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Flexibases: A way to enhance the use of molecular docking methods

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SUMMARY

Specially expanded databases containing three-dimensional structures are created to enhance the utility of docking methods to find new leads, i.e., active compounds of pharmacological interest. The expansion is based on the automatic generation of a set of maximally dissimilar conformations. The ligand receptor system of methotrexate and dihydrofolate reductase is used to demonstrate the feasibility of creating flexibases and their utility in docking studies.

INTRODUCTION

The structure of biological macromolecules can be derived using X-ray crystallography or NMR spectroscopy. For macromolecules that are receptors for drug molecules (i.e., are therapeutic targets), structural information can be used in at least three ways. First, given the structures of a drug–receptor complex, one may rationally modify a drug to more closely complement a receptor [1–4]. Second, one may also design molecules de novo to fit the receptor cavity [5–9]. Third, one can select existing molecules that might bind to the receptor [10–17]. Computational methods that dock smaller molecules into putative receptor sites have shown great potential in the second and third areas.

At the inception of a drug design project, one must discover leads, i.e., novel compounds that bind to a particular receptor. One strategy for suggesting compounds to test is the use of docking techniques to search large chemical databases that have compound samples readily available. One looks for compounds that are complementary to the receptor and yet have a different chemical topology from that of any previously identified leads. Docking methods are unique in that they

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do not require knowledge of at least one active compound beforehand (in contrast to methods that depend on chemical similarity). The criteria for the selection of compounds is based purely on the three-dimensional (3D) structure of the receptor.

The way molecules dock to their receptors is dependent on their conformations. Most molecules are inherently flexible. However, most docking methods, as applied to searches of large chemical databases, investigate but a single molecular conformation – usually the X-ray or a low-energy conformation. If one is to incorporate conformational flexibility into a 3D database, there are two basic approaches. The first approach is to generate the conformations as needed during the search [18,19]. This can be done by either systematic (e.g., rules-based, directed) or random (e.g., distance geometry, Monte Carlo) methods. This approach is attractive in that it provides an opportunity to incorporate additional information that is specific to the problem under study during conformer generation. The disadvantage is the trade-off in time for accuracy; the generated conformations must be reasonable, but the time taken to generate them must be small. The second approach, which is the focus of this work, is to expand an existing 2D database into a *flexibase*, wherein each compound is represented by a set of carefully chosen conformations that are permanently stored. The time expense of conformer generation is incurred only once. A great advantage in generating conformations beforehand is that we are not limited to generation strategies that must be time-competitive with the search method. Omissions or systematic biases that can occur with the first approach are not manifested, because the conformational space mapped by the second approach is arranged to be uniform and representative. Any docking routine that currently searches databases with one conformation per compound can, without modification, search a flexibase, thereby gaining some handle on conformational flexibility.

This strategy has been largely avoided in the past for practical reasons; it seemed prohibitively expensive in computer time and disk space to generate a large number of conformations per compound and store them all. This paper demonstrates that for some applications like docking, the number of conformations that must be generated per compound can be modest, so that the computational and storage expenses, while large, are not prohibitive. The relatively low precision of docking methods as applied to database scans permits us the following rationale: the most important criterion for docking a compound into a receptor site is that of shape complementarity. (Criteria used later in docking, such as matching ancillary physicochemical properties, have more to do with the ranking of hits. Such aspects of docking have been addressed elsewhere [10].) We are interested, then, only in those conformations that have substantially different shapes. Therefore, our tactic is to ensure that the flexibase contains a set of maximally dissimilar conformations for each compound. We illustrate with the example of the dihydrofolate reductase–methotrexate complex how using flexibases improves the chances that interesting ligands can be found in docking studies.

METHODS

Generating a flexibase requires the application and automation of several molecular modeling techniques in a sequence of steps.

Preprocessing of structures and generation of an initial 3D structure

This step can be very problematic in some cases. Most chemical databases are composed of 2D structural drawings. (In our case, structures are retrieved from MACCS databases, usually

in SD file format [20].) Our experience is that the drawings generally have to be cleaned up when they are to be used for applications other than their original purpose as an archive. In many cases, compounds are represented as salts and mixtures which need to be broken into fragments. Each fragment is treated separately as an individual database entry. Small fragments (less than seven nonhydrogen atoms) are deleted, and hydrogens are stripped from each fragment. Another preprocessing step is to set up parameters and restraints necessary for conformer generation.

Automation of the conversion from two to three dimensions has been addressed by several software programs [21,22]. Our approach is to use our own 2D to 3D conversion program. This is essentially a simplified and very robust molecular mechanics program called IDEALIZE [23], which was originally based on BIGSTN-3 [24]. The IDEALIZE force field works without hydrogens and calculates only the repulsive part of the nonbonding interactions. The 2D drawings require some further processing before relaxation by IDEALIZE. Atoms that have been identified as chiral in the original 2D drawings are pushed out of the plane as appropriate. (Atoms with specified chirality are maintained with correct chirality during the 3D conversion and conformer generation. Atoms with unknown chirality will randomly invert.) The initial 3D structure is sensitive to the initial local geometry of the 2D structure, and the automatic perception of geometric restraints used in the next step for conformer generation by distance geometry is, in turn, somewhat sensitive to the initial 3D structure. This requires some chemical groups, in unrealistic local geometries, to be perceived and corrected in the 2D drawing before running IDEALIZE. In particular, acyclic *cis*-esters or *cis*-amides are fixed by reflecting the ester oxygen or the nitrogen across the line drawn between their two attached atoms. This results in a crude trans-configuration that is easily relieved by IDEALIZE. There are some problems with properly projecting highly connected cage-like structures. These have to be corrected by hand.

We prefer IDEALIZE to rule-based 2D to 3D conversion procedures such as CONCORD [21]. CONCORD produces a single, more or less extended, low-energy structure or else it fails to produce any structure. In comparison, IDEALIZE is more robust. From a random sampling of 1000 structures, CONCORD built 84.4% while IDEALIZE converted 99.9% successfully. In terms of local covalent geometry, the quality of structures generated by IDEALIZE is sometimes not as good as those generated by rule-based methods. However, we emphasize that in the context of docking methods, details of the local geometry are of less importance than the overall shape of a molecule.

Conformer generation

There are many methods for generating conformations of a molecule. For our purposes, we require a method that quickly generates conformers which are completely independent of one another and that randomly samples diverse portions of conformational space. Distance geometry satisfies these requirements [25,26]. Automating distance geometry requires the perception of rigid fragments and identification of chiral atoms. For instance, aromatic rings are perceived and the appropriate distances fixed between all the atoms, including those radiating one bond out from the rings. Double bonds, primary amides and imines are similarly perceived and held in their original configuration. Secondary amides are allowed to adopt both cis and trans configurations.

