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On the role of the solvent in biosystems: atomistic insights from computer simulations

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1. ABSTRACT

This review describes recent progress of atomistic simulations to promote the understanding of the role of the solvent in biophysics and biochemistry. Fundamental aspects of aqueous solutions and their importance for the rationalization of biomolecular arrangements and metabolic transport are discussed. Many of these insights were obtained from molecular dynamics simulations performed during the last three decades. In combination with quantum mechanical descriptions this approach nowadays advanced to a reliable tool for the investigation of biochemical reactions. Apart from passive coordination of the reactants, the solvent studies may also address proton transfer over chains of hydrogen bonded molecules.

2. INTRODUCTION

Despite the immense complexity inherent to most biosystems, atomistic computer simulations are becoming an increasingly powerful tool of investigation boosting the understanding of biophysical and biochemical processes. This might be counterintuitive as in such simulations ‘large model systems’ and ‘long simulation times’ still refers to only tens of nanometers and typically less than microseconds, respectively. However, the smart design of the simulation setups allows focusing on specific aspects and particularly interesting events. In parallel to the increasing performance of computer hardware, which shall not be discussed here, the development of efficient algorithms accounts for much of the possibilities open today. Depending on the system under investigation,

different approaches to atomistic modeling proved advantageous and many state-of-the-art methods are discussed in detail in this issue of *Frontiers in Bioscience*.

Strikingly, the discussion of atomistic simulations dedicated to the role of the solvent in biosystems needs to revisit most of these contributions. A large part of the complexity of biological processes is actually due to the complexity of the solvent. While a single H_2O molecule looks rather simple, ensembles of many water molecules exhibit a manifold of peculiar arrangements. The structure and function of biomolecules is closely connected to the interplay with water and/or the integration into lipid assemblies. Much of today's knowledge of this interplay was gained from molecular dynamics (MD) simulations (1). In aqueous solutions, hydrogen bonds form and rearrange on a picoseconds scale. MD simulations offer insights at femtosecond resolution and the atomistic level of detail, and are hence ideally suited for profound investigations in this field.

The present review discusses the different features of aqueous and lipid solutions with a focus to recent MD simulations. Surely, the topic is too broad to provide a full account of the field. We shall hence concentrate on fundamental aspects which form the basis of solvation. The next section is dedicated to water in the liquid state and describes MD studies of the hydrogen bonded network and proton transfer reactions. Thereafter, insights into hydrophilic and hydrophobic/lipophilic solvation are discussed. Conclusions and perspectives are outlined in the final section.

3. AQUEOUS SOLUTION

3.1 Hydrogen bonded networks

In the condensed states water exhibits strong intermolecular interactions. Each water molecule offers two donors and two acceptors for hydrogen bonding. The formation of multiple hydrogen bonds leads to network structures which can be of considerable complexity. Apart from a manifold of different polymorphs of ice this also applies to liquid water. In the liquid state, each water molecule is mobile and can diffuse by micrometers within just a second. Nevertheless, the connection of nearest neighbors largely prevents uncorrelated motions. The investigation of liquid water is an ongoing challenge which motivated an immense number of both experimental and theoretical studies (2). Among the latter, MD simulations date back to as early as 1971 (3).

The rationalization of liquid water at the molecular scale requires a dynamical picture. This can be illustrated by comparing snapshots taken from a MD simulation at intervals of 1 picosecond. Each of the obtained configurations reflects a hydrogen bonded network in which each water molecule participates to an average of four hydrogen bonds. From combined experimental and theoretical efforts two of these hydrogen bonds were characterized as strong, while the remaining two hydrogen bonds were found to be comparably weak. The overall network may hence be interpreted in terms of chains and rings formed by strong hydrogen bonds and weaker connections between adjacent chains (4). At ambient pressure and room temperature, this temporary ordering changes frequently. This particularly applies to the formation and dissociation of weak hydrogen bonds which occurs a picosecond time scale (2,4).

