Water Mediation in Protein Folding and Molecular Recognition

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Abstract

Water is essential for life in many ways, and without it biomolecules might no longer truly be biomolecules. In particular, water is important to the structure, stability, dynamics, and function of biological macromolecules. In protein folding, water mediates the collapse of the chain and the search for the native topology through a funneled energy landscape. Water actively participates in molecular recognition by mediating the interactions between binding partners and contributes to either enthalpic or entropic stabilization. Accordingly, water must be included in recognition and structure prediction codes to capture specificity. Thus water should not be treated as an inert environment, but rather as an integral and active component of biomolecular systems, where it has both dynamic and structural roles. Focusing on water sheds light on the physics and function of biological machinery and self-assembly and may advance our understanding of the natural design of proteins and nucleic acids.

Contents

Funneled energy landscape: because of minimal frustration, folding can be described as a progressive organization of an ensemble of partially folded structures

TSE: transition state ensemble

PERSPECTIVES AND OVERVIEW

The funneled energy landscape of evolutionarily selected proteins governs their robust ability to efficiently organize the polypeptide chain into a specific structure (16, 86, 105, 106). Theoretical and simulation strategies based on the funnel concept impressively reproduce the experimental characterization of folding mechanisms, intermediate(s), transition state ensemble (TSE), and folding rates (22, 28, 43, 51, 80). The experimental mechanisms and kinetics of protein associations have been recently obtained using a funneled energy landscape (89, 94), illustrating that binding, similar to folding, follows the principle of minimal frustration (17). The funnel landscape idea implies the notion, now well accepted as a general guideline, that because energetic frustration for folding/binding is relatively small in natural sequences, the native topology determines the mechanism of biological self-organization processes. However, it is obvious that the appropriate environment is conditional to all recognition processes in the cell. This review presents a survey of the many ways water properties are exploited in biology with a special emphasis on the dynamic role water has in gating folding and binding, where it actively assists the search through the funneled landscape.

Water is a remarkable chemical compound that has been long appreciated to be absolutely fundamental for life. Indeed, water is one of the four Aristotelian elements and its importance to life is well reflected by its sanctity in many religions and myths. Water is life's true and unique medium, and without it life simply cannot be sustained. It is therefore of high interest to biologists, chemists, physicists, as well as cosmologists (6). Water is the fluid that lubricates the workings of the cell, transporting the materials and molecular machinery and facilitating the chemical reactions. Yet, water also plays an active and complex role in the life of the cell, to the extent that water itself can be considered a biomolecule because without it the cell function would cease to exist. Without water, biomolecules such as proteins and nucleic acids might no longer truly be biomolecules. When dealing with proteins and genes in modern molecular biology, we should not ignore that it is all about the interactions of such molecules in and with water. Truly, water's function in the cell is far beyond that of an inert solvent.

Water has had an active role in the evolution of life, constitutes about 70% of the human body, and covers about 75% of the earth. An astronomer or a nuclear physicist

would not find the ubiquity of water in biochemistry a total surprise. Our understanding of the origin of the elements is consistent with their observed abundances in the universe. Clearly, hydrogen (whose name means "water former") and oxygen, which are the most and third-most abundant elements, respectively, are major candidates for chemical combination. The second-most abundant element, helium, is not reactive. Hydrogen peroxide, H_2O_2, is rather unstable. Hydrogen oxide, H_2O, must be present on planets. Yet its abundance alone cannot explain its role in life. This curious molecule has rather extraordinary properties, which are quite different at low and high temperatures. It has often been stated that life depends on these anomalous properties of water (a few examples include its unusually large heat capacity, high melting and boiling points, high thermal conductivity and surface tension, and shrinking on melting). Fluidity seems to be essential for active life, and completely solid-phase life, if it exists, must be very slow in its actions. It is unclear if other liquids (e.g., other hydrides, oxides, or hydrocarbons) can replace water and be compatible with the existence of complex molecules, maintaining their integrity to bear information and self-organize. Clearly, some media seem to present fundamental problems from a physical point of view: For molecular life we need a fluid in which chemical bonds are stable. So far no other milieu has been demonstrated as a viable alternative.

Despite the simple structure of water and its obvious importance, it is still poorly understood and many of its aspects, either as a pure substance or as a solvent, are controversial. An infamous example that highlights both our interest and ignorance of water is the mistaken discovery of "polywater" to explain its perplexing properties. Some have even suggested the notion that water molecules have memory (32, 65). Beyond pure water, the properties of water in the cytoplasm are also a matter of debate. It is usually believed that water in the cell is like bulk water; however, others think that its structure is modified by the presence of many macromolecules and surfaces. Some have postulated that cell water is more like a gel. Others believe that it is strongly inhomogeneous owing to the presence of dissolved ions. Recently, it has been proposed that water very near hydrophobic surfaces is vapor-like. This may suggest that such surfaces are relatively dry and provide an explanation for the long-ranged attraction between hydrophobic surfaces.

The interactions between water and proteins and nucleic acids at the molecular level are also a topic of a major interest with the ultimate goal of understanding cellular function. A variety of experimental methods have been used to study the weak interactions between water and proteins. For example, differential scanning calorimetry, neutron diffraction (125), femtosecond fluorescence (109, 110), NMR spectroscopy (36, 45, 107, 135), and X-ray crystallography measurements are often used to study the binding sites, structure, and dynamics of water. However, some of the methodologies probe water indirectly or have other shortcomings. For example, X-ray crystallography detects only structured and localized water molecules. At present the crystal structures of biological macromolecules are determined after rapid cooling to cryogenic temperatures at which artifacts may be present in the hydration pattern (60). Theoretical and computational approaches can aid and complement the experimental efforts to decipher the interplay between water and biomolecules by providing the microscopic and physical details.

In this review, we discuss the active role that water has in the structures and dynamics of proteins and nucleic acids. Then we discuss the role water has in biological self-assembly processes such as protein folding, protein-protein recognition, and protein-DNA binding. The presented discussion on these biological processes in the context of the interaction of the biomolecules with water is twofold. First, we use these biological processes to illustrate that water is not just an "environment" for biochemical reactions but

Principle of minimal frustration: natural protein sequences are evolutionarily selected to minimize interactions that are in conflict

Frustration: conflicting interactions arising from competition between two or more states that minimize a local part of the free energy

rather it is often an active player, justifying the treatment of water as a "biomolecule." Second, we discuss that by adding the water to the description of these processes, a better understanding of their physical principles can be achieved. Focusing on biomolecules alone while ignoring the environment likely is not sufficient to capture all their properties and binding capabilities.

WATER AND BIOMOLECULE STRUCTURES AND FUNCTIONS

Although not proven, it is widely accepted that water is essential to life. On the basis of this notion water has been called the matrix of life, but it is still questioned whether water has been "fine-tuned" for life and which of its unique properties are essential for life (7, 9). Nevertheless, there is no question that water plays an important role in biomolecular structures, dynamics, and functions, a fact nicely illustrated by treating water as the "twenty-first" amino acid. Indeed, water seems to be key in understanding the interplay between structure and function, which is a central goal in protein science.

Water and Protein Structure and Stability

The hydration forces are responsible for the packing and the three-dimensional structure of proteins, which in many cases is invaluable to the protein bioactivity. The aqueous solution (55 M concentration of water) dictates the hydrophobic force, which is the driving force for protein folding and other biological processes (e.g., aggregation of amphiphilic lipids into bilayers) (15, 37, 68, 116, 130). The conflict between the hydrophobic side chains and the polar nature of the water guides these groups to collapse and be shielded from water by forming a tightly packed core that contains more than 80% of the nonpolar side chains of a typical protein. This conflict is central for protein folding. The hydrophobic interactions, as proposed by Kauzmann (74), are driven by the unfavorable structural entropy decrease that can be caused by forming a large surface area of nonpolar groups with water. Water can additionally drive protein folding by the gain in translational entropy of water molecules bound to the protein in the unfolded state upon their release (62).

