PROTEINS: Structure, Function, and Genetics 19:174–182 (199

RESEARCH ARTICLES

Molecular Dynamics Simulation of the Docking of Substrates to Proteins

Alfredo Di Nola,[1] Danilo Roccatano,[1] and Herman J.C. Berendsen[2]
[1]*Diparimento di Chimica, Università di Roma, P.le A.Moro 5, 00185 Rome, Italy, and* [2]*Department of Biophysics and BIOSON Research Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, the Netherlands*

ABSTRACT A simple method is described to perform docking of subtrates to proteins or probes to receptor molecules by a modification of molecular dynamics simulations. The method consists of a separation of the center-of-mass motion of the substrate from its internal and rotational motions, and a separate coupling to different thermal baths for both types of motion of the substrate and for the motion of the receptor. Thus the temperatures and the time constants of coupling to the baths can be arbitrarily varied for these three types of motion, allowing either a frozen or a flexible receptor and allowing control of search rate without disturbance of internal structure. In addition, an extra repulsive term between substrate and protein was applied to smooth the interaction. The method was applied to a model substrate docking onto a model surface, and to the docking of phosphocholine onto immunoglobulin McPC603, in both cases with a frozen receptor. Using translational temperatures of the substrate in the range of 1300–1700 K and room temperature for the internal degrees of freedom of the substrate, an efficient nontrapping exploratory search ("helicopter view") is obtained which visits the correct binding sites. Low energy conformations can then be further investigated by separate search or by dynamic simulated annealing. In both cases the correct minima were identified. The possibility to work with flexible receptors is discussed.

Key words: molecular dynamics, docking, computer simulation, substrate docking, immunoglobulin, rational drug design

INTRODUCTION

Modern structural biochemistry based on high-resolution X-ray diffraction provides detailed information on drug–receptor complexes. This information enables us to visualize and understand interactions between macromolecules and ligands, and suggest ligand modifications to improve its binding affinity.

Drug design based on crystal structures relies at present largely on chemical intuition, aided by computer graphics. Thus one may suggest small changes to the ligand, but it is extremely difficult to design new chemical structures. More detailed computational techniques to predict binding energies—and free energies—are available for a given receptor–probe complex. The basic problem that remains to be adequately solved is the *docking problem:* the determination of the thermodynamically most favorable modes of binding between a receptor of known structure and a given, flexible or rigid, probe. The general docking problem shares two major difficulties with the protein folding problem: the (in)accuracy of the molecular force field and the search for a global free energy minimum in a very high-dimensional space.

In the case of a rigid probe and a rigid receptor, the docking problem essentially reduces to a six-dimensional search over translational and rotational degrees of freedom in some selected region around the binding site. Many different approaches have been employed, using scoring or penalty functions involving steric complementarity and distance geometry as well as pair potential functions, often in connection with Monte Carlo (MC) and simulated annealing techniques.[1–8]

The manual docking methods currently in widespread use[9–11] only explore a limited subset of conformations because of their need for intensive user interaction. Automated methods, using exhaustive search[12–15] or distance geometry methods,[1,16] allow

Received May 27, 1993; revision accepted March 29, 1994.
Address reprint requests to Herman J.C. Berendsen, Department of Biophysics and BIOSON Research Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, the Netherlands.

the exploration of a large area of conformational space, but require the use of simplified energetics models. The subject matter has been reviewed by Wodak et al.[17] and Rashin.[18]

Although some results are promising, we wish to emphasize that the development of general automatic docking methods is still in an early stage. In this article we investigate some modifications of dynamic simulations that allow a probe molecule to perform an efficient positional and conformational search on a rigid receptor surface, while preventing the molecule from being trapped in one of its local energy minima. The method, described in the next section, consists of a dynamic simulation in which the center of mass motion is handled separately from the internal motion of the probe molecule, and both types of motion are separately coupled to their own thermal baths. In addition, extra repulsive terms are added to provide a smoothing of the hard contacts. We apply the method both to a simple model system and to the binding of phosphocholine to an immunoglobulin (McPC603), which has been studied earlier by Goodsell and Olson[7] by Monte Carlo and simulated annealing techniques using affinity potentials on a grid.[19] Lee et al.[20] have recently calculated the free energies of binding of phosphorylcholine analogs to McPC603.

THE METHOD OF MOLECULAR DYNAMICS DOCKING

We consider a large receptor surface, such as part of a protein, and a smaller probe molecule, that we wish to dock onto the surface by a dynamic technique. That is, we shall perform molecular dynamics simulations on the system as a whole, but shall manipulate the *temperature* of various dynamic subsystems separately in order to allow fast search (high temperature) in some degrees of freedom while cooling those degrees of freedom that we do not wish to change. As subsystems we consider the translation of the probe, the internal motion in the probe, and the motion in the receptor.

The kinetic energy of a system with respect to the laboratory frame is given by $E_{kin} = 1/2\ \Sigma_i m_i v_i^2$, where v_i is the velocity with respect to the laboratory frame. In a coordinate system with origin in the center of mass (*c.o.m. frame*), the velocities are

$$v_{c,i} = v_i - V$$

where $V = \Sigma_i m_i v_i / M$ is the velocity of the c.o.m. in the laboratory frame (M is the total mass). The total momentum $\Sigma_i m_i v_{c,i}$ in the c.o.m. frame is zero.

