Modal Dynamics of Proteins in Water

J. ELEZGARAY, Y. H. SANEJOUAND

Centre de Recherche Paul Pascal, Avenue Schweitzer, 33600 Pessac, France

Received 10 April 2000; accepted 18 May 2000

ABSTRACT: We studied a dynamical model for the motion of the large scales of proteins in water. The model was obtained by projecting the (averaged) Newton equations onto some set of harmonic modes. We compared the statistics of the so-obtained trajectories with those obtained by standard techniques, and concluded that our dynamical model is able to fairly reproduce the average properties of the large-scale motion of the protein, and at the same time allow time steps one order of magnitude larger than the standard ones. © 2000 John Wiley & Sons, Inc. J Comput Chem 21: 1274–1282, 2000

Keywords: dynamic model; proteins in water

Introduction

Molecular dynamics constitutes today an important tool in the study of protein dynamics.[1, 2] Despite the considerable progress made both in the computer power and the algorithmic approaches to molecular simulation, the longest time scales available for usual runs (based on fully atomistic descriptions) are limited to a few nanoseconds. On the other hand, models based on coarse-grained versions of macromolecules and their interactions are useful in describing "macroscopic" properties on sometimes macroscopic time scales. However, they obviously fail at describing specific properties of individual molecules.

Correspondence to: J. Elezgaray; e-mail: elez@crpp.u-bordeaux.fr

Contract/grant sponsor: Pôle MNI

Contract/grant sponsor: IDRIS; contract/grant number: 990871

In the present work, we describe an alternate way in which an approximate description of macromolecules and their interactions, based on projections onto some set of normal modes, are used to generate trajectories that are shown to share many properties with those of fully atomistic simulations. One of the few parameters determining the model is the ratio $c = N'/3N_a$ between the effective number N' of simulated degrees of freedom and those ($3N_a$) of the full system, the solvent being excluded. We show that for such low values as $c \sim 10^{-2}$, the trajectories generated still reflect several properties of the full ($c = 1$) simulation.

The use of normal modes in the context of protein dynamics simulations is far from new.[1] It has been shown,[3] for instance, that the hinge-bending motion characteristic of several proteins can be analyzed in terms of the lowest frequency modes (up to 10 cm^{-1}). More generally, it is well known[4–9] that protein motion is confined to some subspace (reduced set of the phase space) of a very small dimension, corresponding typically to a few percent of the total number of degrees of freedom of the

protein. The basic idea of the present approach is to take advantage of this fact, to obtain an efficient algorithm for the simulation of the degrees of freedom describing this subspace. The present method will be shown to be efficient (in terms of speed-up with respect to more traditional methods) and fairly accurate in capturing statistical features of protein dynamics.

The central question to be adressed is the possibility of deriving evolution equations for the degrees of freedom corresponding to soft harmonic modes. Of course, these equations should involve only these coordinates. To the best of our knowledge, this question has only been adressed by two different authors in the context of protein dynamics (see ref. 10 for the application of similar ideas in the simulation of "small" molecules). In ref. 11, J. Durup introduced a change of variables to describe the protein motion in a hierarchycal way. No modifications of the interactions are involved in this method. Although this appears to be a rather promising approach, it has not been tested either for sufficiently long trajectories or in the presence of water. The second reference is the so-called MBO(N)D method.[12] In this method, the protein is partitioned into several groups of atoms. Each group can be described at different levels of detail, ranging from strictly rigid to fully flexible bodies. Alternatively, each group of atoms can be described in terms of a truncated set of harmonic modes, in the same spirit of references.[10, 13] From this point of view, MBO(N)D generalizes the approach described in refs. 10 and 13, as well as that of the present article. However, in ref. 12, only vacuum simulations are reported and, more importantly, no modification of the interactions is implied by the projection. It will become apparent in the following that this last point is a key issue to take into account the neglected degrees of freedom involved in the projection procedure. Let us notice that this idea is not completely new. It appears, for instance, in the context of smoothed molecular dynamics[14, 15] (described below), or in the so-called "blocking technique" for emulating very large polyelectrolytes,[17] where it is shown that taking into account the electrostatic interactions between groups ("blocks") of atoms requires the addition of a corrective nearest-neighbor interaction, as well as the rescaling of the bonded interactions.

This article is organized as follows. In Section 2, we present the theoretical basis of the method. The next section shows the results obtained in several simulations of the CTF protein. Concluding remarks are addressed in the last.

