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Automatic superposition of drug molecules based on their common receptor site

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SUMMARY

We have previously developed a new rational method for superposing molecules in terms of submolecular physical and chemical properties, but not in terms of atom positions or chemical structures as has been done in the conventional methods. The program was originally developed for interactive use on a three-dimensional graphic display, providing goodness-of-fit indices on molecular shape, hydrogen bonds, electrostatic interactions and others.

Here, we report a new unbiased searching method for the best superposition of molecules, covering all the superposing modes and conformational freedom, as an additional function of the program. The function is based on a novel least-squares method which superposes the expected positions and orientations of hydrogen bonding partners in the receptor that are deduced from both molecules. The method not only gives reliability and reproducibility to the result of the superposition, but also allows us to save labor and time. It is demonstrated that this method is very efficient for finding the correct superposing mode in such systems where hydrogen bonds play important roles.

INTRODUCTION

In the case that the receptor structure is unknown, superposition of known drug molecules gives a good starting point for further drug development [1,2]. Many attempts of molecular superpositions have been done in order to extract the common structural features from molecules that show similar biological activities. The most popular method for superposing molecules is that by a least-squares calculation which optimizes the matching of atom positions between molecules. The method can yield the least-squares residual as an index of the similarity of the two structures, but it requires an explicit assignment of atom correspondences before the similarity can be assessed. So it is very difficult by this method to reach the correct superposing model for dissimilar

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molecules where atom correspondences are not clear, although this problem does not occur for isosteric structures since the correspondences are clear. On the other hand, the interactive method, which superposes molecules on a 3D computer graphic display based on a visual judgement, can be applied to any molecule because it does not require advance assignments of atom correspondences. However, with this method, degrees of structural similarities cannot be easily assessed by numeral indices.

Both superposing methods described above are not satisfactory for the purpose of drug design. It is necessary to develop a new method which can find, without preconception, a correct superposition model as regards atom correspondences or superposing modes between molecules. Recently, Dean et al. have reported a new method for unbiased search for an optimal molecular superposition based on simulated annealing and cluster analysis [3–6]. Although the method is based on the matching of atom positions, it does not require explicit assignment of atom correspondences in advance. If a set of atoms in the first molecule is specified, the second molecule is searched combinatorially by optimizing the difference-distance matrix for both molecules. Poorly matched atoms are rejected from the matching as null correspondences. The method seems to be useful to search the best superposition model based on molecular shapes.

However, the matching of atom positions is not necessarily essential for the specific binding to the common receptor although the similarity of molecular shapes is very important. For the specific binding to the receptor site, intermolecular interactions such as hydrogen bonding, electrostatic, and hydrophobic interactions are thought to be important. Therefore, molecules should be superposed in terms of physical and chemical properties involved in the interaction with the receptor, but not in terms of chemical structures or atom positions themselves. Moreover, for the purpose of extracting the essential structural requirement for the biological activities, superpositions of molecules with dissimilar structures are much more significant than those with similar structures.

We have previously developed a rational method of molecular superposition and implemented it in a computer program, called RECEPS [7–10] for the purpose of superposition of dissimilar molecules. With the RECEPS program, one can superpose molecules manually by making use of 3D grid points for evaluating the similarity of the spatial arrangements of submolecular physical and chemical properties of molecules. Molecules are manipulated interactively on the graphic display by rotation, translation and bond rotation, so as to get better goodness-of-fit indices for the various properties that are updated in real time during the superposing operation. Based on the superposed structures of multiple active molecules, a 3D receptor model can be constructed rationally. The main advantages of the RECEPS program are as follows: (1) Superposition based on the assumption of the common receptor; (2) Applicability to molecules with quite different structures; (3) No need for assignment of atom correspondences between molecules; (4) Various goodness-of-fit indices available for assessing similarities of various properties; (5) Various graphics representations of the receptor model.

