MOLECULAR DYNAMICS PROVIDES INSIGHTS INTO AN ENGINEERED OXIDOREDUCTASE WITH ALTERED COFACTOR SPECIFICITY

Josef Sperl, Chair of Chemistry of Biogenic Resources, Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany
josef.sperl@tum.de
Tobias Gmelch, Chair of Chemistry of Biogenic Resources, Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany
Volker Sieber, Chair of Chemistry of Biogenic Resources, Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany

Key Words: cofactor specificity, oxidoreductase, semirational enzyme engineering, molecular dynamics

The aldehyde dehydrogenase from *Thermoplasma acidophilum* is one of the key enzymes in a previously established synthetic cell-free reaction cascade for the production of alcohols. In order alter the cofactor specificity of this enzyme from $NADP^+$ to NAD^+, we applied the CSR-salad tool and investigated further amino acid positions based on its crystal structure. Introduction of five point mutations reduced the K_m for NAD^+ from 18 mM to 0.6 mM and simultaneously increased the activity for D-glyceraldehyde from 0.4 U/mg to 1.5 U/mg. In order to understand the structural basis of the beneficial mutations, we performed molecular dynamics simulations that showed a significant flexibility gain at the cofactor binding site of the final variant. This increased flexibility facilitates a loop movement that largely contributes to the gain in activity and cofactor specificity. We envision a future optimization potential for aldehyde dehydrogenases based on our results.