Computational Design of Proteins

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Enzymes are natural catalysts of living organisms, which find broad use in biotechnological processes, biopharmaceuticals and biomaterials. Modern enzymes evolved for catalysis in aqueous environment under mild conditions and therefore often do not meet requirements of practical applications. Engineering projects typically focus on enzyme catalytic activity, enantioselectivity or stability. Two major streams in protein engineering are the rational protein design and directed evolution. These two approaches can be combined in a single workflow employing rational design for selection of "hot spot" positions and directed evolution for their combinatorial mutagenesis.

We have recently proposed that the enzyme engineering strategies should focus both on the active sites as well as on the access pathways connecting buried active sites with the surrounding solvent [1,2]. We have demonstrated using the model enzymes haloalkane dehalogenases that the concept of engineering access tunnels may lead to proteins with improved catalytic properties [3] and stabilities [4]. *In house* software tools Caver [5] and HotSpot Wizard [6,7] can be used for analysis of protein tunnels and rational identification of "hot spots" for mutagenesis. Several case studies demonstrating the applicability of computational design for engineering proteins of practical utility in biocatalysis and stem cell research will be discussed [8,9].

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