Basis of the Method of Projections

Molecular dynamics simulations usually assume the Newton equations

$$m_i \frac{d^2 X_i}{dt^2} = F_i(X_i), \qquad i = 1, \ldots, N_a \tag{1}$$

where N_a is the number of atoms of the system, X_i is the position of the i-th atom, m_i its mass, and $F_i(X)$ the i-th component of the force exerced on this atom. The assumption that the motion of some molecule is well described by a "few" modes can be written as

$$X_i(t) \sim X_i^0 + \frac{1}{\sqrt{m_i}} \sum_{n=1}^{N'} c_n(t)\varphi_n(i). \tag{2}$$

Notice that this is a somewhat restraining assumption on the motion of the molecule, as no (solid) rotation of the ensemble is allowed. Taking into account this fact considerably complexs the description, and will be considered elsewhere. Expression (2) becomes an equality if the set of modes $\{\varphi_n(i)\}$ forms a basis of the configuration space of the protein. Thus, the approximation made in (2) consists in neglecting the projection onto the subset $\{\varphi_n(i),\ n > N'\}$, which will be considered as the "ensemble of high-frequency modes." The number N' of retained modes is a free parameter of the present model.

A first approach to obtain evolution equations of the restrained configuration (2) is the standard Galerkine approximation, which leads to the set of equations (the projected equations):

$$\frac{d^2 c_n}{dt^2} = \sum_i \varphi_n(i) \frac{F_i}{\sqrt{m_i}} \left(X_i^0 + \frac{1}{\sqrt{m_i}} \sum_{m=1}^{N'} c_m \varphi_m(i) \right), \qquad n = 1, \ldots, N'. \tag{3}$$

This is a closed set of equations that can be integrated in time, yielding a trajectory in which the molecule shows very reduced fluctuations. This is a puzzling situation, first pointed out in ref. 16. In fact, we know that $X_i(t) \sim X_i^0 + \sum_{m=1}^{N'} c_m(t)\varphi_m(i)$ is a very good approximation; still, $F_i(X_i(t))$ and $F_i(X_i^0 + \sum_{m=1}^{N'} c_m(t)\varphi_m(i))$ yield very different dynamical properties. A first hint to the solution of this question is suggested by an estimate of the error made in eq. (3):

$$\frac{d^2}{dt^2}\left(c_n^{true} - c_n^{approx}\right) = \sum_i \varphi_n(i) \left[\frac{1}{\sqrt{m_i}} F_i \left(X_i^0 + \frac{1}{\sqrt{m_i}} \sum_{n=1}^{3N_a} c_n(t)\varphi_n(i) \right) \right.$$

$$- \frac{1}{\sqrt{m_i}} F_i \left(X_i^0 + \frac{1}{\sqrt{m_i}} \sum_{n=1}^{N'} c_n(t)\varphi_n(i) \right) \Bigg]$$

$$= \sum_i \varphi_n(i) \sum_j \frac{1}{\sqrt{m_i}} \frac{\delta F_i}{\delta X_j} \Bigg|_{X_i^0 + (1/\sqrt{m_i}) \sum_{n=1}^{N'} c_n(t)\varphi_n(i)}$$

$$\times \frac{1}{\sqrt{m_j}} \sum_{N'+1}^{3N_a} c_m \varphi_m(j) + O(c_m^2).$$

In the last expression, while c_m, $m \geq N' + 1$ can be thought as being a small quantity [this is equivalent to the assumption that the approximation in eq. (2) is good], the multiplying factor $\delta F_i / \delta X_j$ can be extremely large, mainly due to the existence of stiff (short-range) forces. In others words, as soon as the projected configuration is slightly out of the equilibrium values for the bond lengths and valence angles, the restoring force is tremendous, and the molecule cannot fluctuate. The conclusion is that the approximate equations (3) are actually very bad approximations of the evolution equations (1) because the r.h.s. in eq. (1) is a very rapidly varying force.

A remedy for this problem consists in projecting a smoothed (in time) version of the Newton equations (1). Smoothed evolution equations are obtained in the limit of infinitely stiff bond and angle energy terms.[14, 15, 18] If the latter are noted by $\epsilon^{-2} U_{bond}$, and

$$E = \epsilon^{-2} U_{bond} + U_{soft} \tag{4}$$

denotes the total potential energy of the system, it can be shown[15, 18] that, in the limit $\epsilon \to 0$ (infinitely stiff system), the dynamics of the molecule is given by a constrained Hamiltonian system with a force term of the form

