Development and Evaluation of a Generic Evolutionary Method for Protein–Ligand Docking

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Abstract: We have developed a generic evolutionary method with an empirical scoring function for the protein–ligand docking, which is a problem of paramount importance in structure-based drug design. This approach, referred to as the GEMDOCK (Generic Evolutionary Method for molecular DOCKing), combines both continuous and discrete search mechanisms. We tested our approach on seven protein–ligand complexes, and the docked lowest energy structures have root-mean-square derivations ranging from 0.32 to 0.99 Å with respect to the corresponding crystal ligand structures. In addition, we evaluated GEMDOCK on crossdocking experiments, in which some complexes with an identical protein used for docking all crystallized ligands of these complexes. GEMDOCK yielded 98% docked structures with RMSD below 2.0 Å when the ligands were docked into foreign protein structures. We have reported the validation and analysis of our approach on various search spaces and scoring functions. Experimental results show that our approach is robust, and the empirical scoring function is simple and fast to recognize compounds. We found that if GEMDOCK used the RMSD scoring function, then the prediction accuracy was 100% and the docked structures had RMSD below 0.1 Å for each test system. These results suggest that GEMDOCK is a useful tool, and may systematically improve the forms and parameters of a scoring function, which is one of major bottlenecks for molecular recognition.

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Key words: empirical scoring function; generic evolutionary method; protein–ligand docking; hybrid-solution docking method; structure-based drug design

Introduction

A computer-aided docking process, identifying the lead compounds by minimizing the energy of intermolecular interactions, has greatly advanced an understanding of the molecular recognition phenomenon, and has been demonstrated to play an important role for structure-based drug design.[1,2] Protein–ligand docking simulations need to yield the binding energy of the bound complex crystal structure. In general, solving a protein–ligand docking problem involves two critical elements:[3] a good scoring function and an efficient search algorithm for finding a global minimum on the binding energy landscape of a simulation scoring function that is often complex and rugged funnel shapes.[4]

A good scoring function should be fast and simple for screening large potential solutions and effectively discriminating between correct binding states and nonnative docked conformations. Various scoring functions have been developed for calculating binding free energy, such as empirical-based,[5,6] knowledge-based,[5,7,8] physic-based,[9–11] solvent-based scoring functions,[12] and consensus scoring function.[13] A search algorithm should consist of global and local search strategies for covering the conformation and orientation spaces fast and efficiently, including the deterministic,[14,15] stochastic,[5,16,17] and hybrid approach.[18]

Many automated docking approaches have been developed and can be roughly divided into rigid docking, flexible ligand docking, and protein flexible docking methods. The rigid-docking methods[14] treated both ligands and proteins as rigid. In flexible ligand docking methods, such as evolutionary algorithms,[5,11,17,19] simulated annealing,[16] and fragment-based approach,[15] the ligand is flexible and the protein is rigid. For reasonably addressing protein flexible problems, where both ligands and proteins are flexible, most of the docking methods often allowed a limited model of protein variations, such as the side-chain flexible or small motions of loops in the binding site.[20] Among these search algorithms, an evolutionary-based approach is a very promising direction.

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Main procedure proceeds following steps:

1. Prepare the protein binding site and assign the atom formal charge (Table 1) and the atom type (Table 2).
2. Fix the location of the receptor and Let $g = 1$. Randomly generate initial population, $P(g)$, with N solutions by initializing the orientation and conformation of a ligand related to the receptor.
3. Evaluate the scoring fitness of each solution in the population $P(g)$.
4. Generate a new quasi-population, $P_1(g)$, with N solutions by applying FC_Adaptive with $P(g)$ and *decreasing-based Gaussian mutation* (M_{dg}).
5. Generate a new quasi-population, $P_2(g)$, with N solutions by applying FC_Adaptive with $P_1(g)$ and *differential equation* (M_{dg}).
6. Generate a new quasi-population, $P_{next}(g)$, with N solutions by applying FC_Adaptive with $P_2(g)$ and *self-adaptive Cauchy mutation* (M_c). Let $g = g + 1$ and $P(g) = P_{next}$.
7. Repeatedly execute from step 4 to step 6 until the terminal criteria are satisfied.

FC_adaptive procedure proceeds the following steps with two parameters, working population (P) and working mutation (M_{dg}, M_{DE}, or M_c):

1. Let C be an empty set ($C = \emptyset$). For each solution a, called *family father*, in working population (P) executes following steps: {*family competition*}
   - (a) Generate L docked ligand solutions (the orientation and conformation), denoted as $c^1, \cdots, c^L$, by applying the recombination, rotamer mutation, and working mutation.
   - (b) Select the one, c^{best}, with the lowest scoring value from the union set (e.g., a and $c^1, \cdots, c^L$).
   - (c) Add the c^{best} into the set C.
2. Return the set C with N solutions.

Figure 1. The main steps of GEMDOCK for flexible ligand docking.

Here, we developed a generic evolutionary approach with an empirical scoring function, referred to as the Generic Evolutionary Method for molecular DOCKing (GEMDOCK), to address several issues of protein–ligand docking problems. First, we have reported the validation and analysis of GEMDOCK on various search spaces and scoring functions to understand which factors (e.g., search algorithms, scoring functions, or experimental errors) are mainly responsible for the molecular docking errors. Second, we have analyzed the nature and influences of a hybrid-solution docking method, which evolves simultaneously both rigid and flexible docked conformations, because most of the current methods are either flexible or rigid docking methods. Finally, our approach is likely to help in making a good choice or in improving the scoring function which is one of the major bottlenecks in protein–ligand problems. The GEMDOCK is an extended work of our recently developed evolutionary algorithm which was more robust than three standard evolutionary approaches, including genetic algorithms,[21] evolution strategies,[22] and evolutionary programming.[23]

Table 1. Atom Formal Charges of GEMDOCK.

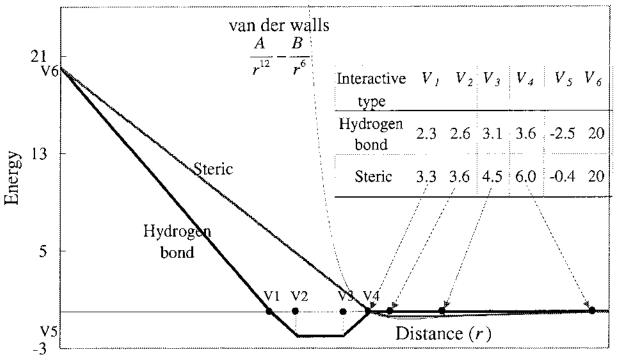
Formal charge	Heavy atom name
Receptor:	
0.5	N atom in His (ND1 & NE2) and Arg (NH1 & NH2)
−0.5	O atom in Asp (OD1 & OD2) and Glu (OE1 & OE2)
1.0	N atom in Lys (NZ)
2.0	metal atoms (MG, MN, CA, ZN, FE, and CU)
0	other atoms
Ligand:	
0.5	N atom in —$C(NH_2)_2^+$
−0.5	O atom in —COO—, —PO_2^-, —PO_3^-, —SO_3^-, and —SO_4^-
1.0	N atom in —NH_3^+ and —$N^+(CH_3)_3$
0	other atoms

We have now substantially enhanced the original method, and there are four main differences in methodology between the present work and our previous study.[24] First, GEMDOCK could be a flexible or a hybrid-solution docking method. Second, we developed an empirical scoring function, which was specifically designed for fast docking applications. Third, GEMDOCK combines both continuous and discrete search mechanisms to improve the

Table 2. Atom Types of the GEMDOCK.

Atom type	Heavy atom name
Donor	primary and secondary amines, sulfur, metal atoms, and atom with positive formal charge[a]
Acceptor	oxygen and nitrogen with no bound hydrogen, and atom with negative formal charge[a]
Both	water and hydroxyl groups
Nonpolar	other atoms (such as carbon and phosphorus)

[a] The atom formal charge defined in Table 1.



Interactive type	V_1	V_2	V_3	V_4	V_5	V_6
Hydrogen bond	2.3	2.6	3.1	3.6	-2.5	20
Steric	3.3	3.6	4.5	6.0	-0.4	20

Figure 2. The linear energy function of the pair-wise atoms for steric and hydrogen bonds in GEMDOCK (bold line) with a standard Lennard–Jones potential (light line).

performance via two new genetic operators. Finally, GEMDOCK is an automatic system that is able to prepare all required materials, such as the atom formal charge, the atom type, and the ligand binding site of a protein.

To evaluate the performance and limitations of GEMDOCK, we tested it on seven protein–ligand complexes. The docked lowest energy structures have root-mean-square derivations ranging from 0.32 to 0.99 Å with respect to the corresponding crystal ligand structures. GEMDOCK was compared to five stochastic approaches applying the very similar scoring function.[25] In addition, GEMDOCK was tested on two crossdocking ensembles of protein structures, 10 complexes of the dihydrofolate reductase,[26] and six complexes of the trypsin,[15] to evaluate GEMDOCK on a problem in which a protein structure is small motion during docking processing. Experimental results indicate that GEMDOCK is robust and the empirical scoring function is simple and fast to recognize compounds. Furthermore, it may be used to systematically evaluate and thus improve scoring functions.

Method

Here, we present the details of our GEMDOCK for the protein–ligand docking (Fig. 1). GEMDOCK, an automatic docking tool, is able to generate all experimental variables and serve as a flexible or hybrid-solution docking tool. We designed a new rotamer-based mutation operator for reducing the search space of ligand structure conformations, and used a differential evolution operator[27] for reducing the disadvantages of Gaussian and Cauchy mutations. First, we specified the coordinates of ligand and protein atoms, the ligand binding area, atom formal charge (Table 1), and atom types (Table 2). Crystal coordinates of the ligand and protein atoms were taken from the Protein Data Bank, and were separated into different files. The size and location of the ligand binding site was determined by considering the protein atoms located <10 Å from each ligand atom when preparing the proteins. GEMDOCK then automatically determined the center of the receptor and the search cube of a binding site according to the maximum and minimum of coordinates of these selected protein atoms.

