Assessing Energy Functions for Flexible Docking

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ABSTRACT: A good docking algorithm requires an energy function that is selective, in that it clearly differentiates correctly docked structures from misdocked ones, and that is efficient, meaning that a correctly docked structure can be identified quickly. We assess the selectivity and efficiency of a broad spectrum of energy functions, derived from systematic modifications of the CHARMM param19/toph19 energy function. In particular, we examine the effects of the dielectric constant, the solvation model, the scaling of surface charges, reduction of van der Waals repulsion, and nonbonded cutoffs. Based on an assessment of the energy functions for the docking of five different ligand–receptor complexes, we find that selective energy functions include a variety of distance-dependent dielectric models together with truncation of the nonbonded interactions at 8 Å. We evaluate the docking efficiency, the mean number of docked structures per unit of time, of the more selective energy functions, using a simulated annealing molecular dynamics protocol. The largest improvements in efficiency come from a reduction of van der Waals repulsion and a reduction of surface charges. We note that the most selective potential is quite inefficient, although a hierarchical approach can be employed to take

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advantage of both selective and efficient energy functions. J Comput Chem 19: 1612–1622, 1998

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Introduction

In structure-based drug design one would like to be able to predict the orientation of a drug candidate in the active site as well as estimate its binding affinity. The former is a problem of molecular recognition—a field of great importance in molecular biology. Molecular docking is a computational approach to molecular recognition and a key technique in computer-aided rational drug design.[1,2] The aim of docking is to predict the structure of a ligand–receptor complex given the structures of the free ligand and free receptor.[3] The first ligand docking program, DOCK,[4] employed shape information and a hydrogen bonding term. Since then, docking techniques have been developed with a variety of energy functions and different search strategies.[3,5,6] The number of docking strategies continues to rise with increasing interest in structure-based drug design. Simulated annealing Monte Carlo,[7] molecular dynamics (MD) and, recently, genetic algorithms[5,9–11] have been employed. In the studies we report here, we introduce and apply metrics for systematically assessing and developing docking algorithms. In this article, we systematically study the energy function used in docking and, in the accompanying article, we compare the search strategies for molecular docking. Preliminary results of these studies have been summarized elsewhere.[12] The macromolecular modeling software, CHARMM, provides a convenient means of introducing modifications to the energy functions, and we have implemented several docking protocols within the CHARMM environment as a step toward the development of an integrated toolbox for structure-based drug design.

A successful docking methodology requires an energy function capable of selecting the correct ligand–receptor structure; that is, one consistent with the crystallographic structure of the complex. A good energy function should also be efficient, meaning that the landscape on which the ligand moves should be relatively smooth with an absence of large energy barriers separating different structures, to allow the desired minimum to be located reasonably rapidly. Both components, selectivity and efficiency, are of importance and must coexist in a successful docking algorithm. For a given energy function, the efficiency of the docking process will also depend on the search algorithm. In this study, we concentrate on the energy function and aspects of selectivity and efficiency. We describe the selection of the search algorithm in the accompanying work.[13] The influence of the energy function on the docking efficiency, as measured by the computer time required to find the correct structure of the complex, is investigated by means of an MD simulated annealing protocol. The energy functions evaluated in this work are derived from the CHARMM param19 parameter set,[14] with some modifications that are introduced to achieve good selectivity and high docking efficiency. Here the docking efficiency is discussed only in the context of the MD methodology.

In the majority of docking approaches, the location of the active site is assumed to be known and the receptor is kept rigid.[3,7–11,15,16] Many test studies on docking published in the last 15 years have used the rigid receptor structure taken from the exact complex that was to be predicted, even though, in many cases, the receptor active site differed substantially from its free form (e.g., HIV protease[17] and streptavidin[18] to name just two). The idea behind this approach, which we also adopt in our study, is to test the strengths and weaknesses of various docking algorithms in the ''best case scenario,'' which assumes that the free and loaded forms of the receptors are identical. In only a few cases[19] has the structure of the native unbound form of the receptors been employed. In practical applications, the receptor structure is taken from either an experimentally determined structure of the free form or from a structure of a complex with another ligand.

The organization of this study is as follows. First, we introduce the five ligand–receptor complexes that form the basis of the study. General features of the energy functions, together with the parameterization of the ligands, are presented. In the next section, we introduce our modifications to

the CHARMM force field and the metrics used to assess the selectivity and efficiency of the energy functions. The modifications that result in selective and efficient energy functions are presented. We conclude with the implications of our findings for the development of fast and effective docking strategies.

