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Perspective

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New Insights into the Role of Water in Biological Function: Studying Solvated Biomolecules Using Terahertz Absorption Spectroscopy in Conjunction with Molecular Dynamics Simulations

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Supporting Information

ABSTRACT: In life science, water is the ubiquitous solvent, sometimes even called the "matrix of life". There is increasing experimental and theoretical evidence that solvation water is not a passive spectator in biomolecular processes. New experimental techniques can quantify how water interacts with biomolecules and, in doing so, differs from "bulk" water. Terahertz (THz) absorption spectroscopy has turned out to be a powerful tool to study (bio)molecular hydration. The main concepts that have been developed in the recent years to describe the underlying solute-induced sub-picosecond dynamics of the hydration shell are discussed herein. Moreover, we highlight recent findings that show the significance of hydrogen bond dynamics for the function of antifreeze proteins and for molecular recognition. In all of these examples, a gradient of water motion toward functional sites of proteins is observed, the so-called "hydration funnel". By means of molecular dynamics simulations, we provide new evidence for a specific water−protein coupling as the cause of the observed dynamical heterogeneity. The efficiency of the coupling at THz frequencies is explained in terms of a two-tier (short- and long-range) solute−solvent interaction.

1. INTRODUCTION

It is now widely recognized that hydration water in the proximity of protein surfaces plays an essential role in the structure, stability, and dynamics of proteins. It has been proposed that hydrogen bond networks stabilize intermediates during protein folding or protein aggregation, and they have recently been visualized forming extended water channels, for example, in photosystems or pockets filled with up to 1000 water molecules.[1,2] Yet it is part of an ongoing scientific debate whether solvent dynamics influence or even "enslave" the dynamics of biomolecules[3,4] or, more speculatively, whether solvent fluctuations actually contribute to biomolecular function (or malfunction). Going beyond individual proteins, the active role of water in protein interactions, protein aggregations, and molecular recognition is also a topic of intense and even controversial debate.[5] Today, solvent molecules are more and more considered as functional units to be employed as active species in solvent-mediated and solvent-controlled processes, rather than being just inert and passive spectators. *Solvation science* aims to provide a unifying framework for understanding and predicting solvent processes. This Perspective focuses on the hitherto existing contribution and the future potential of terahertz (THz) absorption spectroscopy in this field.

Only in recent years, new sources have accessed the so-called "terahertz gap", i.e., the region of the electromagnetic spectrum from 0.3 to 20 THz (from 10 to 600 cm^{-1}, with 1 THz = 33.33 cm^{-1}), between the dielectric and the infrared regimes. This has offered a unique view of water and its properties, the understanding of which has required the development of new concepts, because the ones applied in other regimes are not suitable at THz frequencies.

The significance of the spectroscopic information in this frequency range stems from the hydrogen bond network of water, which exhibits vibrational resonances exclusively in the frequency range from 1 to 6 THz,[6] due to the numerous processes occurring in water on the picosecond (ps) time scale, e.g., hydrogen bond rearrangements, translational relaxation, and rotational relaxation. More specifically, this technique directly probes the fluctuating orientation of molecular dipoles and the collective intermolecular vibrations of the hydrogen bond network, both of which may be fundamentally different in hydration water when compared to bulk. Our group has pioneered THz absorption spectroscopy as a sensitive tool to detect solute-induced sub-picosecond (sub-ps) solvent dynamics.[7−9]

Hydration dynamics involves motions on a wide range of time scales: whereas hydrogen bond breaking occurs on the ps time scale, diffusion processes occur on time scales of 100 ps.[10] Experimental techniques that are able to probe hydration dynamics are Raman and infrared (IR) spectroscopies,[11] nuclear magnetic resonance (NMR) spectroscopy,[12] neutron spectroscopy,[13] and dielectric relaxation spectroscopy.[14] In addition to the development of new experimental techniques, these advances have gone hand-in-hand with molecular dynamics simulations,[15,16] and we refer to previous review papers for an extensive list.[10,17−20]

Hydration shells can be defined with respect to the distinct deviation seen in their quantitative properties around biomolecules when compared to the properties of bulk water, as probed by a specific technique. Although at first glance this seems to be trivial, various experimental techniques yield contradictory results on the size and physicochemical proper-

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ties of "the" hydration shell. However, most of these seemingly controversial results can be rationalized in a unifying picture: "Hydration shell" is then no longer considered to be just as a static property but can be defined with respect to water molecules that differ by (frequency-dependent) changes in hydrogen bond dynamics. As a consequence, the size of the hydration shell depends on the time scale being probed. Using THz absorption spectroscopy, we defined a "dynamical hydration shell", which includes all water molecules that show sub-ps water network dynamics distinct from those of the bulk, i.e., a distinct THz absorbance. Notably, the influence of the solute on the water dynamics can extend beyond the first water layer and include more water molecules than those that are actually statically H-bonded to the solute.

