Recent advances in in-silico approaches for enzyme engineering

Author : Muniba Faiza

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Enzymes are natural biocatalysts and an attractive alternative to chemicals providing improved efficiency for biochemical reactions. They are widely utilized in industrial biotechnology and biocatalysis to introduce new functionalities and enhance the production of enzymes. In order to be proved beneficial for the industrial purposes, the enzymes need to be optimized by applying protein engineering. This article specifically reviews the recent advancements in the computational approaches for enzyme engineering and structural determination of the enzymes developed in recent years to improve the efficiency of enzymes, and the creation of novel functionalities to obtain
products with high added value for industrial applications. Enzyme engineering strategies aimed at forming enzymes with novel and improved activities, specificities, and stabilities which is greatly influenced by in silico methods. *In-silico* approaches in enzyme engineering can be applied in three main forms: structure analysis, molecular modeling, and *de novo* design. A detailed investigation of engineered enzymes provides valuable information about their structural origin, biochemical catalysis, and natural protein evolution [1]. A large number of enzymes have been widely utilized in biotechnology, pharmaceutical and
industrial processes. Due to the capability of accelerating the reaction speed by a factor up to 10^17 even in mild environments [2], many research are focused on making enzymes applicable in different fields such as academic, industrial and commercial fields, which resulted in the rapid progress of enzyme engineering in recent years.

Databases and tools for engineering enzyme activity

A web server named ZEBRA has been developed for analyzing enzyme functional subfamilies [3]. This web server attempts to systematically identify and analyze adaptive mutations. These subfamily specific positions (SSPs) are conserved within the subfamily differing from each other. The implemented statistical analysis evaluates the significance of SSPs, which can then be modified by rational design or focused directed evolution. The method has been tested with the ?/?-hydrolase superfamily [4]. SSPs calculated for the amidases were integrated into the sequence of the lipase CALB and the library of

mutants was constructed. In silico screening of the library for the reactive enzyme–substrate complexes resulted in the selection of lipases with significantly improved amidase activity.

Another method named JANUS analyzes multiple sequence alignments to predict mutations which can be used for interconversion of structurally related but functionally distinct enzymes [5]. This method has been verified by applying for the interconversion of aspartate aminotransferase into tyrosine aminotransferase. The incorporation of 35 mutations resulted in a protein with the desired specificity but low catalytic activity, which had to be optimized by DNA back-shuffling [1]. Another similar approach has been made by Yang et al., (2012), they proposed a computational approach to engineer allosteric regulation [6]. They performed a statistical comparison between the catalytic and allosteric binding sites, which showed that allosteric sites are evolutionarily more variable and comprise more hydrophobic residues than the catalytic sites.

Tools for molecular modeling and structural analysis of enzymes

ROSETTA and ORBIT are the most widely used web- based tools for *de novo* prediction of enzymes. A new algorithm has been developed by Hallen et al., (2013) known as Dead-End Elimination with Perturbations (DEEPer), which calculates the global minimum-energy conformation of structures with large backbone perturbations [7]. This algorithm attempts to generate more flexible enzymes structures. A computational method has been developed by Khare et al., (2012) which redesigns the active site to catalyze many reactions [8]. Keedy et al., (2012) proposed a novel algorithm for modeling local backrub motions, which are subtle backbone adjustments and participate in natural protein evolution taking place during amino acid substitutions resulting in increased model accuracy [9].

Algorithms for engineering enzyme stability

Most of the consensus-based algorithms for designing thermostable proteins uses the information from multiple sequence alignments to predict the most suitable and most often naturally occurring amino acid at a particular position. But it was pointed out by Sullivan et al., (2012) that consensual approaches are not much reliable as the consensus mutations at more conserved positions were more likely to be stabilizing in the model protein triose phosphate isomerase, while mutations at highly correlated positions were destabilizing [10]. Another algorithm was developed by Wang et al., (2012) which was based on the opposite principle from the Sullivan et al., (2012). This method called combinatorial coevolving-site saturation mutagenesis (CCSM) is used for identifying hotspots for mutagenesis [11].

The molecular modeling approaches greatly benefit from growing computational power and parallelized calculations on graphical cards. Molecular modeling studies offer the combination of several in silico methods, including bioinformatics analysis, to describe structure–function properties and predict beneficial mutations. For example, the prediction of thermostable proteins by combining the calculation of Gibbs free energies with evolutionary analyses. Current challenges include the quantitative modeling of enzyme selectivity and activities, which require the precise estimation of binding energies and reaction activation barriers. Since enzyme engineering has become an important aspect for biotechnological, bio-catalysis, and industrial purposes, it has become the focus of research. Designed enzymes need to be improved by many rounds of directed evolution, and this will not change in the near future.

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