Method

The selectivity and computational efficiency of energy functions have been evaluated with respect to the flexible docking of five diverse ligand–receptor complexes. The complexes include a small, rigid ligand in an open active site, benzamidine/trypsin[20]; flexible ligands in open active sites, phosphocholine/FAB McPC-603[21] and sialic acid/hemagglutinin; and flexible ligands in relatively inaccessible active sites, glycerol 3-phosphate/triose phosphate isomerase,[23] and biotin/streptavidin.[18] The ligands are shown in Figure 1. No crystallographic water molecules were retained, which may have some consequences for the complexes in which water molecules mediate the ligand–receptor interaction. We believe that the five complexes are sufficiently diverse for our conclusions about the selectivity and efficiency of energy functions to be of general applicability. For the complexes phosphocholine/FAB, benzamidine/trypsin, glycerol 3-phosphate/tim, and sialic acid/hemagglutinin, the active site conformation is similar, within 0.4-Å root-mean-square deviation (RMSD) on heavy atoms, to the unbound form of the receptor. For biotin/streptavidin, the changes in the active site are more substantial. Because this article focuses on the assessment of energy functions for docking, and not on the prediction of binding modes, we used the receptor structures present in the complexes.

The receptors were described using the CHARMM param19/toph19 parameter set.[14] For the ligands, charges were generated by the template method in QUANTA,[24] with smoothing of charges over all atoms. The charges on the carboxylic group of sialic acid were taken from aspartic acid. The bond, angle, and improper dihedral angle parameters were also generated by QUANTA, as were the nonbonded van der Waals (vdW) parameters. The ligand charges and parameters for the angles, bonds, dihedrals, and nonbonded interactions are accessible through anonymous ftp.[25] For the nonbonded interactions, switching functions[14] for the vdW and electrostatic interactions were used.

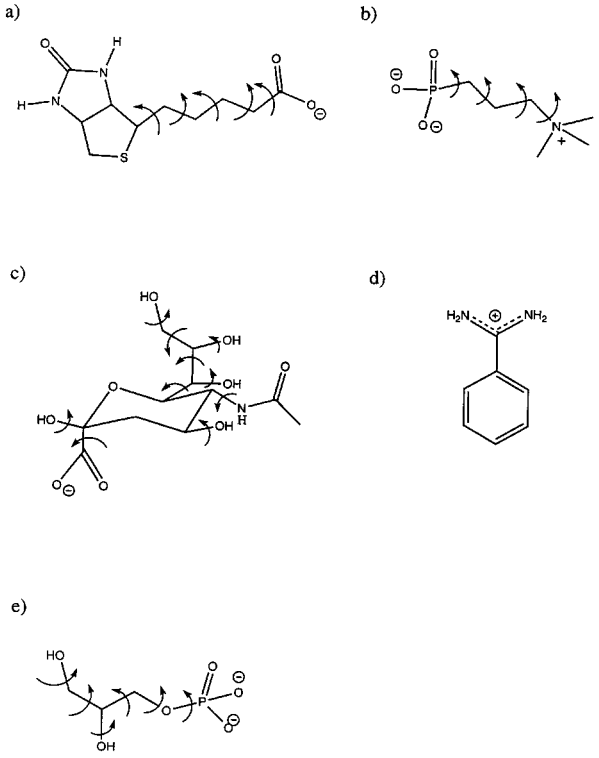

FIGURE 1. The five ligands used this study. Arrows show rotatable bonds: (a) biotin / streptavidin; (b) phosphocholine / FAB McPC-603; (c) sialic acid / hemagglutinin (for hemagglutinin only residues 61 –269 were used, because they account for the neighborhood of the active site); (d) benzamidine / trypsin; and (e) glycerol 3-phosphate / triose phosphate isomerase.

To prevent ligands escaping from the neighborhood of active sites, a harmonic restraining potential was applied. It acted only when the distance, d, between the ligand atom closest to the center of mass of the ligand and the receptor atom closest to this atom in the crystal structure was further than 11 Å. Thus, the center of the search space is about 3.5 Å (the typical distance between the ligand atom and closest receptor atom) from the center of the active site. The restraining potential, R, has the following form:

$$R = \begin{cases} 0 & ; d < 11 \text{ Å} \\ k(d-11)^2 & ; d > 11 \text{ Å} \end{cases} \quad (1)$$

where k is the force constant set to 50 kcal/mol Å^2. The restraining potential is implemented through the NOE module in CHARMM. The restraining potential effectively confines ligands to

the neighborhood of the active site. The volume accessible to the center of mass of a ligand is roughly 5500 Å^3. The search space used in this realization is much larger than in many other docking protocols that employ a search space for the center of mass on the order of 8 Å^3.[3, 15]

In the following subsections, we present the various modifications of the CHARMM force field[14] that will be evaluated. The energy functions that we consider in this article have the form, E(solv, ε, SCR, vdWR, cutoffnb), where solv refers to solvation model, ε refers to four different dielectric constants, SCR refers to the reduction (or not) of surface charges, vdWR refers to soft versus hard core nonbonded interactions, and cutoffnb (r_{off}) refers to three different values of the nonbonded interaction truncation. Different combinations of these modifications give rise to a total of 144 distinct energy functions. We assess the selectivity of each one. Subsequently, the most promising selective energy functions are tested for their efficiency.

