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Dynamics of water interacting with biomolecules

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1 INTRODUCTION

1.1 WATER

The most familiar liquid to humanity is undoubtedly water. It is also a liquid that continues to be the subject of intense study. Despite its chemical simplicity, a single oxygen atom with two hydrogen atoms, water has some unusual chemical properties. It has an anomalously high melting and boiling point, high surface tension, high heat capacity and a maximum density in the liquid phase, to name a few of the more than 70 anomalies of water^{1,2}. Exactly these peculiarities (or rather, the underlying reasons for these) make water such an excellent solvent, reactant and energy transporter, and very important in biology, as we will see further on.

Most anomalous properties of water arise from water's tendency to form hydrogen bonds. This tendency in turn arises from the distribution of electrons in a water molecule. A water molecule has four pairs of valence electrons, two of which are shared between the oxygen and the hydrogens to form covalent bonds, and two lone pairs. Since all electrons repel each other, the most stable configuration is a tetrahedron, with the hydrogen atoms residing in two corners, and the lone pairs smeared out over the other two (fig. 1.1A). (Because of the slightly higher electrostatic repulsion between the lone pairs, the angle between the two OH bonds is 106° in liquid water³, which is smaller than the 109° of a perfect tetrahedron.) While a water molecule has no net charge, the greater electronegativity of oxygen causes the electrons to be distributed unequally, resulting in a partial positive charge on the hydrogen atoms and a partial negative charge on the oxygen atom. As a consequence, the hydrogen atom of one water molecule is attracted to the oxygen lone pairs of another water molecule:

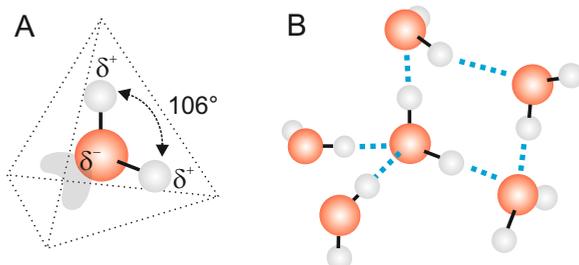


FIGURE 1.1. (A) Structure of a water molecule. (B) Hydrogen-bond network of water.

water forms hydrogen bonds (fig. 1.1B). A hydrogen atom can donate a single hydrogen bond, and an oxygen atom can accept two (one at each lone pair), such that each water molecule can participate in up to four hydrogen bonds. Each bond is highly directional, with the $\text{OH}\cdots\text{O}$ hydrogen bond being parallel to the O-H bond, leading to the formation of an interconnected tetrahedral network of water molecules. This hydrogen-bond network explains the extraordinary properties of water mentioned earlier: the strong attraction between the molecules raises the surface tension and the melting and boiling points, and the large amount of energy that can be stored in the hydrogen-bond network leads to a larger heat capacity.

In ice, each water molecule forms exactly four hydrogen bonds. In liquid water, however, the hydrogen-bond network is more disordered, with each water molecule forming on average 3.6 hydrogen bonds⁴. These hydrogen bonds are by no means static: water molecules move around on a picosecond (10^{-12} s) timescale, and hydrogen bonds are continuously being broken and reformed⁵⁻¹⁰. This process involves rapid switching of hydrogen-bond partners (<200 fs) and re-arrangement of the larger hydrogen-bond network^{5,11-13}. As a result of these rapid motions, water can quickly adjust to changes in environment, which makes it an excellent solvent for reactions¹⁴.

In fact, water is an excellent solvent in general. Water interacts most favorably with compounds that it can form hydrogen bonds to, that is, molecules with hydrogen atoms covalently bound to a strongly electronegative atom (O, N or F). In addition, water forms hydrogen bonds to charged species like ions and - to a lesser extent - to polar molecules in general, as a result of its large dipole moment of 1.85 D¹⁵. Compounds that interact favorably with water are called hydrophilic, and are easily solvated by water. In contrast, compounds that water can hardly interact with, like apolar molecules, are called hydrophobic, and tend to cluster in an aqueous environment. The unfavorable hydrophobic interaction arises from the fact that in order to accommodate apolar molecules, water needs to make room for them¹⁶. For small hydrophobic solutes (<1 nm) the hydrogen-bond network can fold around the solute, and the energy penalty is mainly entropic. To accommodate larger hydrophobes, hydrogen bonds need to be broken and the energy penalty is larger and mainly enthalpic, with a cost of ~ 2.5 kJ/mol per hydrogen bond^{17,18}. As a result, hydrophobes like to stick together: water and oil do not mix.