The collective rearrangements of hydrogen bonded chains gives rise to the formation of new connections and should be considered as a change from one network to another. This reorganization conserves the average number of hydrogen bonds and hence also tends to conserve the interaction energy U. The driving force for the frequent rearrangement of the hydrogen bonded network is entropy S. Liquid water hence represents a balance of two competing phenomena: i) the tendency to potential energy minimization by ordering the water molecules in a regular hydrogen bonded network and ii) the gain in entropy by including irregular arrangements to the configurational manifold. This competition of both contributions to free energy $F = U - T \cdot S$ (with T being the temperature) generally governs the characteristics of order-disorder phenomena. A prominent example from biophysics is represented by protein folding/denaturization which is discussed by Bolhuis in this issue of *Frontiers in Bioscience* (5).

The appropriate description of water molecules in atomistic simulations represents a considerable challenge. To tackle large simulation systems the use of simple and numerically efficient models is imperative. On the other hand, the peculiar characteristics of water impose a need of profound accuracy. The manifold of different approaches may be divided in three groups with different range of application. i) Implicit solvent models are computationally most efficient, but also the least accurate (6). Typically only the electrostatic effect is mimicked. ii) Explicit solvent description based on empirical interaction models is used most widely. Electrostatic interactions and hydrogen bonding is treated by simple potential energy functions. Most models are based on point charges and Lennard-Jones terms to account for induced dipole-dipole interactions and to avoid atomic overlap (7,8). iii) Quantum mechanical treatment offers better accuracy and is particularly important for the investigation of reacting systems as described in the following (9-12).

3.2. Proton transfer along hydrogen bonds

While weak hydrogen bonds may be interpreted by simple the Coulombic attraction of the partial charges located at the interacting atoms, a proper account of strong hydrogen bonds also involves covalent binding. The latter issue may be nicely illustrated by proton transfer along chains of water molecules. In the last ten years, quantum MD simulations offered detailed insights into the mechanisms of proton transfer reactions.

Along this line, the autoprotolysis of liquid water was identified as a multi-step process which is illustrated in Figure 1. The dissociation of two water molecules into a contact pair of OH^- and H_3O^+ ions is disfavored and artificially prepared $OH^-{\cdot\cdot}H_3O^+$ pairs undergo immediate proton transfer yielding two water molecules (13).