Although the RECEPS program has been constructed based on the rational concept as described above, two serious problems remain that are common to all superposing methods. For truly rational superposition, the best superposing mode should be chosen without preconception from all the possible modes. At the same time, the conformational freedom of both molecules should be taken into account. In most of the previous molecular superposition studies, molecules have been superposed in the conformation found in crystals or that of the energy minimum, al-

though any superposition is meaningless unless the molecules are superposed in their active conformations. If the active conformation of the molecule is known or if the molecule has a rigid structure, the best superposing mode can be searched by systematic rotations of the molecule in 3D space, although it will require a vast amount of computation. But, there are many cases where we have no rigid active molecule appropriate for use in the superposition. Furthermore, since it is very difficult to determine the active conformations, the superposed structures of bioactive compounds are often used conversely in speculative attempts to identify these conformations. The superposing modes and conformations of the molecules are thus tightly interlinked, and neither can be neglected. Although the manual-fit function of the RECEPS program allows one to change arbitrarily both the conformation of the molecule to be superposed (that of the template molecule is fixed) and the superposing modes, it is quite difficult to examine all the possible conformations and superposing modes with the manual method. Moreover, there is no guarantee that the best superposition model obtained by the manual method is actually the best among all possible ones.

Dean et al. have also developed another method which searches best superposition of molecules not in terms of atom positions [11–13]. In their method, the goodness of the superposition is evaluated by pattern matches on molecular surfaces. A trial molecule is rotated in 3D space and highly scored orientations are selected by the objective function of the pattern match. It does not require any atom correspondences between molecules. The method would become a powerful tool for superposition of quite dissimilar molecules, if the calculation is so fast as can be used for a vast number of combinatorial sets which arise from conformational freedom of molecules. Their pattern-matching scheme is based on the shapes and the electrostatic potential of the molecular surfaces. However, the electrostatic potential seems to be rather insensitive for distinguishing different superposition modes sharply, compared to the hydrogen bonds. For the molecules where hydrogen bonds appear to be important for the binding to the receptor, it seems to be effective to use matches of hydrogen-bonding patterns as a first clue to the automatic search of the superposition modes.

Thus, we have developed a new method for searching the best superposition model automatically while covering all the possible superposing modes and conformational freedom. The method is based on the hydrogen-bonding characters of molecules. The method has been implemented as a new function, namely AUTOFIT, of the RECEPS program.

METHOD

The overall procedures in the AUTOFIT function are shown in the flow chart in Fig. 1. For the purpose of covering all possible superposing modes, we have adopted a method which performs combinatorial search for all possible correspondences of hydrogen-bonding functional groups between molecules, instead of searching the whole space by systematic 3D rotation and translation of the molecule. The correspondence means that a pair of hydrogen-bonding heteroatoms of two molecules can interact with a common hydrogen-bonding heteroatom in the receptor and does not mean the matching of their atom positions. The expected positions of hydrogen-bonding heteroatoms in the receptor are calculated as dummy atoms from all hydrogen-bonding functional groups in both molecules, considering the positions and orientations of X–H or X–lone-pair electrons (X = hydrogen-bonding heteroatom). The program calculates the positions of dummy atoms automatically according to the type of hydrogen-bonding heteroatoms that are defined as

shown in Table 1. The type number should be assigned to every hydrogen-bonding heteroatom in the molecules in advance.

If the molecule A has n_a hydrogen-bonding groups and the molecule B has n_b groups, and m groups are used to make pairs among them ($m \geq 2$ is preferable), $n_aCm \times n_bCm \times m!$ combinations are produced as the starting superposing modes to be considered. The denotation nCm is the number of combinations of extracting subsets with m elements out of a set with n elements and is calculated by $nCm = n!/[(n-m)!m!]$.

For each member of the combinations of heteroatom correspondences, various conformers of both molecules are generated systematically by torsional rotations of the rotatable bonds. Instead of the systematic generation of conformers, atomic coordinates of the conformers prepared in advance can be optionally read from files. If the numbers of conformers considered for both molecules are N_a and N_b, respectively, the number of total combinations to be examined in the later calculation becomes $n_aCm \times n_bCm \times m! \times N_a \times N_b$.

For each member of the combinations described above, an iterative least-squares procedure is applied. Figure 2 schematically shows the concept of the least-squares calculation, which optimizes the matching of both positions (Y_{Ai} and Y_{Bi}) and direction vectors (V_{Ai} and V_{Bi}) of the expected hydrogen-bonding sites in the receptor deduced from the heteroatoms of both molecules (X_{Ai} and X_{Bi}). The quantity F to be minimized in the least-squares calculation is shown in Eq. 1.