In this Perspective we highlight the key advances made in the field of *solvation science* by THz absorption spectroscopy, encompassing the unraveling of molecular details in the fast (sub-ps) dynamics of the dynamical hydration shell and a better understanding of its role in biomolecular processes. Furthermore, we will focus on future applications in this exciting, emerging field.

2. LIQUID WATER

Hydrogen bond network dynamics in liquid water can be probed by means of several experimental and theoretical techniques. To name but a few: single-particle techniques such as IR pump−probe spectroscopy, a powerful tool to probe water reorientation dynamics with a femtosecond (fs) time resolution that allows scientists to follow the very fast water reorientation motions;[20−22] NMR spectroscopy, which can also investigate reorientation dynamics by determining rotational correlation times in the order of picoseconds;[23] quasi-elastic neutron scattering, which allows researchers to investigate diffusive motions and molecular reorientation by following the motions of individual hydrogens;[24,25] and inelastic neutron and X-ray scattering that can probe high-frequency coherent collective dynamics associated with density fluctuations by measuring the dynamical structure factor.[26,27] Classical and *ab initio* molecular dynamics (MD) simulations have made a large contribution toward guiding and interpreting the experiments.[28−30]

THz absorption spectroscopy directly probes molecular details of changes in the fast collective (sub-ps) hydrogen bond network dynamics, and thereby provides information complementary to the above-mentioned techniques. One of its main advantages is that it is a label-free method to detect subtle changes in the dynamical orientation of water molecules that are otherwise washed-out in radial distribution functions. A second advantage, when comparing to neutron scattering methods that also probe collective motions, is the possibility to carry out studies in real time during a biological function (i.e., changes of the solvation dynamics after a microsecond to second time delay time) using a fs or ps THz laser.

THz (far-IR) absorption spectroscopy probes the reorientation of permanent and induced dipole moments. The isotropic absorption coefficient is defined as[30]

$$\alpha(\omega) = F(\omega) \int_{-\infty}^{\infty} \mathrm{d}t \, \exp(i\omega t) \langle \vec{M}(0)\vec{M}(t) \rangle$$

with

$$F(\omega) = \frac{1}{4\pi\varepsilon_0} \frac{2\pi\beta\omega^2}{3Vcn(\omega)}$$

Here, $\vec{M}(t)$ is the total dipole moment of the sample of volume V with refractive index $n(\omega)$ and temperature $T = 1/k_B\beta$. Thus any fluctuating dipole moment $\vec{M}(t)$ will give rise to an absorption $\alpha(\omega)$ which is proportional to the Fourier transform of the correlation function of $\vec{M}(t)$. A schematic representation of this concept is illustrated in Figure 1. The absorption

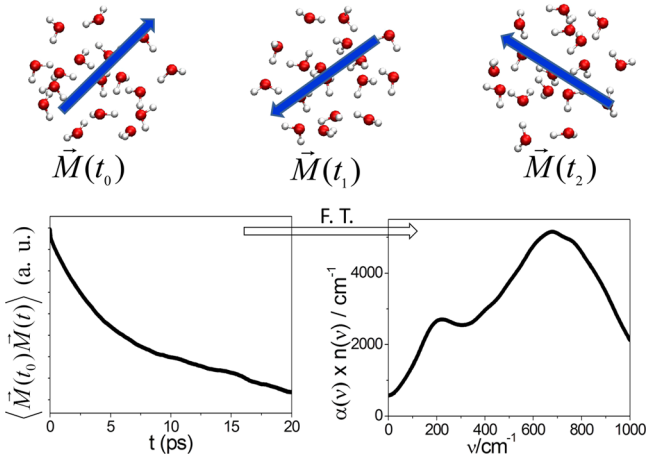

Figure 1. Schematic figure of the total dipole moment of liquid water at different times. Its correlation function in the time domain (left) gives rise to the absorption spectrum in the frequency domain (right).[30]

coefficient of pure water amounts to α = 240 per centimeter optical path length (cm^{-1}) at 1 THz[31] and 420 cm^{-1} at 2.4 THz[32] at a temperature of 21 °C. Since THz absorption probes the fluctuations of the hydrogen bonds, the absorption coefficient is temperature-dependent, with increased absorption α for fluxional water networks at high T and decreased absorption α for ice, where the hydrogen bonds are fixed.

