PCCP

PAPER

View Article Online
View Journal | View Issue

Cite this: *Phys. Chem. Chem. Phys.*, 2017, **19**, 5441

Received 22nd August 2016,
Accepted 26th January 2017

DOI: 10.1039/c6cp05788h

rsc.li/pccp

How water mediates the long-range interactions between remote protein molecules

Anna Kuffel

The high crowding of macromolecules in the cytoplasm affects the processes that occur inside a living cell. It can, for example, promote the forming of various loosely connected structures of proteins. It also means that bulk water is, essentially, not present there. The relatively thin layer of solvent that separates macromolecules may be able to participate in the long-range interactions between them and make them respond to each other. Herein, we propose a mechanism in which water molecules can mediate inter-protein interactions. The origin of this phenomenon emanates from the unique properties of water.

Introduction

The solvation water of biomolecules differs from bulk water in terms of its structure and dynamics.[1–3] The behavior of water depends on the properties of the hydrated surface. Therefore, the properties of solvation water inform us, indirectly, about the interactions between the molecule and the solvent.

Simultaneously, the presence of water is required to maintain the biological activity of biomolecules.[4,5] Proteins are, generally, inactive until a threshold hydration level is reached.[6] The role of solvation water for biological processes has been extensively studied in recent years. The conclusion that can be drawn from these investigations is that water is definitely "more than a bystander".[4,7–10] Water influences, for example, the folding of proteins, the process of association of protein subunits, and enzymatic reactions. The realization of the importance of water has led to the forging of the controversial term "biological water".[11]

The cytoplasm is crowded and the macromolecules within are separated by a layer of water as thin as 1–2 nm,[5] not taking into account small molecules and ions. It is often said that, inside living cells, bulk water is practically not present, although the views on that matter are split.[12]

As a result of the high crowding, one protein molecule may be influenced by quite a few other proteins in its vicinity. One example of this influence is the fact that the proximity of other macromolecules can affect the stability of a protein.[13–15] Furthermore, the motions of the macromolecules can become correlated as a result of hydrodynamic interactions.[16] Moreover, there is an increasing interest in the so-called "quinary structures" of proteins. This term was proposed by McConkey[17] and relates to protein assemblies that are held loosely together by various weak interactions.[18,19] Awareness of the importance of these structures for the functioning of a living cell has recently been increasing.[19,20]

The spatial span of the weakly connected network of proteins may be huge. We can hypothesize that the constituent proteins may take part in some sort of communication between different parts of a cell. Signal transduction might be possible through vibrational motions. So far, though, this hypothesis lacks an experimental confirmation. In our recent papers, we described a process of this kind – the influence of the presence of a protein molecule on the internal vibrations of a molecule close to it, but separated by a layer of water.[21,22] The vibrations of the proteins may participate in signalling not only by mechanical means. It was proposed that electrically polar, vibrating molecules or larger cellular structures may generate a signal-transducing electromagnetic field.[23]

A little more information can be found on the possible propagation of signals through the vibrational motion within one molecule, probably because it has been acknowledged for a long time that for numerous proteins, flexibility is a key player in relation to function.[24] Iakhiaev and Iakhiaev[25] claim that information or energy can be transferred between different parts of a protein molecule in the form of a "perturbation wave". They used molecular dynamics simulations, but long-range protein vibrational modes have also been observed experimentally.[26] They can play a role, for example, in protein folding and in allosteric response.[27,28] They may also be involved in conformational changes that propagate from a specific site to the rest of the molecule.[29] It has even been suggested that vibrations of proteins may participate in the complex information processing systems of the brain.[30]

Each molecule vibrates with its own characteristic frequencies. The binding of another molecule changes its dynamic pattern and,

Faculty of Chemistry, Department of Physical Chemistry, Gdansk University of Technology, Narutowicza 11/12, 80-233 Gdansk, Poland.
E-mail: anna.kuffel@pg.gda.pl

as a consequence, can modify its biological activity.[31] The dynamical coupling between the bound ligand and the residues of the protein can occur, which may have very specific, function-related implications.[32]

In the phenomenon described by us,[21,22] the properties of solvation water play a decisive role in mutual "communication", since the molecules in question are not in direct contact. We observed: (1) a relatively weak, though noticeable synchronization of the motions of protein molecules and (2) a change in the pattern of the internal vibrations of the protein molecule induced by the presence of an additional protein molecule in close proximity. The second effect is much more pronounced than the first one.

Both of these effects were observed in the low-frequency range, up to about 2 THz, which is the range that is especially important for the biological function of proteins.[33] Moreover, the effects were noticeable at distances even up to 2 nm, which correspond to the aforementioned mean distance between the molecules inside a cell. Because of that, we believe that this phenomenon might constitute a meaningful input to the immensely complex network of interactions between the constituents of a cell.

Therefore, herein we would like to extend the discussion published previously with a broader look at the properties of solvation water and the molecular origin of the described phenomena.

In our previous paper[22] that discussed the second phenomenon, we described results for quite an unusual protein – a hyperactive antifreeze protein from *Choristoneura fumiferana* (*Cf*AFP). It is relatively small (90 amino acids) and its structure is well-defined and rigid; it is prism-shaped, with planes formed by β-sheets, and linked together with disulfide bonds. One of the planes is flat and binds to the surface of ice. Our model system contained one, two or three molecules of this protein, carefully arranged so that their planes faced each other.

