Virtual Screening of DNA Minor Groove Binders

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The utility of the two docking programs DOCK and AutoDock in studying the binding of small molecules to the minor groove of B-DNA is examined. The AutoDock program is found to be more effective in both pose prediction and ranking of known binders over random compounds, and this superior performance is shown to be because of the scoring functions rather than the sampling algorithms.

Introduction

Ligands that can bind noncovalently in the B-DNA minor groove[1−3] can show a wide range of anticancer, antiviral, antiprotozoal, and anti-infective properties.[4−6] Some are in current clinical use with, for example, pentamidine active against antimony-resistant leishmaniasis, primary stage human African trypanosomiasis, and AIDS-related *Pneumocystis carinii* pneumonia.[4] An orally active prodrug of the bis-[amidino-phenyl]-furan compound furamidine is currently in clinical trials against both malaria and *Pneumocystis carinii* pneumonia.[7] The mechanism of action of these compounds is not fully elucidated, but numerous studies have implicated active transport into target cells, followed by DNA binding as a key step, then inhibition and disruption of binary DNA−protein complexes, possibly involving host-specific topoisomerases and/or generalized transcription factors. The crystal structures of a number of DNA complexes with these drugs have been determined[8] and constitute a significant resource for further drug design[9] in this area, where there are a number of major worldwide unmet clinical needs.

Virtual, or in silico, screening of compounds for their interactions with a macromolecular target is a popular technique in drug discovery.[10,11] Docking a ligand into a receptor binding site involves the use of a sampling algorithm and a scoring function to assess which orientation the molecule is most likely to adopt, and the correct identification of the binding pose of one or more related ligands can be important in establishing a structure−activity relationship in lead optimization.[12,13] The second use of scoring functions is to rank different ligands to predict their relative experimental activity. Scoring functions that are effective in this regard can be used to find new active compounds from libraries of random molecules.

The vast majority of in silico studies have examined protein receptors, but the minor groove of DNA is also an important target, as discussed above. However, there are only a small number of DNA-small molecule docking studies,[14−21] even though DNA has an exceptionally well-characterized structure. There are two broad families of noncovalent minor groove binding compounds—those that bind without a significant increase in groove width from the free DNA, which typically contains aromatic rings and charged end groups, and the polyamide hairpin and related compounds, which broaden the groove to accommodate side-by-side chains of aromatic rings joined with amide bonds, to give a high level of sequence selectivity.[22] This study focuses on the use of the DOCK and AutoDock programs to study molecules in the first category, both for pose prediction and ranking purposes. Both of these programs use a scoring function derived from molecular mechanics potentials (specifically AMBER94[23]) and as such can be used more readily with DNA than with functions that are derived from structural information on protein−ligand interactions, such as PMF Score,[24] BLEEP,[25,26] and DrugScore.[27] We use 28 ligand−DNA complexes (see Figure 1 for molecular structures) obtained from the Protein Data Bank[28] as a test set to assess the ability of DOCK and AutoDock to predict the experimental binding poses. We also assess four different commonly used methods for ligand charge assignment for use with the pose prediction with DOCK and AutoDock. We use the same ligand test set to investigate whether the scoring functions can discriminate known binders from random compounds, chosen from the ZINC database.[29]

Results

Pose Prediction. The results for DOCK are presented in Figure 2a. These demonstrate a success rate of around 40% in predicting the crystal structure pose to within 2 Å RMSD. No model shows a systematic improvement in the results as the sampling (n_c and n_o) is increased. The results for AutoDock are presented in Figure 2b. These demonstrate a success rate of around 55% in predicting the crystal structure pose to within 2 Å RMSD for the AMSOL charge models, with slightly poorer results for the other models. The improvement in results with increased sampling is somewhat more systematic.