The absorption spectrum of pure water at THz frequencies[33] is shown in Figure 2. It is characterized by two pronounced maxima at approximately 200 and 650 cm^{-1}, which have been respectively attributed to hydrogen bond stretching vibrations (i.e., hindered longitudinal translations) and librations (i.e., hindered rotational motions) of water molecules in the hydrogen bond network.[6,34] Additionally, Raman[6] and optical Kerr-effect[35] spectra feature a prominent absorption peak around 50 cm^{-1} (1.5 THz) that originates from the hydrogen bond bending vibrations—note that this peak is less pronounced in the far-IR absorption spectrum.[36] Most recently, two-dimensional Raman measurements revealed an echo in the Raman response, indicating a heterogeneous distribution of hydrogen bond networks on the time scale of 100 fs.[3]

A recent *ab initio* MD simulations study made it possible to dissect the THz spectrum of liquid water via correlations in time and space, providing new insight for comprehending the underlying intermolecular motions.[30] Most importantly, the sensitivity of THz absorption spectroscopy could be traced back to characteristic distance-dependent modulations of absorption intensities for bulk water.[30]

It has been shown that the 200 cm^{-1} resonance is dominated by stretching motions of hydrogen bonds within the first solvation shell. In particular, the distinct hydrogen bond donor/acceptor topologies accessible in the first solvation shells are

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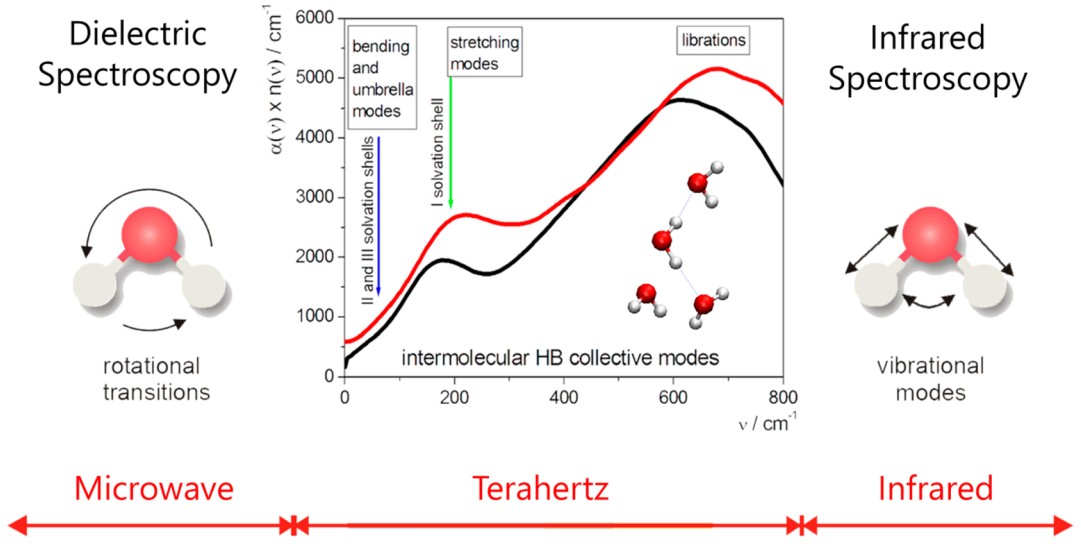

Figure 2. THz absorption spectrum of liquid water from experiments (black line)[33] and *ab initio* MD calculations (red line),[30] describing the intermolecular hydrogen bond collective modes. The THz regime is located between the dielectric and infrared regimes, which correspond to single-particle rotational transitions and intramolecular vibrations, respectively.

characterized by different average frequencies (160, 220, and 290 cm^{-1}) and contribute to the line shape of the band at 200 cm^{-1}.[30]

At a lower frequency, about 80 $cm^{-1} \approx 2.4$ THz, where the absorption revealed long-range effects in the solvation dynamics of various molecules using THz laser spectroscopy[7,8] (as will be described in the following section), a concerted motion involving the second solvation shell has been shown to contribute most significantly to the absorption spectrum.[30] This motion has been described in terms of an umbrella-like motion of two hydrogen bonded tetrahedra along the connecting hydrogen bond axis.[30] Hydrogen bond bending modes mainly contribute between 75 and 80 cm^{-1}, but their tails extend deep into the 200 cm^{-1} maximum.[30] Moreover, the distance-dependent absorption at 80 cm^{-1} is characterized by correlations at distances corresponding the third solvation shell or even beyond.[30]

Overall, these results reveal that, in the THz regime, strongly correlated intermolecular motions characterized by their collective nature and delocalized character dominate the dynamics; in contrast, the intramolecular modes that dominate the IR regime (above 1000 cm^{-1}) show no significant correlation in particle motions beyond the first solvation shell, but instead a pronounced coupling of electronic origin.[30] Therefore, these results reveal that the IR and THz regimes are governed by distinctly different mechanisms and cannot be described by means of the same concepts. The "THz concepts", describing collective translational and sub-ps reorientational motions involving more than one water molecule, are also distinct from those of the dielectric regime (GHz regime), which probes the slower rotational reorientation of the dipole moment.

