

## Enzymes in Non-Conventional Reaction Media

### Rational Analysis of Structural and Functional Changes of Oxidoreductases in Non-Conventional Reaction Media

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#### Kurzgefasst

- Establishing ADH-catalyzed bio-reduction in water-deficient reaction media
- Atomistic resolution molecular dynamics (MD) simulations for a deeper understanding of the protein-solvent interactions
- First evaluation of the catalytic performance of HLADH in the DES composed of choline chloride and glycerol (1:2) by molecular dynamic simulations
- Increase in enzyme flexibility and change in protein structure explains the onset of the catalytic activity at 10 % of water (volume fraction)
- Screening of additional solvents and further characterization of the HLADH structural changes and catalytic performance in non-conventional reaction media

This project is a close collaboration of the Institute of Thermal Separation Processes (Project leader Dr.-Ing. Sven Jakobtorweihen) at the Hamburg University of Technology (TUHH) and the Department of Engineering – Biocatalysis and Bioprocessing (Prof.-Assoc. Selin Kara) at the Aarhus University (AU). Whereby, the latter group will conduct experimental investigations and only the group at the TUHH will carry out simulations at the HLRN. The project is funded by the 'Deutsche Forschungsgemeinschaft' (DFG, Project-ID: 391127961). 1 The central goal of this project is the characterization and quantitative evaluation of the impact of non-conventional solvents on the redox catalysis performed by alcohol dehydrogenases (ADH). Therefore, molecular dynamics (MD) simulations of the protein in several organic solvents and varying water content will be carried out.

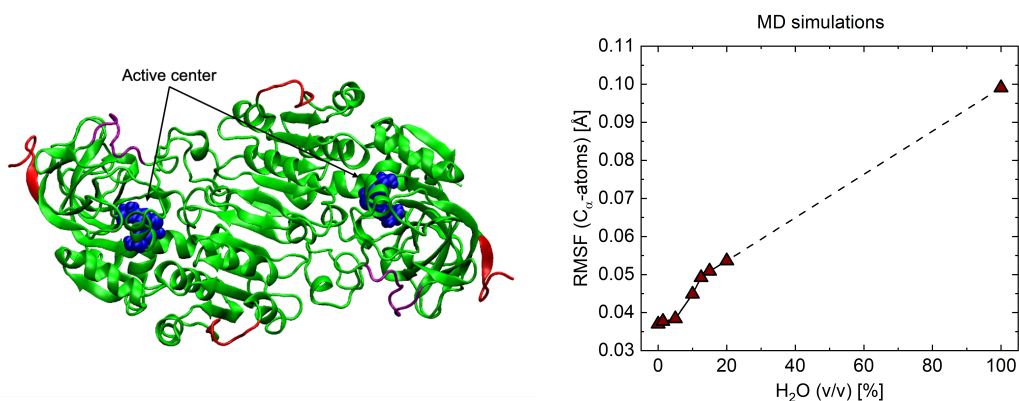
Bio-catalysis may be considered as 'green chemistry', as it takes place in aqueous solution, is performed by biodegradable enzymes and often runs under environmentally benign conditions. However, the usage of water as reaction environment has been debated in the bio-catalysis community due to (1) limited solubility of hydrophobic reagents in aqueous solution, (2) water-induced site reactions

and (3) water-induced enzyme denaturation. 2 Establishing industrial relevant oxidation/reduction reactions performed by horse liver alcohol dehydrogenase (HLADH) in non-conventional media demands a systematic investigation of their behavior in these reaction environments. The HLADH structure is shown in Fig. 1 (left). Thereby, molecular dynamics (MD) simulations are an essential tool to gain deep understanding of the protein-solvent interactions and can relate the solvent properties (e.g., hydrophobicity, molecular structure, water activity) to the HLADH catalysis characteristics (e.g., activity, stability, selectivity). The in-depth investigation of the structural properties of HLADH in non-conventional reaction media as well as the predominant interactions between the protein, solvent and water, demands atomistic resolution of the protein system. This will result in simulations with a large number of atoms requiring parallel calculations.

