Karlsruhe Institute of Technology

Institute for Biological Interfaces
IBG-1 | Biomolecular Micro- and Nanostructures

# Formulation of Enzyme-based Biomaterials for Flow-biocatalysis

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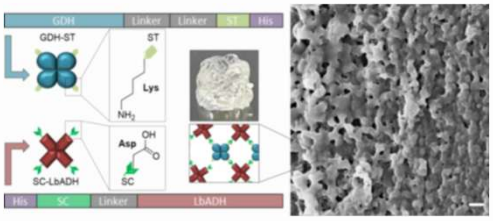
## Motivation

Formulation of protein-based biomaterials that enable an efficient and stable integration of biocatalysts in flow biocatalysis for a 'greener' production of chemicals in industrial processes.

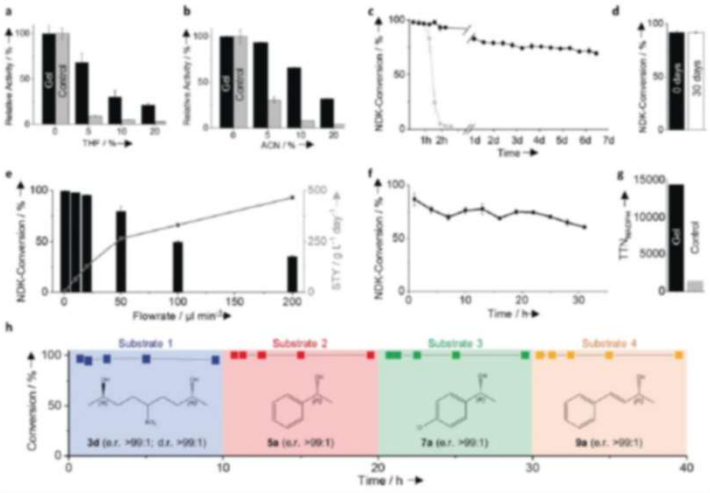
## All-Enzyme Hydrogel (AEH)

All-enzyme hydrogels are covalently crosslinked upon mixing via the genetically encoded SpyCatcher/SpyTag system. They represent a simple and efficient system of carrier-free immobilization of enzymes in microfluidic bioreactors.[1]



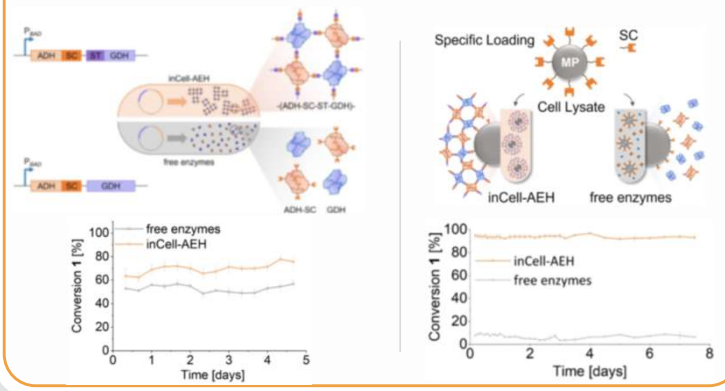

### Flow-biocatalysis

The gels showed excellent stereoselectivity, stable conversion rates and high STY for more than 7 days. The sequential perfusion with different ketones and numbering up of the microreactors demonstrate the potential use for industrial processes.



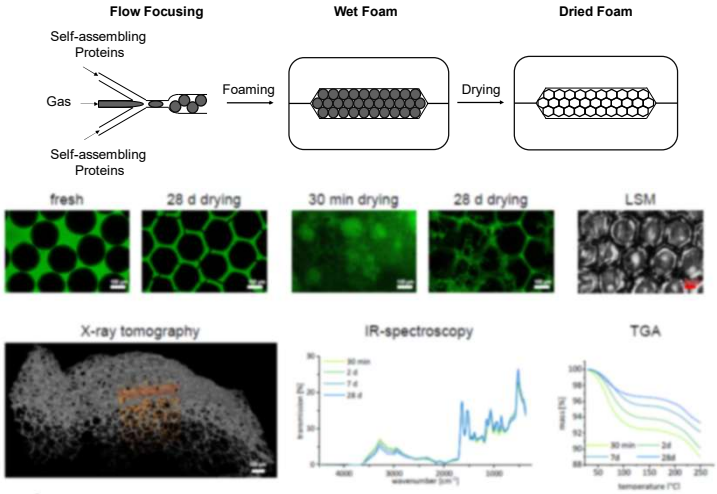

## inCell-AEH

With an *E. coli*-based in vivo system AEHs could be produced intracellularly, resulting in highly active and stable biocatalytic materials that can be used for whole-cell flow-biocatalysis or easily be purified from crude bacterial lysates.[2]



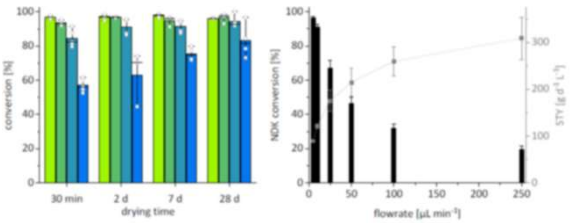

## Enzyme Foam

Biocatalytically active enzyme foams produced via microdroplet foaming of self-assembling enzymes exhibit a regular hexagonal honeycomb network with an average pore diameter of 160 µm. After the drying process, the remaining water amount is between 3 to 7 %.[3]



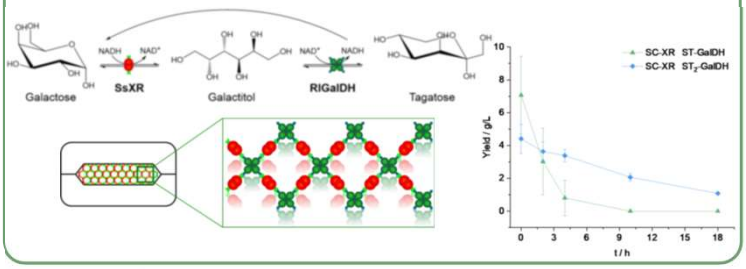

### Flow-biocatalysis

The enzyme foams showed higher resistance to high flow rates and pressures with increased drying time, resulting in a highly stable and catalytically active biocatalyst.




### Application to a D-Tagatose Producing System[3,4]

Attachment of multiple binding sites allowed the production of the rare sugar D-tagatose by a foam consisting of SC-SsXR and $ST_2$-RIGalDH over 18 h.




## References

[1] T. Peschke, P. Bitterwolf, S. Hansen, J. Gasmi, K. S. Rabe, C. M. Niemeyer, *Angew. Chem. Int. Ed.*, **2018**.
[2] P. Bitterwolf, A. E. Zoheir, J. Hertel, S. Kröll, K. S. Rabe, C. M. Niemeyer, *Chem. Eur. J.*, **2022**
[3] J. S. Hertel, P. Bitterwolf, S. Kröll, A. Winterhalter, A. J. Weber, M. Grösche, L. B. Walkowsky, S. Heißler, M. Schwotzer, C. Wöll, T. van de Kamp, M. Zuber, T. Baumbach, K. S. Rabe, C. M. Niemeyer, *Adv. Mater.*, **2023**.
[4] J. Liu, G. Zhang, S. Kwak, E. J. Oh, E. J. Yun, K. Chomvong, J.H.D. Cate, Y. Jin, *Nat. Commun.*, **2019**.