Water is essential for protein structure, not only with regard to defining the collapse of the hydrophobic core, but also in maintaining its stability and structure. Affecting the network of hydrogen bonds between water molecules influences the protein stability. Increasing the ordering of water by decreasing the temperature can result in protein denaturation (i.e., cold denaturation). Indeed, the first hydration shell around proteins is ordered and exhibits a density 10% to 20% higher than that exhibited by bulk water (23, 98). These water molecules have longer residence time than water molecules outside the first hydration shell (13, 46, 63, 87, 110, 123). Some of the water molecules are bound at specific locations and can be identified crystallographically and thus are an integral part of the protein structure. Protein crystals, which normally contain substantial amounts of water (up to 70%), show a wide range of nonrandom hydrogen-bonding environments. About 55% of the first hydration shell water is bound to the backbone and the rest to charged side chain. Some of these waters are in fixed positions and are observed every time the structure is determined, whereas others are in nonunique positions and reflect an ensemble of water-protein interactions that hydrate the entire surface and sometimes the protein core (see **Figure 1*a***). The water network around the protein links secondary structure elements and not only determines the fine detail of the structure but also explains how particular molecular vibrations may be preferred. An example of the importance of solvent dynamics and hydrogen bonding to proteins is the capability of some sugars (sucrose and trehalose) to replace water molecules upon dehydration of a variety of microscopic organisms that can

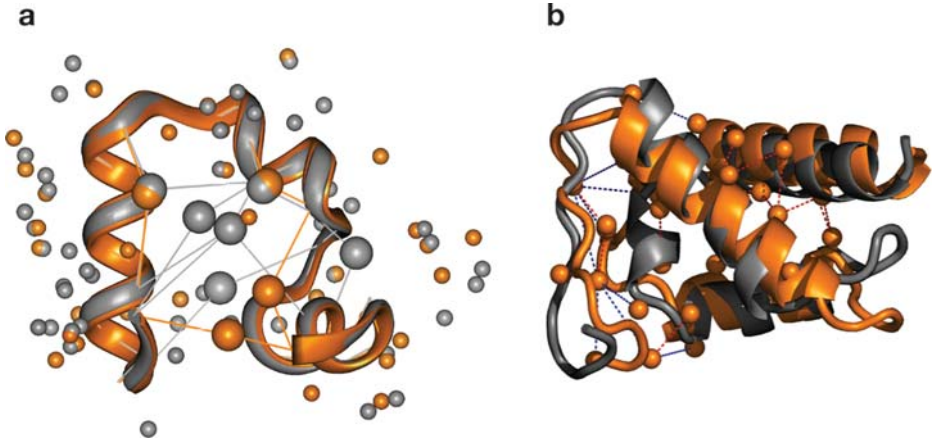

Figure 1

Water maintains protein three-dimensional structures. (*a*) High-resolution X-ray crystal structures of the villin headpiece subdomain at pH 5.1 (*orange*, 1.55 Å resolution, PDB code 1WY4) and pH 7.0 (*gray*, 0.95 Å resolution, PDB code 1WY3) (25). Waters that mediate ternary contacts (*thin lines*) are shown as large spheres, indicating that water is an integral part of the structure and that some waters are conserved in both crystallization conditions. (*b*) The prediction of the structure of CASP5 target T0170 is improved when using an optimized energy function that includes water-mediated interactions. The native and predicted structures are gray and orange, respectively. The virtual waters (defined by distance and the residue solvent accessibility) are shown as spheres.

restore their activity when rehydrated (a phenomenon known as anhydrobiosis) (30). The observation that glucose, for example, is inferior to trehalose in protecting proteins in dry conditions indicates that the preservation ability not only is a consequence of hydrogen bonds but also correlates with their glassy dynamics, which is important for maintaining internal water (124).

Not only do waters interact with the protein surface, but a few water molecules are often found trapped inside internal cavities of the protein (100). These water molecules can interact directly with the protein backbone and side chains in the protein interior (113) or even form clusters of two or more water molecules in hydrophobic cavities (45). The mean residence time is much longer for buried water molecules than for water in the first hydration shell (~500 ps for water in the first hydration shell and 10 ns to 1 ms in internal cavities) (35, 56, 107). Because bound water molecules make important interactions with groups that would otherwise make none, the waters in fixed positions should be considered an integral part of the tertiary structure, and any detailed structural description that does not include them is incomplete. Internal water sites in structurally homologous proteins are highly conserved (140), indicating that introducing buried water may improve the prediction of protein structures. Mutations can affect the number of structural water molecules within the core and disrupt the essential main chain interaction network mediated by ordered water contacts (29), resulting in destabilization. Yet, interior water molecule can escape to the bulk and be replaced by water from the hydration shell (56).

Disrupting the balance between intramolecular interactions within a protein and the hydrogen bond network with the solvent can result in protein destabilization. This principle is routinely used to denature proteins by adding substances such as urea and guanidinium chloride at high-enough concentrations that can unfold proteins (108, 141, 143). The molecular mechanism of protein

destabilization by denaturant is a matter of controversy. It is not our goal in this review to cover the different proposals but to argue that the solvent plays an important role in this process. Urea was suggested to promote unfolding by direct hydrogen bonding to the protein's polar groups, which can lead to the screening of intramolecular hydrogen bonds (18, 101, 144). Another scenario is one in which urea interacts with the nonpolar groups to displace a few water molecules from the solvation shell, resulting in a net entropy gain for the water and later unfolding of the protein (82, 150). Several lines of evidence exist for an indirect unfolding mechanism in which urea perturbs the water structure and dynamics by weakening the hydrogen bond network of the water and thus disorders the water structure so that hydrophobic molecules are more easily solvated (10, 55). In contrast to denaturants that destabilize folded proteins, some small organic solutes (osmolytes) are used in nature by a variety of organisms to increase protein stability upon osmotic or water stress, high hydrostatic pressures, and dehydration. The osmolyte trimethylamine-N-oxide, TMAO, was found in molecular dynamics study to stabilize the native state indirectly by ordering and strengthening the water structure, thereby inhibiting unfolding (3, 11, 145).

Water and Protein Dynamics and Function

In addition to being fundamental to protein structure, water is needed for protein function. Increasing hydration was reported to improve the catalytic activity of enzymes (39, 54). Water was suggested to play a role in allosteric regulation at the interface of complex subunits by acting like transmission units. Water as a proton donor and acceptor can also act as a reagent in biochemical processes, illustrating that it can play more than a purely structural role. Water's property as both donor and acceptor of protons is used in nature by the formation of "proton wires" from a chain of water molecules that is used in a variety of proteins (1). However, water's hydrolytic power and high nucleophilicity is disadvantageous in the context of the cell, as it can destroy and oxidize many functional groups. For example, some enzymes shield their substrates from aqueous solvent by taking advantage of conformational changes that close off the active site from contact with bulk solvent. The cell therefore must develop a strategy to prevent water from interfering in certain biochemical reactions such as protein synthesis. Water thus introduces design demands of the cellular machinery that control water activity.

The interplay between the protein environment and its activity likely corresponds to the flexibility of the protein, which is central to the conformational changes required for enzymatic activity. In solution, proteins possess a conformational flexibility that encompasses a wide range of hydration states not seen in the crystal. Water acts as a lubricant, easing the necessary changes of the hydrogen-bonding patterns responsible for fast conformational fluctuations (8, 142). There is high coupling between the protein motions and water dynamics, and it has been suggested that fluctuations of the hydration water can slave the protein dynamics and thus affect its function (2, 48, 99). The interplay between the protein and solvent complexity is an intriguing open question. Simulations and experiments suggest that the glass-like transition of a protein coincides with dynamical changes characteristic of a glass transition in the solvent. The solvent and the protein motions may be intimately coupled, such that as a protein is warmed through its glass transition temperature, the dynamics of the hydration shell awakens motions in the protein. For lysozyme it was suggested, on the basis of molecular dynamics simulations, that water coverage of about 50% of its surface, which corresponds to about 66% coverage of the purely hydrophilic regions, is needed to achieve its dynamics (104). Simulations have shown that adding water to the cavities of bovine pancreatic trypsin inhibitor and

barnase makes the proteins more flexible and increases the coupling between the motions of the water and the protein (103). Simulations have shown differences in the hydration between the redox states of cytochrome *c* with larger fluctuations upon oxidation, suggesting that a change in water structure in the hydrophobic pocket is important for undocking after oxidation (4).