It could be possible that the observed effect is limited to this type of specific protein and system. Therefore, herein we would like to take the opportunity to test the validity of the model described previously[22] for more complex and less conformationally restrained proteins. We now use a more realistic system consisting of a molecular motor called kinesin (more specifically, its head) and a tubulin dimer (consisting of α- and β-tubulin). Kinesin moves along microtubules and during its working cycle its head alternately binds and unbinds from the track. Therefore, this system is more realistic than two *Cf*AFP molecules in the sense that bringing the proteins close together occurs in cells and has important biological implications, contrary to *Cf*AFP (as far as we know). We analyze the head of kinesin at different distances from the tubulin dimer, which are supposed to represent the various stages of association of the head to the binding site at the surface of the dimer. The kinesin head is significantly more conformationally labile than the *Cf*AFP molecule and its surface is also more rugged. The same applies to the tubulin. Moreover, the motor head includes an attached ADP molecule, therefore this aggregate represents a more complex system than a single AFP molecule.

If our conclusions remain the same for such a different system, then we have a very strong basis to claim that the mechanism is universal to a wide variety of proteins and, most probably, to other macromolecules. As we are going to demonstrate below, the model is generally applicable to the kinesin–tubulin system. However, our main goal is to propose a full and consistent explanation of the role of solvation water in inter-protein interactions, and to describe the mechanism of these interactions at the molecular level.

Methods

The investigations were carried out using molecular dynamics simulations. We investigated three systems composed of the catalytic domain of kinesin and a tubulin dimer. The proteins were separated by three different distances (0.8, 1.2 and 2.0 nm) and immersed in an SPC/E water model,[34] which was also used in previous studies,[21,22,35] and is known for reproducing the properties of liquid water quite well,[34,36] including the diffusion coefficient.[37] This last property is especially interesting for us, because we are predominantly interested in the dynamics of water and proteins.

The data obtained on the performance of any water model during investigations into solvation are hardly ever unambiguous and that includes the data obtained for the SPC/E model.[36,38,39] Anyhow, Hess and van der Vegt[40] concluded that the SPC/E water model is well suited for use with the Amber ff99 force field, which is the predecessor of the one used by us.

Preparation of the systems

Three structures from the Protein Data Bank were used (PDB ID: 1BG2,[41] 1JFF[42] and 2P4N[43]). The 1BG2 structure is the kinesin catalytic domain, the 1JFF structure is the tubulin dimer, and the 2P4N structure consists of the kinesin catalytic domain resting on the tubulin dimer. Details concerning the preparation of the simulation-ready structures of the kinesin head and the tubulin dimer from the PDB files can be found in our previous paper.[35]

Kinesin from the 2P4N structure was moved above the tubulin dimer by 0.8, 1.2 and 2.0 nm. The previously equilibrated structures made up of 1BG2 (kinesin) and 1JFF (tubulin) were fitted to the coordinates of kinesin and tubulin separated by these distances. Na^+ ions were added to make the systems electrically neutral.[35] The three systems were solvated with SPC/E water, with the minimal distance between the proteins and the walls of the octahedral boxes set equal to about 1.2 nm. A system consisting solely of the kinesin catalytic domain in water was used for comparative purposes.

We made sure that the structure of the kinesin head with the tubulin dimer and that of the kinesin head without the tubulin dimer were the same. We also made sure that the equilibration period was the same and that the structure was fully relaxed every time. All of these factors may influence the dynamic pattern of the proteins. The different protocol used for the preparation of the systems explains the fact that the results of the principal component analysis reported by us now are a bit different than the results published previously in the Supporting Information for ref. 21. As it turned out, the present, improved

procedure ensured better comparability of the results between different systems.

Computer simulations

Computer simulations were carried out using the molecular dynamics package Amber12[44] and using the ff03 force field,[45] which is appropriate for protein simulations.

After the short procedure of energy minimalization (during which all protein residues were restrained, k = 10 and then 5 kcal mol^{-1} Å^{-2}), there was a preliminary period of equilibration, lasting for about 1.3 ns. At the beginning of the equilibration, all α-carbon atoms were restrained to equilibrate water first and to prevent the proteins from displacing and changing the distance between them (k = 5 and then 1 kcal mol^{-1} Å^{-2}). Later, the restraining potential was applied only to the α-carbon atoms (the force constant was decreased to 0.5 kcal mol^{-1} Å^{-2}). After the steps with restraints (lasting for about 700 ps), the restraints were turned off.

A particle-mesh Ewald method was used for analysis of the electrostatic interactions, and the lengths of the chemical bonds involving hydrogen atoms were fixed using SHAKE; a 1.2 nm cutoff for nonbonding interactions was used. A constant temperature (298 K) was maintained by weak coupling to an external bath (τ_T = 1.0 ps), and a constant pressure (1 bar) was maintained by the weak coupling method (τ_p = 1.0 ps).

Several dozen independent simulations were performed. The atom velocities for each of these runs were generated *de novo* from the Maxwell distribution (at 298 K), using different seed values obtained from a pseudo-random number generator. There are several advantages to this procedure; first of all, a larger phase space can be probed in a relatively short time. Moreover, we have more results to calculate the averages from. All results are for a comparable inter-protein distance; in the case of one long simulation, the distance between the proteins would be changed more at the end compared to the beginning.

