MOLECULAR DYNAMICS WITH STOCHASTIC BOUNDARIES: APPLICATION TO THE ACTIVE SITE OF PROTEINS IN SOLUTION

by

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Abstract

A new approach to study localized chemical events in solvated proteins is developed. It provides a simple and convenient method for reducing the total number of interacting sites explicitly included in molecular dynamics simulations of localized processes while decreasing spurious edge effects. The effects of distant atoms on the dynamics of the region of interest are approximated by the introduction of boundary forces and a stochastic heat bath region. The method has been tested by comparing the results with conventional molecular dynamics simulations for a sphere of argon atoms, a sphere of water molecules in the ST2 model and a portion of a protein (BPTI). Good agreement for structural and dynamic properties is obtained. An application of the method to the active site of a solvated protein is outlined.

1. Introduction

The active sites of most enzymes are partially accessible to solvent molecules. This accessibility allows the substrate or ligand to approach the active site by diffusion, modulated by interactions with the enzyme. The details of the coupling between the solvent and the enzyme or substrate during the reaction depend on the specific system. The enzymes citrate synthetase, hexokinase A or liver alcohol dehydrogenase, undergo a conformational change upon substrate binding so that the reactions of the enzyme substrate complex are shielded from the solvent (1). In other systems like ribonuclease the active site remains accessible to the solvent throughout the reaction (2). Theoretical investigation of enzymatic activity by molecular dynamics or Monte Carlo methods must take account of the influence of the solvent. However, the explicit inclusion of solvent by treating the entire protein in solution would be a rather inefficient way to simulate the localized chemical events in the active site. Instead, it is desirable to study the catalyzed reaction by focusing on a localized region including the active site, the substrate and the surrounding solvent. To accomplish this spatial reduction of the system, stochastic boundary techniques for molecular dynamics or activated dynamics simulations have been developed.

An essential feature of the stochastic boundary methods is a partitioning of the system into several regions. This partitioning is illustrated schematically for an enzyme-substrate system in Fig. 1. The system is partitioned into two major regions, a reaction zone where all atoms are treated explicitly and the surrounding "reservoir" region composed of atoms distant from the volume of primary dynamical interest. Atoms in this region are eliminated from the calculation and their effect on atoms in the reaction zone is replaced by explicit boundary forces and stochastic terms. The reaction zone which contains the site of major dynamical interest is chosen large enough to include the substrate, the residues that interact with the substrate and some surrounding residues. It is subdivided into a large inner volume reaction region and a buffer region (typically a 2 Å shell) near the boundary. The reaction region atoms are treated by standard molecular dynamics. The buffer region atoms represent a heat bath and are treated as Langevin particles subject to the same empirical energy function as the reaction region particles; for protein buffer atoms, a constraining potential is added to obtain fluctuations in accord with independent estimates. The buffer region allows energy fluctuations to occur in the reaction region and thus *should approximate* the many body dissipative effects that would be produced by the surroundings in the complete system. The partitioning of the protein atoms into different regions is kept fixed throughout the simulation. The water molecules are confined to the reaction region by an appropriate boundary force (see next section), but they are allowed to diffuse freely in it, e.g. they can enter into or exit from the buffer region.

The present stochastic boundaries are only a first order approximation to a complete description of the system. For instance, no attempt has been made to incorporate electrostatic effects due to charges outside the region of interest. In that regard the model should be comparable to conventional molecular dynamics simulation with periodic boundary conditions and a distance cutoff for the nonbonded interactions. The results obtained so far indicate that the stochastic boundaries produce only minor surface effects with regard to the more complete conventional simulations.

In the following applications stochastic boundary techniques are summarized for water (ST2 model), for a small protein in vacuum (PTI) and for the active site of ribonuclease A in solution. In the last case use of the stochastic boundary approach greatly reduces the number of atoms that have to be included in the simulation relative to that required for the full protein in solution. The gain in computing time is expected to be a factor somewhere between 10 and 100, depending on the specific system.

