MS09-P02

On the versatility of CLECs for biotechnological applications, from micro to macro-fluidics devices

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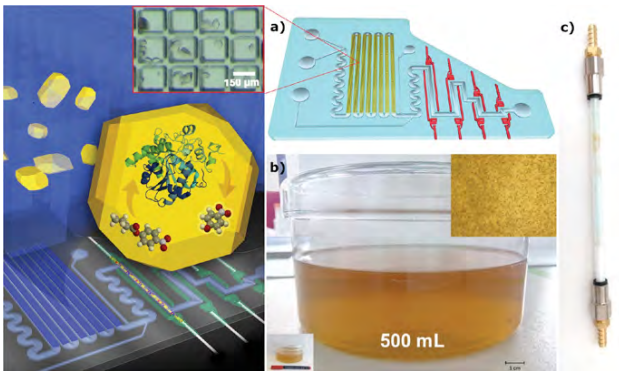
Biocatalysts make use of the versatility, selectivity and specificity of enzymes to catalyze a variety of processes for the production of relevant compounds under mild conditions. One of the most common strategies to extend the lifetime under extreme conditions and to increase the efficiency of enzymes is their immobilization in different materials or the auto-immobilization by cross-linking. In this context, Cross-Linked Enzyme Crystals (CLECs), yet proven to be a better solution to enhance catalyst lifetime or recoverability when compared with Cross-Linked Enzyme Aggregates (CLEAs), were almost abandoned [1]. However, a look into the literature reveals a renaissance in the interest on this methodology, probably boosted by the extensive knowledge gained during the last two decades in protein crystallization, highly decreasing its cost and laboriousness [2]. We have recently demonstrated that the use of CLECs-based microreactors shows unprecedented self-storage capability and stability as compared to standard biosensors, which cannot be stored for long periods due to quick denaturation of the enzymes (with lifetimes of weeks in the best case) [3]. We have further extended this concept by combining an enzymatic lipase microreactor, operating in continuous mode, with an optofluidic detection system [3] (Fig. a). The use of enzymatic catalytic reactions under microfluidic flow conditions reveals a promising technology with a number of strategic advantages: improvement of surface/volume ratios, enhanced energy consumption and mass transport, fundamental for the fabrication of CLECs-based biosensor systems. Furthermore, we have produced a packed 10 cm-chromatographic column with Reinforced Cross-Linked Lipase Crystals (RCLLCs), demonstrating a scale-up representation of the microfluidic approach that also operates in continuous flow (Fig. b-c). In this work, we will summarize our most recent results in the biotechnological application of enzyme crystals using both the micro and macro scale flow systems.

The author would like to acknowledge the support of the MICINN (Spain) project BIO2013-4297-P.



References:

[1] Brady, D. et al. (2009) J. Biotech. Letters, 31, 1639-50.
[2] Lopez, S. et al. (2017) J. Am. Chem. Soc., 2017, 139 (49), 17994–18002 & Yan E.-K. et al. (2016) Sci Rep. 6:34770.
[3] Conejero-Muriel, M. et al. (2015) Lab Chip, 15, 4083-4089 & Conejero-Muriel, M. et al., (2016) Anal. Chem. 88, 11919−11923.

Keywords: CLECs, Reinforced-cross-linked-enzyme-crystals, lipase