Eur Biophys J (1999) 28: 478–488

ARTICLE

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Constructing the suitable initial configuration of the membrane-protein system in molecular dynamics simulations

Received: 10 September 1998 / Revised version: 19 March 1999 / Accepted: 19 March 1999

Abstract A method for constructing the suitable initial configuration of the membrane-protein system for molecular dynamics (MD) simulations is presented. This method could provide some hydrated initial configurations and help us to determine the best surface area of the system by contracting the surface area and comparing the optimized lowest energy of the system by energy minimization. The gramicidin A (GA) channel in the fully hydrated dimyristoylphosphatidylcholine (DMPC) bilayer was used as our model. Three configurations with different surface areas were selected and applied for one 400 ps and two 300 ps MD simulations at constant pressure and temperature. All simulations were fairly stable without any constraints. Through analysis of the MD trajectories we found that the system with the best surface area was more stable than the other two systems, whose sizes were changed in the simulations. Further analysis of the bilayer normal length and the order parameters of the lipid alkyl tails indicates that the system with the best surface area shows some characteristics of the L_α phase, while both the smaller and the larger size systems have distinct deviations from the L_α phase that we expect. This illustrates that the correct surface area and the suitable initial configuration have an important influence on the phase of the membrane in the MD simulation. In addition, by comparing the root mean square differences of GA relative to the initial structure and interaction energy between different components of the system for all three systems, we find that the state of the DMPC bilayer has exerted a significant influence on the structure of GA. All these results demonstrate the validity of our method for constructing the initial configuration of the membrane-protein system for MD simulations.

Key words Gramicidin A · Dimyristoylphosphatidylcholine · Ion channel · Lipid bilayer · Membrane-protein interaction

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Introduction

In recent years, molecular dynamics (MD) simulation has developed rapidly and is used widely as a powerful tool for study of the structure and dynamics of macromolecules (van Gunsteren 1988; Karplus and Petsko 1990). Many studies of soluble proteins in vacuum and in solution have demonstrated its validity in the fields of structure refinement, free energy calculation, and others (Karplus and Petsko 1990). However, in the case of membrane systems such as lipid bilayers and membranes with proteins embedded, the application of MD simulations only appeared at the end of 1980s owing to the large size of the systems and the long time scales of the motions (Egberts et al. 1994). In the following 10 years, numerous workers have been involved in this rapidly developing research field with studies on the properties of pure bilayers and on the interactions between small molecules and lipid bilayers, as well as on the interactions between transmembrane helices and lipid bilayers (Pastor et al. 1991; Bassolino et al. 1993; Venable et al. 1993; Marrink and Berendsen 1994; Pastor 1994; Woolf and Roux 1994; Chiu et al. 1995; Damodaran et al. 1995; Edholm et al. 1995; Woolf and Roux 1996; Merz

1997; Shen et al. 1997). However, in spite of this rapid progress, two problems prevent further applications of MD simulations in this field (Pastor 1994; Woolf and Roux 1996; Merz 1997). First, the extension of the force field, which was originally used in the simulations of soluble proteins in solution or vacuum, to membrane systems still needs more examination and careful consideration. Particularly in the past two years the correct treatment of long-range electrostatic interactions for lipid bilayers is of particular concern since the head groups of many lipids are highly charged (Feller et al. 1996; Merz 1997). Second, the construction of a suitable initial configuration for the membrane system is critical to the success of the MD simulation (Heller et al. 1993; Pastor 1994; Woolf and Roux 1994, 1996). The membrane system is a very large and flexible system in which all components are tightly packed together and the bilayer structure is maintained only by the non-bonded interactions. Therefore, an incorrect initial configuration may result in an unstable or disordered state of the bilayer and affect the structural and dynamic properties of the lipids and proteins in MD simulations (Heller et al. 1993; Pastor 1994).

