OVERCOMING CHALLENGES IN ORGANOFLUORINE BIOSYNTHESIS BY ENGINEERED FLUORINASES

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Organofluorines find widespread usage as pharmaceuticals, agrochemicals and diagnostics. An estimated 20-30% of all drugs currently in the market contains fluorine. However, the unique physico-chemical properties of fluorine make chemical incorporation challenging, usually with the need of harsh synthetic reagents with varying chemo- or stereo- selectivity. Therefore, enzymatic fluorination offers a greener alternative and can also be scaled up to meet industrial requirements. One such method is by use of a fluorinase enzyme which converts S-adenosyl-L-methionine (SAM) and fluoride ions into 5'-fluoro-5'-deoxyadenosine (5'-FDA) and L-methionine (L-Met) through nucleophilic attack on SAM by fluoride. F^- enters first to the active site and then is followed by SAM. Fluorine is stabilized by forming a hydrogen bond with Ser-OH, Ser-N-H and Thr-OH. Fluorine is a highly electronegative molecule, which requires a dehydrated state in the binding site. F^- will enter in the active site by forming hydrogen bonds with the water molecules. Entry of SAM should displace the water molecules. Thr-OH forms another hydrogen bond with F^- to stabilize it. Diffusion of F^- to the active site is proposed rate-limiting step. We have conducted diffusion studies on the enzyme-fluoride complex by placing F^- 13.3 Å away from the binding site. Probable high-energy regions, as well as probable hydrogen bonds between F-, surrounding water molecules and amino acids, were identified. Quantum chemical calculations revealed four energy barriers along the F^- diffusion path into the active site (**Figure 1**). The F^- ion tends to stabilize itself by forming four hydrogen bonds with the adjacent water molecules that lie in a plane. 7D grid technology was used to engineer an energetically favourable F^- diffusion path. 7D grid technology is an artificial intelligence-based method which is used for designing industry-ready enzymes. Designed mutants showed improved K_M and k_{cat} with respect to wild-type fluorinase. Through optimized biosynthetic strategies, incorporation of fluoride *in vivo* could open up multiple green avenues for the synthesis of many fluorinated anticancer drugs, polyketides, terpenoids and steroids.

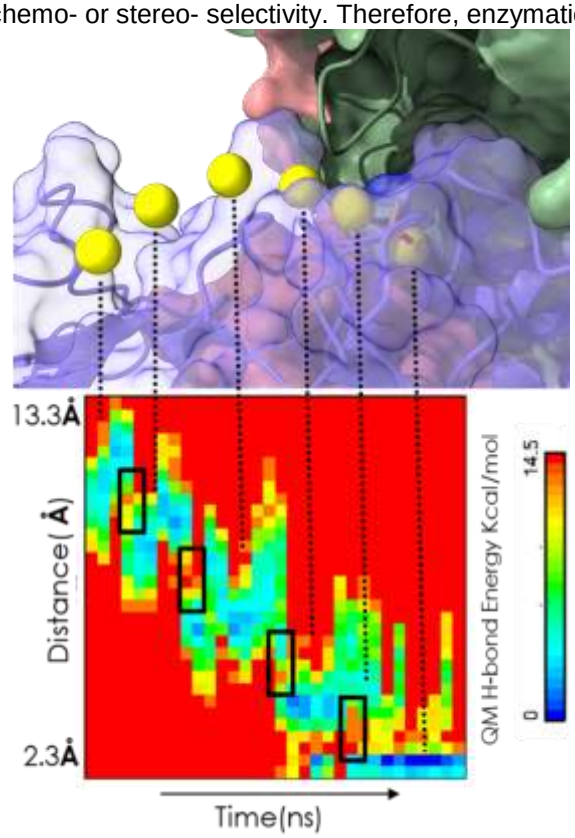

Figure 1. Diffusion study of fluoride and the corresponding energetics involved in the diffusion by means of 2D QM H-bond energy of a single F^- ion as a function of distance (Å) and time (ns). Fluoride ions in yellow spheres and active site residues in orange sticks. Protein molecule is colored by chain.