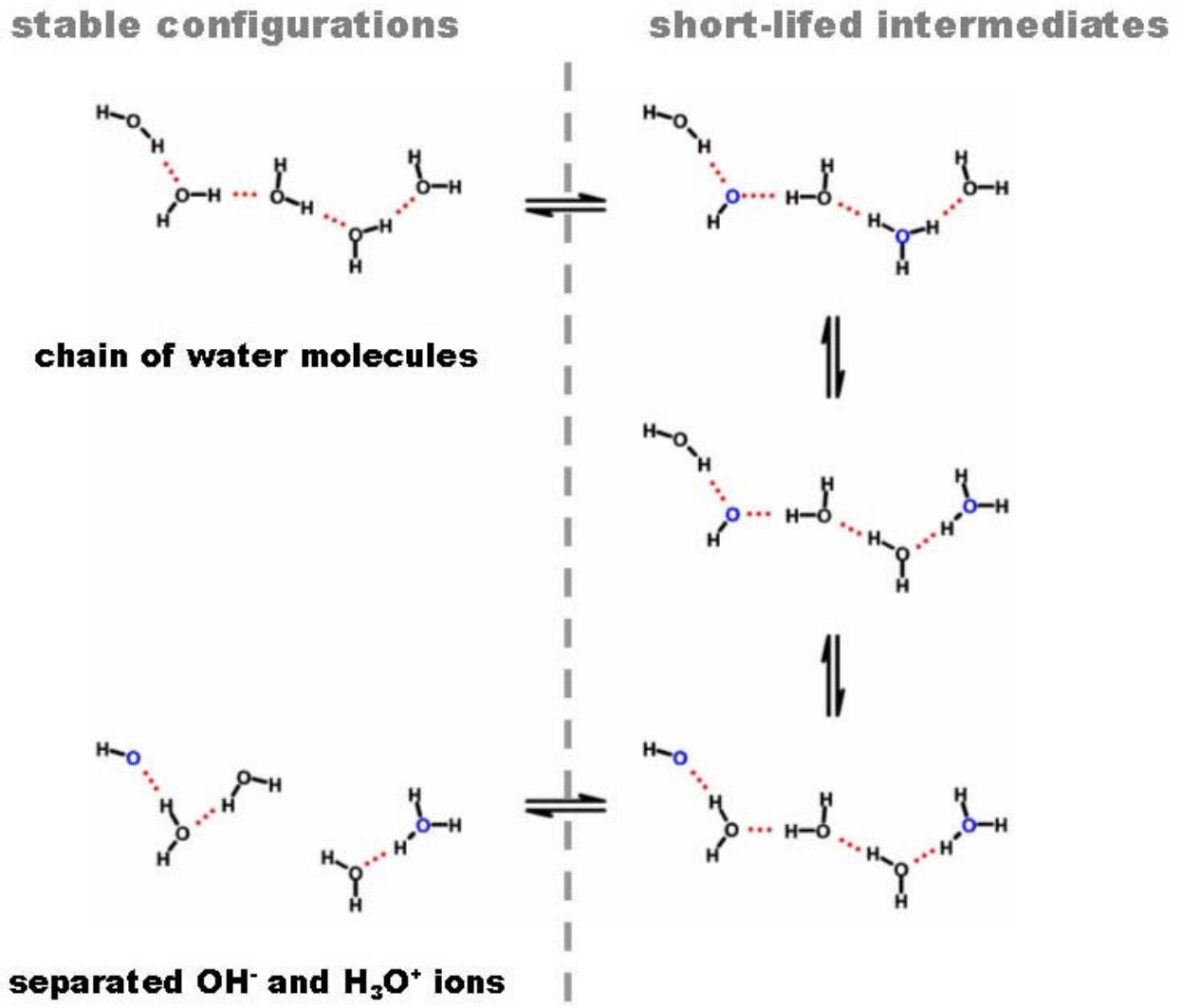

Figure 1. Multi-step mechanism of the autoprotolysis of liquid water.

However, a chain of strong hydrogen bonds may used for a series of proton transfer reactions leading to a yet unstable $OH^- \cdots H_2O \cdots n\text{-}H_2O \cdots H_2O \cdots H_3O^+$ constellation (n≥1). To stabilize the separated charges, the chain needs to be broken such that the hydrogen bonded network no long offers a route to immediate recombination (14).

It appears reasonable to expect this mechanism to be of general relevance to acid-base reactions in aqueous solutions. Recent work on the water-assisted hydrolysis of amides supports this picture (15-17). Therein the dissociation of a water molecule leading to the association of an OH group to the amide and the formation of H_3O^+ was observed to follow similar proton transfer chains. In full analogy to the autoprotolysis in liquid water, the dissociation of hydrogen bonded chains was found to be necessary to avoid the reverse reaction (15).

The migration of protons represents a fundamental issue of the metabolism. While the details of proton transfer via porins depends on the specific protein structure (18), a key principle may be studied on the basis of a much simpler system. In bulk water, the transport of an excess proton is accompanied by a change of the coordination of the involved water molecules (19-21). The preferential coordination of a H_3O^+ molecule involves three hydrogen bonds. Therein each of hydrogen atoms of H_3O^+ acts as a proton donor to one such bond. On the other hand, the coordination of a water molecule involves four hydrogen bonds (two donor and two acceptor type hydogen bonds, see also section 3.1). The reaction $H_3O^+ + H_2O \rightarrow H_2O + H_3O^+$ is therefore driven by specific fluctuations in the local network of hydrogen bonds.

The transport mechanism as identified from the quantum MD simulations of Marx *et al.* is illustrated in Figure 2 (19). While the highlighted molecules are three-fold (H_3O^+-like) coordinated, all other molecules exhibit four-fold hydrogen bonding according to liquid water. As a transition state, two water molecules are H_3O^+-like