$$F = \sum_{i=1}^{m} w_i \left\{ \sum_{j=1}^{3} [Y_{Ai(j)} - Y_{Bi(j)}]^2 + \sum_{j=1}^{3} [V_{Ai(j)} - V_{Bi(j)}]^2 \right\} \tag{1}$$

m : number of pairs of hydrogen-bonding sites to be considered,
j : 1 for X-coordinate, 2 for Y-coordinate and 3 for Z-coordinate,
X_{Ai}: position of the i-th heteroatom in molecule A,
X_{Bi}: position of the i-th heteroatom in molecule B,
Y_{Ai}: position of the hydrogen-bonding site in receptor expected from X_{Ai},
Y_{Bi}: position of the hydrogen-bonding site in receptor expected from X_{Bi},
V_{Ai}: direction vector from X_{Ai} to Y_{Ai} ($V_{Ai} = Y_{Ai} - X_{Ai}$),
V_{Bi}: direction vector from X_{Bi} to Y_{Bi} ($V_{Bi} = Y_{Bi} - X_{Bi}$),
w_i : weight for the i-th pair (calculated from the result of the previous iteration).

The weight w_i is expressed as a product of the weight ws_i (0 or 1) for switching the use of each pair of hydrogen-bonding sites and the weight wb_i (varies from 0 to 1) for taking balance between pairs of hydrogen-bonding sites which belong to the same heteroatom.

$$w_i = ws_i wb_i \tag{2}$$

The weight ws_i is determined to favor pairs of hydrogen-bonding sites with good fit. At the beginning of the iteration, ws_i is set to 1 for all pairs. If hydrogen-bonding characters of a pair of sites do not agree (for example donor character is expected from the molecule A and acceptor character is expected from the molecule B), the weight ws_i for the pair is reset to zero and the pair is treated as a null correspondence. At every iteration of the least-squares calculation, disagreements of the positions (Y_{Ai} and Y_{Bi}) and the vectors (V_{Ai} and V_{Bi}) are evaluated by using the coordinates from the previous iteration and the weight ws_i is reset to zero if either of them exceeds predefined limits.

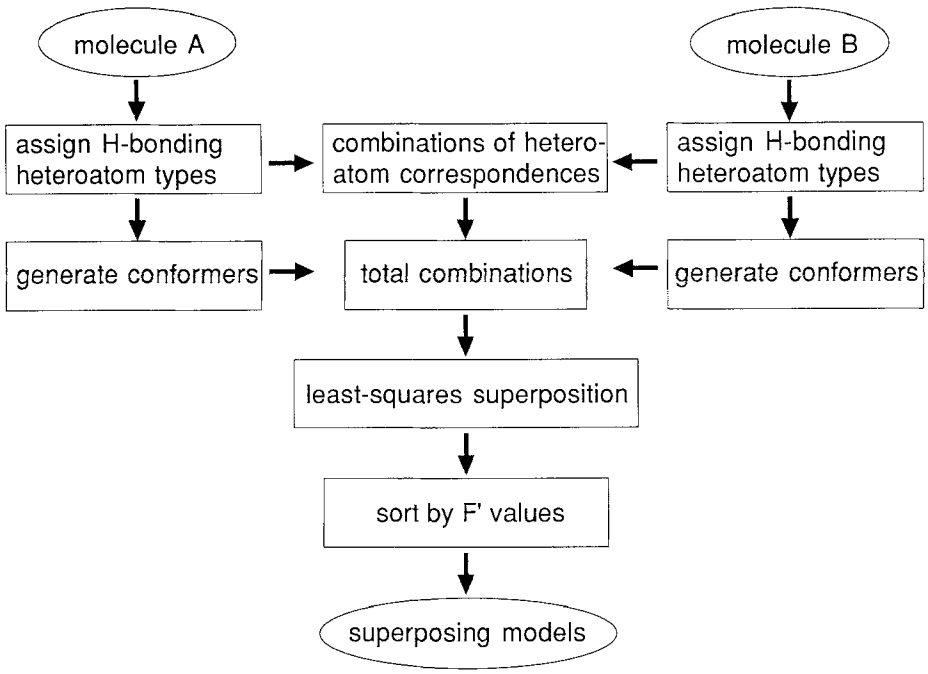


Fig. 1. A flow chart of the AUTOFIT function.

$$wb_i \propto 1/\left\{ \sum_{j=1}^{3} [Y_{Ai(j)} - Y_{Bi(j)}]^2 + \sum_{j=1}^{3} [V_{Ai(j)} - V_{Bi(j)}]^2 \right\} \tag{3}$$