1.2 WATER AND BIOMOLECULES

Life as we know it takes place in liquid water; the human body, for example, contains on average 65% of water. In fact, water seems so essential for life that astrophysicists get excited whenever liquid water is found somewhere in the universe. The fact is that water is not merely a passive solvent, but plays an active role in many biological processes^{19,20}. Understanding the subtle interplay between water and biomolecules is therefore crucial for understanding the mechanics and chemistry of life. A few cases, which will feature later in this

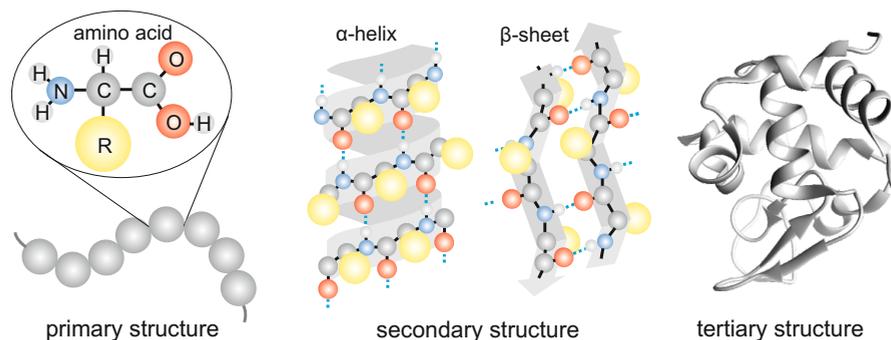


FIGURE 1.2. Structural levels of a protein. Each amino acid is defined by its side chain R. The sequence of amino acids (primary structure) determines the formation of specific hydrogen-bonded structures like α -helices and β -sheets (secondary structure), which in turn can fold into a compact shape (tertiary structure).

thesis, are highlighted here.

LIPID STRUCTURE AND ASSEMBLY

The interaction between water and phospholipids drives the self-assembly of phospholipid bilayers that make up the membrane of cells²¹. The phospholipids arrange themselves such that their hydrophilic head-groups are solvated by water, while their hydrophobic tails are clustered together, shielded away from the water. This results in an effective barrier between the inside and the outside of the cell, and enables cells to regulate in- and outward transport of materials. Another major class of lipids is the triglycerides, which are consumed as foods and constitute the main part of body fat, where they are responsible for energy supply and storage²². In analogy to phospholipids, it is expected that the interaction with water influences triglyceride structure, however this interaction has hardly been studied yet.

PROTEIN FOLDING AND FUNCTION

Water is crucial for the folding and function of proteins^{23,24}. Proteins are components of every living cell that perform many specific tasks, ranging from catalysis to material transport and the formation of a structural framework. The specific task of a protein is determined by its three-dimensional structure: Each protein consists of a linear chain of amino acids, the primary structure, which can form particular hydrogen-bonded segments in its secondary structure, and these segments in turn can pack in a specific way to form the tertiary structure of a protein (fig. 1.2). Nature has constructed an enormous amount of proteins, each with their specific function, from just 20 types of amino acids. The molecular mechanism by which proteins adopt their functional fold remains one of the fundamental questions in biochemistry²⁵.

One of the main drivers of the protein folding process is the unfavorable interaction between water and hydrophobic groups of the protein²³. Folding allows

the burial of hydrophobic groups inside the core of the protein, shielded away from the water, which is energetically favorable. In many cases, water plays a role via more specific interactions as well, for example by bridging different protein residues^{24,26}. These internal water molecules can contribute to the folding speed, stability and structure of the protein.

Besides influencing the protein structure, water can also contribute to the function of a protein directly. Many proteins need a minimal amount of hydration water in order to retain their normal catalytic activity^{27,28}. It has been suggested that this is due to the dynamics of hydration water driving protein motion²⁹⁻³¹. In addition, water can interact more specifically with proteins, for example to assist in molecular recognition and binding, allostery, and proton conduction³²⁻³⁴.

OSMOLYTES

Osmolytes are small organic molecules that enable cells to maintain a healthy amount of water³⁵. These molecules can strongly affect the three-dimensional structure of proteins as well; some osmolytes unfold proteins, whereas others stabilize the folded protein structure^{36,37}. Hence, many organisms use osmolytes to counteract chemical factors that might promote protein unfolding. Marine animals, for example, use trimethylamine N-oxide (TMAO) to deal with extreme deep-ocean pressures, and seeds of desert plants can survive in dry conditions due to the presence of certain sugars.