These initial *NPT* runs were followed by 140 ps-long simulations under *NVE* conditions (and without any restrains). The trajectories were saved after each step, namely, every 2 fs. The terminal 120 ps of these runs were used to calculate the covariance matrix for the principal component analysis and the velocity correlation functions for the spectral analysis. The details of these calculations can be found in ref. 22. The total analyzed time period (the summed times of all runs) was equal to about 10 ns. Of course, we did not want to use any restraining forces during the production run because they could affect the protein dynamics.

Results and discussion

The system selected for our studies consisted of a kinesin head and a tubulin dimer. We considered three different distances between them, equal to about 0.8 nm, 1.2 nm and 2.0 nm. As the reference, we used a system with a single kinesin head. The analyzed distances represent the typical distances between macromolecules in a crowded environment inside a living cell, as was mentioned in the introduction.

The internal vibrations of the protein molecules were analyzed using principal component analysis. Upon input of the analysis, there was a covariance matrix with mass-weighted elements. All the coordinates of the atoms and the displacements from their mean positions were calculated after removing the translational and rotational motion of the molecule as a whole. This was done by fitting the protein molecule to the indicated reference structure. The dynamic properties of solvation water were analyzed using the velocity autocorrelation function. The details of the calculation methods are described in our previous paper.[22]

The assumption of harmonicity allowed us to estimate, very roughly, the frequencies ν_n of the vibrational modes of the protein, according to the relation:

$$\nu_n = \sqrt{\frac{k_B T}{4\pi^2 m_p \lambda_n}} \quad (1)$$

where λ_n is the eigenvalue obtained as a result of principal component analysis, k_B is the Boltzmann constant and m_p is the mass of a proton.

This method of calculating frequencies may seem controversial. However, we were more interested in the changes between the systems and in the general shapes of the distributions rather than in the exact values of the frequencies. The assumption of harmonicity is very problematic, especially for very small frequencies.

This and other assumptions and their validities are also thoroughly discussed in our previous paper.[22]

In Fig. 1, we can see how the frequencies of the consecutive modes of vibrations change with the diminishing distance between the proteins.

The changes in frequency can be, in our opinion, partially explained by a simple model – a damped harmonic oscillator with many degrees of freedom and subject to an external random force, with known spectral characteristics.[22] The collisions with the molecules of the solvent are assumed to be the source of the external random force. The spectral characteristic of the force is therefore the velocity power spectrum, $S(\nu)$, of the solvation water. The damping is associated with the viscosity.

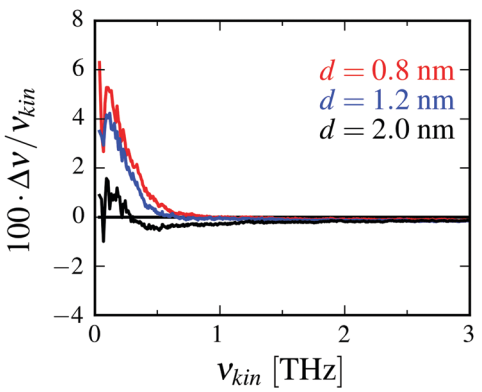

Fig. 1 The relative change of the frequencies of the normal modes of the kinesin head in the presence of the tubulin dimer. The relative change is plotted as a function of the frequencies of the protein without any neighbors (ν_{kin}). The change $\Delta\nu$ is equal to $\nu_d - \nu_{kin}$, where d = 0.8, 1.2 or 2.0 nm.

The velocity power spectrum $S(\nu)$ of solvation water was calculated from the velocity autocorrelation function $C(t)$ according to the relation:

$$S(\nu_n) = \frac{1}{T/2}\int_{-T/2}^{T/2} C(t)\cos(2\pi\nu_n t)\mathrm{d}t \qquad (2)$$

where $T/2$ is the analyzed correlation time (4 ps).

According to this model, the changes in the velocity power spectrum, $\Delta S(\nu)$, should influence the frequency characteristics of the internal fluctuations of the proteins. Every one of the many vibrational modes can be affected differently. The $S(\nu)$ spectrum is prone to external perturbations. It changes when the kinesin head approaches the tubulin dimer (as is illustrated in Fig. 2). Therefore, the model can be validated by comparison of the theoretical calculations with the actual distribution of the changes of the amplitudes of the inner motions of the kinesin head.

When the change of the frequency dependence of the external force $\Delta S(\nu)$ is known, then the change of the mean square displacement $\overline{y^2}$ that characterizes each normal mode (with frequency ω) can be calculated as:[22]

$$\Delta\left(\overline{y^2(\omega)}\right) = \int_{-\infty}^{\infty} \frac{\Delta S(\nu)}{\left(\omega^2-\nu^2\right)^2 + 4\xi^2\nu^2}\mathrm{d}\nu \qquad (3)$$

This comparison is presented in Fig. 3. Again, the amplitudes and the relationship between amplitude and frequency are estimated using the harmonicity assumption.[22] As we can see, the calculations and the estimations lead to roughly comparable distributions, but not for the first nodes of the highest amplitudes (lowest frequencies). For those, the harmonicity assumption is almost certainly not fulfilled.[22] The calculated values depend on the chosen friction parameter ξ. The best suited value of ξ differs from system to system and would depend, among other things, on the internal friction of the protein in question. To simplify the calculations, we assumed that its value is constant.[22]

As was already mentioned, the system investigated herein is very different to the one used previously.[22] The confirmed validity of the model (excluding the lowest frequencies) indicates that the discussed effect might be a general property and might apply to the majority of biomolecules in water.