Although water is important for function, there are several cases in which enzymes are functional in the absence of bulk water (e.g., halophiles that have adapted to life in high-salt solutions) (77, 78, 85). Addressing whether water is absolutely necessary for any form of life or if water is replaceable is difficult, mainly because life on earth has evolved in the presence of water (7).

Water and Nucleic Acids

As with proteins, the aqueous solution is critical to the conformation and function of nucleic acids (98) (**Figure 2**). Water constrains the conformation of a DNA molecule, as reflected by the transition from B-DNA to A-DNA upon dehydration. DNA undergoes conformational transitions in some polar solvents. The hydration of DNA depends not only on the DNA conformation but also on its sequence (42). The C-G base pairs were found to be more hydrated than T-A base pairs in both A and B conformations in both simulations and experiments (44). As for proteins, the DNA interior is mainly hydrophobic and stabilized by the stacking interactions between the consecutive base pairs, and its surface is rich with hydrophilic groups from the phosphates and sugars (19). While proteins can have hydrophilic residues in the core or hydrophobic residues at the surface, the core of nucleic acids is more uniform, as it is composed of the aromatic bases of each nucleotide. The fundamental forces that cause proteins and nucleic acids to fold to unique structures are the same; however, the energetic contributions from free energies of solvation for DNA are stronger.

Without water to screen the electrostatic repulsions between phosphate groups, the classic double-helical structure of DNA is no longer stable. In addition to hydrating the backbone phosphates, the waters in the grooves are ordered and vital to stability. Because of the regular repeating structure of DNA, hydrating water is held in a cooperative manner along the double helix in both the major and minor grooves. At high humidity at least 25 water molecules per base pair are tightly bound to the DNA. The water molecules are held relatively strongly in the first hydration shell, with residence times of about 1 ns. The water density in the first hydration shell is much larger than that in bulk water and is the outcome of the many strongly solvated sites. Changes in the hydrogen-bonding network between the hydration shell and DNA can assist ligand binding or release of ions. RNA molecules have a greater extent of hydration than DNA because of their extra oxygen atoms (i.e., ribose O2′) and unpaired base sites, suggesting an important role for structured water in RNA-RNA and RNA-protein recognition (40).

Water and Protein Structure Prediction

Physical approaches for predicting protein structure often focus on some heuristic potentials that simply acknowledge the existence of hydrophobic interactions. These models can be called dry models. Wolynes and coworkers (111) have recently incorporated a knowledge-based potential for water-mediated interactions in a Hamiltonian for structure prediction (described below). The many-body water knowledge-based potential revealed that water can stabilize proteins by bridging two hydrophilic or charged residues separated by relatively large distance. There is a substantial improvement in the predicted structures of several α-helical proteins when these water-mediated contacts are included, mainly for those with more than 115 residues.

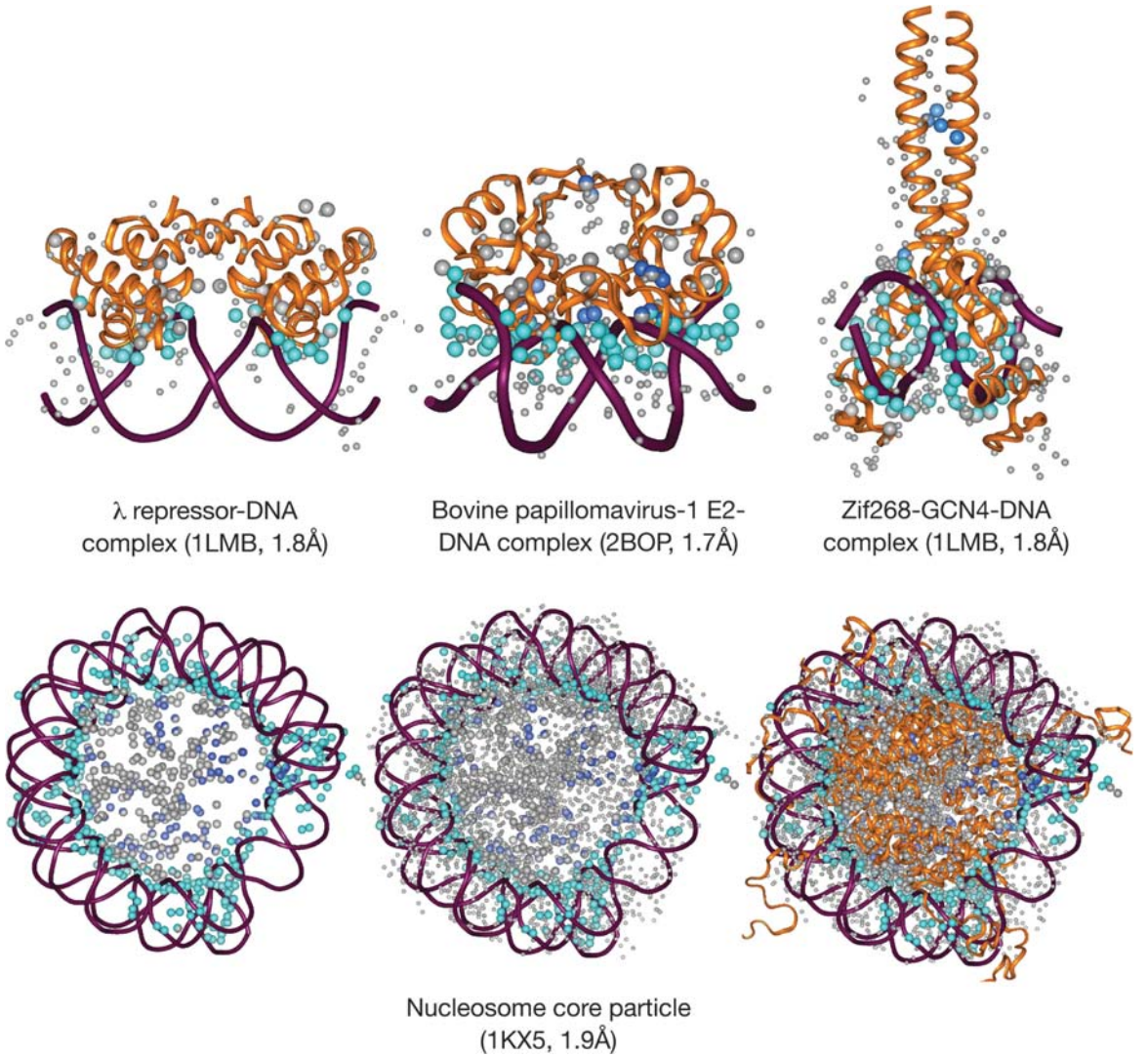

Figure 2

Waters mediate contacts in monomeric proteins, as well as protein-protein and protein-DNA interfaces. The proteins and DNAs are colored orange and purple, respectively. Waters that mediate protein-DNA interactions are shown as cyan spheres and those that mediate protein-protein interactions are shown as blue spheres. Water molecules that bridge intramolecular contacts within a single-protein chain are shown as large gray spheres and all other crystallographic waters are depicted as small spheres. Water is ubiquitous at protein-DNA interfaces (*top panel*) and may contribute to high specificity. The nucleosome is shown three times (*bottom panel*) illustrating the importance of mediation by water (*bottom*, *left*), its highly hydrated state (*bottom*, *center*), and the assistance of water in packing the histones (*bottom*, *right*). These examples nicely illustrate that water molecules are significant in mediating intra- and intermolecular interaction and in particular are extensive in protein-DNA interfaces.

