Atomistic investigation of enzyme immobilization

A Molecular Dynamics investigation of enzyme immobilization on responsive gels

M. Fiedler, K. M. Eckert, K. R. Dittmer, P.A. Gurumoorthi, S. Müller*, Institute of Thermal Separation Processes, Hamburg University of Technology*

In Short

- Immobilization of enzymes on stimuli-responsive gels with distinct monomeric units
- Determination of the relative free energy of adsorption
- Atomistic resolution molecular dynamics (MD) simulations for a deeper understanding of the protein adsorbent-interaction
- Investigation of causes of inactivation during immobilization

Stimuli-responsive hydrogels are known to undergo macroscopic swelling or shrinkage in response to external stimuli, including temperature, pH, light, pressure, or changes in solvent composition [

]. Their responsiveness arises from the monomeric units within the polymer network. While *N*-isopropylacrylamide is well known for its temperature- and chemo-responsiveness, functional groups such as carboxylic acids in acrylic acid causes pH-responsiveness of the porous matrix.
Previous research has primarily focused on applications in medicine or as actuators, an emerging field involves their use in smart reactor systems, particularly in biocatalysis [

]. Over the last decades, these smart carriers are increasingly employed due to their porous network, which changes throughout the swelling and shrinkage. While in the shrunken state, small pore sizes increase mass transport limitation inside the gel, in the swollen state, enlarged pores reduce diffusion limitations. While commercial carriers are often characterized by large surface areas for increase enzyme loading after immobilization[

]. However, this is mostly achieved by decreasing pore sizes, which subsequently increase the mass transport limitations. These distinct mass transport properties in carriers with larger or smaller pores are both occuring in responsive gels as well. Employing these smart gels as biocatalyst carriers, in-line reaction control can be reached without external actuation by modulating mass transport.
The enzyme studied in this work is formate dehydrogenase (FDH), which catalyzes the industrially relevant reduction of the cofactor NAD^+ to NADH. In addition to its importance for NADH regeneration, the reaction constitutes a sustainable carbon dioxide fixation pathway, linking industrial cofactor recycling to CO_2 utilization strategies. For this sensitive enzyme, immobilization on suitable carrier matrices can preserve catalytic activity under specific process conditions [

]. However, inappropriate carrier matrices or immobilization strategies may lead to a significant loss of biocatalytic activity. Therefore, optimized immobilization conditions are essential for successful industrial application. These conditions include suitable binding mechanisms, such as adsorption or binding, as well as sufficiently strong enzyme-carrier interactions to achieve high immobilization yields.
Importantly, successful reactor integration requires not only high immobilization yields but also high catalytic activity of the immobilized enzyme. The residual activity after immobilization strongly depends on structural changes of the enzyme during the immobilization process and on the accessibility of the enzymes active center to the substrate. In this context, the binding configuration of the enzyme on the carrier matrix is critical, as unfavorable orientations may limit substrate diffusion toward the active site.
By combining biocatalysis with smart hydrogels, this work employs molecular dynamics simulations to investigate enzyme-matrix binding mechanisms and the accessibility of enzymes after immobilization on responsive surfaces. While in experimental studies the distinct polymers can be synthesized, characterized and the catalytic performance of the immobilized enzyme investigated, the underlying mechanisms and principles of interactions and conformational effects cannot be determined. By using a free energy approach to calculate the free energy of adsorption of the enzyme on these carriers, the occurring interactions and binding configurations of the enzyme can be characterized. For this a two-dimensional free energy approach was used to sample the whole surface of the enzyme. Based on this analysis, favorable binding configurations can be identified, and the suitability of different carrier materials can be compared. Furthermore, the observed enzyme-carrier interactions can indicate matrices in which reduced enzyme loading or increased leaching are likely to occur. The system is represented as an simplified system with immobilized functional groups acting as the gel surface and just enzyme in water representing the enzyme interaction as shown in .