It is important to ask whether the scoring function or the sampling algorithm is responsible for the failure of the methods to predict the correct docked pose. The score of the PDB ligand poses was calculated with each program, after the geometry had been optimized in the receptor structure using the same scoring function and local minimization algorithm used in the docking runs. We then define the discrimination factor metric (D_f)

$$D_f = \frac{E_{\mathrm{dock}} - E_{\mathrm{pdb}}}{\sigma(E_{\mathrm{pdb}})} \qquad (1)$$

where E_{pdb} is the score of the crystallographic pose, E_{dock} is the score of the top-ranked (lowest energy) pose found in the docking run, and $\sigma(E_{\mathrm{pdb}})$ is the standard deviation of E_{pdb} across all 28 complexes. If $D_f < 0$, the scoring function erroneously

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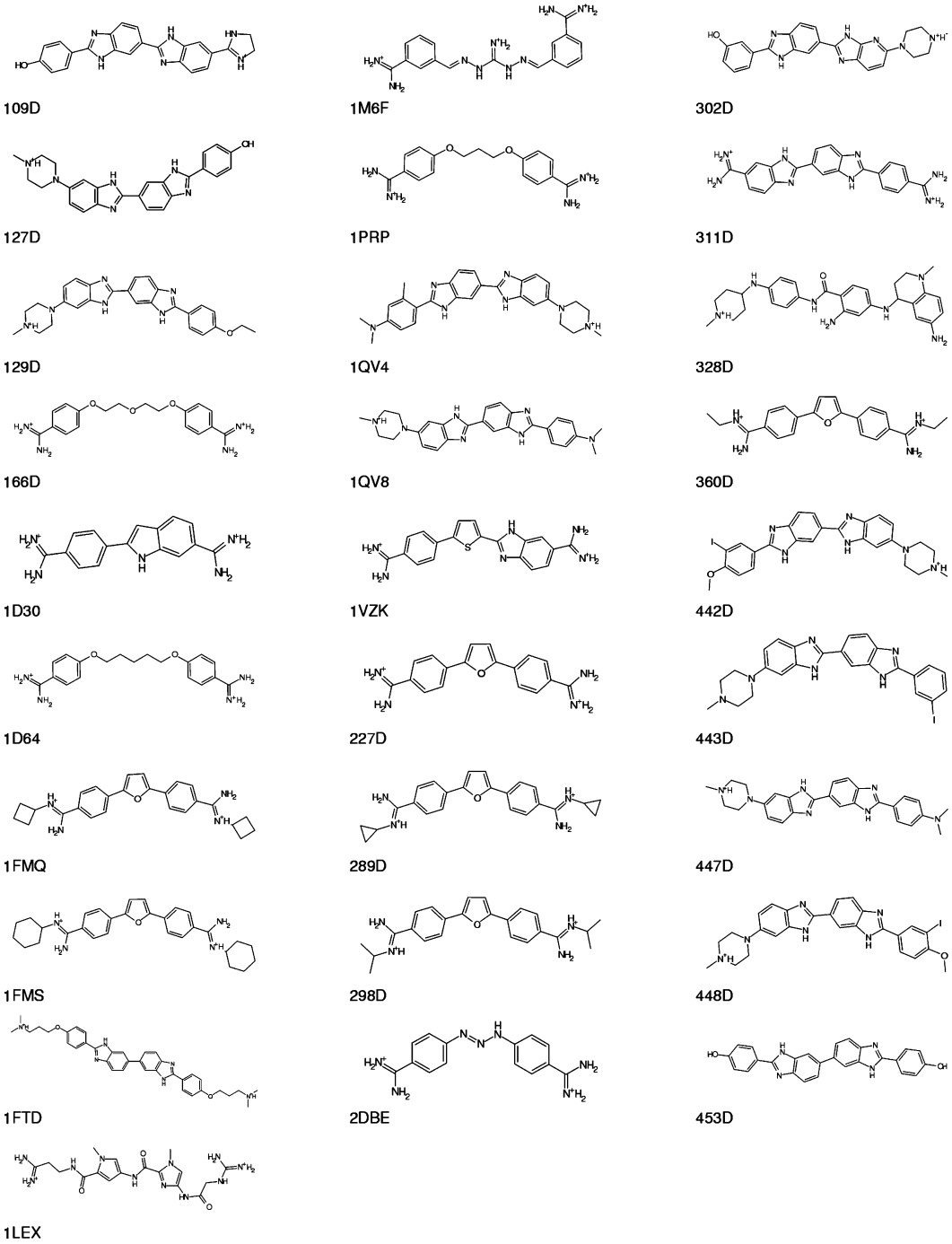

Figure 1. The 28 ligands used as a test set, labeled by the PDB ID of the DNA−ligand crystal structure from which they were extracted.

predicts that a pose exists, which is lower in energy than the experimentally observed conformation but is significantly structurally distinct. If $D_f > 0$, the sampling procedure has failed to locate the experimental structure, even though it is lower in energy than the predicted pose.