The kinetic energy in the laboratory frame can be expressed as

$$E_{kin} = \frac{1}{2}\sum_i m_i v_i^2 = \frac{1}{2}\sum_i m_i v_{c,i}^2 + \frac{1}{2} MV^2$$

Let us call these two energies *internal energy* and *c.o.m. energy*, respectively. For an isolated molecule these two energies are completely uncoupled, as the motion of the center of mass can be changed only by external forces.

When the molecule (let's say substrate) interacts with another molecule (receptor), the force of the substrate due to the receptor affects both internal and c.o.m. energies of the substrate and effectively produces a coupling between them and, of course, between substrate and receptor. As a consequence, there is an energy flow between these three pools of kinetic and potential energies.

Let us now imagine a substrate approaching a receptor with a certain velocity. What happens is that it gets trapped and the kinetic energy of the c.o.m. is converted into internal energy of both substrate and receptor. For a molecule with several internal degrees of freedom there is a very low probability of a back conversion of energy. In this respect the external force, due to the receptor, acts as a friction force on the substrate.

In order to permit a large exploration of the receptor surface we have coupled the internal kinetic energy and the c.o.m. kinetic energy to two different baths with different coupling times. The coupling is effected by a first-order response function to a given bath temperature, according to the method of Berendsen et al.[21] This method provides a smooth coupling to a thermal bath with an exponential decay of the kinetic energy towards a prescribed value. It is one of the possible ways to impose constraints on the temperature of (parts of) a system of particles. The *extended system* methods of Nosé[22,23] and Hoover[24] are less useful in this case, because they involve a second-order response with possible oscillatory behavior.[25] The extended system methods have the advantage that they provide a proper canonical ensemble, but this property is not relevant in our case.

The time constant for the exchange of energy between the c.o.m. motion and the internal motion depends on the external forces and cannot be fixed a priori. We consider the receptor as rigid and hence couple its motion strongly to a bath of very low temperature in the present application. If we express the kinetic energies in temperature units we have an internal temperature of the substrate T_{int} and a temperature of the c.o.m. of the substrate T_c.

Whether trapping in a local minimum occurs depends on the bath temperatures and coupling constants. Trapping is often caused by detailed local minima produced by fine-grained hard contacts. Therefore we also add an additional repulsive force at a larger radius than the atomic repulsions to smooth the repulsive surface. This is one method of smoothing (with the disadvantage that it makes the substrate "thicker" and may prevent it from entering a narrow cleft), which may be replaced by other methods, such as a soft core repulsion.[26]

The advantages of this procedure are that

1. It permits an extensive exploration of the receptor surface.
2. It is possible to freeze the internal motion of the substrate and perform the so called "rigid docking."
3. It is possible to give high energy to the center of mass, so that it can climb over high barriers, while maintaining the molecule at room temperature and avoiding unreasonable internal structural changes.

In principle the same procedure should be applied to the three rotational degrees of freedom around the center of mass, but trial experiments have taught us that this does not give any additional advantages.

COMPUTATIONAL PROCEDURE

The programs for the molecular dynamics simulations were taken from the GROMOS87 library[27] and were adapted to perform separate scaling of temperature for receptor, c.o.m. of substrate, and internal degrees of freedom of the substrate. The temperature rescaling was obtained by coupling the systems to external baths.[21] The potential energy function in GROMOS87, describing the interactions between atoms in crystals, has been used. The parameters describing the van der Waals interactions in the model system were those used for carbons: $\epsilon = 0.406$ kJ/mol and $\sigma = 0.336$ nm. The dielectric permittivity was $\epsilon = 1$. The time step was 0.002 psec. The bond lengths of the substrates were kept rigid by application of the SHAKE method.[28] The receptor atoms were constrained in their starting position by a harmonic force with a force constant $k = 4000$ kJ mol^{-1} nm^{-2}. No intramolecular interactions in the receptor were calculated and the temperature of the receptor was kept at $T = 10$ K. An extra half-harmonic repulsive term in the interatomic potential function was used:

$$V(r) = \frac{1}{2} k(r - r_0)^2 \qquad \text{if } r \leq r_0$$

with $k = 1000$ kJ mol^{-1} nm^{-1}. Its purpose is to avoid too short contacts between substrate and receptor (i.e., to smooth the potential surface) and to prevent the protein and substrate from bumping into one another due to the high temperature of the latter.

The crystal structure of the phosphocholine–immunoglobulin complex was taken from the Brookhaven Protein Data Base.[29] It was first energy-refined by 10 ps of MD simulation at $T = 300$ K followed by a 10 ps annealing of the temperature down to 10 K. The docking simulation was performed by taking into account all atoms of the receptor included in a sphere of 2.0 nm radius, centered at the c.o.m. of the substrate in the crystal structure. A cutoff radius of 0.8 nm was used for intermolecular interactions.

