Predicting activated conformations of substrates in enzyme binding sites

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Enzymes are proteins that catalyze metabolic reactions in the cell. While a number of approaches for studying the metabolic network have been suggested, no high throughput approaches for studying the way that proteins catalyze reactions are yet available. Theoretical approaches rely on quantum chemical calculations, which are too resource intensive to realize at the genome level. Experimentally, the identification of possible catalytic residues takes place either by mutation analysis or by structural analysis of the complex of an enzyme with a transition state analogue (TSA). TSAs are molecules that have similar structure to the putative transition state of the reaction. Their binding mode in the protein can often lead to hypotheses for the actual stabilization of the true transition state by the enzyme, and thus shed light on the catalytic mechanism.

Here we suggest a simple method for describing the ensemble of activated conformations of the proteins substrates. Activated conformations, are physically allowed conformations of the substrates which foster direct reaction between the reactive atoms, under minimal nuclear displacement. We discuss the accuracy of the predictions by comparing our results to the structure of transition state analogues alone (Fig.1) and in complex with the enzyme (Fig. 2).

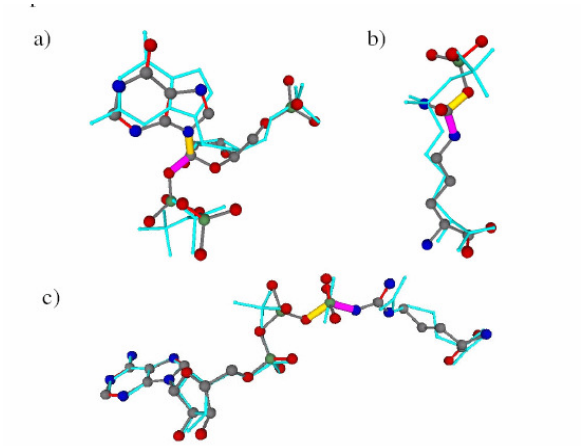

Fig.1 Comparison between transition state analogues from the literature (light blue) and the superposed activated conformation of the substrates. Magenta and yellow bonds denote bonds that are broken or formed by the reaction.

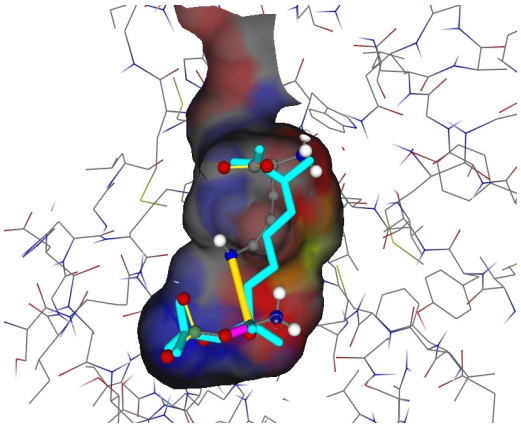

Fig.2 Substrates docked in their active conformation into the active site of ornithine transcarbamoylase (1DUV). The structure of the cocrystallized TSA is shown in light blue. The active sight is shown as solvent accessible surface.

The approach is tested on 40 complexes taken from the PDB, where transition state analogues were cocrystalized with an enzyme. In 25% of cases a model with an RMSD <2.5 A is found on the top rank. In 50% of the cases a model with an RMSD below 2.5A was found on 1 of the top 5 ranking predicted poses. Adaptation of the scoring function can lead to further improvement of prediction rate at the top rank. Furthermore, the prediction of the activated conformation of the products of the reaction, can be combined with the results from the substrate docking.

The automatic definition of the active state ensemble and the docking itself take minutes, making this method a good choice for high throughput model generation for enzyme substrate interactions. We will report our results from the application of the method on approximately 1000 enzyme structures, and describe the picture these provide of the way that enzymes interact with the metabolome. Finally, two examples of applications on inhibitor screening and enzyme specificity studies from our current work will serve to highlight the technical applications of the suggested approach.