There are two methods commonly used to construct an initial configuration for a membrane system with a protein embedded (Woolf and Roux 1996). The first is the standard overlay method used for constructing the initial configuration for proteins in solution, which inserts a protein into the bulk solvent and removes the overlapping solvent. The second is to pack the protein and lipids together and assemble the lipid bilayer around the protein. No matter which method is used, how to choose the surface area (also called the cross-sectional area) of the model system is a serious problem. Prior studies for a pure dimyristoylphosphatidylcholine (DMPC) bilayer have illustrated the important effect of the surface area on the state of the membrane system (Heller et al. 1993; Damodaran and Merz 1994; Pastor 1994). Both overestimation and underestimation of the surface area will cause phase deviation of the lipid bilayer. This point is also demonstrated in the present study. However, in spite of extensive investigations by experimental and theoretical studies, the correct value of the surface area per lipid in a pure bilayer is still unclear (Woolf and Roux 1996; Shen et al. 1997). A range from 0.52 to 0.74 nm^2 for the surface area of a dipalmitoylphosphatidylcholine (DPPC) lipid is obtained by different methods (Pastor et al. 1991; Nagle 1993; Chiu et al. 1995; Feller and Pastor 1996; Nagle et al. 1996; Tieleman and Berendsen 1996; Tu et al. 1996; Woolf and Roux 1996). As for the surface area of a protein in a bilayer, the value is even more confusing. For instance, the surface area of gramicidin A (GA) has a range of 1.1–3.05 nm^2 (Urry 1971; Mau et al. 1987; He et al. 1993; Woolf and Roux 1996). Therefore, for our model system (GA dimer with 16 DMPC lipids), the possible value of the total surface area could be between 5.26 and 8.97 nm^2. Obviously, this range is too wide for us to directly generate a starting configuration with the correct surface area.

The goal of this work is to present a method for constructing a suitable initial configuration for the membrane-protein system. The initial configuration can be obtained, and a reasonable value for the total surface area of the model system can be selected, by using a special contraction process. Although the problem considered here is somewhat similar to one studied by Woolf and Roux (1996), the method used in the present work is different. Woolf and Roux used the global system search method and the CHARMM force field (Mackerell et al. 1992) plus the TIP3P water potential (Jorgensen et al. 1983) to select the initial system and perform MD simulations. In the present study, we have used the method of combining the configuration construction with contraction of the surface area to choose the best surface area of the system. The GROMOS 96 force field (van Gunsteren et al. 1996) and SPC/E water model (Berendsen et al. 1987) were used in our work. Even though both approaches and procedures are quite different, our results are very consistent with those obtained by Woolf and Roux.

The GA protein and DMPC lipids were used as a model system. GA is a linear antibiotic pentadecapeptide (Wallace 1990) and can form the voltage-gated ion channel in a membrane with a head-to-head dimer conformation by two single-stranded right-handed helices (Wallace 1990; Roux and Karplus 1994). The high-resolution structure of the GA channel has been determined by NMR experiments (Arseniev et al. 1985; Chiu et al. 1991; Ketchem et al. 1993; Cross and Opella 1994; Hu et al. 1995). Because of its small size and exceptionally well defined structure and function, GA has been studied widely as a model for ion channels and lipid-protein interactions in both experimental and theoretical work in the past 20 years (Andersen 1984; Wallace 1990; Xing and Scott 1992; Roux and Karplus 1994; Koeppe and Andersen 1996).

The following is the outline of this paper. The method for building up the model system and the computational details, especially the details of the generating process and the contraction process, are presented in the next section. Then, the results of the construction procedure and the MD simulation are reported and discussed. Finally, some important points are summarized in the conclusions.

Methodology

Generation of the starting configuration

The simulation system consisted of one GA dimer channel and 16 DMPC lipid molecules plus bulk water molecules. This model has been also used in NMR experiments and other MD simulation work (Ketchem et al. 1993; Woolf and Roux 1994; Hu et al. 1995; Woolf and Roux 1996). The initial coordinates of the GA channel used in this study were obtained from the entry