Osmolytes affect the structure of proteins by shifting the equilibrium between the folded and unfolded state. Protecting osmolytes like TMAO drive the equilibrium towards the native folded state, whereas denaturing osmolytes like urea and guanidinium favor the unfolded state. Even though this process is well understood from a thermodynamical viewpoint^{37,38}, a consistent picture of the molecular mechanism by which some osmolytes interact with proteins is still lacking. Proposed mechanisms include direct interactions between osmolyte and protein³⁹⁻⁴¹, as well as indirect effects, where osmolytes primarily modify the properties of the water solvent⁴²⁻⁴⁵. In both mechanisms, the effect of osmolytes on the structure of proteins cannot be understood without including the role of water.

1.3 SPECTROSCOPY OF WATER

To study the dynamics of water on the molecular scale, one can use spectroscopy: the interaction of water with light. While water is mostly transparent in the visible region, it strongly absorbs UV light due to electronic resonances, and mid-infrared light due to molecular vibrations. At room temperature, a water molecule vibrates; the hydrogen and oxygen atoms wiggle around like balls connected by springs. The vibrational resonances of water are of particular interest as they can supply information on the hydrogen-bond structure and dynamics of water. The frequencies of the main vibrational modes, the bend vibration and the OH stretch vibration, are very sensitive to hydrogen-bond strength^{8,46-48} (fig. 1.3). Formation of an OH \cdots O hydrogen bond weakens the

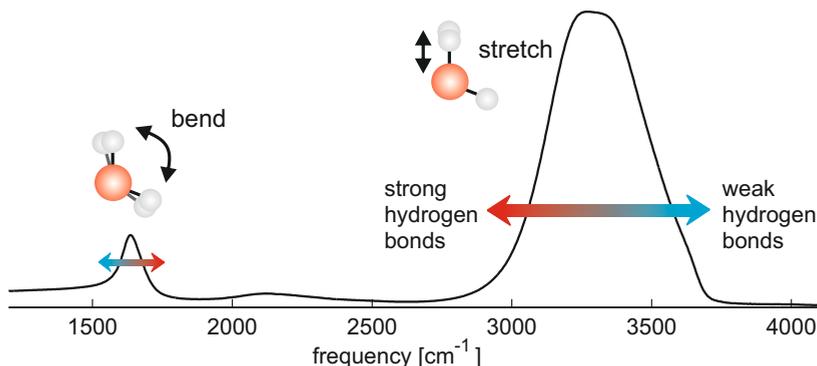


FIGURE 1.3. Infrared absorption spectrum of liquid water at 25°C in the infrared spectral region, indicating the main vibrational modes and their correlation with hydrogen-bond strength.

covalent OH bond, causing the OH stretch vibration to shift to lower frequencies. This is why the OH stretch frequency of liquid water, centered around 3404 cm^{-1} , is so much lower than the OH stretch frequency of water in the gas phase at 3657 cm^{-1} (symmetric stretch⁴⁹); in the former case water molecules are hydrogen-bonded while in the latter case they are not.

An additional difference between the spectrum of liquid water and that of water in the gas phase, is the broad spectral width. In the liquid phase, water molecules exist in many different configurations with different hydrogen-bond strengths, and as a result, the absorption spectrum of the OH stretch vibration is the sum of many different vibrational frequencies: it is inhomogeneously broadened^{9,48,50,51}. Because of the rapid movement of water molecules, these different hydrogen-bonded water molecules quickly interconvert, and the water absorption spectrum presents a time-averaged picture of the hydrogen-bond network of water. To investigate the time-evolution of the hydrogen-bond network, one has to use nonlinear spectroscopy. In this case the water molecules interact with multiple light pulses, making it possible to excite particular vibrations with one pulse and follow their behavior over time with another.

1.4 OUTLOOK

This thesis focuses on the interactions between water and different biomolecules, studied by vibrational spectroscopy. The next three chapters take a closer look at vibrational spectroscopy, with chapter 2 highlighting the theory, chapter 3 focusing on the experimental implementation, and chapter 4 describing the analysis of spectra. After that, we get to the core of the thesis. Chapter 5 describes the effect of sugars on the reorientation dynamics of water. Chapter 6 focuses on water reorientation dynamics in solutions of globular proteins. In

these solutions, a fraction of the water is strongly slowed down by the interaction with the protein surface, which we use to follow the exposure of the protein to water upon urea and temperature-induced unfolding. In chapter 7 we describe the interaction between water and a special type of protein: the antifreeze glycoprotein, which enables the survival of arctic fish by preventing the growth of ice crystals. Finally, in chapter 8 and 9, water is no longer the solvent, but a solute in a matrix of triglyceride oil, which we find to lead to specific water hydrogen-bond configurations and dynamics.