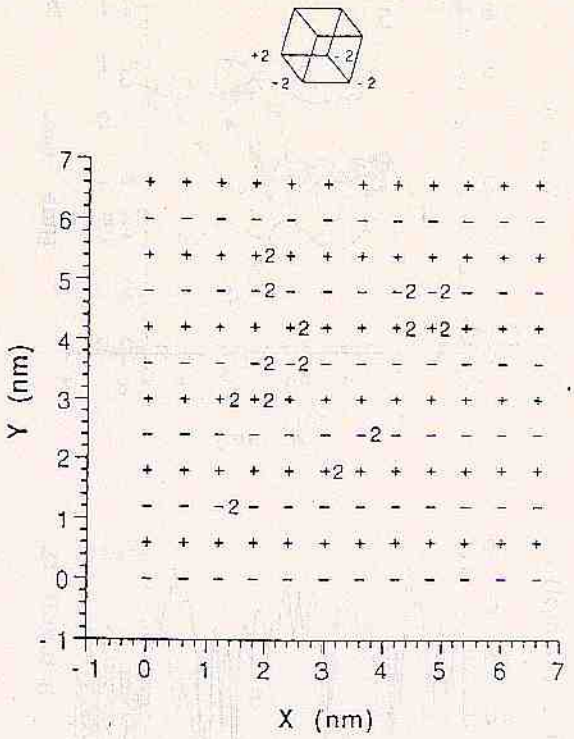

Fig. 1. Charge distribution in the receptor and in the substrate in a.u. + and − signs stand for +0.1 and −0.1 and 2 for 0.2, respectively.

The charges in the phosphocholine were the following: CH3 = +0.248, N = +0.008, (N)CH2 (CH2) = +0.248, (CH2)CH2(O) = 0.000, (CH2)O (P) = −0.300, P = +0.630, (P)O = −0.600, hydroxyl oxygen (P)O(H) = −0.528, H = +0.398. The charges of the immunoglobulin were taken from the GROMOS87 package.

RESULTS

Application to a Model System

We have first applied the method to a very simple model system (Fig. 1), in order to check the effects of the different parameters and to select the best procedure. The receptor is a plane surface obtained by 144 nonbonded atoms with Lennard–Jones parameters taken from carbons and with charges of ±0.1 and ±0.2 a.u. The distance between atoms were fixed at 0.6 nm and the positions were restrained. The substrate is a cube of 0.6 nm length, with Lennard–Jones parameters taken from carbons and with charges of ±0.2 a.u. on 4 atoms. This system presents two well-defined minima, one with the lowest energy and the other with slightly higher energy but with larger area. There are in addition several local minima.

Several different simulations were performed dif-

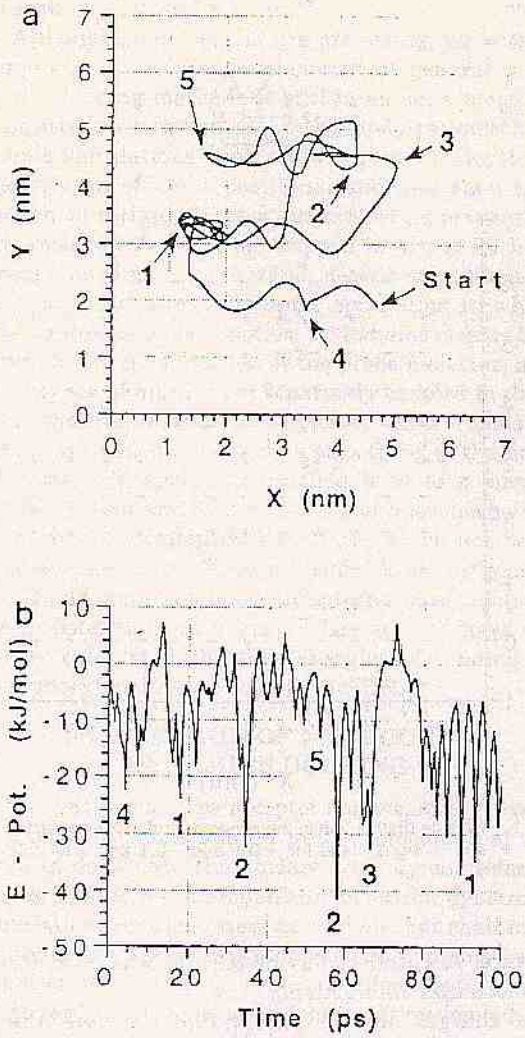

Fig. 2. (a) Trajectory of the center of mass of the substrate in the x,y plane. (b) Electrostatic + van der Waals potential energy of the substrate.