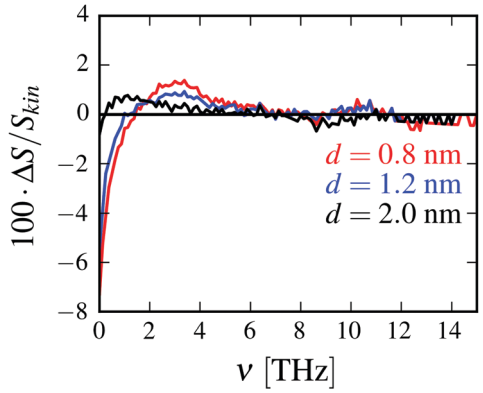

Fig. 2 The relative change of the velocity power spectrum of solvation water. The change ΔS is equal to $S_d - S_{kin}$, where d = 0.8, 1.2 or 2.0 nm and S_{kin} is the power spectrum of solvation water of the protein without any neighbors. The molecule of water was assumed to be in the solvation shell if its distance from the protein did not exceed 5 Å.

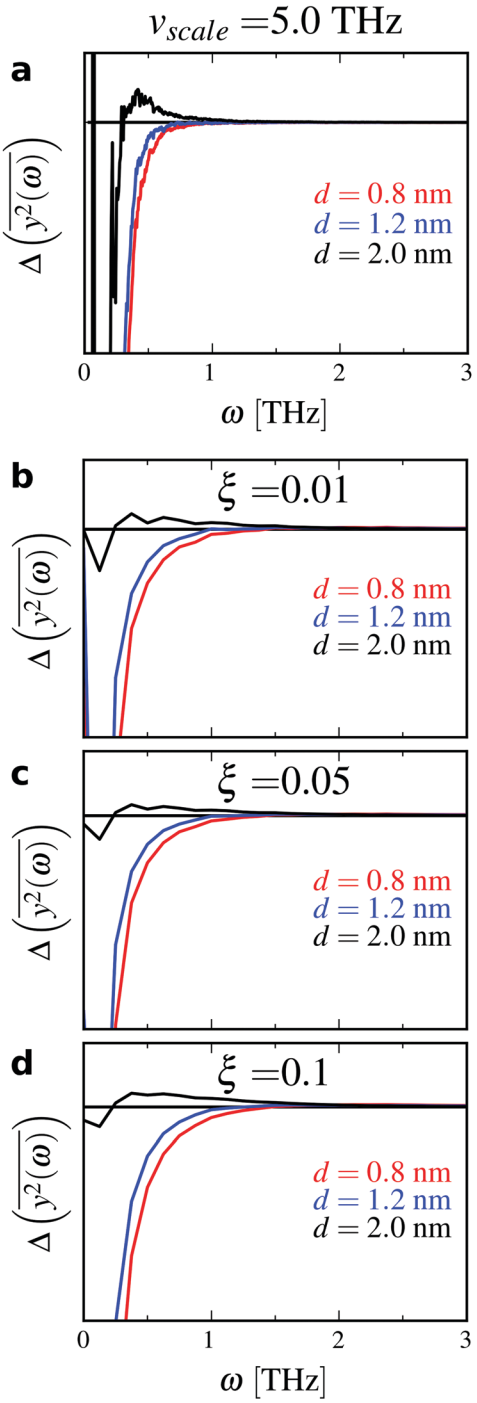

Fig. 3 (a) The differences of the amplitudes $\Delta y^2(\omega)$ of the normal modes of the kinesin head obtained from the simulations. The results are scaled to remove a small deviation of the $\Delta\nu/\nu_{kin}$ values from zero for high frequencies (similar to how it was done in ref. 22). Herein, the data are scaled so that the deviation is equal to zero for ν_{scale} = 5 THz. (b–d) The differences of the amplitudes $\Delta y^2(\omega)$ calculated theoretically according to eqn (3). The friction parameter ξ used in ref. 22 was equal to 0.05. The two other plots are added for comparison. The units of $\Delta y^2(\omega)$ are arbitrary, therefore the values on the axes are not displayed.

To explain the far-ranged influence of one protein molecule on another, it is necessary to take into account the properties of solvation water. We perceive it as a medium that links the distant molecules and is able to make them feel the presence of each other. This opinion is justified by the fact that there is a tight interdependence between the dynamics of the protein and the dynamics of the solvent.[2,5,46]

In the literature, two kinds of protein motions have been described, large-scale motions slaved to the bulk solvent fluctuations, and inner motions slaved to the fluctuations in the hydration shell.[47–51] It should be noted, however, that the term slaving sometimes raises some controversy,[52,53] because it assumes an absolute domination of solvent fluctuations over protein motions.

The dynamic properties of solvation water are important for the two effects associated with the mutual influence of proteins separated by a layer of water on each others dynamics, as was mentioned in the introduction: (1) the weak synchronization of the movement of the surface atoms and (2) the changes in the frequency of the internal vibrations of the proteins. Both of these effects should be explained separately, because we believe that their mechanisms are different.