After GEMDOCK prepares the ligand and protein, GEMDOCK randomly generates a starting population with N solutions by initializing the orientation and conformation of the ligand relating to the center of the receptor according to the search cube. Each solution is represented as a set of three n-dimensional vectors ($\boldsymbol{x}^i$, σ^i, ψ^i), where n is the number of adjustable variables of a docking system and $i = 1, \ldots, N$. The vector x represents the adjustable variables to be optimized in which x_1, x_2, and x_3 are the three-dimensional location of the ligand; x_4, x_5, and x_6 are the rotational angles; and from x_7 to x_n are the twisting angles of the rotatable bonds inside the ligand. σ and ψ are the step-size vectors of decreasing-based Gaussian mutation and self-adaptive Cauchy mutation, respectively. In other words, each solution x is associated with some parameters for step-size control. The initial values of x_1, x_2, and x_3 are randomly chosen from the search box, and the others (x_4 to x_n) are randomly chosen from 0 to 2π in radians. The initial step sizes σ is 0.8 and ψ is 0.2. The ligand conformations (i.e., twisting angles of the rotatable bonds inside a ligand) are randomly generated if GEMDOCK is a flexible docking method. If GEMDOCK works as a hybrid-solution docking method, the initial ligand conformations of rigid solutions ($0.2N$) are set to the conformation of the ligand crystal structure in PDB and the others (flexible solutions with $0.8N$) are randomly generated.

GEMDOCK enters the main evolutionary loop which consists of three main stages in every iteration: decreasing-based Gaussian mutation, differential equation, and self-adaptive Cauchy mutation after GEMDOCK initializes the solutions. Each stage is realized by generating a new quasi-population (with N solutions) as the parent of the next stage. As shown in Figure 1, these stages apply a general procedure "FC_adaptive," with only different working population and the mutation operator.

The FC_adaptive procedure (Fig. 1) employs two parameters, namely, the working population (P, with N solutions) and mutation operator (M), to generate a new quasi-population. The main work of FC_adaptive is to produce offspring and then conduct the family competition. Each individual in the population sequentially becomes the "family father." With a probability p_c, this family father and another solution that is randomly chosen from the rest of the parent population are used as parents for a recombination operation. Then the new offspring or the family father (if the recombination is not conducted) is operated on by the rotamer mutation and then by the working mutation, i.e., decreasing-based Gaussian mutation (M_{dg}), differential equation (M_{DE}), or self-adaptive Cauchy mutation (M_c). For each family father, such a procedure is repeated L times called the family competition length.

Table 3. Parameters of the GEMDOCK.

Parameter	Value of parameters
Initial step sizes	$\sigma = 0.8$, $v = \psi = 0.2$ (in radius)
Family competition length	$L = 2$
Population size	$N = 400$
Recombination rate	$p_c = 0.3$
No. of the maximum generation	60

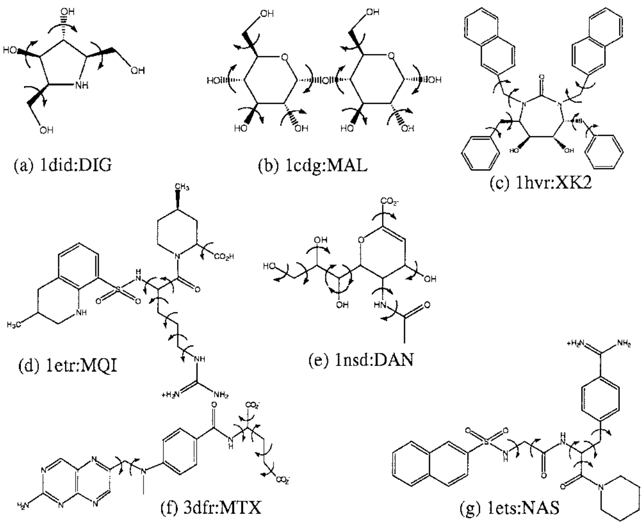

(a) 1did:DIG (b) 1cdg:MAL (c) 1hvr:XK2 (d) 1etr:MQI (e) 1nsd:DAN (f) 3dfr:MTX (g) 1ets:NAS

Figure 3. The ligands used for docking in this article with rotatable bonds are indicated. The lower-case four-letter and upper-case three-letter symbols are the PDB code and ligand code in PDB, respectively.

Among these L offspring and the family father, only the one with the lowest scoring function value survives. Because we create L children from one "family father" and perform a selection, this is a family competition strategy. This method avoids the population prematureness but also keeps the spirit of local searches. Finally, the FC_adaptive procedure generates N solutions, because it forces each solution of the working population to have one final offspring.

When GEMDOCK is a rigid docking, these values of x_7 to x_n, conformations of rotatable bonds inside a ligand, are fixed and set to the ligand conformations of the crystal bound complex. GEMDOCK is a flexible docking tool if it evolves the conformation variables $(x_7, \ldots, x_n)$ of each solution in a population. GEMDOCK is a hybrid-solution approach if the conformation variables of part of solutions (e.g., ηN solutions) are set to the values of the crystal bound complex. In this article, η is 0.2 when GEMDOCK is a hybrid-solution method.

In the following, genetic operators are briefly described. We use $a = (x^a, \sigma^a, \psi^a)$ to represent the "family father" and $b = (x^b, \sigma^b, \psi^b)$ as another parent. The offspring of each operation is represented as $c = (x^c, \sigma^c, \psi^c)$. The symbol x_j^s is used to denote the jth adjustable optimization variable of a solution s, $\forall j \in \{1, \ldots, n\}$.

Recombination Operators

A recombination operator selected the "family father (a)" and another solution (b) randomly selected from the working population. GEMDOCK implemented both modified discrete recombination and intermediate recombination.[22] The former generates a child as follows:

$$x_j^c = \begin{cases} x_j^a \text{ with probability } 0.8 \\ x_j^b \text{ with probability } 0.2. \end{cases} \tag{1}$$

The generated child inherits genes from the "family father" with a higher probability 0.8. Intermediate recombination works as:

$$w_j^c = w_j^a + \beta(w_j^b - w_j^a)/2, \tag{2}$$

where w is σ or ψ based on the mutation operator applied in the FC_adaptive procedure. The intermediate recombination only operated on step-size vectors, and the modified discrete recombination was used for adjustable vectors (x).

Mutation Operators

After the recombination, a mutation operator, the main operator of GEMDOCK, is applied to mutate adjustable variables (x). Gauss-

Table 4. Test Systems Used in Docking Experiments.

			Ligand			Interaction between ligand and receptor		
Protein/ligand complex	PDB code	Search Cartesian volume (Å)	No. of torsion	No. of polar atoms[a]	No. of charge atoms[b]	No. of hydrogen binding[c]	No. of electrostatic interaction[c]	Energy of native binding[c]
D-xylose isomerase/D-glucitol	1cdg	28 Å × 23 Å × 28 Å	10	11	0	11	0	−91.33
Cyclodextrin glycosyltransferase/ maltose	1did	29 Å × 29 Å × 31 Å	4	5	0	8	0	−66.13
Thrombin/Argatroban	1etr	56 Å × 37 Å × 43 Å	7	10	4	12	4	−154.63
Thrombin/NAPAP	1ets	56 Å × 37 Å × 42 Å	6	8	2	9	4	−198.27
HIV-1 protease/XK263	1hvr	29 Å × 32 Å × 38 Å	8	3	0	6	0	−187.61
Influenza virus neuraminidase/ DANA	1nsd	28 Å × 31 Å × 35 Å	10	9	2	14	8	−157.27
Dihydrofolate reductase/Methotrexate	3dfr	34 Å × 32 Å × 32 Å	7	12	4	12	7	−215.67

[a] and [b] are defined in Table 1 and Table 2, respectively.

[c] Statics are derived from the native crystal conformations of test systems according to our scoring function [eq. (12)].

Table 5. GEMDOCK Results on the Test Cases Presented in Table 4.

	Selected binding site[a]									
	Flexible docking[c]				Hybrid-solution docking[d]			Whole protein[b] with flexible docking		
PDB code	Minimum energy	Best RMSD (Å)	Average RMSD (Å)	Success rate[e]	Best RMSD (Å)	Average RMSD (Å)	Success rate	Best RMSD (Å)	Average RMSD (Å)	Success rate
1cdg	−107.81	0.99	1.16	100%	0.60	0.97	100%	0.85	1.67	90%
1did	−78.83	0.40	1.80	55%	0.38	0.75	75%	0.67	2.72	45%
1etr	−163.93	0.58	3.14	55%	0.53	2.77	65%	0.49	4.09	55%
1ets	−194.55	0.63	4.85	25%	0.46	4.49	30%	0.79	6.79	40%
1hvr	−192.55	0.30	1.28	85%	0.22	1.11	85%	0.34	2.42	80%
1nsd	−151.53	0.32	0.44	100%	0.30	0.38	100%	0.34	4.18	85%
3dfr	−222.77	0.35	0.97	90%	0.30	0.65	95%	0.43	2.20	85%

All results are derived from 20 independent docking runs, and the docked lowest energy conformation is considered to calculate the best RMSD and average RMSD.

[a] and [b] the selected binding site and the whole protein are considered as the search binding areas, respectively.

[c]GEMDOCK evolves a population with N flexible solutions.

[d]GEMDOCK evolves a population with $0.2N$ rigid solutions and $0.8N$ flexible solutions.

[e]The percentage of the docking runs that find a docked lowest energy structure within 2.0 Å RMSD with respect to the crystal ligand structure.

ian and Cauchy Mutations are continuous search operators and the rotamer mutation is a discrete operator.

