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Flexible Ligand Docking: A Multistep Strategy Approach

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ABSTRACT **A flexible ligand docking protocol based on a divide-and-conquer strategy is investigated. This approach first separates total search space into conformation and orientation space. It uses a grid-based method to sample the conformation of an unbound ligand and to select the low-energy conformers. Rigid docking is then carried out to locate the low-energy binding orientations for these conformers. These docking structures are subsequently subjected to structure refinement including molecular mechanics minimization, conformational scanning at the binding site and a short period of molecular dynamics-based simulated annealing. This approach has been applied to twelve ligand-protein complexes with three to sixteen rotatable bonds. The docked lowest-energy structures have root mean square deviations ranging from 0.64 Å to 2.01 Å with respect to the corresponding crystal structures. The effect of atomic charges and van der Waals parameters on the docking results, and the role of the dielectric constant in the conformation sampling are discussed in detail. A fragment-based docking approach that takes advantages of the divide-and-conquer strategy has also been explored and the results are compared with those produced by a whole molecule-based approach. Proteins 1999;36:1–19.** © 1999 Wiley-Liss, Inc.

Key words: docking; molecular mechanics; molecular dynamics

INTRODUCTION

Predicting how small molecules interact with biological macromolecule targets is of fundamental importance. As three-dimensional structures of new therapeutically relevant macromolecules are becoming available at a dramatically increasing rate, computer-aided structure-based ligand design plays an increasingly important role in the discovery of new therapeutic molecules. Several cases where structure-based ligand design has lead to the development of compounds that are currently in clinical trials or in the market have been documented.[1–3] However, despite recent advances, both in molecular simulation methodologies and in computer hardware, the prediction of the binding affinities for a set of compounds in a specific macromolecular target is still a major challenge. Even the simpler problem of docking a flexible ligand into a rigid protein requires a powerful search technique to explore the conformation and orientation space available to the binding site, and a scoring function that can reliably predict the correct binding modes from all the putative modes. The purpose of this paper is to present a new protocol for the flexible ligand docking problem.

In general, strategies for docking a ligand to a macromolecular target fall into two classes. The first class uses a whole ligand molecule as a starting point and then employs a particular search algorithm to explore the energy landscape of the ligand at the binding site, searching for optimal solutions for a given scoring function. The search algorithms include geometric complementary match, simulated annealing (SA), molecular dynamics and genetic algorithms. DOCK3.5,[4] AutoDock,[5] and GOLD[6] are among the successful examples in this class. The second class, sometimes called "fragment-based" docking, starts with placing one or several base fragments of a ligand into a binding pocket, and then constructs the rest of the molecule in the site. DOCK4.0,[7] FlexX,[8] LUDI,[9] Hammerhead,[10] GROWMOL,[11] and HOOK[12] are programs in this class. The fragment-based approach is often an order of magnitude faster than the whole molecule-based docking method. However, only the local interactions between the receptor-binding site and the ligand fragments under construction are taken into account during the docking procedure; thus it is difficult to evaluate whether the global energy minimum structure has been found. The results from the fragment-based approach are also often sensitive to the choice of the base fragment and its placement. If the binding site has a deeply buried pocket with hydrogen bond donors or acceptors, this approach

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works well. However, if the binding pocket is dominated by hydrophobic residues or characterized by a shallow pocket, not only is the placement of the base fragment often not definite for the fragment-based approach, but also it is difficult to decide where to add the next fragments. Consequently, the resulting docking structures can deviate from the real binding mode significantly. While the docking results from whole-molecule-based methods are not dependent on the choice of base fragment, they require much more computation to find an optimal solution in general. For a large flexible ligand, the whole-molecule based docking approaches often cannot find a good binding mode due to the vast search space of the problem.

The current performance of flexible ligand docking programs is illustrated by the results using two recently published programs: GOLD and FlexX. Among 100 selected ligand-protein complexes, GOLD docked 71 ligands having structures in "good" agreement (rmsd $\leq$ 2.0 Å) with the experimentally observed ones.[6] When considering 19 ligand-protein complexes, FlexX found 14 lowest-energy ligand structures that have rmsd less than 2.0 Å in comparison with the experimental results, i.e., its success rate is ~74%.[8] While these results are very encouraging, new ideas and methods for docking a flexible ligand to binding sites are still of interest. Moreover, as computers become more powerful, one needs approaches that can draw upon optimization techniques to improve the docking results.

The total search space is very large in docking a flexible ligand into a rigid receptor-binding site, because it consists of the six-dimensional orientation space (translation and rotation) as well as the ligand conformation space. For instance, for an average size of binding site ($\sim 15 \times 15 \times 15$ Å^3) and a ligand with ten rotatable bonds, the effective translational space for a 0.50 Å grid is around $(15/0.5)^3 \cong 10^4$ grid points and the rotation space is about $(360^o/10^o)^3 \cong 10^4$ grid points. So the number of points to be sampled in the total orientation space is on the order of 10^8 ($10^4 \times 10^4$). If we assume that the conformation space of a ligand consists only of its torsional angle space and each torsional angle is explored on a grid of 60^o (which is often too crude), the grid points for the conformational space for the 10-rotatable bond ligand are $6^{10} \cong 10^7$. Hence, the total search space of the ligand is on the order of 10^{15} grid points. Obviously, even if we had a perfect scoring function, it would still not be feasible to use a brute force method to search this many-dimensional energy map comprehensively in order to find the global minimum energy structure.

One important question to ask is how much sampling one needs to obtain an optimal binding structure. In a simulated annealing study, Olson et al have docked two10-rotatable bond flexible ligands into binding sites using their AutoDock program.[5] They have found that $\sim 10^8$ steps of Monte Carlo simulated annealing are necessary to obtain the lowest-energy structure with a success rate in the range from 5 to 20 percent. In the other words, at least 10^{-7} of the search space ($10^8/10^{15} \cong 10^{-7}$) needs to be sampled in order to obtain reliable results. Since the search space grows exponentially with respect to the number of rotatable bonds, docking large flexible ligands presents a considerable challenge.

Here we present a divide-and-conquer protocol to tackle the docking problem. Instead of randomly selecting one conformation of a ligand and then searching for an optimal solution at the binding site, we start with separating the total search space into conformation and orientation spaces. We systematically scan the conformation of an unbound ligand using a grid-based method and select an ensemble of low-energy conformers. After that, we employ the idea of geometric complementary to search the orientation space for each conformer at the binding site and score each binding orientation based on the AMBER force field. A key part of a divide-and-conquer approach is to include an optimization mechanism to systematically improve the low-resolution structures filtered by the previous coarse sampling. The structure refinement in our approach consists of three components. The first is molecular mechanics (MM) geometry optimization. The MM minimization serves as a fast tool to locate the local energy minimum structure of the ligand at the binding site. The second component is a torsional-angle-driven refinement. It scans the torsional angle space of the ligand at the binding site for a given orientation. The purpose of this refinement is to partially overcome local energy barriers. And, finally, we introduce a short molecular dynamics simulated annealing method to shake the structures near their local energy minima and to see if we can find lower energy solutions. The idea underlying this protocol is to divide the total search space into a set of subspaces and then to select a small set of subspaces using energy as criterion, and finally to carry out a fine-resolution search in each selected subspace. This protocol has been tested on a set of the difficult ligand-protein complexes that were reported using AutoDock[5] and FlexX.[8] Encouraging results have been obtained. For this set of ligand-protein complexes, the docked lowest-energy structures have root mean square deviations (rmsd) of 0.64 Å$-$2.01 Å with respect to the experimentally observed structures.

METHODS AND COMPUTATIONAL DETAILS

The basic idea of our approach is illustrated in Figure 1. In order to find a global energy minimum point on a simplified two-dimensional energy map (Fig. 1a), we first divided the whole map into a set of submaps (Fig. 1b). After selecting a set of low-energy submaps, we start to search each submap using a high resolution grid. Again, the search is focused on identifying low-energy points in the sub-submaps (Fig. 1c). For any low-energy points identified, an attempt is made to expand the search space around these points to see if there are any other points which have lower energies. In this way, the search is not confined to the starting submaps (Fig. 1d). The whole search procedure can be iteratively repeated several times

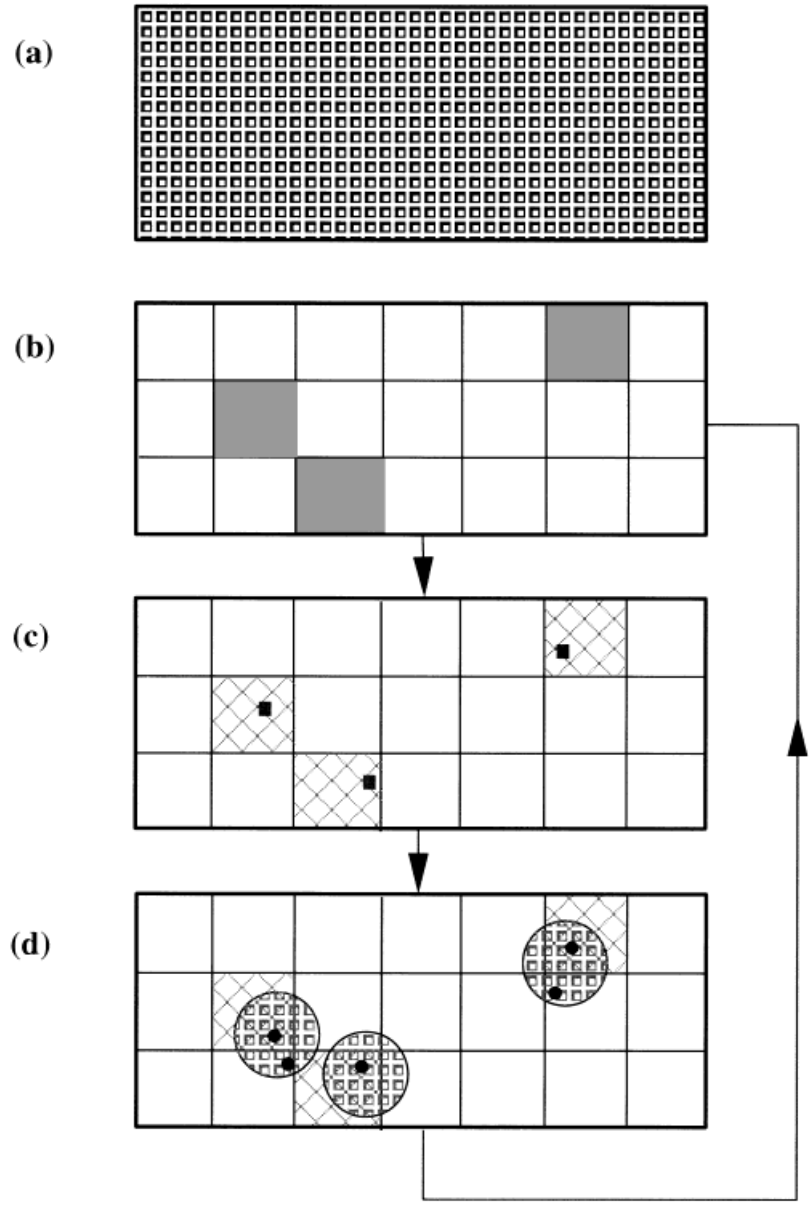


Fig. 1. A schematic description of a divide-and-conquer approach to search for global energy minimum points on a two-dimensional energy map.

until no lower energy points have been found. The global energy minimum point is assumed to be the lowest-energy point among all low-energy points. Following this idea, we illustrate the flowchart of our docking protocol in Figure 2. The technical details of each step are listed in Table I and explained in the following sections.