We have written our own version of the distance geometry algorithm that incorporates this automatic restraint perception [27] and uses the latest advances in sampling [27,28] and regularization [27] techniques. A guideline was developed for determining the number of trial conformations to

be generated by testing the procedure on a number of diverse structural types. The most radical conformational changes tend to occur around single bonds in the structure. Therefore, we enumerate the number of cyclic and acyclic single bonds in the structure. (Single bonds within rings greater than seven atoms are regarded as acyclic.) Empirically we find that the following formula works well for estimating the number of trial projections necessary to cover conformational space:

$$\text{Trials} = 8 * \text{Acyclic} + 4 * \text{Cyclic} \quad (1)$$

There is little precedence for this. Typically, for global search methods that work in torsion angle space, it is found that grid-based explorations with a resolution of 60° are effective for most molecules [29]. This would require that Trials be set at six raised to the power of the number of freely rotating torsions – many more than what is estimated by Eq. 1. Notwithstanding this apparent undersampling, we later show that this heuristic is sufficient for sampling diverse regions of shape space. We set a cap of 300 on the total number of distance geometry trials. This prevents spending large amounts of time on very large or flexible structures.

Crude energy-based regularization and selection

The crude structures projected by distance geometry need to be relaxed to remove bad local atomic interactions. (This does not mean that they are optimized to the nearest local energy minimum; we will return to this point in the discussion section.) Again we use IDEALIZE, as described in the first step. Some conformers, even after relaxation, can still be considered too high in energy. Those conformers that have a potential energy higher than a certain threshold above the lowest energy conformer are eliminated. In our implementation, only the repulsive nonbonding potential is monitored and the threshold is 16 kcal/mol (around 67 kJ/mol). We always retain the initial 3D reference structure among the conformations, regardless of its energy. In our implementation, this is the original extended structure IDEALIZE'd from 2D, but it may equally well be any *special* structure, for example the CONCORD conformation or a known receptor-bound conformation.

Selection of a maximally dissimilar subset of conformers

The selection of maximally dissimilar conformers bears a more detailed explanation and provides the most unique aspect to the methods used in this work. The conformers are compared using the minimized root-mean-square deviation in distance between corresponding atoms for two structures [30]. This fitted superposition will be referred to as the mrmsd. A zero value for the mrmsd means that the conformers are the same. The merit of the mrmsd is that it measures global shape similarity for the structures if all the atoms are used in the comparison. Also, chiral differences are accounted for, unlike comparisons made by inspecting distances alone.

Later on, during our docking studies, we refer to the quantity rmsd. This is the root-mean-square deviation in distance between corresponding atoms for two structures, a quantity which is not minimized with respect to rotation and translation. It therefore reflects the relative spatial dispositions of two structures – the similarity of the two structures is probably not so apparent from the rmsd.

The algorithm for selecting a maximally dissimilar set is straightforward. Two parameters are required: *maxsim*, the minimum mrmsd any two conformers can bear to one another; and *max-*

kept, the limit on the number of conformers to be selected. For the first conformer in the list, all conformers are eliminated that have an mrmsd less than *maxsim*. The algorithm then moves down the list to the next retained conformer and eliminates conformers further down the list in the same manner. This procedure is carried out until the list is exhausted. All that remains are conformers that are dissimilar to one another by at least *maxsim*. If the number remaining is less than *maxkept*, then we have obtained the required subset of maximally dissimilar conformers. If more conformers than *maxkept* were selected, then *maxsim* must be adjusted and the process repeated until exactly *maxkept* structures remain. This is accomplished by bracketing the value of *maxkept* and refining the value of *maxsim* by the bisection method.

Experience has shown that a minimum allowable mrmsd of 1.2 Å works well (this is the starting value of *maxsim*; it can be adjusted upward by the algorithm). We chose this value because: (i) it selects structures that, to the eye, smoothly fill the 3D volume available to a molecule; (ii) any smaller value would generate too many configurations – a practical limitation; (iii) energetically reasonable conformations generated by systematic rotations around acyclic bonds are generally more than 1.2 Å mrmsd apart; (iv) the value is large enough not to make too much distinction between conformations which are the same except for topological symmetries – see Discussion section; (v) our docking program is generally not too sensitive to differences in conformations that are similar by less than this value.

The value *maxkept* = 25 structures is a practical limit that avoids storing of, and searching over, an excessive number of conformations.

The set of maximally dissimilar conformers selected by the above method is not unique; it is dictated by the order of conformers in the list. It may not be optimal either in the sense that there may be other sets, with the same number of structures, that are on average more dissimilar to one another. Addressing this subtlety is not necessary for the intended application of the flexibases. Sorting by energy has been suggested [31] as a way to uniquely order the list; this way the lowest energy structure will always be selected. However, except for the initial 3D structure, which is always first on the list, we choose not to order the conformations. As will be shown below, at the level of approximation required for our application, it is not necessary that the set of conformations be unique or supremely optimal.

Typing of atoms

Atom typing is necessary to calculate a physicochemical complementary score for docking. Each atom in each conformation is assigned to one of seven atom types depending on its local bonding environment: cation, anion, H-bond donor, H-bond acceptor, polar (generally both an H-bond acceptor and donor), hydrophobic, and other. Details of how this is done have been given elsewhere [32].

Hardware implementation issues

We have dubbed the procedure for generating diverse conformations FLXGEN. It consists of a collection of programs which carry out the tasks sequentially by a shell script on a CRAY YMP and a proclib on an IBM 3090. Keeping the programs loosely coupled to one another allows us to substitute modules that perform specific tasks (e.g., distance geometry, structure minimization, atom typing, etc.) and extends the scope and utility of the FLXGEN protocol beyond the generation of flexibases. Alternative programs to the ones we use in this paper for

minimization, distance geometry, etc., could be used to obtain similar results.

It requires 3–8 CPU minutes on a Cray YMP to generate a final set of conformations from the 2D chemical structure of a typical drug-like molecule. Using the 1.2 Å mrmsd criterion, the average set contains eight dissimilar conformations selected from a larger set of 40 to 80 energetically acceptable ones.

Presently we have constructed four flexibases, containing approximately 1.2 million conformations of 150 000 structures. The conformations are currently stored as flat ASCII files.

Docking studies

The use of flexibases described here is for docking studies. We carry out all docking studies using our program FLOG [10]. As with most docking algorithms, the binding-site cavity is filled by a set of previously defined *match centers*. Initial dockings of a particular conformation of a ligand are generated by finding sets of ligand atom-match center pairs, such that the atom–atom distances match the corresponding center–center distances within a specified distance tolerance. The initial orientations are scored on a grid that represents the interaction energy from the receptor. The interaction score includes van der Waals, electrostatic, hydrogen-bonding and hydrophobic potentials. Higher scores indicate a better fit of the ligand within the receptor. FLOG has a feature where the initial dockings are refined by a rigid-body optimizer, such that the score is maximized. For any conformation there may be many binding modes. Usually only the mode with the highest score after optimization is considered. FLOG runs are much more efficient if one can define *essential points*. These are match centers that must be paired with a ligand atom. Details on how FLOG runs are conducted have been given elsewhere [10].