(1) The partial synchronization of the collective movements of the surface atoms of the proteins

Previously, we determined a cross-correlation function of the vectors of the collective velocity of surface atoms of proteins separated by a layer of water.[21,22] A correlation was observed in the low-frequency range for *Cf*AFP, as well as for the kinesin head accompanied by the tubulin dimer.[21,22] It was also very weak; the order of magnitude of the cross-correlation coefficient was 10^{-5}.

In a crowded environment, hydrodynamic interactions can lead to the correlation of the motions of macromolecules.[16] The concerted movement of two protein molecules would of course result in the correlation of movement of the surface atoms of these molecules. As we argued when we discussed the motions of the surface atoms of *Cf*AFP,[22] concerted diffusion of the two proteins can take place, but on its own it cannot fully explain the observed correlations between the motions. The kinesin head and the tubulin dimer are significantly larger, therefore their rates of diffusion are much slower. This difference in size may explain the difference in the spectral characteristics of the correlation functions of the collective velocities of the surface atoms of neighboring proteins, that can be observed when the results from ref. 22 and 21 are compared.

The occurrence of the cross-correlation of motion of the surface atoms belonging to separate proteins had been hypothesized by us based on the results of Heyden and Tobias.[54] Those authors described the cross-correlation between the movement of atoms of a protein molecule and the movement of water molecules present at a distance from the surface of the protein. This collective movement of protein atoms and water molecules was explained by them by the propagation of the sound wave. In this light, it might have also been expected that the motions of the two groups of atoms belonging to neighboring proteins and separated by a sufficiently thin layer of water could be correlated to some extent.

The existence of this correlation was indeed detected by us.[21,22] Additionally, we suggested that the network of hydrogen bonds spanning from one protein surface to the other may be involved in this process.[21] Ahmad *et al.*[55] demonstrated that two hydrophilic planes of associating proteins are connected with many chains of hydrogen bonds, that run through interfacial solvation water. It might have been expected that these links could contribute to the inter-protein interactions. This supposition was supported by our results obtained when using modified water models.[21] One modified water model that was used, on average, created more hydrogen bonds which led to the higher cross-correlation coefficients. This emphasizes the role of the properties of water in intermolecular interactions. Nevertheless, this effect is relatively weak, therefore the question as to whether it is of any real significance remains open.

(2) The change of the frequency pattern of internal vibrations of a protein molecule caused by the presence of another molecule

This effect was also observed mainly in the low-frequency range.[21,22] In contrast to the first phenomenon, this one is much more pronounced – the frequency changes by as much as several percent. Since the functioning of proteins strongly depends on the dynamics of the internal movements,[56] this effect may have some important biological implications. It is said that the most important and characteristic features of protein dynamics can be described by a limited number of modes (up to several dozen out of many thousands).[57–59] Such modes are the modes of the highest amplitudes (and lowest frequencies). This frequency range includes the one that is modified by the presence of additional proteins.

The fundamental question is what is the factor responsible for this change and how exactly does it lead to the modification of the characteristic pattern of the vibrations of a macromolecule?

Our model of a damped harmonic oscillator, as described in ref. 22, can be related to a protein in water as follows. Let us assume, hypothetically, that we immersed in water a protein molecule that initially does not move at all – neither as a whole, nor are there any internal vibrations. As a result of constant collisions with the surrounding molecules of the solvent, the protein will be stimulated to oscillate, but different vibrational modes will be stimulated in different degrees, depending on the spectral characteristics of the translational motions of the water molecules from the solvation shell.[60] If the spectral characteristics are changed, then the pattern of vibration of the oscillator is changed as well.

To explain this phenomenon better, we should start with an explanation as to why the translational velocity power spectrum is changed in solvation water in the first place. The spectrum of the solvation shell is shifted to greater frequencies (blue shift)[35] than that of bulk water. The highest band of the spectrum has a maximum at about 50 cm^{-1}. This is explained by the "cage effect" – a molecule of water moves in a cage created by surrounding water molecules. The remaining bands, at about 150, 200 and 250 cm^{-1} are commonly associated with the existence of hydrogen bonds between water molecules.[61–63] The blue shift is usually best visible

for the first band, which is attributed to "caging". As a result of the shift, the value at the frequency equal to zero also diminishes. Because this value is a direct measure of the translational diffusion coefficient, we can say that the slowed-down diffusional motion is the best known manifestation of this shift. The molecular origin of the enhanced "caging" can be understood if we remember that the solvation water of proteins is, generally, said to be more dense than bulk water, although the data are not always unanimous in this regard.[64–67]

The number of hydrogen bonds between water molecules in solvation shells can be greater (after we account for the excluded volume occupied by the protein) than in bulk water.[68] Also, hydrogen bonds tend to become, on average, a little shorter.[35]

These features explain the blue shift of the translational velocity power spectrum. The increase of the density of solvation water means that the potential energy of interactions is higher. This results in the increased "stiffness" of the structure of water.

