PluriZymes: new enzymes for new times

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Extended Abstract

For the last decades, enzymes have been understood as globular proteins hosting one reactive region, called the active site. This state of fact has limited the enzymatic applications compared to heterogeneous catalysis, where a functionalized surface can host thousands of active sites. But, recent research has proved otherwise, enzymes can hold multiple active sites [1].

Applying a state-of-the-art modelling software, Protein Energy Landscape Exploration (PELE), detection of non-catalytic active sites is possible and, instead of blocking them (a classical approach), functionalize the region introducing the required mutations. The first attempt was done with esterases, because the catalysis requires only from three amino acids, the catalytic triad (serine, histidine and aspartic acid). Using a well-known esterase (PDB code: 5JD4) as initial model, a PluriZyme was *in silico* designed and *in vitro* tested (Fig. 1).

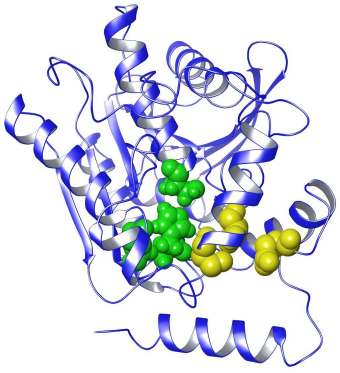

Figure 1: PluriZyme structure with an original active site (green) and an engineered one (yellow).

This first design succeed, both active sites (the natural and the engineered) were active and fully functional. Despite the positive results obtained, the final output was bittersweet, so the active site addition did not have the expected catalytic performance. The aftermatch was to be decided in further optimization rounds.

PluriZyme's engineered active site was deeply visual inspected aiming for potential mutation hotspots. Position 23 was found, an arginine that was close to the catalytic triad, reducing reaction space and potentially kidnapping the aspartate residue. An *in silico* saturated mutagenesis protocol was applied and for position 23 all short amino acids were tested, resulting glicine the most promising. Following *in vitro* validation proved a 7000 times activity fold in the artificial active sites and the obtention of an overall improved enzyme through additivity gain when both active sites are working at the same time. Furthermore, the analysis was extended to another esterases proving reproducibility.

Next natural step is the addition of different catalysis in the same enzyme scaffold and go through challenging problems. With this approach chain reaction can be placed together saving tremendous costs in industrial processes and reducing environmental harms. For example, enhanced PluriZymes for polyethylene terephthalate (PET) or auto-feeding unspecific peroxidases (UPOs), capable to produce their own required hydrogen peroxide.

References

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