Figure 1*b* shows the predicted structure of target T0170 from the CASP5, indicating the network of water-mediating interactions at the protein surface. Most notably is the improvement in the prediction of the loop structure using the water potential. Water, therefore, may play an important role in stabilizing loop structures and as such may be involved in the recognition of natively disordered proteins, which are often rich with hydrophilic residues. A recent all-atom simulation suggests that fluctuations in loop conformations can strongly affect protein hydration (31). In addition, a statistical study showed that internal water molecules in globular proteins preferentially reside near residues of loops and random coil regions (113). Long-range water-mediated interactions are vital in predicting α/β proteins as well (C. Zong, G. Papoian, J. Ulander, P. G. Wolynes, manuscript in preparation). Upon adding water that bridges solvent-exposed residues, the improved predicted structures suggest that incorporating water-mediated contacts between hydrophilic residues in protein design may result in proteins with enhanced stability.

The wet potential suggests that water does not only entropically drive the interactions of hydrophobic residues but also enthalpically promotes interactions between hydrophilic residues or charged residues, even of like charges. These interactions are important in the early stage of folding to guide the structural search by the formation of long-range contacts. Late events include the formation of short-range contacts and the exclusion of water from the protein interior. Water molecules can guide folding and facilitate packing of supersecondary structural elements by mediating long-range interactions between polar and charged amino acids, highlighting its role for folding and stabilizing large and multi-domain proteins. The water bridges polar groups on the protein surface. The water constrains the conformational freedom of the polypeptide chain and "smoothes" the funneled landscape.

THE DYNAMIC ROLE OF WATER IN PROTEIN FOLDING

Folding mechanisms, TSE, and even intermediates (at least for small- and medium-sized proteins) can be predicted using structure-based (Go) models that include a renormalized effect of solvent free energy (22, 27, 28, 80). This model removes energetic frustration and therefore extracts only the contributions from topological frustration (131). The excellent agreement between theory and experiments suggests that the native fold, or topology, plays the primary role in determining the folding mechanism and kinetics. These models hold true because natural proteins have a sufficiently reduced level of energetic frustration. Nevertheless, without an appropriate solvent description, one cannot explore the microscopic gating of folding by solvent.

Solvent Models in Molecular Dynamics Simulations: Implicit Versus Explicit Models

Introducing solvent effects into molecular dynamics simulations is mandatory to obtain a realistic understanding of biomolecules. The energy landscape of a protein can be drastically affected by changing the environment properties, suggesting different structure and altering the folding thermodynamics and kinetics (90, 138). The direct approach to incorporate water into the simulations of biomolecules is to explicitly include water molecules, which can be modeled in various ways. This approach significantly increases the size of the system by about one order of magnitude compared with the size of the solute alone, making the sampling of the conformation space under physiological conditions on a sensible timescale a nontrivial task. Nevertheless, applying elaborate sampling techniques (e.g., replica exchange sampling) can overcome the sampling limitation in explicit solvent simulations. Using the replica exchange method, Garcia & Onuchic (57) mapped the free-energy

Desolvation model: In this model any tertiary native contact can be either direct or mediated by a water molecule with a free-energy barrier separating them

landscape for folding of fragment B of protein A of *Staphylococcus aureus* in explicit water, suggesting that folding studies using explicit solvent are difficult but not impossible. The use of explicit water is valuable to gain insight into the discrete role of water in folding; however, the large computational demand imposed by such models does not necessarily equate to higher accuracy, as they include several shortcomings. For example, the water is often represented by rigid three-point charge models such as TIP3P, which has been parameterized to a single temperature (~298 K) and therefore poorly captures the temperature dependence of its properties. Introducing more elaborate models for water (e.g., adding polarization, more charge sites, and bond stretching and bending) (38) will naturally increase the computational demands.

In addition to developing new sampling methodologies of simulation with explicit solvent, another approach is to mimic water effects using simplified models (24, 47, 111, 122, 137). Implicit solvent models yield significant solvent efficiency because the solvent is modeled effectively as a function of the solute configuration alone, and therefore the need to average over the solvent degrees of freedom is overcome. Efforts to develop theories of implicit solvation have been ongoing for some time (47). These models are often based on Poisson-Boltzmann theory (i.e., continuum dielectric models such as generalized Born models) (5, 47), dielectric screening functions (84), or solvent-accessible surface area (41). In recent years, many efforts have been made to improve implicit solvent models, yet large differences still seen between implicit and explicit solvent model calculations question the degree to which implicit solvent models mimic the solvent environment (102, 119, 148).

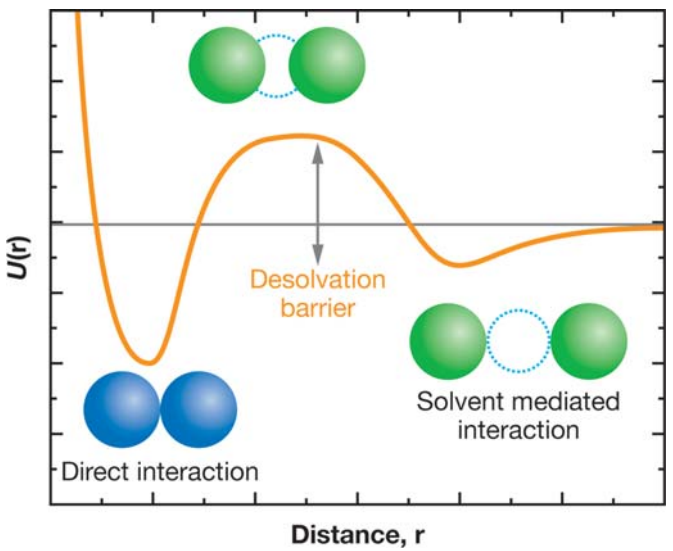

Figure 3

Schematic representation of the potential energy function, $U(r)$, in the desolvation model. In this model, any native interaction between two residues (*spheres*) can either be direct or separated by a water molecule (*light blue dashed circle*). The Cα-Cα distance of two residues that directly interact is defined by the native structure, and when a water molecule separates them the optimal distance increases by the diameter of the water molecule. At the desolvation barrier the water overlaps with the two residues.

It is beyond the scope of this review to cover the different approaches used to implicitly represent solvent effects on biomolecule dynamics and thermodynamics, but we discuss two reduced solvent models designed to incorporate mediating tertiary interactions via water. The desolvation and the water knowledge-based models focus on contact gating by water, but they also show several differences. The desolvation model focuses on the free-energy cost of bringing two nonpolar solutes into contact (24, 64, 114, 117). This free-energy penalty of contact formation is a direct result of the granularity of water molecules in the first hydration shell. Using this idea, Hillson et al. (64) have explained the non-Hammond pressure dependence of folding rates. This model suggests that the solvent gates the formation of individual pairwise contacts in folding. Accordingly, the phenomenological potential for each tertiary contact includes two minima of direct and mediated interactions, which are separated by the desolvation barrier (**Figure 3**). The desolvation model has been applied to protein folding in several studies that are discussed below.

Another approach to model water gating in biomolecular self-organization processes

is to construct a knowledge-based potential based on the occurrence of water-mediated interactions in a large nonredundant dataset of biomolecular structures. Because the number of water molecules in X-ray structures is underestimated (even at high resolution), the knowledge-based water potential cannot rely on crystallographic waters, but rather on a more general definition (91). Wolynes and coworkers (111, 112) have recently constructed such wet potentials for folding and binding using a physical bioinformatic approach. In this model, two residues can interact directly or indirectly via a water molecule that serves as a bridge between them. Tertiary interactions mediated by a water molecule exist if the distance between the C_β atoms of the two residues, which are exposed to solvent, is in the range of 6.5 to 9.5 Å (a typical distance between C_β atoms that directly interact is 4.5 to 6.5 Å). As the C_β-C_β distance between residues with large side chains can exceed 6.5 Å, the model also allows each pair of residues to interact directly, with a C_β-C_β distance of 6.5 to 9.5 Å (in a dry environment). The bioinformatics-derived occurrences of residue-residue contacts are optimized by maximizing the ratio of the folding/binding temperatures to the glass-transition temperature (i.e., minimizing frustration effects) (58).