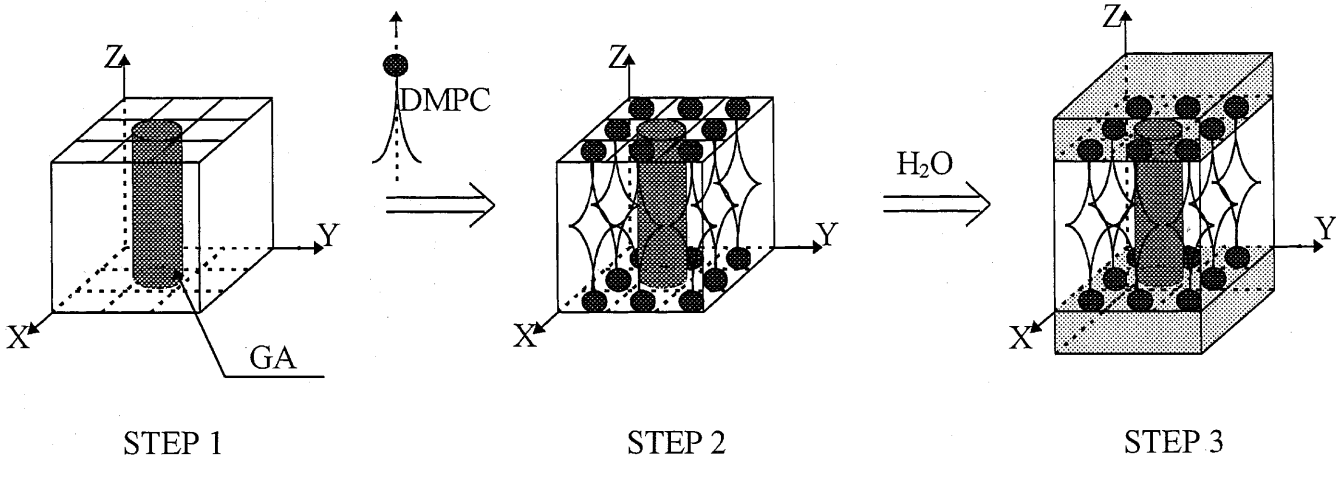
1MAG of the Protein Data Bank (Ketchem et al. 1993). The initial coordinates of DMPC were taken from the equilibrium structure of the previous MD simulation for the DPPC bilayer in the L_α phase (Egberts et al. 1994). The only difference between the DPPC and DMPC lipids is that there are two more CH_2 groups at the end of each DPPC alkyl chain, so we deleted these groups from DPPC to form the DMPC configurations. The DMPC lipid is often used as a model for a lipid bilayer. Its lecithin head-group is a zwitterion but it is overall neutral, with a positive choline group and a negative phosphate group. The two alkyl tails of the DMPC molecule, which had 12 CH_2 groups each, are designated as Sn-1 and Sn-2 in this paper.

The method used to construct the initial configuration of the model system for the MD simulation consisted of two processes: a generation process and a contraction process. The generation process was used to generate the starting configuration for the contraction process, which is similar to the method proposed by Venable et al. (1993) for constructing a pure lipid bilayer and developed by Woolf and Roux (1994, 1996) for constructing the GA-DMPC starting configuration. Figure 1 shows the schematic view of the generation process. A rectangular box was equally divided into nine blocks in its *X*-*Y* plane (see step 1 of Fig. 1). The initial configuration of the GA dimer was placed in the central block of the box with its channel axis parallel to the *Z*-axis. Then, 16 pre-equilibrated DMPC lipid configurations, which were randomly selected from the library of lipid configurations, were placed in eight peripheral blocks in a bilayer form with their normal direction along the *Z*-axis (see step 2 of Fig. 1). Although it is more natural to use a hexagonal box for this channel-membrane system rather than a rectangular box (Woolf and Roux 1996), the periodic boundary condition and completely free MD simulation make it also reasonable to use a rectangular box in our simulations. In step 3, two layers of bulk water were added on both sides of the bilayer. Since there were no water molecules in the hydrophobic region of the membrane, the water molecules which entered into the region between $Z = \pm 1.4$ nm were all removed.

Since the DMPC and GA molecules are packed together artificially, a number of bad contacts between molecules are formed and should be taken into account. In the present study, two ways were used to reduce these bad contacts. One way was to take a large total surface area (12 nm^2 in our work), because a large surface area was demonstrated to be very effective in decreasing bad contacts (Woolf and Roux 1996). The later contraction process also required a large surface area (see below). The other way was to let DMPC and GA have a random rotation around their axis (parallel to the *Z*-axis) and make a random position for the phosphorus atoms (P atoms) of DMPC in the *X*-*Y* plane of each block. The experimental data indicated that the bilayer normal length of DMPC (the distance between P atoms of two layers) is about 3.6 nm in the L_α phase (Engelman and Lewis 1983; Watnick et al. 1990). In addition, recent MD simulation work illustrates that the membrane-solvent interface is as broad as 1.5 nm (Woolf and Roux 1996). Therefore, the *Z*-coordinates of the P atoms of DMPC were set at ± 1.8 nm with a random shift within ± 0.2 nm from this value in this work.