$$-\nabla U_{soft} - \lambda \nabla U_{constraint} - F_{Fixman}.$$

The term $\lambda \nabla U_{constraint}$ is a Lagrange multiplier constraining the dynamics to the manifold $U_{bond} = 0$. Practically, this means that the dynamics takes place only on dihedral space. The F_{Fixman} term arises as a correcting term (second order in the limiting process of taking infinitely stiff bonded terms). It can be shown[15] to be strictly zero for the bond terms, but yields nonzero contributions for the angle energy terms. Several expressions have been given in the literature for the F_{Fixman} term. Probably one of the first to note the need of including such a correcting term was Fixman, in the context of polymer dynamics.[19] He argued that such a term is necessary to correct time averages possibly biased by the constraints imposed on the dynamics. Another line of reasoning has been followed since (at least) the work of Takens,[18] who gave a more rigorous discussion of the origin of this term. Interestingly, the two approaches give different results. Indeed, it can be shown[15] that, at least in certain low-dimensional cases (small molecules), the Fixman's approach is not correct.

As stated above, one could expect that projecting the smoothed evolution equations, the problem related to the existence of rapidly varying forces disappears. This will be shown to be true in the following. However, in doing so, two different questions arise. First, is the use of smoothed evolution equations realistic in the context of molecular dynamics? Second, how to effectively compute the projections of these smoothed equations? The use of smoothed evolution equations instead of the original ones has been suggested by several authors.[14, 15] The idea seems very appealing, as smoother forces mean larger time steps and, consequently, larger integration times. The problem is that the computation of the correcting potentials is, *a priori*, extremely expensive. On the other hand, the noninclusion of this correcting terms has been shown to be not quite appropriate.[20] In the following, we will make the (strong) hypothesis that the correcting terms are negligible when projected onto large-scale, soft modes. In ref. 13, we assumed that the projected contribution of the Lagrange multiplier (constraining the dynamics to dihedral space) is small. This second hypothesis seems rather plausible, as soft normal modes are expected to be orthogonal to the directions corresponding to stiff motions. However, it will be shown below that as the number of slow retained modes N' increases, the contribution of the Lagrange multipliers becomes important, and that it has to be taken into account in some way. Instead of explicitly computing the Lagrange multipliers, we have used the following set of equations:

$$\frac{d^2 c_n}{dt^2} = - \sum_i \varphi_n(i) \frac{1}{\sqrt{m_i}} \frac{\partial U_{soft} + \mu \epsilon^{-2} U_{bond}}{\partial X_i} \left(X_i^0 + \frac{1}{\sqrt{m_i}} \sum_{m=1}^{N'} c_m \varphi_m(i) \right), \tag{5}$$

with the parameter μ chosen so that $\mu \epsilon^{-2} \ll 1$.

In ref. 13, we investigated the consequences of a similar hypothesis ($\mu = 0$) for the dynamics of BPTI in vacuum, using the so-called anharmonic modes. The latter are obtained as the eigenmodes of the correlation matrix of the atomic positions. Thus, their computation assumes the existence of some computed trajectory, long enough to ensure the convergence of the averaging procedure. As

noted in ref. 21, this can be quite a slow process, and doubts can be cast as to the relevance of the so computed modes to describe the phase space of the protein. The situation is different when using harmonic modes, as only a configuration of minimal energy is needed for their computation. Still, it could be said *a priori* that soft modes do not contain all the information needed to describe the phase space. The results of ref. 9 (and references therein), concerning the description of transition between closed and open forms of several proteins, as well as the success of the phase-space exploring algorithm of ref. 22 tend, however, to provide some support to this hypothesis.

The last point to be taken into account to perform the simulation of the projected protein is how to treat the solvent molecules. Several points need some discussion. First, is it the sole protein that has to be projected, or could one project more generally a partially solvated protein? Based on the results of ref. 23, it seems that some water molecules stay glued to the outer part of the protein, and could participate in the large-scale motion of interest here. Second, if large time steps are being looked for, how can one ensure the stability of the computed trajectory as far as the solvent molecules are concerned? In this work, we will be using the approach of ref. 24, in which the authors artificially increased by a factor of 10 the hydrogen mass of the water molecules. The resulting solvent is, as expected, slightly more viscous, but displays significantly the same structural correlation functions as ordinary water.

To summarize, the following basic steps have been taken in each of the (projected) simulations:

1. Generate a starting configuration by minimizing the set protein plus water (periodic boundary conditions for the solvent have been used throughout).
2. Extract a subset of atoms containing the protein plus the water molecules distant by less than 4 Å. Minimize this subset. The resulting configuration is actually very close to that obtained in the previous step. However, the next step requires a rather accurate approximation of the minimum; thus, step 2 is necessary.
3. Compute the harmonic modes (of the set proteine + close water) with frequencies up to some cutoff (typically 80 cm^{-1}), and orthogonalize the protein components of these modes.
4. Generate a new initial configuration by adding water to the minimum obtained in step 2.
5. Integrate in time the projected eq. (5) for the protein and the usual ones for the solvent with the hydrogen mass increased by a factor of 10.