The knowledge-based potential accounts for several central features of water-protein properties: inducing folding by mediating long-range interactions, protein stabilization by the first hydration shell, and the contribution of water to folding cooperativity. **Figure 4** shows the optimized potentials for forming all possible direct and indirect interactions in folding and binding for the 20 amino acids. The folding potential was calculated on the basis of a dataset of monomeric proteins and the water-mediated interactions are between two residues at the protein surface. For binding, however, a dataset of protein complexes has been used and the water mediates interfacial interactions. These potentials, for both folding and binding, illustrate that gating by water is highly favorable for polar and charged residues. This gating by water can increase specificity and stability.

The desolvation model and the wet knowledge-based potential for water-mediated interactions complement each other. Both models are pairwise potentials; however, the knowledge-based model includes cooperativity effect and therefore local frustration. The knowledge-based approach allows both native and nonnative interactions to be mediated by water, and in the current desolvation model only native interactions are treated. Moreover, the desolvation model focuses on the energy penalty in expelling a water molecule that bridges two residues, whereas the knowledge-based potential describes the enthalpic effect of water in stabilizing the native state. Note that both models do not account for buried waters in internal cavities.

Water Expulsion Versus Drying Effects in Folding

Water has a dynamical role in protein folding. A detailed investigation on the dynamical role of water in folding is available for the SH3 domain, for which both minimalist model and atomistic simulations have been used to explore the microscopic properties of water during folding. The desolvation model, which focuses on the energy cost of expelling waters that mediate any native contact, suggests that the folding of the SH3 protein is a two-step process: First, the fully solvated SH3 protein undergoes an initial structural collapse to an overall native topological conformation (funneling landscape), followed by a second transition in which water molecules are cooperatively squeezed out from the hydrophobic core region, resulting in a dry and packed protein (24). Folding, thus, is achieved through a TSE that is highly hydrated but has a native-like structure. The water acts as a lubricant that enables the hydrophobic core to find its optimally packed state, and it can play a role in preventing nonnative contacts from forming.

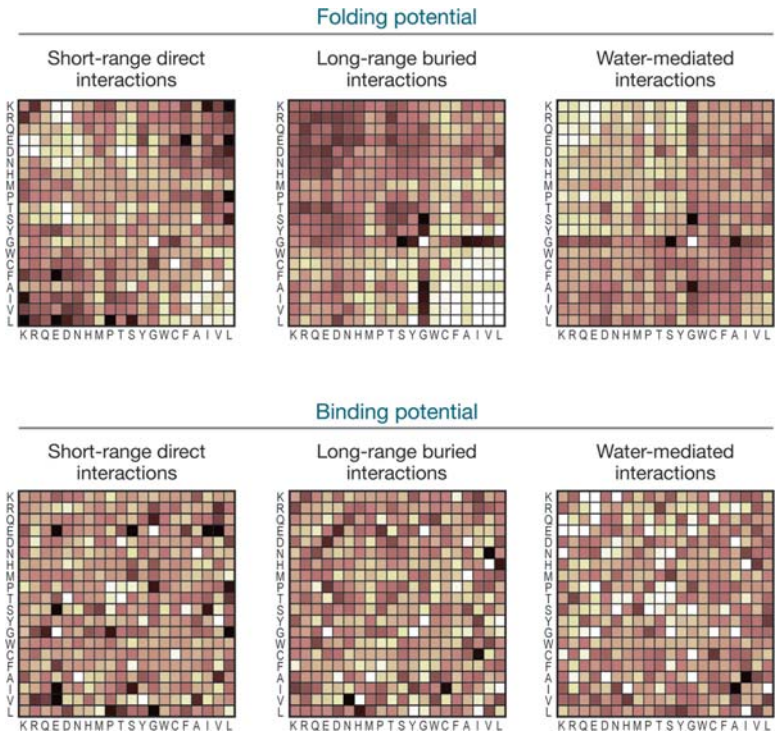

Figure 4

Optimized knowledge-based potentials for folding and binding. Each pair of residues can interact directly or indirectly via a water molecule. Lighter color indicates a more favorable interaction. The potentials illustrate that water-mediated interactions are dominant for both folding and binding yet with a stronger signal for binding processes. Short- and long-range direct contacts occur between residues when the distance between their C_β atoms is 4.5 to 5 Å and 6.5 to 9.5 Å, respectively. Similarly, a water-mediated contact was set to a distance of 6.5 to 9.5 Å with the additional demand of high solvent accessibility.

The desolvation model has correctly reproduced the folding rate of SH3 mutants and provides a microscopic explanation of destabilizing core mutations on the folding rate. Mutating valines 44 and 53, which participate in the folding nucleus, result in slower folding rates because the V44T mutation disrupts the structural search collapse while the V53T mutation hinders the desolvation of the hydrophobic core of the TSE (50).

Adding the pairwise additive desolvation term to the native structure–based model results in increasing the stability of the native conformation (128) and in slowing the folding kinetics for both the SH3 domain (24) and chymotrypsin inhibitor 2 (75). These folding rates are more similar to the experimental folding rates than to those obtained by a model that does not take solvent effects into account. Yet, nonadditivity must likely be incorporated to introduce the many-body nature of solvent-mediated interactions to achieve the level of cooperativity often observed in experiments (20). An initial effort in this direction has been performed by introducing small intrachain pairwise desolvation barriers, which is independent of the interaction stability (96), or increasing the desolvation barrier height (76), resulting in a relatively high enthalpic barrier for folding.

The atomistic simulations of the folding of the SH3 protein domain agree with the folding mechanism of SH3 found using the desolvation model. These studies show that the

folding depends on a gradual, few molecules at a time expulsion of water from the collapsed interior and can involve a lubricated hydrophobic core at the late stage of folding (59, 132). Atomistic simulation studies of protein A three-helix bundle (57), protein G (133), and protein L (73) support the role of water as a lubricant for the packing of the hydrophobic core after the formation of the transition state (**Figure 5**). A similar mechanism has been observed in the folding of a 23-residue peptide (119). The latter simulation study has suggested that although water is trapped in the core at the TSE, the TSE is completely defined by the protein and not by the geometry of the water. Accordingly, the folding probability of a given conformation of small peptide was found to be independent in the configuration of the water (119). Moreover, these fully atomistic simulations (57, 119, 132, 133), which are not biased toward direct contacts between the residues, point out that the folded state is not completely dry but that a few core water molecules form hydrogen bonds with the protein backbone.

Thus, water molecules can mediate the search for the protein native topology, in which waters serve a structural role as backbone hydrogen bond bridges between the residues connecting the hydrophobic residues and as water molecules simply residing inside the core. Some of these waters are gradually expelled from the formed core in a later stage after the initial funneling. The active role of water in folding is an outcome of the size of a water molecule, their discrete nature, and the flexibility of proteins as well as the hydrophilicity of the backbone chain. Water might assist the association of two rigid hydrophobic objects by the so-called dewetting (drying) effect, which is characterized by the collective emptying of space around the nucleating sites and the formation of a large vapor bubble (21, 67, 97). Protein hydrophobic collapse via the dewetting mechanism is characterized by a decrease in water density and then by the spontaneously collapse of the core to stabilize the protein by reducing the solvent-accessible area of the core residues. On the other hand, in the expulsion mechanism, core compaction precedes water expulsion. The validity of the dewetting

