Fitting flexible ligands into flexible active sites

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Understanding binding affinity is arguably the ultimate contribution of structural knowledge and computer simulation to the process of drug design: i.e. prediction of the affinity of hypothetical drugs for target receptors, to confer potency; the affinity of known drugs for known or unknown mutant receptors, in the study of resistance; or the affinity of drugs for non-target receptors, for building in specificity, safety and bioavailability. Computational affinity prediction for large systems is currently not possible, primarily due to the enormous volume of conformational space occupied by these systems, and in part due to inadequate representation of the energetics of atomic interactions. Rapid progress has been made, however, in simplifying the problem into manageable portions in order to gain qualitative insight into specific complexes of interest [for a review, see Lybrand, T. *Curr. Opin. Struct. Biol.* **5**, 224-228 (1995)].

Determining the energetically preferred orientation of a ligand in the active site of an enzyme has received a great deal of attention recently. A number of methods have been developed that take advantage of the high speed of rigid and grid-based docking, or allow the greater accuracy and thermodynamic analysis of full dynamics simulations [for a review, see Kuntz, I.D.; Meng, E.C.; and Shoichet, B.K. *Acc. Chem. Res.* **27**, 117 (1994); Humblet, C. and Dunbar, J.B. *Ann. Rep. Med. Chem.* **28**, 275 (1993)]

Recently we reported a dynamics simulation that combines speed and accuracy by allowing full conformational freedom of the ligand and the enzyme residues within a fixed radius of the active site, constraining residues at an intermediate distance, and representing the furthest residues as fixed points on a grid (Luty et al., *J. Comp. Chem.* **16**, 454-464 (1995). An atom-based solvation term to simulate the effects of water without adding the computational burden of explicit water is also included. Applying this method to "fly" a flexible molecule (benzamidine) into an enzyme (trypsin) rapidly generates the crystallographically observed complex starting from random orientations outside of the active site. Of 100 independent 20-picosecond simulations, 23 regenerated the crystal structure, and these were well-separated energetically from the other conformational solutions. The method is proposed to be useful in ligand-receptor systems in which the approximate location of the binding site is known, the ligand is placed in reasonable proximity to the binding site, and no large-scale rearrangements of the receptor are expected upon formation of the complex.

We have since extended this method to fit hydroxamate and aminocarboxylate inhibitors into zinc proteases. In one case study, leucine hydroxamic acid was able to reproducibly regenerate its crystallographic orientation in thermolysin when positioned as far as 8 Å from the catalytic zinc [Wasserman, Z.R. and Hodge, C.N., *Proteins: Structure, Function and Genetics,* in press (1995)]. The method is fast enough to allow statistical sampling and analysis of the docking probabilities of multiple congeneric inhibitors. This poster will detail these studies, which use the Amber forcefield, as well preliminary results on docking cyclic urea inhibitors [Lam et al., *Science* **263**, 380-384 (1994)] into HIV protease using the cff91 or cvff forcefield in Discover 3.0 (Biosym/MSI, 9685 Scranton Road, San Diego, CA 92121-3752).