7D QSAR BASED GRID MAPS GENERATED USING QUANTUM MECHANIC PROBES TO IDENTIFY HOTSPOTS AND PREDICT ACTIVITY OF MUTATED ENZYMES FOR ENZYME ENGINEERING

Roopa L, Kcat Enzymatic Pvt Ltd, Bengaluru, India
lalitha.p@kcat.co.in
Pravin Kumar R, Kcat Enzymatic Pvt Ltd, Bengaluru, India
Gladstone Sigamani, Kcat Enzymatic Pvt Ltd, Bengaluru, India

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Use of Quantum Mechanics hybridized with Molecular Mechanics (QM/MM) in Enzyme studies have greatly accelerated the finding of intermediate states of enzymatic reactions. The gaps in the conventional methods are in the identification of hot spots and screening enzyme variants. As a proof of concept, for the first time, receptor dependent – 4D Quantitative Structure Activity Relationship (RD-4D-QSAR) to predict kinetic properties of enzymes was demonstrated by Pravin Kumar et al, presented in Enzyme Engineering XXII, 2013, Toyama. We have extended this methodology to study enzymes using 7D-QSAR based grid maps. Induced-fit scenarios were explored using QM/MM simulations, which was then placed in a grid that stores interactions energies derived from QM parameters (QMgrid). The novelty of this method is that the mutated enzymes are immersed completely inside the QMgrid and this is combined with solvation models to predict descriptors; the grid captures the accurate electronic details of the reaction at very high resolution. Every grid point here is a probe, which are atoms that mimic atoms of the substrate interacting with the atoms of the enzyme, also atoms of the enzyme interacting with itself. The probes with its reaction coordinates are mapped on the ES complex conformations derived from ES, enzyme-transition and enzyme-product stages. The statistically relevant conformations are derived after screening using knowledge-based energy scoring matrices. The grid map shows high energy and low energy reactions across the ES system, which is used to pick hotspots. A substitution matrix is automatically constructed on the chosen hotspots using an evolutionary based scoring matrix coupled with statistical modelling process that gives the suited amino acids for a specific hot spot. We have tested this on a specific transaminase and QSAR models showed >90% specificity and >85% sensitivity towards the experimental activity with enzyme variants. Mapping descriptors on the enzyme structure revealed hotspots important to enhance the enantioselectivity of the enzyme. The method is efficient to design enzymes and proteins with minimum of double extending upto seven mutations on its own.
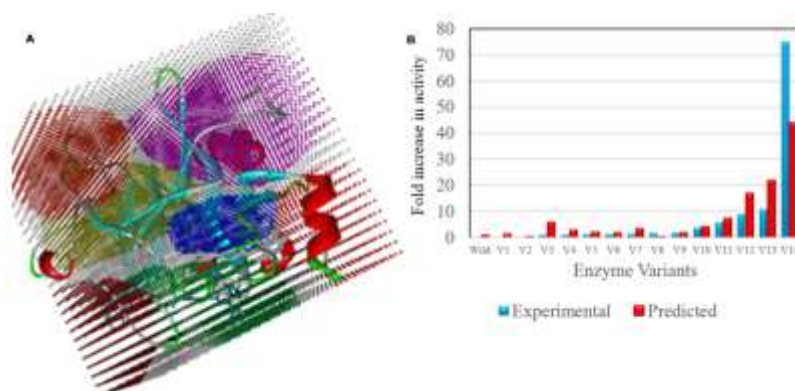

Figure 1A.QM grid where every gridpoint represents a probe. B. Comparative results of experimental and predicted activity of wild and variant transaminase