Molecular Simulation Tools for Biotransformation Enzymes

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Molecular simulation tools are rapidly taking their place in the arsenal of modelling tools used for the study of enzyme systems in general, as well as for biotransformation enzymes and Cytochromes P450 (CYPs) in particular. Examples are studies on substrate access, product exit and water channels in CYPs by Wade and co-workers, and numerous homology modelling studies where molecular dynamics (MD) simulations are used to assess model stability. Recent applications aim to deal with more subtle effects like dynamics enzyme-substrate interactions, or refinement steps in the course of the homology modelling process.

In CYP102-BM3, a Cyt.P450 from *Bacillus megaterium*, MD simulations have been applied to characterize differences in enzyme dynamics caused by point mutations in the active site region, as well as statistically characterizing the dynamics of substrate binding in the active site cavity. From Essential Dynamics (ED) analysis of the MD trajectories, differences between wild-type and mutants were detected in dynamical behaviour for the active site region, but not in the dynamics for the whole enzyme. Statistical analysis of substrate dynamics in the active site, revealed differences in preferred orientation between wild-type and mutants, more so for the substrate octane, and less for the fatty acid substrates that are thought to be 'anchored' in the active site by binding to a glutamine and arginine sidechain.

For the flavo-enzyme Styrene Mono-Oxygenase (SMO), MD simulations have been performed for refinement of a homology model built on the basis of the para-hydroxybenzoate hydroxylase crystal structure (1pbe, 15% overall identity). In a 'controlled release' procedure, position restraints were removed from subsequent parts of the enzyme during room-temperature MD simulations, in order to relax strain from the model with minimal effect on the active site three-dimensional structure. Several candidate models were extracted from subsequent 'free' MD simulations, and screened using automated docking for optimal substrate binding properties. One model was found to perform best, and show predictive quality with respect to binding orientation of a range of substrates and with respect to substrate binding affinity.

In summary, MD simulations are used successfully for optimization of enzyme homology models, study of enzyme-substrate interactions and assessment of the effect of mutations on enzyme dynamical behaviour. Critical aspect in application of this method are the use of multiple simulations to minimize bias from starting conditions, and to form a statistically solid basis for the extraction of average properties.