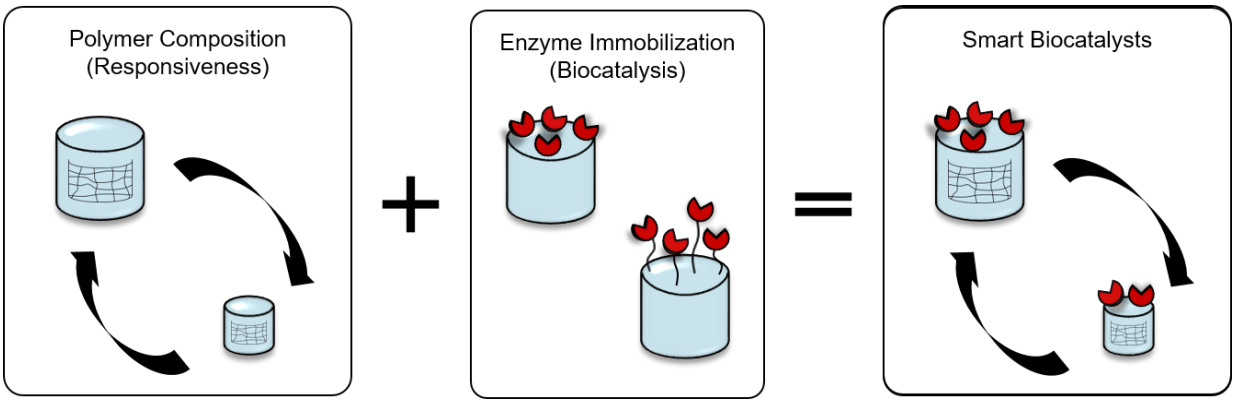

Figure 1: *Schematic representation of the combined approach of stimuli-responsive gels with biocatalysis. From left to right: responsivity, biocatalysis, smart biocatalysis.*

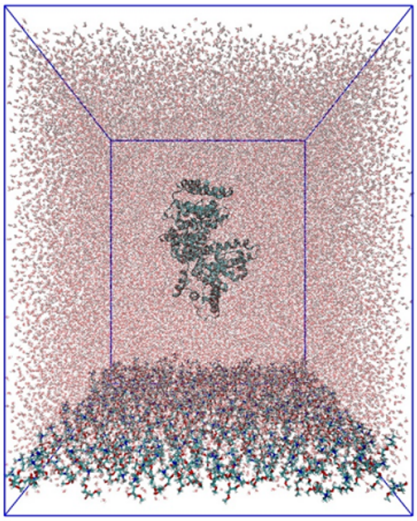

Figure 2: *The molecular dynamics simulation box with the enzyme in a water phase (top) and the gel phase represented with immobilized functional groups (bottom).*

The simulative framework employed in this work offers broad applicability to a wide range of systems. The surface of the gel can be readily exchanged to represent distinct polymer formulations, such as carboxylic acid or amide functionalities characteristic of pH-responsive polymers. Moreover, the simulation principle enables the combined variation of both polymer surfaces and enzymes, within box sizes constrained by computational cost. Beyond enzyme immobilization, this approach can be extended in future studies to model interactions between responsive polymer matrices and complex protein mixtures, thereby providing an approach for the design of smart hydrogel-based separation systems on a molecular level for protein or enzyme purification from fermentation broths in downstream processing.

WWW

https://www.tuhh.de/v8/de/people/professors-and-group-leaders/simon-mueller

More Information

[1] E. M. Ahmed, *J. Adv Res.* **6**, 105-1 (2015). doi: 10.1016/j.jare.2013.07.006

[2] D. Eixenberger, A. Kumar, S. Klinger, N. Scharnagl, A. W. H. Dawood, A. Liese *Catalysts* **13**, 1130 (2023). doi: https://doi.org/10.3390/catal13071130

[3] A. Liese, L. Hilterhaus *Chemical Society Reviews* **42**, 6236 (2013). doi: https://doi.org/10.1039/c3cs35511j

[4] S. Rouf, Y, E. Greish, B. Van der Bruggen, S. Al Zuhair *Int. J. Hydrogen Energy* **142**, 1067-1074 (2025). doi: https://doi.org/10.1016/j.ijhydene.2024.12.492

Project Partners

Institute of Technical Biocatalysis, Hamburg University of Technology

Funding

This project is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - SFB 1615 - 503850735.

DFG Subject Area

421-04