In all the following simulations, the time step we have been using is 10 fs, and the SHAKE algorithm[25] for the heavy water molecules. In simulations with projected proteins in vacuum, 20 fs time steps or even higher gave satisfactory results. This was not the case for the present system. It should also be mentioned that, in the heating stage of the dynamics, a time step of 10 fs leads to unstable trajectories. We have used a simple time adaptive algorithm to cope with this problem. All the simulations were done with the CHARMM (c24g2) program,[26] using a 13 Å cutoff for the electrostatic forces, and an all-hydrogen force field (version 22). The CTF has been surrounded by TIP3 water molecules in a cubic box of length 40 Å. The nonprojected (reference) trajectories are of a length of 200 ps.

Results

We will consider in the following the particular case of the carboxy terminal fragment (CTF) of the L7/L12 ribosomal protein of *E. coli*.[27, 28] It has previously been shown by several authors[29, 30] that the CTF dimer shows a collective motion between the oligomers that reflects a low-frequency motion (of a period of around 5 ps) that is performed by two of its (sub)domains: this motion appears as a fluctuation of relatively large amplitude of the $\alpha\alpha$ domain with respect to the β-sheet domain. Moreover, this motion seems to be quite insensitive to the particular details of the simulation, such as the existence of effective solvent, choice of the force field, etc. Our purpose here is to show that the projections onto normal modes and the approximations involved in eq. (5) preserve this large-amplitude motion.

Let us first look at the rms (root mean square) fluctuations of the position of the C^{α} atoms. In Figure 1, we represent the measurements made on (a) the standard trajectory (no projection, time step 1 fs); (b) a projected dynamics trajectory (dotted line) with 150 harmonic modes (time step 10 fs) and $\mu\epsilon^{-2} = 0.01$; and (c) the previous standard trajectory projected itself onto the same 150 vectors (dashed line). Although the rms of the projected dynamics trajectory (b) is slightly lower than that of the original trajectory (a), the overall agreement is satisfactory, particularly when compared to the rms of the projection of the original trajectory. By

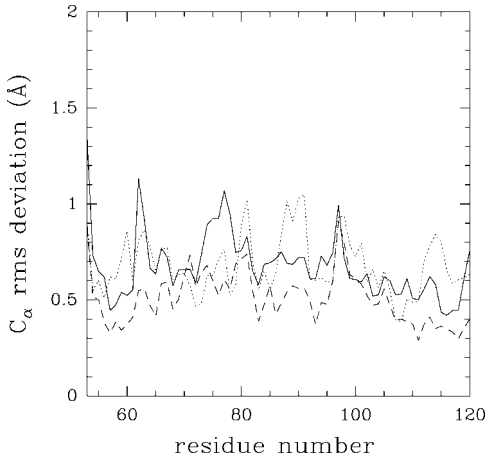


FIGURE 1. The rms of the position of the C^α atoms as a function of the residue number for the standard trajectory (continuous line) and for the trajectory projected into 150 normal modes and $\mu = 0.01$ (dotted line). The dashed line corresponds to the projection onto the same 150 modes of the standard trajectory.

