2.5.7 Research Area "Biomolecular Solvation" (M. Heyden)

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Objective: Fundamental biomolecular processes involve major changes of the solvation for the involved molecular species: aggregation, folding, unfolding or conformational changes of proteins, complex formation of enzymes with their substrates, and the binding of ligand or drug molecules to receptors. Consequently, solvation free energies are a major driving force that determines thermodynamic equilibrium as well as kinetic barriers. Here, we develop novel simulation and analysis procedures to study microscopic contributions to the solvation free energy, which can be utilized for enzyme and drug design, as well as to understand a particular biomolecular system. Further, we utilize this information to improve implicit solvent simulations of concentrated biomolecular solutions on the *meso*-scale, which can describe realistic *in vivo* environments of biochemical processes.

Results: Atomistic molecular dynamics simulations with explicit solvent molecules contain, in principle, all the information required to analyze the influence of a molecular solute on the solvent energetics and structure. However, extracting this information, in particular separating energetic and entropic contributions to the total free energy, is often a challenging task. We have now developed a novel technique (3D-2PT), which provides not only total solvation energies and entropies of a given molecule, but resolves their local contributions in the three-dimensional environment of a molecule. A main challenge is the spatial resolution of the local solvent entropy, which we obtain from analyzing the local vibrational density of states (VDoS) of the solvent. The latter is obtained from time-dependent fluctuations of atomic velocities. Figure 18 shows an example analysis for a small model solute, which exhibits the chemical features of a peptide bond. Of particular importance for the analysis are low-frequency modes in the

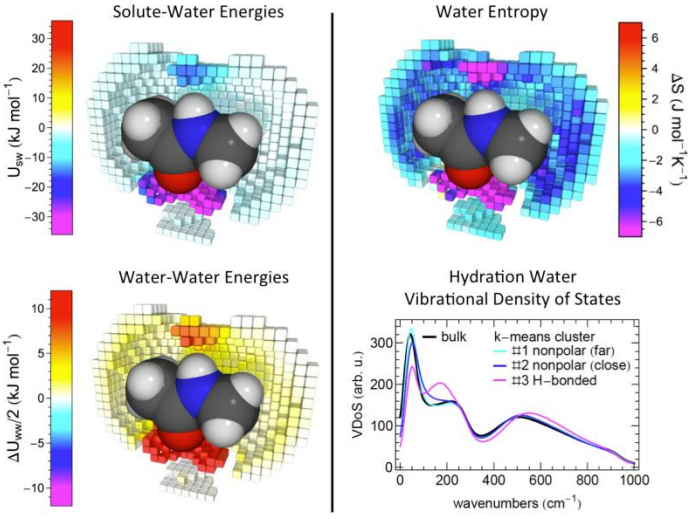

Fig. 18. Solvation free energy contributions in the hydration shell of N-methylacetamide. Local hydration water entropies are derived from spectra of thermally excited intermolecular vibrations (bottom right).

far-infrared range (0-330 cm^{-1} or 0-10 THz) of the vibrational spectrum, which are primarily characterized by intermolecular vibrations in the solvent, e.g. vibrations of the water hydrogen bond network. These vibrations are thermally accessible (k_BT/h=6 THz at 300K) and therefore carry the main part of the entropic information (Figure 19). The method can be applied for simulations of large biomolecular systems containing ~100,000 of atoms (Figure 20) and non-aqueous solvents.

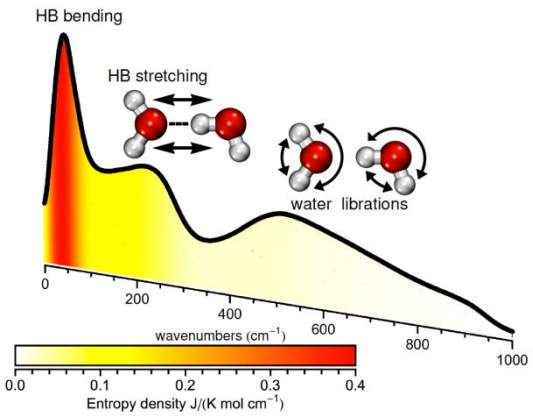

Fig. 19. Entropy information of characteristic intermolecular vibrations in the vibrational density of states of water.

A thermodynamic analysis based on the spectrum of intermolecular vibrations is particularly powerful in combination with spectroscopy experiments, which can follow changes in the solvent spectrum. This is realized by our cooperation partner Martina Havenith at the Ruhr-University Bochum, who develops time-resolved terahertz absorption spectroscopy methods. This technique allows for studies of non-equilibrium processes during a triggered reaction on a millisecond timescale, i.e. enzymatic catalysis in a stopped-flow experiment and protein unfolding/refolding after a laser-induced T-jump. Here, simulations of the biomolecular systems at different stages of the process can reproduce the observed spectral changes, in particular shifts of vibrational frequencies, and provide the microscopic information for the interpretation in terms of changes in the solvation energy, entropy and free energy.