Scoring Function

One important component in docking is the scoring function. A good scoring function should be able to distinguish a correct binding mode from the other putative modes. In this work, we use the AMBER force field as our scoring function. The pairwise potential energy function of the AMBER force field has the form:

$$U_{\text{pair}} = \sum_{\text{bonds}} k_r(r - r_{\text{eq}})^2 + \sum_{\substack{\text{bond}\\ \text{angles}}} k_\theta(\theta - \theta_{\text{eq}})^2 + \sum_{\text{torsions}} \frac{V_n}{2}[1 + \cos(n\varphi - \varphi_0)] + \sum_{i<j} \epsilon_{ij}\left[\left(\frac{R^*_{ij}}{r_{ij}}\right)^{12} - \left(\frac{R^*_{ij}}{r_{ij}}\right)^{6}\right] + \sum_{i<j} \frac{q_i q_i}{\epsilon(r_{ij}) r_{ij}} \quad (1)$$

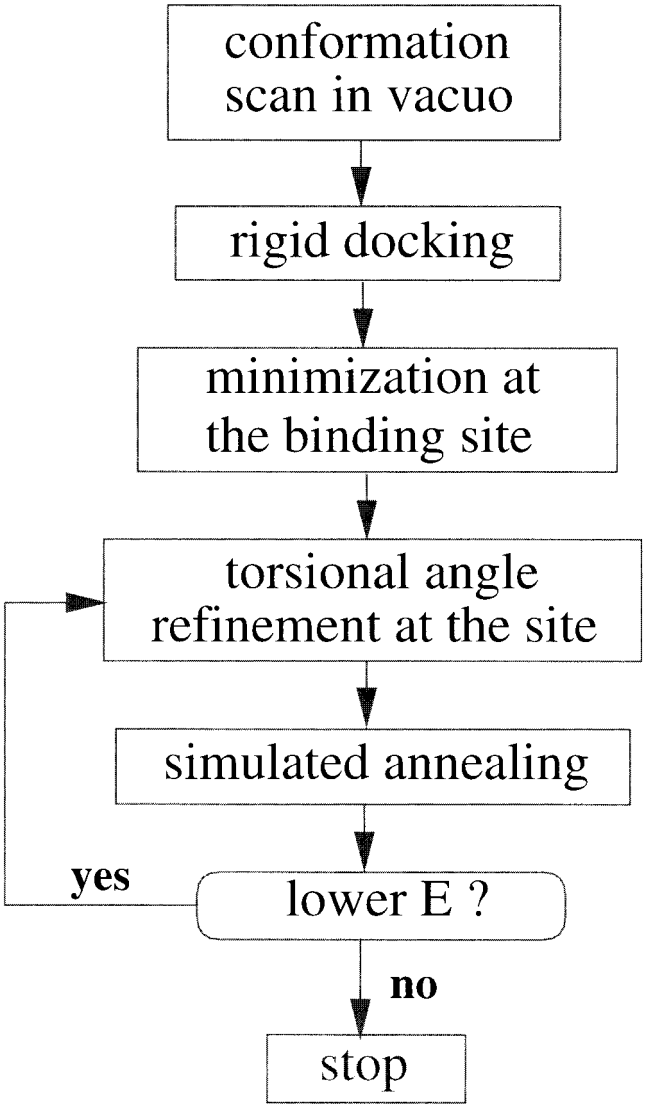


Fig. 2. Flowchart of the flexible ligand docking approach implemented in this work.

where k_r, k_θ, V_n are empirical parameters relating to bond stretching, bond angle bending and torsion angle, ϵ_{ij} and R^*_{ij} are van der Waals (VDW) parameters, q_i are atomic charges, r_{ij} is the distance between atom i and j, and $\epsilon(r_{ij})$ is the distance-dependent dielectric constant. Since we did not include crystal water molecules or add solvent water molecules in our calculations, the distance-dependent dielectric constant serves to mimic the water solvent environment during the calculations.

In detail, the AMBER(86) force field[13,14] was used as the scoring function in rigid docking as implemented in DOCK3.5.[15,16] During the calculations of the conformational scanning, torsional angle refinement, geometry optimization, and simulated annealing, we use the updated AMBER(95) energy.[17] In selecting MM minimized structures or structures generated from grid-based conformational sampling, we excluded the first three terms in Eq. 1, i.e., the terms related to the bonded internal energies, although these three terms were explicitly included during the molecular mechanics minimization and simulated annealing calculations. The AMBER(95) force field without the first three terms has been found to perform slightly better in terms of selecting "good" binding modes for large flexible ligands, as discussed in later sections. Two atomic charge models, Gasteiger[18] and AMSOL[19] charges, are investigated in this study.

We should point out that the structure selection hereafter was purely based on energy, rather than free energy. This is because there is no reliable way to describe the free energy given the complexity of the problem.

Conformation Sampling of an Unbound Ligand Molecule

When separating the total search space into conformation and orientation spaces, one important question is: What is the most likely conformation of a ligand at the binding site? Obviously the global minimum energy conformation of a ligand in vacuum or in solution does not always correspond to the experimentally observed bound conformation. The ligand bound conformation is determined by the intraligand energy and the interaction energy between the ligand and its surrounding environment including the receptor and the solvent. The purpose of the conformation sampling at this stage is to generate structures close enough to the bound conformation and to serve as useful starting points for later refinement.

There are many ways to sample conformation space. Distance geometry and simulated annealing are among the most widely used methods. Distance geometry[20,21] can sample conformation broadly, but it often lacks energy information. Simulated annealing approaches,[22] on the other hand, provide energy information for each conformer, but the generated structures in general do not cover broad regions of conformation space unless an additional procedure is used. Thus, we decided to use a coarse-grid-based systematic search method to scan the torsional angle space of a ligand. The basic idea of the algorithm is outlined in Figure 3.

First, we break a molecule into fragments. A fragment is defined as a part of the molecule associated with only one unsymmetric rotatable bond. Starting with the nth fragment of a molecule, we carry out a bump check to see if the fragment has any van der Waals clashes with previous fragments for a given torsional angle value. If a steric collision occurs, we change the angle value until a nonclashing conformation is generated. Then the interaction energy between this fragment and previous fragments is calculated. This energy, denoted as the energy for the nth fragment, E^n_{inter} is compared with the lowest-energy for the nth fragment ($E^n_{\min}$). If E^n_{inter} is smaller than $E^n_{\min}$, $E^n_{\min}$ is updated. On the other hand, if the energy difference, $|E^n_{inter} - E^n_{\min}|$, is within a predetermined threshold (δE), this fragment and the corresponding torsional angle value is saved and the next fragment is examined. Otherwise, the search procedure alters the torsional angle or tracks back to the previous fragment to start the search again.

TABLE I. Details in Docking Procedures Used in This Paper

Step	Energy function[a]	Scoring function[b]	Dielectric constants	Number of conformers[c]
Conformational sampling of an unbound ligand	AMBER(95)	Nonbonded interaction energy[d]	1.5	$<E_{min} + 30.0$[e]
Rigid docking	AMBER(86)	Ligand-protein nonbonded interaction energy[f]	4r	50% of the lowest DOCK-scored structures[g]
MM minimization	AMBER(95)	Nonbonded interaction energy[h]	4r	6
Conformational scan at binding site	AMBER(95)	Nonbonded interaction energy[h]	4r	6
Simulated annealing	AMBER(95)	Nonbonded interaction energy[h]	4r	6

[a]The force fields used in the calculations.
[b]The scoring function used to select structures.
[c]The number of conformers kept for the next level of structural refinement.
[d]The intraligand nonbonded energy.
[e]Using the energy criterion to select compounds.
[f]The VDW well depth is scaled by 1/1000.
[g]Based on DOCK energy to select compounds.
[h]The interaction energy includes the intraligand and ligand-protein nonbonded interaction energies.

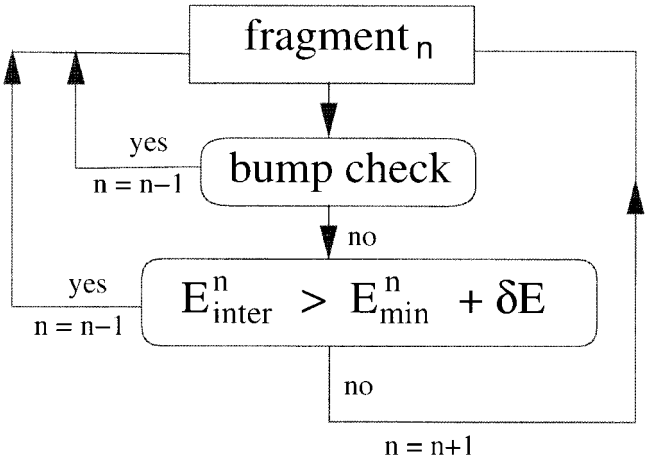


Fig. 3. Flowchart of the conformation search algorithm.

The bump check speeds up the search by a factor of 3–5. The energy threshold (δE), for each fragment growth serves as a threshold for the greedy search algorithm. Overall, the search algorithm is a combination of depth-first search and greedy search algorithms, which can effectively avoid the exponential growth problem. In general, we used a very coarse grid of 120° in the conformation sampling. For fragments larger than phenyl, a 60° grid can be used. The ring conformations in present study are taken from relevant experimental values. Using this algorithm, the conformation sampling for ligands with 5–10 rotatable bonds takes less than a few minutes of the CPU time on a SGI/R10000/195Mhz below:

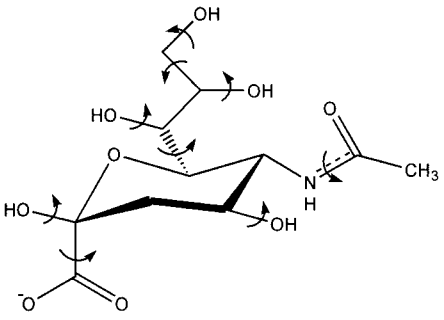
This grid-base sampling is a process of dividing the total conformation space into a set of subspaces. The selection of "interesting subspaces" was based on the energies of these conformers. All conformers within 30 kcal/mol of the lowest-energy were selected for the next step of docking. The energy criterion of 30 kcal/mol is found to lead to good final docking results with the minimum computational cost. In our sampling, we used a dielectric constant of 1.5. Our experience indicates that the energy threshold of 30 kcal/mol and the dielectric constant of 1.5 lead to good coverage of the conformation space, as discussed below and yield good final results.