The weight wb_i is used to balance between more than two hydrogen-bonding sites that are expected from a heteroatom. In the case where only one hydrogen-bonding site is expected from a heteroatom, the weight wb_i is set to 1. If a heteroatom has more than two possible hydrogen-

TABLE 1
HYDROGEN-BONDING HETEROATOM TYPES IN THE RECEPS PROGRAM

1. primary amine N (sp2)	13. carboxylate anion O
2. primary amine N (sp3)	14. carboxylic acid O (with hydrogen)
3. ammonium ion N	15. phosphate O
4. amide N	16. water O
5. secondary amine N	17. thiol S
6. aromatic N	18. thioether S
7. aromatic N protonated	19. hydroxyl O (hydrogen position fixed)
8. tertiary amine N	20. thiol S (hydrogen position fixed)
9. tertiary amine N protonated	21. hydroxyl O (explicit H, acceptor only)
10. hydroxyl O	22. hydroxyl O (explicit H, donor and acceptor)
11. ether O	23. hydroxyl O (explicit H, donor only)
12. carbonyl O	

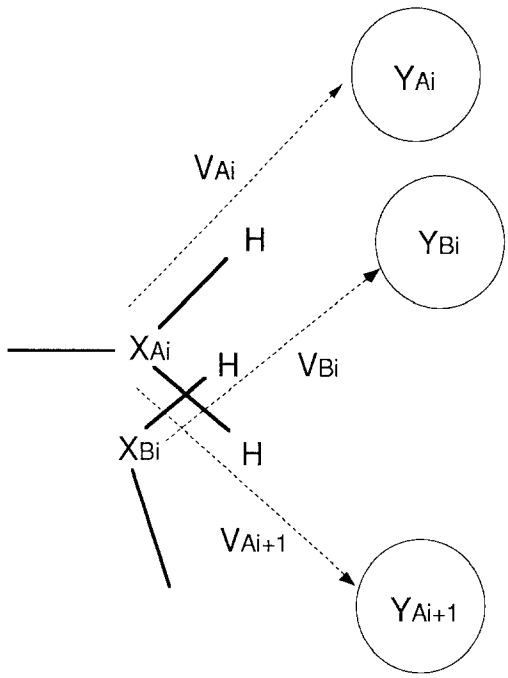


Fig. 2. Schematic expression of the least-squares method of the AUTOFIT function. Positions (Y_{Ai} and Y_{Bi}) and direction vectors (V_{Ai} and V_{Bi}) of hydrogen-bonding sites expected in the receptor are superposed.

bonding sites, the weights wb_i for those sites are calculated by Eq. 3 at every iteration by using the coordinates from the previous iteration. The weight wb_i is normalized so that the sum of the weights for the sites that are expected from the same heteroatom becomes 1. The weight wb_i is set to 1 for the site where the denominator in Eq. 3 is smaller than a predefined limit. In the schematic example of the superposition shown in Fig. 2, the program appropriately superposes the position and the vector of the molecule B to either of two possible sites of the molecule A by this weighting scheme.

The program repeats the least-squares fitting and the re-evaluation of the weights until a root-mean-square value of the shifts of atom coordinates becomes less than a predefined limit. After the convergence of the calculation, a score value F′ is calculated by Eq. 4. The second term is introduced for adjusting the least-squares residual value F to be used as an index of the goodness of superposition. If a superposition model has greater numbers of site pairs with favorable fit, this term biases F′ to a more negative value.

$$F' = F - \sum_{i=1}^{m} w_i R_{HB}^2 \tag{4}$$

R_{HB}: hydrogen-bonding distance (here assumed to be 3.1 Å).

For each member of the total number of combinations, the program outputs a set of atomic coordinates of superposed molecules and the score value F′. The more negative the F′ value, the

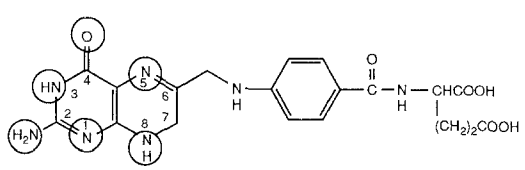
better the matching of hydrogen-bonding patterns between the molecules. The obtained superposition models can be analyzed by sorting with the F′ value and by grouping with patterns of heteroatom correspondences.

