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Computational Drug Design Accommodating Receptor Flexibility: The Relaxed Complex Scheme

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Computational structure-based drug design is a multidisciplinary research area and is still challenging in many respects. While ligand flexibility has been incorporated in many docking schemes, most programs still treat the receptors as rigid objects.[1] In general ligands may bind to conformations of the receptor that occur infrequently in the unliganded receptor; therefore, this rigid body assumption will fail to find correct ligand−receptor binding modes. Inspired by two recent successful experimental methods for the rapid discovery of ligands that bind strongly to a receptor, namely the ''SAR by NMR" method[2] and the "tether" method,[3] here we present a novel computational approach, called the "relaxed-complex" method, which incorporates receptor flexibility.

This method recognizes that ligands may bind to conformations that occur only rarely in the dynamics of the receptor and that strong binding often reflects multivalent attachment of the ligand to the receptor. Like the "dynamic pharmacophore" method,[4] in the "relaxed complex" scheme a long molecular dynamics (MD) simulation of the unliganded receptor will first be conducted to extensively sample the protein's conformations. The second phase of the "relaxed complex" method involves rapid docking of mini-libraries of candidate inhibitors to a large ensemble of the enzyme's MD snapshots.

The experimentally well-characterized system FK506 binding protein, FKBP, was considered. The immunophilin FKBP is the soluble receptor for the natural immunosuppressant drug FK506 (tacrolimus).[5] Immnuophilins, when complexed to immuno-suppressive ligands, appear to inhibit signal transduction pathways that result in exocytosis and transcription.[6] Substantial efforts have been made to search for strong-binding ligands that can substitute for the natural FK506.[7] The target of FK506 is the hydrophobic pocket formed by Y26, D37, F36, F46, Q53, E54, V55, I56, R57, W59, E60, Y82, H87, I90, L97, and F99.

The new X-ray structure in the unliganded form[8] and the SANDER module of the AMBER program[9] were used for the MD simulation. The duration of the simulation was 2 ns. The AMBER force field (parm99),[10] explicit aqueous solvent, and the particle-mesh Ewald methods[11] were used in the simulations to yield accurate sampling of the conformational space. It is shown in Figure 1 that the side chains of the aromatic residues at the active site wobble rapidly, whereas both the radius of gyration and the secondary structures are rather stable within this time scale. Details of the system setup, simulation protocols, and basic MD analyses are provided as Supporting Information.

With the advent of a new docking algorithm (the Lamarckian genetic algorithm) and a very successful empirical free energy function, AutoDock 3.0.5[12] is able to perform very efficient docking of large, flexible ligands and was adopted in the "relaxed complex" scheme. An automation procedure was developed to both prepare the molecular files for AutoDock and to perform the docking. When this procedure is used in conjunction with the MD simulations, it allows for the direct accommodation of a receptor's flexibility.

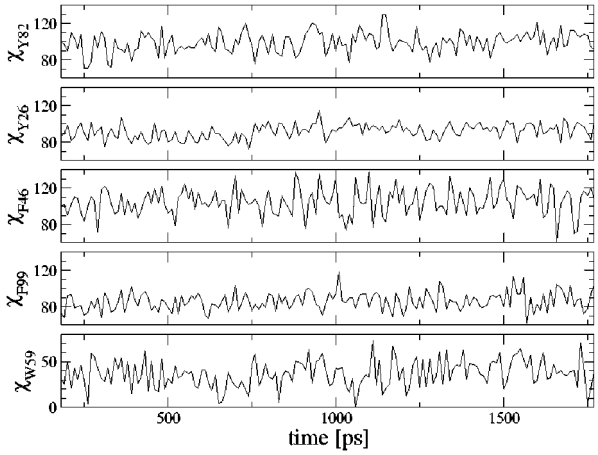

Figure 1. Librational motion of aromatic side chains in the active site. The side chain torsional angle χ (in degrees) is defined by the dihedral of $C_\alpha-C_\beta-C_\gamma-C_{\delta 1}$.

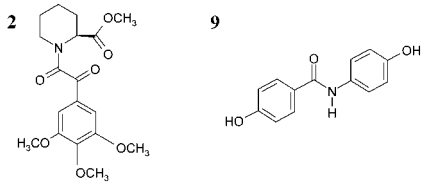

Figure 2. Chemical structures of the compounds **2**, trimethoxyphenyl pipecolinic acid derivative, and **9**, 4-hydroxy(1-hydroxy)benzanilide.