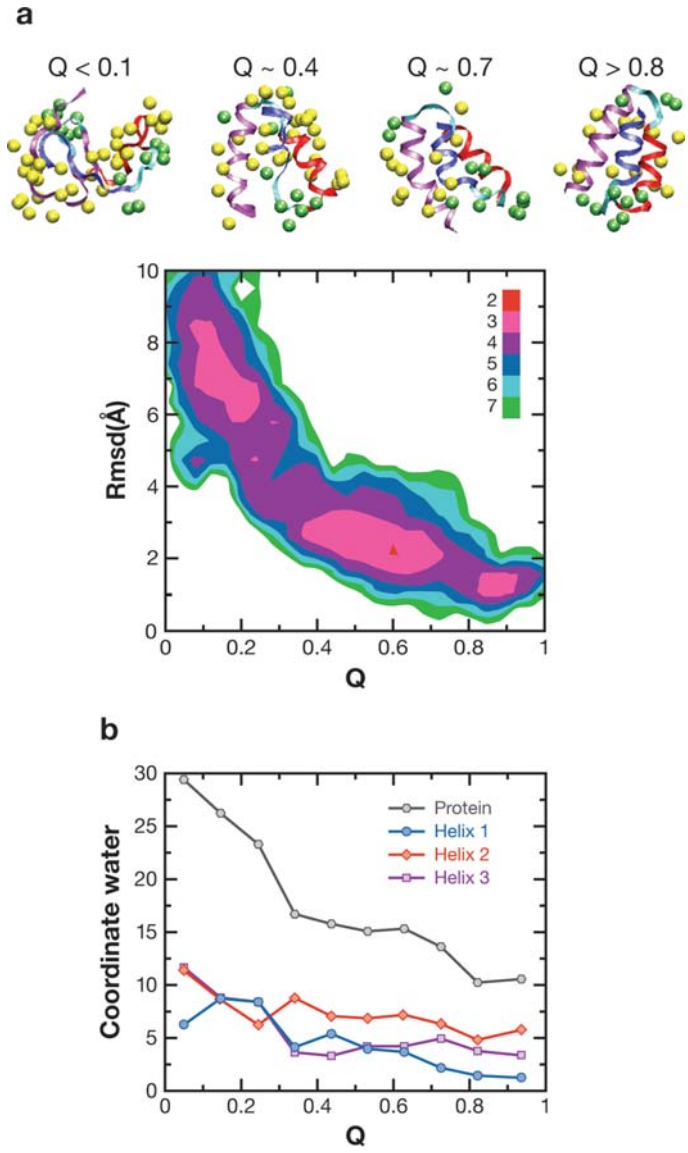

Figure 5

Folding of protein A through a hydrated native-like intermediate. (*a*) Free-energy surface at the transition temperature. (*b*) Average coordination number of water molecules in the helices and the whole protein as a function of Q. The selected conformations at the top illustrate the water expulsion as the folding progresses along the reaction coordinate Q.

Dewetting (drying) effect: water density is decreased and a vapor bubble is formed that drives hydrophobic assembly

Water expulsion: water is gradually expelled from the collapsed interior and assists protein folding by mediating interactions

scenario for proteins has been directly questioned recently by exploring the hydrophobic collapse of several multidomain proteins with hydrophobic simple interfaces using atomistic simulations (66, 95, 149). A signal for the possible drying effect was seen only when the intrinsic properties of the protein chain were turned off. In the association of the two almost-rigid domains of the BphC enzyme, a drying effect was observed only when the electrostatic protein-water forces or attractive van der Waals forces were turned off (149). Similarly, a signal for a drying effect was detected when protein flexibility was drastically suppressed in the association of melittin tetramer and $\alpha_2 D$ homooligomers, although their assembly is experimentally described as coupled folding/binding processes (66, 95).

Water plays an active role in the folding of nucleic acids as well. All-atom molecular dynamics simulations of a RNA hairpin-loop motif showed that, similar to protein folding, RNA folding occurs by hydrophobic collapse via the expulsion mechanism of desolvating central hydrophobic regions after initial nucleation of several base pairs (139). Water-mediated interactions in the folding of small nucleic acids appear to be essential for capturing the correct hydrophobic collapse among other nonspecific collapse events, therefore constituting a structural role. Nucleic acids have more uniform hydrophobic cores than do proteins owing to the aromatic group of the bases compared with hydrophobic residues that are sparsely located along the sequence, suggesting higher cooperative collapse and less trapping of water in the folding of nucleic acids.

WATER IN PROTEIN-PROTEIN BINDING

The forces that drive protein-protein binding are similar to those that drive protein folding, and thus polar and hydrophobic interactions as well as hydrogen bonding dominate both processes. Water, however, as is evident more from their abundance at the interfaces of protein complexes than from the interior of a monomeric protein, is likely to be more dominant for binding and recognition than for folding.

Water in Protein Interfaces

Water is abundant in protein-protein interfaces. Upon assembly, the interfaces of many formed complexes are hydrated and consist of about 10 water molecules per 1000 $Å^2$ of interface area (121). Water molecules at interfaces form hydrogen bonds with the backbone polar groups or charged side chains. Although common, interface hydration is not uniform (87). Protein-protein interfaces exhibit different degrees of solvation and also different spatial distribution patterns. The level of hydration obviously depends on the polarity and geometry of the interface. It was also observed that homooligomers have more hydrated interfaces than do heterooligomers (121). In some complexes the waters are only at the interface rim, whereas in others they cover the entire interface area. The higher hydrophilic nature of interfaces formed when two folded monomeric proteins associate, compared with those formed in coupled folding-binding process (72, 94, 147), may suggest a different role of solvent. Accordingly, the interfaces of complexes formed between subunits that are natively disordered are expected to be dryer than those formed between folded proteins, exhibiting similarities to protein cores. Nonspecific crystal packing interfaces, which are more polar, are often 50% more solvated than the interfaces of protein complexes. Although immobilized solvent is widely observed in X-ray structures, it is likely that the interface solvation is currently underreported, as their detection demands extremely high-resolution structures.

Binding Mechanism Is Governed by the Protein Topology

Protein topology, currently well accepted as a pivotal factor in determining

unimolecular folding, also determines many aspects of protein assembly. This notion has been obtained from native topology-based (Go) models, which include only interactions that stabilize the native structure as determined by NMR or X-ray crystallography and thereby capture the protein topology. These models are energetically unfrustrated models (i.e., they do not include nonnative contacts) and correspond to a perfectly funneled energy landscape.

The native topology-based model has been applied recently in several studies to examine the mechanism of protein association (89, 93, 94) and successfully reproduce the experimental classification of homodimers regarding whether monomer folding is prerequisite to monomer association (**Figure 6**). Obligatory homodimers that exhibit two-state thermodynamics are formed by a coupled folding and binding reaction. Transient homodimers, which bind via a thermodynamic intermediate, are formed by the association of already folded monomers. In general, we found that most of the gross and many of the finer features of binding mechanisms can be obtained by Go model simulations. The need to understand the mechanism of binding and its main determinants is well illustrated by our study on the association pathway of dimeric HIV-1 protease (88). These studies have indicated that the monomeric HIV-1 protease is relatively folded in its free form. The binding by association of prefolded monomers suggests a new way to inhibit the protease activity by designing an inhibitor that binds to the monomer and thus prevents dimerization rather than by designing an inhibitor that blocks the active site but eventually becomes ineffective owing to drug resistance.

Native topology-based model: a solvent-averaged energy potential defined by attractive native state interactions and repulsive nonnative interactions; corresponds to a perfectly funneled energy landscape; also called Go model