Gaussian and Cauchy Mutations

Gaussian and Cauchy Mutations are accomplished by first mutating the step size (w) and then mutating the adjustable variable x:

$$w_j' = w_j' A(\cdot), \tag{3}$$

$$x_j' = x_j + w_j' D(\cdot), \tag{4}$$

where w_j and x_j are the ith component of w and x, respectively, and w_j is the respective step size of the x_j where w is σ or ψ. If the mutation is a self-adaptive mutation, $A(\cdot)$ is evaluated as $\exp[\tau' N(0, 1) + \tau N_j(0, 1)]$ where $N(0, 1)$ is the standard normal distribution, $N_j(0, 1)$ is a new value with distribution $N(0, 1)$ that must be regenerated for each index j. When the mutation is a decreasing-based mutation $A(\cdot)$ is defined as a fixed decreasing rate $\gamma = 0.95$. $D(\cdot)$ is evaluated as $N(0, 1)$ or $C(1)$ if the mutation is, respectively, Gaussian mutation or Cauchy mutation. Our decreasing-based Gaussian mutation uses the step-size vector σ with a fixed decreasing rate $\gamma = 0.95$ and works as

$$\sigma^c = \gamma \sigma^a, \tag{5}$$

$$x_j^c = x_j^a + \sigma^c N_j(0, 1). \tag{6}$$

The self-adaptive Cauchy mutation is defined as

$$\psi_j^c = \psi_j^a \exp[\tau' N(0, 1) + \tau N_j(0, 1)], \tag{7}$$

$$x_j^c = x_j^a + \psi_j^c C_j(t). \tag{8}$$

We set τ and τ' to $(\sqrt{2n})^{-1}$ and $(\sqrt{2\sqrt{n}})^{-1}$, respectively, according to the suggestion of evolution strategies.[22] A random variable is said to have the Cauchy distribution $[C(t)]$ if it has the density function:

$$f(y;\, t) = \frac{t/\pi}{t^2 + y^2}, \quad -\infty < y < \infty,$$

where t is set to 1.

Differential Evolution

An offspring of differential evolution is generated as

$$x_j^c = \begin{cases} u_j^m, & \text{if rand}[0, 1) \le CR \\ x_j^a, & \text{otherwise} \end{cases} \tag{9}$$

and

$$u_j^m = x_j^a + F(x_j^b - x_j^c), \tag{10}$$

where a is the "family father"; b and c are two solutions randomly selected from the working population subjected to $a \neq b \neq c$. In this work, F and CR are set to 0.5 and 0.9, respectively.

Rotamer-Mutation

This operator is only used for x_7 to x_n to find the conformations of the rotatable bonds inside the ligand. For each ligand, this operator mutates all of the rotatable angles according to the rotamer distribution and works as:

$$x_j = r_{ki} \text{ with probability } p_{ki}, \tag{11}$$

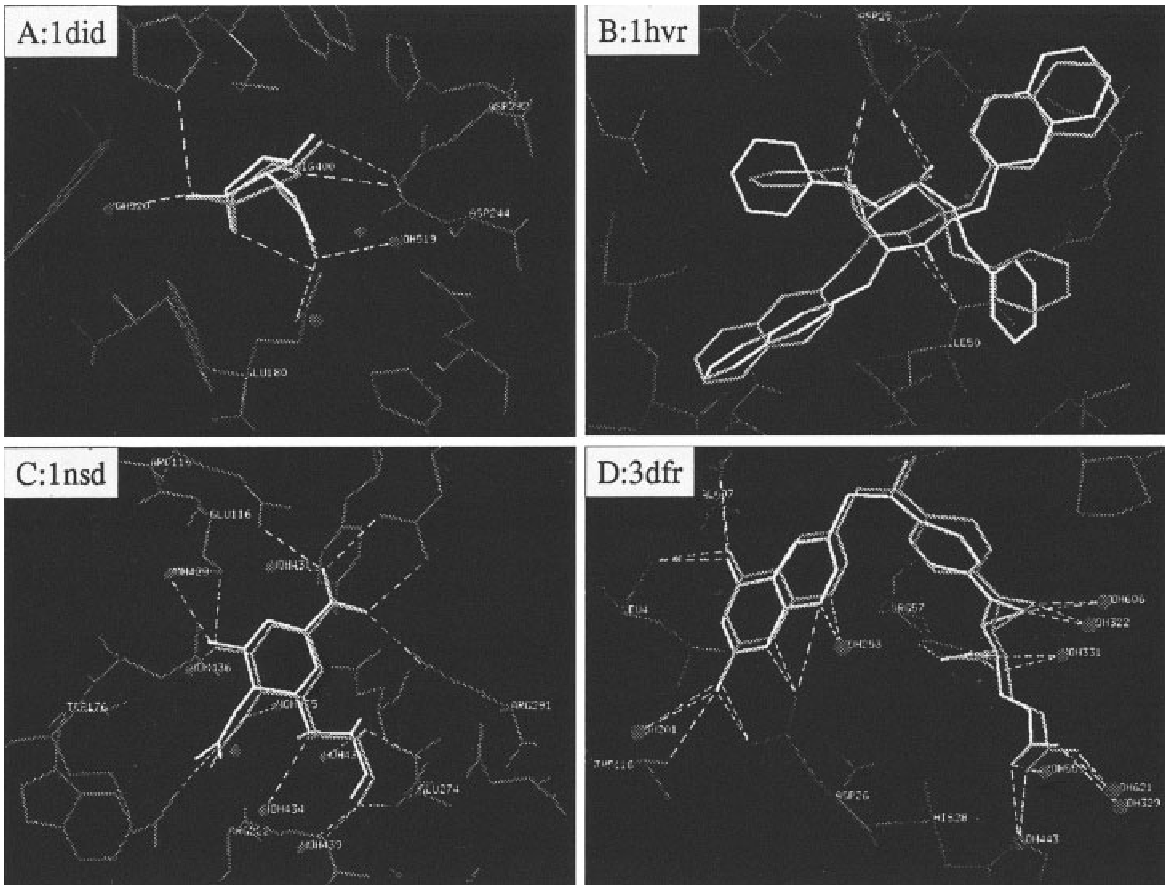


Figure 4. GEMDOCK results of four protein–ligand complexes. The RMSD values of these four complexes are less than 1.0 Å, and most of the docked ligand groups (white) are identical with the crystal ligand structures (gray). The white dotted lines are hydrogen bonds.

where r_{ki} and p_{ki} are the angle value and the probability, respectively, of ith rotamer of kth bond type including sp^3—sp^3 and sp^3—sp^2 bonds. The values of r_{ki} and p_{ki} are based on the energy distributions of these two bond types.

Scoring Function

In this work, we used an empirical scoring function given as

$$E_{\text{tot}} = E_{\text{inter}} + E_{\text{intra}} + E_{\text{CO}}, \tag{12}$$

where E_{inter} and E_{intra} are the intermolecular and intramolecular energy, respectively, E_{CO} penalizes a solution if the relative contract order between ligand and receptor is less than a predefined value.

The intermolecular energy is defined as

$$E_{\text{inter}} = \sum_{i=1}^{\text{lig}} \sum_{j=1}^{\text{pro}} \left[F(r_{ij}^{B_{ij}}) + 332.0 \frac{q_i q_j}{4r_{ij}} \right], \tag{13}$$

where r_{ij} is the distance between the atoms i and j, q_i and q_j are the formal charges and 332.0 is a factor that converts the electrostatic energy into kilocalories per mol. The lig and pro denote the numbers of the heavy atoms in the ligand and receptor, respectively. The formal charge of atom type of receptor and ligand are defined in Table 1. $F(r_{ij}^{B_{ij}})$ is a simple atomic pair-wise potential function (Fig. 2) modified from previous works[5,28] and given as

$$F(r_{ij}^{B_{ij}}) = \begin{cases} V_6 - \dfrac{V_6 r_{ij}^{B_{ij}}}{V_1}, & \text{if } r_{ij}^{B_{ij}} \le V_1 \\ \dfrac{V_5(r_{ij}^{B_{ij}} - V_1)}{V_2 - V_1}, & \text{if } V_1 < r_{ij}^{B_{ij}} \le V_2 \\ V_5, & \text{if } V_2 < r_{ij}^{B_{ij}} \le V_3 \\ V_5 - \dfrac{V_5(r_{ij}^{B_{ij}} - V_3)}{V_4 - V_3}, & \text{if } V_3 < r_{ij}^{B_{ij}} \le V_4 \\ 0, & \text{if } r_{ij}^{B_{ij}} > V_4 \end{cases}. \tag{14}$$

$r_{ij}^{B_{ij}}$ is the distance between the atoms i and j with bond type B_{ij} which is the interaction bonding type forming by the pair-wise heavy atoms of a ligand and a protein. B_{ij} is either hydrogen binding or steric state. The values of parameters, $V_1, \ldots, V_6$, are given in Figure 2. In this atomic pair-wise model, the interactive types are only hydrogen binding and steric potential which have the same function form but with different parameters, $V_1, \ldots, V_6$.

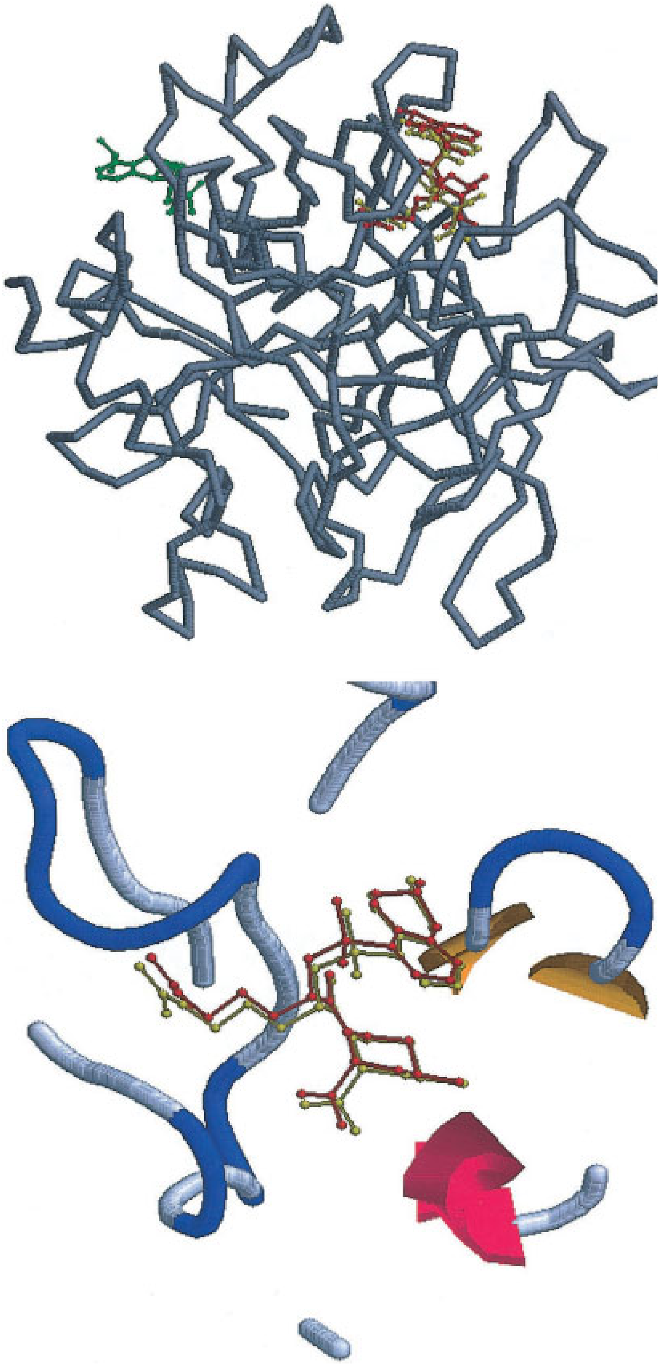


Figure 5. Results of docking the ligand argatroban (MQI) into thrombin (1etr) with (a) the while protein and (b) the selected binding site as the search binding areas. The docked ligand conformations are yellow, and the crystal ligand structures are red. (a) Shows three main clusters of the docked ligand conformations, 55% results are near the native binding state (yellow), 30% results are near the pocket (green), and 15% results are in the other positions.