For each starting configuration of the GA-DMPC system in water, which was built with the method mentioned above, 20 steps of steepest descent energy minimization (EM) were performed for this system and the final energy was recorded. At last, 10 configurations with the lowest energies were selected from hundreds of starting configurations and subjected to further extensive EM (200 steps with the steepest descent method and 600 steps with the conjugate gradient method). All of these configurations reached minimal energy within 800 steps. Throughout all of these EM procedures the harmonic position restraints were applied for all the GA

Fig. 1 Schematic view of the generation process. The normal axis of the bilayer and GA channel axis are in the *Z*-axis direction. The *X*-*Y* plane of the box is considered as the total surface area and is divided equally into nine blocks. GA is in the central block and eight DMPC lipid molecules are in the surrounding eight blocks. The gray regions in step 3 are bulk water layers



atoms and for the P atoms of the DMPC lipids to maintain a reasonable conformation of the GA and the normal length of the bilayer membrane. These optimal configurations were then used in the contraction procedure.

The contraction process for selecting the best surface area

The right choice of surface area for the membrane system is a very important factor in a successful MD simulation (Heller et al. 1993; Pastor 1994). In order to solve this problem, a special contraction procedure is proposed in our method. The main idea of this procedure is to decrease the total surface area of the system step by step and obtain the minimal energy of the system at every step by the extensive EM. Thus, comparing the minimal energies, we could decide which was the best surface area for a given model system. The contraction process can be represented in detail as follows. First, the *X*- and *Y*-coordinates of the DMPC lipid were contracted by multiplying by a factor of 0.99 while the *Z*-coordinates were retained. However, all the atom coordinates of GA were fixed since its structure was taken from experimental data and could represent the right configuration for GA in the L_α phase DMPC bilayer. Second, in order to retain the density of water, we removed those water molecules which were located outside the new boundary of the contracted system. Then, a 600 step EM with the conjugate gradient method was performed for the new configuration, in which all the atoms of GA and the P atoms of DMPC were still constrained. Such a contraction step was repeated 38 times and the total surface area was reduced to 5.47 nm^2 from 12.00 nm^2. Comparing the final energies with different surface areas of the system, we could choose the best surface area associated with the lowest energy. Thus, we not only obtained a series of optimal initial configurations for the model system with different surface areas, but also determined which was the suitable initial configuration with the best surface area for our model in MD simulations.

Computational details of the MD simulations

The GROMOS 96 force parameters were used for all calculations of the EM and MD simulations. Since there was no parameter set for DMPC in the GROMOS 96 force field, we referred to the data reported by Egberts et al. (1994) and updated them to the GROMOS 96 force field. The ultimate parameters used in this work for DMPC are listed in the Appendix. Non-polar hydrogen atoms were included in the carbon atoms (united atom) while polar hydrogen atoms were treated explicitly. The Ryckaert-Bellemans potential (Ryckaert and Bellemans 1975; see Appendix) was used in this work for the dihedral angles of the alkyl chains of DMPC, such as the CH_2—CH_2—CH_2—CH_2 and CH_2—CH_2—CH_2—CH_3 dihedral angles. This potential was demonstrated to be very suitable for MD simulations of lipid bilayers in the L_α phase (Egberts and Berendsen 1988; Pastor et al. 1991). The SPC/E model was used for water molecules (Berendsen et al. 1987). In all the EM and MD procedures the bond lengths were constrained with the SHAKE algorithm (Ryckaert et al. 1977; van Gunsteren and Berendsen 1977). The cut-off radius was 0.8 nm for the short-range interactions and 1.3–1.4 nm for the long-range interactions. Because the model systems in the present study contained strongly polar groups (the headgroups of the DMPC lipid), careful handling of the electrostatic interaction is very important. Therefore, we incorporated the reaction-field calculation in all the simulations since it has been showed that the inclusion of the reaction field had a significant improvement on the correction of the long-range calculation of the electrostatic interaction (Hunenberger and van Gunsteren 1998). The periodic boundary conditions were applied along all three dimensions. All MD simulations were performed with constant temperature and constant pressure (Berendsen et al. 1984). By being weakly coupled to a temperature bath and a pressure bath, the system was kept at 340 K, which was above the transition temperature of the gel-liquid crystal phase, and in a pressure of 1 atm. We have noticed that in the past two years there has been a considerable debate about the proper choice of the normal pressure (NTP ensemble) or the surface tension ($NP_n\gamma T$ ensemble) as the boundary condition for the bilayer system (Jakobsson 1997; Merz 1997). Since the recent studies demonstrated that the use of a NTP or $NP_n\gamma T$ ensemble made little practical difference in the simulation (Tieleman and Berendsen 1996), we chose the NTP ensemble in our simulation work. The MD time step was set to 2 fs, and trajectories were stored every 50 steps (0.1 ps). The starting 60 ps simulation was carried out for the equilibrium process with harmonic restraints to all atoms of GA and P atoms of DMPC. These restraints were gradually reduced by decreasing the parameters of the harmonic restraints potential step by step. Therefore, the DMPC and GA were completely free after 40 and 60 ps MD simulations, respectively. No restraints were included in the systems in the following simulations.