THz spectroscopy is therefore a direct measure of the collective dynamics of water molecules on a spatial scale spanning several solvation shells. THz absorption constitutes a method to probe even small modifications of the hydrogen bond network, induced by the presence of a solute. These include water molecules that exhibit a changed, albeit generally retarded, hydrogen bond dynamics. This dynamical hydration shell can extend far beyond the first shell (with bound water molecules) or the distance at which density fluctuations can be observed.

In the following section we will report the key advances made by THz absorption toward understanding solute-induced solvent dynamics by means of THz absorption and the open questions that still need to be addressed.

3. SOLVATION OF BIOMOLECULES

In bulk, any water molecule is, on average, coordinated by about four neighbors in a very dynamic tetrahedral hydrogen bond network. Any solute will perturb this fluctuating network locally, but, vice versa, the solvent might affect the solute. Revealing the influence of a solute on the hydration shell dynamics is a main aim of *solvation science*. This is currently addressing some pressing questions: What are the parameters of solute−solvent interfaces that determine solvation dynamics? Is topology, e.g., steric hindrance in pockets, of major importance? What role does chemical and topological heterogeneity of a solute/solvent interface play? Is the hydration in the vicinity of a protein homogeneous or heterogeneous? Do the solvent dynamics play a role in molecular recognition or enzymatic catalysis? Does the solvent contribute to biological function?[38]

Moreover, THz includes the frequency range of the large-amplitude modes of proteins, involving skeleton motion during conformational changes.[39,40] In initial studies of hydrated films and protein solutions, a two-component model could be fitted to the data (bulk water and protein absorption). As a result, a broad, protein-concentration-dependent increase or decrease in the THz absorption was found, but it was not possible to obtain characteristic spectroscopic features for the protein itself.[41,42]

In Bochum, we 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 temperature.[32] Solute-concentration-dependent THz absorption measurements revealed a significant deviation from a (linear) two-component model and uncovered the necessity of including a third component: hydration water.

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MD simulations provide a microscopic picture of changes in water network motions associated with the solute−solvent interaction. MD analyses have revealed that water in the vicinity of proteins that are affected in their hydrogen bond dynamics, i.e., water within the "dynamical hydration shell", is characterized by retarded hydrogen bond rearrangement dynamics. This is accompanied by a blue-shift of vibrational modes centered at ∼1.5 THz in the vibrational density of states, due to strengthened interactions among water molecules.[9] As a result, we predict an increase of THz absorption above ca. 1.5 THz and a decrease below ca. 1.5 THz.

In most solutions, the THz absorption (between 1 and 3 THz) 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 (e.g., for sugars α_{solute} is 10 times smaller than $\alpha_{solvent}$ in the THz range). This implies that, to a first order, a linear decrease of the THz absorption with increasing solute concentration is expected. However, for several biomolecules in solution,[8,43] a "THz excess" and an onset of nonlinearity at specific concentrations have been found between 2.4 and 2.7 THz (see Figure 3). These can be explained when taking into account that the water molecules in the 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:[8,43,44]

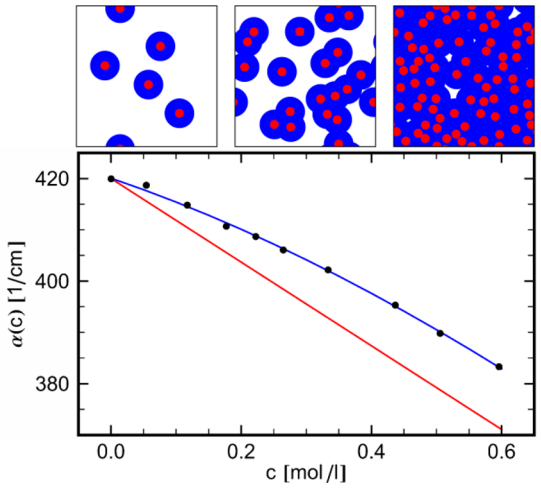

Figure 3. Concentration dependence of the THz absorbance of a biomolecule solution. (Top) Solute (red) and its dynamical hydration shell (blue) at different solute concentrations. At high concentration, the hydration shells overlap. When the solutes start to "share" hydration shells, we see a deviation from linearity. (Bottom) THz absorption coefficient as expected for a two-component model (red) and a three-component model (blue) that reproduce the experimental data (black circles) well for lactose. Reproduced with permission from ref 18. Copyright 2010 John Wiley & Sons Ltd.

$$\alpha_{total}(\omega) = \frac{V_{solute}(c)}{V_{total}}\alpha_{solute}(\omega) + \frac{V_{shell}(c, \delta R)}{V_{total}}\alpha_{shell}(\omega) + \left(1 - \frac{V_{shell}(c, \delta R)}{V_{total}} - \frac{V_{solute}(c)}{V_{total}}\right)\alpha_{bulk}(\omega)$$

where α_{total}, α_{solute}, α_{shell}, and α_{bulk} are the absorption coefficients of the solution, the solute, the solvation water, and the absorption coefficient of the bulk water, respectively. V_{total}, V_{solute}, and V_{shell} are the total volume, the volume of the solute molecules, and the volume occupied by the dynamical hydration shell, i.e., the part of the water around the solute that is affected by the solute, respectively.

