APPLICATION AND EVALUATION OF THE AUTOMATED DOCKING METHOD

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The method of automated searching for stable docking structures of protein-ligand complex , which is reported in the preceding paper, was applied to a dihydrofolate reductase–inhibitor system. The usefulness of the method was confirmed by the fact that the most stable docking models accurately reproduced the crystal structures of two enzyme–inhibitor complexes.

KEYWORDS docking simulation; protein-ligand interaction; ligand conformation; dihydrofolate reductase

In the preceding paper,[1] we reported the development of a new automated docking method. Here, we describe the application and the evaluation of our method.

Dihydrofolate reductase (DHFR) is a good system for testing the efficiency of our method, because it is one of the most extensively studied enzymes as a target of clinical drugs[2] and many crystal structures of complexes with various inhibitor molecules have been solved.[3] Atomic coordinates of the binary complex of *E. coli* DHFR and methotrexate (MTX),[3a] which was solved crystallographically with the highest resolution, were taken from the Protein Data Bank (PDB).[4] As the allowable region for the ligand to be docked, the substrate-binding site of the enzyme was prepared by removing the MTX molecule from the complex. A three-dimensional (3D) grid with an interval of 0.4 Å was generated inside the region and various data were tabulated at each grid point. All the water molecules were also removed, except for two molecules strongly bound to the enzyme at the bottom of the binding site. There were 10 hydrogen bonding (H-bonding) groups exposed in the region, which produced 13 dummy atoms.

Trimethoprim (TMP) and MTX molecules were docked to the enzyme, starting from the crystal structures of the isolated molecules[5] taken from the Cambridge Crystallographic Database.[6] For MTX, the structure without the terminal acetate moiety was used for simplicity, as shown in Fig. 1. Atomic charges in both ligands were calculated using the MNDO method[7] in the MOPAC program (version 6.0). H-bonding heteroatoms used for estimating possible H-bonding schemes and the rotated bonds are shown in Fig. 1.

Docking of TMP H-bonding schemes forming 3 H-bonds were searched, between 4 heteroatoms in TMP and 13 dummy atoms in the protein. The number of combination sets searched was $N(3) = {}_{13}P_3 \times {}_4C_3 = 6864$. As the

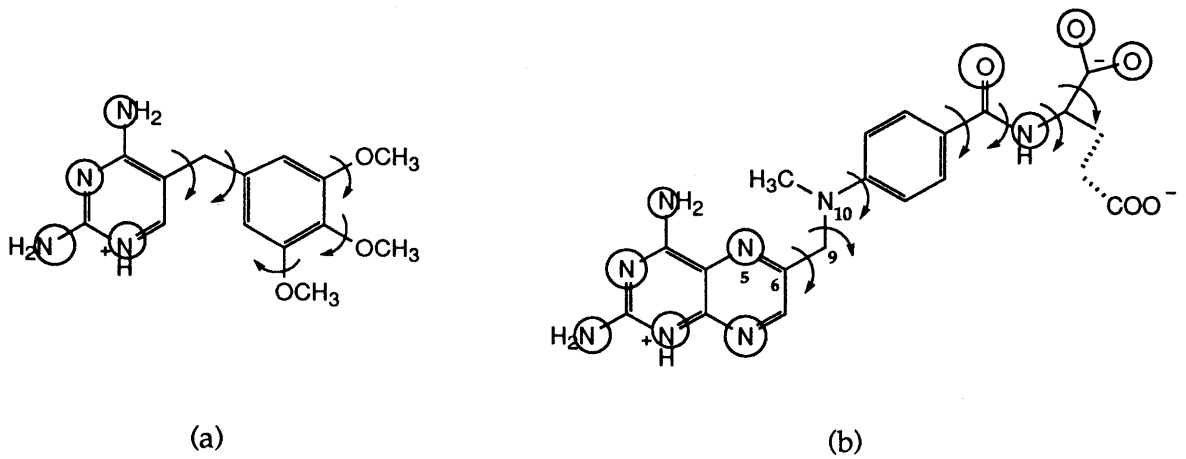


Fig. 1. Chemical Structures of (a) Trimethoprim (TMP) and (b) Methotrexate (MTX)

Heteroatoms used for predicting possible H-bonding schemes are encircled. Rotated bonds are shown by arched arrows. The acetate moiety in MTX which is neglected in this work is shown by dotted lines.

Table I. Summary of the Docking Results

Ligand	No.	Initial docking model		Final docking model		
		No. of H-bonds	Total energy (kcal/mol)	No. of H-bonds	Total energy (kcal/mol)	rms with crystal structure (Å)
TMP	1	1	–51.62	5	–71.15	
	2	1	–47.63	4	–59.21	
	3	2	–43.94	5	–76.18	
	4	2	–43.72	3	–57.99	
	5	2	–41.52	5	–75.63	
MTX	1	4	–85.01	8	–176.71	0.26
	2	3	–67.32	6	–158.15	1.15
	3	3	–61.27	6	–141.91	1.04
	4	3	–56.01	6	–159.06	1.18
	5	4	–53.53	6	–126.85	1.71