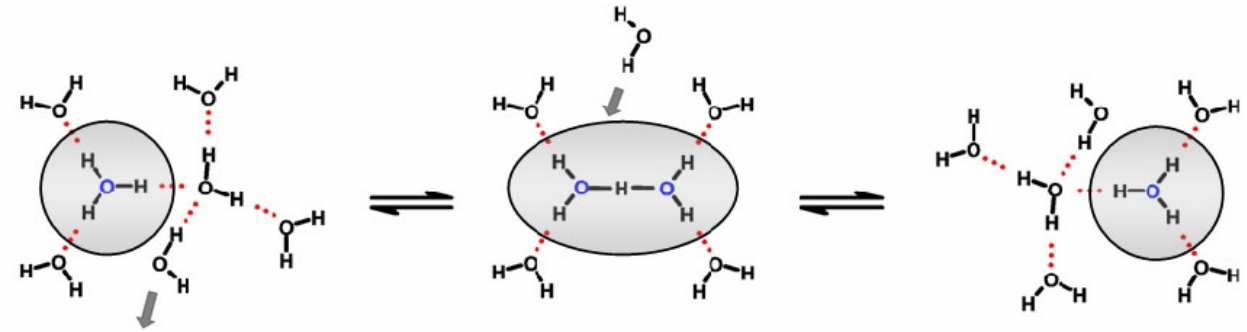

Figure 2. Migration of an excess proton in water.

coordinated. The hydrogen bond between these two molecules is used for forward and backward proton transfers on a scale of only tens of femtoseconds (21). Finally the $H_5O_2^+$ complex is dissociated into H_2O and H_3O^+ by another change in the hydrogen bonded network. The right hand side of Figure 2 illustrates association of a neighboring water molecule and the formation of an additional hydrogen bond.

3.3 Hydrophilic and hydrophobic solvation

Aqueous solutions of molecules or ions are characterized by the different types of solute incorporation into/exclusion from the hydrogen bonded network of water. For studying solvation from atomistic simulations, it is educative to calculate the respective free energy terms of solute-solute, solute-solvent and solvent-solvent interactions separately. The solute interactions with the water molecules may involve hydrogen bonds, electrostatic and Lennard-Jones type interactions. While the strength may vary considerably, in principle the solute-water interactions tends to be attractive. This attraction however refers to the association of single water molecules to isolated molecules/ions of the solute species. In bulk solution, every solute corresponds to a defect in the hydrogen bonded network which negatively affects the water-water interactions. The sum of all interaction terms eventually determines whether solvation is hydrophilic or hydrophobic.

Hydrophilic solvation may nicely be illustrated by aqueous solutions of Na^+ and Cl^- ions. Figure 3 shows a snapshot of a MD simulation which corresponds to a slightly supersaturated solution (22). The dotted lines indicate electrostatic $H_2O{\cdot}Na^+$ and $HOH{\cdot}Cl^-$ binding. These interactions are particularly strong and over-compensate the less favorable water-water interactions resulting from the required rearrangement of the hydrogen bonded network. For many compounds, the electrostatic ion-water interactions give rise to the formation of compact solvent shells. Ion migration in water may then be interpreted as the diffusion of a complex of the ion and the coordinating water molecules (23). The latter issue is of particular relevance for the study of ion transport through protein channels. Limited space and the different abilities to substitute ion-water by ion-protein interactions dictate species-dependent ion permittivity (23,24). Examples for MD simulations of transport processes are given by the review of Ceccarelli (25).

Hydrophobic solvation basically reflects the exclusion of a solute from the hydrogen bonded network of water. The involved rearrangement of the water molecules strongly depends on the size of the hydrophobic solute. By means of MD simulation studies, small voids in the hydrogen bonded network of liquid water were found to be implemented without changes of the coordination number of the involved water molecules (26-28). As the net balance of the number of hydrogen bonds is neutral, the potential energy of the water-water interactions experiences only marginal changes. Nevertheless, an increase in free energy results from a reduction of entropy. Locally, the hydrogen bonded network is forced to 'enwrap' the void which decreases the configurational manifold. The solvation of hydrophobic molecules which imply large voids is more akin to a water-vapor interface. Such interfaces exhibit dangling hydrogen atoms which leads to a lack of abot one hydrogen bond per surface water molecule (29,30).

The two different aspects of hydrophobic solvation form a competition between entropic and energetic disfavoring. The latter is proportional to the number of surface water molecules and hence scales with the square of the diameter of a hydrophobic solute. The entropic disfavoring applies to the water molecules which were excluded by the void. The corresponding contribution to free energy is therefore proportional to the volume of the solute. Chandler used these considerations to apply classical nucleation theory to the assembly of hydrophobic molecules in liquid water (27,28). From this, the critical radius for the segregation of hydrophobic molecules was estimates as about a nanometer. Accordingly, sufficiently small hydrophobic molecules prefer aqueous solvation as isolated solutes. Segregation only occurs after crossing the nucleation barrier related to the spontaneous formation of an assembly exceeding the critical radius.

