Deracemization of monoalkyl glyceryl ethers by spatially organized heterogeneous biocatalyst

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The need to meet techno-economical requirements for industrial process has opened the search for finely organization of enzymes performing enzymatic cascades, aiming to mimicking cellular networks[1]. Spatial organization of enzymes enables substrates and intermediaries to be sequentially delivered to the next step or enzyme active site without its diffusion into the bulk, minimizing side reactions, inhibition, among other phenomena.

As enantiomerically pure molecules are of most importance in the pharma industry, we herein decide to spatially organize an enzymatic cascade performing an oxidoreductive deracemization of alkyl glyceryl ethers, building blocks already present in commercial drugs.

We design a system comprising a tailored NAD^+ dependant glycerol dehydrogenase (tGlyDH) to selectively oxidize from a racemic mixture the (*S*)-ethyl glyceryl ether (EGE), to ethyl hydroxyketone (EHK); and a ketoreductase (KRED) to asymmetrically reduce back the EHK to (*R*)-EGE. After testing the system in a soluble non-organized manner deracemizaton only achieved 62% yield and 42% enantiomeric excess due to inhibition phenomena.

By finely tuning the intraparticle organization of the enzymes in a porous carrier[2], we design heterogeneous biocatalysts (HB) capable to outperform the soluble system, achieving 100% yield and >99% e.e. Remarkably, we found an optimal spatial assembly able to ameliorate the inhibition phenomena experimented by the system and increases the deracemization rate by 4-fold.

Finally, integrating an enzymatic cofactor (NAD+) regeneration system to the heterogeneous biocatalyst[3], we intensified the process by scaling the reaction up to 250 mM substrate (10-fold), achieving 100% yield and e.e. > 99%. This HB herein design has a high potential for the synthesis of building blocks useful for drug fabrication in a greener manner, matching the current requirements in the industry

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[1] Schmid-Dannert C., López-Gallego F, *Curr Opin Chem Biol* **2019**, *49*, 97-104.

[2] Bolivar JM, Hidalgo A, Sánchez Ruiloba L, Berenguer J, Guisán JM, López Gallego F. *J. Biotechnol,* **2011**, *155*, 412-420 .

[3] Velasco-Lozano S, Roca M, Leal- Duaso A, Mayoral J, Pires E, Moliner V, López Gallego F. *Chem Sci,* **2020**, *11*, 12009-12020.