Project title

Rigidity theory-based prediction of protein stability in ionic liquids and seawater for guiding protein engineering

HPC System(s) and corresponding centre(s)

JUWELS Booster

Project ID

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Principal investigator

Prof. Dr. Holger Gohlke
Department of Mathematics and Natural Sciences, Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany.
and
Institute of Bio- and Geosciences (IBG-4: Bioinformatics), John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC), and Institute of Biological Information Processing (IBI-7: Structural Biochemistry), Forschungszentrum Jülich, Jülich, Germany

Project contributor(s)

Till El Harrar
John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC), Institute of Bio- and Geosciences (IBG-4: Bioinformatics), and Institute of Biological Information Processing (IBI-7: Structural Biochemistry), Forschungszentrum Jülich, Jülich, Germany
and
Institute of Biology VI (Biotechnology), RWTH Aachen, Aachen, Germany.

In this project, Prof. Dr. Holger Gohlke and Till El Harrar aimed to understand how certain solvents affect the activity of enzymes. Specifically, they investigated the influence of ionic liquids (IL: salts in a liquid state), aqueous ionic liquids (aIL), and seawater on the stability of a lipase and aIL-induced molecular changes in the biotransformation of a pyruvate decarboxylase. To achieve this, they used advanced computational techniques, including extended biomolecular simulations and configurational free energy computations, requiring the efficient use of GPUs on the JUWELS Booster system. The results of this project can be applied to improve enzyme resistance to such solvents and modify the biotransformations of enzymes in biotechnological approaches.

In this project, Prof. Dr. Holger Gohlke and Till El Harrar investigated the influence of certain ionic solvents, such as ionic liquids (IL), aqueous ionic liquids (aIL), and seawater, on the activity of two biotechnologically relevant enzymes. IL are salts that are liquid at low temperatures and represent ecologically attractive choices for biotechnological applications, but at the same time influence the activity of an enzyme in a complex manner, which is not fully understood at the molecular level. As a result, a general approach to guide protein engineering towards enzymes with improved IL and salt resistance has remained elusive.

Specifically, the team studied the effect of aIL on the enzyme *Bacillus subtilis* Lipase A (*Bs*LipA) as well as the molecular mechanism of aIL-induced changes on the product formation in the biotransformation of a thiamine diphosphate-dependent pyruvate decarboxylase from *Acetobacter pasteurianus* (*Ap*PDC). To gain a fundamental understanding of the complex aIL-enzyme systems, the team used advanced computational techniques, including biomolecular simulations and configurational free energy computations. The large size of the systems, exceeding 165,000 atoms, in conjunction with the outstandingly long simulation time of in total more than 1 millisecond, required the efficient use of Graphics Processing Units (GPUs) on the JUWELS Booster system.

During the project, the team achieved several key objectives, which they present in three publications. Firstly, they investigated aIL-enzyme interactions and their influence on the structure and dynamics of *Bs*LipA and revealed previously underexplored long-range perturbation effects of aIL affecting important structural regions of the enzyme (https://doi.org/10.1016/j.csbj.2021.07.001). Secondly, they evaluated the influences of aIL on individual intramolecular enzyme interactions on a mechanistic and energetic level. Their findings revealed complex solvent-, interactions-, conformation-, and concentration-specific effects of aIL, which however, can be exploited for deriving substitution patterns for efficiently improving an enzymes' stability towards specific aIL (https://doi.org/10.1021/acs.jcim.2c01123). **Figure 1** shows a comparison of interaction profiles for two such interactions, one of which (left) is heavily affected by the presence of various aIL, whereas the other one (right) is resistant to their effects. Thirdly, the team systematically evaluated the performance of currently available literature approaches and physicochemical/evolutionary properties to increase enzyme resistance to aIL by scrutinizing an experimental complete site-saturation mutagenesis library of *Bs*LipA. This resulted in the generation of a cost-and time-efficient approach for improving the enzyme resistance to aIL (https://doi.org/10.1016/j.csbj.2021.12.018). Finally, the team examined aIL-induced changes in the product formation by *Ap*PDC upon incubation in certain aIL, revealing that these aIL displace the substrate from its usual position by entering the region where the biotransformation occurs.

The insights gained in this project using multiple computational techniques and large-scale experimental data in an integrative manner expand our current understanding of complex aIL-enzyme systems on a molecular and thermodynamic level. The knowledge can be practically applied for two purposes. Firstly, it can be used to improve enzyme resistance to aIL for the use in biotechnological applications. This can be achieved by either targeting the regions affected by interactions of enzyme and aIL, as well as by improving commonly used computational tools by aIL-specific terms, thereby accounting for aIL-induced changes in the strength of intramolecular forces. Secondly, the knowledge can be used to modify the biotransformation properties of enzymes in biotechnological approaches. The insights gained in this project also stress the importance of considering a priori information and evaluating approaches for improving aIL resistance on large and diverse datasets in the future.

In the granting period, the following publications were published:

El Harrar, T., Frieg, B., Davari, M. D., Jaeger, K.-E., Schwaneberg, U., Gohlke, H. (**2021**). *Aqueous ionic liquids redistribute local enzyme stability via long-range perturbation pathways*. Computational and Structural Biotechnology Journal, pages 4248-4264. https://doi.org/10.1016/j.csbj.2021.07.001.