THz absorption spectroscopy thus allows a direct investigation the dynamical hydration shell and determination of its size, i.e., just how far-reaching the influence of the biomolecule on the solvent is.[8]

THz studies of solvated sugars yielded influences of the solute on the collective water network motions of up to 3−4 Å for the monosaccharide glucose and 6−7 Å for the disaccharides lactose and trehalose.[7,43] The increase in THz absorption could be correlated with the number of hydrogen bonds formed between the solute and the solvent.

More-precise THz absorption measurements of hydrated proteins (five-helix bundle protein, lambda repressor, λ^*_{6-85}) showed a clear nonlinear absorbance versus concentration, which could be attributed to an extended hydration shell of 15−20 Å, or even beyond, i.e., up to several hydration layers from the protein surface.[8] On the other hand, structural parameters of hydration water (i.e., density changes measured by diffraction methods) differ from those of bulk water only up to 3−6 Å since these probe another time scale of hydration bond dynamics.

This is not a contradiction but a reflection of the fact that different techniques will "see" different aspects of solvation. Solvent density changes are mostly restricted to the first or second shell. "Hydration water" and "bulk water" are constantly exchanging on a time scale of 100 ps; therefore, any method that probes static solvent properties, or motions which are slower than this diffusional exchange time, will "see" smaller sizes of the hydration shell, just as in case of too long exposure times. As THz absorption probes the change in solvation dynamics of (sub-ps) hydrogen bond network motion, it can reveal changes that are not observed when probing properties averaged on a time scale of 100 ps. The hydrogen bond lifetime in the vicinity of a protein is retarded over 1−2 nm distances compared to the hydrogen bond lifetime of bulk water (ca. 1 ps). While the effect of retardation is small (ranging from a factor of 10 to a factor of 2) and is smaller at larger distances from the protein, the number of water molecules rapidly increases with the diameter d of the solvation shell ($\sim d^3$), such that even small effects add up to a clearly observable effect.

THz spectroscopy has revealed that water network dynamics differ upon structural changes of the protein: native protein (λ^*_{6-85} at pH 7.3) shows an extended hydration shell, while denatured proteins show a distinct influence on the solvent.[45] Similarly, protein mutants were found to influence water dynamics to a lesser extent when compared to wild-type proteins.[45] Future studies will have to find out the reason behind this and must focus on the contribution of these changes in the solvent dynamics to protein function.

A remarkable example of the correlation between protein function and hydration dynamics is provided by the antifreeze proteins (AFPs) and antifreeze glycoproteins (AFGPs). AFPs enable the survival of organisms in sub-freezing environments by binding to nano-sized ice crystals and preventing the further growth of these seed crystals:[46] after docking of the protein at a so-called ice-binding site (IBS), ice growth in the normal low radius of curvature is prohibited, thereby restricting growth to

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regions between the adsorbed AFPs in a high radius of curvature front, until the temperature is lowered further to the hysteretic freezing point. This preferred binding of the protein to the nano-sized ice crystal instead of water constitutes one of the toughest recognition problems.[47] Initially, the underlying molecular mechanism has been explained exclusively by a short-range hypothesis, i.e. in terms of finely tuned hydrogen bonding of polar threonine hydroxyl groups directly to ice surfaces.[46] However, a study of several mutants of the protein did not provide the anticipated decrease in antifreeze activity that was expected for the threonine as the single cause of AF activity.[48] Recent work[49,50] on AFPs and AFGPs revealed at least two mechanisms that contribute to the antifreeze activity: (a) short-range interactions such as hydroxyl bond formation by threonine and (b) long-range interactions that correlate with heterogeneous hydration dynamics.

THz spectroscopic studies combined with MD simulations have recently allowed us to prove the additional long-range mechanism by discovering the presence of a dynamical hydration shell—extending up to 20 Å from the protein surface—a necessary condition for all antifreeze active proteins.[50,51] The retardation of the hydrogen bond dynamics shifts the vibrational density of states of water in the hydration layer to higher frequencies, resulting in an increase of THz absorption between 2.4 and 2.7 THz.[49] We have therefore proposed that the antifreeze activity, while requiring a short-range direct interaction of the protein with the ice face, is also assisted by long-range perturbation of the solvent's collective dynamics.[50,51] Different AFPs have optimized either their short-range interaction or their long-range effect or both, as is the case for hyperactive proteins. Studies of a recently discovered AFP retaining ordered waters from the core to the surface and binding to its ligand through the ordered surface waters, could shed more light on the mechanisms responsible for the antifreeze activity.[2]