Rigid Docking: Sampling of Orientation Space

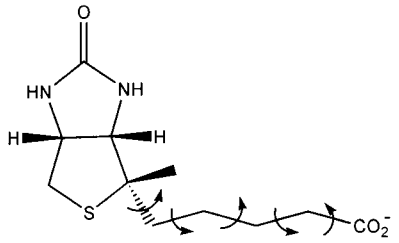
The next step in our procedure is rigid docking of the lowest-energy conformers selected from the previous step. We used the DOCK3.5 program.[15,16] In DOCK, the shape characteristics of a binding site are represented by a minimum set of sphere centers calculated with the SPHGEN subroutine. To orient a ligand within the binding site, some of the sphere centers are matched with ligand atoms. The orientation of the ligand is evaluated with the AMBER(86)-based energy scoring function and a distance-dependent dielectric constant ($\epsilon = 4r$). Because DOCK3.5 uses a longest-distance heuristic as a filter, it dramatically prunes the orientation space at the binding site. DOCK has been proven to be efficient for rigid docking and database searching.

Since the starting conformers at this early stage are of low resolution, many conformers might have bad van der Waals (VDW) contacts with the receptor and thus have high DOCK scores (high energy) if the conventional AMBER parameters were used. For this project, we introduce a soft VDW well depth for the ligand during the rigid docking step, scaling down the VDW well depth (ϵ) by a factor of 1,000. This allows about 1 Å extra displacement into the VDW repulsive curve. Consequently, the DOCK score is dominated by the electrostatic energy between the ligand and receptor. We are assuming that a favorable electrostatic interaction mainly determines the orientation of a bound-like conformer at the binding site, whereas the bad VDW contacts should hopefully be corrected during the later stages of optimization.

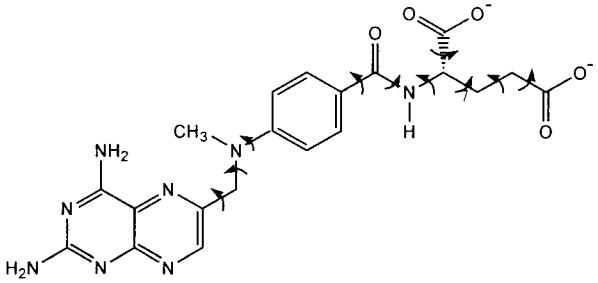
The single mode option was used in the rigid docking, and only the single best DOCK orientation was saved for each conformer.[16] The maximum energy score to save a ligand orientation during the rigid docking is +50 kcal/



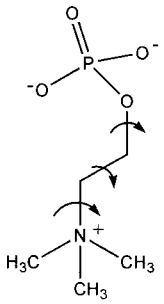
Sialic acid/hemagglutinin (4hmg)



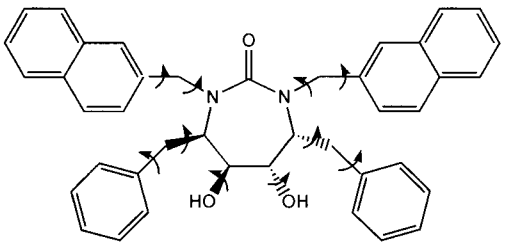
Biotin/streptavidin (1stp)



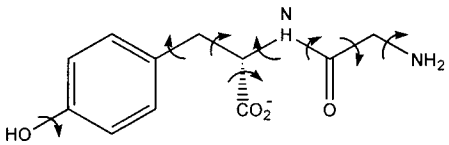
Methotrexate/dihydrofolate reductase (3dfr)



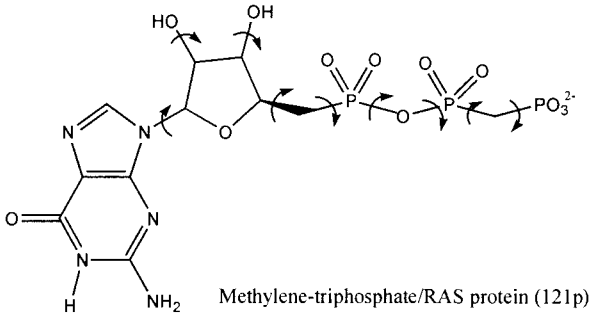
Phosphocholone/Fab McPc-603 (2mcp)



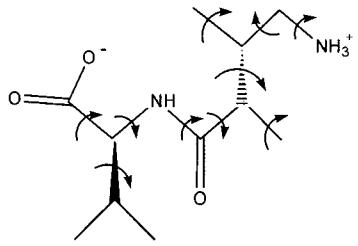
XK263/HIV-1 protease (1hvr)



TYR-GLY/carboxypeptidase (3cpa)

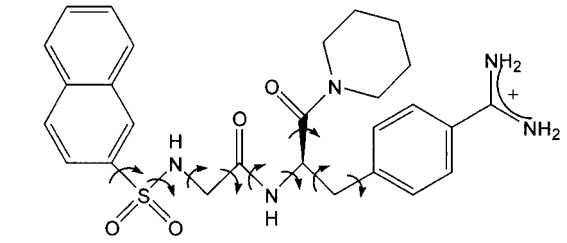


Methylene-triphosphate/RAS protein (121p)

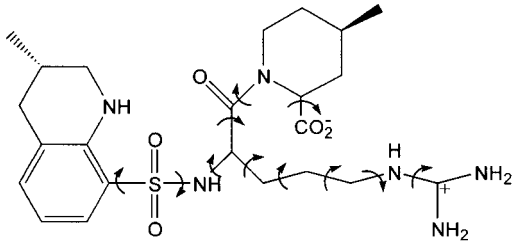


VAL-ILE/trypsinogen (3tpi)

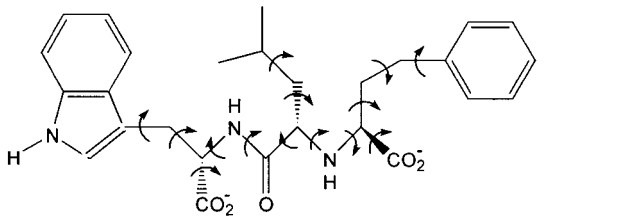
Fig. 4. Chemical formula of the twelve ligand-protein complexes.



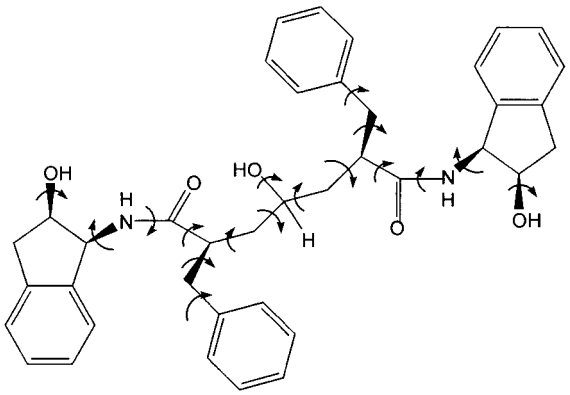
NAPAP/thrombin (1dwd)



Argatroban/thrombin (1dwc)



N-(1-carboxylato-3-phenylprop-1-yl)-leucyl-tryptophan/thermolysin (1tmn)



VAC/hiv-1 protease (4phv)

Figure 4. (Continued.)

mol. Among all docked structures, only the 50% of the lowest-energy structures were selected for the next level of optimization.

Molecular Mechanics Minimization

Conformational sampling and rigid docking can be regarded as a global scan of the energy landscape. Each low-resolution structure identified is then subject to structural refinement. The structural refinement here is of a local character, serving to find lower energy structures near the starting structures. The global energy minimum structure is assumed to be among or near to one of these low-energy structures. Several steps of refinement are used as described below:

For a starting structure, energy minimization at the binding site using a molecular mechanical force field is an efficient way to find a local energy minimum structure. Fifty (50) steps of steepest-descent minimization were used first, followed by conjugate gradient minimization. A distance dependent dielectric constant ($\epsilon = 4r$) was employed to mimic the solvent effect. The energy convergence criterion was 0.50 kcal/mol together with a maximum of 300 steps of minimization. The distance cutoff between atom pairs was 6 Å, while the nonbond pairs were updated every 25 steps. A short distance cutoff speeds up the minimization process significantly; however, no extensive investigation has been carried out in this respect. The receptor conformation was held rigid during the minimization using the BELLY option in the AMBER 4.1 program.[23] The VDW parameters at this stage are the conventional AMBER ones in order to correct the possibly bad VDW contacts between the ligand and receptor generated in the "soft" rigid docking.

Ligand Torsional Scanning at the Binding Site

The molecular mechanics minimization can simultaneously optimize bond lengths, bond angles, and torsional angles of a ligand at the binding site. However, it cannot overcome a local energy barrier in the vicinity of its starting structure. To do so, we added a step of torsional angle scanning for the previously selected structures at the binding site. In detail, we systematically rotated each rotatable bond on a 30° grid at a time, if the resultant structure has bad VDW contacts with the receptor, the next rotatable bonds were adjusted near their initial angles and so on in order to obtain a "good" (no VDW clash) structure. A subsequent MM minimization was then carried out.

The six lowest-energy structures from MM minimization of the rigid docking structures were selected for the torsional angle scanning. The choice of six structures was found to permit all necessary conformers that lead to the final good docking results.

Simulated Annealing (SA)

The final step of the refinement is designed to make small changes in the structures selected from previous steps. The simulated annealing procedure permits both conformation and orientation sampling. For small molecules with less than four rotatable bonds, SA is not necessary. However, it is useful for large flexible ligands, as shown in the next section. A moderate temperature range was used in the SA simulation to keep the structure near its starting point. The temperature ranged from 0 to 500°C and from 500 to 200°C in two runs of 2 ps MD simulation. Again, the BELLY option was turned on to keep the receptor binding site rigid. A distance-dependent

TABLE II. Docking Results (Energy in kcal/mol and rmsd in Å)

	Minimized experimental results[a]		This work							
			Lowest energy		Closest structure			FlexX[b]	GOLD[c]	AutoDock[d]
System	E_{AMBER}	rmsd	E_{AMBER}	rmsd	E_{AMBER}	rmsd	Rank	rmsd[e]	rmsd[e]	rmsd[e]
121p	−14.8	0.43	−13.3	1.02	−13.3	1.02	1	2.00	NA	NA
1dwc	−65.3	0.19	−75.5	1.15	−75.5	1.15	1	2.66	NA	NA
1dwd	−94.3	0.33	−89.0	2.01	−87.8	1.40	2	2.12	1.71	NA
1hvr	−94.2	0.16	−95.2	1.16	−84.2	1.14	6	0.82	NA	0.86
1stp	−54.5	0.25	−54.6	1.02	−54.6	1.02	1	0.81	0.69	0.89
1tmn	−55.4	0.30	−70.1	1.93	−68.7	1.73	1	0.87	1.68	NA
2mcp	−33.3	0.53	−11.2	1.71	−11.2	1.71	1	>2.00	4.37	0.96
3cpa	−68.7	0.36	−80.6	0.62	−76.9	0.59	4	3.08	1.58	NA
3dfr	−66.7	0.34	−66.3	1.39	−66.3	1.39	1	1.34	1.44	NA
3tpi	−75.6	0.26	−75.5	1.73	−75.5	1.73	1	0.58	0.80	NA
4hmg	−42.9	0.18	−45.4	0.76	−45.4	0.76	2	0.80	NA	1.40
4phv	−131.8	0.13	−110.6	1.97	−100.7	1.80	2	1.04	1.11	NA

[a]Structures minimized from crystal structures.
[b]FlexX[8].
[c]GOLD[6].
[d]AutoDock.[5]
[e]Based on the lowest energy structures.

dielectric constant was used throughout the MD simulations.

