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Enzymes are versatile proteins which catalyse most of reactions in our body. They structure was optimised during natural evolution to perform catalysed reactions efficiently, with high activity and selectivity. At the end of XIX century, the key-lock mechanism was proposed to explain outstanding performance of enzymes. Close fitting of substrate molecule (key) to active centre of the enzyme (lock) has been introduced to describe enzymes properties. This simply and elegant theory works successfully in popular science till today however, it fails when active site is hidden deep in protein core. Such enzymes, widely spread around protein world, are equipped with tunnels which properties can additionally regulates enzyme activity and selectivity. Those new constrains provide an opportunity for reactivity control and makes enzymes with buried active site an ideal candidates for industrial and medical applications.

Most of strategies proposed for enzyme redesign are focused on reengineering of active site vicinity. Such methods quite often provides loss of enzyme activity due to rearrangement of residues crucial for enzyme catalytic properties. Modification of residues that builds tunnels can provide safe alternative for existing protein design protocols, however they require deep understanding of the transport phenomena through the tunnel network. Such task is quite difficult to achieve, due to lack of proper experimental methods which can explore ligand transportation inside protein core.

In our project problem we are employing combination of modern computational tools which can provide picture of the product exits and substrate entry to the enzyme buried active site. State of the art methodology will be used to support experimentalists and to precisely describe ligand transportation phenomena. Due to enlargement of studied ligands into set of 30 different compounds we will identify subtle differences responsible for enzyme selectivity.

Moreover to facilitate our research we have constructed enzyme equipped in a switch located inside the tunnel. By the change of redox conditions we are able to open or close the tunnel. Our enzyme can be seen as a prototype of the enzyme in which we can switch on/off the activity on request or activate it in particular part of the living cell. During the project we will validate the possibility of our system grafting into other enzymes and the possibility of further switch modification to enhance control ability.

Our project runs with co-operation with one of the best protein engineering group – the Loschmidt Laboratory in Brno in Czech Republic.