NONBONDED CUTOFFS AND DIELECTRIC CONSTANT

We assessed energy functions with values of 8 Å, 9 Å, and 10 Å for the truncation of the nonbonded interactions (r_{off}). The values for the nonbonded list were 9 Å, 10 Å, and 11 Å, respectively. A short nonbonded list decreases the number of nonbonded interactions to compute and hence the computational cost of the docking. In addition, short values for the nonbonded interaction truncation should favor tightly packed structures and highly localized electrostatic interactions in the active site over more loosely packed structures with screened electrostatic interactions.

Both distance-dependent and constant dielectrics were tested. The dielectric constant was also treated as a parameter and values between 1 and 4 were explored. Low dielectric constants should favor strong electrostatic interactions, but shifting the balance of electrostatic versus vdW interactions may effectively decrease the importance of tight packing in active sites. In addition, a low dielectric constant may also generate kinetic traps and not discriminate well between active site cavities and binding sites on the protein surfaces.

SURFACE CHARGE REDUCTION

One of the problems that can arise in the docking of a ligand to a rigid protein is preferential binding of the ligand at surface sites instead of active site cavities. Another issue that needs to be accounted for is the flexibility of the surface side chains and resulting surface charge delocalization due to this flexibility. As a means of avoiding these possible problems, we test a model in which the surface side chain charges are reduced:

$$C_{new} = \left(1 - \frac{\sigma_{protein}}{\sigma_{tripeptide}}\right) C \tag{2}$$

where C_{new} is the reduced charge of the side chain atom, $\sigma_{protein}$ is the surface exposure of side chain X, $\sigma_{tripeptide}$ is the surface exposure of this side chain in a Gly—X—Gly trans tripeptide and C is the original charge of the side chain atom. This modification reduces the surface side chain charges, but does not modify the charges of buried side chains, thereby providing a ''smeared-out'' representation of the surface charges accounting for both aspects of side chain mobility and solvent screening. The largest observed reduction of a side chain charge was 50%. This modification of the energy function is implemented through the SCALAR module in CHARMM.

REDUCTION vdW REPULSION

The next modification involves the introduction of a vdW potential with a soft core repulsion. The following function was employed in the docking simulations:

$$E_{vdW} = \begin{cases} \displaystyle\sum_{i \neq j} \left(\frac{12\,A_{ij}}{(f\sigma_{ij})^{13}} - \frac{6\,B_{ij}}{(f\sigma_{ij})^{7}} \right) (f\sigma_{ij} - r_{ij}) \\ \quad + E_{vdW}(f\sigma_{ij}) \qquad ; r_{ij} < f\sigma_{ij} \\ \displaystyle\sum_{i \neq j} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right) sw\left(r_{ij}^2, r_{on}^2, r_{off}^2\right) \\ \qquad\qquad ; r_{ij} \geq f\sigma_{ij} \end{cases} \tag{3}$$

where E_{vdW} is the vdW energy; A_{ij} and B_{ij} are nonbonded parameters, and sw is a switching function[14]; σ_{ij} is the sum of the vdW radii for atoms i and j; r_{ij} is the distance between atoms i and j, and r_{on} is the inner switching distance (6 Å in our case). The parameter f denotes the fraction of σ_{ij} at which the soft core potential starts (f = 0.885 was used in our applications). The essence of this modification is that, for distances shorter than

$f\sigma_{ij}$, the force acting on atoms i and j is the same as at $f\sigma_{ij}$, and the energy is a linearly decreasing function of r_{ij}. This modification makes the vdW repulsions very small (the maximum observed value is on the order of hundreds of kilocalories per mole) and the resulting conformational transition barriers much smaller than with the unmodified vdW potential. An analogous modification is made to the electrostatic potential and forces. However, its influence on the electrostatics should be negligible relative to the effect on the vdW interactions. Thus, most of our discussion of the soft core nonbonded terms will concern the modification of the vdW potential and forces. The soft core modification is similar in principle to approaches in other docking programs,[26,27] and allows a ligand to penetrate the interior of the protein with a relatively small energetic penalty. In other words, the local energy barriers are very small. As a result, MD sampling is no longer disadvantaged with respect to Monte Carlo sampling and can be used as an effective search strategy. The only negative effect of this modification is that the tight packing of ligands in the active site is less favored compared with a regular vdW potential. However, we note the soft core potential can be turned on or off during different stages of the docking protocol. This modification is similar in principle to the soft core potential used in the early stages of NMR refinement protocols[28] and in some versions of simulated annealing. The major influence of a soft core potential is expected to be on the efficiency of the docking process, without degrading the selectivity of the energy function.[30] This modification of the energy function will be available in a future CHARMM release.