RESULTS AND DISCUSSION

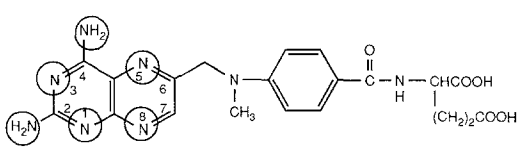
In order to show the usefulness of the AUTOFIT function, we first attempted to search for the best superposition of the pteridine ring of dihydrofolic acid (abbreviated as DHF) (**1**) onto that of methotrexate (MTX) (**2**), based on the assumption that they bind to a common receptor. DHF is the true substrate of the enzyme dihydrofolate reductase, whereas MTX is a potent inhibitor of the enzyme. Although the two structures are very similar, the carbonyl group at C4 in DHF is replaced by an amino group in MTX. The 6 hydrogen-bonding functional groups on the pteridine ring of both molecules, which are encircled in the chemical formula, make 720 combinations in total (derived from ${}_6C_6 \times {}_6C_6 \times 6!$). Although crystal structures of dihydrofolate reductase are known, we have tested all the 720 superposing modes without any preconception on the receptor environment.

For each member of the 720 combinations, the two pteridine rings were superposed by the least-squares calculation of the AUTOFIT function. For comparison, the conventional least-squares calculation which optimizes the matching of the atom positions of 6 heteroatoms between molecules was also performed. Table 2 shows the results obtained by both methods that are ordered either by the residual value of the conventional least-squares calculation or by the score value F′ of the AUTOFIT function. In the results of the conventional method, the superposing mode with the best residual value is tentatively named model A and that with the second best value is named model B. By the AUTOFIT function, model B was the best, and model A was ranked 156th from the best among 720 combinations.

The superposed structures of model A and model B are shown in Fig. 3. The two pteridine rings are just fitted in model A, whereas the pteridine ring in DHF is reversed in model B. Which is the correct superposition, model A or model B? This question can be answered by considering the stereospecificity of the enzymic reaction of DHF. It has been proved that the β-hydrogen atom at C6 in tetrahydrofolic acid (**3**), which is produced by the reaction, originates from the cofactor NADPH [14]. Although the binding mode of DHF to the enzyme is not yet experimentally known, the correct binding mode of the DHF pteridine ring can be presumed by the required relative position between the DHF pteridine ring and NADPH for this β-hydrogen outcome. Since the crystal structure of the ternary complex of enzyme–NADPH–MTX has been elucidated [15] as shown in Fig. 4, we know from which side of the pteridine ring of MTX the hydride ion from NADPH is coming. If the DHF pteridine ring binds to the enzyme in the same binding mode as that of MTX (as shown in Fig. 5A), the enzyme would produce tetrahydrofolic acid with the op-



(**1**) dihydrofolic acid (DHF)



(**2**) methotrexate (MTX)

TABLE 2
THE RESULTS OF THE TWO SUPERPOSITION METHODS CALCULATED FOR 720 COMBINATIONS OF 6 HETEROATOMS OF PTERIDINE MOIETIES OF DHF AND MTX

Conventional method[a]				AUTOFIT function			
Order	rms residual	Model no.		Order	F′	Model no.	
1	0.013	1	model A	1	−95.752	270	model B
2	0.075	270	model B	2	−79.560	156	
3	2.926	567		3	−76.736	317	
4	3.009	577		4	−76.663	577	
5	3.093	305		5	−76.637	269	
6	3.110	120		6	−76.634	509	
7	6.411	451		7	−76.573	268	
8	6.478	720		8	−70.144	533	
9	7.039	144		9	−69.586	579	
:				:			
:				156	−38.440	1	model A
:				:			
720				720			

[a]The result of the conventional least-squares method which minimizes $F = \sum_{i=1}^{m} w_i \sum_{j=1}^{3} [X_{Ai(j)} - X_{Bi(j)}]^2$ where X_{Ai} and X_{Bi} are the positions of heteroatoms.

posite stereochemistry at the C6 position (**4**). The binding mode A corresponds to the superposition model A, whereas the superposition model B corresponds to the binding mode B shown in Fig. 5B. In the binding mode B, the pteridine ring is reversed by rotation around the C6–C9 bond. Five hydrogen bonds stabilize the interaction between DHF and the enzyme in the binding mode B, whereas in the binding mode A, only 3 hydrogen bonds are formed and severe electrostatic repulsion is expected between the carbonyl oxygen of Leu[4] in the enzyme and the carbonyl oxygen