The energy value of hydrogen binding should be larger than the one of steric potential. In this model, the atom is divided into four different atom types (Table 2): donor, acceptor, both, and nonplar. The hydrogen binding can be formed by the following atom-pair types: donor–acceptor (or acceptor–donor), donor–both (or both–donor), acceptor–both (or both–acceptor), and both–both. Other atom-pair combinations are to form the steric state.

The intramolecular energy of a ligand is

$$E_{\text{intra}} = \sum_{i=1}^{\text{lig}} \sum_{j=i+2}^{\text{lig}} [F(r_{ij}^{B_{ij}})] + \sum_{k=1}^{dihed} A[1 - \cos(m\theta_k - \theta_0)], \quad (15)$$

where $F(r_{ij}^{B_{ij}})$ is defined as eq. (14) except the value is set to 1000 when $r_{ij}^{B_{ij}} < 2.0$ Å for penalizing the unreasonable ligand conformations and *dihed* is the number of rotatable bonds. We followed the work of Gehlhaar et al.[5] to set the values of A, m, and θ_0. For the sp^3—sp^3 bond A, m, and θ_0 are set to 3.0, 3, and π; and $A = 1.5$, $m = 6$, and $\theta_0 = 0$ for the sp^3—sp^2 bond.

The relative contract order is defined as $R_{\text{CO}} = f/T$, where f is the frequency of the atom-pair distance less than 8 Å, and T is the total number of interactions between the ligand and receptor. The penalty E_{CO} is based on R_{CO} and is given

$$E_{\text{CO}} = \begin{cases} 1000(K - R_{\text{CO}}), & \text{if } R_{\text{CO}} \leq K \\ 0, & \text{if } R_{\text{CO}} > K \cdot \end{cases} \quad (16)$$

In this article, the K is set to 0.025 and 0.075 when the whole protein and the selected binding site as the search binding areas, respectively.

Results and Discussions

Parameters of GEMDOCK

Table 3 indicates the setting of GEMDOCK parameters, including initial step sizes, family competition length ($L = 2$), population size ($N = 400$), and recombination probability ($p_c = 0.3$) in this work. The GEMDOCK optimization stops when either the convergence is below certain threshold value or the iterations exceed a maximal preset value which was set to 60. Therefore, GEMDOCK generated 2400 solutions in one generation and terminated after it exhausted 144,000 solutions in the worse case. These parameters were decided after experiments conducted to recognize complexes of test docking systems with various values.

Test Complexes and Docking Protocols

We chose seven protein–ligand complexes shown in Figure 3 and Table 4 to illustrate the effectiveness of our approach and to allow comparison with other docking approaches,[6,25] which used the similar empirical scoring functions. These ligands have between 4 and 10 rotatable bonds, between 3 and 12 polar atoms, and between 0 and 4 formal charge atoms. The native binding energy of a crystal complex is calculated by using our scoring function [eq. (12)], which consists of three major kinds of protein–ligand interactions (i.e., the hydrophobic interactions, electrostatic interactions, and hydrogen bindings). The number of the hydrogen bonds ranges between 6 and 14 and the number of the electrostatic interactions ranges between 0 and 8. These statics are derived from

Table 6. Comparisons GEMDOCK with Different Energy Functions on Test Cases.

	E_{inter} [eq. (13)] without electrostatic energy			E_{tot} [eq. (12)] without penalty E_{CO}			RMSD [eq. (17)] scoring function		
PDB code	Best RMSD (Å)	Average RMSD (Å)	Success rate[a]	Best RMSD (Å)	Average RMSD (Å)	Success rate	Best RMSD (Å)	Average RMSD (Å)	Success rate
1cdg	0.97	1.15	95%	0.80	1.15	95%	0.01	0.02	100%
1did	0.43	1.86	50%	0.43	2.00	50%	0.01	0.02	100%
1etr	0.48	3.22	45%	0.58	7.98	10%	0.03	0.05	100%
1ets	0.89	6.72	15%	1.58	9.53	5%	0.09	0.14	100%
1hvr	0.27	1.40	85%	0.32	1.86	85%	0.03	0.08	100%
1nsd	0.35	0.67	95%	0.38	0.45	100%	0.02	0.03	100%
3dfr	0.32	1.42	85%	0.32	1.48	80%	0.04	0.10	100%

All results are derived from 20 independent docking runs and the docked lowest energy conformation is considered for each test case.

[a]The percentage of the trials that find a docked lowest energy structure within 2.0 Å RMSD with respect to the crystal ligand structure.

the crystal binding conformations and the distant thresholds of a hydrogen bond and an electrostatic interaction are set to 3.2 and 4.5 Å in this article, respectively. GEMDOCK was also tested on two crossdocking ensembles of protein structures, 10 complexes of the dihydrofolate reductase, and six complexes of the trypsin (serine proteinase) complex, to evaluate it on unbound docking problems and on the problem in which a protein structure is small motion during docking processing.

Crystal coordinates of ligand and protein atoms were taken from the Protein Data Bank, and were separated into different files. The protein atoms are selected if they are located less than 10 Å apart from each ligand atom. We followed the work[25] to retain the metal atoms and water molecules. GEMDOCK automatically decided the cube of a binding site based on the maximum and minimum of atom coordinates of a selected binding site. Among these seven test systems, the minimum cube is 28 × 23 × 28 Å (1cdg) and the maximum cube is 56 × 37 × 44 Å (1etr). Our program also automatically assigned the formal charge (Table 1) and the atom type (Table 2) of each atom in the ligand and protein. The bond type (sp^3—sp^3, sp^3—sp^2, or others) of a rotatable bond inside a ligand is also assigned. The energies of the native crystal conformations of test systems are indicated for referring based on our scoring function [eq. (12)].

Accuracy of Docking Prediction

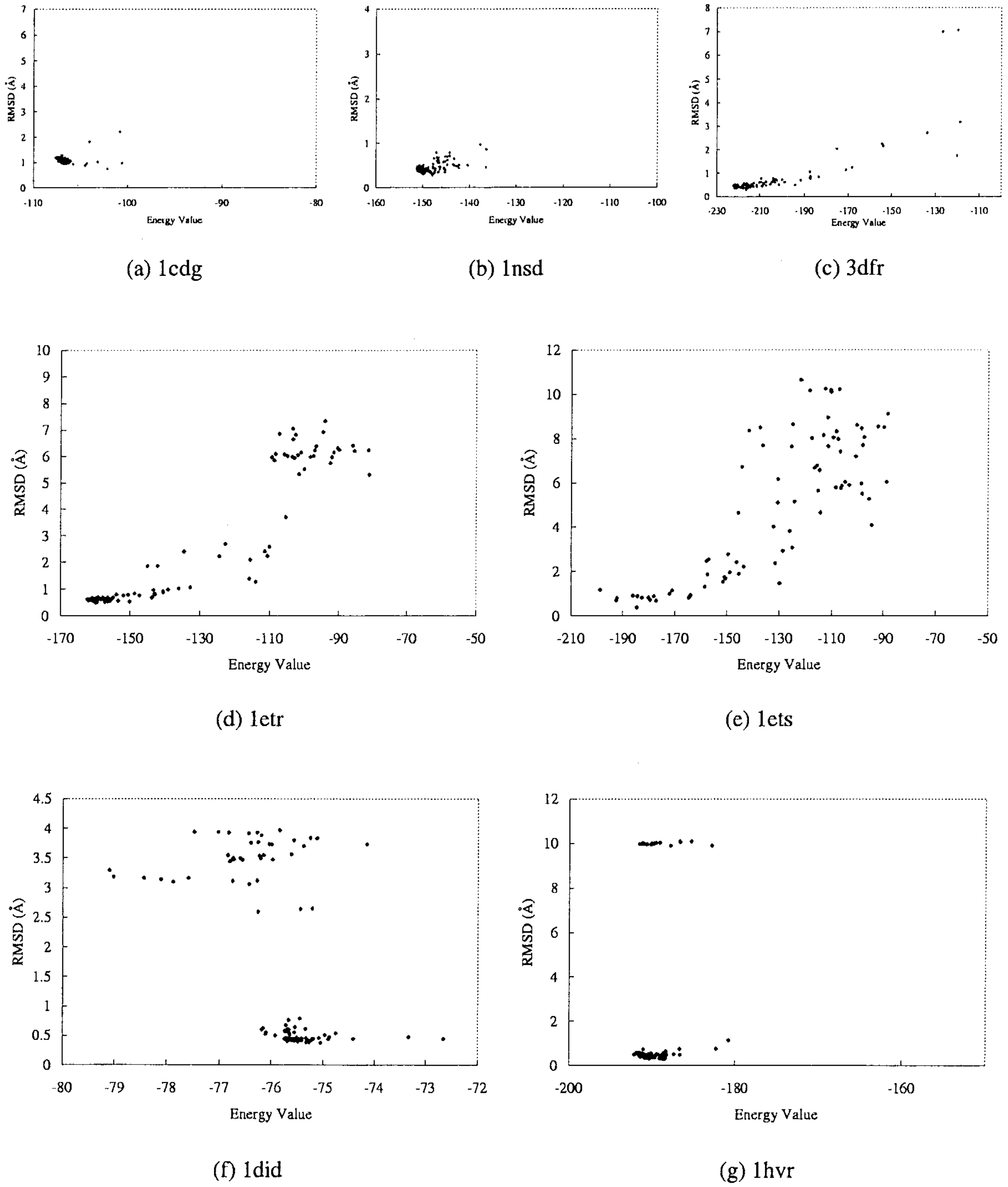
The overall accuracy of GEMDOCK in predicting the docked conformations of seven test cases is shown in Table 5. We used two performance criteria to evaluate the accuracy and robustness of a docking method. The first is the root-mean-square deviation (RMSD) error in ligand heavy atoms between the docked conformation and the crystal ligand structure. The second criterion is the success rate, which is the percentage of the trials that find a solution within 2.0 Å RMSD. The RMSD is commonly used and is given