Notably, heterogeneous hydration dynamics in AFPs have been revealed with a gradient of retardation toward the so-called ice binding plane, i.e., the site that preferentially binds to the nano-sized ice crystals (see Figure 4). This gradient of retardation, which we will call a "hydration funnel", seems to be involved in the molecular recognition effect. The simulation supports an entropically favorable docking of a nano-sized ice crystal near the binding plane, where the water is retarded considerably more than it is along the rest of the protein surface.[50]

Notably, a very similar gradient of fast-to-slow coupled protein−water motions has been observed in the catalytic domain of a model enzyme (human membrane type-1 matrix metalloproteinase (MT1-MMP), the solvent dynamics being mostly retarded at the metalloprotease active site.[52] Importantly, this evidence suggests the existence of a heterogeneous hydration dynamics paired with a less flexible water network when approaching a functional site: the hydration funnel. A schematic sketch of the enzyme (gray) substrate (green) complex which shows the gradient of hydrogen bond dynamics towards the active site is reported in Figure 5. We speculate that this heterogeneous hydration assists protein function.

In the following we will discuss which specific water−protein dynamical coupling is responsible for the observed dynamical heterogeneity.

We have performed MD simulations of a model enzyme (the catalytic domain of MT1-MMP) and two model substrates with different structures and flexibility, a collagen-like substrate and a gelatin-like substrate (details of the simulations and analyses can be found in the Supporting Information). The former is a biologically active triple-helix peptide with an optimized recognition sequence for MT1-MMP, while the latter is a single-chain unstructured peptide, forming a random coil in solution. In both enzyme−substrate complexes, we observe that the vibrational modes of water at the catalytic site (water within 6 Å catalytic zinc ion) are blue-shifted with respect to the

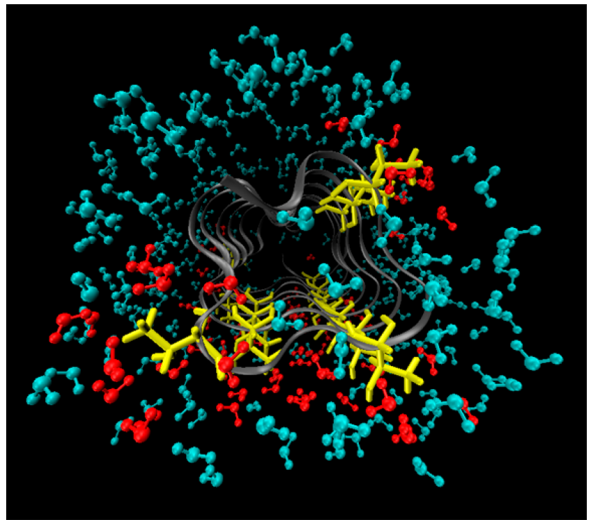

Figure 4. Heterogeneous hydration in the antifreeze protein DAFP-1.[50] The β-helical right-handed helix of the protein is shown in gray, and threonine residues located at the ice-binding site are shown in yellow. Water molecules are colored in cyan and the strongly retarded ones in red. The most significant retardation is found at the ice-binding site.

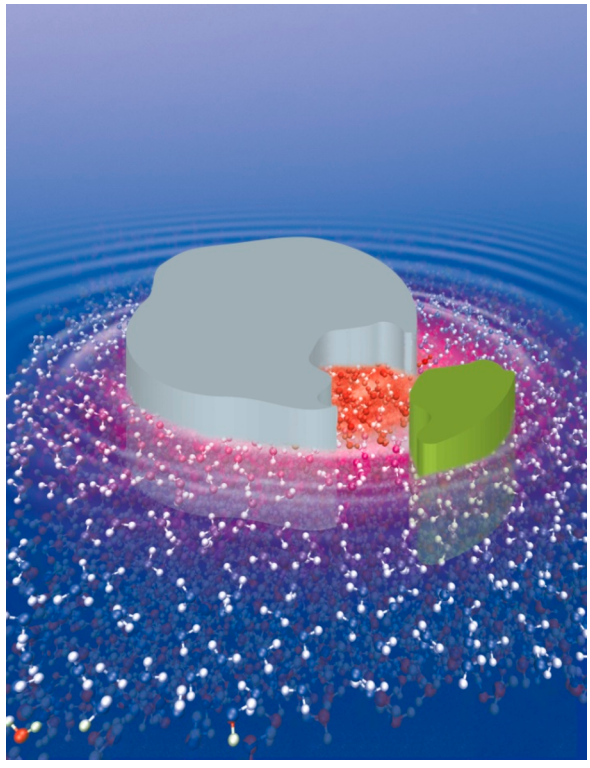

Figure 5. Schematic representation of the hydration funnel in an enzyme−substrate complex (see text for a detailed description).