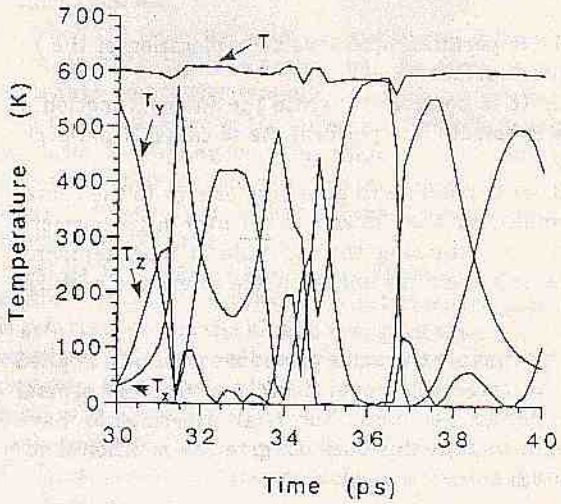

Fig. 3. Kinetic energy expressed as temperature, of center of mass of the substrate, x,y, and z components and total, during 10 ps.

fering in the temperature of the c.o.m. (from 600 to 1200 K) and in the time constant of the coupling with the external bath (from 0.005 to 0.5 ps). In addition, in order to avoid too close contacts, a repulsion was applied between atoms of receptor and substrate that are closer than r_0. The value of r_0 was varied in the range 0.4–0.6 nm. Finally, to confine the search to the proper region, reflections were put at the system boundaries.

We report the results obtained with the following values of the parameters: T_c = 600 K, τ_c = 0.01 ps, r_0 = 0.5 nm, simulation time 100 psec. The results have been analysed in terms of trajectory of the c.o.m. of the substrate and potential energy of the substrate.

Inspection of the trajectory of the c.o.m. in the xy plane (Fig. 2a) shows that all the minima have been explored. The substrate spends, as expected, more time in the broader second minimum (nr 1 in Fig. 1a) than in the deepest minimum (nr 2 in Fig. 1a) and less time in the others. The potential energy of the substrate (Fig. 2b) shows several well recognizable minima that correspond to the "binding sites." The two lowest points correspond to the minima; after energy minimizations from these points the values of −60 and −87 kJ/mol were found for minima nr 1 and 2, respectively (In all quoted energy values the added repulsive terms are not included.)

In Figure 3 the translational kinetic energy of the c.o.m. is reported in terms of temperature over 10 ps. The total kinetic energy is almost constant but there is an exchange between the three components.

Application to the Binding of a Substrate to a Protein

We applied the method to the binding of phosphocholine to the immunoglobulin McPC603, which has been studied before.[4] The binding depends on both steric and electrostatic interactions. The substrate binds in a hydrophobic pocket, with its choline group interacting favorably with a glutamic acid at the base of this pocket. The phosphate end forms hydrogen bonds with tyrosine and arginine groups.

Several simulations were performed. The starting position of the receptor was obtained by translating the X-ray coordinates in a range between 0.6 and 1.0 nm, rotating the crystallographic coordinates with respect to the crystallographic x or y axis by 180°, and rotating the two internal torsion angles by 60°.

The results obtained in two simulations will be presented in detail and the effects of different values of the parameters will be briefly discussed. The results will be presented in terms of the rms deviation of the skeletal atom coordinates of the substrate

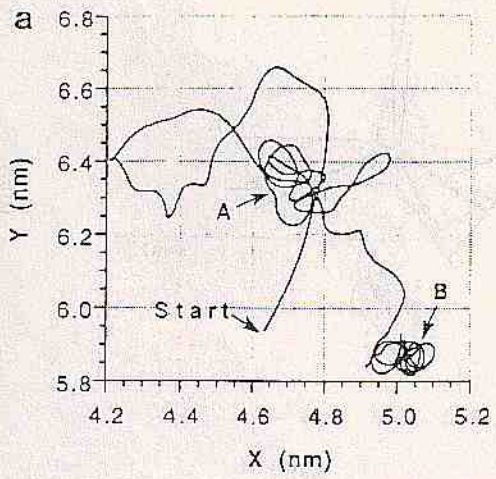

Fig. 4. Trajectories of the center of mass of the substrate in the x,y (a) and x,z (b) planes. The cartesian axes coincide with the crystallographic axes.