$$\left\{\sum_{i=1}^{M}[(X_i-x_i)^2+(Y_i-y_i)^2+(Z_i-z_i)^2]/M\right\}^{1/2}, \quad (17)$$

where M is the heavy atom number of a ligand; (X_i, Y_i, Z_i) and (x_i, y_i, z_i) are the coordinates of the ith atom of X-ray crystal and docked structures, respectively. All results were derived from 20 independent docking runs and the docked lowest-energy structure was considered for each test case. In this work GEMDOCK runs on Pentium 1.4 GHz personal computer with single processor. On average GEMDOCK took 410 s, the maximum time was 652 s for the complex, 1ets, and the shortest time was 130 s for 1did. When we analyzed the characteristics of GEMDOCK and compared GEMDOCK with other methods, GEMDOCK executed 100 and 500 docking runs and the docked lowest energy structure was considered for each test system, respectively.

As shown in Table 5, GEMDOCK yielded the best RMSD values ranging between 0.30 Å (1hvr) and 0.99 Å (1cgd) and the average RMSD values ranging between 0.44 Å (1nsd) and 4.85 Å (1ets) when GEMDOCK worked as a flexible docking and the selected binding site was considered as the binding search area. The success rates range between 100% (1cdg) and 35% (1ets). Figure 4 shows four docked solutions (i.e., 1did, 1hvr, 1nsd, and 3dfr) in which GEMDOCK predicted correct positions for most of the ligand groups. The docked and crystal ligand conformations are white and gray, respectively, and the white dotted lines indicate hydrogen bonds. The RMSD values of these four docked conformations are less than 1.0 Å. According to these docked conformations, we observed that GEMDOCK often yielded more number of hydrogen bonds than native states to minimize the docking energy based on our energy function [eq. (12)]. The energy of the docked conformation (Table 5) obtained by GEMDOCK was often lower than the energy of the crystal conformation (Table 4). Although GEMDOCK worked as a hybrid-solution docking method, evolving 260 flexible ligand solutions and 40 rigid ligand solutions, it consistently yielded lower RMSD values and higher success rates than the ones of the flexible docking method for all test cases.

Although the whole protein was considered as the search binding area, GEMDOCK used the same parameter values, shown in Table 3, except that the population size was 700 to improve the success rates and reduce the average RMSD values. Table 5 indicates that GEMDOCK yields slightly different performance on



(a) 1cdg

(b) 1nsd

(c) 3dfr

(d) 1etr

(e) 1ets

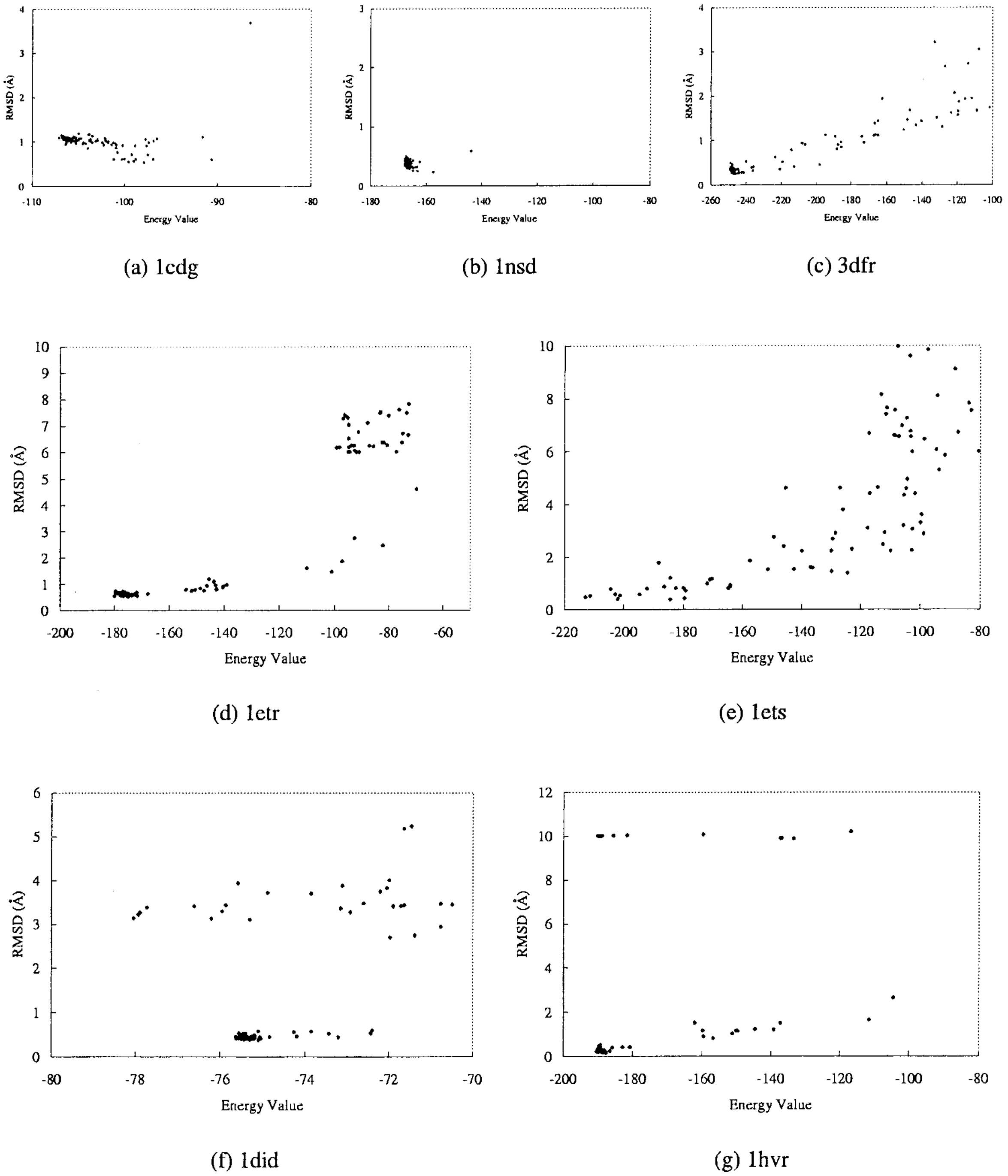
(f) 1did

(g) 1hvr

Figure 6. GEMDOCK results are divided into three categories (see text) for the flexible docking in seven complexes with 100 docking runs: (a) 1cdg, (b) 1nsd, and (c) 4dfr are the first class; (d) 1etr and (e) 1ets are the second class; (f) 1did and (g) 1hvr are the final class.

these two kinds of the search binding sites, the selected binding site, and the whole protein. Figure 5a and b shows the results of docking the argatroban (MQI) into the thrombin (1etr) with the whole protein and selected binding site as the search binding areas, respectively. The docked ligand conformations are yellow and the crystal ligand structures are red. The docked lowest energy conformation is identical with the crystal structure for most of ligand groups. Figure 5a shows that the docked ligand conformations can be divided into three main clusters: the first (55% solutions) is near the native binding state (yellow), the second (30% solutions) is near the pocket (green), the final (15% solutions) is in the other locations.

In the following subsections, we validated and analyzed the characteristics of GEMDOCK, including energy functions, the



(a) 1cdg (b) 1nsd (c) 3dfr

(d) 1etr (e) 1ets

(f) 1did (g) 1hvr

Figure 7. GEMDOCK results are divided into three categories (see text) for the hybrid-solution docking in seven complexes with 100 docking runs: (a) 1cdg, (b) 1nsd, and (c) 4dfr are the first class; (d) 1etr and (e) 1ets are the second class; (f) 1did and (g) 1hvr are the final class.

search spaces, and docking materials, and should therefore help to understand the error-free prediction of docked conformations.