Here again, six lowest-energy structures from previous steps of refinement were selected for the SA calculations. This number was arrived from the overall performance of the SA calculations on the twelve complexes.

Clustering

The structures at each stage of rigid docking, torsional angle scanning at binding site and simulated annealing are minimized and clustered based on the root mean square deviations of their Cartesian coordinates. In detail, we use a single linkage method, starting with an available lowest-energy structure, denoted as the anchor structure here. Any unclustered structure that has a rmsd less than a predetermined threshold from the anchor structure is assigned to the same cluster as the anchor. This process is continued until all structures were clustered. The purpose of clustering is to reduce the redundant structures. The rmsd threshold in clustering is 1.0 Å as used by AutoDock.

RESULTS

In order to test our program, we started with a set of ligand-protein complexes that were investigated in two well-known programs, AutoDock[5] and FlexX.[8] We chose a total of 12 ligand-protein complexes that represent all the difficult ligands tested in these two programs. Figure 4 shows the chemical structures of these ligands. These ligands have between 3 and 16 rotatable bonds. Some of the ligand-protein interactions are of a hydrophobic character, while others are dominated by electrostatic interaction including hydrogen bonds. Hence this set of ligands provides a good test for a new docking program.

Table II lists the docking results. The energies in Table II are nonbonded energies including the intraligand energies and ligand-protein interaction energies. The root mean square deviations (rmsd) in Table II indicate the difference between the docked structures and the observed crystal binding structures. Table II also lists the results minimized directly from the crystal complex structures and the available results from AutoDock, FlexX, and GOLD.

If we start with the experimental binding structures, the molecular mechanics minimization alone leads to structure deviations in the range of 0.13 to 0.5 Å relative to their initial structures. The small shifts of these structures indicate that experimentally observed structures are in the vicinity of local energy minimum of the AMBER(95) force field. These displacements set the minimum expected deviation with this choice of force field.

Following conformation sampling, rigid docking, minimization, torsional angle scanning at the binding site, and simulated annealing, the lowest-energy structures have rmsds in the range from 0.64 Å to 2.01 Å with respect to the experimental results. The best-predicted structures, on the other hand, have rmsds ranging from 0.61 Å to 1.18 Å. Of course, the lowest-energy structures are the appropriate set to evaluate a docking method since a scoring function is the only way to select binding modes when the experimental binding structures are not known. If one uses a rmsd less than 2.0 Å as a criterion of a good structure, the docking results based on the lowest-energy are encouraging for this set of ligand-protein complexes. Figure 5 illustrates the lowest-energy results together with the corresponding experimental crystal structures for each compound. Overall, our docking program can reproduce the experimentally observed the binding modes well, as has also been achieved with AutoDock, Flex X and GOLD.

Looking at the docking structures in detail, we observed that some hydrogen bonds in the ligand-protein complexes were misplaced. For example, the hydroxyl hydrogen in

121p rotates about 60° in comparison with the respective crystal structure. One hydroxyl hydrogen in 1hvr rotates 180° relative to the experimental structure. The same phenomenon is observed for 4hmg. The misplacement of the hydrogen atoms can be partially attributed to the scoring function used in our study. In our scoring function, the regular VDW 12–6 terms (Eq. 1) were used, instead of the VDW 12–10 terms which are often used to describe the hydrogen bonds.

The docking structure for 1dwd complex has a rmsd of 2.01 Å mainly owing to the naphthyl group, which is more exposed to the solvent than in the crystal structure. The presence of waters might push this part of ligand closer to the protein-binding pocket due to the hydrophobic effect. The lowest-energy docking structure for 1dwd has a rmsd of 2.12 Å from the FlexX produced structure; the deviation is mainly from the naphthyl group and the sulfonyl group of the ligand as well. A slightly smaller deviation is seen in the GOLD results (rmsd: 1.71 Å). This indicates that the scoring functions used in the present docking study cannot handle well large hydrophobic fragments exposed to the solvent. A new scoring function that takes into account desolvation energy of the system is certainly needed.

With a rmsd of 1.97 Å for the docked structure of 1tmn, the two end groups of the ligand are twisted relative to the experimental structure, although, overall, the docked structure in this study is consistent with the crystal structure. FlexX yields an excellent docked structure in this molecule (rmsd: 0.87 Å), while GOLD produce a docking structure with rmsd of 1.65 Å, as listed in Table II.

The binding of phosphocholine to the Fab McPC-603 (2mcp) is predominantly of an electrostatic character. The three methyl groups make close contact with the pocket. The hydrogen bonds between the ligand oxygen and Tyr9 and Arg13 are correctly reproduced in our docking approach. AutoDock also has yielded a structure close to the experimental results. FlexX and GOLD, however, cannot reproduce the corresponding crystal binding mode.

In 4phv, the second part of our ligand docking structure is shifted in comparison with the experimental results and resulted in a structure with rmsd of 1.97 Å. No crystal water molecules were used in present study. FlexX cannot reproduce the corresponding experimental binding structure unless these crystal water molecules are included in docking. GOLD yields a "good" docking structure with a rmsd of 1.11 Å.

One interesting observation in this study is that eight out of the twelve docked structures have lower energies than those directly minimized from the experimental structures. Some docked structures have energies lower than the directly minimized ones by more than 10 kcal/mol. In other two complexes, 121p and 3dfr, our docked structures have comparable energies to the directly minimized ones. For 2mcp and 4phv, however, the docking structures have energies much higher than the corresponding experimental ones. This indicates that our search algorithm works well for only ten out of twelve molecules, which is probably an upper limit of sampling coverage of this approach. We should also mention that the scoring function used here is based on the interaction energy (enthalpy), instead of free energy. The crystal structures are expected to correspond to the global minimum free energy structures, instead of global minimum energy structures.

DISCUSSION

In this section, we discuss some technical aspects of our docking protocol. We first discuss conformational sampling of an unbound ligand and examine the effect of dielectric constant on sampling. We then investigate the scoring functions used in rigid docking and in ranking the structures generated from the MM minimization. The effects of atomic charges and structural refinement on rigid docking results are also discussed. Finally we discuss the results from a fragment-based docking approach.

Conformation Sampling

Conformation sampling of an unbound ligand is the first step in our docking protocol. Ideally, the conformers generated from the conformation sampling should be as close to the experimentally-observed bound conformation as possible. Also, since the docking time is related to the number of the starting conformers, a small number of conformers is desirable.

Table III lists the results from conformation sampling for this set of ligands. The conformation sampling was done using the grid-based algorithm as discussed in the "Methods and Computational Details" section. The number of generated conformers range from ~10 to ~100 except for 2mcp and 3dfr. 2mcp has only five conformers while 3dfr has more than 1,000 conformers. The number of generated conformers is usually dependent on size of the fragments associated with the rotatable bonds. The larger the fragment, the fewer the generated conformers owing to the VDW clashes between the bulky fragment and the previous fragments. Since the conformers at this stage have different internal energies, a threshold of 30 kcal/mol energy was used to select low-energy conformers. As demonstrated in Figure 6, the energy differences between the lowest-energy conformers and the conformers that lead to the lowest-energy docking structures are in the range from 0.2 to 26.0 kcal/mol. Consequently, the 30 kcal/mol threshold was used. The number of the selected conformers is shown in the third column of Table III. The energy criterion effectively prunes down the number of conformers by 10–30 %, as seen in Table III.

Note that some molecules such as 121p, 2mcp, and 3dfr have a large magnitude of the energy in the conformational sampling, as shown in Table III. This is probably due to the coarse torsional angle grid used in the sampling.

Table III also illustrates the coverage of conformation sampling with respect to the experimentally-observed bound structures of the ligands. The rmsd results reported in Table III were calculated by superimposing heavy atoms of the calculated and experimental structures. Overall, the conformation sampling covers a quite broad range of conformation space. For most ligands, there are some

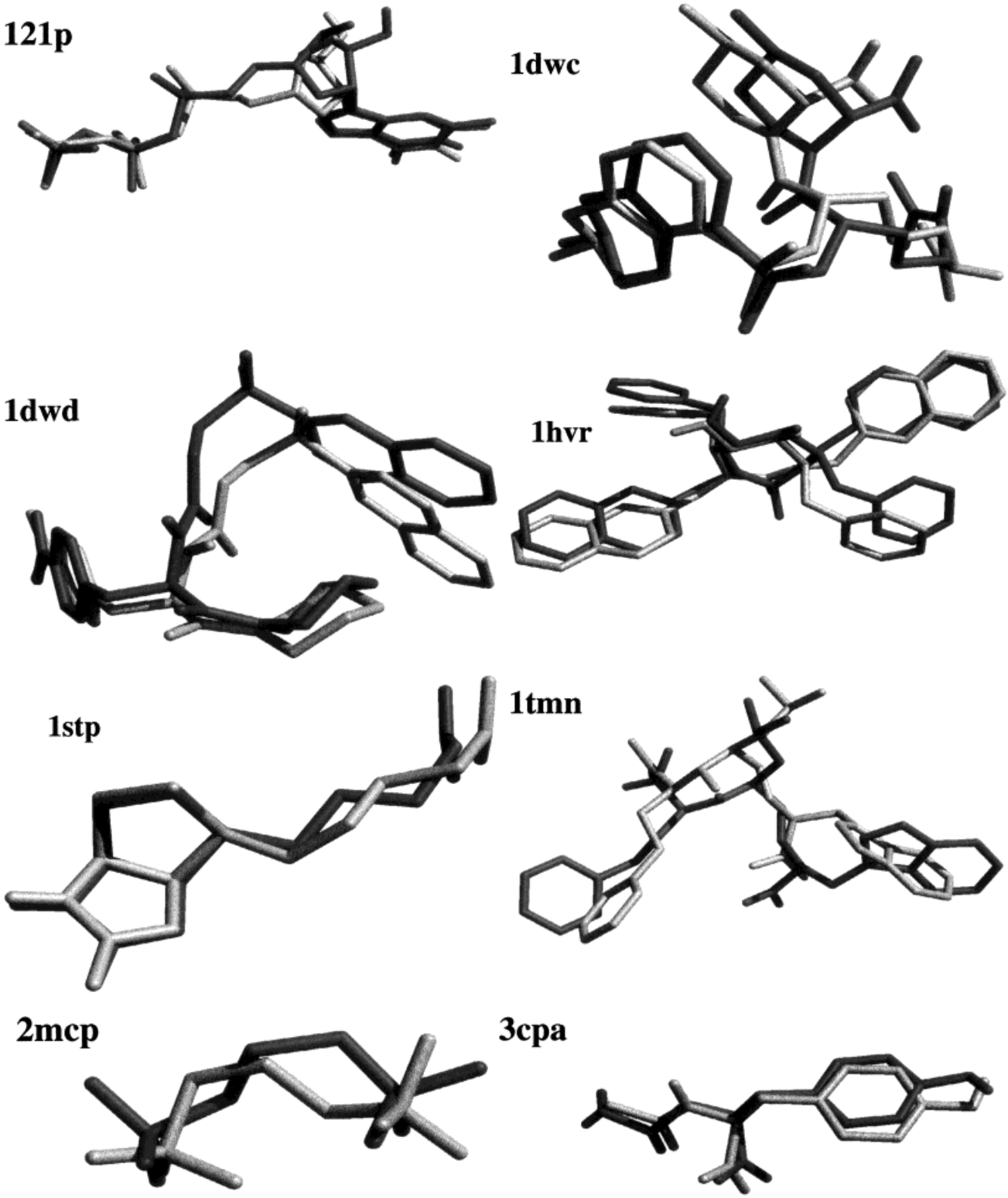


Fig. 5. The calculated (black) and crystal (grey) three-dimensional structures of the ligands at the binding sites.

conformers which are close to the bound conformation (rmsd near 1 Å), while others are quite different from the bound ones (rmsd larger than 4 Å).

