Exploring the basis of enzyme catalysis to computationally design biocatalysts of medical and industrial interest

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Enzymes are the most efficient, specific and selective catalysts known up to date. Despite the enzyme advantages, not all synthetic processes present a natural enzyme to catalyze and accelerate the reactions. Hence, the design of new stable enzymes for those processes where no biocatalyst is known represents a major challenge for protein engineering and a stringent test to understand how natural enzymes work. In addition to that, the ability of designing specific active enzymes is of great interest due to the potential applications in biotechnology, biomedicine and industrial processes.

In this communication, the *Inside-out* computational protocol for designing enzymes will be described together with its main advantages and current limitations. This will be demonstrated with two related projects. First, the computational design of an enzyme for the production of the type 2 anti-diabetes drug known as Sitagliptin (Januvia®) will be presented. It is worth emphasizing that the actual industrial method of production is far from ideal because of its inadequate stereoselectivity and product contamination with the rhodium catalyst. Finally, a multisdisciplinary project to unveil the structural basis of improved catalysis achieved by the experimental directed evolution (DE) technique will be described. This project is of extreme relevance for understanding the reason for the low efficiency of the computationally designed enzymes (k_{cat}/k_{uncat} values that range from 10^2 to 10^5 in contrast with average k_{cat}/k_{uncat} of ~10^{11} for natural enzymes) and for the future improvement of the actual *Inside-out* protocol.