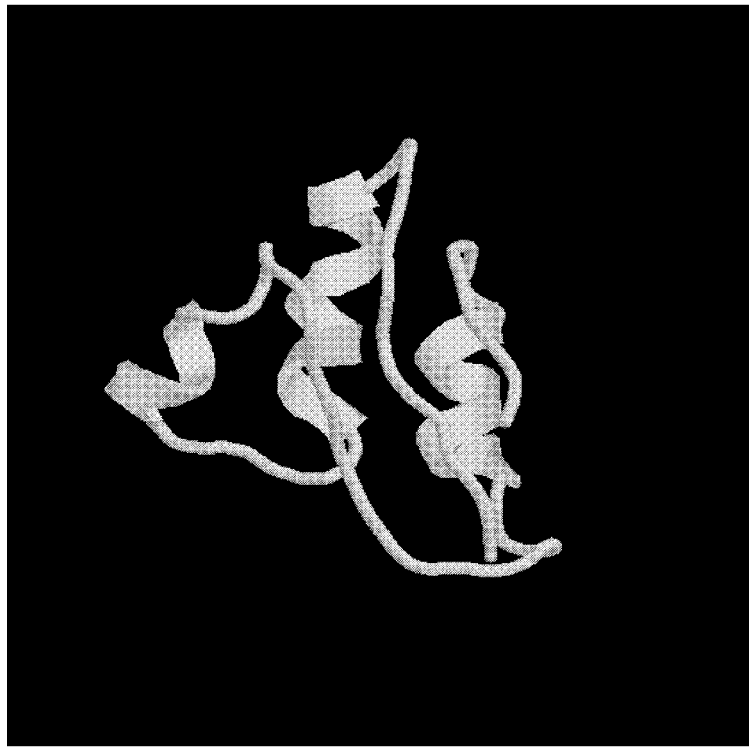
comparison with the results obtained in ref. 13, the projection onto the modes of the quasiharmonic analysis seem to perform better than the harmonic modes. That should be expected, as the former are, by construction, optimal in the description of the position fluctuations in the rms sense. Note, however, that in our previous work the temperature in the projected subspace was a free parameter of the model. The particular value $N' = 150$ chosen here comes from a compromise between accuracy of the projected dynamics and the possibility of using a 10 fs time step. It turns out that twice the number of harmonic modes leads to a trajectory where very often the change in total energy is larger than 20 kcal, thus requiring shorter time steps. The $\mu\epsilon^{-2}E_{bond}$ term is of crucial importance in this context. We have initially integrated the model equation (5) with $\mu = 0$ and $N' = 50$, obtaining satisfactory results for the rms of the C^α carbons (not shown in Fig. 1). However, when increasing from $N' = 50$ to $N' = 100$, while keeping $\mu = 0$, the protein very rapidly loses its main structural properties, as can be seen in the ribbon representation of Figure 2.

Let us now turn to the consideration of the large amplitude motion observed in refs. 29 and 30, between the $\alpha\alpha$ and the β-sheet domains. More precisely, following the method proposed in ref. 29, we measure the angle between the α-B and α-C helices, defined, respectively, by residues 80–88 and 100–113, using the program *helix* of CHARMM. The resulting time series and corresponding Fourier spectra are displayed in Figures 3 and 4, respectively, for simulations done with no projections and projections with 150 ($\mu\epsilon^{-2} = 0.01$) and 150 ($\mu = 0.0$) harmonic modes. The time series (Fig. 3) shows that the average angle ($\sim$70°) is well conserved upon projection for the trajectories (b) and (c), all of them being started from the same configuration and performed with the same heavy water model. Figure 3a shows that a different starting configuration together with the use of standard water yields an average angle around 75°. This probably corresponds to the existence of several basins of attraction for CTF. The trajectory (d) shows the angle variation for $N' = 150$, $\mu = 0.0$ (the asymptotic value of this angle is $\sim$30°), which corroborates the undesirable effect of increasing N', while keeping the evolution eq. (5) with $\mu = 0$.

The Fourier spectra (Fig. 4) show two interesting features. First, all the spectra show a typical power law decay that is quite well preserved by the projections, independently of the number of harmonic modes. Second, the characteristic frequency of the time series (here identified with the maximum of the spectra) depends on the number of harmonic modes retained in the projection. Here, a number of around 50 modes and $\mu = 0$ seems to yield satisfactory results concerning the position of the characteristic frequency (4.5 ps vs. 6 ps). Increasing this number to 150 and adding the bond corrections with $\mu\epsilon^{-2}$ yields a low-frequency component not present in the nonprojected trajectory, but also a secondary frequency quite similar to that of the reference trajectory. Notice that a similar variations of the characteristic frequencies are observed upon changes on the parameters used in the electrostatic computations (cf., for instance, Figs. 5 and 9 of ref. 29 obtained by changes in the electrostatic cutoff distance). We, therefore, conclude that the present model dynamics seems to fairly reproduce the angle variations between the α and β domains.