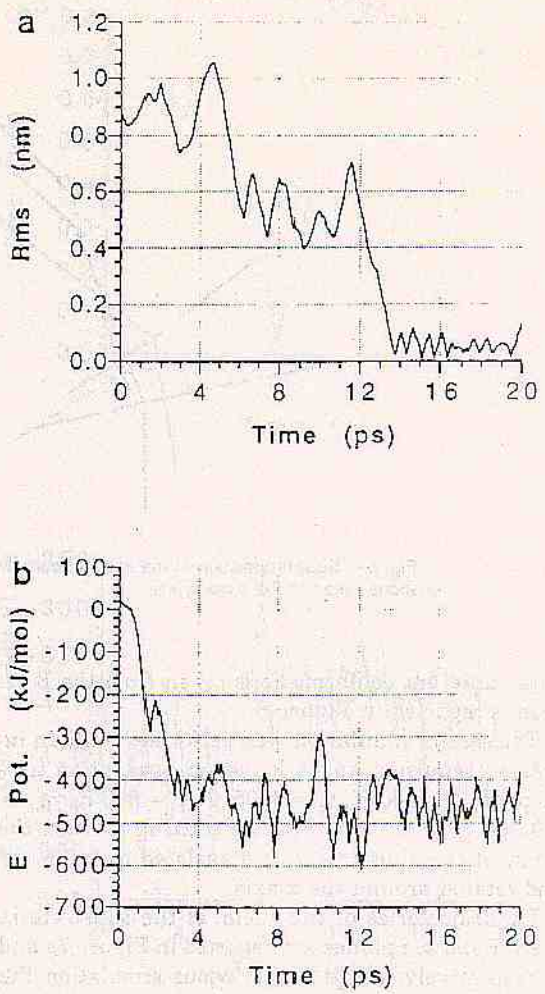

Fig. 5. (a) Rms deviation of the skeletal atoms of the substrate with respect to the energy refined crystal structure. (b) Electrostatic + van der Waals potential energy of the substrate. The temperature of the c.o.m. of the substrate was 1800 K in the first half of the simulation and 1000 K in the second half.

with respect to the energy-minimized crystal structure, the potential energy of the substrate, and the trajectory of the c.o.m. of the substrate.

The first simulation was performed in two phases. First phase: simulation time 10 ps, T_c = 1800 K, τ_c = 0.01 ps, T_{int} = 300 K, τ_{int} = 0.01 ps, repulsion reference distance r_0 = 0.35 nm. This step was followed by a second phase of 10 ps length, in which T_c was gradually lowered to 1000 K. For the starting position the c.o.m. of the substrate was translated over 0.9 nm away from the active site, and rotated around the y axis.

The trajectories of the c.o.m. of the substrate in the x, y and x, z planes are reported in Figure 4a and b, respectively. The figure shows that the c.o.m. explores a surface of 0.7 and 0.8 nm^2 in the two planes and that it spends most of the time in two regions (A and B) centered at A = (4.7, 6.3, −3.6) and B = (5.0, 5.9, −3.6), respectively. They correspond to two different minima.

The rms deviations of the skeletal atoms of the substrate and the potential energy of the substrate in the simulation are reported in Figure 5a and b, respectively. Figure 5a shows that region A corresponds to a minimum at a distance of 0.6 nm from the crystal structure, while region B coincides with the crystal structure. Figure 5b shows that both minima have comparable depth, but it is not possible to make a quantitative comparison from this simulation as the temperature has been changed from 1800 to 1000 K. The average internal temperature of the substrate was 434 K due to the coupling with the c.o.m. temperature. The superposition of the energy-refined crystal structure (in black) and

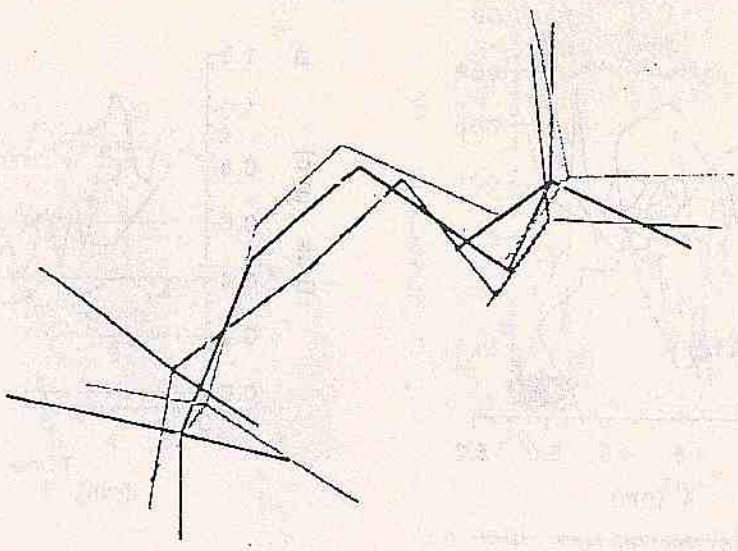

Fig. 6. Superimposition of the energy refined crystal structure (black) of the substrate with three conformations taken in the binding site.

three different conformations taken from the B region is reported in Figure 6.

The second simulation was performed with an increased repulsion and a longer τ_c: simulation time 20 ps. T_c = 1500 K, τ_c = 0.05, T_{int} = 300 K, τ_{int} = 0.01 ps, r_0 = 0.4 nm. For the starting position the c.o.m. of the substrate was translated over 0.6 nm and rotated around the x axis.

The trajectories of the c.o.m. of the substrate in the x, y and x, z planes are reported in Figure 7a and b, respectively. As in the previous simulation the substrate spends most of the time in regions A and B. Contrary to the previous simulation it flips between the two regions and is not trapped in a local minimum. The rms deviation and the potential energy of the substrate, reported in Figure 8a and b, respectively, show that several well-defined minima are detectable in the two regions. Region A seems to have a somewhat lower energy.

The superimposition of the energy-minimized crystal structure (in black) and three different conformations taken from the B region is reported in Figure 9.

The potential energy of the substrate in the two regions was minimized by the same procedure used to refine the crystal structure, i.e., 10 ps of simulation at T_c = 300 K, followed by an annealing of the temperature in a further 10 ps. The final potential energy and rms values in the two regions were −640 kJ/mol and 0.66 nm in A and −610 kJ/mol and 0.09 nm in B.

