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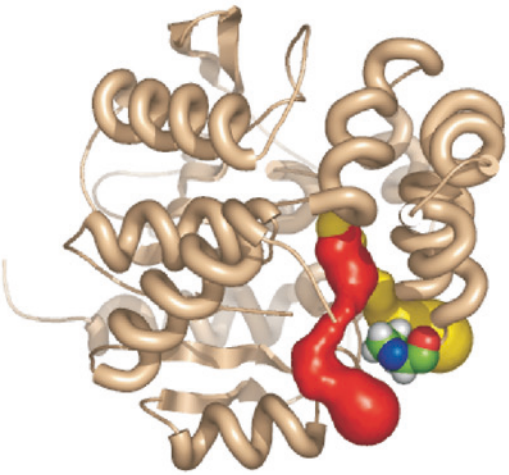

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Book of Abstracts

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Protein-solvent Interaction and Simulation Studies of Solvent Stable and Thermostable Lipase from *Bacillus* strain 42 in Water-solvent Mixtures

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A purified solvent stable and thermostable recombinant lipase, Lip 42, isolated from *Bacillus* sp. strain 42 was previously shown to be stable in polar organic solvents such as dimethyl sulfoxide (DMSO) and methanol. Stabilities in different solvent compositions were studied based on 40°C pre-incubation in solvent and the purified lipase was shown to retain at least 100% residual activity in up to 45% v/v DMSO and 45% v/v methanol. In 60% v/v DMSO, 68% of residual activity was retained, however, this dramatically reduced to 6.5 % at 65% v/v DMSO. Activity enhancement was recorded at lower solvent composition (less than 45% v/v solvent), whereby, at 30% v/v DMSO, enhancement was recorded to be as much as 35%. Enhancement tends to increase as temperature increases. Based on these solvent stability margin, molecular dynamic simulations were then carried out in the presence of water, 60% v/v DMSO + 40% v/v water and 100% v/v DMSO, by using a structure model predicted from a highly homologous (97%) lipase (PDB:1JI3). Results showed that the Lip 42 structure was retained and the flexibility of polypeptide backbone decreased or increased depending on the location of loop regions. Flexibility changes in the helix-loop-helix-motif covering catalytic triad were found to be associated with a hydrophobic cluster region. The presence of 60% v/v DMSO resulted in the disorganization of the cluster, accompanied with non-native H-bonds formations. However, the cluster still presents in 100% v/v DMSO and resembles to that of water simulation. Mutant form of lip 42 V174S contains residue substitution near the cluster and within helix-loop helix motif. At 50°C pre-incubation, the mutant lost as much of high temperature enhancement commonly observed in low DMSO composition. This indicates the potential role of hydrophobic residues in helix-loop-helix motif and the cluster in interfacial activation.