COMPUTER-AIDED ENZYME ENGINEERING

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Aniline polymers are conductive chains of oxidized aniline monomers produced under very harsher conditions using strong chemical products. Laccases can also produce this polymer but in low yields. However, applying our computer-aided rational design technologies a laccase variant obtained by directed evolution was improved. The new mutant variant has increased the polyaniline production a 35% and totally avoid strong chemicals used.

Recently, we have challenged our "in silico" method even further, developing monomeric enzymes with multiple active sites: the PluriZymes. Protein Energy Landscape Exploration (PELE), a Monte Carlo based software, allows a fast enzymatic surface exploration to identify not only the active site but non-catalytic binding regions. Once the surface area is mapped and the local energy minima identified, we introduce the needed mutations to create a serine-hydrolase catalytic triad. These artificial active sites (tested in three different enzymes, two esterases, and one LPMO) have not only substantial catalytic activity (comparable to the wild-type) but additivity (when both sites are acting towards the same reaction) or enantioselectivity. Solved crystal structures proved the presence of the two catalytic triads and the expected/predicted binding elements. Furthermore, covalent inhibitors (with chemo-catalytic properties) can be used to design cascade reactions in a single monomer, combining chemo- and biocatalysis in the same enzyme.