The dependence of the conformation distributions on dielectric constant is illustrated in Figure 7 for 1dwc. There is noticeable change in conformation distribution

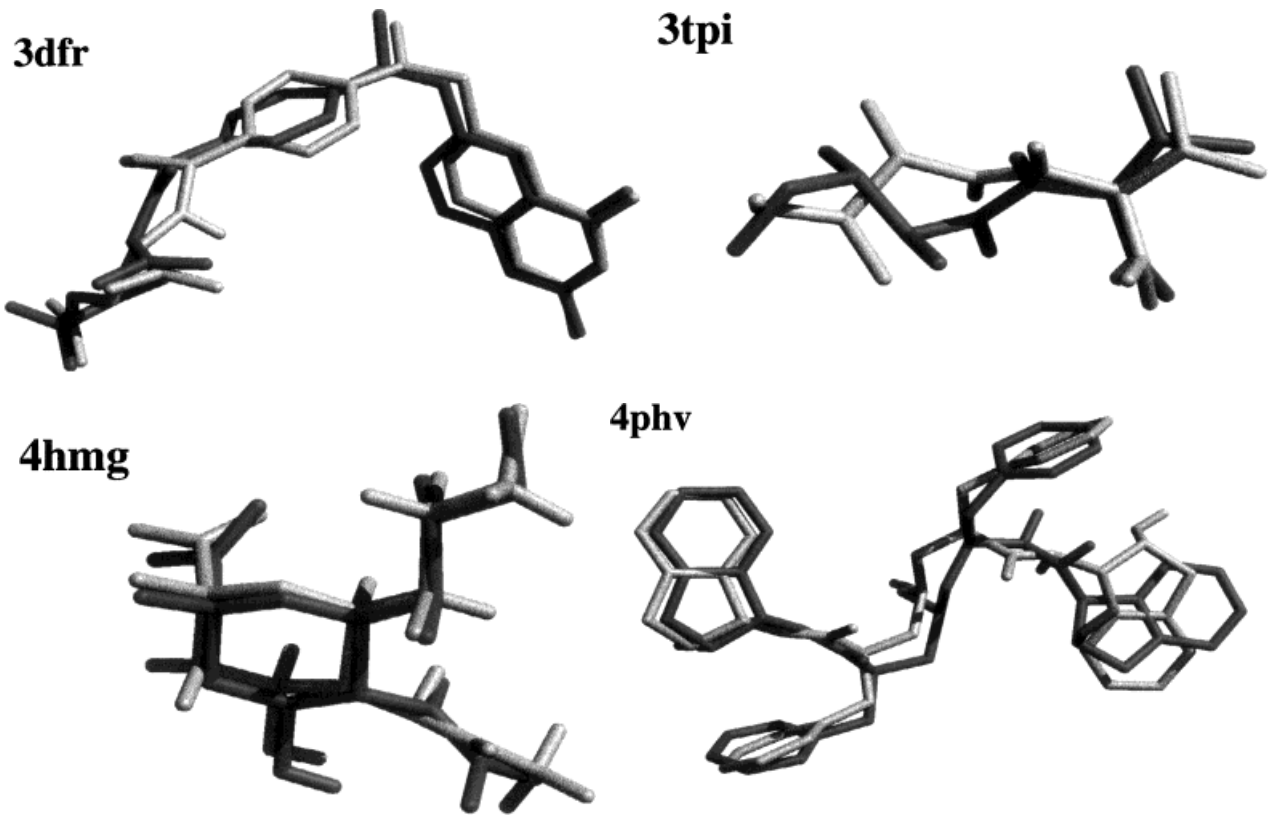


Figure 5. (Continued.)

going from dielectric constant of 1 to 4 (Fig. 7). The distribution, however, is almost identical for dielectric constants beyond 4. In terms of population percentage, small dielectric constants yield larger percentage of the conformers close to the bound conformation than the large dielectric constants. Also, note that small dielectric constant corresponds to a small number of conformers, whereas a large constant produces a large number of conformers. This can be understood from the last term in Eq. 1. The dielectric constant shields the interaction between charged groups; as a result, a large dielectric constant flattens the energy landscape, permitting a large number of conformers to have similar energies. Considering the coverage of conformation sampling and number of relevant conformers generated, we choose the dielectric constant of 1.5 in our conformation sampling. There is no strong physical reason to recommend this value, but choices between 1 and 2 are sensible for gas phase systems and justified by the overall docking results.

Rigid Docking

Finding the relevant conformations is only the first step in our docking procedure. The next important step is to locate the orientation for each low-energy conformer. Rigid docking using the DOCK program serves for this purpose. Since the starting conformers were generated from low-resolution torsional angles, these conformers were not expected to fit particularly well into the binding pocket. If the conventional van der Waals parameters were applied in rigid docking, many of these conformers would not survive, owing to the bad VDW contacts between them and receptor binding sites. Therefore, a soft VDW well depth (scaling factor of 1/1,000) for the ligand molecules was employed during the rigid docking runs. Table IV illustrates these docking results.

Starting with an ensemble of the low-energy conformers, DOCK found some orientations with rmsds less than 3.5 Å relative to the experimentally observed structures. Not only did DOCK find some good orientations, but it could also rank them well. In 1hvr, for instance, starting with 280 conformers, DOCK found that 153 conformers have orientation energies under 50 kcal/mol at the binding site. The rest of 135 conformers could not fit to the binding pocket well and consequently, were discarded from consideration in the later stages of optimization. Among the 153 orientations, 13 have a rmsd less than 3.5 Å. Among the 50% of the low-energy orientations, 9 orientations have the rmsd less than 3.5 Å. If we start with the 25% of the lowest orientations, there are 6 orientations close to the experimental results (rmsd less than 3.5 Å). The even distribution of the good orientations among all orientations is an important feature for information enrichment. Taking 1hvr as an example again, the probability to find three good orientations among all 153 orientations is $C_{13}^3/C_{153}^3 = 0.488 \times 10^{-3}$ whereas the probability to find three good orientations among top 25% DOCK scored orientations is

TABLE III. Conformation Sampling of the Unbound Ligands†

System	$N_{generated}$	$N_{selected}$	rmsd among $N_{selected}$[a]	E_{min}[b]	Rank of the best scored conformer[c]
121p	51	34	1.39 ~ 3.16	−486.3	24
1dwc	134	67	2.31 ~ 4.28	−70.9	27
1dwd	65	58	1.77 ~ 4.98	−61.6	53
1hvr	288	280	2.17 ~ 4.94	−4.1	197
1stp	105	77	0.39 ~ 2.20	−9.2	56
1tmn	380	334	1.05 ~ 4.35	34.6	257
2mcp	5	3	1.42 ~ 1.58	−149.1	3
3cpa	83	83	0.85 ~ 2.83	29.1	93
3dfr	1082	768	1.25 ~ 3.81	116.9	27
3tpi	5	4	2.06 ~ 2.69	−46.4	3
4hmg	96	96	0.87 ~ 2.97	−25.8	29
4phv	122	59	2.10 ~ 5.40	−40.9	28

†Using dielectric constant of 1.5 in the calculation.
[a]rmsd were calculated by superimposing the generated conformer over the observed bound conformer of the ligands.
[b]The lowest energy found among the generated structures.
[c]The conformer which leads to the lowest energy docking result.

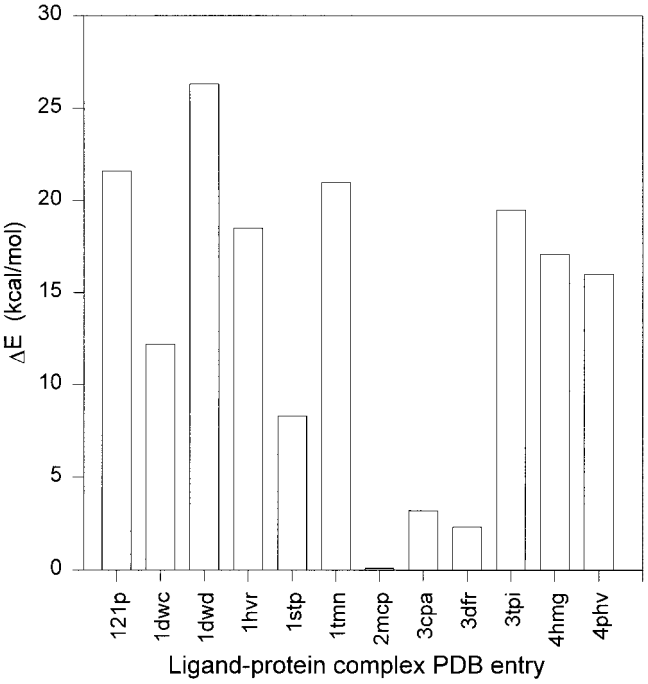


Fig. 6. The energy differences (kcal/mol) between the lowest-energy structures generated in the conformation sampling of the unbound ligands and the structures that lead to the lowest-energy docking structures.

$C_6^3/C_{38}^3 = 2.37 \times 10^{-3}$. The probability of finding three good orientations thus has been increased by a factor of about 5. The similar results are observed in other systems as shown in Table IV.

We should point out that DOCK could not find good orientations for the same set of conformers if the conventional VDW parameters were used. Other scaling factors such as 1/10 and 1/100 have been investigated as well (data not shown). The general observation is that smaller scaling factors lead to better results in rigid docking. The scaling factor of 1/1,000 is found to be optimal for this set of test compounds.

Atomic Charge Effect on the Docking Results

In our scoring function, van der Waals (VDW) and electrostatic interactions dominate the binding energies between ligand and receptor (Eq. 1). The VDW terms are related to the shape complementary between ligand and receptor binding site, while the electrostatic interaction is the Coulombic interaction between their partial atomic charges. The atomic charges thus play an important role in determining the binding energy and binding structures.