RESULTS

We will take for an example the classic docking benchmark of dihydrofolate reductase (DHFR) with the folic acid antagonist methotrexate (MTX). The structure of this complex is known from X-ray crystallography [33–35] and is available as the Brookhaven dataset 3DFR. MTX (Figs. 1 and 2) binds in a bent conformation, with the pteridine ring in a deep cylindrical pocket and the benzoate and glutamate portions in a surface groove.

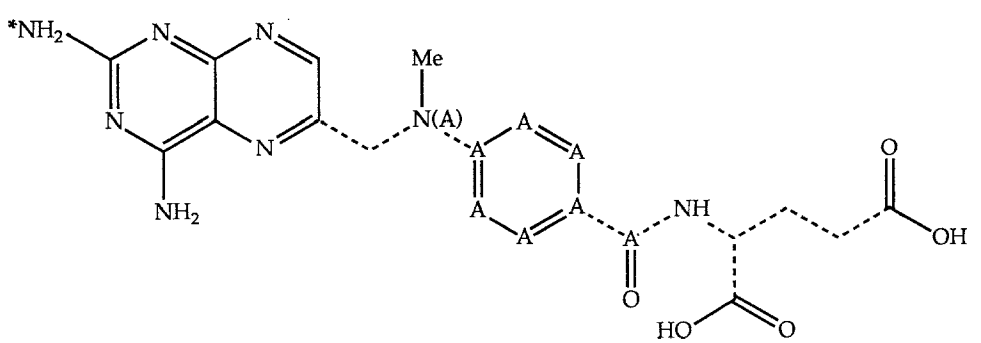

Fig. 1. Structure of methotrexate. Dashed bonds were identified as acyclic single bonds and determined the number of trials for the sampling of conformational space. The nitrogen indicated with an asterisk was selected as a docking essential point. Atoms labeled (A) were used in the alignment of conformers in Figs. 2–4.

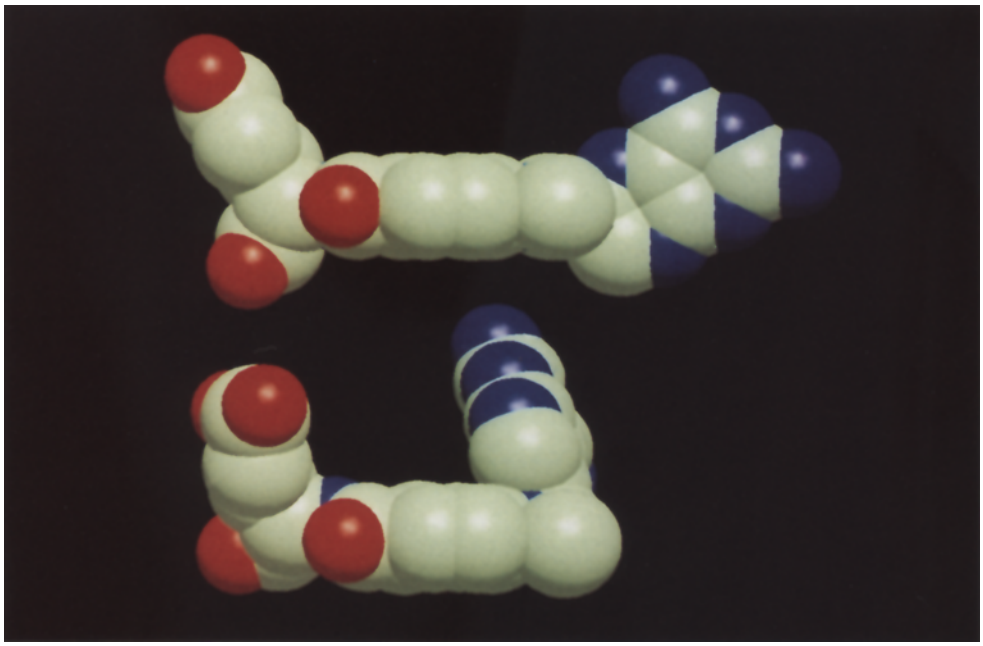

Fig. 2. Spacefilling representation of CONCORD (top) and X-ray (bottom) conformations of methotrexate.

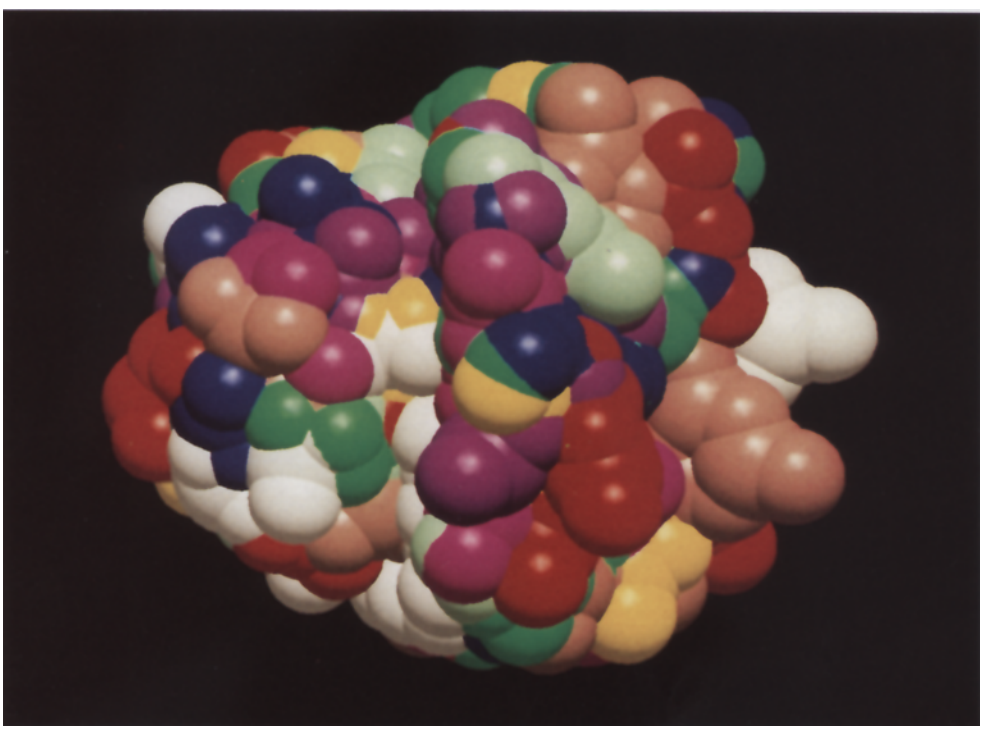

Fig. 3. Spacefilling representation of all 80 conformers of MTX. The conformers were aligned by superimposing the atoms labeled A in Fig. 1. Each molecule is shaded a different color.