Comparison between the two simulations shows that different values of temperature, time constant and repulsion reference distance give different behavior: in the first simulation the substrate is trapped (in 20 ps), while in the second one it flips between A and B minima. The longer time constant τ_c, used in the second simulation, allows the substrate to explore the minima in a better way, as is evidenced by the narrow minima in the potential energy profile.

Some other simulations were performed in order to check the effects of different values of the parameters. The analysis of the results suggests that the repulsion reference distance should not exceed 0.4 nm and the coupling time to the c.o.m. τ_c should be taken in the neighborhood of, but not exceeding, 0.05 ps. The temperature of the c.o.m. can be varied in a range 1300–1700 K.

Possible Inclusion of Receptor Flexibility

A crucial issue in the docking problem is how to work with flexible ligands and flexible receptors. This problem has not yet been solved and most of the methods in widespread use cannot easily be modified to include some sort of flexibility. The present method easily allows the inclusion of flexibility in any part of the system, but its application must be subjected to further study. The feasibility of introducing receptor flexibility does not depend on the method itself, but on the complexity of the problem. If the docked conformation of the receptor is very different from the undocked one, the "brute force" simulation of the conformational change many require computational efforts far beyond the presently available computational resources. Even in the case of small changes in the receptor's conformation it is necessary to work with care and with a realistic system that includes solvent molecules, at least in the region of the binding site. Without solvent the re-

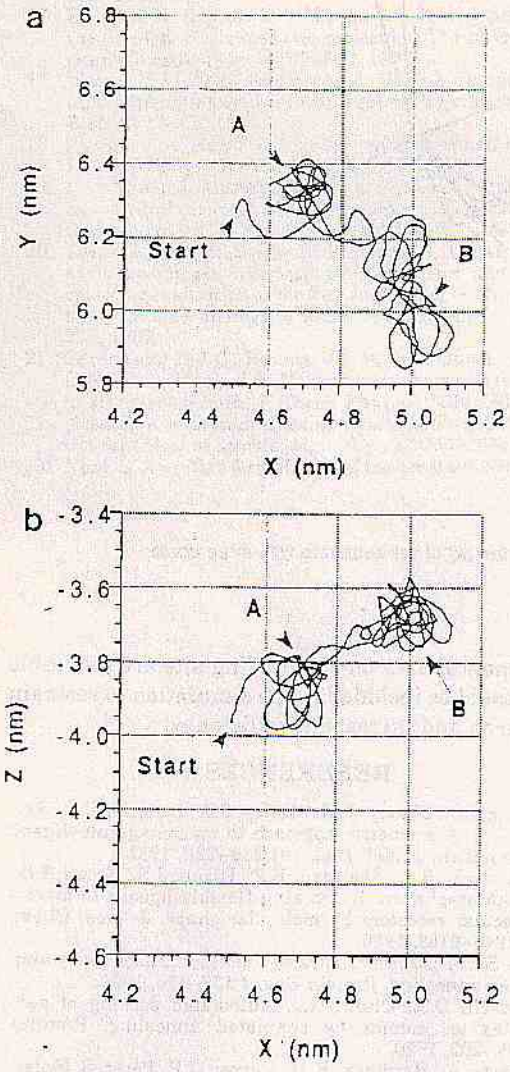

Fig. 7. Trajectories of the center of mass of the substrate in the *x,y* (a) and *x,z* (b) planes. The cartesian axes coincide with the crystallographic axes.

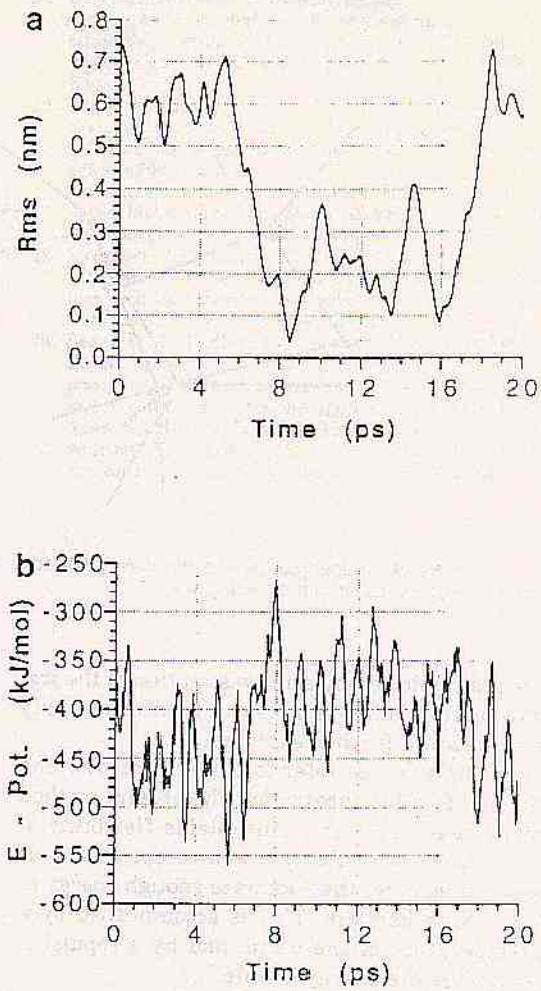

Fig. 8. (a) Rms deviation of the skeletal atoms of the substrate with respect to the energy refined crystal structure. (b) Electrostatic + van der Waals potential energy of the substrate.

ceptor tends to maximize the intramolecular interaction and to close the pocket of the binding site. The fast motions of the ligand could cause unrealistic local modifications of the receptor structure, which can be limited by inclusion of (weak) position restraints in the receptor. Finally it has to be considered that a flexible receptor removes energy from the ligand, which may change the required values for the temperatures and coupling time constants.

