## Spatial organization of multi-enzyme systems in porous materials

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Combination of several enzymes in one-pot opens a very attractive pathway to access more complex synthetic schemes to manufacture high added-value products in a more sustainable manner. Nowadays, chemical biomanufacturing is dominated by microbial fermentations that use native pathways to mainly produce alcohols, acids or aminoacids. In the last decade, cell-free multi-enzyme systems have emerged as an alternative to whole cell biotransformations in chemical manufacturing<sup>1</sup>. However, enzyme isolation faces some challenges (productivity, robustness and costs) for implementing cell-free multi-enzyme systems into the industrial context. Scaling-up cell-free bioprocesses involving cofactor-dependent enzymes are even more challenging because they demand for exogenous cofactors that hamper the downstream processing and limit their economic feasibility of the process. Inspired by the spatial organization and the molecular confinement of metabolic pathways within the living cells, our group has exploited several immobilization techniques to spatially organized a variety of multi-enzyme systems (oxidoreductases, oxidases, transaminases) confined with their corresponding cofactors (NAD(P)H, PLP, FAD...) across the surface of synthetic porous carriers<sup>2,3</sup>. Our work aims at creating in vitro and confined enzymatic pathways as selfsufficient and multi-functional heterogeneous biocatalysts, where cofactors and enzymes are successfully recycled and reutilized during several operational cycles to increase their total turnover numbers (TTN). The performance of these immobilized multi-enzyme systems has been expanded to flow-biocatalysis and accompanied by some metrics that quantify the sustainability of the processes.

## REFERENCES

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## FIGURES



**Figure 1:** Spatial organization of enzymes across the porous surface of solid carriers for step-wise biocatalysis