TABLE 1
SUMMARY OF DISSIMILARITY SELECTIONS AND DOCKING SCORE FOR MTX

A	B	C	D	E	F	Remarks
X-ray	**2.99**	**0.00**		**0.00**	**94**	
Xmin	3.32	1.45		4.96	40	Energy −411 kJ/mol
Gmin	4.05	2.36		7.97	59	Global −429 kJ/mol
Nmin	2.97	2.54		2.57	60	Next −420 kJ/mol
A56	**2.52**	**1.72**	**********	**2.23**	**75**	
A04	3.14	2.64	**|******	2.85	75	
A15	**2.62**	**3.02**	**********	**3.17**	**72**	
A53	2.71	2.54	*|**|*|**|	2.83	70	
A06	3.15	1.90	|*|||*|||	2.51	69	
A76	**3.23**	**1.47**	**|||*|||||	**1.86**	**68**	nearest X-ray
A11	3.16	2.71	||||||||*	3.12	68	
A70	3.57	1.83	|||||||*|	2.04	68	
A17	2.70	2.31	****||***|	2.59	66	
A52	2.78	2.41	|||||****	2.56	65	
A34	3.12	2.18	|*||*||||	3.46	64	
A46	1.94	3.12	*****|***|	4.25	64	
A35	2.87	2.08	|||*|||||	8.95	64	
A29	**2.50**	**1.50**	**|***||*	**1.76**	**62**	2nd nearest X-ray
A51	3.22	1.91	*||**|||*	2.18	62	
A27	3.21	2.61	|*|||||||	2.74	62	
A62	3.78	2.91	|||||||||	3.57	62	
A05	4.22	2.68	**||||||*	–	–	
A79	3.51	2.80	||||**||*	3.35	60	
A37	2.31	2.66	|**|*||***	4.95	59	
A49	4.00	1.93	|||||||||	7.91	59	
A75	3.53	2.69	|||*||||	8.50	59	
A44	2.39	2.00	*****|***|	3.02	59	
A32	3.18	3.18	*|*|*||||*	3.68	59	
A42	3.43	2.53	|||||*|||	2.92	58	
A55	3.25	2.59	*|********|	2.74	58	
A67	3.01	1.65	***||*****	2.10	57	6th nearest X-ray
A08	3.38	2.23	*|**|||||*	2.82	57	
A72	3.05	1.67	||||||||*	2.39	56	8th nearest X-ray
A09	3.11	1.99	|||***|||	9.11	56	
A63	4.08	3.17	|*|||*|||	8.01	55	
A07	4.07	1.96	|||||||||	8.35	55	
A48	2.15	3.23	||*|**||*	10.71	55	
A26	2.48	1.92	|**||**||	3.58	54	
A03	1.81	2.25	||||*|||*	5.13	54	
A38	3.70	3.03	||||||*||	8.41	53	
A41	3.79	2.13	**|||*|||	2.74	53	
A59	3.81	3.07	|||||||||	4.26	53	
A31	3.91	2.26	***||****|	7.37	52	
A10	4.08	2.72	**|***|***	3.43	51	
A00	**0.00**	**2.99**	**********	**5.98**	**51**	CONCORD structure
A77	3.88	2.79	||||||||*	6.61	50	
A71	2.45	2.37	|****|||**	3.44	49	
A14	3.36	1.62	*||*||||**	6.73	49	3rd nearest X-ray
A57	4.22	2.52	|||**||**|	8.40	49	
A68	3.74	2.58	|||||||||	3.58	47	
A01	1.87	2.69	*****|***|	3.30	47	
A65	3.76	3.17	||||*||*|	7.64	46	

TABLE 1
(continued)

A	B	C	D	E	F	Remarks
A19	3.91	2.84	| | | * | | | | |	3.31	46	
A28	3.76	2.59	| | | | | | | | |	3.22	46	
A25	3.41	1.66	| | * | * * | * | |	2.31	45	7th nearest X-ray
A73	3.74	2.51	| | | * | | | | *	–	–	
A02	3.27	2.17	* | | | | | * | |	2.64	44	
A64	3.74	1.70	| | | | | | | | |	8.36	43	
A22	2.23	2.39	* | | | | * * | |	3.54	42	
A18	3.88	2.83	| | * | | * | | *	7.59	42	
A45	3.87	2.95	| | | | | | | * |	–	–	
A40	3.87	1.62	| * * | | | | | |	2.38	41	4th nearest X-ray
A23	3.22	2.53	| | | | | | | | |	7.94	41	
A60	3.98	2.67	| | | * * | | | |	5.88	40	
A24	3.44	2.46	| * | | | | | | |	8.12	40	
A54	3.85	2.97	| | | | | | | | |	7.46	37	
A43	2.84	1.73	| | * * | | | * |	8.81	36	
A50	4.30	3.35	| | | * | | | | |	6.07	36	
A74	3.63	2.22	| | | | | | | * *	2.87	35	
A33	3.44	2.02	| | * | * * | * | *	9.47	35	
A36	3.38	1.63	| | | | | | | | |	11.92	34	5th nearest X-ray
A78	3.20	2.99	| * | | | * | | *	3.54	33	
A39	3.53	2.33	| | | | | | * | |	8.42	33	
A16	3.61	2.53	| | | | * | | * |	8.92	32	
A12	3.04	2.72	* | | | * | | | |	7.01	29	
A13	3.97	2.95	* | | | | | | | |	7.56	27	
A20	4.26	2.80	| | * * * | | | |	–	–	
A30	3.95	3.00	| | * * | | | | |	7.55	18	
A58	3.94	2.79	| | | | | | | | |	7.23	8	
A69	4.40	2.24	| | | * * | | * | *	7.49	6	
A47	3.75	1.86	| | | | | | | | | *	–	–	
A66	4.69	2.64	| | | | | * * | | |	6.84	5	
A21	3.04	2.92	* | * * | * | | * |	10.14	1	
A61	3.80	2.49	| * * | | * * | | |	–	–	

Rows in bold type are discussed explicitly in the text. Column A: conformer label; B: mrmsd (in Å) between CONCORD structure and conformers; C: mrmsd (in Å) between X-ray structure and conformers; D: 10 random dissimilarity selections (each selection is represented as a vertical string of asterisks and vertical bars, an asterisk indicating a selected conformer); E: rmsd (in Å) between X-ray and docked structure; F: docking score using atom coordinates of X-ray structure as matching centers (a dash indicates that the conformer did not dock at all).