CONTINUUM SOLVATION CONTRIBUTION

The final modification of the force field involves the representation of the solvation contribution. This contribution is based on an approximation to the finite difference Poisson–Boltzmann (PB) continuum solvent model.[31] Because we are using a fixed receptor model, we propose that the receptor is the only low dielectric medium that needs to be considered and the additional contribution of a ligand to the polarization and the potential is negligible. To examine this, we generated a number of ligand conformations of glycerol 3-phosphate around the active site of triose phosphate isomerase. For each position of the ligand, the ''true'' solvation energy, E_{solv}, is computed in the following way:

$$E_{solv} = \sum_{j=1}^{N} (\varphi_{j,80,int} - \varphi_{j,int,int}) q_j \qquad (4)$$

where N is the number of ligand atoms; q_j is the atomic charge; $\varphi_{j,80,int}$ refers to the electrostatic potential generated by the complex with an interior dielectric, int = 1, 2, 3, or 4 (depending on the value of dielectric constant chosen) in an exterior dielectric of 80; $\varphi_{j,int,int}$ refers to the potential generated by the complex in the reference state where exterior dielectric is the same as the interior dielectric. A similar calculation is performed using the approximate method, where the electrostatic potentials are generated from the receptor charge distribution only; that is, the receptor is the only low dielectric medium and the ligand is absent. A grid size of 0.7 Å was used in the calculations. The two solvation energies are compared in Figure 2 for ten different positions of glycerol 3-phosphate in the triose phosphate isomerase binding sites. These ten positions have a mean RMSD of 9 Å from one another. The correlation coefficient is $r^2 = 0.92$. It varies from 0.92 to 0.97 for the five

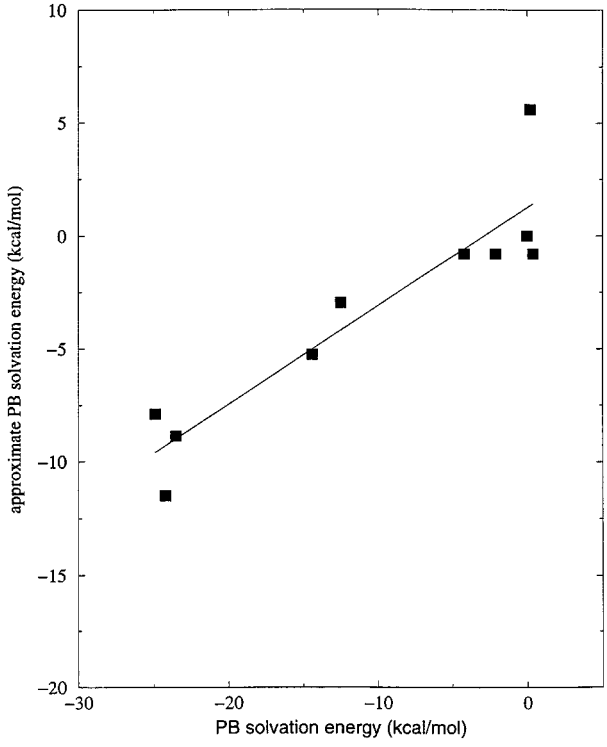

FIGURE 2. Correlation between finite difference PB solvation energies of ligands and approximate solvation energies.

complexes tested. The high correlation allows us to use a static solvation potential around the protein and a fast computation of the approximate electrostatic contribution to the solvation energies for ligands in the different positions around the protein. The finite difference PB equation[31] has to be solved only twice to provide the potential on the grid. The forces are computed as the electrostatic potential gradients on the grid using a central difference derivative approximation. We propose this simplified treatment of the system's electrostatics as an approximation of the solvation effects.

ASSESSMENT OF SELECTIVITY

The selectivity of a given energy function or its ability to discriminate between docked and misdocked structures was assessed using a database of docking trials. This database was generated using the 12 energy functions (out of 144), which have a distance-dependent dielectric and $\varepsilon = 3$. For each ligand–receptor complex, 240 positions of the ligand around the active site were generated from 20 trial simulations using each of the 12 energy functions. The 20 initial conformations of the ligands for each complex were generated randomly and had a mean RMSD from the crystallographic ligand position of 9 Å. An additional 50 simulations, initiated from the crystallographic ligand position, were carried out to increase the sampling of docked structures. The average ratio of docked:misdocked structures was roughly 1:1. This database of 290 structures for each complex was used to evaluate the energy distributions of each energy function.