While MD or Monte-Carlo simulations may form a detailed basis for the investigation of solvation, the large computational efforts needed yet prevent a fast scan of many solutes at multiple configurations. As an alternative, molecular modeling approaches often use empiric considerations to estimate the solvation characteristics. For this purpose, the solvent accessible surface may be

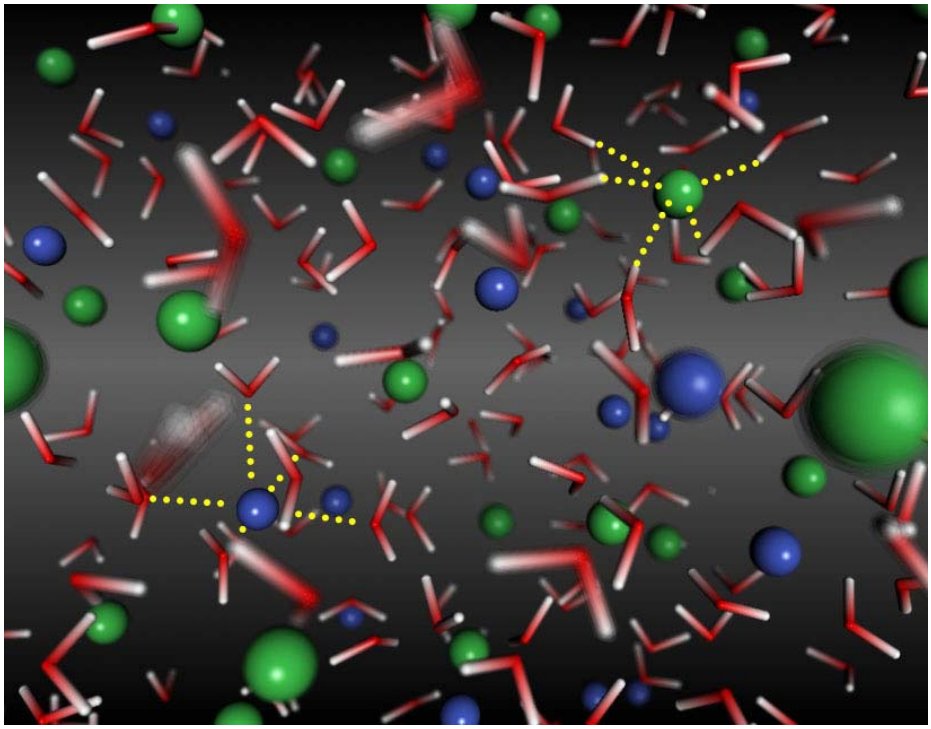

Figure 3. Snapshot taken from a molecular dynamics simulation of an aqueous solution of Na^{+} (blue) and Cl^{-} (green) ions.