There are many ways to calculate atomic charges. The electrostatic potential-fitted (ESP) charges are derived from fitting to the electrostatic potential of a molecule around its van der Waals surface.[24,25] The potential is determined by either ab initio quantum mechanical calculations at the Hartree-Fock/6–31G* level or semiempirical quantum mechanical calculations such as AM1 or MNDO. The ab initio calculations are often expensive for large ligands, whereas the ESP charges based on semiempirical quantum mechanical calculations have not been extensively tested; therefore, no ESP charges were investigated in this work. The charge models investigated here are AMSOL charges[19] and Gasteiger charges.[18]

The AMSOL charges, referred to as Class IV charge models by Cramer and Truhlar et al., were parameterized to reproduce physical observables such as the molecular dipole moment.[19] Using semiempirical electronic wave functions like AM1 and PM3 to map experimental dipole moments for a large number of small molecules, the AMSOL charges can reproduce the dipole moments with the root mean square deviation of 0.3 D. Gasteiger charges are calculated based on the partial equalization of orbital electronegativity.[18] In the calculation only the connectivity of the atoms is considered, thus only the topology of a molecule is of importance. Through an iterative procedure, partial equalization of orbital electronegativity is reached and atomic charges are obtained. These two charge models are fast to compute and are widely used in molecular modeling community.

Table V tabulates the final docking results with respect to the two charge models. Among the 12 ligand-protein complexes, Gasteiger charges result in five ligand structures with rmsd larger than 2.0 Å, whereas the AMSOL charges have one ligand structure having rmsd larger than 2.0 Å. The AMSOL charges in general seem to be better than Gasteiger charges for our test cases.

Figure 8 shows the charges calculated from the two different models in ligands 121p and 1dwc. We observe that AMSOL charges generally have larger magnitude than Gasteiger charges. The better representation of charge distribution and the large magnitude in AMSOL charges helps the ligand orient inside the binding pocket. As discussed in the preceding section, the orientation of a low-resolution conformer was mainly determined by elec-

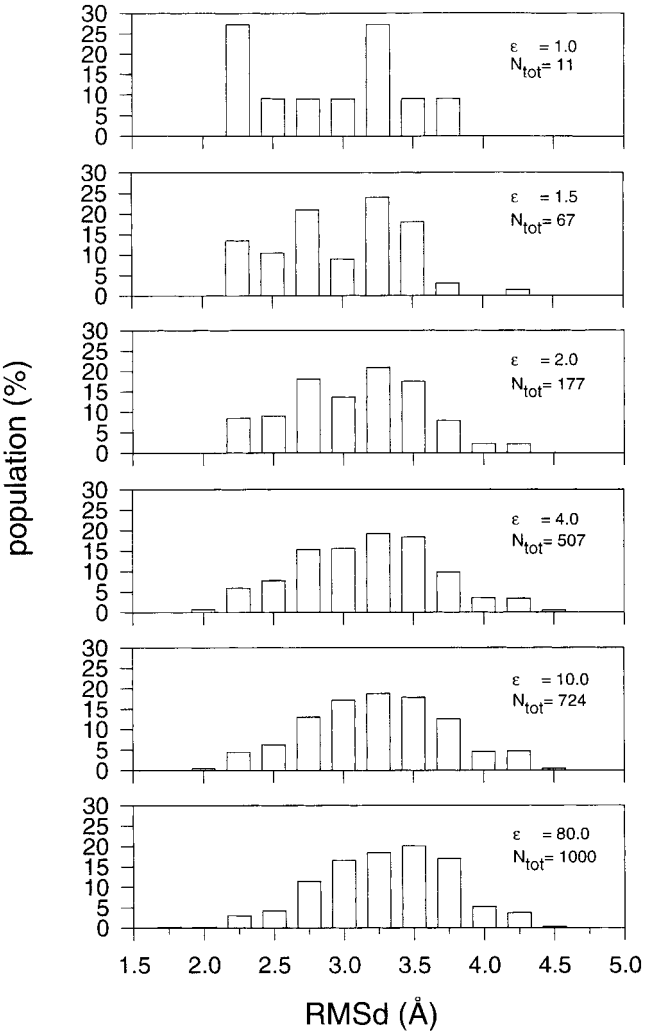


Fig. 7. The conformational distribution of argatroban (1dwd) with respect to the dielectric constant.

TABLE IV. The Rigid Docking Results for the Structures Less Than 3.5 Å rmsd From the Respective Crystal Structures

System	Top 25%[a]	Top 50%	100%
121p	2 (8)	2 (17)	6 (34)
1dwc	3 (16)	7 (33)	7 (64)
1dwd	1 (14)	1 (29)	2 (58)
1hvr	6 (38)	9 (76)	13 (153)
1stp	13 (19)	28 (38)	59 (77)
1tmn	5 (74)	9 (149)	14 (298)
2mcp	1 (1)	3 (3)	5 (7)
3cpa	8 (57)	18 (114)	27 (228)
3dfr	23 (192)	63 (384)	111 (768)
3tpi	1 (1)	2 (2)	4 (4)
4hmg	5 (27)	12 (55)	31 (111)
4phv	1 (8)	1 (16)	1 (33)

[a]The number of 25% of the lowest DOCK scored structures with rmsd less than 3.5 Å. The numbers in parenthesis are 25% of the total numbers of the docked structures. The similar definition is applied to column 3 and 4.

TABLE V. The Lowest Energy Docking Results With Respect to Different Charges

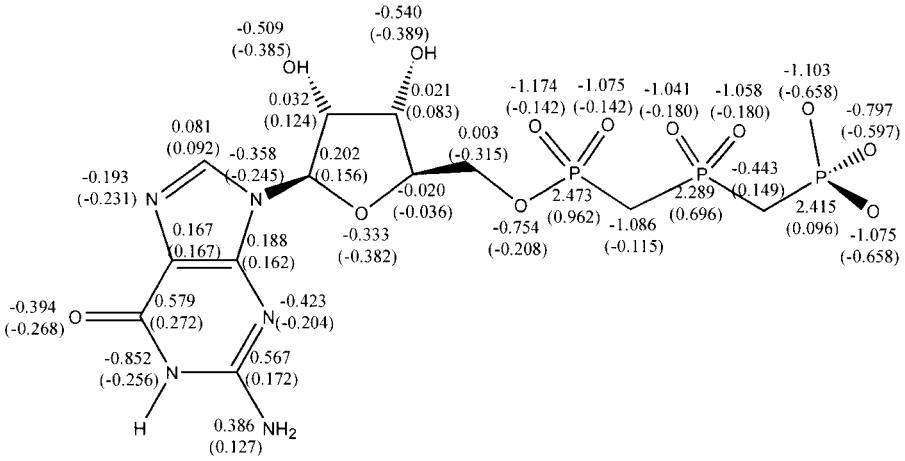
	Gasteiger		AMSOL	
System	E_{AMBER}	Rmsd	E_{AMBER}	Rmsd
121p	−9.5	2.15	−13.3	1.02
1dwc	−75.9	2.81	−75.50	1.15
1dwd	−77.9	7.01	−88.95	2.01
1hvr	−88.1	1.01	−95.2	1.16
1stp	−53.8	0.58	−54.6	1.02
1tmn	−56.1	1.92	−70.1	1.93
2mcp	−36.9	1.48	−11.2	1.71
3cpa	−65.1	7.88	−80.6	0.62
3dfr	−77.8	3.46	−66.3	1.39
3tpi	−71.4	1.65	−75.5	1.73
4hmg	−46.6	0.90	−45.4	0.76
4phv	−99.8	1.62	−110.2	1.97

trostatic interactions between ligand and receptors in our rigid docking protocol. The large charges—in particular the charges at hydrogen bond donors and acceptors—help the ligand orient itself to the receptor through the strong local hydrogen bonds.

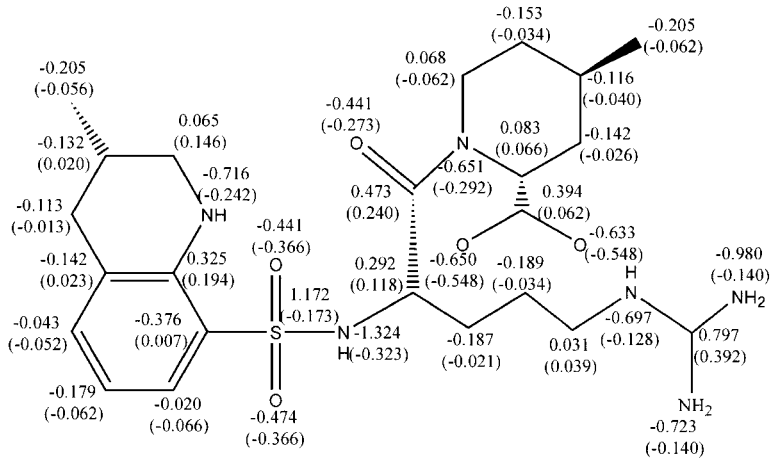
Scoring Functions in Molecular Mechanics Minimization

The molecular mechanics minimizations were used after rigid docking, local torsion angle refinement, and simulated annealing calculations. Due to the simplified representation of the receptor binding site and approximation introduced in the force field, the AMBER force field is not always able to distinguish or identify low rmsd structures, in particular with the structures from the rigid docking. Table VI shows the minimization results for several complexes. The initial structures were taken from rigid docking. Using the AMBER energy as scoring function, the resultant three-lowest-energy structures have rmsd of 7.50 ~ 7.84 Å for 1dwd, 9.34 ~ 12.03 Å for 1hvr, and 1.11 ~ 2.19 Å for 4hmg. The AMBER energy here consists of the internal energy of a ligand including bond stretching, bond-angle bending, torsional angle energies and nonbonded energies between intraligand atoms, and the interaction energies between ligands and receptor atoms. The AMBER energy excluding the first three terms in Eq. 1, termed hereafter as the "nonbonded energy," does a better job in terms of ranking structures. For example, it ranks the three lowest-energy structures having rmsd of 2.93 ~ 7.50 Å for 1dwd, 1.59 ~ 11.19 Å for 1hvr, and 1.11 ~ 1.70 Å for 4hmg.

The better performance of the nonbonded energy as a scoring function can be understood as following. The structures generated from rigid docking were still not in their best bond angles and torsional angles due to the low-resolution starting structures used in the rigid docking. The large internal energy penalty from these two terms excluded these structures from the lowest-energy list. On the other hand, the scoring function using only the nonbonded energies avoids these internal energy terms,



121p



1dwc

Fig. 8. The atomic charges calculated from the AMSOL and Gasteiger models (the number in parentheses).

and consequently it can still score the structures that are close to the crystal structures well.

We observed that the scoring function had a more pronounced effect on the rigid docking structures than those from torsion angle refinement at the binding site or simulated annealing calculations. For simplicity, we use the nonbonded energy as our scoring function throughout this work. It should be pointed out that the scoring functions here were only used to rank the order of the structures. The complete AMBER force field was used during geometry minimization and simulated annealing calculations.