The reasons for the increased density can be proposed by resorting to the discussion on the unusual structure of bulk water. This vast subject has been tackled from many points of view. One of the approaches, that is able to explain many properties of this liquid, was proposed by Tanaka.[69–72]

Tanaka postulated that the structure of water should be considered as a mixture of ordered, symmetrical, high-volume structures stabilized by hydrogen bonds and high-density structures, without any particular geometry. Molecules of water interact with each other in a directional (anisotropic hydrogen bonds) and nondirectional (isotropic) manner. The "local number density of locally favored HB structures"[69] depends on the temperature. When the temperature drops, two opposing tendencies emerge. On one hand, there is a tendency to pack the molecules closely (thus maximizing the density). On the other hand, the number of hydrogen bonds increases, which results in an increase in the number of ordered structures and leads to a decrease in density. As a result of these two competing tendencies, the physical properties of water are unique. One of these properties is, for example, the well-known density maximum of water at 4 °C.

The Tanaka model does not precisely describe what the high-volume structures look like. It is suspected that they resemble little seeds of the ice phase.[71] These could be, for example, six-membered rings or octameric units.[71,73]

In our previous paper[67] we proposed a mechanism that used some concepts from the Tanaka model and explained the increase in the density of solvation water. According to this mechanism, near the surface of the protein, the number of high-volume structures diminishes. The factors that may lead to the disruption of these structures include: the electric field of the protein, the interactions with the surface of the protein, and, especially, the excluded volume occupied by the protein. The creation of high-volume ordered structures near the surface of the protein, where some space is unavailable for water molecules, may be less probable than elsewhere.

The results obtained recently by Jahn and Gekle[74] support this hypothesis. The authors found that there are many loops in bulk water. The loops created by six and seven molecules were more numerous than the loops of any other size. However, near the model hydrophilic surface, the most represented rings were those created by five molecules. There was also an increase in the number of rings created by four molecules. The authors also observed that the rings tended to arrange parallel to the solvated surface.

When we introduce another biomolecule to the system, the delicate balance between order and disorder in water is distorted. The nearby presence of another protein can lead to the overlapping of two solvation shells of two separate biomolecules[35] and to the modification of the local electric field.[35,66] These factors are able to modify the spectral characteristics of the translational motion of solvation water.

Solvation water between the kinesin head and the tubulin dimer also displays increased density,[35] and the dynamics of solvation water can be changed more significantly than in the case of a single protein. These changes were observed, on average, even when the proteins were 2 nm away from each other. In this case, the factor that contributes to the decrease of the number of high-volume structures may also be the electric field generated by the protein molecules.[35]

In ref. 35, we also described the results of calculations of differential local ordering parameters, as defined by us. These parameters were calculated for the entire interfacial water present between the kinesin head and the tubulin dimer (the exact definition of this solvation shell can be found in ref. 35). The parameter that described the differences in the conformational ordering relative to bulk water indicated that the ordering of the solvation water increased. This result may seem to contradict with the discussion on the Tanaka model, but it does not. The increased ordering can be associated with the increased stiffness of the structure. It does not mean, however, that the structure of water in this region is more ice-like and that its density is lower than in bulk water.

The density increase between two proteins is not always observed. In the case of water between associating hydrophobic proteins, sometimes a density decrease is reported.[75] For hydrophilic proteins, such as the ones investigated here, an increase in water density is observed.[55] It is possible that because of the different properties of solvation water, a very hydrophobic macromolecule would exert a different influence on the dynamics of the neighboring protein. This may be a question for a future study. In any case, the structure and dynamics of the solvation water of a protein molecule will be affected if another macromolecule approaches.

Conclusions

Our aim was to explain as fully as possible how water can mediate interactions between proteins present at a set distance from each other and make them feel the presence of each other. The long-range influence manifests itself by characteristic changes in the dynamics of two neighboring proteins, observed for low-frequency vibrations. In the discussion, we used new results obtained for the kinesin head and the tubulin dimer. We also referred to some previously published results obtained for these proteins, as well as for a hyperactive antifreeze protein

*Cf*AFP, in an attempt to logically link them together. Our previously described[22] model of a damped harmonic oscillator subjected to an external random force turned out to be roughly applicable to the motions of the kinesin head in the presence of the tubulin dimer.

We were able to propose a molecular mechanism that explains the role of water in this phenomenon of interdependent dynamics by taking into account various structural and dynamical properties of solvation water. In liquid water, an equilibrium exists between two tendencies: the first one is to maximize the packing of the molecules and the second one is to maximize the ordering of water molecules by creating hydrogen bonds. The second tendency leads to the creation of symmetrical, ordered microstructures of water molecules. As a result, in water there exists a very subtle balance between order and disorder. This balance depends not only on temperature but also on many other external (with regard to the protein molecule) factors, such as the electric field or the presence of biomolecules. When the balance is disturbed, the result is a change in density and a change in the structure of solvation water, which is also associated with a change in the dynamics of water.

Of course, the degree of disturbance depends on the properties of the solvated surface, such as its geometry and chemical character.[68] It also depends on the electric field generated by the surrounding macromolecules or the overlapping of two solvation shells (of two separate molecules).

As a result, the inner dynamics of two protein molecules change when the molecules are brought closer to each other. The effect is noticeable even at a 2 nm distance between the proteins. The medium that enables these long-range interactions is solvation water present at the interface. The electric field generated by the biomolecules and the overlapping of the solvation shells lead to a change in the number of high-volume structures in water. The change in the density has implications on the changes of the spectral characteristics of the translational motion of water. This factor influences the dynamics of the protein vibrations. It may be said that this effect is in fact a consequence of the special properties of liquid water.