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frequencies of bulk water (~1.5 THz[9]). However, the magnitude of this effect is substrate specific. A deeper analysis reveals that the water dynamics at the active site carry the specific fingerprints of the substrate: when comparing the vibrational density of states for water in the first shell and the substrate, we observe a strict correlation between them (see Figure 6).

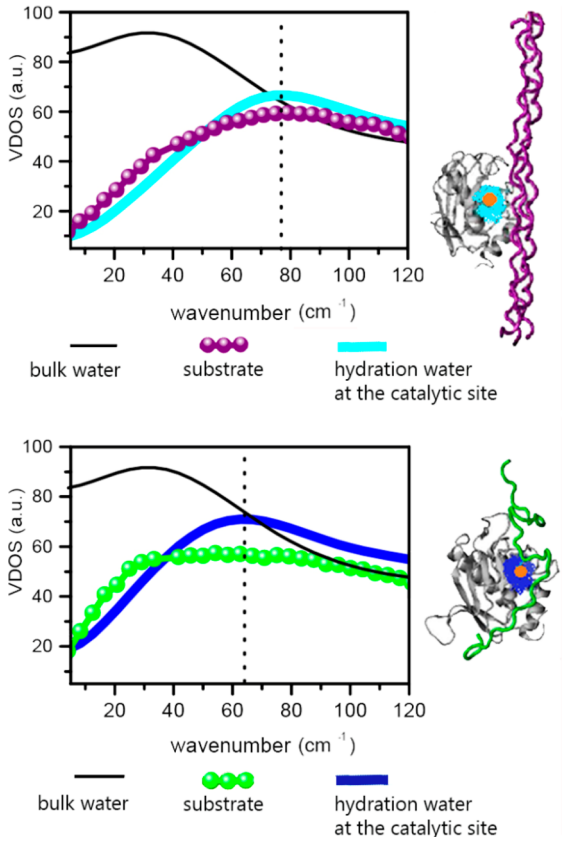

Figure 6. Specific solvent–substrate coupling at the catalytic site of MT1-MMP. (Top) Vibrational density of states of water at the catalytic site (<6 Å from the catalytic zinc ion; cyan line) and the collagen substrate (purple circles). (Bottom) Vibrational density of states of water at the catalytic site (<6 Å from the catalytic zinc ion; blue line) and the gelatin substrate (green circles). The dashed black line indicates that the vibrational density of states of the water at the catalytic site is blue-shifted with respect to the bulk (>12 Å from the protein surface, black line), more for the MMP-collagen complex than for the MMP-gelatin one. The simulated systems are shown in a cartoon representation: enzyme, gray; catalytic zinc ion, orange; water at the catalytic site, cyan for the MMP-collagen, blue for the MMP-gelatin; collagen, purple; gelatin, green.

This provides evidence for strong, substrate-specific water–protein coupling as the cause of the solvent dynamical heterogeneity at the functional site. It is worth noting that the triple-helix substrate affects water motions more strongly compared to the unstructured single-chain substrate. The coupling observed may assist enzyme–substrate interactions and play a specific role in molecular recognition.

Why is the coupling between water and solute so effective, and why does it show such a long-range correlation in the THz range compared to other frequency range e.g. IR[30]? A driven MD study of a small solvated peptide[53] has provided an explanation for the efficiency: since the water spectrum itself has a high density of states involving collective modes in this frequency range, a rapid flow of energy from the low-frequency modes of peptide to the collective water modes (see Figure 7)