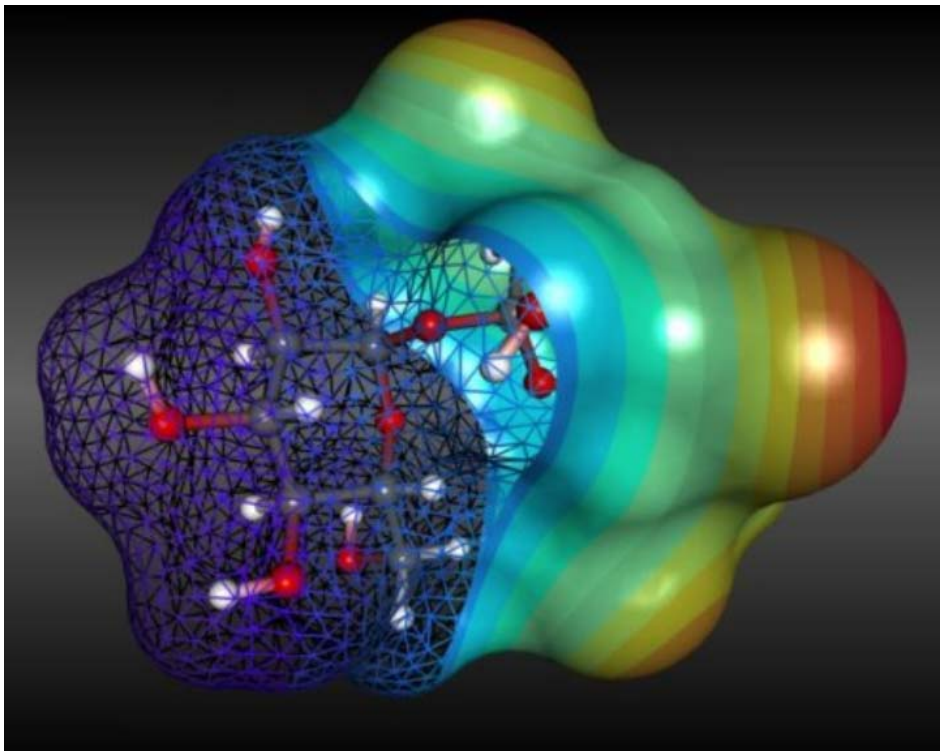

Figure 4. Modeling of a sucrose-derivate using a ball-and-stick representation of the atoms (left) and a geometric mash for describing the solvent accessible surface. The latter is used to locally indicate the molecules lipophilicity potential. The picture is a courtesy of the Molcad Corporation (31).

approximated by mimicking all atoms as hard spheres. Moreover, local patches of the solute surface can be used for highlighting the properties of the atoms below (31,32). An example for the construction of such three-dimensional shapes which coloring accounts for local binding capabilities is illustrated in figure 4.

Such simplified models are particularly useful for the investigation of the docking of rigid molecules. While the shape of the molecular surface accounts for sterical fitting, proper matching of the local atomic properties may be illustrated by the surface coloring. The latter issue involves complementary partial charges, the matching of hydrogen bond donors to acceptors and the assembly of hydrophobic domains (32). During the last two decades, this approach to the modeling of protein-ligand complexes became a standard tool which is accessible to common PCs and interactive computation. In contrast to this, flexible docking requires MD or Monte-Carlo simulations and, in particular when considering explicit solvent models, implies dramatically larger computational costs and the application of specialized simulation strategies (33).

3.4. Lipid Assemblies and lipophilic Solvation

Lipid molecules are characterized by a polar head group combined with a hydrophobic tail. In aqueous solution such amphiphilic molecules form aggregates in terms of micelles and membranes. Within such assemblies, the individual lipid molecules are mobile and may be interpreted in terms of a two-dimensional liquid. The third dimension is confined as the hydrophobic tails avoid the water phase.

Membrane proteins take use of the peculiar characteristics of the lipid-water interactions. Indeed, the arrangement of a membrane protein results from the specific configuration of hydrophilic and hydrophobic domains. The incorporation of hydrophobic domains into the hydrophobic interior of a lipid membrane involves only weak interactions between the hydrophobic moieties. The driving force for the lipophilic solvation arises indirectly from the reduction of unfavorable water-water interactions.

Modern computers allow MD simulations of the spontaneous formation of vesicles and membranes from aqueous solutions (34,35) including protein incorporation into lipidic aggregates (36). However, the more common approach to modeling proteins in lipid solutions is to place the protein in an artificially prepared membrane pore (37). This can be achieved by several techniques which range from cutting according to simple geometric considerations to automated insertion procedures (38). The resulting model systems can account for proteins embedded in nm-sized membrane patches which are in contact to aqueous solution – realistic all-atom models of membrane proteins in their functional environment (37).

4. CONCLUSIONS AND PERSPECTIVES

The complexity of aqueous and lipid solutions will surely continue to inspire atomistic simulation studies over many more years to come. So far, our understanding of fundamental aspects benefitted considerably from theoretical modeling. The detailed account of hydrogen bonded networks and lipophilic solvation opens the way to new insights into important biophysical processes. Surely, the simulation community is just at the beginning of the rationalization of protein folding, ligand docking and metabolic transport. However, the number of investigations dedicated to detailed models comprising millions of atoms is increasing dramatically. This issue of *Frontiers in Bioscience* reviews a series of examples for such successful employment of computers. The obtained atomistic scale insights range from the biophysics of biomolecular folding, molecular transport and biochemical reactions (5,25,39).

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