El Harrar, T., Jaeger, K.-E., Schwaneberg, U., Gohlke, H. (**2021**). *Critical Assessment of Structure-based Approaches to Improve Protein Resistance in Aqueous Ionic Liquids by Enzyme-wide Saturation Mutagenesis*, Computational and Structural Biotechnology Journal, pages 399-409. https://doi.org/10.1016/j.csbj.2021.12.018.

El Harrar, T. & Gohlke, H. (**2023**). *Millisecond-long sampling for a comprehensive energetic evaluation of aqueous ionic liquid effects on amino acid interactions*, Journal of Chemical Information and Modeling, pages 281–298. https://doi.org/10.1021/acs.jcim.2c01123.

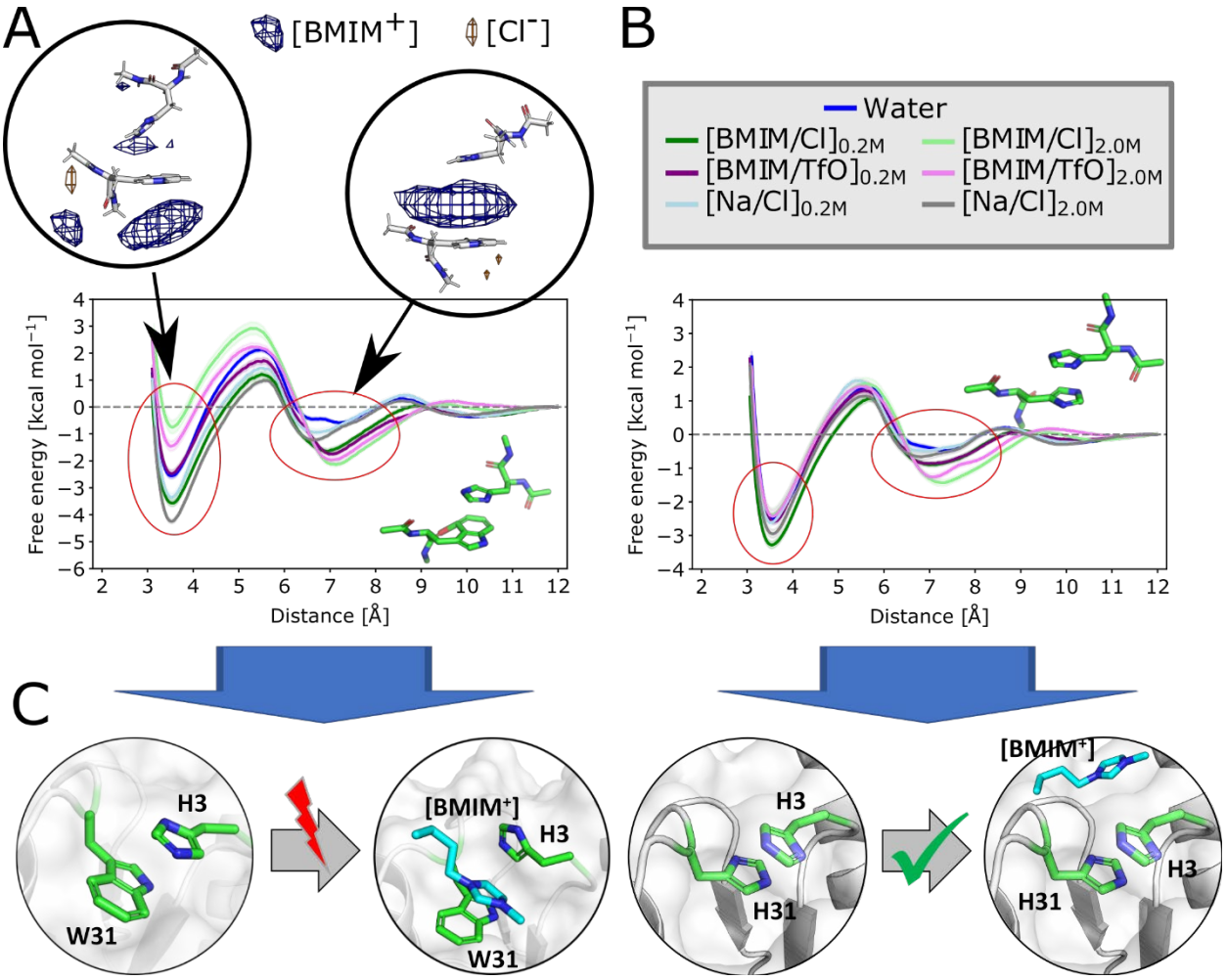

Figure 1: PMFs of π-stacking interactions reveal a novel mutation pattern for rational mutagenesis approaches. A-B: PMFs of Trp-His (A) and His-His (B) in water (blue), aIL (0.2 M [BMIM/Cl]: light green; 2 M [BMIM/Cl]: green; 0.2 M [BMIM/TfO]: purple; 2 M [BMIM/TfO]: pink), and [Na/Cl] (0.2 M: light blue and 2 M: grey). In A, average densities for representative states are shown for the Trp-His interaction in [$BMIM^+$] (blue meshes) and [Cl^-] (orange meshes) at 3.5 Å and 7 Å, respectively. Data is shown as mean ± standard error of the mean. **C:** Simplified representation of substitution pattern for the perturbed W31-H3 interaction and the presented His-His substitution pattern in wild type *Bs*LipA. Figure taken from https://doi.org/10.1021/acs.jcim.2c01123 .