The mean values of D_f for ligands where the docking methodology failed to predict the crystallographic pose to within 2 Å RMSD are plotted in Figure 3a and b. For DOCK, the mean values of D_f are consistently negative, indicating that a poor scoring function is responsible for docking failure. For AutoDock, the mean values of D_f are positive for low sampling but become negative as the sampling is increased, indicating that the AutoDock scoring function is also ultimately at fault in the cases where the program failed. Looking at the results for success or failure for each individual ligand, rather than the average over ligands (data not shown in full), reveals only one exception from these general rules: for the 1FMS ligand in the DOCK program, the program failed to dock the ligand accurately but had positive D_f values for all of the charge methods. One possibility is that this could be a consequence of the large hydrophobic substituent at each end of the 1FMS molecule.

We note that information on the performance of the sampling algorithm could also be obtained by comparing the RMSD of every trial pose generated during each docking run with the crystallographic pose, but this would require technical modifications to the DOCK and AutoDock programs to output all of the poses, which are beyond the scope of this study. Also, since sampling is guided by the energy function, such a measure would not test the independent performance of the sampling. A positive D_f score is a definite indication that sampling failed to locate the global minimum of the potential, (although $D_f > 0$ does not prove that the energy function is not also incorrect).

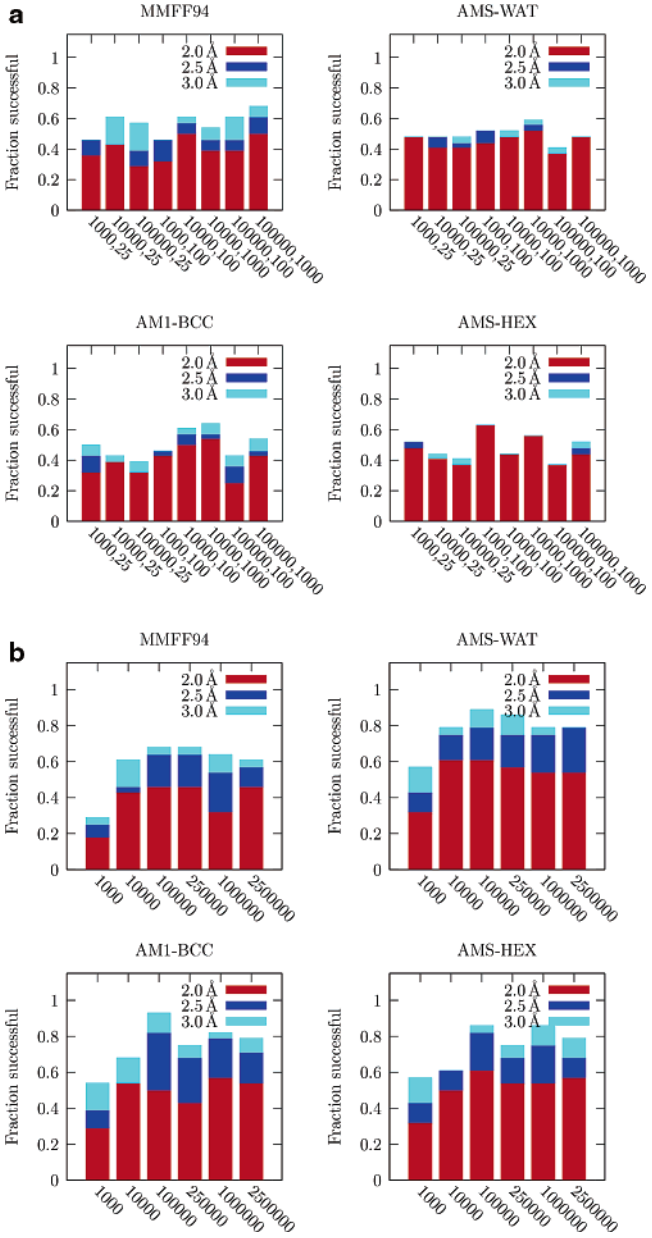

Figure 2. (a) DOCK pose prediction results, showing for each charge model the fraction of the 28 ligands that were successfully docked to within 2.0, 2.5, and 3.0 Å of the crystal structure pose, using each combination of n_o and n_c (see *x*-axis label). (b) AutoDock pose prediction results, showing for each charge model the fraction of the 28 ligands that were successfully docked to within 2.0, 2.5, and 3.0 Å of the crystal structure pose, using each value of n_e (see *x*-axis label).

