

Understanding Alcohol Dehydrogenase in Deep Eutectic Solvents - a molecular dynamics study

Design of Redox Biocatalysis in Deep Eutectic Solvents (DESiRE)

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In Short

- Establishing ADH-catalyzed bioreduction in Deep Eutectic Solvents (DESs)
- Atomistic resolution molecular dynamics (MD) simulations for a deeper understanding of the enzyme behavior and enzyme-solvent interactions
- Calculation of free-energy (FE) profiles of a substrate molecule along the substrate-binding tunnel of horse-liver alcohol dehydrogenase (HLADH)
- Screening of tunable DESs with varying thermo-physical properties

Solvent design and selection with a particular emphasis on their greenness is currently a main focus both in chemo- and biocatalysis. Biocatalysis has traditionally relied on either aqueous- or classical organic media (mainly for hydrolases), or biphasic systems thereof. In this respect, Deep Eutectic Solvents (DESs) have been identified as „the solvents of the 21st century“ and offer a new dimension as ‘Safe and Green-by-Design’ solvents for biocatalysis. In a nutshell, DESs’ assets are based on their often biogenic origin, and their properties such as melting points below room temperature, low volatility, high thermal stability, tuneability analogous to ionic liquids, biodegradability, large availability at acceptable costs, and straightforward preparation. In particular, the high degree of freedom in designing DESs from a variety of (biogenic) substances enables the creation of a sustainable solvent platform. The enormous potential of DESs for biocatalytic applications has been mainly explored for hydrolases (EC3), after their first application demonstrated in 2008. Different than EC3 enzymes, oxidoreductases (EC1) have only rarely been employed in DESs at predominant amounts. Few examples for the use of alcohol dehydrogenases (ADHs) have been documented (mainly from our previous DFG-funded project) either to demonstrate the use of DESs for organic synthesis while using a DES component as substrate or to understand the effect of different DESs on the activity and stability of a redox enzyme. Redox biocatalysis is still underrepresented for DES-based applications which will be addressed deeply by the

interdisciplinary setup of DESiRE. Overall, the here presented follow-up project [1] represents a clear strategy to elucidate the protein-DES-water interactions, which possess a considerable potential for understanding the ADH-catalysis in DESs. Especially the close collaboration between experimental methods and molecular simulations will lead to new insights on different scales. Moreover, this follow-up project [1] will further open new possibilities to evaluate other enzyme classes for the effects of DESs on catalytic performance (activity, stability and selectivity) of enzymes.

This project is thereby a close collaboration of the Institute of Thermal Separations Processes (project leaders: Prof. Dr. Irina Smirnova and Dr. Sven Jakobtorweihen) at the Hamburg University of Technology and the Institute of Technical Chemistry at the Leibniz University Hannover (project leader: Prof. Dr. Selin Kara). While the group of Prof. Dr. Selin Kara performs the experimental investigation, the MD simulations at the NHR will be carried out by the group of Dr. Sven Jakobtorweihen. The MD simulations will be utilized to gain a deep understanding of the enzyme-solvent and enzyme-substrate interactions on the molecular level.

In the last DFG project (grant numbers: KA 4399/3-1 and JA 2500/5-1) and NHR project (hhi00032) we investigated the behavior of HLADH in the presence of different organic solvents, an organic-aqueous interface and different DESs at varying water contents by means of experimental analysis and molecular simulations. [2–4] Thereby, the main focus was laid on unraveling structural changes of the enzyme and studying solvation effects in different non-conventional reaction media, which resulted in various publications. [2–4] During that analysis we identified structural changes of HLADH in particular at the active center, which may impact its interaction with the substrate molecule and, hence, its catalytic performance. To further understand the influence of different non-conventional solvents on the active cavity, FE profiles along the designated substrate-binding path should be calculated from the MD simulations. This allows to identify free-energy barriers for a potential substrate molecule (e.g., cyclohexanone) and to calculate the binding free-energy for the substrate, which can subsequently be compared with experimentally derived values.

In general, quantifying the interaction of the substrate molecules with the active center of HLADH delivers an in-depth analysis of the catalytic capabili-

ties of HLADH in different media and in combination with the experimental analysis will shed light on future solvent selection.

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<https://www.tuhh.de/v8/tuhh-thermische-verfahrenstechnik.html>

More Information

- [1] DFG - Design of Redox Biocatalysis in Deep Eutectic Solvents (DESiRE). <https://gepris.dfg.de/gepris/projekt/391127961>
- [2] J. P. Bittner, L. Huang, N. Zhang, S. Kara, S. Jakobtorweihen, *J. Chem. Theory Comput.* **17**, 5322-5341 (2021). doi: 10.1021/acs.jctc.1c00274
- [3] J. P. Bittner, N. Zhang, L. Huang, P. Domínguez de María, S. Jakobtorweihen, S. Kara, *Green Chem.* **24**, 1120-1131 (2022). doi:10.1021/acs.jctc.1c00274
- [4] N. Zhang, J. P. Bittner, M. Fiedler, T. Beretta, P. Domínguez de María, S. Jakobtorweihen, S. Kara, *ACS Catal.* **12**, 9171-9180 (2022). doi:10.1021/acs.jctc.1c00274

Project Partners

Group of Prof. Selin Kara at the Institute of Technical Chemistry - Gottfried Wilhelm Leibniz University Hannover (LUH)

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