The previous points can be studied with the present method that is simple enough to include water, position restraints, and different temperatures for both molecules. When available, experimental data on the receptor structure, even if this represents very limited information, can restrict the search at the conformational hypersurface of interest and make the inclusion of receptor flexibility treatable. Another very promising but thus far untested approach is restricting the conformational search to the low-dimensional hyperplane of "essential degrees of freedom" dervied from a correlation analysis of atomic fluctuations.[30] Experience thus far has shown that these essential degrees of freedom indeed describe the conformational space in which proteins can perform their functions, including the binding of substrates, without running into high energy barriers (Amadei and Linssen, personal communication).

CONCLUSIONS

The method reported here shows that the use of different temperatures for the center of mass (c.o.m.) of the substrate, for the internal degrees of freedom of the substrate, and for the receptor allows one to explore the receptor surface for the determination of possible binding sites by a simple modification of standard molecular dynamics techniques. A docking procedure should consist of a nontrapping ("helicop-

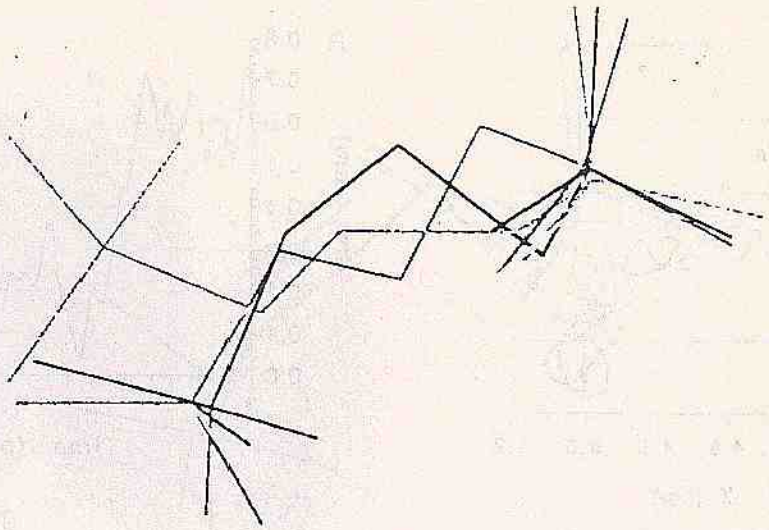

Fig. 9. Superimposition of the energy refined crystal structure (black) of the substrate with three conformations taken in the binding site.

ter") search, followed by an investigation of the trajectory. Possible binding sites can then more easily be identified and further explored.

The examples given refer to a frozen receptor and to rigid and flexible substrates. The present method permits a reasonable and controllable flexibility of the molecules (both substrate and receptor) and, at the same time, gives the substrate enough energy to pass over high barriers. This is accomplished by a high temperature of the c.o.m. and by a repulsion that smoothes the energy profile.

The present results show that very short time constants are required for the coupling to external baths, both for the receptor and for the interal degrees of freedom of the substrate. This is necessary to remove the heat flow from the c.o.m. motion into the internal degrees of freedom. The c.o.m. temperature should be kept high, in the range 1300–1700 K, and its coupling time constant should be larger than those used for the internal motions to permit large fluctuations in the translational motion.

The usefulness of the extra repulsion is still to be further investigated. The smoothing was in this case obtained by an additional semi-harmonic repulsive potential. Large values of the reference distance avoid a deep exploration of the active site. High values of the force constant provoke a reflection and low values cause the substrate be trapped for a long time. It would be worthwhile to investigate the use of other smoothing techniques such as soft core potentials.

One advantage of the present method is that no scoring functions or grid search methods are required. The method is general enough to include solvent molecules and flexible receptors as well. When experimental data on the binding site are available they should be included in the simulation to restrain the search and increase its efficiency.

