

## Enzymes in Non-Conventional Reaction Media

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

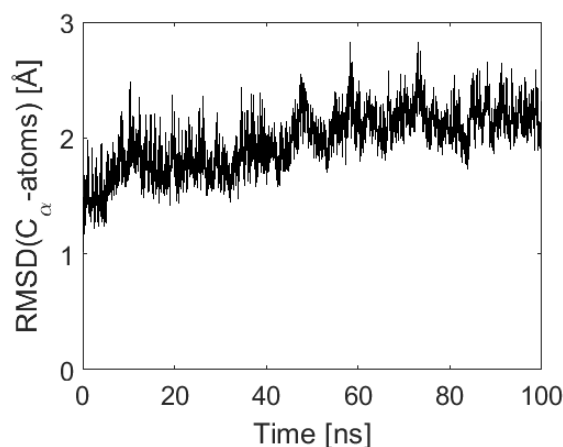
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#### In Short

- Determination of the range of organic solvent properties to optimize the catalytic activity and stability of oxidoreductases in low-water media
- Characterization of the interactions and molecular complexes formed between oxidoreductases and the solvents
- Development of a 'Solvent Selection Guide'

This project is a close collaboration of the Institute of Thermal Separation Processes (Project leader Dr.-Ing. Sven Jakobtorweihen) at the Technical University of Hamburg (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' [1] (DFG, Project-ID: 391127961). The central goal of this project is the characterization and quantitative evaluation of the impact of organic 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.

Establishing biotransformation in non-aqueous media is recognized as a hot-topic in the biocatalysis community owing to several advantages: (1) high-solubility of synthetically interesting hydrophobic substrates, (2) reduced risk for water-induced protein denaturation, and (3) elimination of undesired side reactions. Whereas a high number of research studies on the application of hydrolases (EC3) in low-water media are published, very few publications are available on the use of oxidoreductases (EC1) in this non-conventional environment. This is unfortunate as oxidoreductases catalyses synthesis of valuable chemical compounds which are of high interest for pharma and agro, but as well as for bulk chemical industries. Among the oxidoreductase enzymes (e.g., dehydrogenases/reductases, oxygenases, oxidases, and peroxidases), alcohol dehydrogenases (ADHs) are predominantly applied in organic synthesis not



**Figure 1:** Root-mean-square-deviations of the  $C_{\alpha}$ -atoms of an ADH with respect to the crystal structure in water at 1 bar and 298.15 K

only at laboratory but also at industrial scale. However, these applications are mainly based on the use of aqueous media. Due to the lack of an understanding for the effects of organic solvents, which would replace the water as the reaction environment, in the present project proposal we focus on a rigorous evaluation of these effects on the ADH-catalysis. For this purpose, two model ADHs will be characterized in commonly used organic solvents (e.g., ethyl acetate and methyl tert-butyl ether), in 'greener' ones (e.g., 2-methyltetrahydrofuran) as well as in deep-eutectic-solvents, which are a new generation of solvents with more sustainable characteristics compared to some ionic liquids. Thereby, the focus will be on relating solvent properties (e.g., hydrophobicity, molecular structure, and water content) to ADH catalysis characteristics (e.g., activity, stability, and selectivity).

Molecular dynamics (MD) simulations will be performed for a deep understanding of the interactions between the protein, solvents, and water. This investigation demands atomistic resolution of the protein systems resulting in simulations with a large number of atoms, requiring parallel calculations. To understand the influence of the different solvents on bio-catalysis, the interactions and molecular complexes formed between the model enzyme and the surrounding solvents should be determined. For investigating the behavior of enzymes in different solvents, a variety of properties can be calculated from MD simulations. Structural changes of the enzyme can be characterized by the root-mean-square

deviations (RMSD). For example, Fig. 1 shows that an ADH structure is stable in MD simulations. In addition, the radius of gyration, which indicates protein unfolding, can be analyzed over the simulation time. In case of enzymes dissolved in organic solvents, it was found that the enzyme flexibility is decreased compared to aqueous solutions [2]. In MD simulations the enzyme flexibility is often related to the B-factors or root-mean-square fluctuations. As the location of every molecule is monitored over time in MD simulations, these quantities can easily be calculated and flexible regions of the protein can be identified. Another important factor for bio-catalysis in water-deficient media is the amount of water bound to the enzyme, as it can ensure its flexibility and stability. Whereas the experimental determination of the 'bound water' is difficult [2], its calculation using MD simulations is straightforward. Furthermore, a determination of the spatial distributions of water molecules in the proximity of the enzyme as well as an identification of water clusters and networks is possible in MD simulations [3]. Accordingly, the in-depth investigation of the water binding sites on the surface of the protein demands the usage of a force field with atomistic resolution, this results in computational demanding simulations. Having elucidated and understood the effects of the organic solvent properties on the enzyme catalysis, our final goal is to develop general solvent selection rules taking into account the solvent properties.

Overall, the here presented project topic has not been investigated yet and it represents a clear strategy to elucidate the protein-solvent-water interactions, which possess a considerable potential for understanding the ADH-catalysis in non-aqueous media. Moreover, this research project will open new possibilities to evaluate other enzyme classes for the effects of organic solvents on enzyme characteristics.

## WWW

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

## More Information

- [1] <http://gepris.dfg.de/gepris/projekt/391127961>
- [2] W. Kroutil, Organic Synthesis with Enzymes in Non-Aqueous Media. Edited by Giacomo Carrea and Sergio Riva., *ChemSusChem* **2008**, 1, 467–467. doi:10.1063/1.3382344
- [3] R. Wedberg, J. Abildskov, G. H. Peters, Protein Dynamics in Organic Media at Varying Water Activity Studied by Molecular Dynamics Simulation, *J. Phys. Chem. B* **2012**, 116, 2575–2585. doi:10.1021/jp211054u

## Project Partners

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

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