Examining the data for each ligand (not shown in full) also demonstrates a general consistency across the charge sets within either DOCK or AutoDock. If a ligand was successfully docked with <2 Å RMSD accuracy with the best performing charge set in DOCK (MMFF94, 47% success rate at the highest sampling), there is a 71% chance it was also docked accurately in all three other charge sets and a 76% chance it was docked accurately in at least two of the three other charge sets. If a ligand was successfully docked with <2 Å RMSD accuracy with the best performing charge set in AutoDock (AMS-HEX, 57% success rate at the highest sampling), there is a 67% chance it was also docked accurately in all three other charge sets and a 100% chance it was docked accurately in at least two of the three other charge sets.

In contrast, comparing results from DOCK and AutoDock, there is only a 50% chance that a ligand docked correctly with

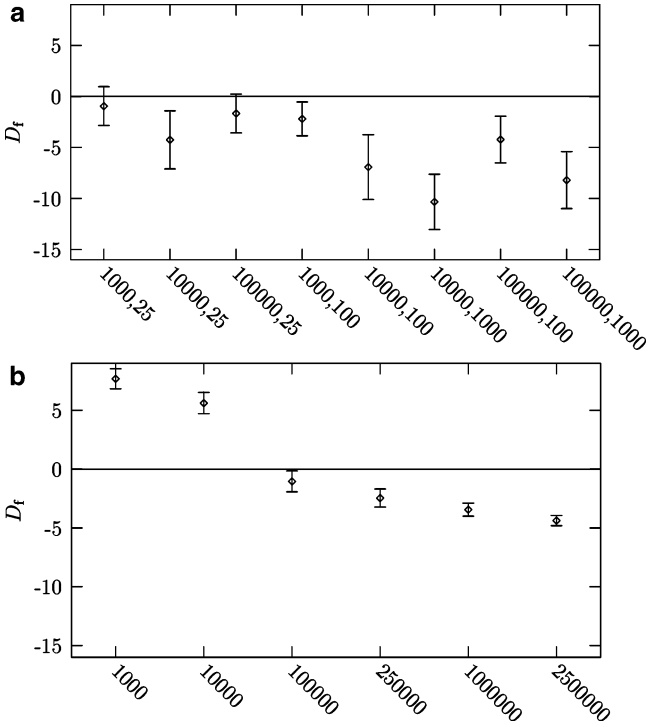

Figure 3. (a) DOCK D_f values for the MMFF94 charge model, using each value of n_o, n_c (see *x*-axis label). The values plotted are the average over all ligands that were not docked to within 2 Å RMSD of the crystallographic pose, and the error bars are the standard deviation. The results for the other three charge models (not plotted) are very similar. (b) AutoDock D_f values for the AMS−HEX charge model, using each value of n_e (see *x*-axis label). The values plotted are the average over all ligands that were not docked to within 2 Å RMSD of the crystallographic pose, and the error bars are the standard deviation. The results for the other three charge models (not plotted) are very similar.

DOCK and MMFF94 charges will also be docked correctly by AutoDock and AMS-HEX, and conversely, there is only a 47% chance that a ligand docked correctly with AutoDock and AMS-HEX charges will also be docked correctly by DOCK and MMFF94.

To address the question of why certain ligands docked successfully and others did not, we considered receptor flexibility and the possibility that molecules that crystallized in a complex where the DNA structure was significantly different from the 1VZK structure used as the receptor would be more likely to fail. However, we found that the maximum RMSD between the atoms of the 1VZK structure and the other DNA duplexes was only 1.47 Å and that there was no correlation between this RMSD and the RMSD of the docked ligands from their crystal pose.

We also examined two unliganded structures of the same sequence (355D and 9BNA) and found that both had an RMSD of <1Å from the 1VZK structure, further suggesting that there is no significant induced fit in these complexes.

