From Surface Analysis to Process Intensification: Data Driven Immobilization Workflow of Ene-Reductases

Cristina Lía Fernández Regueiro[1], Lauriane Pillet[2], Markus Richard Busch[2], David Roura Padrosa[1], Francesca Paradisi[2]

[1]Inseit AG (Gesellschaftsstrasse 42, Bern, Switzerland)
lia.fernandez@inseit.ch
[2]DCBP, University of Bern (Freiestrasse 3, 3012, Bern, Switzerland)

Immobilization is often the bottleneck in transitioning enzymes from the lab to industrial flow processes.[1] While traditional methods rely on inefficient "trial-and-error" screening, inseit AG employs a rational design workflow to streamline this transition. By combining computational surface analysis with targeted reactivity profiling, we rapidly identify optimal immobilization strategies without extensive experimental screening.

In this work, we demonstrate this workflow on a panel of ene-reductases (ERs). The surface topologies of McOYE, TtENR, and the novel HeOYE were analyzed using CapiPy and ImmoFinder.[2,3] These tools map functional clusters and predict the reactivity of surface amino acids toward specific support and immobilization chemistries, enabling an initial in silico screening to replace random testing.

Guided by these predictions (72 conditions screened), we selected 1 chemistry for TtENR and 2 chemistries McOYE, and 2 chemistries for HeOYE, to test and validate in the lab. TtENR and McOYE achieved >99% Immobilization Yield (IY) and >75% Retained Activity (RA). In contrast, HeOYE showed fewer exposed reactive amino acids on the surface, making it more difficult to immobilize, but still achieved an Immobilization Yield (IY) of 60% and a Recovered Activity (RA) of 23%.

As an example of the utility of immobilized enzymes in flow, the rationally immobilized HeOYE was co-packed with a cofactor-recycling enzyme (immobilized BmGDH) in a continuous flow reactor. This optimized heterogeneous system enabled the continuous reduction of cinnamaldehyde with a 62-fold process intensification (2173.9 mg L-1h-1) compared to the batch process.

This study confirms that characterizing protein surface topology before experimental screening significantly streamlines the development of robust biocatalysts for industrial flow applications.

[1] Fernández Regueiro, C. L. & Padrosa, D. R. CHIMIA 79, 411–416 (2025).
[2] Pillet L., Fernández Reguerio C. L., Busch M. R., Padrosa, D. R., & Paradisi F., RSC Adv. 15, 43974–43982 (2025).
[3] CapiPy by inSEIT | Protein Surface Analysis. https://www.capipy.ch.