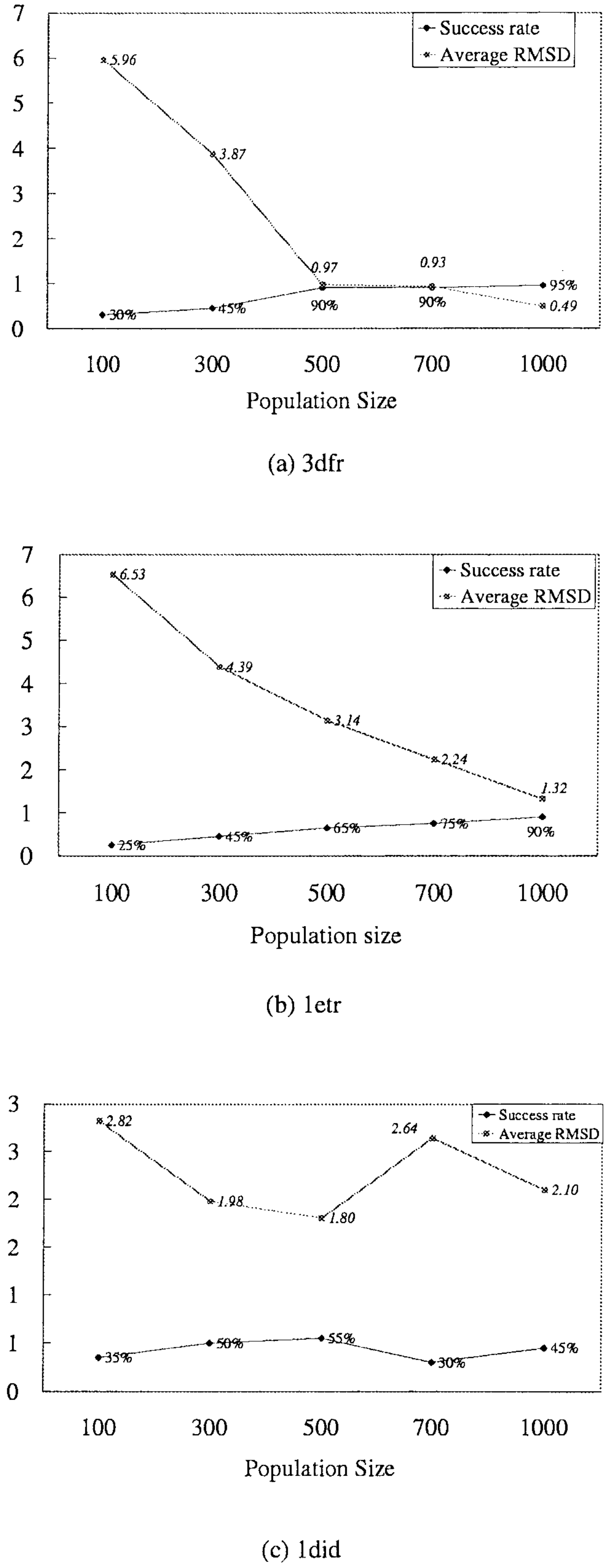
Evaluation of the Energy Function Used

One of main objectives of this study was to evaluate whether our empirical scoring function was robust for molecular docking. To simplify the task, we tested GEMDOCK with various uses and parameter values of our scoring function [eq. (12)] on test complexes. The overall accuracy is shown in Tables 5 and 6. GEMDOCK generally improved the docked quality by considering the electrostatic energy if the protein–ligand interaction has the electrostatic energy, such as the 1etr, 1ets, 1nsd, and 3dfr. For complex

1nsd, eight electrostatic interactions were formed between the atoms **O** of CO_2^- (ligand) and Arg115 N^{ζ}, Arg291 N^{ζ}, and Arg373 N^{ζ} (receptor). For the complex 1etr, three electrostatic interactions were formed between the atoms **N** of $(NH_2)_2^+$ (ligand) and Asp189 O^{δ} (receptor) and one electrostatic interaction was formed between the atoms **O** of CO_2^- (ligand) and His57 N^{ε} (receptor). The E_{CO} is useful for large search Cartesian volume, such as 1etr and 1ets. According to these experimental results, the element, $F(r_{ij}^{B_{ij}})$, of the E_{inter} [eq. (13)] was the main element of our scoring function. In contrast, the E_{intra}, E_{CO}, and electrostatic energy were minor elements that influenced some specific docking cases.

Figures 6 and 7 show the relationships between binding energies and RMSD values of the docked lowest energy structures for flexible and hybrid-solution docking methods with 100 independent docking runs for each complex. For these two docking methods, GEMDOCK has similar search behaviors and results that are roughly classified into three kinds of typical categories. For the first category, GEMDOCK yielded high success rates (>90%) and our scoring function [eq. (12)] is able to discriminate between native and nonnative conformations for complexes 1cdg (Figs. 6a and 7a), 1nsd (Figs. 6b and 7b), and 3dfr (Figs. 6c and 7c). For the second category, GEMDOCK yielded medium success rates (<60%) and may trap into local optimal, such as 1etr and 1ets. Figures 6d, 7d, 6e, and 7e show that our scoring function is also able to discriminate between correct binding states and nonnative conformations. In general, GEMDOCK is able to improve the success rate and docked accuracy by enlarging the population size for this category (Fig. 8b). For the final category, including 1did (Figs. 6f and 7f) and 1hvr (Figs. 6g and 7g), our scoring function may be unable to discriminate between correct binding states and incorrect conformations, for example, the lowest energy structures cannot promised to produce good docked conformations. In summary, GEMDOCK is able to achieve good predictions for the complexes of categories 1 and 2 by increasing the population size (Figs. 8a and b) or lengthening the family competition length. In contrast to the protein–ligand complexes of the third category, a modified scoring function is required to improve solution quality.

However, with uncertainty in the scoring function, the robustness of GEMDOCK was difficult to assess. To address this question, we made use of the high adaptability of GEMDOCK and simply replaced the empirical scoring function with a RMSD scoring function (i.e., one that would produce zero RMSD in heavy atom positions). As shown in Table 6, using the RMSD scoring function [eq. (17)], GEMDOCK could achieve the best RMSD and the average RMSD of docked structures were below 0.09 and 0.14 Å for each test complex, respectively. It is also worthy of note that GEMDOCK converges much faster with the RMSD scoring function (<4 s for a docking run). These results may suggest that the flexible of GEMDOCK should allow us to begin to systematically

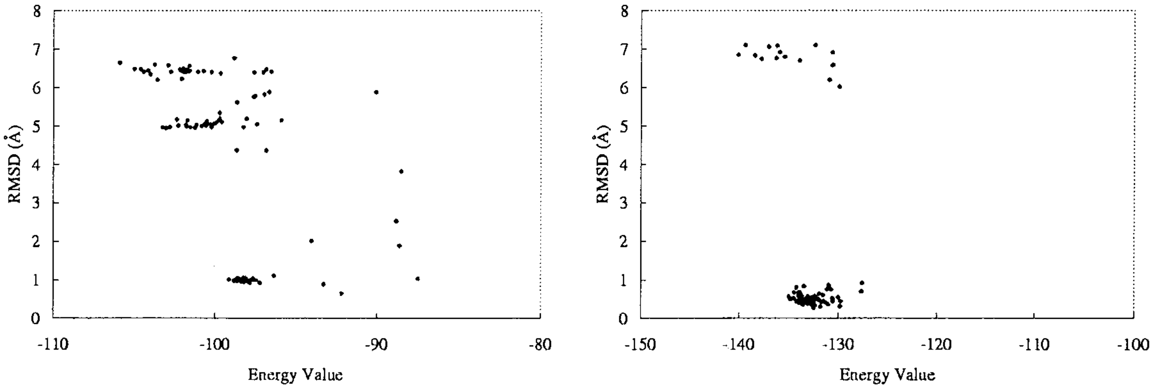


(a) 3dfr

(b) 1etr

(c) 1did

Figure 8. The relationships between the solution quality (the success rate and average RMSD value) and the population size. GEMDOCK is able to improve the solution quality of docking (a) MTX into 3dfr and (b) thrombin into 1etr when the population size increases. In contrast, the solution quality of docking (c) cyclodextrin glycosyltransferase into 1did is unrelated to the population size.



(a) 1cdg

(b) 1nsd

Figure 9. GEMDOCK results for removing the structure water molecules in the complexes (1cdg and 1nsd) with 100 independent runs.

improve the forms and parameters of energy function for molecular recognition.

Evaluation of Search Spaces Used

Table 5 shows the accuracy of GEMDOCK with various search spaces and environments, including ligand size (i.e., number of heavy atoms), ligand flexibility (i.e., the number of rotatable bonds), sizes of the binding areas (i.e., selected binding site and whole protein), hetero atoms (i.e., water molecules and metal ions), and ligand polarity (i.e., numbers of hydrogen binding and electrostatic interactions between ligands and proteins). When GEMDOCK is a hybrid-solution docking method (Fig. 6), it yielded slight better docked conformations than the ones of the flexible docking method (Fig. 7). These results show that the GEMDOCK performance was somewhat influenced by ligand size and ligand flexibility. For the large search cube (i.e., 1etr and 1ets) or the whole protein as the search binding area, GEMDOCK often yielded a low success rate and a large average RMSD value (Table 5). Fortunately, Figure 8 shows that GEMDOCK is able to improve the docked accuracy by enlarging the population size if the scoring function can discriminate between native and nonnative conformations.

When hetero atoms in the binding site were retained, GEMDOCK generally improved the predicated accuracy for complexes 1did, 1nsd, 1etr, and 3dfr. For the complex 1cdg, Figures 9a and 6a show that the success rates are 31 and 99% for removing and retaining water molecules, respectively. For the complex 1nsd, Figures 9b and 6b show that the success rates are 86 and 100% for removing and retaining water molecules, respectively. The reasons can be attributed to the additional steric, hydrogen binding, and electrostatic interactions between ligands and hetero atoms, which are "both (water molecules)" or "donor (metal ions)" (Table 2). As shown in Figure 4, a water molecule is often able to form hydrogen bonds with ligand atoms and become the search space constraint to reduce the possible docked orientations. For example, the ligand DIG forms two hydrogen bonds with the 519th and 520th water molecules in the complex 1did (Fig. 4A); the ligand DAN forms five hydrogen bonds with the 429th, 431th, 434th, 435th, and 437th water molecules in the complex 1nsd (Fig. 4C). This observation was also supported by the work of Westhead,[25] which showed that the removal of the waters could lead to considerable errors.

Comparison with Other Approaches

Table 7 shows the results of comparing GEMDOCK with five different search methods, which were tested on the very similar scoring function [eq. (12)].[25] In general, it is neither straightforward nor completely fair to compare the results of different molecular docking methods because different accuracy measures, energy functions, and test complexes. Most of docking methods, with the exception of the studies of Jones et al.[17] and Kramer et al.,[15] have performed on a rather small set of complexes. Despite this, here we compared GEMDOCK with several molecular docking methods including simulated annealing (SA), evolutionary programming (EP), Tabu search (TS), genetic algorithm (GA), and random search (RS). These methods were studied by Westhead et

Table 7. Comparison GEMDOCK with Some Heuristic Approaches Based on Success Rate.[a]

PDB code	GEMDOCK	SA[b]	EP[b]	TS[b]	GA[b]	RS[b]
1etr	55%	30%	21%	39%	13%	3%
1ets	21%	3%	9%	8%	11%	2%
1hvr	86%	65%	54%	58%	59%	2%
1nsd	98%	40%	64%	88%	57%	6%
3dfr	92%	90%	76%	93%	76%	9%

[a] The percentage of 500 docking runs that find a docked lowest energy structure within 1.5 Å RMSD with respect to the crystal ligand structure.
[b] These results were summarized from ref. 25. SA is simulated annealing, EP is evolutionary programming, TS is Tabu search, GA is genetic algorithm, and RS is random search.

