined in ref 287), for the aquaporins AQP1, AQPZ, and GlpF from ref 287 and for nanotubes from ref 297. The figure is reproduced with permission from ref 287. Copyright 2015 American Association for the Advancement of Science.