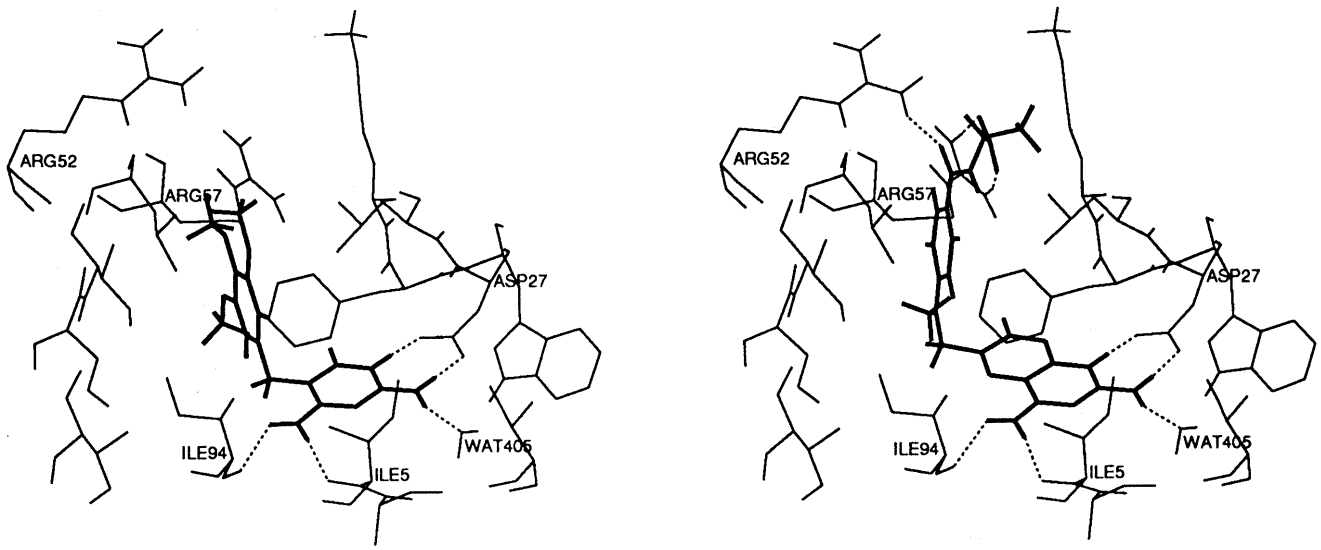
H-bonding part of TMP (the 2,4-diaminopyrimidine moiety) does not include any rotatable bond, conformations in the 5 rotatable bonds were considered only for possible H-bonding schemes of the moiety. Each bond was rotated by 120° first, and by 15° for the likely conformations. The whole docking process proceeded automatically to give 9 initial docking models.

Docking of MTX Between 10 heteroatoms in MTX and 13 dummy atoms in the protein, 6 or 5 H-bonds were attempted to be formed, and conformations for 7 rotatable bonds were considered for each of a great number of combination sets ($N(6) + N(5) = {}_{13}P_6 \times {}_{10}C_6 + {}_{13}P_5 \times {}_{10}C_5 = 269\ 619\ 840$). In order to cover such an enormous number of possibilities in a short computational time, the PP procedure was adopted. As a partial structure for the PP procedure, the structure from the pteridine ring to the benzene ring was indicated. The number of combination sets was reduced to 3168, by excluding unused heteroatoms and impossible H-bonding schemes. Finally, the program output 11 initial docking models.

The initial docking models were energy-minimized using the AMBER program.[8] For each ligand, energies and numbers of H-bonds of the five high-ranking models are summarized in Table I. It can be seen that both energy values and numbers of H-bonds were greatly improved by the AMBER minimization. In both cases, changes of ranking were seen in some models. These facts suggest that the influence of local adaptation of protein structure is not so small that it can be neglected. It should be noted that the results are influenced by the conditions used in the calculation such as force field, dielectric constant, and positions and number of water molecules.

The structure of the most stable final docking model for TMP (model no. 3 in Table I; Fig. 2(a)) well reproduced the crystal structure of the enzyme–TMP complex.[3b] The H-bonding scheme as well as the position and orientation of the TMP molecule were very similar to those of the crystal structure in the literature, although the similarity cannot be shown numerically because the crystal structure of the complex is not yet available in the PDB. The TMP conformations in the three most stable models (model no. 3, no. 5 and no. 1) were very similar to that in the crystal except for three methoxy groups. The variations in methoxy conformations, which were also observed in the two independent molecules in an asymmetric unit in the crystal, might be ascribed to exposure to the external environment.

The most stable docking model of MTX (model no. 1 in Table I; Fig. 2(b)) was also very similar to the crystal structure.[3a] The structural similarities of the models are shown by the root-mean-squares (rms) values to the crystal structure (Table I). The value for the most stable model was by far the best, 0.26 Å. The total energy of the model (–176.71 kcal/mol) was almost the same as that obtained by energy minimization of the crystal structure under the same conditions (–174.34 kcal/mol). Eight intermolecular H-bonds found in the crystal were reproduced in the model. The MTX conformation in the most stable model was very much like that in the crystal structure, but far from the input conformation. The torsion angle of N5-C6-C9-N10, which most clearly shows the conformational



(a) (b)

Fig. 2. Structures of the Most Stable Final Docking Models for (a) TMP and (b) MTX
The ligand molecules are shown by thick lines. Dotted lines represent intermolecular H-bonds.

differences, was −159.5° in the model, −162.2° in the crystal, and 42.0° in the input structure.

The effectiveness of our new docking method was shown by the fact that the crystal structures of two enzyme-ligand complexes were well reproduced automatically. The correct binding mode and ligand conformation were obtained, without using any presumptions, starting from arbitrary positions and conformations of the ligands. The final highest ranking model was the one corresponding to the observed docking structure.

One of the advantages of our method is the accuracy of the docking results, which owes to the successive energy minimization or structure optimization steps. The minimization steps consume more than 90 percent of the computational time required for docking, but as a result, the correct ligand conformation can be reproduced accurately. Even so, the search by our method is sufficiently fast. It took 19 minutes for docking TMP and 30 minutes (using the PP procedure) for MTX on an Iris 4D workstation (40 MHz, R3000).

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