Our analysis of spatially resolved thermodynamic properties of water in the hydration shell of proteins further allows detailed studies of the complex and non-additive effects that govern the interactions of biomolecules with their solvent, as well as their binding partners, i.e. ligands, substrates, or potential drug molecules. Biomolecular surfaces feature a heterogeneous mix of functional groups as well as various convex and concave surface curvatures. Both, surface topology and the chemical nature of solvent-accessible groups affect the solubility of a protein interface and the binding of other molecules. This results in broad distributions of hydration water properties, i.e. binding energies and local entropies.

Equally broad distributions are observed for hydration water dynamics, i.e. hydrogen bond network fluctuations, rotational relaxation and translational diffusion. These dynamic processes can be characterized in simulations via time correlation functions,

which is a routine application in our lab. A combined analysis of local thermodynamic and dynamic properties of water in the hydration shell of proteins allows us to study fundamental correlations between both. In particular, we can demonstrate that the molecular entropy of water is related to translational and rotational dynamics. Studying these correlations in detail provides the key for the thermodynamic interpretation of experimental observables that are sensitive to local hydration water dynamics at various sites of a biomolecular surface. For example, Overhauser dynamic nuclear polarization (ODNP) can be used to study local hydration water mobility in the vicinity of spin-labels attached to selected sites in the protein. These experiments are carried out in the group of our partner Songi Han at UC Santa Barbara. Our simulations are able to reproduce the observed variations in hydration water mobility, while providing at the same time a semi-quantitative ruler to translate experimentally detectable variations of local dynamics into variations in entropy.

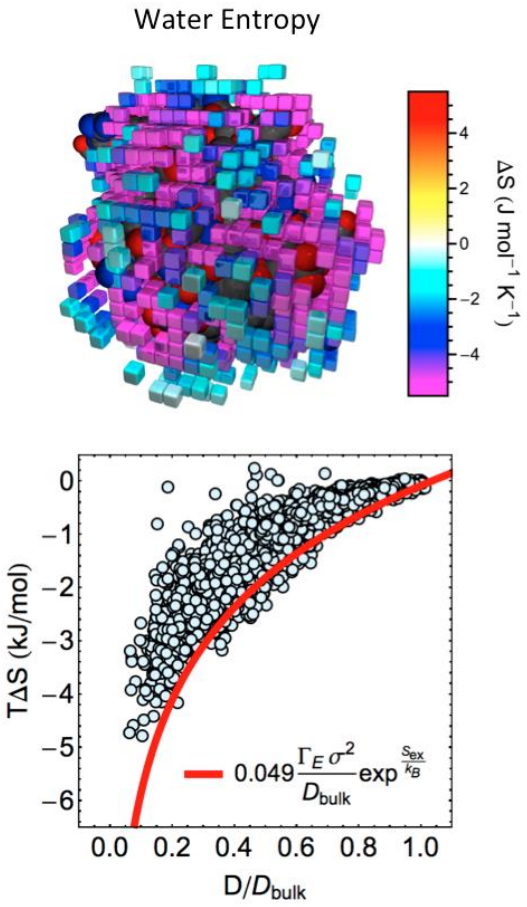

Fig. 20. Local hydration water entropies in the hydration shell of ubiquitin (top) are partially determined by local diffusion (bottom). The universal scaling law (red line), derived previously for bulk liquids, provides a lower limit for the local entropy (Dzugutov, M. *Nature* **1996**, *381*, 137-139).

A new target application of spatially resolved solvation free energies is their use as effective desolvation potentials in implicit solvent simulations of *meso*-scale systems of multiple interacting proteins and biomolecules. Together with the group of Douglas Tobias at UC Irvine, we recently developed a novel Monte Carlo simulation technique that includes efficient sampling of protein flexibility in simulations of complex protein solutions (Figure 21). We could show that this treatment significantly improves predictions of protein-protein interactions; however, the empirical treatment of (de)solvation free energies in the implicit solvation model remains a source of error. Our 3D-2PT approach enables us to derive tailored desolvation free energy potentials for the studied proteins directly from explicit solvent simulations, which then allow accurate simulations of biomolecular aggregation and molecular recognition in conditions resembling realistic biomolecular environments.

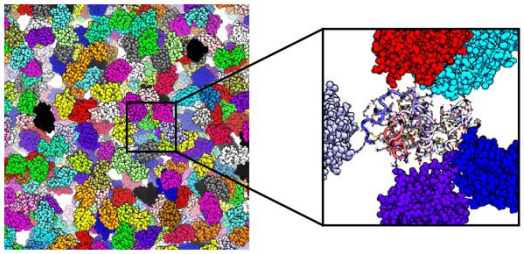

Fig. 21. Multiple-Conformation Monte Carlo simulation of a concentrated solution of lysozyme (169 mg/ml).

Future directions: We plan to extend the 3D-2PT methodology to mixed solvents, to develop a machine-learning procedure for the fast prediction of solvation free energies based on datasets generated by 3D-2PT simulations, and to derive computational tools to optimize the solvation free energy of lead compounds and ligand complexes.

Publications resulting from this research area: 124, 125, 128, 129, 132, 133, 134

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