To illustrate this new scheme, the binding of compounds **2** and **9** from the work by Shuker et al.[2] (Figure 2) was modeled by docking them to an ensemble of MD conformations. The snapshots at each 10 ps interval were targeted. The RESP scheme[13] was used to derive the partial charges on the atoms of the compounds. The measured inhibition constants are 2 μM and 0.1mM for **2** and **9**, respectively.[2] As shown in Figure 3, the binding free energy of **2** to these MD conformations varies by over 3 kcal/mol. This wide distribution indicates the sensitivity of docking results to the different MD conformations. Note that a 3−4 kcal/mol difference in the binding free energies represents a (100−1000)-fold difference in dissociation constants. Due to the current accuracy of the AutoDock scoring function, it was not feasible to discriminate between the two different orientations of **2** at its binding site purely from the binding free energies. But the best complexes, ranked by free energy, had **2** in the correct binding site, and the correctly

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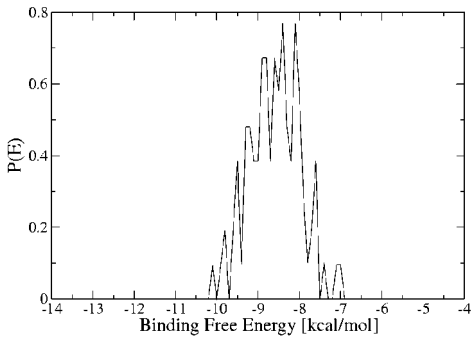

Figure 3. Probability distribution of the binding free energies of **2**.

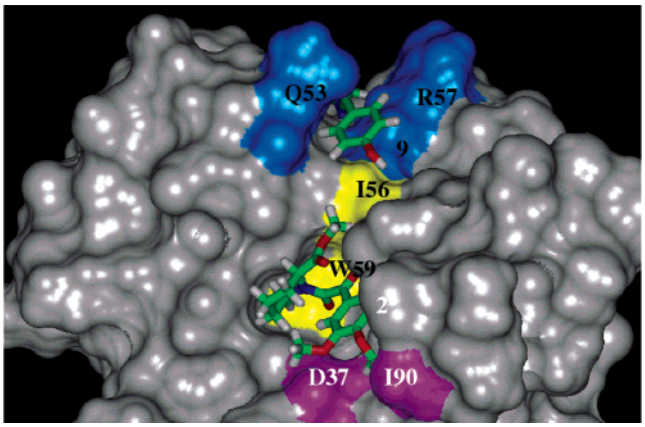

Figure 4. Location of **2** and **9** in the docked complex. **9** was docked in the presence of **2**.

oriented compound was included among these best complexes. It should also be noted that due to the dynamic conformational changes of the receptor, **2** did not always dock to the active site. However, the binding modes of **2** outside the active site consistently ranked poorly.

Similar to the "SAR by NMR" and "tether" methods, the "relaxed-complex" scheme permits a building block approach for constructing a very potent drug by combining two or three ligands with weak affinities. At this step the docked **2** is considered as part of the enzyme, and a spatially limited, or "focused", docking (within a cube of 20 Å in length, centered at the backbone nitrogen atom of ILE56) of **9** was conducted. This "focused" docking enables a more extensive search for binding modes of **9** that are within a possible linker distance to **2**, while automatically excluding any unproductive binding modes. The final docked ternary complex is in very good agreement with experimental structure.[2] The relative binding free energy ($\Delta\Delta G = \Delta G_{9} - \Delta G_{2}$) is 2.10 kcal/mol, which is also very close to the experimental value of 2.33 kcal/mol.

It should be noted that the binding of the first ligand could influence the binding of the second ligand; thus, the combination of the best-scoring ligands for respective binding sites does not necessarily produce the best composite compound. Our sequential approach avoids problems due to such circumstances. On the other hand, the presence of the first ligand would also introduce specificity in the orientation of the second ligand. Indeed, in the divalent docking studies, we found that most of the best-docked conformations of the second ligand had the same orientation. In principle, ligands with higher affinity to the active site (or other targets) should be used in the first phase of docking. Reversing the order may be inappropriate for lead optimization, because the weaker or less specific ligand could also occupy the target site of the more specific ligand and thus introduce unanticipated hindrances.

In summary, here is demonstrated a computational approach that can help elucidate complex binding relationships with atomic details, that does not require the synthesis and purification of proteins, that is not limited by the sizes of the molecules, and that does not require mutagenesis.