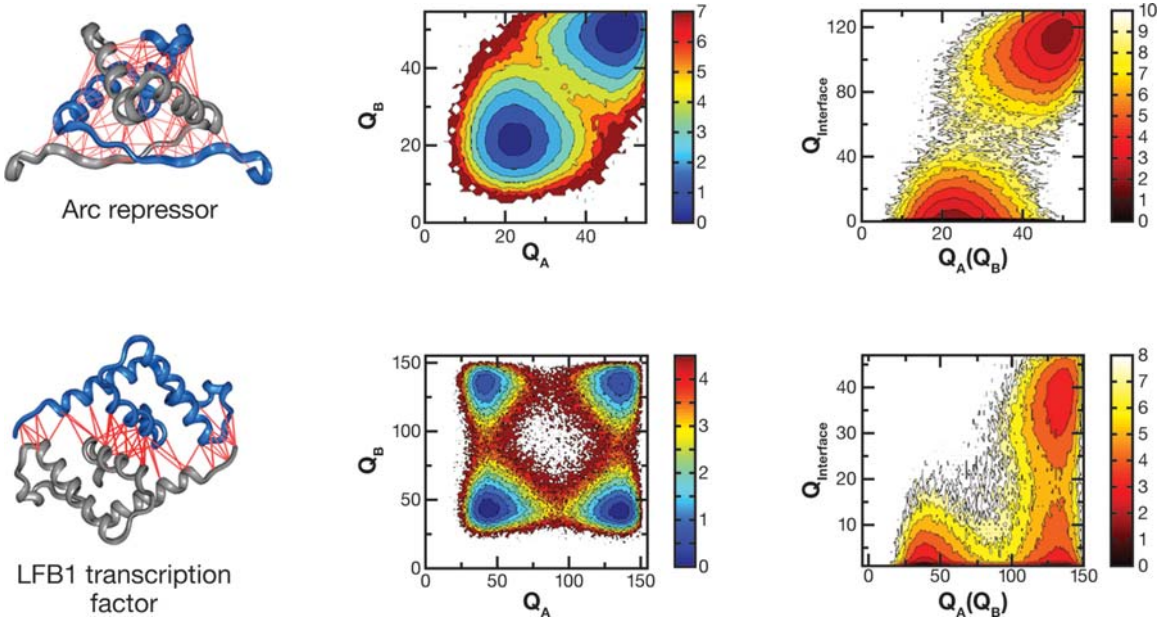

Figure 6

The association mechanism of the dimerization of Arc-repressor (an obligatory homodimer) and LFB1 transcription factor (a transient homodimer). The binding free-energy landscapes are plotted against Q_A and Q_B (the native contacts in monomers A and B, respectively) and $Q_{Interface}$ (the interfacial native contacts). Q_A and Q_B correspond to folding/unfolding events, and $Q_{Interface}$ corresponds to binding/unbinding events. The free-energy surfaces indicate that the native topology-based model reproduced their experimentally determined binding mechanism.

The native topology-based models agree with the experimentally determined binding mechanism regarding the existence of a monomeric intermediate. The validity of the model in studying protein binding is reflected by the good correlation obtained between the computational and experimental Φ values, which measure the degree of structure at the TSE at the residue level. For Arc-repressor and the tetramerization domain of p53 (p53tet), a direct comparison between the simulated and experimental Φ values is available and indicates that the simple Go models capture the nature of the TSE reasonably well. For Arc-repressor there are detailed deviations between the simulated and experimental Φ values of particular residues (reflected by a correlation coefficient of 0.31), but there is an agreement about the overall structure of the TSE. For p53tet, which was experimentally classified as a dimer of dimers, not only did the native-centric model reproduce the association mechanism, but the computational Φ values for the dimerization and tetramerization reactions are in agreement with the experimental ones (**Figure 7**). Note that recently an all-atom molecular dynamics study was done on the dimerization reaction of p53tet (26). The Φ values for the binding TSE from that study, which includes explicit water and nonnative interactions, displays results qualitatively similar to those obtained from the native topology-based model.

The ability of native topology-based models to reproduce the features of binding mechanisms is significant and suggests that the binding TSE and binding mechanism can be obtained by the knowledge of the final complex's structure alone. We have recently found that protein complexes formed by the association of already folded subunits have structural and topological properties different from those with intrinsically unfolded subunits. More specifically, these two classes of complexes differ in the topological properties (i.e., connectivity of residues, average clustering coefficient, and mean shortest path length) of the monomers and the interfaces (89). Nonetheless, one may expect that adding water to binding simulations may reveal the existence of additional binding TSEs that are relatively hydrated as well as high-energy intermediates.

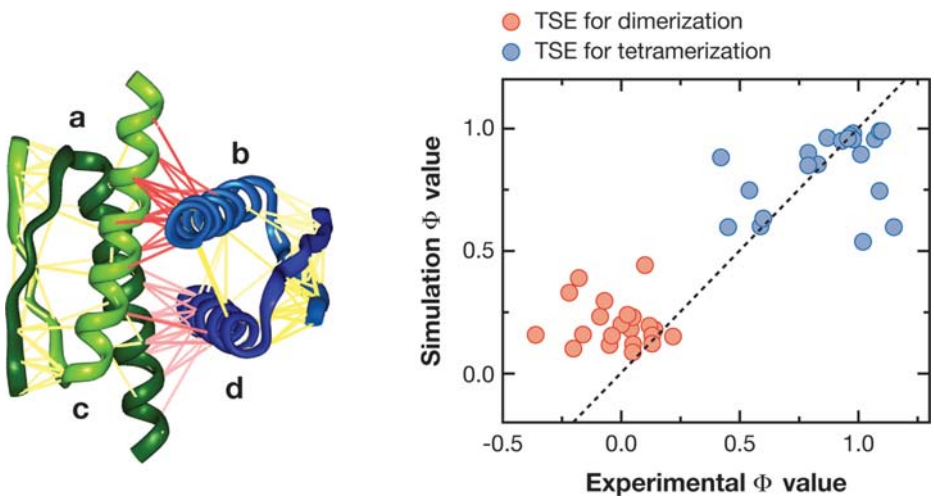

Figure 7

Comparison of the structure of the TSE of the tetramerization of p53tet from simulation and experimental Φ value analysis. The two TSEs in the assembly of the tetramer (dimerization of monomers to form *ac* and *bd* and the dimerization of the dimers) were detected in the native topology-based model.

The Role of Water in Biological Associations and Aggregation

In addition to topology, other factors such as nonnative interactions, electrostatics, and water interaction can affect binding mechanisms and kinetics (92). The abundance of water in complex interfaces, as discussed above, indicates its potential role in binding. Kinetically, water molecules can guide a fully solvated protein to recognize another fully solvated protein (or nucleic acids) by a gradual expulsion of water layers. The native topology-based model effectively takes into account structural water molecules but not dynamic water molecules and thus cannot address the desolvation mechanism of bringing two solvated proteins to form a specific and tight assembly. We had conjectured that our simulations of antibody-antigen complex using the topology-based model poorly reproduced the binding TSE because of lack of water molecules in our model (89). The abundance of water in mediating contacts in other forms of the complex explained the discrepancy between the experimental and computational characterization of the binding transition state. Solvent molecules thus can assist the initial association to form the encounter complex. Alternatively, the main binding transition state, which is squeezed out at a later stage and results in a dry interface, is stabilized by shape complementarity (126). A wet encounter complex and transition state suggests that, similar to folding, proteins bind by a gradual expulsion of the solvent molecules, which is even less complete owing to the hydrophilic nature of many complex interfaces.

Because water is essential for folding and binding, it is certainly important to aggregation as well; however, it is still unclear if its effect is direct or indirect. Dehydration can affect the intricate balance between the protein internal interactions and the interaction with the hydration shell. Destabilization of the weak water-protein interactions can affect protein stability and flexibility and therefore supports conformational changes (81). Molecular dynamics simulation study of the amyloidgenic $A\beta_{16-22}$ peptides has shown that the monomer adopts a β-strand conformation in urea, suggesting that urea at low concentrations may facilitate amyloid formation (61, 79). It was also hypothesized that proteins involved in conformational diseases have a large number of hydrogen bonds not protected against solvent interactions (34, 49). This solvated region of the protein surface was suggested to be structurally more labile with a consequent potential for aggregation.

Water Is Central for Recognition

The common wet nature of protein-protein interfaces may suggest that water is part of the recognition code, as it mediates interactions that are less favorable in its absence. It is plausible that water assists two proteins not only in improving their binding interface but also in discriminating between the potential binding sites. Accordingly, the water smooths the binding funnel. A coarse-grained folding potential showed limited success in describing binding (112). Water can make single or multiple regions of the protein surface more adaptable for binding than other patches. This scenario suggests that water is a kind of molecular glue between protein subunits and can eliminate the number of possible binding modes by contributing to exquisite specificity (12). The water molecules that were part of the hydration shell of the free subunits are much more localized when placed at the interface and can be treated as an integral part of the structure.

The knowledge-based potential for direct and water-mediated interactions for protein binding shows that with the assistance of water molecules some residues are likely to interact (**Figure 4**). These potentials indicate that water-mediated interactions are more central in bimolecular than in unimolecular recognition. These water molecules capture structural information for the formation of the protein complex, or alternatively, they edit empty

spaces between the complex subunits and act as extensions of the protein chain. The proposed role of water in binding suggests that water must be included in methods designed to predict protein-binding sites and the complex formed between two or more proteins. Currently, most docking algorithms for predicting protein complexes starting from the free monomers ignore hydration effects upon binding. A docking approach that incorporates discrete water molecules has reported a significant improvement in some cases (118).