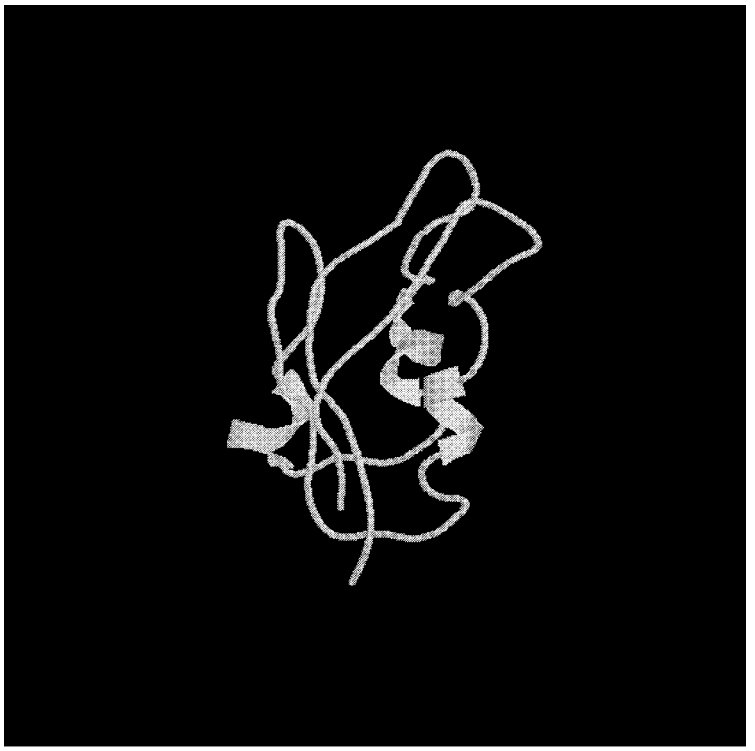
In Figure 5 are shown the dihedral fluctuations observed during the projected and nonprojected trajectories, as well as their mean averages. As before, we also consider the variations of the reference trajectory projected onto the first 50 harmonic modes. From this figure, it is clear that, although the average values are (globally) well preserved upon projection, the fluctuations are reduced by almost a factor 2. As before, the projected dynamics are able to quite well reproduce the projected dihedral fluctuations. The reduction of the dihedral fluctuations can be easily understood by saying that the projection acts as a filter, keeping only the collective motions, all the local movements being discarded.

(a)

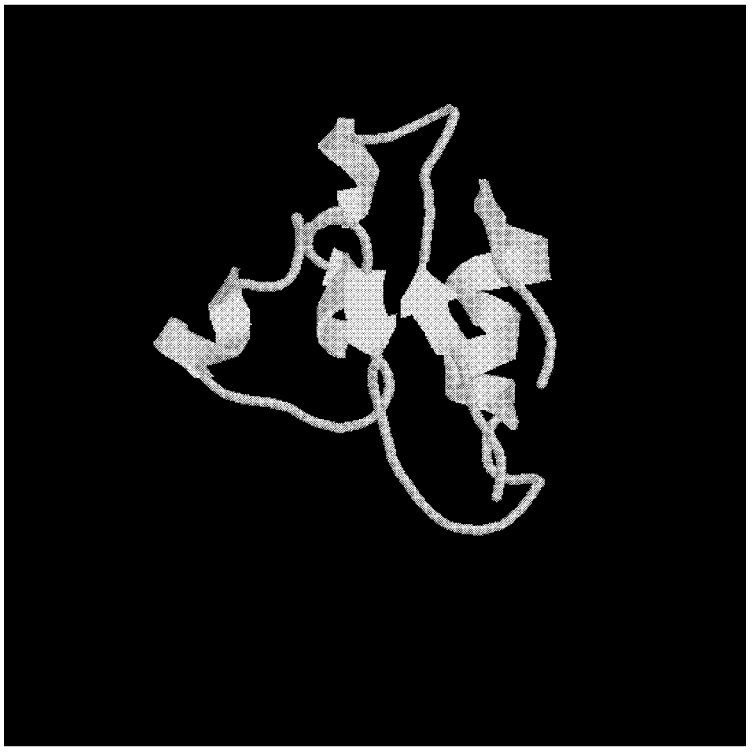


(b)

FIGURE 2. (a) Ribbon representation of CTF after 200 ps of standard dynamics. (b) Ribbon representation of CTF after 200 ps of projected dynamics with $N' = 50$ and $\mu = 0$. (c) Ribbon representation of CTF after 200 ps of projected dynamics with $N' = 150$ and $\mu = 0$. (d) Ribbon representation of CTF after 200 ps of projected dynamics with $N' = 150$ and $\mu\epsilon^{-2} = 0.01$.