REFERENCES

1. Kuntz, I.D., Blaney, J.M., Oatley, S.J., Langridge, R., Ferrin, T.E. A geometric approach to macromolecule–ligand interactions. J. Mol. Biol. 161:269–228. 1982.
2. DesJarlais, R.L., Sheridan, R.P., Dixon, J.S., Kuntz, I.D., Venkataraghavan, R. Docking flexible ligands to macromolecular receptors by molecular shape. J. Med. Chem. 29:2149–2153, 1986.
3. Yue, S.Y. Distance-constrained molecular docking by simulated annealing. Protein Eng. 4:177–184, 1990.
4. Goodsell, D.S., Olson, A.J. Automated docking of substrates to proteins by simulated annealing. Proteins 8:195–202, 1990.
5. Imberty, A., Hardman, K.D., Carver, J.P., Pérez, S. Molecular modelling of protein-carbohydrate interactions. Docking of monosaccharides in the binding site of concanavalin A. Glycobiology 1:631–642, 1991.
6. Hart, T.N., Read, R.J. A multiple-start Monte Carlo docking method. Proteins 13:206–222, 1992.
7. Caflish, A., Niederer, P., Anliker, Monte Carlo docking of oligopeptides to proteins. Proteins 13:223–230, 1992.
8. Goodsell, D.S., Lauble, H., Stout, C.D., Olson, J. Automated docking in crystallography:analysis of the substrates of aconitase. Proteins 17:1–10, 1993.
9. Beddell, C.R., Goodford, B.J., Norrington, F.E., Wilkinson, S., Wootten, R. Compounds designed to fit a site of known structure in human hemoglobin. Br. J. Pharmacol. 57:201–209, 1976.
10. Freudenreich, C., Samama, J.P., Biellmann, J.F. Design of inhibitors from the three-dimensional structure of alcohol dehydrogenase. Chemical synthesis and enzymatic properties. J. Am. Chem. Soc. 106:3344–3353, 1984.
11. Blaney, J.M., Jorgensen, E.C., Connolly, M.L., Ferrin, T.E., Langridge, R., Oatley, S.J., Burridge, J.M., Blake, C.C.F. Computer graphics in drug design: Molecular modelling of thyroid hormone-prealbumin interactions. J. Med. Chem. 25:785–790, 1982.
12. Wodak, S.J., Janin, J. Computer analysis of protein-protein interaction. J. Mol. Biol. 124:323–342, 1978.
13. Santavy, M., Kypr, J. A fast computational algorithm for finding an optimum geometrical interaction of two macromolecules. J. Mol. Graphics 2:47–49, 1984.

14. Goodsell, D., Dickerson, R.E. Isohelical analysis of DNA groove-binding drugs. J. Med. Chem. 29:727–733, 1986.
15. Rashin, A.A., Yudman, B.H. Calculation of the quaternary structure of α-chymotrypsin dimer. Studia Biophys. 72: 123–126, 1978.
16. Billeter, M., Havel, T.F., Kuntz, I.D. A new approach to the problem of docking two molecules: The ellipsoid algorithm. Biopolymers 26:777–793, 1987.
17. Wodak, S.J., De Crombrugge, M., Joël, J. Computer studies of interactions between molecules. Progr. Biophys. Mol. Biol. 49:29–63, 1987.
18. Rashin, A.A. Aspects of protein energetics and dynamics. Progr. Biophys. Mol. Biol. 60:73–200, 1993.
19. Goodford, P.J. A computational procedure for determining energetically favorable binding sites on biologically important molecules. J. Med. Chem. 28:849–857, 1985.
20. Lee, F.S., Chu, Z., Bolger, M.B., Warshel, A. Calculation of antibody-antigen interactions: Microscopic and semi-microscopic evaluation of the free energies of binding of phosphorylcholine analogs to McPC603. Protein Eng. 5:215–228, 1992.
21. Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., DiNola, A., Haak, J.R. Molecular dynamics with coupling to an external bath. J. Chem. Phys. 81:3684–3690, 1984.
22. Nosé, S. A molecular dynamics method for simulations in the canonical ensemble. Mol. Phys. 52:255–268, 1984.
23. Nosé, S. A unified formulation of the constant temperature molecular dynamics method. J. Chem. Phys. 81:511–519, 1984.
24. Hoover, W.G. Canonical dynamics: equilibrium phase space distributions. Phys. Rev. A31:1695–1697, 1985.
25. Berendsen, H.J.C. Transport properties computed by linear response through weak coupling to a bath. In "Computer Simulation in Material Science." Meyer, M., Pontikis, V., eds. NATO ASI Series E 205:139–155. Dordrecht: Kluwer, 1991.
26. Sok, R.M., Berendsen, H.J.C., van Gunsteren, W.F. Molecular dynamics simulation of the transport of small molecules across a polymer membrane. J. Chem. Phys. 96: 4699–4704, 1992.
27. van Gunsteren, W.F., Berendsen, H.J.C. GROMOS: Groningen Molecular Simulation package manual. BIOMOS. Nijenborgh 4, 4747 AG Groningen, the Netherlands.
28. Ryckaert, J.P., Ciccotti, G., Berendsen, H.J.C. Numerical integration of the cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. J. Computat. Phys. 23:1311–1327, 1977.
29. Segal, D.M., Padlan, E.A., Cohen, G.H., Rudikoff, S., Potter, M., Davies, D.R. The three-dimensional structure of a phosphorylcholine-binding mouse immunoglobulin Fab and the nature of the antigen binding site. Proc. Natl. Acad. Sci. U.S.A. 71:4298–4302, 1974.
30. Amadei, A., Linssen, A.B.M., Berendsen, H.J.C. Essential dynamics of proteins. Proteins 17:412–425. 1993.