Conformer generation and selection

There are 10 acyclic single bonds in MTX (see Fig. 1). Therefore, according to Eq. 1, the dissimilarity selection should be based upon 80 distance geometry trials. This includes the CONCORD structure as the first conformation (labeled conformer A00) and 79 conformers generated by distance geometry (A01–A79). Of all the possible pairings of the conformers, the most similar pair has an mrmsd of 0.70 Å and the most dissimilar has an mrmsd of 4.69 Å. Columns B and C in Table 1 show the mrmsd of each conformation to the CONCORD and the observed X-ray conformation, respectively.

To measure the effects of order dependence in the selection procedure, we performed the procedure 10 times with the order randomized each time. For all trial selections a maximum of 25 representative configurations was chosen; *maxsim* varied from 1.71 to 1.76 Å over the 10

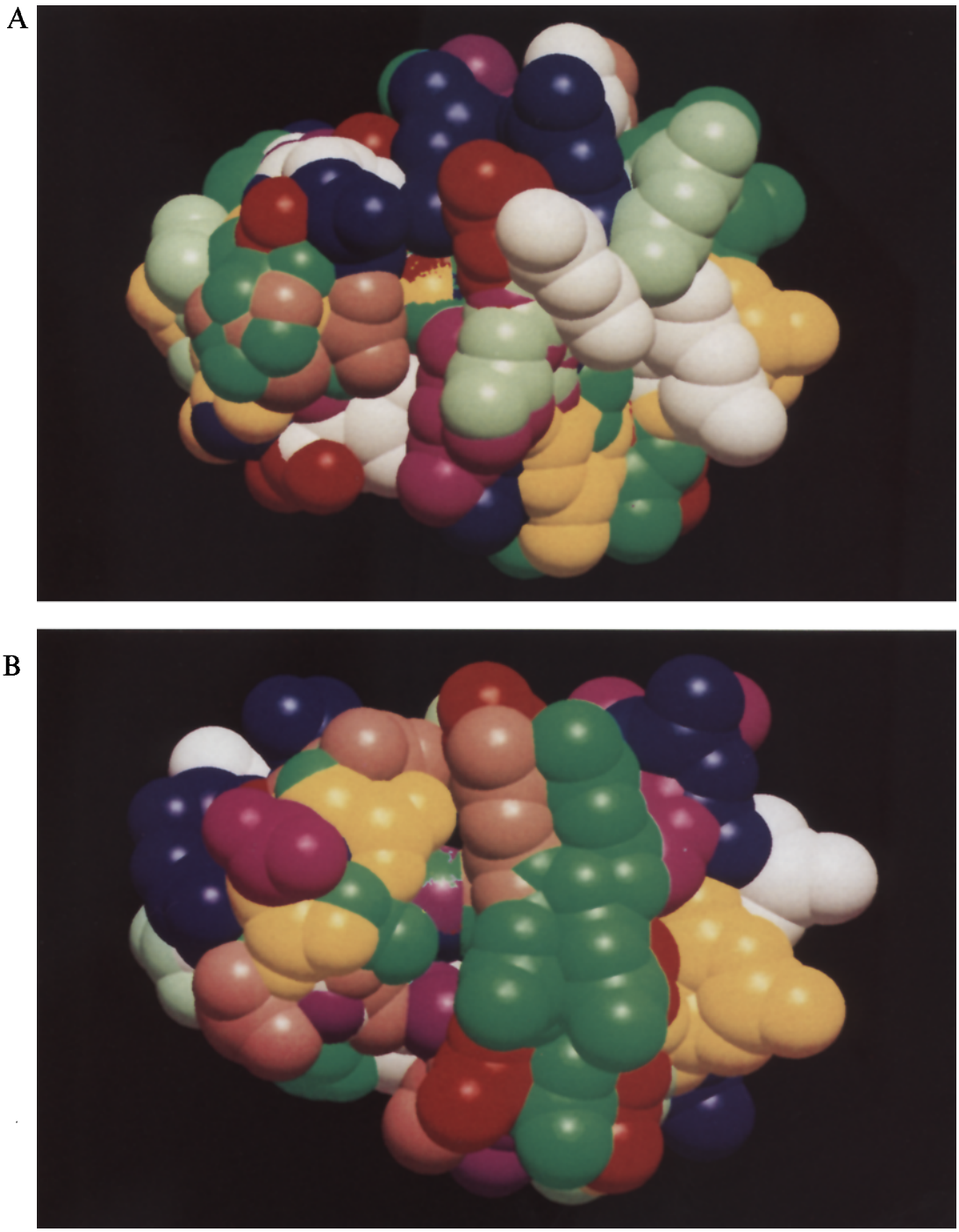

Fig. 4. Spacefilling representations of two aligned trial subsets, comprising 25 dissimilar conformations each. Each molecule is shaded a different color. Comparison of Figs. 3 and 4 should give the impression that conformational space is being sampled sufficiently but at a lower density for the subset trials.

trials, with a mean of 1.74 Å. In column D of Table 1, the conformations that were selected by each trial are indicated with an asterisk. The set of selected conformers is indeed different from trial to trial. However, certain conformers were preferentially selected – in particular, conformers A56, A15 and the CONCORD structure A00. The reason is that these conformers have no neighbors nearer than 1.74 Å.

Figures 3 and 4 show that shape space is sufficiently covered by the selected sets. All the MTX conformations were aligned by superposing the atoms labeled A in Fig. 1. Thus, it is the relative disposition of the acid and pteridine terminal groups that is displayed. The parent amorphous blob comprising all 80 conformations in Fig. 3 is similar in size to two example dissimilarity selections in Figs. 4A and B. Furthermore, the uniform distribution of colors indicates that shape space is being adequately represented by different conformers.

For a further detailed analysis we generated 2000 distance geometry structures to assess the likelihood and necessity of projecting a structure sufficiently similar to the X-ray conformer and to analyze our criteria for selecting dissimilar structures.