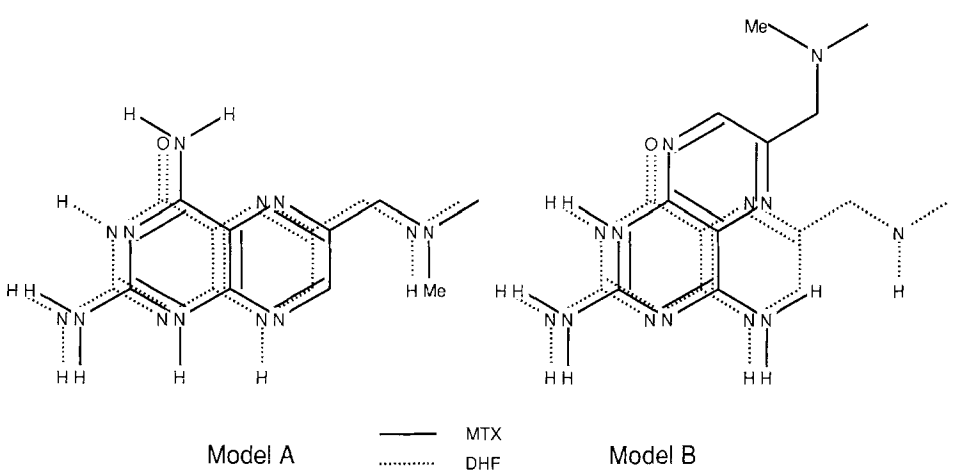
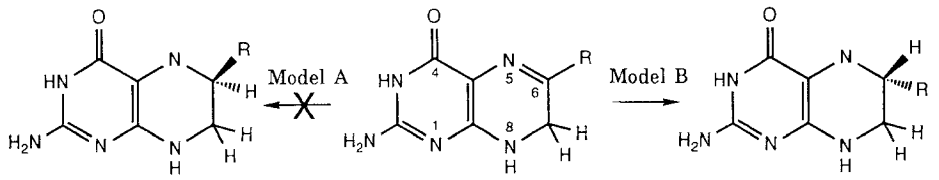


Fig. 3. Two superposing models of pteridine moieties of DHF and MTX.



at C4 of the DHF pteridine ring, as can be seen in Fig. 5A. There seems to be no steric hindrance between the enzyme and DHF in both binding modes. Thus, binding mode B has been accepted as the preferred binding mode of DHF [15]. Recently, crystal structures of *E. coli* [16] and human [17] dihydrofolate reductase complexed with folic acid were published. In these structures, folic acid, which is closely related to DHF, binds to the enzyme in the orientation similar to that of the binding mode B.

As regards the conformational degrees of freedom of both molecules, the program can automatically test all the conformations that are generated by systematic rotations of several torsion angles combinatorially. In order to test this function, we extended the range of the superposition of DHF and MTX to the amide bond of the glutamate moiety. In addition to the 6 heteroatoms of the pteridine rings, carbonyl oxygen of the amide bond was included as one of the heteroatoms to be used. The conformation of MTX was fixed to that in the crystal structure of the enzyme–NADPH–MTX ternary complex. The conformation of DHF was changed systematically by rotations of two bonds with an angle step of 30°. The side chain and carboxylate group of the glutamate moiety of both molecules were omitted in the calculation. The total number of trial combinations of hydrogen-bonding sites and conformations was 725760 ($= {}_7C_7 \times {}_7C_7 \times 7! \times 12^2$) and the calculation took about 20 700 s on an IRIS 4D/220GTX workstation (CPU 25 MHz R3000). Figure 6A shows the best superposition model that was selected by the F′ value of the AUTOFIT function. For comparison, an 'experimental' superposition model of MTX and folic acid, which

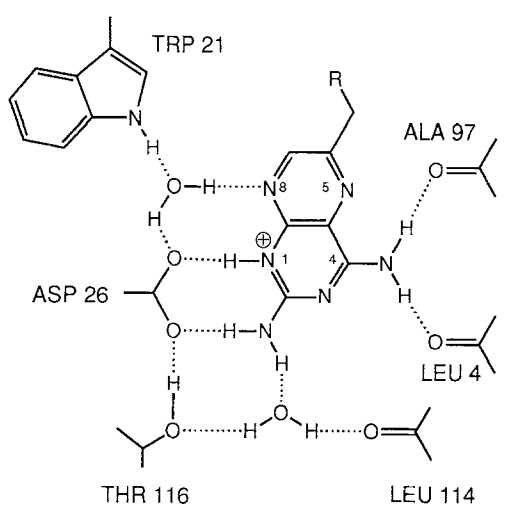


Fig. 4. Binding mode of MTX to *L. casei* dihydrofolate reductase found in the crystal structure of the enzyme–NADPH–MTX ternary complex [15].