Enrichment. The results for DOCK with the standard energy function are presented in Figure 4a. This graph shows a very high enrichment rate of known binders over the random compounds. The results improve markedly on moving from the lowest sampling rate (n_c = 1000, n_o = 25) to the next highest rate (n_c = 10 000, n_o = 100). We also tested the generalized Born surface area (GBSA) scoring functions of DOCK by rescoring the docked poses generated with the standard energy function. Results for DOCK with the $\Delta G_{binding}{}^{ZSK}$ function (eq 2) are shown in Figure 4b (the results with $\Delta G_{binding}{}^{KSK}$ are

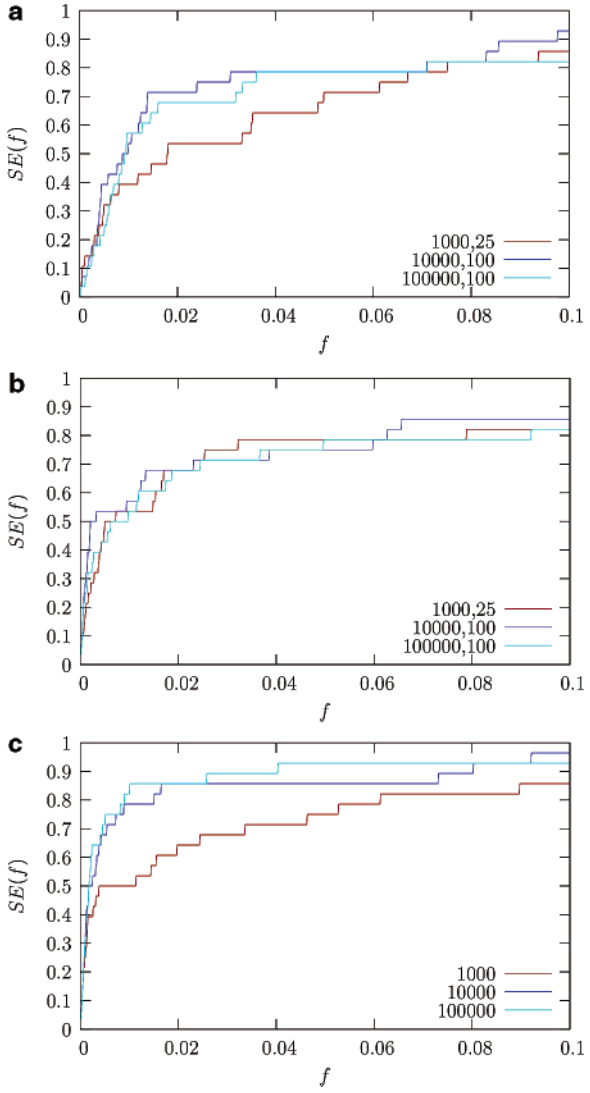

Figure 4. (a) DOCK enrichment plots with an energy score of *SE*(*f*) for $0 < f < 10\%$ of the 9216 random and 28 known binding molecules for three different sampling rates (n_c, n_o). The MMFF94 charge model was used. (b) DOCK enrichment plots with $\Delta G_{binding}{}^{ZSK}$ scoring function (see eq 2) of *SE*(*f*) for $0 < f < 10\%$ of the 9216 random and 28 known binding molecules for three different sampling rates (n_c, n_o). The MMFF94 charge model was used. (c) AutoDock enrichment plots of *SE*(*f*) for $0 < f < 10\%$ of the 9216 random and 28 known binding molecules for three different sampling rates (n_e). The MMFF94 charge model was used.

very similar). These results show some improvement over the standard energy function at $n_c = 1000$ and better performance at $f < 0.5\%$, but no systematic improvement as sampling was increased, which is perhaps a result of the poses not being minimized with the GBSA function, a procedure that was too time consuming.

The results for AutoDock are presented in Figure 4c and show excellent enrichment rates that increase systematically with sampling. Enrichment and AUC values are compared in Tables 1 and 2. AutoDock is clearly the better performer, despite the fact that the results for pose prediction with MMFF94 charges were roughly equivalent between the two programs.

We also attempted to assess the importance of accurate pose prediction in producing good ligands scores by recalculating the *SE*(*f*) and AUC values, treating only those ligands that were shown to successfully dock to <2 Å RMSD accuracy as known binders and also only using those ligands that were shown not to dock successfully. In all cases, there is an improvement in the *SE*(*f*) and AUC values when only successfully docked ligands are considered (and a deterioration for unsuccessfully docked ligands). These observations are equivalent to noting that successfully docked ligands have on average a better ranking (more favorable binding energy) than unsuccessfully docked ligands. This point potentially could be exploited to improve confidence estimates for pose prediction results, though it is also noticeable that as the sampling increases the differential between the enrichment of the successfully and unsuccessfully docked ligands decreases.