Docking studies using FLOG

Docking studies with FLOG were conducted using the various conformations of MTX as trial ligands. FLOG results are somewhat sensitive to the choice of match centers. The coordinates of the X-ray structure of bound MTX were used as the match centers for this study. There are two reasons for doing this. First, analysis of the docking study is made easier by simply calculating the rmsd to the match centers to get a measure on how well the conformer docked geometrically. Second, using the X-ray coordinates as site points almost guarantees that, if the MTX conformer can possibly be docked near the X-ray binding mode, a docking will be found. The

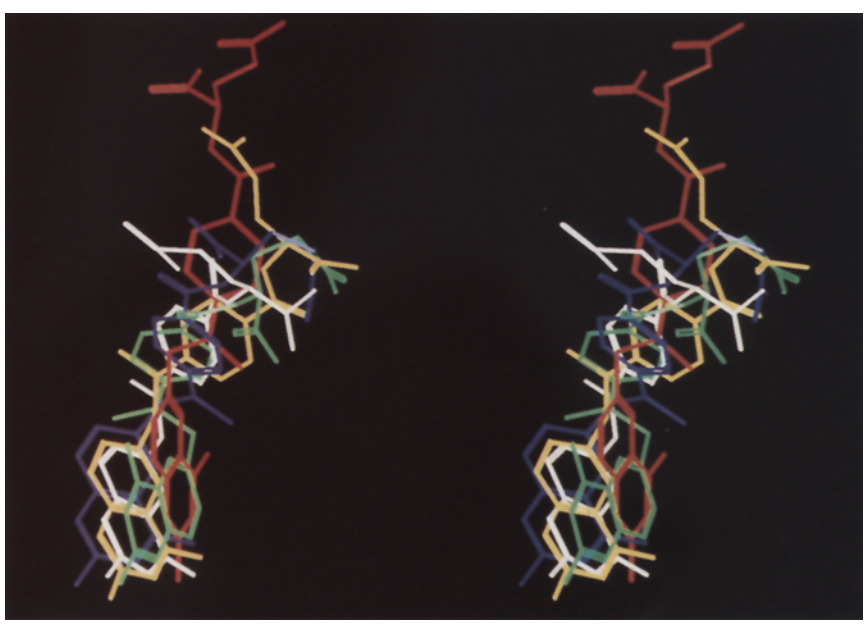

Fig. 5. Stereoview of docked conformers relative to the X-ray structure (white), the CONCORD conformation (red), conformer A56 (yellow), conformer A04 (green) and conformer A15 (blue).

center corresponding to the 2-amino nitrogen (indicated with an asterisk in Fig. 1) was taken as the essential point. This choice of essential point restrains the search to binding modes where the ligand penetrates deeply into the active site. (Of course, the use of the MTX atoms as match points would probably be too biased if the goal were to search over libraries of diverse ligands. Our earlier paper [10] showed an unbiased method of generating match points for such searches.)

Column F in Table 1 shows the FLOG scores for the set of 80 conformations. Table 1 is sorted, beginning at the row labeled A56, according to the score in column F. The scoring units should be regarded as a semiquantitative measure of ligand–cavity complementarity, not as a measure of interaction energy. The first row of Table 1 shows the FLOG score for the X-ray conformation. The score for the rigid-body-optimized binding mode of the X-ray conformation is 94, not surprisingly better than the scores of any of the other conformations of MTX that were generated without reference to DHFR; it is essentially superimposed with the X-ray binding mode.

TABLE 2
FLOG SCORES FOR THE BEST FITTING CONFORMERS OF MTX

Conformer	Score	$mrmsd_{min}$	$mrmsd_{max}$	Difference	MaxDiff	rmsd
A00613	86	1.27	1.52	0.25	0.50	1.65
A00575	86	1.33	1.68	0.35	0.48	1.61
A00105	85	1.52	1.86	0.34	0.44	1.86
A01889	84	0.98	1.44	0.46	0.59	1.50
A00390	84	1.05	1.49	0.44	0.57	1.41
A01044	84	1.24	1.53	0.29	0.51	1.43
A01527	83	2.04	2.16	0.12	0.34	2.33
A01765	82	1.37	1.56	0.19	0.47	1.64
A00384	82	2.49	2.68	0.19	0.29	2.71
A00937	81	1.73	1.95	0.23	0.39	2.08
A01840	81	0.99	1.49	0.50	0.59	1.58
A00208	80	1.06	1.30	0.24	0.56	1.70
A01610	80	1.32	1.57	0.25	0.48	1.76
A01712	80	1.11	1.49	0.38	0.55	1.98
A01806	**80**	**0.90**	**1.38**	**0.49**	**0.62**	**1.34***
A01889	84	0.98	1.44	0.46	0.59	1.50*
A01840	81	0.99	1.49	0.50	0.59	1.58*
A00288	79	1.00	1.42	0.43	0.58	1.37
A00110	76	1.03	1.50	0.47	0.58	1.72
A00390	84	1.05	1.49	0.44	0.57	1.41*
A00208	80	1.06	1.30	0.24	0.56	1.70*
A01701	39	1.08	1.40	0.32	0.56	8.25
A01712	80	1.11	1.49	0.38	0.55	1.98*
A00397	76	1.14	1.59	0.45	0.54	1.78
A00621	60	1.16	1.54	0.38	0.53	1.87
A01824	76	1.17	1.49	0.33	0.53	1.69
A00930	74	1.19	1.46	0.27	0.52	1.82
A00300	78	1.20	1.58	0.38	0.52	1.87
A01742	46	1.21	1.54	0.33	0.52	6.07

Shown are the behavior of the top FLOG scores, the best fitting mrmsd values (minimum and maximum of the eight local topology permutations) and the rmsd to X-ray. Also shown are the observed differences between minimum and maximum mrmsd and a maximum difference (MaxDiff) computed as follows: $\text{MaxDiff} = \sqrt{(mrmsd_{min}^2 + 8 \times 2.5^2/33)} - mrmsd_{min}$. The upper section shows the 2000 conformers ordered by FLOG score, the lower section is ordered by $mrmsd_{min}$. The asterisks in the lower section indicate that these conformers are common to both sections.

Figure 5 depicts the best scoring conformers (A56, A04 and A15) along with the CONCORD structure (A00), docked in the active site of DHFR. Obviously, structure A00 intrudes into the enzyme whereas the others bend around it. In general, much of the positive score results from the correct fitting of the pteridine moiety of MTX. A15 (blue) is slightly too bent for the cavity. A04 (green) explores the *inverted* ring binding mode used by dihydrofolic acid. All the best docked conformations also superimpose a carboxylic acid oxygen on an oxygen of the α- and γ-carboxylates of the X-ray structure, which are hydrogen-bonded to Arg^{57} and His^{28} on DHFR, respectively.

Examining the FLOG scores for the 2000 conformers, 135 had scores ≥ 70 and 15 had scores ≥ 80. The two parts of Table 2 show the relationship between score, mrmsd and rmsd for the highest scoring conformations and for the conformations closest to the X-ray conformation.