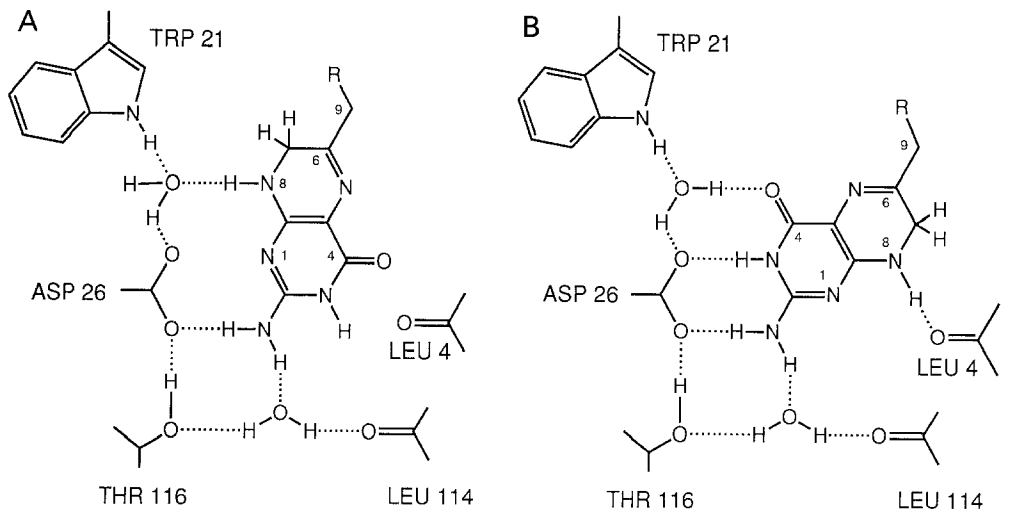


Fig. 5. Two presumed binding modes of DHF to *L. casei* dihydrofolate reductase. (A) Binding mode A; (B) Binding mode B.

is extracted from the superposed crystal structures of dihydrofolate reductase complexed with MTX [15] and folic acid [16], respectively, is shown in Fig. 6B. The superposition model by the AUTOFIT function is very similar to the 'experimental' superposition model as regards the positions and directions of functional groups and the conformations of molecules.

Introduction of the conformational degrees of freedom explosively increases the total number of combinations to be tested, and the total number of combinations practically allowed is limited according to the speed of the calculation. The current version of the RECEPS program is written for interactive use on the 3D graphics of the IRIS workstation. Without the overhead of graphics processing, the computation would be much faster. Furthermore, filtering of trial combinations prior to the least-squares calculation by some methods, such as that by applying distance constraints to the pairs of expected sites, would reduce the amount of computation. The improvement of the algorithm for the acceleration of the calculation is now in progress.

If we want to use the program in a limited way so that the corresponded hydrogen-bonding heteroatoms must have the same hydrogen-bonding character (donor vs. donor or acceptor vs. acceptor), the number of combinations of atom correspondences subjected to the least-squares calculation could be greatly decreased. But we cannot neglect the case in which the interacting heteroatom in the receptor has ambivalent hydrogen-bonding character, such as an oxygen atom in the side chains of serine or threonine. In such cases, opposite hydrogen-bonding characters can be permissible at the receptor heteroatom. Instead of excluding superposing modes that have pairs of heteroatoms with mismatched hydrogen-bonding characters, our method assigns zero weight to such pairs during the least-squares procedure. Although our treatment might not be the best, it seems to be very difficult to deal with this problem of the ambiguity of hydrogen-bonding characters in a general way.

The AUTOFIT function was originally developed for the purpose of generating superior starting structures for the manual superposition method of the RECEPS program, covering all the possible superposing modes. In the AUTOFIT function, molecules are superposed in terms of hy-

drogen bonding without using 3D grid point data of the RECEPS program. In the example of the superposition of MTX and DHF, the score value F′ could be used as a good index for choosing the correct superposing mode. This seems to be a rather straightforward case, because the binding modes of MTX and DHF to the target receptor, namely dihydrofolate reductase, are mainly determined by the hydrogen bonding. In many cases, high-ranked superposition models obtained by the AUTOFIT function should be used as starting structures for more detailed manual superposition by using various goodness-of-fit indices such as for molecular shape, electrostatic potential and hydrogen bonds, that are evaluated by the 3D grid point data. The superposed structures thus obtained can be further refined by minimizing a combined index from the appropriately weighted goodness-of-fit indices by the Simplex algorithm.