Acknowledgements

This work was supported by the Polish National Science Centre within the research grant no. DEC-2013/09/D/NZ1/01087. The calculations were carried out at the Academic Computer Center (TASK) in Gdańsk. This research was supported in part by the PL-Grid Infrastructure.

References

1 M.-C. Bellissent-Funel, A. Hassanali, M. Havenith, R. Henchman, P. Pohl, F. Sterpone, D. van der Spoel, Y. Xu and A. E. Garcia, *Chem. Rev.*, 2016, **116**, 7673–7697.
2 V. Helms, *ChemPhysChem*, 2007, **8**, 23–33.
3 A. R. Bizzarri and S. Cannistraro, *J. Phys. Chem. B*, 2002, **106**, 6617–6633.
4 P. Ball, *Chem. Rev.*, 2008, **108**, 74–108.
5 P. Ball, *ChemPhysChem*, 2008, **9**, 2677–2685.
6 J. A. Rupley and G. Careri, *Adv. Protein Chem.*, 1991, **41**, 37–172.
7 P. Ball, *Nature*, 2011, **478**, 467–468.
8 Y. Levy and J. N. Onuchic, *Annu. Rev. Biophys.*, 2006, **35**, 389–415.
9 P. Mentré, *J. Biol. Phys.*, 2012, **38**, 13–26.
10 S.-H. Chong and S. Ham, *Angew. Chem., Int. Ed.*, 2014, **53**, 3961–3964.
11 P. Jungwirth, *J. Phys. Chem. Lett.*, 2015, **6**, 2449–2451.
12 E. Persson and B. Bertil, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 6266–6271.
13 Y. Wang, M. Sarkar, A. E. Smith, A. S. Krois and G. J. Pielak, *J. Am. Chem. Soc.*, 2012, **134**, 16614–16618.
14 A. C. Miklos, M. Sarkar, Y. Wang and G. J. Pielak, *J. Am. Chem. Soc.*, 2011, **133**, 7116–7120.
15 A. C. Miklos, C. Li, N. G. Sharaf and G. J. Pielak, *Biochemistry*, 2010, **49**, 6984–6991.
16 T. Ando and J. Skolnick, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 18457–18462.
17 E. H. McConkey, *Proc. Natl. Acad. Sci. U. S. A.*, 1982, **79**, 3236–3240.
18 A. J. Wirth and M. Gruebele, *BioEssays*, 2013, **35**, 984–993.
19 A. Gershenson, *J. Mol. Biol.*, 2014, **426**, 4–6.
20 P. Chien and L. M. Gierasch, *Mol. Biol. Cell*, 2014, **25**, 3474–3477.
21 A. Kuffel and J. Zielkiewicz, *Phys. Chem. Chem. Phys.*, 2015, **17**, 6728–6733.
22 A. Kuffel and J. Zielkiewicz, *Phys. Chem. Chem. Phys.*, 2016, **18**, 4881–4890.
23 O. Kučera and M. Cifra, *J. Biol. Phys.*, 2016, **42**, 1–8.
24 K. Teilum, J. G. Olsen and B. B. Kragelund, *Cell. Mol. Life Sci.*, 2009, **66**, 2231–2247.
25 M. A. Iakhiaev and A. V. Iakhiaev, *Comput. Biol. Chem.*, 2013, **47**, 89–95.
26 G. Acbas, K. A. Niessen, E. H. Snell and A. G. Markelz, *Nat. Commun.*, 2014, **5**, 3076.
27 H. M. Müller-Werkmeister and J. Bredenbeck, *Phys. Chem. Chem. Phys.*, 2014, **16**, 3261–3266.
28 K. A. Niessen, M. Xu and A. G. Markelz, *Biophys. Rev.*, 2015, **7**, 201–216.
29 R. Brust, A. Lukacs, A. Haigney, K. Addison, A. Gil, M. Towrie, I. P. Clark, G. M. Greetham, P. J. Tonge and S. R. Meech, *J. Am. Chem. Soc.*, 2013, **135**, 16168–16174.
30 J. Smythies, *Front. Mol. Neurosci.*, 2015, **8**, 38.
31 T. Yonetani and M. Laberge, *Biochim. Biophys. Acta*, 2008, **1784**, 1146–1158.
32 M. G. Galinato, J. G. Kleingardner, S. E. Bowman, E. E. Alp, J. Zhao, K. L. Bren and N. Lehnert, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 8896–8900.
33 I. Bahar, T. R. Lezon, A. Bakan and I. H. Shrivastava, *Chem. Rev.*, 2010, **110**, 1463–1497.
34 H. J. C. Berendsen, J. R. Grigera and T. P. Straatsma, *J. Phys. Chem.*, 1987, **91**, 6269–6271.
35 A. Kuffel and J. Zielkiewicz, *Phys. Chem. Chem. Phys.*, 2013, **15**, 4527–4537.
36 D. van der Spoel, P. J. van Maaren and H. J. C. Berendsen, *J. Chem. Phys.*, 1998, **108**, 10220–10230.