We define correctly docked conformations as those less than 2 Å RMSD (on all heavy atoms) from the crystallographic structure of the ligand in the ligand–receptor complex. Structures with a RMSD of larger than 4 Å are considered incorrectly docked (or misdocked). Structures with RMSD between 2 and 3 Å are considered partially docked, whereas those with RMSD between 3 and 4 Å are partially misdocked. Both partially docked and partially misdocked structures are disregarded in our assessment of selectivity, because they are, in most cases, in the binding site, but often with an incorrect orientation. This left, on average, about 220 structures for each complex.

The measure of selectivity we use is similar to those used in the design of parameters for protein folding algorithms,[32] sequence design studies,[33,34] and inverse folding approaches.[35–37] In these problems, energies are converted to so called Z-scores to take into account the nature of energy distributions. The Z-score is defined as:

$$Z(E) = \frac{(E - \bar{E})}{\sigma} \tag{5}$$

where E is the energy of the ligand–receptor complex, $\bar{E}$ is the mean energy and σ is the standard deviation of the energy distribution. The energy computed is the sum of the internal energy of the ligand and its interaction energy with its receptor. The selectivity, SG, of an energy function is defined as:

$$SG = \begin{cases} \dfrac{1}{N}\displaystyle\sum_{i=1}^{N}(Z^i_{D,m} - Z^i_{M,m})f_i & ; \displaystyle\prod_{i=1}^{N} f_i \neq 0 \\ 0 & ; \displaystyle\prod_{i=1}^{N} f_i = 0 \end{cases} \tag{6}$$

where i refers to the different complexes; and $Z^i_{D,m}$ and $Z^i_{M,m}$ are the minimum values of the Z-score for the docked and misdocked conformations (the Z-scores of the lowest energy docked and misdocked structures), respectively; f_i is the fraction of the docked structures with Z-scores lower than those of the misdocked structures. N is the number of complexes (in this work $N = 5$). The selectivity of an energy function is taken to be zero if, for any complex, the lowest energy misdocked structure is lower in energy than the lowest energy docked structure. The lower the value of SG, the more selective is the energy function. The metric SG defines a selective energy function as one that maximizes the energy gap between the energy distributions of the docked and misdocked structures, while minimizing the overlap of the distributions.[39]

ASSESSMENT OF EFFICIENCY

To estimate the efficiency of each energy function, we carried out 20 docking simulations for each of the five complexes. The search strategy used a version of the multiple-copy simultaneous search method,[40] which employed an MD-simulated annealing search protocol. The initial conditions were the same for each energy function, as were the cooling schedules. The annealing schedule for the docking simulations depends on the nature of the vdW potential. For energy functions

with a soft core vdW potential, the annealing was accompanied by a gradual hardening of the vdW potential, as described previously. The annealing protocol for energy functions with a hard core vdW was identical, but without hardening. First, 75 picoseconds (ps) of dynamics, with a timestep of 1.5 femtoseconds (fs), was performed, while the system was cooled from T = 700 K to T = 300 K, with a cooling frequency of 150 steps and a cooling decrement of 1 K. The short-range nonbonded interactions were modified, as described in eq. (3), with $f = 0.885$ and the nonbonded list was updated every 0.3 ps. In the second stage, the velocities were generated from the Boltzmann distribution and 75 ps of annealing from 400 K to 300 K was performed with a cooling frequency of 100 steps and a temperature decrement of 5 K. At this stage, the short-range vdW and electrostatic interactions were modified using $f = 0.6$. This was followed by 10 ps of cooling by 1 K every 150 steps, from 400 K to 300 K using a reduction of the nonbonded interactions with $f = 0.4$. The initial temperature of the second stage was higher that the final temperatures for the first stage to compensate for the hardening of the vdW potential. The final stage consisted of 6 ps of quenching from 300 K to 50 K with a temperature decrement of 5 K every 100 steps and unmodified short-range nonbonded interactions.