Time is an important factor in planning virtual screening experiments, and so we note here that sampling with DOCK and parameters $n_c = 10\,000$ and $n_o = 100$ took an average of 40 s per ligand, and with AutoDock and $n_e = 10\,000$, it took an average of 8 s per ligand (on a 3.0 GHz Intel ×86−64 CPU). At these values of the parameters, the enrichment performance appears to be near maximal, although this is only on the basis of three sampling rates per program (Figure 4a and c, Tables 1 and 2), and it would be necessary to use a larger number of intermediate rates to fully establish which program was more efficient in computer time.

Table 1. *SE* ($f = 1\%$) Values for the Docking Methodologies, as Also Shown Graphically in Figure 4a and c[a]

DOCK energy score					AutoDock			
		SE ($f = 1\%$)				*SE* ($f = 1\%$)		
		total	success	failure		total	success	failure
1000	25	0.39	0.80	0.17	1000	0.50	0.80	0.43
10000	100	0.54	0.64	0.43	10000	0.79	0.92	0.69
100000	100	0.57	0.55	0.59	100000	0.86	0.92	0.80

[a]Total refers to all of the known binding compounds, success to those that were docked to <2 Å RMSD accuracy with the relevant docking methodology, and failure to those that were not docked to <2 Å RMSD accuracy.

Table 2. AUC Values for the Docking Methodologies, as Also Shown Graphically in Figure 4a and c[a]

DOCK energy score					AutoDock			
		AUC				AUC		
		total	success	failure		total	success	failure
1000	25	0.93	0.99	0.90	1000	0.96	0.99	0.95
10000	100	0.97	0.98	0.96	10000	0.98	0.99	0.98
100000	100	0.91	0.96	0.88	100000	0.97	1.00	0.96

[a] Total refers to all of the known binding compounds, success to those that were docked to <2 Å RMSD accuracy with the relevant docking methodology, and failure to those that were not docked to <2 Å RMSD accuracy.

Conclusions

The separate but related problems of pose prediction and assessing binding affinity for DNA-binding ligands have been addressed with both DOCK and AutoDock. The AutoDock program with the default empirical free energy scoring function and AMS-HEX charges is shown to give the best performance for accuracy of pose prediction, with 57% of the 28 ligands docked to <2.0 Å RMSD accuracy. Via the D_f metric introduced here, the failure of pose prediction in all cases (except one ligand with the DOCK sampling procedure) is shown to be because of deficiencies in the scoring functions.

The AutoDock program was also shown to give the best enrichment of known binding compounds in a screen of 9216 randomly chosen molecules with an enrichment value *SE*($f =$ 1%) = 86%. This value might be expected to improve if the mol2 files with AMS-HEX charges become available from the ZINC database because this charge set is demonstrated to give

the best pose prediction, and accurate pose prediction is also shown to be correlated to enrichment. Nonetheless, the current performance is very encouraging and should enable screens of larger numbers of compounds to detect unknown DNA-binding molecules. The GBSA scoring function in the DOCK program was employed as the post-docking filter and not shown to give a large improvement in enrichment over the standard DOCK energy score, except at very low *f*.

As noted earlier, the application of in silico virtual screening techniques to DNA-ligand binding is relatively uncommon, and we are not aware of a similar study since the early work of Grootenhuis et al.[15,16] It is interesting to consider whether these methods are more or less effective for DNA than for the protein systems for which they are principally used. A recent study on the pose prediction of 189 different protein−ligand complexes with different scoring functions showed that success rates of 70−90% were typical for DOCK and AutoDock scores with the exception of a few protein families.[30] Another recent study on 5 protein targets and a total of 49 known ligands, which focused on enrichment, showed that average DOCK $SE(f = 2\%)$ values were around 20%.[31] The GOLD scoring function[32] performed significantly better in the same study with $SE(f = 2\%)$ values averaging 40% but reaching 80% for some targets; AutoDock was not used in this work. Therefore, it would appear (although it is impossible to summarize all of the available protein−ligand docking information) that the pose prediction performance in the present study is slightly disappointing, but the observed enrichment exceeds what one would expect from similar protein−ligand studies. It is interesting that the AutoDock scoring function, which was parametrized with experimental protein−ligand inhibition constants, performs better than the DOCK scoring function, which is more closely matched to the original AMBER94 force field. It would thus appear that the parametrization is transferable from proteins to DNA.