37 P. Mark and L. Nilsson, *J. Phys. Chem. A*, 2001, **105**, 9954–9960.
38 D. P. Tieleman and H. J. C. Berendsen, *J. Chem. Phys.*, 1996, **105**, 4871–4880.
39 D. S. Cerutti, P. L. Freddolino, R. E. Duke, Jr. and D. A. Case, *J. Phys. Chem. B*, 2010, **114**, 12811–12824.
40 B. Hess and N. F. A. van der Vegt, *J. Phys. Chem. B*, 2006, **110**, 17616–17626.
41 F. J. Kull, E. P. Sablin, R. Lau, R. J. Fletterick and R. D. Vale, *Nature*, 1996, **380**, 550–555.
42 J. Löwe, H. Li, K. H. Downing and E. Nogales, *J. Mol. Biol.*, 2001, **313**, 1045–1057.
43 C. V. Sindelar and K. H. Downing, *J. Cell Biol.*, 2007, **177**, 377–385.
44 D. A. Case, T. A. Darden, T. E. Cheatham, III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, R. C. Walker, W. Zhang, K. M. Merz, B. Roberts, S. Hayik, A. Roitberg, G. Seabra, J. Swails, A. W. Götz, I. Kolossváry, K. F. Wong, F. Paesani, J. Vanicek, R. M. Wolf, J. Liu, X. Wu, S. R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M.-J. Hsieh, G. Cui, D. R. Roe, D. H. Mathews, M. G. Seetin, R. Salomon-Ferrer, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko and P. A. Kollman, *AMBER 12, Reference manual*, University of California, San Francisco, 2012.
45 Y. Duan, C. Wu, S. Chowdhury, M. C. Lee, G. M. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T. Lee, J. Caldwell, J. M. Wang and P. Kollman, *J. Comput. Chem.*, 2003, **24**, 1999–2012.
46 V. Conti Nibali, G. D'Angelo, A. Paciaroni, D. J. Tobias and M. Tarek, *J. Phys. Chem. Lett.*, 2014, **5**, 1181–1186.
47 P. W. Fenimore, H. Frauenfelder, B. H. McMahon and F. G. Parak, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 16047–16051.
48 P. W. Fenimore, H. Frauenfelder, B. H. McMahon and R. D. Young, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 14408–14413.
49 H. Frauenfelder, P. W. Fenimore, G. Chen and B. H. McMahon, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 15469–15472.
50 H. Frauenfelder, G. Chen, J. Berendzen, P. W. Fenimore, H. Jansson, B. H. McMahon, I. R. Stroe, J. Swenson and R. D. Young, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 5129–5134.
51 B. H. McMahon, H. Frauenfelder and P. W. Fenimore, *Eur. Phys. J.: Spec. Top.*, 2014, **223**, 915–926.
52 N. Shenogina, P. Keblinski and S. Garde, *J. Chem. Phys.*, 2008, **129**, 155105.
53 S. Khodadadi, J. H. Roh, A. Kisliuk, E. Mamontov, M. Tyagi, S. A. Woodson, R. M. Briber and A. P. Sokolov, *Biophys. J.*, 2010, **98**, 1321–1326.
54 M. Heyden and D. J. Tobias, *Phys. Rev. Lett.*, 2013, **111**, 218101.
55 M. Ahmad, W. Gu, T. Geyer and V. Helms, *Nat. Commun.*, 2011, **2**, 261.
56 K. Henzler-Wildman and D. Kern, *Nature*, 2007, **450**, 964–972.
57 A. Ramanathan and P. K. Agarwal, *J. Phys. Chem. B*, 2009, **113**, 16669–16680.
58 A. Amadei, A. B. Linssen and H. J. Berendsen, *Proteins*, 1993, **17**, 412–425.
59 D. Vitkup, D. Ringe, G. A. Petsko and M. Karplus, *Nat. Struct. Mol. Biol.*, 2000, **7**, 34–38.
60 S. H. Crandall and W. D. Mark, *Random Vibration in Mechanical Systems*, Academic Press, New York, London, 1963.
61 M. Cho, G. R. Fleming, S. Saito, I. Ohmine and R. M. Stratt, *J. Chem. Phys.*, 1994, **100**, 6672–6683.
62 A. Idrissi, F. Sokolić and A. Perera, *J. Chem. Phys.*, 2000, **112**, 9479–9488.
63 A. Idrissi and P. Damay, *J. Non-Cryst. Solids*, 2006, **352**, 4486–4489.
64 D. I. Svergun, S. Richard, M. H. Koch, Z. Sayers, S. Kuprin and G. Zaccai, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 2267–2272.
65 N. Smolin and R. Winter, *J. Phys. Chem. B*, 2004, **108**, 15928–15937.
66 F. Merzel and J. C. Smith, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 5378–5383.
67 A. Kuffel and J. Zielkiewicz, *J. Phys. Chem. B*, 2012, **116**, 12113–12124.
68 A. Kuffel and J. Zielkiewicz, *Phys. Chem. Chem. Phys.*, 2012, **14**, 5561–5569.
69 H. Tanaka, *Phys. Rev. Lett.*, 1998, **80**, 5750–5753.
70 H. Tanaka, *J. Phys.: Condens. Matter*, 1999, **11**, L159–L168.
71 H. Tanaka, *Europhys. Lett.*, 2000, **50**, 340–346.
72 H. Tanaka, *J. Chem. Phys.*, 2000, **112**, 799–809.
73 F. H. Stillinger, *Science*, 1980, **209**, 451–457.
74 M. Jahn and S. Gekle, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2015, **92**, 052130.
75 R. Zhou, X. Huang, C. J. Margulis and B. J. Berne, *Science*, 2004, **305**, 1605–1609.