Protein Protein	1dhj	1dra	1drb	1jol	1dyh	1dyi	1dyj	2drc	3drc	4dfr
1dhj	0									
1dra	0.17	0								
1drb	0.15	0.12	0							
1jol	0.54	0.25	0.29	0						
1dyh	0.32	0.25	0.28	0.1	0					
1dyi	0.28	0.21	0.25	0.14	0.16	0				
1dyj	0.19	0.17	0.19	0.22	0.23	0.18	0			
2drc	0.22	0.19	0.22	0.25	0.24	0.2	0.18	0		
3drc	0.2	0.14	0.21	0.26	0.23	0.2	0.18	0.11	0	
4dfr	0.24	0.19	0.26	0.29	0.28	0.24	0.22	0.16	0.13	0

(a) Ten complexes of dihydrofolate reductase

Protein Protein	1tng	1tnh	1tni	1tnj	1tnk	1tnl
1tng	0					
1tnh	0.11	0				
1tni	0.12	0.12	0			
1tnj	0.11	0.11	0.1	0		
1tnk	0.14	0.11	0.12	0	0	
1tnl	0.13	0.13	0.11	0.1	0.1	0

(b) Six complexes of trypsin

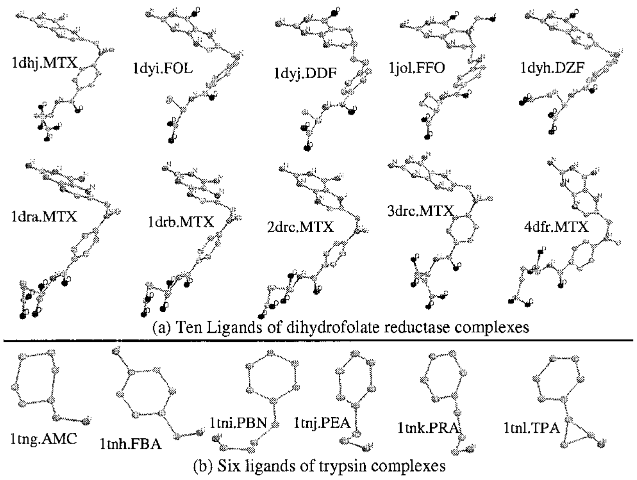
Figure 10. Cross-RMSD matrices of all paired PDB entries for (a) 10 dihydrofolate reductase complexes, and (b) six trypsin complexes.

al.[25] with the similar empirical scoring function and the same test complexes. They calculated the success rate, the percentage of the trials which find a solution within 1.5 Å RMSD, based on 500 independent runs. We followed their criteria to obtain the GEMDOCK results and the results of comparative approaches were directly summarized from the previous study.[25]

As shown in Table 7, our approach was more robust than these comparative approaches on this test set. The random search is the worst and GEMDOCK is the best among these approaches on this test set. GEMDOCK seems good for the complexes 1etr and 1ets and the success rate is approaching to 90% if we enlarged the population size to 1000 (Fig. 8). At the same time, the GEMDOCK approach, as discussed above, can be used to analyze elements of molecular docking approaches, such as search schemes, docking materials, and energy functions. It should help in moving toward error-free prediction of docked conformations and in systematically improving the forms and parameters of a scoring function, which is one of major bottlenecks for molecular recognition.

Crossdocking Results

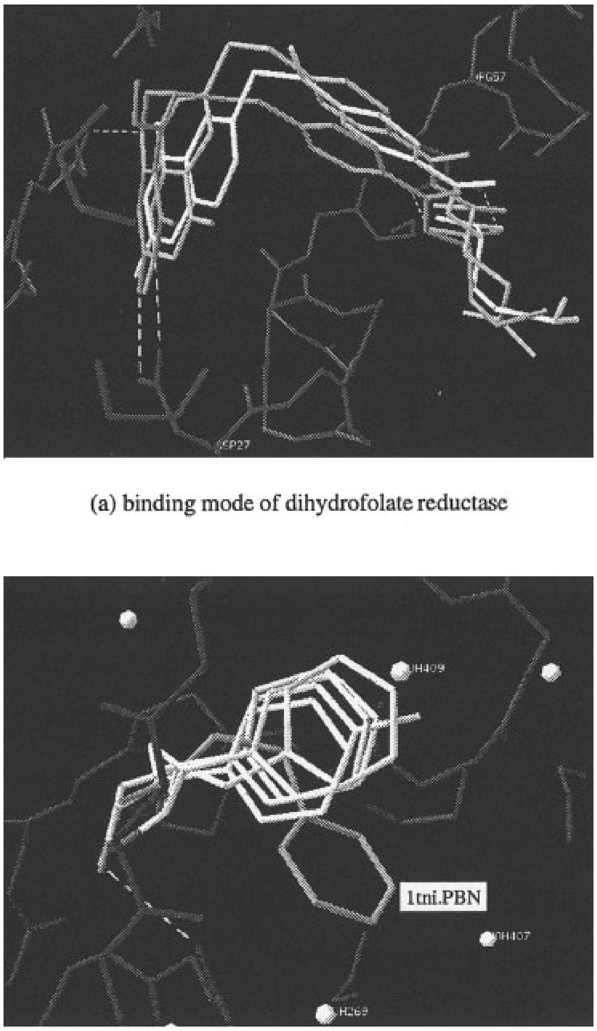
We used two ensembles of protein structures, i.e., 10 complexes of the dihydrofolate reductase and six complexes of the trypsin (serine proteinase) complex,[15] to evaluate GEMDOCK on the unbound docking problem and the problem in which a protein structure is small motion during docking processing. These protein structures differ only on a small variation of side chains and loops on the active site. Figure 10 shows the cross-RMSD matrices (e.g., protein heavy atoms of the binding site) of all paired PDB entries that indicate the protein flexibility in the binding site. The largest RMSD is 0.54 Å and the smallest RMSD is 0.1 Å. Figure 11a and b shows these 10 inhibitors of the dihydrofolate reductase ensemble and six inhibitors of the trypsin ensemble, respectively. The symbol, four lower-case letters with three upper-case letters, was used to denote a ligand. For example the ligand “4dfr.MX,” “4dfr” denotes the PDB code and “MTX” is the ligand name in the Protein Data Bank. Figure 12a and b shows the binding modes of the dihydrofolate reductase and the trypsin complex, respectively. For the dihydrofolate reductase ensemble, the sizes and conformations of all of ligands are similar. Three ligands, 1jol.FFO (5-formyl-6-hydrofolic acid colored dark), 1dyh.DZF (5-deazafolate colored gray), and 4dfr.MTX (methotrexate colored white), are shown in Figure 12a. On the other hand, the binding mode of the trypsin can be divided into two categories, one is the ligand 1tni.PBN (4-phenylbutylamine colored gray) and the other consists of the other five ligands (white).



(a) Ten Ligands of dihydrofolate reductase complexes

(b) Six ligands of trypsin complexes

Figure 11. Ligands bound to (a) 10 dihydrofolate reductase complexes, and (b) six trypsin complexes tested in this work. Coordinates for each complex were obtained from the Protein Data Bank, using the accession codes given here. A four lower-case letter (e.g., 1dhj and 1tng) with a three upper-case letter (e.g., MTX and AMC) denotes a ligand.



(a) binding mode of dihydrofolate reductase

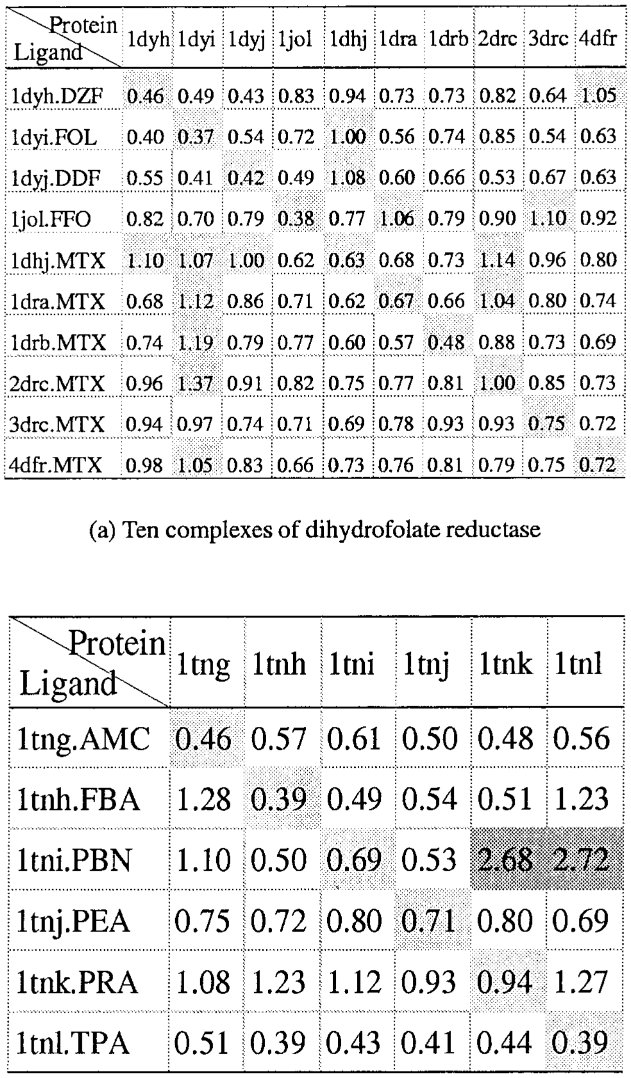
(b) binding mode of trypsin

Figure 12. Binding modes of (a) dihydrofolate reductase and (b) trypsin complexes. Three ligands, 1jol.FFO (dark), 1dyh.DZF (gray) and, 4dfr.MTX (white), of dihydrofolate reductase have a similar binding mode. Six ligands of trypsin have similar conformations (white) except the ligand 1tni.PBN (dark). The dotted lines are hydrogen bonds and white balls are the water molecules.

The dihydrofolate reductase[26] is a small enzyme that plays an essential role, in the building of DNA and other processes. There are over 60 crystal structures with good resolution (≤2.8 Å) in the Protein Data Bank. This reductase with diverse inhibitors, which are similar in size and structure flexible (Fig. 11a), is an important medicinal target, and shows the type of flexibility that can pose significant problems in docking simulation. It has been commonly used to evaluate and compare the performance of various docking methods. The trypsin, a kind of serine proteinase, specifically cleaves the peptide bond on the carboxyterminal side of positively charged residues, namely lysine and arginine. The trypsin binding pocket is only small motion and the structures of several trypsin-inhibitor complexes have been solved in the PDB.