The efficiency, the number of correctly docked structures per unit of time (min), of an energy function is defined as:

$$SE = \begin{cases} \dfrac{1}{T}\displaystyle\sum_{i=1}^{N}\left(f_{i,<2} + 0.5(f_{i,<3} - f_{i,<2})\right) \\ \qquad ; \displaystyle\prod_{i}^{N} f_{i,<3} \neq 0 \\ 0 \qquad ; \displaystyle\prod_{i}^{N} f_{i,<3} = 0 \end{cases} \tag{7}$$

where $f_{i,<a}$, indicates the fraction of structures with RMSD less than a Å from the crystal structure and energy lower than that of the lowest energy misdocked structure. T is the average time required for docking a ligand to its receptor. All timings and fractions of docked structures are given for the same annealing schedules with 108,000 energy evaluations. The efficiency is zero, if $f_{i,<3}$ is zero for any complex. For the efficiency measure, we include 50% of the partially docked structures with energies better than the lowest misdocked in the pool of docked structures.

Results

In the series of calculations presented in what follows, we assess the performance of the energy functions in docking experiments. First, we consider their selectivity. Then, from a pool of selective energy functions, we identify the most efficient one.

SELECTIVITY

Table I ranks the 22 energy functions (out of the 144) that have nonzero selectivity. A variety of energy functions are selective. Most of them have short distances for the truncation of nonbonded interactions, a distance-dependent dielectric, and unmodified vdW and electrostatic interactions. The advantage of the standard nonbonded interactions over the soft core vdW is probably due to better packing of the ligands in their respective active sites. Looser packing in false binding sites may compete more favorably in a soft core vdW potential. Also, a short distance for nonbonded truncation is advantageous for specific interactions in the active sites over nonspecific interactions.

Three of the four most selective energy functions have reduced surface charges. This may reflect the fact that most nonspecific binding sites are located on the receptor surface, or in small cavities, and thus reducing the surface electrostatic interactions favors the more buried active site structures. In addition, a distance-dependent dielectric is almost exclusively present in the selective potentials. A constant dielectric, low dielectric constants, and the approximate continuum solvation model are disadvantageous for selectivity. The poor performance of the energy functions employing a constant dielectric may be explained by the fact that a constant dielectric selects predominantly on the basis of electrostatics and, as in the case for low values of dielectric constants, probably disrupts the proper balance between electrostatics and packing (vdW interactions) necessary for tight and specific interactions. On the other hand, incorporation of the continuum solvation flattens the energy surface and reduces the gap between the energy distributions of the docked and misdocked structures. The general conclusion to be drawn from Table I is that, for the most selective energy function, one should use a distance-dependent dielectric, with $\varepsilon = 3-4$ (3 being

TABLE I.
Ranking of Energy Functions Based on Selectivity.

Rank	Selectivity (SG)	ΔE[a]	f_G[b]	Solvation model[c]	Reduced surface charges (RSC)?	vdW soft core?	NB cutoff[d]
1	−0.78	−10.2	0.77	rdie, 3	YES	NO	8
2	−0.77	−9.6	0.76	rdie, 4	YES	NO	8
3	−0.72	−9.4	0.74	rdie, 4	NO	NO	8
4	−0.70	−11.4	0.76	rdie, 2	YES	NO	8
5	−0.67	−9.8	0.72	rdie, 3	NO	NO	8
6	−0.45	−10.0	0.54	rdie, 2	NO	NO	8
7	−0.36	−5.9	0.46	rdie, 3	NO	YES	8
8	−0.35	−5.4	0.45	rdie, 4	NO	YES	8
9	−0.35	−5.9	0.45	rdie, 3	YES	YES	8
10	−0.33	−5.1	0.44	rdie, 4	YES	YES	8
11	−0.31	−6.4	0.44	rdie, 2	YES	YES	8
12	−0.26	−5.9	0.40	rdie, 2	NO	YES	8
13	−0.23	−4.7	0.43	rdie, 2	NO	NO	10
14	−0.22	−3.5	0.39	rdie, 4	YES	YES	10
15	−0.22	−4.5	0.44	rdie, 2	YES	NO	10
16	−0.21	−3.6	0.37	rdie, 4	NO	NO	9
17	−0.21	−3.5	0.37	rdie, 4	YES	NO	9
18	−0.17	−3.4	0.32	solv, 4	YES	NO	10
19	−0.16	−4.0	0.25	solv, 4	YES	YES	8
20	−0.13	−4.8	0.30	rdie, 1	YES	NO	10
21	−0.09	−5.6	0.20	rdie, 1	YES	YES	8
22	−0.04	−3.5	0.23	rdie, 1	NO	NO	10

[a] Energy gap (kcal / mol) between the lowest energy docked structure and the lowest energy misdocked structure.
[b] Fraction of docked structures with energies lower than that of the lowest energy misdocked structure.
[c] Dielectric parameters. Type of solvation model (rdie, distance-dependent dielectric constant; solv, continuum solvation contribution with constant dielectric) followed by the value of the dielectric constant.
[d] Nonbonded interaction truncation (Å).

the most universal for the short nonbonded cutoffs), short (8-Å) nonbonded interaction truncation, regular vdW interactions, and reduce the charges of surface side chains. Figure 3a shows the energy histograms for the most selective energy function. The fraction of docked structures whose energy is lower than that of the lowest energy misdocked conformation is 0.77. For the most selective energy function, both the separation of the energy distributions and the energy gap between the tails of distributions are satisfactory.

