COMPUTATIONAL PROTEIN DESIGN TO ACCELERATE
THE CONCEPTION OF FINE-TUNED BIOCATALYSTS

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The remarkable properties of enzymes (high catalytic efficiency, regio- and stereo-selectivity) have been
recognized and largely exploited in biocatalysis. Accordingly, enzyme-driven processes should play an
increasing role in the next decades, potentially substituting chemical processes and contributing to the raise of
bioeconomy. However, to foresee a viable future to biocatalysis, advances in R&D are required to accelerate the
delivery of fine-tuned enzymes displaying high chemical specificity on non-cognate substrates, high efficiency
and better stability in reaction conditions. To this end, structure-based Computational Protein Design (CPD) is a
promising strategy to fully rationalize and speed-up the conception of new enzymes while reducing associated
human and financial costs.

By combining physico-chemical models governing relations between protein amino-acid composition and their
3D structure with optimization algorithms, CPD seeks to identify sequences that fold into a given 3D-scaffold
and possess the targeted biochemical properties. Starting from a huge search space, the protein sequence-
conformation space, this in silico pre-screening aims to considerably narrow down the number of mutants tested
at experimental level while substantially increasing the chances of reaching the desired enzyme. While CPD is
still a very young and rapidly evolving field, success stories of computationally designed proteins highlight future
prospects of this field. Nonetheless, despite landmark achievements, the success rate of the current
computational approaches remains low, and designed enzymes are often way less efficient than their natural
counterparts. Therefore, several limitations of the CPD still need to be addressed to improve its efficiency,
predictability and reliability.

Herein, we present our methodological advances in the CPD field that enabled overcoming technological
bottlenecks and hence propose innovative CPD methods to explore large sequence-conformation spaces while
providing more accuracy and robustness than classical approaches. Our CPD methods speed-up search across
vast sequence-conformation spaces by several orders of magnitude, find the minimum energy enzyme design
and generate exhaustive lists of near-optimal sequences, defining small mutant libraries. These new methods,
in rupture with classical approaches are based on efficient algorithms issued from recent research in artificial
intelligence. The performance and accuracy of our computer-aided enzyme design methods have been
evaluated and validated on various types of protein design problems.

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