Mediating residue-residue interactions by water in protein recognition, which may make protein surfaces more adaptable for binding, can also lead to promiscuous binding (83). In such cases, water acts as a buffer that weakens unfavorable interactions, thereby accommodating various substrates with low specificity (136). It is possible that such weak water-mediated interactions are key for transient protein-protein interactions, which are characterized by smaller and less hydrophobic interfaces. The high adaptability and relatively low energetics of water-mediated interactions are in accordance with the observation that residues that contribute the most to the binding free energy (i.e., "hot spots") are placed in a dry environment (15). Promiscuous binding via water can be the basis of the dynamic protein association needed for signal transduction pathways.

Water-mediated interactions in protein interfaces is suggested to be favorable enthalpically and thus enhance stability in a way that compensates the entropic cost that must be paid for immobilizing interfacial waters. In recent molecular dynamics simulations it was found that water can also enhance binding affinity by a gain in free energy resulting from an increased entropy of the trapped water molecules. Water molecules in bulk have limited freedom due to their participation in a water network, while water molecules inside a slightly nonpolar cavity may have more freedom than in bulk, resulting in higher entropy (115).

WATER IN PROTEIN-DNA RECOGNITION

The tightness and order of the DNA hydration shell and that DNA hydration depends on DNA conformation and sequence indicate that water molecules are an integral part of nucleic acids. This suggests that water can be directly involved in protein-DNA recognition. Indeed, many protein-DNA interfaces are highly solvated (69). These interfaces are much more polar than protein-protein interfaces because of the phosphate groups on the DNA side and the abundance of positively charged groups on the protein side. In addition to direct interactions between proteins and DNA base pairs (i.e., direct hydrogen bonds, van der Waals, electrostatic, and hydrophobic contacts), which are important for sequence-specific recognition, in many cases indirect interactions between residues and the DNA bases exist via water molecules (120) (**Figure 2**). These water molecules are not just "filling spaces"; they mediate recognition and specificity mainly by screening unfavorable electrostatic and hydrogen bonding (70, 127). A large number of water-mediated contacts (mainly between protein and DNA but also within and between the histones) have been found in the structure of the nucleosome core particle, enabling additional interactions between the DNA and the histones and within and between the histones themselves (33) (**Figure 2**).

Mutating the DNA target by perturbing a water site affects the protein binding affinity. It was observed that the DNA hydration pattern is similar in the free and bound states, suggesting that recognition is, in part, due to complementarity of surface hydration (129). It was also suggested that protein atoms involved in binding to DNA occupy positions normally occupied by waters in the free DNA (146). In some cases, water at the interface can exchange with bulk solvent and maintain a partially disordered interface, which can be entropically advantageous. Furthermore, the interfacial disorder can facilitate

recognition via the fly-casting mechanism (134), in which the water acts as a molecular glue that increases structural adaptability. In nonspecific protein-DNA complexes more water molecules remain at the interface and lubricate protein sliding on the DNA. Many of these water molecules must be displaced for specific recognition, and the driving force for complex formation would seem primarily entropic (52, 71).

Despite the evidence for the role of water mediation in sequence-specific DNA recognition by proteins, this notion is still underappreciated. Current methods used to decipher protein-DNA recognition codes on a genomic scale rely mainly on the direct contacts between protein residues and the DNA base pairs. An initial approach to construct an optimized knowledge-based potential for protein-DNA binding includes water-mediated interactions in which a virtual water is defined by distance criterion and solvent accessibility of the residue and the base to which it links (**Figure 8**). The wet knowledge-based potential for protein-DNA recognition discriminates between specific and nonspecific DNA sequences (unpublished data). The potential reveals that some water-mediated interactions between the protein residues and the DNA groups are as important as the direct interactions (**Figure 8**). This observation is supported by the finding that perturbing a direct hydrogen bond or a water-mediated interaction at the interface of the papillomavirus E2C–DNA complex results in similar destabilization (53).

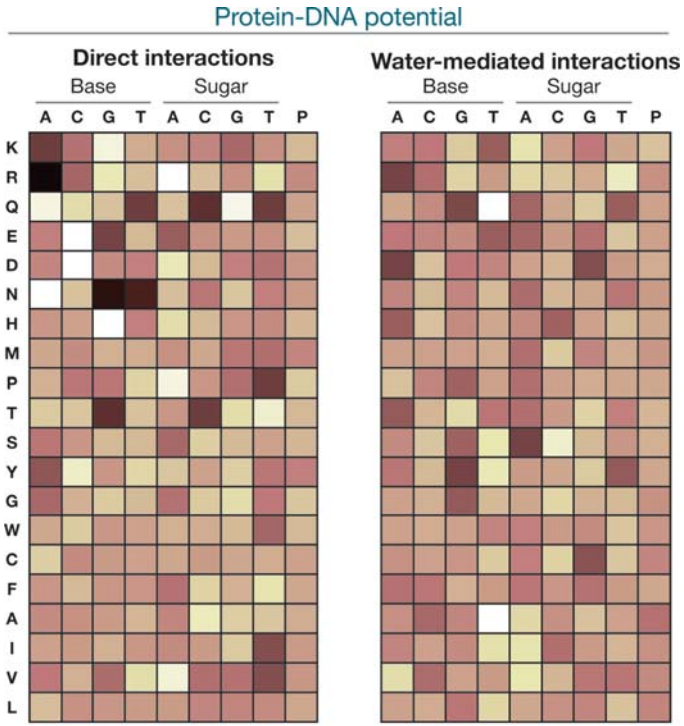

Figure 8

Optimized knowledge-based potentials for protein-DNA binding. Each residue can interact directly or indirectly (mediated by water) with the bases, sugar, and phosphate groups of the four nucleotides. Lighter color indicates a more favorable interaction. A direct contact is defined on the basis of the typical distance found in resolved protein-DNA structures, and the indirect interactions are defined by allowing distances larger by 3 Å than the distances of the direct interactions.

SUMMARY

Whether life can evolve in nonaqueous media is still an open question; however, it is unquestionable that water is central for life on earth. The many roles water plays in biomolecular processes, and particularly the coupling between its motions and the dynamics of proteins and nucleic acids, are currently widely acknowledged, as reflected, for example, by hydrating vacuum simulation, which is done routinely. Nonetheless, water is often treated as an inert environment, yet in many cases it is actually an active player. Therefore, even when we omit water molecules when drawing three-dimensional structures of proteins and nucleic acids for the sake of simplicity, we should keep them in mind.

In this review, we presented a few cases in which water has a dynamic role beyond maintaining the structure of proteins and nucleic acids. For example, water can guide the conformational search in protein folding by gating hydrophobic residues. While our understanding of the role of water solvation in protein folding has improved, the limited successes of implicit solvent models in accurately

representing protein stability and dynamics suggest that the physics of the interaction between biomolecules and the solvent is not completely captured. Developing a battery of models to explore the dynamical and structural features of water in the hydration shells, internal cavities, and complex interfaces is vital to understanding the structures of proteins and nucleic acids and their folding and binding processes. Giving attention to water may therefore be beneficial to folding, docking, and structure design efforts.

SUMMARY POINTS

1. Water is highly acknowledged for playing an important role in the structure, stability, dynamics, and function of biological macromolecules. Yet only recently has water been considered an active component rather than an inert environment.
2. Water guides the conformational search in protein folding by gating hydrophobic residues. Several studies reported the existence of wet native-like intermediates. Thus water has a dynamic role in mediating the collapse of the chain and the search for the native topology through a funneled energy landscape.
3. Water can enhance the stability of biological macromolecules. Water-mediated interactions are favorably enthalpic. Alternatively, water residing in hydrophobic pockets can stabilize entropically because it has higher entropy than in bulk water. Both cases indicate that water molecules are not just "filling spaces" but are integral components of the structure.
4. Water can mediate recognition by discriminating between specific and nonspecific binding.
5. Giving attention to water will shed light on the physics of self-assembly and advance our understanding of the natural design of proteins and nucleic acids.

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