Structure Refinements

The divide-and-conquer algorithm is an efficient way to reduce the complexity of many difficult problems. However, a refinement mechanism must be included in this approach in order to improve the low-resolution structures. In present work, molecular mechanics minimization, torsional angle scanning at the binding site, and simulated annealing were carried out to improve the binding structures that were generated from the rigid docking. The performance of the structure refinement at each stage is shown in Table VII.

Among the three lowest-energy structures minimized by molecular mechanics with the rigid docking structures, eight ligands have binding structures with rmsd less than 2.0 Å relative to the crystal structure. However, the three lowest-energy structures in 1dwc, 1dwd, 2mcp, and 4phv have rmsd larger than 2.0 Å. The torsional angle scanning at the binding site improves the structures in 1dwc, 1dwd and 4phv, yielding some structures with rmsd less than 2.0 Å, as shown in Table VII. The three lowest-energy structures of 2mcp, on the other hand, still have rmsds larger

TABLE VI. Selection of the Molecular Mechanics Minimization Results Over the Rigid Docking Structures

	Conventional AMBER[a]		Non-bonded AMBER[b]	
System	E_{total}	rmsd	$E_{nonbonded}$	Rmsd
1dwd	139.1	7.50	−79.4	2.93
	143.9	7.84	−74.6	7.50
	148.0	7.55	−74.1	2.95
1hvr	1.4	9.34	−60.6	9.34
	14.7	12.03	−55.6	1.59
	16.7	10.92	−53.9	11.19
4hmg	−58.6	2.19	−39.3	1.11
	−58.0	1.54	−38.4	1.54
	−47.9	1.11	−37.9	1.70

[a]The conventional AMBER energy including all internal energies of the ligand and the interaction energy between the ligand and protein was used to select the structures.
[b]The AMBER energies include the intraligand and ligand-protein nonbonded interaction energies was used to select the structures.

than 2.0 Å. The rmsd of the three lowest -energy structures in 3dfr are also larger than 2.0 Å at the torsional angle refinement stage. Overall, after the torsional angle refinement, only two ligands, 2mcp and 3dfr, have rmsd larger than 2.0 Å among the three lowest-energy structures. The binding structures in 2mcp and 3dfr are further improved by simulated annealing. The rmsd of the lowest-energy structure after SA are 1.71 Å for 2mcp and 1.39 Å for 3dfr, respectively. The simulated annealing method in general is able to find the low-energy structures that are in good agreement with the experimentally observed structures. The two exceptions are 1tmn and 1dwd. For 1tmn, the 4ps simulated annealing runs over the six best energy structures generated from torsion angle refinement lead to the lowest-energy structure shifted from 1.93 Å to 6.81 Å in rmsd. However, the structure with rmsd of 6.81 Å has higher energy than the one with 1.93 Å (−69.4 vs −70.1 kcal/mol). In 1dwd, the lowest-energy structure shifted from 1.40 Å to 2.01 Å after simulated annealing. The 2.01 Å structure has a lower energy than the 1.40 Å structure (−89.0 vs. −87.8 kcal/mol).

Timings

All dockings were carried out on a single processor SGI/R10000/195Mhz workstation. The whole molecule docking takes about 20 ~ 40 minutes for a medium size flexible ligand (with 8 ~ 10 rotatable bonds). Table VIII tabulates the timing data at each docking stage for selected molecules. As seen in Table VIII, the rate-limiting steps are in local refinements, including molecular mechanics minimization, torsional angle refinement, and simulated annealing. There are ways to improve the performance, such as using united atoms instead of all atoms, using the top 25% of rigid docking results for the later stage of minimization, and employing a small distance cutoff during the molecular mechanics minimization and simulated annealing. The further tuning of some parameters are needed to improve the performance of our docking approach.

TABLE VII. Docking Results at the Structure Refinement Stages[†]

System	Minutes from rigid docking	Torsional angle refinement	Simulated annealing
121p	1.87	2.10	1.02
	2.29	2.09	2.00
	2.33	1.87	1.31
1dwc	4.18	2.00	1.15
	6.75	4.29	1.30
	3.40	4.14	3.27
1dwd	2.93	1.40	2.01
	7.50	1.74	1.92
	2.95	6.33	8.66
1hvr	9.34	1.14	1.16
	1.59	1.39	10.03
	11.19	10.05	1.61
1stp	0.99	0.94	1.02[a]
	1.04	0.92	
	0.58	0.69	
1tmn	1.95	1.93	6.81
	2.25	3.52	1.73
	6.30	1.99	7.05
2mcp	3.10	4.07	1.71
	4.25	3.10	5.04
	4.23	4.17	2.86
3cpa	0.59	0.56	0.64
	7.95	7.94	1.31
	7.94	7.91	8.11
3dfr	2.72	2.72	1.39
	2.61	2.58	2.45
	1.35	2.72	2.16
3tpi	1.79	1.73	1.73
	2.46	1.82	2.48
	2.96	1.87	2.03
4hmg	1.11	1.04	0.76[b]
	1.54	1.06	
	1.70	0.86	
4phv	9.31	1.80	1.97
	9.64	9.33	8.32
	7.26	9.39	5.39

[†]The rmsds are ordered by energy.
[a]Only one cluster found.

Fragment Docking

Although the conformation sampling in our program is efficient, it is difficult to handle large flexible ligands with more than 20 or 30 rotatable bonds. In this case, we can adopt the ideas of fragment-based docking method in search for optimal binding modes. In conventional fragment-based docking approaches, one first breaks a ligand molecule into many small fragments associated with each rotatable bond, then selects a base fragment and docks it to the binding site. The rest of the fragments are constructed based on the positions of the base fragment. Since we have built a conformation sampling module into our program, we can break a ligand molecule into a few large substructures. Each substructure here is associated with several rotatable bonds. We then dock each substructure into the binding site and finally assemble the rest of the molecule based on the base positions of these substruc-

TABLE VIII. Timings for the Docking Run at Each Stage (in Seconds)

System	Conformation scan in vacuo	Rigid docking[a]	Minimization[b]	Torsion angle refinement[c]	Simulated annealing[d]	Total
121p	2	1.1	1.7	17	58	592
1dwc	6	0.9	2.2	31	66	817
1stp	3	0.8	0.9	30	25	470
3tpi	1	1.0	1.5	26	52	429
4phv	19	2.4	5.4	34	75	991

[a]The timing for docking one conformer.
[b]The average timing for minimizing one ligand-protein complex.
[c]For one structure refinement.
[d]For one 4ps simulated annealing simulation.

TABLE IX. Comparison of the Docking Results From Substructure-Based Docking Approach and Whole Molecule-Based Approach

	Substructure docking		Whole molecule docking	
System	$rmsd_{lowest}$[a]	$rmsd_{best}$[b]	$rmsd_{lowest}$[a]	$rmsd_{best}$[b]
1hvr	1.26	1.02	1.16	1.14
1tmn	1.52	1.13	1.93	1.93
3dfr	1.06	0.72	1.39	1.39
4phv	0.98	0.98	1.97	1.80

[a]The rmsd of the lowest energy docking structures with respect to the corresponding crystal structures.
[b]The rmsd of the best predicted docking structures with respect to the corresponding crystal structure.

tures. Furthermore, the constructed low-energy binding structures are subject to the optimizations of MM geometry minimization, torsional angle scanning, and simulated annealing. This approach is less dependent on the choice of base fragments, because it breaks the whole molecule into a few large substructures and use each of them as base substructure.

The results for several selected ligand-protein complexes are presented in Table IX. For example, the ligand in 1hvr has 10 rotatable bonds. Based on the symmetry of the molecules, we can select either the core of the molecule as substructure or the core attached with other fragments as substructures, as demonstrated in Figures 9b, c, and d. We first sampled conformation for these substructures and selected their low-energy structures for rigid docking. After rigid docking, we constructed the rest of the molecules based on the orientation of these substructures at the binding site. The constructed structures were then subject to the optimization by molecular mechanics, torsional angle scanning, and simulated annealing. The final lowest-energy binding structure has a rmsd of 1.25 Å. The fragment docking results for 1tmn, 3dfr, and 4phv are also listed in Table IX, whereas their selected substructures are illustrated in Figures 10–12. In most cases, we simply break a molecule into two or three substructures based on the symmetry or on the size of the molecule.

One interesting observation is that our substructure-based docking approach in general resulted in slightly

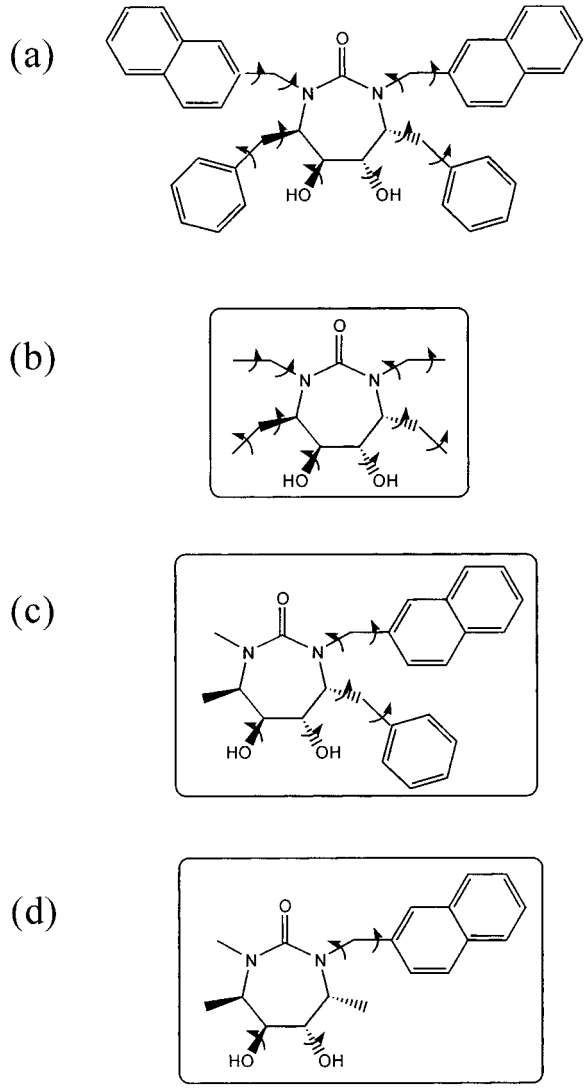


Fig. 9. The base fragments of the ligand in 1hvr. **(a)** Whole molecule; and **(b)-(d)** the three base fragments used in the docking study.

better binding structures than those from whole-molecule-based docking approach. For the few test cases, the cost of this substructure-based docking protocol is also slightly less than the whole-molecule-based docking approach (about 10% less, data not shown), as it still needs the structural refinement steps including minimization, torsional angle scanning at the binding site, and simulated annealing. The structural refinements are the rate-limiting steps in our docking algorithm. However, this approach is expected to be much faster for very large ligand than the whole-molecule-based method, as the conformational space grows exponentially for large flexible molecules.