(c)



(d)

FIGURE 2. (Continued)

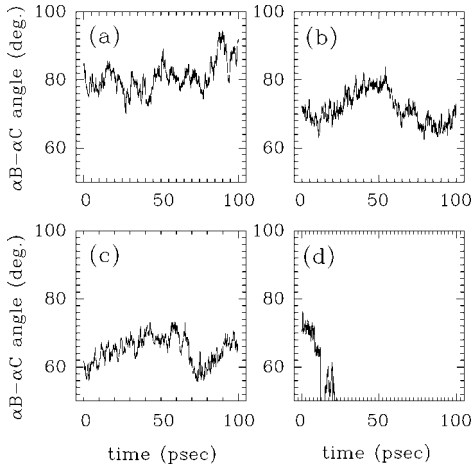


FIGURE 3. Time series of the angle between the α-B and α-C helical axis computed with: (a) standard water trajectory, (b) heavy water nonprojected trajectory, (c) projected trajectory with 150 modes and $\mu\epsilon^{-2} = 0.01$, (d) projected trajectory with 150 modes and $\mu = 0$.

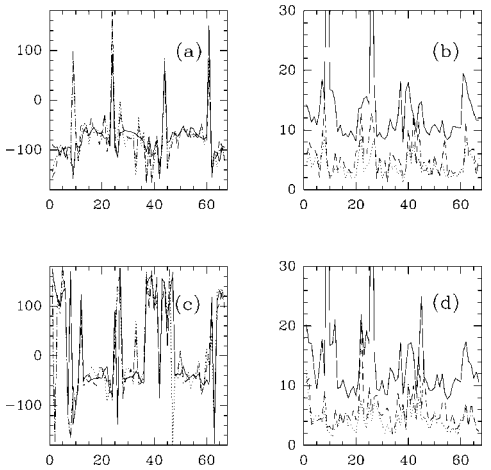


FIGURE 5. Average and fluctuations of dihedral angles for, respectively, the reference trajectory (continuous line), the projected (50 modes) reference trajectory (long dashed line) and the projected dynamics (50 modes) (short dashed line): (a) average of φ dihedrals, (b) fluctuations of φ dihedrals, (c) average of ψ dihedrals, (d) fluctuations of ψ dihedrals.

In Figure 6 are displayed the fluctuations in the c_1–c_2 plane, obtained from the reference and projected trajectories. The agreement between the two plots should be expected from the closeness of the rms fluctuations of the positions of the C^α atoms, as those are mainly due to collective fluctuations. This plot also gives some information on the volume of phase space explored by the considered trajectory.

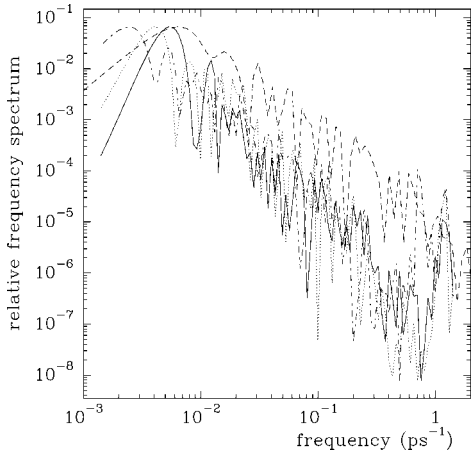


FIGURE 4. Relative power spectrum of the angle between the α-B and α-C helical axis computed with: (a) standard water trajectory (connected line), (b) heavy water nonprojected trajectory (dashed line), (c) projected trajectory with 50 modes and $\mu = 0$ (short dashed line), (d) projected trajectory with 150 modes (long-short dashed line) and $\mu\epsilon^{-2} = 0.01$.

Figure 6 shows that this volume is not significantly reduced by the projected dynamics. It is interesting to note that, here, the φ_1 mode is different from the first quasiharmonic mode, as its fluctuations are lower than those of φ_2.

Finally, it is also of interest to quantify the eventual geometric distorsion of the protein induced by the rescaling of the bonded interactions. To do so, we have measured the average bond distances along the backbone of the protein, as well as the C_γ–C_δ distances in the lateral chains. It turns out that all these lengths are, on average, slightly longer than the corresponding equilibrium distance, the elongation being typically between 2 and 3%, and not exceeding 5% of this distance. Similar effects can be observed for the angles between successive bonds.

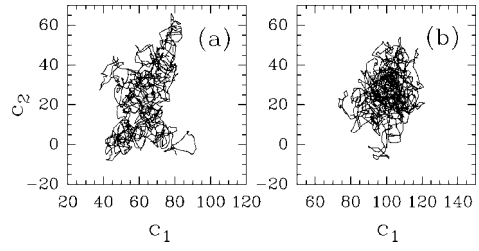


FIGURE 6. Trajectory in the c_1–c_2 plane: (a) projected trajectory with 50 modes, (b) reference trajectory.