EFFICIENCY

To test the efficiency of docking we selected nine energy functions from Table I: ranks 1–2, 7–11, 14, 19, and one energy function not in Table I (with $SG = 0$) that used the continuum solvation contribution. The choice of the first eight was based on ranking and low nonbonded cutoffs. The energy function of rank 14 was chosen as a representative of those with longer nonbonded cutoffs, whereas the energy function of rank 19 was selected as a selective potential with a continuum solvation contribution. From the best 11 energy functions with short nonbonded cutoffs, we do not examine ranks 3–6, as they have the same type of vdW interactions as the first two. As can be seen from the data presented in Table II, these are not likely to affect our final conclusions.

Table II shows the ranking of the ten energy functions based on their efficiency. To get an acceptable efficiency for all five complexes, a soft core vdW potential is clearly necessary. This is due to the fact that for two receptors, streptavidin and triose phosphate isomerase (although less so), the active site is in a cavity and, consequently, rather inaccessible to the ligands. For these two complexes, the efficiency is much lower, in fact zero for streptavidin, if the regular vdW potential is used. Soft core vdW interactions are needed for and result in efficient docking in these cases.

For the other complexes, where the active site is easily accessible, the efficiency does not strongly

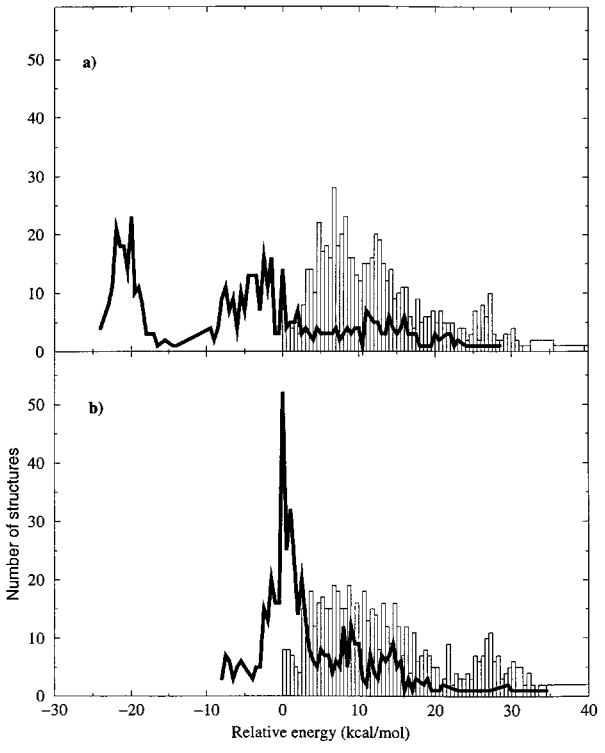

FIGURE 3. Energy distributions for the docked and misdocked conformations averaged over five complexes. Histograms represent the misdocked structures; solid black lines are the correctly docked structures. Energies are relative to the minimum energy of the misdocked structure. (a) Energy distribution for the most selective energy function (Table I, rank 1). (b) Energy distribution for the most efficient energy function (Table II, rank 1).

depend on the form of vdW potential. This is shown in Table III, where we compare the efficiency of the second most selective energy function (which has a standard vdW potential) and the corresponding energy function with a soft core vdW potential. The energy gap between the docked and misdocked structures is, in two cases, substantially reduced by the presence of the soft core vdW potential. We do not see a correlation between efficiency and the size of the energy gap. The energy barriers have a stronger influence on efficiency. This is apparent especially for biotin but also for glycerol 3-phosphate. For the lowest energy structures, the balance between the electrostatic and the vdW interactions varies from almost entirely electrostatic in the phosphocholine/FAB and glycerol 3-phosphate/tim complexes, to almost entirely vdW in the biotin/streptavidin complex. This balance is different for misdocked structures and thus a proper weighting of electrostatics is important for selectivity.