In future work, more accurate evaluations of electrostatic energy (based on solutions of the Poisson−Boltzmann equation), desolvation energy, solute entropy, and conformational energy will first be included in a rescoring scheme and eventually incorporated into the docking program. For systems with large conformational changes, such as those caused by induced-fit effects, other sampling techniques for receptor conformations could also be devised.

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Supporting Information Available: Detailed MD simulation protocols, enzyme structural analyses, derivation of the partial charges, and docking parameters (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Klebe, G. *J. Mol. Med.* **2000**, *78*, 269−281. (b) Carlson, H. A.; McCammon, J. A. *Mol. Pharmacol*. **2000**, *57*, 213-218.
(2) Shuker, S. B.; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. *Science* **1996**, *274*, 1531−1534.
(3) Erlanson, D. A.; Braisted A. C.; Raphael, D. R.; Randal, M.; Stroud, R. M.; Gordon E. M.; Wells, J. A. *Proc. Natl. Acad. Sci*. **2000**, *97*, 9367−9372.
(4) Carlson, H. A.; Masukawa, K. M.; Rubins, K.; Bushman, F. D.; Jorgensen, W. L.; Lins, R. D.; Briggs, J. M.; McCammon, J. A. *J. Med. Chem*. **2000**, *43*, 2100−2114.
(5) Babine, R. E.; Bender, S. L. *Chem. Rev*. **1997**, *97*, 1359−1472.
(6) (a) Marx, S. O.; Ondrias, K.; Marks, A. R. *Science* **1998**, *281*, 818−821. (b) Aghdasi, B.; Ye, K. Q.; Resnick, A.; Huang, A.; Ha, H. C.; Guo, X.; Dawson, T. M.; Dawson, V.; Snyder, S. H. *Proc. Natl. Acad. Sci*. **2001**, *98*, 2425−2430. (c) Jeyakumar, L. H.; Ballester, L.; Cheng, D. S.; McIntyre, J. O.; Chang, P.; Olivey, H. E.; Rollins-Smith, L.; Barnett, J. V.; Murray, K.; Xin, H. B.; Fleischer, S. *Biochem. Biophys. Res. Commun*. **2001**, 979−986.
(7) (a) Muegge, I.; Martin, Y. C.; Hajduk, P.; Fesik, S. W. *J. Med. Chem*. **1999**, *42*, 2498−2503. (b) Gold, B. G.; Zeleny-Pooley, M.; Wang, M. S.; Chaturvedi, P.; Armistead, D. M. *Exp. Neurol*. **1997**, *147*, 269−278. (c) Dubowchik, G. M.; Vrudhula, V. M.; Dasgupta, B.; Ditta, J.; Chen, T.; Sheriff, S.; Sipman, K.; Witmer, M.; Tredup J.; Vyas, D. M.; Verdoorn, T. A.; Bollini, S.; Vinitsky, A. *Org. Lett*. **2001**, *3*, 3987−3990. (d) Clemons, P. A.; Gladstone, B. G.; Seth, A.; Chao, E. D.; Foley, M. A.; Schreiber, S. L. *Chem. Biol*. **2002**, 49−61.
(8) Burkhard, P.; Taylor, P.; Walkinshaw, M. D. *J. Mol. Biol*. **2000**, *295*, 953−962.
(9) Case, D. A.; Pearlman, D. A.; Caldwell, J.W.; Cheatham, T. E., III; Wang J.; Ross, W. S.; Simmerling C.; Darden, T.; Merz, K. M.; Stanton, R. V.; Cheng, A.; Vincent, J. J.; Crowley, M.; Tsui. V.; Gohlke, Radmer R.; Duan, Y.; Pitera, J.; Massova, I.; Seibel, G. L.; Singh, C.; Weiner, P.; Kollman, P. A. *AMBER 7*; University of California, San Francisco, 2002.
(10) Wang, J.; Cieplak, P.; Kollman, P. A. *J. Comput. Chem*. **2000**, *21*, 1049−1074.
(11) Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. *J. Chem. Phys*. **1995**, *103*, 8577−8593.
(12) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olsen, A. J. *J. Comput. Chem*. **1998**, *19*, 1639−1662.
(13) Bayly, C. I.; Cieplak, P.; Cornell, W. D.; Kollman, P. A. *J. Phys. Chem*. **1993**, *97*, 10269−10280.

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