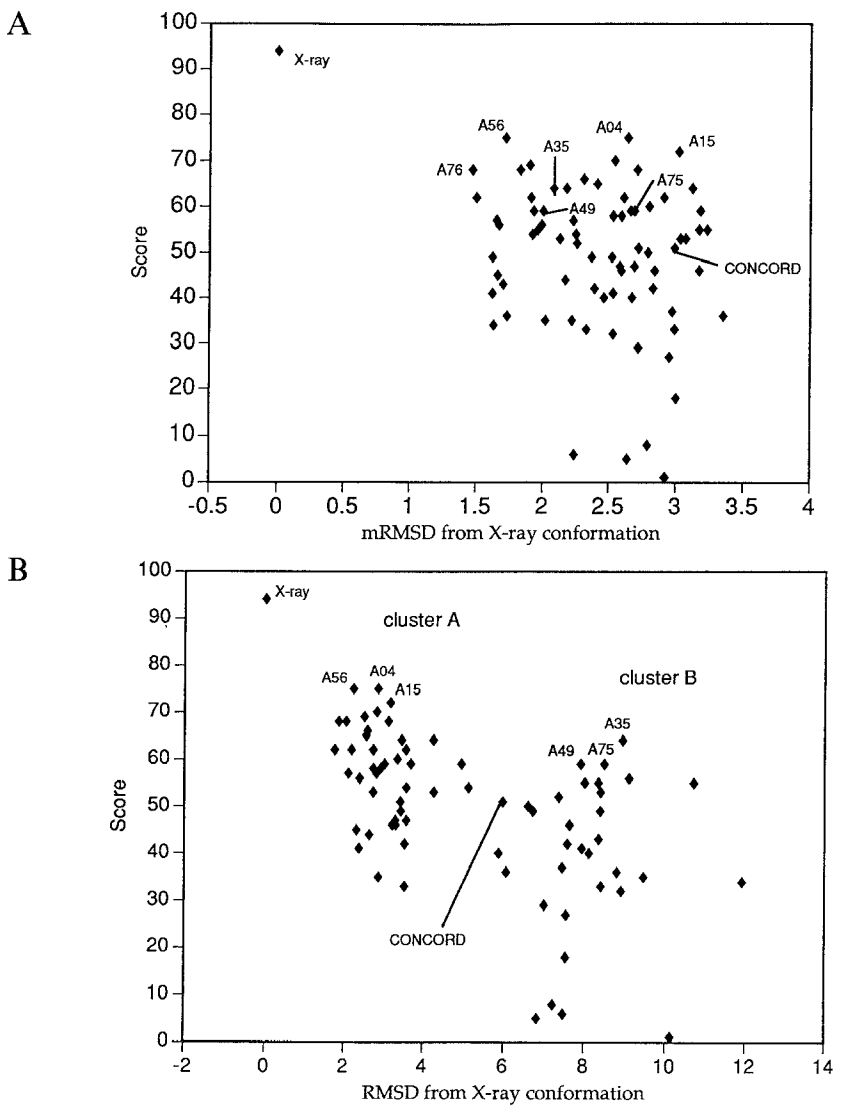

Fig. 6. FLOG score versus (A) mrmsd and (B) rmsd, in Å, from the X-ray conformation.

Mrmsd and score

There is little or no correlation between the docking score in column F and the mrmsd between the X-ray configuration in column C in Table 1 (see Fig. 6A). The top three scores have mrmsd values of 1.73, 2.64 and 3.02 Å, the last one being as dissimilar to the X-ray structure as is the CONCORD configuration. A correlation is slightly more apparent if the rmsd, not the fitted mrmsd, is measured between the docked and X-ray structures (see column E and Fig. 6B). Furthermore, in Fig. 6B, there are clearly two clusters, one slightly tighter than the other. The looser cluster (B) contains some exceptions that have borderline scores (~60 units), in particular, A35, A49 and A75. These are essentially docked in reverse, with the acid groups buried.

Table 2 examines the top scoring and best fitting conformers from the set of 2000. The best fitting conformer had an mrmsd of 0.90 Å and a FLOG score of 80. The table also shows the results of including topological symmetry permutations. It was found that 14 conformers scored better than the best fitting conformer. The best score was 86. Their best mrmsd values varied between 0.90 and 2.49 Å. The reverse correlation holds better – very small mrmsd values lead to good scores.

Energy evaluations

We have chosen to focus on shape as the primary criterion for selecting compounds. An alternative approach is to choose molecular energy as the selection criterion. We investigate the possible effects on selection by energy minimizing pertinent conformations. All the following energy evaluations are conducted in isolation – not in the presence of the receptor. The energy of the X-ray conformation of MTX was optimized to its nearest local minimum using Batchmin [36]. This minimum energy structure, referred to as Xmin, has an energy of −411 kJ/mol (Table 1). Xmin differs from the X-ray conformation by slightly canting the pteridine rings about their long axis and making a marginal twist, about 30°, around the bond between the amide nitrogen and the chiral carbon that attaches the two acid groups. Its fitted mrmsd to the X-ray structure is 1.45 Å. The conformational space for MTX was systematically searched using the SPMC option in Batchmin [37] about the 10 relevant torsions (dashed bonds in Fig. 1). The two lowest energy minima found, labeled as Gmin and Nmin in Table 1, had energies of −429 and −420 kJ/mol, respectively.

DISCUSSION

We try to show in this paper, with a single pertinent example, why moderate expansion to a flexibase is much better than no flexing at all. In particular, our criterion for success is that at least one of a limited number of MTX conformations generated in the absence of DHFR can attain a docking score comparable to that of the bound form of MTX. Our earlier paper [10] demonstrated that at least one conformation of MTX is selected as more complementary to DHFR than nearly all other drug-like molecules.

The discussion centers on the following points: (1) why a maximum dissimilarity criterion for selection rather than using a clustering algorithm; (2) why the raw mrmsd, although not a perfect measure of shape similarity, is sufficient for the dissimilarity selection process; (3) why the relatively coarse exploration of conformational space in our implementation of FLXGEN is sufficient for docking studies; (4) why there is little or no correlation between mrmsd and docking score; (5) why energy minimization and energy selection criteria are not necessary.

(1) An alternative method of selecting conformations from a large set is to cluster the original conformations and then select the conformations at the *center* of each cluster [38]. For clustering algorithms to be effective there must be clusters present. However, based on our mrmsd criterion, conformers tend not to cluster tightly, if at all, even with energy minimization. Moreover, there is the new problem of deciding which conformer to choose as a representative from irregular or chained clusters. The dissimilarity selection algorithm guarantees that no two conformers are more similar than the limiting mrmsd – this cannot be said of selections made from any clustering algorithm.