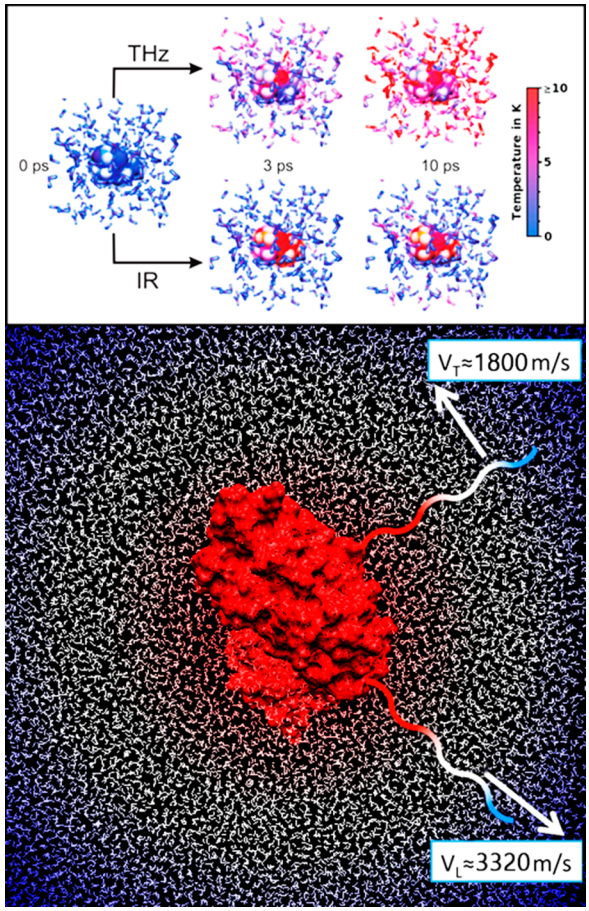

Figure 7. (Top) Kinetic energy flow at THz and IR frequencies for a dialanine molecule and its hydration water before driving and after driving for 3 and 10 ps, respectively, at the frequencies 81.9 cm^{-1} (THz) and 1012.7 cm^{-1} (IR). Reproduced with permission from ref 53. Copyright 2012 American Chemical Society. (Bottom) Illustration of the transverse and longitudinal collective motions in the hydration water of a protein.[54,55]

is facilitated once a solute mode in the THz range is driven. A less efficient coupling has been revealed in the IR range, where most of the energy is stored in the intramolecular vibrations,[53] due to an energetic mismatch with intermolecular water bands; thus, the flow is restricted to the first shell (single-particle dynamics).

MD simulations of hydrated proteins have now provided evidence for a long-range water–protein coupling, as suggested by THz spectroscopy. Optical-like modes originating in the protein have been found in the hydration water, and the result has been interpreted as clear proof of a very effective coupling occurring at the THz frequencies.[54] Furthermore, MD simulations now provide evidence that solute–solvent acoustic-like cross-correlations propagate in the system, extending up to 10 Å from the protein surface and sensitive to the chemical properties of this surface. Notably, the sound velocity in hydration water at momentum transfer $Q > 0.2$ $Å^{-1}$ is ~3320 m/s and it is close to the value of the so called fast sound in bulk water, i.e. we are above the transition from the hydrodynamic to the fast sound.[54]

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This is of special importance given the fact that all protein conformational changes involve large-amplitude motions that are in the "right" frequency range for collective intermolecular hydrogen bond motions.

To us, the following conditions are crucial for the development of this sound wave-like propagation reaching out beyond the first hydration shells into the solvent: (a) A short-range mechanism is necessary to provide an efficient direct coupling (a "hook") between the protein surface and the water molecules in the first shell. (b) The energies of the solute modes should be energetically close to those of the water modes (peaking at 200 cm^{-1} or 6 THz). In the case of efficient coupling, the density of states of the solute and solvent modes is correlated highly in frequency terms. This generally results in a blue-shift of the coupled protein water modes. (c) We speculate that the extension of the dynamical hydration shell depends on the speed of the acoustic wave.

4. FINAL CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, it could be shown that THz absorption provides a sensitive tool to probe subtle changes in hydration dynamics caused by a solute. These studies revealed that an efficient dynamical coupling of the THz dynamics of biomolecules with those of their hydration shells can play a key role in biomolecular mechanisms. A two-tier (short-range and long-range) solute−solvent interaction and a heterogeneous hydration dynamics toward functional sites (hydration funnel) appear to be fundamental elements of this coupling. Further insights into the underlying mechanism of binding could be provided by studies of the entropy and enthalpy of binding of water in the binding pocket.[56]

Finally, given the established role of the dynamical hydration shell in some molecular mechanisms, investigating the solvation dynamics at THz frequencies in real time during kinetic processes constitutes a promising research area. Pioneering kinetic THz absorption studies (KITA), monitoring the changing THz electric field pulse shape on the ps time scale as a reaction proceeds on a longer time scale, e.g., seconds, made real-time detection of hydration water possible.[52,57]

In the future, the KITA investigations of solvation dynamics complemented with picosecond fluorescence or IR studies allowing real-time detection of docking events or protein folding will allow us to probe energy flow in proteins or from the protein into the solvent.

Together with systematic thermodynamic studies, these techniques can provide the missing details for a full comprehension—in terms of its enthalpic and entropic contributions—of the role of the dynamical hydration shell in protein functions.

In addition to its fundamental significance, the concepts that are developed within the new field of *solvation science* should have an impact on the future design of artificial antifreeze proteins and small-molecule drugs directed at biomolecular targets in pharmaceutical applications. A fundamental molecular understanding might be the missing link for the development of new enzyme constructs or for copying molecular recognition in nature. So far, in all these applications, the solvent is still a strongly underestimated and mostly neglected element of the multilateral partnership in biomolecular function.

■ ASSOCIATED CONTENT

Supporting Information

Details of the MD simulations and the analysis carried out for the enzyme−substrate complexes reported in section 3. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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