Methods

Ligand and Receptor Preparation. Twenty-eight crystal structures of the $d(CGCGAATTCGCG)_2$ sequence complexed with small molecule ligands were downloaded from the PDB,[28] via a search of the Nucleic Acid Data Bank (NDB[33]). The ligands with their corresponding PDB IDs are shown in Figure 1. The ligands were extracted, atom types assigned, explicit hydrogens added, and the molecules minimized to 0.1 kcal mol^{-1}/Å root-mean-squared gradient using the Sybyl 7.0 package[34] and the MMFF94 force field. Atomic point charges were assigned according to four different models, MMFF94,[35−39] AM1-BCC,[40,41] and two AM1-CM2 methods,[42] designed to produce charges approriate for an aqueous and nonpolar organic solvent respectively (AMSOL-WAT and AMSOL-HEX). MMFF94 was chosen because the mol2 format files available from the ZINC database[29] (see below) have MMFF94 point charges assigned. AM1-BCC is a semiempirical charge model derived to reproduce the restrained electrostatic potential (RESP) approach to charge fitting used in parametrizing the AMBER94 force field,[23] the force field that is the basis of the scoring functions used in both DOCK and AutoDock. The AMSOL-WAT models are used in the db format files in ZINC, and the charges were assigned here using the approach[43] of Wei et al., as is also the case for ZINC.[29]

The receptor for all of the docking studies was chosen from the 28 ligand−receptor structures to be the DNA duplex closest to the average of these 28 structures, as determined by a script written for the *ptraj* program in the AMBER8 package.[44] This most representative structure was the 1VZK DNA duplex.

DOCK Methodology. The DOCK program (v. 5.1.1) was used.[45] It was slightly modified to introduce a Mersenne twister random number generator (RNG)[46] because the built-in random number generators did not give reproducible results on our systems; the default RNG is adequately random, but identical seeds did not give identical runs, which was felt to be important for a systematic study. DOCK defines the binding site by a cluster of spheres, which was chosen in this case to cover the entire minor groove. AMBER94 charges and van der Waals parameters were assigned to the atoms of the DNA to construct the interaction grids. The DOCK energy score was used as the primary and secondary scoring function in the docking runs. The generalized Born surface area (GBSA) scoring function[47] was found to be too slow to use as a primary or secondary function in docking calculations, but was tested as a post-docking filter (see below). All default parameters were used for the docking runs and grid construction, except for two parameters, which were systematically varied to determine their effect: n_o, the number of orientations of each anchor attempted; and n_c, the number of conformations per cycle of ligand growth. These parameters both relate to the anchor-first flexible ligand algorithm,[48] which involves splitting each ligand into fragments. One fragment is selected as the anchor. This anchor is docked n_o times into the receptor, and the top-scoring n_c orientations taken forward. The remaining ligand fragments are then added one by one with a torsional search to find the best orientation with respect to the anchor, and after each fragment is added, the top-scoring n_c conformations are taken forward to the next stage until the whole ligand has been constructed. Increasing n_o and n_c will increase the sampling carried out in each docking run, which should improve the accuracy of the method if the scoring function is adequate, but naturally, it also increases the run time. It was also found in preliminary testing to be more effective in terms of pose prediction to use united atom descriptions of both the DNA and the ligands.

The accuracy of each docking run was assessed by the root-mean-squared deviation (RMSD) of the heavy atom coordinates of the docked ligand from the heavy atom coordinates of the crystal structure ligand, after the heavy atoms of the two DNA duplexes had been placed in maximal alignment. This procedure was automated via a script written for the VMD program.[49] The symmetry of the DNA sequence was taken into account in determining the RMSD. Only the best-scoring pose from each docking run was considered, which in our opinion is the best measure of the utility of the docking tool in the absence of other information about the binding pose.

AutoDock Methodology. The AutoDock program (v 3.05) was used.[50,51] AutoDock defines the binding site solely in terms of a grid of interaction points. We chose to center this grid at the center of the minor groove. The minor groove center was defined as the point on the line perpendicular to line connecting the N3 atoms of the central purine residues and the line connecting the O2 atoms of the central pyrimidine residues, which is 3.0 Å away from both O2 atoms on the central pyrimidine residues. This position was calculated using a script written for the MMTK package.[52] The grid was extended 30 Å along the DNA axis and 22.5 Å in the two perpendicular directions to encompass the entire minor groove.

