Comprehensive description of interactions of ionic liquid ions with *Bs*LipA

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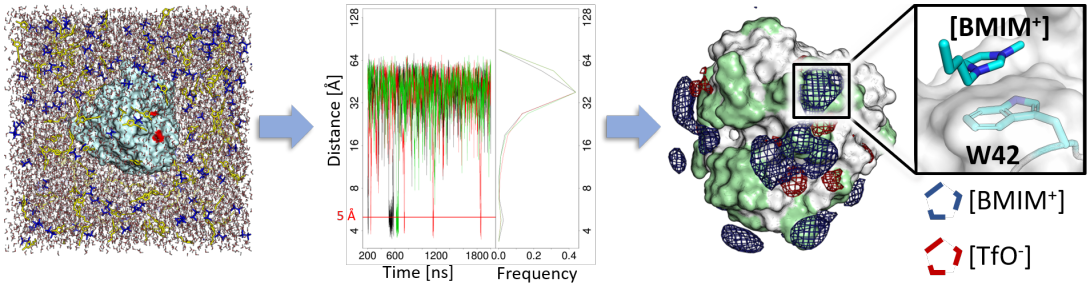

Ionic liquids (IL) and seawater are attractive (co-)solvents for biocatalysis due to their unique properties such as negligible vapor pressure respectively high abundance of seawater on earth [1,2] but, at the same time, influence activity and stability of enzymes in a complex manner [3]. However, this influence is not fully understood at the molecular level, and a general approach to guide protein engineering towards enzymes with improved IL and salt resistance has remained elusive. We aim at establishing a comprehensive description of the interactions of IL-/salt ions with the Lipase A from *Bacillus subtilis* (*Bs*LipA). Therefore, we employ molecular dynamics (MD) simulations to fundamentally understand the influences these solvents pose on proteins.

Our results reveal that MD simulations of *Bs*LipA in IL are feasible and successfully identify interaction sites of the IL ions with *Bs*LipA. We found multiple distinctive interaction sites of both the IL cation and the respective anion, which are in good accordance to recently published MD- and X-ray crystallography studies [4,5]. Interestingly, IL lead to only minor changes of the global protein structure but induce large changes in local intramolecular interactions of *Bs*LipA. This includes deep-reaching perturbations of the intramolecular H-bond network as well as changes in the ratio of active to inactive state conformations of the catalytic site, thus affecting both the catalytic site residues and the protein core. These results give new insights into the molecular effects of IL ions on *Bs*LipA activity in full atomic detail.

The knowledge derived from MD simulations will be applied to adapt a computationally highly efficient graph- and rigidity theory-based approach (Constraint Network Analysis) previously used successfully in the context of protein thermostability for the application with IL [6]. To experimentally validate and refine our model, a complete site-saturation mutagenesis library covering all possible single amino acid mutations of *Bs*LipA is available [7].

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