CONCLUSIONS

A divide-and-conquer flexible ligand docking algorithm has been investigated in this study. This approach first divides the docking search space into conformation and

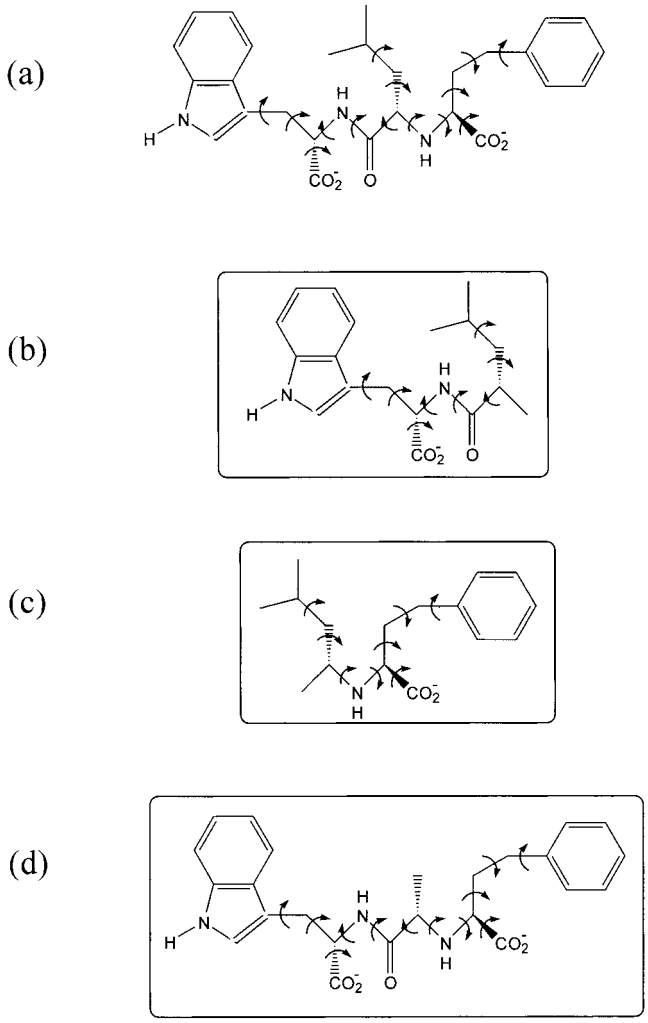


Fig. 10. The base fragments of the ligand in 1tmn. **(a)** Whole molecule; and **(b)-(d)** the three base fragments used in the docking study.

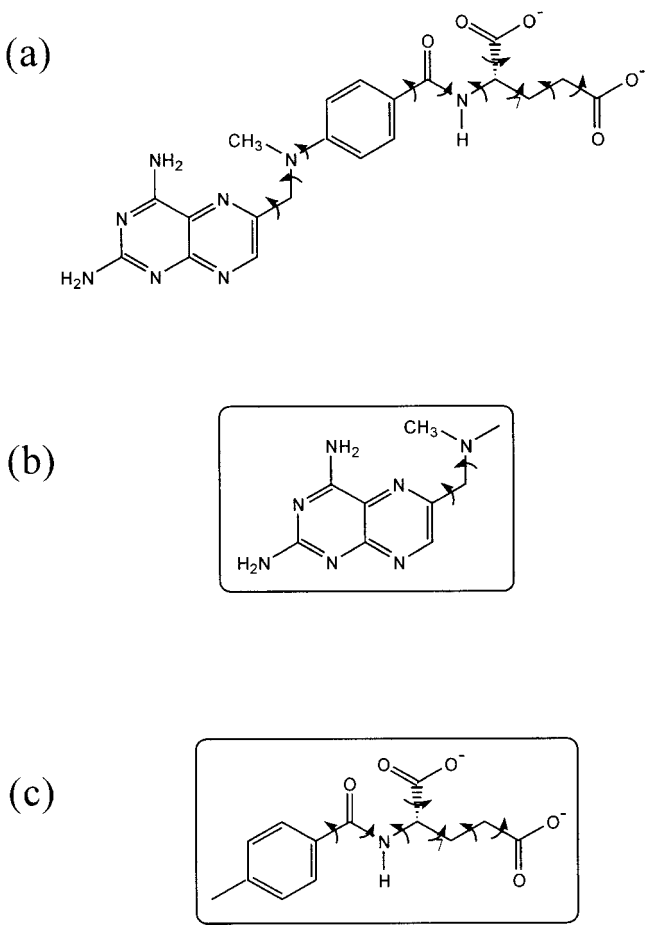


Fig. 11. The base fragments of the ligand in 3dfr. **(a)** Whole molecule; and **(b)-(c)** the two base fragments used in the docking study.

orientation space. For the conformation space, a grid-based program has been used to sample conformation of an unbound ligand and to select an ensemble of low-energy conformers. A rigid docking method that employs geometric complementary match as implemented in the DOCK program is applied to locate orientations for each low-energy conformer. These first two steps serve to globally scan the energy landscape of the ligand at the binding site and find low-energy regions. A subsequent structure refinement is designed to improve these low-resolution structures. The structure refinement comprises of three steps: molecular mechanics geometry minimization, torsional angle scanning at the binding site, and a short period of molecular dynamics simulated annealing. The final docking structure is therefore the lowest-energy structure among all structures generated during the search procedure. This docking protocol has been applied to 12 ligand-protein complexes that represent the difficult systems published in the studies using AutoDock and FlexX programs. Encouraging results have been obtained. For ligands with the sizes of three to 16 rotatable bonds, this method yields ligand-binding structures with rmsds from 0.62 Å to 2.01 Å relative to the corresponding crystal binding structures. The whole docking process takes 10–30 minutes of computation in a standard workstation and thus provides a practical tool to study ligand-receptor interactions. Based on the limited test cases, the approach presented here can achieve at least the performance produced by AutoDock, FlexX, and GOLD.

The success of the present approach for this set of test cases can be attributed to several factors. One is to select a set of bound-like conformers for a flexible ligand, the second is to find low-energy binding orientations for these conformers using rigid docking, and the final one is to refine these low-resolution binding structures systematically. Each of these steps is important for a successful docking result and needs to be handled appropriately based on the resources.

In the conformational sampling of an unbound ligand, a low dielectric constant and the energy criterion of 30 kcal/mol are found to give good results in terms of the conformation coverage over the bound-like conformations and the number of non-redundant conformers generated.

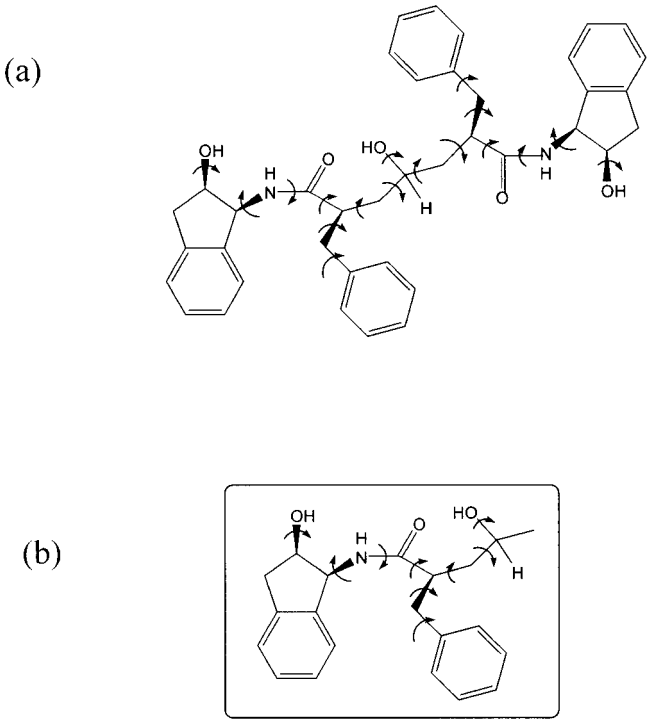


Fig. 12. The base fragments of the ligand in 4phv. **(a)** Whole molecule; and **(b)** the base fragments used in the docking study.

In rigid docking, a soft van der Waals well depth is necessary to generate experimental-like orientations with a set of low-resolution conformers. The geometry optimization using molecular mechanics is proven to be an efficient way to locate the local energy minimum structures at the binding site. The torsional angle scanning at the binding site can partially overcome the energy barriers and thus expand the conformation search space near the starting structures. The short molecular dynamics simulated annealing method could serve as a method to "clean up" the structures both in conformation and orientation space and find the lowest-energy structures that are in good agreement with the experimentally observed binding structures. Several thresholds and parameters involved in these steps such as the dielectric constant in conformation sampling of an unbound ligand, energy criterion to select low-energy conformers, and number of conformers selected for structural refinement, etc., were derived based on the overall docking results so that they lead to the good final results.

The proposed docking approach can also adopt the fragment docking idea to dock large flexible ligands into binding sites. In this case, a large ligand molecule can be broken into a few large substructures that associate with several rotatable bonds. Conformation sampling and rigid docking calculations can be carried out for each of these substructures, then whole molecules can be constructed at the binding site and the resultant lowest-energy structures are subject to structure refinement including minimization and simulated annealing. For a small test set, we found that the fragment-based docking approach yielded comparable or even better results than the whole-molecule based docking method.

The present docking approach is one of the attempts to combine available techniques together to tackle docking problems.[26,27] Some of the ideas, in particular generating an ensemble of low-energy structures and rigid docking, can be immediately applied to generate a set of starting structures for further refinement by other docking protocols such as AutoDock (Monte Carlo simulated annealing) or GOLD (genetic algorithm based optimizations).

In terms of computation, the present approach is still slow to carry out a database search. It is also slower than the FlexX program and other fragment-based docking approaches. However, fragment-based docking constructs the ligand conformation based on the local interaction (i.e., short-range interaction) between fragments and receptor, therefore its results are sensitive to the local environment (shape and hydrogen bond groups) of the receptor. It can often do well in reproducing a known ligand-protein complex structure, because the binding pocket is already adjusted to this particular ligand. However, rank ordering a set of ligands with different sizes against the same pocket is challenging for this approach due to its lower tolerance to the local environment of the binding pocket. The whole-molecule based docking, on the other hand, includes both the long-range and short-range interactions and therefore its results are less sensitive to the shape and hydrogen-bond pattern of the binding pocket. The other nice feature of the present approach is that it can naturally incorporate partial protein flexibility during the late stages of minimization and simulated annealing. Therefore, the present approach can serve as a useful tool to do lead optimization.

One of the needs in flexible docking is to improve the scoring function. A scoring function that can effectively distinguish the correct binding modes from all other putative mode and handle well with large hydrophobic fragments of a ligand is certainly needed. Rarey et al has shown some promising results using the LUDI scoring function.[8] The development of scoring functions that can take into account of the desolvation energy of a ligand is in process (Zou X, Sun Y, Kuntz ID, personal communication, 1998). Efficient sampling of conformational space of an unbound ligand is also an issue to be addressed. Klebe and Mietzner have developed an interesting heuristic approach to sample the bound-like conformation of a ligand based on available experimental crystal structures of small molecules.[29] More studies are certainly needed in this respect.

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