2. A Spherical Boundary for ST2 Water

Neglect of the detailed dynamics of atoms in the boundary region requires that the influence of these atoms be replaced by appropriate mean and stochastic forces. The purpose of the boundary force is to maintain the correct average distribution of atoms in the reaction region; that is, to keep the waters from escaping into the vacuum and to approximate the forces due to the volume of the fluid that has been neglected. For fluid argon, Berkowitz and McCammon (3) proposed an approach which is based on the force field of a static distribution of a shell of argon atoms outside the reaction region. As an alternative, Brooks and Karplus (4) developed a deformable boundary force field

$$\underline{F}_B(\underline{r}_i) = \int P(\underline{r}_j|\underline{r}_i)\, \underline{F}(\underline{r}_j,\underline{r}_i)\, d\underline{r}_j \qquad (1)$$

where $\underline{r}_i$ is the position of the argon atom inside the reaction zone, $P(\underline{r}_j|\underline{r}_i)$ is the probability of finding an atom, j, at $\underline{r}_j$ in the reservoir region when atom i is at $\underline{r}_i$, $\underline{F}(\underline{r}_j,\underline{r}_i)$ is the force of interaction between atom i and atom j, and the integral is extended over the surrounding region. Both approaches were shown to give good results in comparison to conventional simulations for liquid argon. However, when extended to water (5), it was found for the ST2 model (6) that the first approach gave a too structured fluid with the density of molecules increasing towards the boundary. This may be explained by the strong directional forces present due to hydrogen bonding with the fixed surroundings. An approximate numerical boundary force for ST2 water was then obtained in the spirit of the deformable boundary approach (Eq. 1). The ensemble average $P(\underline{r}_j|\underline{r}_i)$ probability which is difficult to obtain for the non-site-decomposable ST2 model was approximated by a spherical averaging over an equilibrated fixed shell of ST2 molecules.

It was shown (5) that the averaged ST2 boundary produces radial distribution functions and an oxygen velocity autocorrelation function for atoms in the reaction zone in good agreement with full periodic boundary simulation results. The self-diffusion coefficient of water was slightly higher than the value obtained by conventional techniques.

3. Stochastic Boundaries for Proteins

To develop models for localized events in proteins (e.g. enzyme reactions), the boundary model was adapted to systems with a well-defined average structure. An early example of this type of approach is the activated dynamics study of tyrosine ring flips where the surrounding region was represented by fixed atoms (7). We recently showed (8) that such a fixed environment may produce artifacts similar to the ones found for the water case. As the protein is an inhomogenous system, an averaging approach analogous to the one for liquids cannot be employed to smear out the directional effects of the surroundings. Fortunately, in protein molecules, and other macromolecules with well-defined average structures, the atoms oscillate around known mean positions. The average amplitude of these oscillations can be used to define a local effective potential. In defining the boundary forces for the macromolecule system we assume for atom i that

$$\underline{F}_B(\underline{r}_i) = -S(\underline{r}_i)\, m_i\Omega_i^2\, (\underline{r}_i - \underline{r}_i^{ref}) = -S(\underline{r}_i)\, m_i\Omega_i^2\, \Delta\underline{r}_i \qquad (2)$$

with

$$m_i\Omega_i^2 = 3k_BT/\langle\Delta r_i^2\rangle \qquad (2)$$

Here $\underline{r}_i^{ref}$ are reference coordinate values and $\langle\Delta r_i^2\rangle$ are mean square fluctuations known from experimental X-ray studies or simulations. The function $S(\underline{r}_i)$ is introduced to scale the force constants to avoid effects in Eq. (2) due to the fact that the constrained atoms also interact directly with other atoms in the buffer and reactions regions.

The stochastic forces on the buffer region atoms are designed to mimic the fluctuating dynamics of the neglected region. A simple Langevin equation is used with the friction coefficient determined from velocity autocorrelation functions.