Concluding Remarks

In this work, we have considered some aspects of the extension of our previous work to simulate proteins in vacuum to the more realistic situation of proteins surrounded by a solvent. Furthermore, we have extended the validity of our method to bases constituted by harmonic modes, instead of the quasiharmonic used in ref. 13. This reduces the *a priori* information needed to run the projected dynamics to the determination of some particular minimum, and to the computation of the corresponding harmonic modes. With the help of methods such as those developed in ref. 9, this is significantly less demanding than the computation of quasiharmonic modes.

The main conclusion of this study is the feasability of computing molecular dynamics trajectories using rather large time steps, at the same time keeping the properties related to collective motions, such as the angle fluctuations considered here. The effective speedup obtained both in parallel and scalar architectures is close to optimal (a factor 9 with a time step of 10 fs), indicating that the additional overhead implied by the projection onto normal modes is fairly negligible. The major deficiency of the present model is the use of a "viscous" solvent (obtained through the artificial increase of the water hydrogen mass). This point can probably be circumvented through the use of implicit solvent models. Finally, the extension of the present method to the more general situation where the reference configuration of the protein is allowed to rotate and translate is clearly needed to uncover interesting situations such as the dynamical behavior of transmembrane proteins.

References

1. Brooks, C. L.; Karplus, M.; Pettit, B. M. Adv Chem Phys 1988, 71.
2. McCammon, J. A.; Harvey, S. C. Dynamics of Proteins and Nucleic Acids; Cambridge University Press: Cambridge, 1987.
3. Brooks, B.; Karplus, M. Proc Natl Acad Sci USA 1985, 82, 4995.
4. Horiuchi, T.; Go, N. Proteins 1990, 10, 106.
5. Kitao, A.; Hayward, S.; Go, N. J Chem Phys 1991, 158, 447.
6. Amadei, A.: Linssen, B. M.; Berendsen, H. J. C. Proteins: Str Funct and Gen 1993, 17, 412.
7. van Aalten, D. M. F.; Findlay, J. B. C.; Amadei, A.; Berendsen, H. J. C. Proteins 1995, 22, 45.
8. Garcia, A. E. Phys Rev Lett 1992, 68, 2696.
9. Marques, O.; Sanejouand, Y.-H. Proteins 1995, 33, 557.
10. Askar, A.; Space, B.; Rabitz, H. J Phys Chem 1995, 99, 7330.
11. Durup, J. J Phys Chem 1991, 95, 1817.
12. Chun, H. M.; Padilla, C. E.; Chin, D. N.; Watanabe, M.; Karlov, V. I.; Alper, H. E.; Soosar, K.; Blair, K. B.; Becker, O. M.; Caves, L. S.; Nagle, R.; Haney, D. N.; Farmer, B. L. J Comp Chem 2000, 21, 159.
13. Elezgaray, J.; Sanejouand, Y. H. Biopolymers 1998, 46, 493.
14. Reich, S. Physica D 1995, 89, 28.
15. Schütte, C.; Bornemann, F. A. Physica D 1997, 57.
16. Sanejouand, Y. H. Ph.D. Thesis, Paris (1990).
17. Peterson, C.; Sommelins, O.; Söderberg, B. Phys Rev Lett 1996, 76, 1079.
18. Takens, F. In Global Theory of Dynamical Systems, Evanston 1979; Nitecki, Z.; Robinson, C., Eds.; Springer-Verlag: Berlin, 1980.
19. Fixman, M. J Chem Phys 1978, 69, 1527; 1978, 69, 1538.
20. van Gunsteren, W. F.; Karplus, M. Macromolecules 1982, 15, 1528.
21. Balsera, M. A.; Wriggers, W.; Oono, Y.; Shulten, K. J Phys Chem 1996, 100, 2576.
22. Kolossvary, I.; Guida, W. C. J Am Chem Soc 1996, 118, 5011.
23. Ma, J.; Karplus, M. J Mol Biol 1997, 274, 114.
24. Pomes, R.; McCammon, J. A. Chem Phys Lett 1990, 166, 425.
25. van Gunsteren, W. F. J Comp Phys 1977, 23, 327.
26. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. J Comp Chem 1983, 4, 187.
27. Moller, W. In Ribosomes; Nomura, M.; Tissieres, A.; Lengyel, P., Eds.; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1974, p. 711.
28. Moller, W.; Groene, A.; Terhorst, C.; Amons, P. Eur J Biochem 1972, 25, 5.
29. Sanejouand, Y.-H.; Tapia, O. J Phys Chem 1995, 99, 5698.
30. Aqvist, J.; Leijonmarck, M.; Tapia, O. Eur Biophys J 1989, 16, 327.