In this paper, we have shown that the AUTOFIT function is a very powerful tool for searching the correct superposition mode in molecular systems in which hydrogen bonding plays a predominant role in molecular recognition. For systems in which the importance of hydrogen bonding is rather small, it seems to be necessary to develop another method or to use the manual method. The AUTOFIT function offers a new concept of molecular superposition, for a truly rational ap-

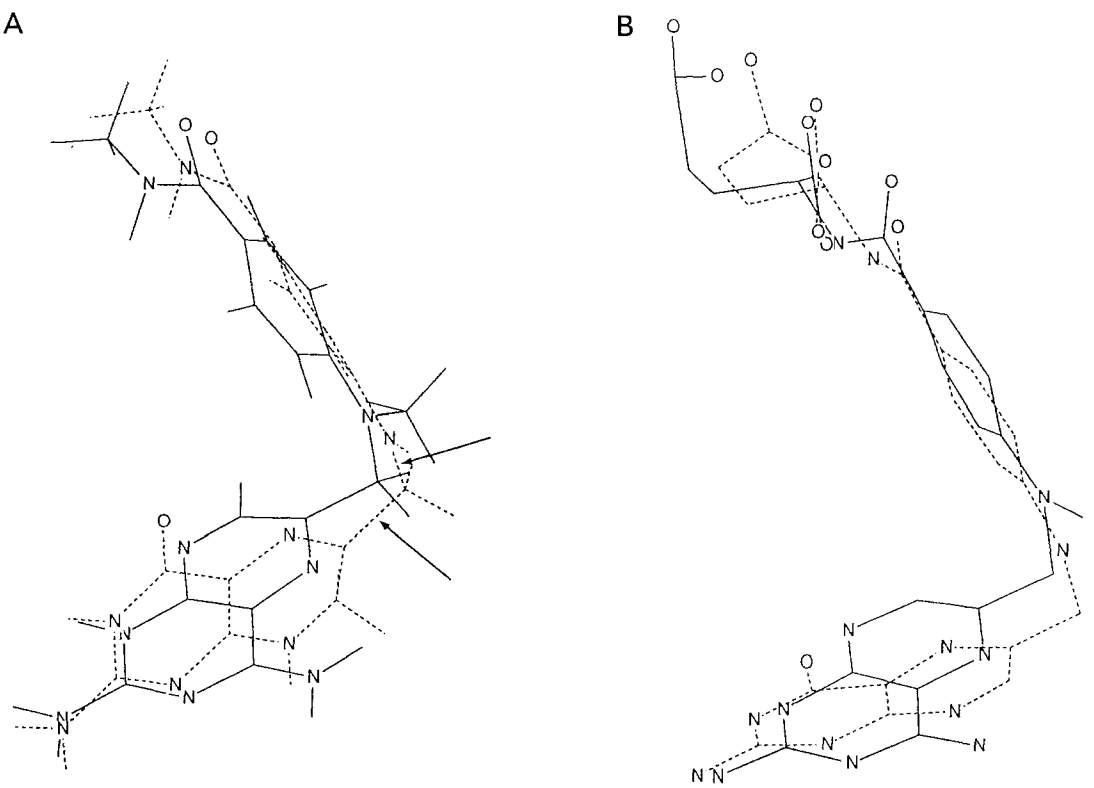


Fig. 6. Superposition including the conformational degrees of freedom. (A) The superposition model by the AUTOFIT function. Two bonds of DHF (marked with arrows) were rotated with an angle step of 30° (MTX: solid lines, DHF: dashed lines). (B) The 'experimental' superposition model obtained by superposing the crystal structures of *E. coli* dihydrofolate reductase complexed with MTX (solid lines) and folic acid (dashed lines), respectively. Hydrogen atoms are not elucidated by the X-ray analyses.

proach for drug design. It should save labor and improve the efficiency of the superposition processes, while providing reliable, objective and reproducible results. Papers on further applications of the method to more complicated systems with conformational freedom are now in preparation.

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