The results obtained by applying this model to a tyrosine ring of BPTI show that the dynamical and structural properties are in satisfactory agreement with conventional simulations of the full system (8).

4. Application: The Active Site of Ribonuclease A

The ST2 water boundary and the protein stochastic boundary are combined for the simulation of the dynamics of the active site of ribonuclease A, including the local solvent environment (9). The correct generation of an initial structure involves the adjustment of the number of water molecules in the active site (c.f. Fig. 1). The number of water molecules is chosen such that the density in a region distant from the protein corresponds approximately to the density of bulk water. Stochastic boundary simulations are being performed on a series of ribonuclease-substrate-complex structures which correspond to the important steps during the reaction catalyzed by ribonuclease A (2).

The atomic fluctuations of the protein atoms relative to their average positions are in good qualitative agreement with temperature factors determined by X-ray crystallography. Correlated fluctuations of protein atoms confirm some of the interactions postulated in the catalytic mechanism of ribonuclease A. The stabilization of a set of positively charged groups in the active site by a network of water molecules is observed. The hydrogen bonding network in the active site deviates somewhat from the X-ray structures for side chains with large disorder or for side chains for which alternative conformations have been found in different X-ray structure determinations.

The application of stochastic boundaries to dynamics in the active site of ribonuclease A and its substrate bound complexes represents a step toward understanding the dynamics of enzyme catalysis on an atomic level. Further, the methods outlined in this paper may serve to aid in the interpretation and refinement of X-ray crystallographic data.

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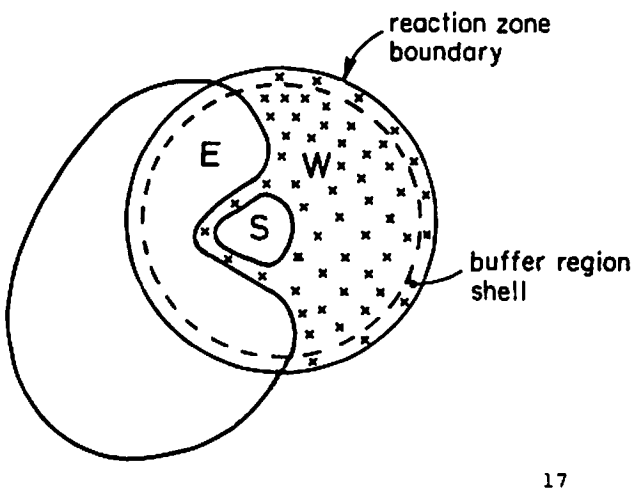
References

1. R. Huber and W. S. Bennett, Jr., Biopolymers 22, 261-279 (1983).
2. W. A. Gilbert, A. L. Fink, and G. A. Petsko, Biochemistry, in press (1985).
3. M. Berkowitz and J. A. McCammon, Chem. Phys. Lett. 90, 215 (1982).
4. C. L. Brooks, III, and M. Karplus, J. Chem. Phys. 79, 6312 (1983).
5. A. Brünger, C. L. Brooks, III, and M. Karplus, Chem. Phys. Lett., 105, 485 (1984).
6. F. H. Stillinger and A. Rahman, J. Chem. Phys. 60, 1545 (1974).
7. S. H. Northrup, M. R. Pear, C.-Y. Lee, J. A. McCammon, and M. Karplus, Proc. Natl. Acad. Sci. USA 79, 4035 (1982).
8. C. L. Brooks, III, A. Brünger, and M. Karplus, Biopolymers, in press (1985).
9. A. Brünger, C. L. Brooks, III, and M. Karplus, Biochemistry, to be published.



Schematic partitioning of an enzyme (E) - substrate (S) - water (W) system into a spherical reaction zone and the surroundings. A subdivision of the reaction zone into a large inner volume (reaction region) and a surrounding shell (buffer region) is also indicated.