(2) The mrmsd we use in conformation selection depends on atom labels, and does not take into account that some atoms might be topologically equivalent. This is also true for the rmsd comparison of the FLOG binding modes to the observed binding mode. For instance, in MTX there are four sets of atoms that are equivalent by a twofold local symmetry transformation, i.e., two sets of carbons in the phenyl group and two sets of carboxylic acid oxygens. It is possible to calculate an $\text{mrmsd}_{\text{min}}$, the minimum mrmsd for all permutations of equivalent atoms. Thus, mrmsd might overestimate $\text{mrmsd}_{\text{min}}$ and can indicate that conformations are different when in fact they are completely equivalent. The problem with using the mrmsd versus the $\text{mrmsd}_{\text{min}}$ then, is that equivalent conformations can be saved in the selection process. Should the total number of conformers, including the equivalent ones, exceed 25, the limiting mrmsd could be raised too high. The worst case for mrmsd is $\text{mrmsd}_{\text{max}}$, where each set of equivalent atoms is mismatched. Table 2 lists the $\text{mrmsd}_{\text{min}}$ and $\text{mrmsd}_{\text{max}}$ for MTX conformers from the set of 2000 and shows that the relative difference is not very great. Thus, the calculation of $\text{mrmsd}_{\text{min}}$, the expense of which increases rapidly with the number of equivalent atoms, is not necessary and mrmsd will suffice.

(3) We now address the question whether our implementation of FLXGEN covers conformational space sufficiently to be useful in docking studies. The idea of FLOG is to generate a list of compounds that are potentially complementary to a receptor. The score of a given compound is taken as the maximum score over all its conformations, including mirror reflections. (Which conformation is the most complementary and in what mode it binds are of lesser importance.) We expect known ligands to have a higher score than almost all the compounds in a diverse flexibase. Normally a user would inspect only the highest scoring few percent of the compounds.

In an earlier paper [10], we presented the distribution of FLOG scores on DHFR for the MINDEX [39] flexibase, which contains about 60 000 conformations of 7600 compounds. Many known folate analogs within MINDEX docked, with raw scores (scores not normalized by molecular size [10]) varying from 69 to 82; these are within the top 50 compounds. From the overall distribution of scores for DHFR, we conclude that for a molecule the size of MTX any score above 70 should be considered good, any between 60 and 65 could be considered marginal (i.e., picking up only a few of the important binding points within the site) and any below 60 might be considered poor.

Clearly, having multiple conformations of MTX is important. Most docking studies have been done with a single CONCORD conformation (see Fig. 2, which compares the X-ray and CONCORD conformations). The CONCORD conformation of MTX is too extended to fit into the active site of DHFR (hence its low score of 51). If the CONCORD structure were the only representative conformation of MTX, it might be missed as a candidate ligand.

Are the FLOG results dependent on particular selections of maximally dissimilar conformers? For MTX to be discovered by FLOG, at least one conformer of MTX must have a score ≥ 70. The fact that at least one such conformer is found in all 10 random dissimilarity selection trials

(Table 1) indicates that, at this level of precision, the ability of FLOG to find MTX is not sensitive to the particular subset of selected conformers.

What are the chances that no conformer of MTX scoring above 70 would be produced by our conformation generation procedure? Examining the FLOG scores for the 2000 conformers, 135 scored 70 and above (6.75% of the conformers). Therefore, the probability of *not* finding a single conformer in 80 distance geometry projections with such a score is 0.37%. This is acceptable, so the empirical parameter settings for Eq. 1 are justified in this context. If 25 conformers were *randomly* selected, then the probability of *not* selecting one that scores 70 and above is 17%; fairly low but possible.

(4) The fact that there is no correlation between mrmsd and docking score may be somewhat surprising – even the best scoring conformers still had mrmsd values that varied substantially; see the top half of Table 2. One would expect that the more similar a conformation is to the X-ray structure, the more likely it will bind and score like the X-ray structure. This is probably true in the limit when the mrmsd is in the range of the resolution of the scoring grid (usually 0.25 to 0.50 Å). The explanation of this lack of correlation has to do with precisely what the mrmsd and docking score measure. The mrmsd is an average distance between corresponding atoms which we take as a sufficient measure of structural similarity. However, even at a value of 1.0 Å, the individual distances at any local point between conformers may vary much more than this value. In general, the scoring function is very sensitive to shape (and property) complementarity between ligand and receptor. A few distances can influence the score dramatically; picking up hydrogen bonds or a small set of localized distances that may describe a hydrophobic interaction are highly beneficial to the score. Alternatively, one or two atoms from the ligand that penetrate too far into the receptor are extremely deleterious, but the mrmsd is not sensitive to displacements of single atoms. Conversely, different conformations may interact with the same binding points on the receptor, for instance the α- and γ-carboxylates of MTX may exchange places. Moreover, different conformations may also find very different but equally satisfactory binding modes with the receptor.

(5) There are several points to make regarding energy evaluations. It is apparent, at least according to the MM2 energy parameterization, that the X-ray conformation does not possess the lowest possible energy in the absence of DHFR. It is at least 18 kJ/mol higher in energy than its nearest local minimum. There is precedence for this observation: the antifolate drug trimethoprim shows similar behavior with respect to DHFR [40]. For a relatively minor change in conformation, the docking of the Xmin was dismal (it scored 40 dock units, Table 1). The two low-energy minima both dock with barely acceptable scores (around 60, Table 1); in particular, Gmin, the global minimum, docked backwards with the acid groups deep within the enzyme pocket, as seen by the large value of 7.97 Å for the rmsd (column E in Table 1). Certainly, selecting conformations based solely on a minimum energy criterion will not improve the potential of finding a complementary fit and may even hinder it. Therefore, especially in the context of database scans, the CPU time invested in finding these minimum-energy configurations is not merited.

CONCLUSIONS

In this paper we have demonstrated the following: (i) A carefully constructed flexibase is a viable way of introducing conformational flexibility into docking methods that assume rigid ligands. (ii) Many possibilities are overlooked in using databases that contain a single repre-

sentative conformation. In the case of methotrexate, the CONCORD structure was inadequate. (iii) Rigorous energy minimization of the conformations was unnecessary and even deleterious in finding a docking conformer. This is understandable, since the bound form, when taken out of the context of the receptor, is unlikely to be in a minimum-energy configuration. This was the case with methotrexate. (iv) The dissimilarity algorithm for selecting representative conformations using the mrmsd similarity criterion (fitted rmsd) was sufficient to ensure shape space coverage. (v) The mrmsd describes molecular shape differences adequately, in spite of inaccuracies due to local topological symmetries. (vi) There was little or no correlation between the mrmsd and the dock score at reasonable conformer sampling densities. One need not have mrmsd values close to the X-ray structure to find a conformer that fits the receptor well. In short, there is no simple molecular feature that can be used which will a priori correlate with quality of fit.

To find new leads using our docking program FLOG [10], we chose our flexibase construction criteria to achieve a practical compromise: that of adequate sampling of molecular shape space and search time considerations. We have illustrated the benefit of this database expansion by docking methotrexate, a flexible molecule, into dihydrofolate reductase. This example demonstrates that, using a flexibase, this known folate analog would always be identified.

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