Point charges were assigned to the DNA according to the AMBER94 force field, as with DOCK. The default van der Waals interaction parameters (based on a smoothed united atom AMBER force field) were used. The default behavior of using united atom representations of both ligand and receptor was adopted, as had been shown to be most successful in DOCK.

Table 3. $SE(f = 1\%)$ and AUC Values for the DOCK GBSA $\Delta G_{binding}{}^{ZSK}$ Methodology, as Also Shown Graphically in Figure 4b

DOCK GBSA $\Delta G_{binding}{}^{ZSK}$ Score			
n_c	n_o	$SE(f = 1\%)$	AUC
1000	25	0.54	0.93
10000	100	0.57	0.95
100000	100	0.54	0.91

The AutoDock free energy scoring function,[50] which is based on these force field terms plus additional solvation and entropic terms, was used for all calculations. It was necessary to add solvation terms for phosphorus atoms; these were adopted from a recent study that used AutoDock to examine RNA−ligand interactions.[53] Ten runs of the default Lamarkian genetic algorithm for searching ligand conformations were used per ligand; the only parameter adjusted from the default was n_e, the number of energy evaluations allowed for each run of the genetic algorithm. The n_e value was adjusted systematically to investigate the effect of increased sampling on the effectiveness of the procedure. The best scoring solution from all 10 runs was used as the predicted binding conformation.

The accuracy of each docking run was assessed according to the RMSD of the ligand heavy atom coordinates from the crystal structure, using the same procedure as used for the DOCK runs, described above.

ZINC Database and Enrichment Calculations. We wished to investigate the effectiveness of the two programs in discriminating known minor groove binders from random compounds. The same twenty-eight compounds as used for the pose prediction testing formed the test set of known binders. Using the ZINC web-based database of commercially available compounds[29] as the source, we searched for compounds with a net charge $\geq +1$ and molecular weight (MW) within one standard deviation of the mean of the test set (i.e., $336 < MW < 510$). These criteria returned 29 6573 compounds from the ZINC database (on 13/08/2005), from which we chose 9216 purely randomly (each compound with an equal probability of selection) to form our random set. We chose these search criteria because all but one of the test set of known binders are positively charged and because computed binding affinities have been shown to increase disproportionately with molecular weight.[54]

All compounds in the test set and the random set were docked using the DOCK and AutoDock programs as described above. The compounds were ranked according to the scoring functions of the two programs. The GBSA scoring function of DOCK was also tested for rescoring docked poses. United atom models of the DNA were used in constructing the GB and SA scoring grids and default parameters applied.[47] The GBSA function was used in a recent study of DNA-minor groove binders by Kang et al.,[55] where all-atom models of the DNA were used. Unfortunately, it appears to be impossible to rescore poses generated using a united atom representation with an all-atom grid without atom overlap occurring, and therefore, we could not test the previous authors' exact methodology with our docked poses. We tested two parametrizations of the total score as a function of different interaction components:

$$\Delta G_{binding}{}^{ZSK} = \Delta G_{pol} + 0.6\,\Delta G_{vdw} + 0.025\,\Delta SA_{HP} + 0.02\,\Delta SA \quad (2)$$

$$\Delta G_{binding}{}^{KSK} = 0.1413\,\Delta G_{pol} + 0.0787\,\Delta G_{vdw} + 0.004\,\Delta SA \quad (3)$$

$\Delta G_{binding}{}^{ZSK}$ is the parametrization according to Zou et al.,[47] where ΔG_{pol} is the polar (GB) contribution to binding, ΔG_{vdw} the van der Waals interaction, SA_{HP} the hydrophobic surface area, and SA the total surface area. $\Delta G_{binding}{}^{KSK}$ is the parametrization according to Kang et al.[52]

The results studies were assessed according to the enrichment or sensitivity $SE(f)$:[42]

$$SE(f) = \frac{N_{\text{selected known binders}}}{N_{\text{total known binders}}} \quad (4)$$

This calculation refers to a scenario where the top fraction f of the compounds as ranked by docking are selected and one wishes to know what fraction of the total number of known binders have been found. Similarly, the specificity $SP(f)$ is defined as

$$SP(f) = \frac{N_{\text{discarded random compounds}}}{N_{\text{total random compounds}}} \quad (5)$$

and the area under the curve (AUC) of a plot of $SE(f)$ versus $(1 - SP(f))$ is another useful measure of the overall performance of a virtual screening procedure.[56]

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