Figure 13 shows the results for the crossdocking experiments in which all ligands of a protein ensemble were docked into each protein of this ensemble. For example, we obtained 100 crossdocked results when each of 10 ligands was docked into each of 10 complexes of the dihydrofolate reductase. For the trypsin ensemble, we obtained 36 crossdocked solutions. When preparing the proteins for crossdocking experiments, the size and location of the ligand binding site was determined by considering the protein atoms that are located less than 12 Å apart from each ligand atom. We removed all water molecules from the binding sites.

Figure 13a shows the crossdocking results of the dihydrofolate reductase ensemble. All of the diagonal results, docking the ligand



Protein / Ligand	1dyh	1dyi	1dyj	1jol	1dhj	1dra	1drb	2drc	3drc	4dfr
1dyh.DZF	0.46	0.49	0.43	0.83	0.94	0.73	0.73	0.82	0.64	1.05
1dyi.FOL	0.40	0.37	0.54	0.72	1.00	0.56	0.74	0.85	0.54	0.63
1dyj.DDF	0.55	0.41	0.42	0.49	1.08	0.60	0.66	0.53	0.67	0.63
1jol.FFO	0.82	0.70	0.79	0.38	0.77	1.06	0.79	0.90	1.10	0.92
1dhj.MTX	1.10	1.07	1.00	0.62	0.63	0.68	0.73	1.14	0.96	0.80
1dra.MTX	0.68	1.12	0.86	0.71	0.62	0.67	0.66	1.04	0.80	0.74
1drb.MTX	0.74	1.19	0.79	0.77	0.60	0.57	0.48	0.88	0.73	0.69
2drc.MTX	0.96	1.37	0.91	0.82	0.75	0.77	0.81	1.00	0.85	0.73
3drc.MTX	0.94	0.97	0.74	0.71	0.69	0.78	0.93	0.93	0.75	0.72
4dfr.MTX	0.98	1.05	0.83	0.66	0.73	0.76	0.81	0.79	0.75	0.72

(a) Ten complexes of dihydrofolate reductase

Protein / Ligand	1tng	1tnh	1tni	1tnj	1tnk	1tnl
1tng.AMC	0.46	0.57	0.61	0.50	0.48	0.56
1tnh.FBA	1.28	0.39	0.49	0.54	0.51	1.23
1tni.PBN	1.10	0.50	0.69	0.53	2.68	2.72
1tnj.PEA	0.75	0.72	0.80	0.71	0.80	0.69
1tnk.PRA	1.08	1.23	1.12	0.93	0.94	1.27
1tnl.TPA	0.51	0.39	0.43	0.41	0.44	0.39

(b) Six complexes of trypsin

Figure 13. Crossdocking results of all-pair experiments for (a) 10 dihydrofolate reductase complexes and (b) six trypsin complexes. The color-coded table shows the gray-scaling of RMSD values for each ligand (row) docked into each protein (column) of a protein ensemble.

back into its respective complex, are less than 1.0 Å, and most of off-diagonal results, crossdocking examples, GEMDOCK also yielded good results that the RMSD values are less than 1.5 Å. The largest RMSD value is 1.37 Å when the ligand 2drc.MTX was docked into the complex 1dyi, and the average is 0.77 Å. As shown in Figure 13a and Figures 3 and 4 in ref. 29, GEMDOCK is significantly better than FlexX[15] and FlexE[29] on the dihydrofolate reductase ensemble. The largest RMSD values of docked conformations predicted by FlexE and FlexX were 5.37 and 7.55 Å, respectively. FlexX and FlexE yielded 44 and 83.3% docked conformations with RMSD less than 2.0 Å, respectively. For six ligands, including 1dhj.MTX 1dra.MTX 1drb.MTX 2drc.MTX 3drc.MTX 4dfr.MTX, both FlexE and FlexX worked well for predicting the docked conformations. On the other hand, FlexX was unable to obtain good enough solutions for other ligands, such as 1dyh.DZF 1dyi.FOL 1dyj.DDF 1jol.FFO. FlexE also yielded little poor predictions for these four ligands, especially, FlexE obtained wrong docked conformations for the ligand 1jol.FFO. By contrast, GEMDOCK achieved 100% correct docked conformations, i.e., the RMSD values less than 1.5 Å, for all 100 crossdocking experiments.

For all 36 docked conformations of the trypsin ensemble, GEMDOCK also obtained good and stable results except when the ligand 1tni.PBN was docked into the complexes 1tnk and 1tnl. The largest RMSD value is 2.72 Å, and the average is 0.82 Å (Fig. 13b). Our approach was more stable than FlexX[15] on this trypsin ensemble. As shown in Figure 12b, the binding mode of the ligand 1tni.PBN was significantly distinct from other ligands. Although this ligand was docked into six trypsin complexes, GEMDOCK obtained lower successful rates (≤20%) than the rates (≥60%) of other crossdocking experiments in the trypsin ensemble. If water molecules were retained in the binding site, GEMDOCK was able to find corrected conformations, which the RMSD value is less than 0.6 Å and the successful percentage is more than 60%, for docking the ligand 1tni.PBN into other systems. These results show that GEMDOCK may be able to address the problem in which the protein structure makes a slight variation during docking processing.

Conclusions

We have developed a robust evolutionary approach with an empirical fitness function for the flexible protein–ligand docking. GEMDOCK seamlessly blends local search and global search to work cooperatively by the integration of a number of genetic operators, each having unique search mechanism. We have validated GEMDOCK on seven test cases and on two crossdocking experimental sets by using various search spaces and scoring functions. Experimental results have demonstrated the robustness and adaptability of GEMDOCK for exploring the conformational space of a molecular docking problem and efficiently finding the solution under the constraint of the fitness function used. GEMDOCK could indeed yield 100% docking accuracy if the RMSD scoring function was used.

Despite the GEMDOCK's apparent success, there are a number of problems with the methodology in general. First, our approach is somewhat time-consuming; second, the binding site of the protein is essentially rigid; and finally, some protein–ligand interactions were not considered in our fitness function. In the future, we will investigate three directions to reduce above disadvantages: (1) developing a rapid energy evaluation with grid-based potentials for drug screening; (2) considering the side-chain flexibility in the protein active site; (3) incorporating important function–group interactions between ligands and proteins;[17] the solvent effect,[12] and the hydrogen bond strength for calculating a hydrogen bonding energy[6] into our empirical scoring function.

References

1. Kuntz, I. D. Science 1992, 257, 1078.
2. Wlodawer, A.; Vondrasek, J. Annu Rev Biophys Biomol Struc 1998, 27, 249.
3. Halperin, I.; Ma, B.; Wolfson, H.; Nussinov, R. Proteins 2002, 47, 409.
4. Miller, D. W.; Dill, K. A. Protein Sci 1997, 6, 2166.
5. Gehlhaar, D. K.; Verkhivker, G. M.; Rejto, P.; Sherman, C. J.; Fogel, D. B.; Fogel, L. J.; Freer, S. T. Chem Biol 1995, 2, 317.
6. Verkhivker, G. M.; Bouzida, D.; Gehlhaar, D. K.; Rejto, P. A.; Arthurs, S.; Colson, A. B.; Freer, S. T.; Larson, V.; Luty, B. A.; Marrone, T.; Rose, P. W. J Comput Aided Mol Design 2000, 14, 531.
7. Gohlke, H.; Hendlich, M.; Klebe, G. J Mol Biol 2000, 295, 337.
8. Verdonk, M. L.; Cole, J. C.; Watson, P.; Gillet, V.; Willett, P. J Mol Biol 2001, 307, 841.
9. Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. J Am Chem Soc 1984, 106, 765.
10. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. J Comput Chem 1983, 4, 187.
11. Taylor, J. S.; Burnett, R. M. Proteins Struct Funct Gene 2000, 41, 173.
12. Shoichet, B. K.; Leach, A. R.; Kuntz, I. D. Proteins Struct Funct Gene 1999, 34, 4.
13. Paul, N.; Rognan, D. Proteins Struct Funct Gene 2002, 47, 521.
14. Kuntz, I. D.; Blaney, J. M.; Oatley, S. J.; Langridge, R.; Ferrin, T. E. J Mol Biol 1982, 161, 269.
15. Kramer, B.; Rarey, M.; Lengauer, T. Proteins Struct Funct Gene 1999, 37, 228.
16. Sherman, C. J.; Ogden, R. C.; Freer, S. T. J Med Chem 1995, 38, 466.
17. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. J Mol Biol 1997, 267, 727.
18. Wang, J.; Kollman, P. A.; Kuntz, I. D. Proteins Struct Funct Gene 1999, 36, 1.
19. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. J Comput Chem 1998, 19, 1639.
20. Österberg, F.; Morris, G. M.; Sanner, M. F.; Olson, A. J.; Goodsell, D. S. Proteins Struct Funct Gene 2002, 46, 34.
21. Goldberg, D. E. Genetic Algorithms in Search, Optimization and Machine Learning; Addison-Wesley Publishing Company, Inc.: Reading, MA, 1989.
22. Bäck, T. Evolutionary Algorithms in Theory and Practice; Oxford University Press: New York, 1996.
23. Fogel, D. B. Evolutionary Computation: Toward a New Philosophy of Machine Intelligent; IEEE Press: New York, 1995.
24. Yang, J.-M.; Kao, C.-Y. J Comput Chem 2000, 21, 988.
25. Westhead, D. R.; Clark, D. E.; Murray, C. W. J Comput Aided Mol Design 1997, 11, 209.
26. Feeney, J. Angew Chem Int Ed 2000, 39, 290.
27. Storn, R.; Price, K. V. J Global Optimizat 1997, 11, 341.
28. Knegtel, R. M. A.; Antoon, J.; Rullmann, C.; Boelens, R.; Kaptein, R. J Mol Biol 1994, 235, 318.
29. Claussen, H.; Buning, C.; Rarey, M.; Lengauer, T. J Mol Biol 2001, 308, 377.