The best energy function, in terms of efficiency, seems to be characterized by a distance-dependent dielectric with $\varepsilon = 3$, reduced surface charges, a soft core vdW potential, and an 8-Å nonbonded truncation. The most efficient potential is the ninth most selective and gives a reasonable separation of the energy distributions of the docked and misdocked structures (see Fig. 3b). We consider the overall best docking energy function as the most efficient one, for two reasons. First, to consider selectivity at all, at least one ligand has to dock to the active site, and only an efficient energy function can achieve this condition. Second, during our annealing schedule we gradually change the soft core to a hard core, so the final energy evaluation is performed on the unmodified short-range vdW and electrostatic interactions. Thus, the importance of the soft core vdW potential is emphasized in early stages of docking, in which ligands are finding the active site. Once the active site is found, the hard core potential will optimize the binding mode. In this way, our annealing protocol combines the desirable features of efficiency and selectivity.

Conclusions

In this article, we presented metrics for assessing the selectivity and efficiency of docking energy functions. Some key features of our approach are borrowed from methodology used for designing potentials applied to protein folding problems. We have identified a number of energy functions that discriminate correctly docked structures from misdocked structures.

A soft core nonbonded repulsion was found to be critical for the kinetic accessibility of the binding site. In a receptor with a relatively small entrance to the active site, the use of the regular vdW potential precludes ligands from entering. For all receptors with closed active sites, such as streptavidin or triose phosphate isomerase, a soft core potential appears to be essential for successful docking. Thus, in general, the most discriminating energy functions are not the best in terms of efficiency. No correlation is observed between the size of the energy gap between the best docked and the best misdocked structures and docking efficiency. Thus, we see a separation of kinetic effects (efficiency) and thermodynamic effects (selectivity). To take advantage of both a selective energy function with hard core vdW interactions and the effi-

TABLE II.
Efficiencies of Some Selective Energy Functions.

SE rank	Efficiency (*SE*)	$f_{<2}$[a]	$f_{<3}$[b]	*SG* rank	f_G	Solvation model	RSC	vdW soft core?	NB cutoff
1	0.060	0.45	0.55	9	0.45	rdie, 3	YES	YES	8
2	0.057	0.39	0.53	10	0.44	rdie, 4	YES	YES	8
3	0.057	0.41	0.55	8	0.45	rdie, 4	NO	YES	8
4	0.057	0.43	0.53	7	0.46	rdie, 3	NO	YES	8
5	0.054	0.39	0.52	11	0.44	rdie, 2	YES	YES	8
6	0.033	0.43	0.56	14	0.39	rdie, 4	YES	YES	10
7	0.032	0.40	0.57	144	0.35	solv, 3	YES	YES	10
8	0.00	0.28	0.32	2	0.76	rdie, 4	YES	NO	8
9	0.00	0.20	0.22	1	0.77	rdie, 3	YES	NO	8
10	0.00	0.19	0.21	19	0.25	solv, 4	YES	YES	8

[a] Average fraction of docked conformations.
[b] Average fraction of partially docked structures. All other abbreviations are explained in the footnotes to Table I.

TABLE III.
Per-Complex Comparison of Efficiency for Two Selective Potentials (Selectivity Ranks 2 and 10) Differing Only in Short-Range Interactions.

Ligand (vdW potential)	*SE*	$f_{<1}$[a]	$f_{<2}$	$f_{<3}$	Time[b]	ΔE
g3p (hard core)	0.02	0.1	0.15	0.15	7.2	−7
g3p (soft core)	0.09	0.25	0.50	0.65	6.7	−8
Sialic acid (hard core)	0.02	0.05	0.20	0.20	10.3	−2
Sialic acid (soft core)	0.01	0.00	0.0	0.15	10.7	−3
Biotin (hard core)	0.00	0.00	0.00	0.00	7.2	−11
Biotin (soft core)	0.05	0.15	0.20	0.50	6.9	−6
Phosphocholine (hard core)	0.05	0.00	0.25	0.45	7.3	−5
Phosphocholine (soft core)	0.09	0.00	0.45	0.75	6.8	−5
Benzamidine (hard core)	0.10	0.15	0.80	0.80	8.2	−23
Benzamidine (soft core)	0.10	0.15	0.80	0.80	8.1	−3

[a] Fraction of ligands with RMSD on all heavy atoms less than 1 Å.
[b] Average computer time (minutes) for a single docking simulation. Other columns as in Table I.

ciency of the corresponding energy function with a soft core vdW, our annealing protocol gradually changes the vdW potential from soft to regular, yielding a useful tool for molecular docking.

In contrast to the impression given by the relative rarity of MD as a docking tool, we have shown that MD can be successfully used when paired with a smooth energy surface that has a clear global minimum. In the subsequent study, we present a comparison of different algorithms based on docking efficiency. Much remains to be done to apply our approach to more general cases of docking using unbound receptor structures and to larger search spaces when the active site location and the binding mode are unknown. Nevertheless, this work provides guidelines that could be useful in developing solutions to various docking problems.

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