The hydration of the enzyme is one of the key factors in order to maintain enzymatic activity in non-conventional media. The catalytic activity of enzymes can be maintained in organic media, however, for an optimal enzyme activity water has to be present in order to allow the enzyme to follow conformational changes during the formation of enzyme-substrate complexes. Whereby, the present water will form a hydration layer around the enzyme ensuring its flexibility and stability. As long as the so-called 'enzyme-bound water' is present, the rest 'bulk water' can be replaced with an organic solvent without enzyme deactivation. How much water is needed on the protein surface of HLADH to ensure its activity in organic media, is still unknown. Thus, determining the appropriate amount of water for each solvent becomes an additional task that can be addressed by MD simulations.

Deep eutectic solvents (DESs) have recently emerged as a new solvent class for bio-catalytic applications as they often offer diminished toxicity and better biodegradability. Mostly composed of an ionic liquid and a hydrogen bond donor (HBD), DESs owe tailored characteristics and therefore promising opportunities in tuning the solvent effects for bio-catalysis. Recent developments of molecular methods allow to characterize not only the formation of DESs but also their effect on bio-molecular systems. 3 Herein, we present novel investigations of HLADH in different DES-water mixtures. 4

In order to elucidate the effects of the DES composed of choline chloride and glycerol in a ratio of



**Abbildung 1:** Schematic representation of the HLADH crystallographic structure (left). The two active centers of HLADH including the active zink ion are displayed in blue. Flexible regions of the protein structure are displayed in purple and red. 4 Time averages of the root mean square fluctuations (RMSF) of the C<sub>α</sub>-atoms of HLADH in the DES composed of choline chloride and glycerol in a ratio of 1:2 with different water content from MD simulations (right). 4

1:2 (ChCl-Gly) on the HLADH-catalyzed reaction, experimental measurements and MD simulations have been performed for DES-water mixtures ranging from 0% to 20% of water (volume fraction). Experimental measurements show no enzymatic activity in the mixtures with a water concentrations below 10%. 4 The MD simulations were used to quantify the HLADH's molecular flexibility, hydration layer and the intra-protein hydrogen bond network. Whereby, a reduced flexibility of the enzyme in DESs solutions with a low water content compared to the pure aqueous environment was observed (see Fig. 1, right). 4 The onset of catalytic activity of HLADH in mixtures of ChCl-Gly and water could be explained by an increase in its flexibility as well as structural changes observed by monitoring the root mean square deviations (RMSD) of HLADH and the intra-protein hydrogen bond network. 4

High attractive interactions between water and the DES components were quantified by determining the thermodynamic water activity in the DES-water mixtures via experiments and MD simulations. 4 Whereby, the water activity is calculated by thermodynamic integration for one water molecule that has been decoupled from its surrounding. These strong attractive interactions between the ChCl-Gly molecules and water cause a preferential solvation of water in the bulk phase and therefore inhibit the hydration of the protein surface. In addition the DESs molecules can mimic the hydrogen bond interaction with water and compete with water for interactions with the protein. This can further explain that HLADH is not active in solutions with a low water content. However, it is yet to be shown if the minimal hydration layer, which is needed for enzymatic activity of HLADH in ChCl-Gly, is similar for different solvents. 4

Understanding the behavior of the enzyme on the atomistic level is the crucial part for predicting the

catalytic performance in different non-conventional media. Therefore, MD simulations provide valuable insights for understanding these effects and guiding the selection of the solvent as well as the necessary water content. In future studies, we will explore additional solvents to further understand the effect of low-water media on the catalytic behavior of HLADH.

### WWW

<https://www.tuhh.de/v8>

### Weitere Informationen

- [1] DFG - GEPRIS - Rational Analyse der strukturellen und funktionellen Veränderungen von Oxidoreduktasen in unkonventionellen Reaktionsmedien. <http://gepris.dfg.de/gepris/projekt/391127961>
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- [4] L. Huang, J. P. Bittner, P. D. d. María, S. Jakobtorweihen, S. Kara, *ChemBioChem* **21**, 811-817 (2020). doi:10.1002/cbic.201900624

### Projektpartner

Group of Prof.-Assoc. Selin Kara at the Department of Engineering - Biocatalysis and Bioprocessing at the Aarhus University (AU)

### Förderung

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