In this work, three initial configurations were selected for the MD simulations. The main difference of these configurations was in their initial surface areas: one system with 7.84 nm^2 (medium size system), one with 6.55 nm^2 (small size system), and another one with 9.41 nm^2 (large size system). Since the 7.84 nm^2 was the best surface area obtained by our method, the 400 ps MD simulation was performed for this system. The other two 300 ps MD simulations were performed for the large and the small size systems, and the corresponding results were used to compare with those obtained from the medium size system and to examine the validity of our method for constructing the suitable initial configuration of the membrane-protein system.

Results and discussion

Selection of the suitable surface area

As stated above, the choice of the surface area of the membrane system has an important influence on the structural and dynamic properties of a lipid bilayer and the nature of the membrane-protein system in the MD simulation (Heller et al. 1993; Pastor 1994). Furthermore, the artificial estimate of this area for the membrane-protein system is very difficult owing to the wide range of possible values (Woolf and Roux 1996; Shen et al. 1997). Therefore, the contraction process mentioned above has been used to help us to choose the best surface area. The Lennard-Jones (L-J) interaction energy between solute molecules of the system was chosen as the criterion to select the best surface area since the L-J interaction has more sensitivity to the change of surface area owing to its short-range behavior.

To choose the best surface area, 10 starting configurations of the GA-DMPC system obtained from the generating process were contracted from 12.00 to 5.47 nm^2. Figure 2 shows the relation of the L-J interaction energy between solute molecules to the surface area for three configurations. For the other seven configurations there exists a similar trend to that shown in Fig. 2 except that the quantities of the L-J energy are somewhat different. It is clear from Fig. 2 that the surface areas corresponding to the lowest energy are all located in the region from 7.69 to 8.13 nm^2. Therefore, these surface areas are the best surface areas obtained with our method and the values are similar to those obtained by Woolf and Roux (1994, 1996). These results support the idea that taking the L-J interaction as a criterion for selecting the best surface area is reasonable. The fact that all profiles have the similar best surface area indicates that this result is independent of the specific starting configuration and displays the intrinsic property of the system. However, we must emphasize that the best areas correspond to the normal length of a certain bilayer, which is used as an index for the bilayer phase. As stated above, we set the normal length of the bilayer to 3.6 nm since we want the bilayer to be in the L_α phase. This normal length was retained by position restraints of the P atoms of DMPC in the contraction process so that the structure with the best area obtained here is only suitable for the MD simulation of GA in the L_α phase of DMPC.

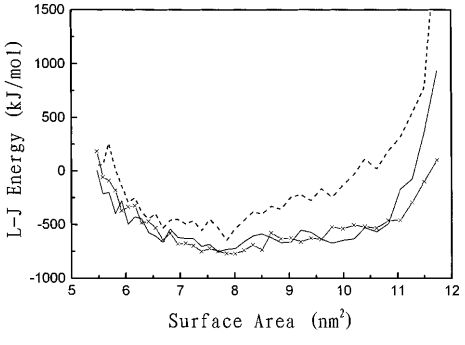


Fig. 2 The total L-J interaction energies between solute molecules of the GA-DMPC system are plotted as a function of the total surface area of the system in the contraction processes for three different starting configurations

At last, a model system with an area of 7.84 nm^2 was selected as the best model system and simulated for 400 ps. For comparison, two other systems with areas of 6.55 nm^2 and 9.41 nm^2 were also used to perform 300 ps MD simulations.

Changes of system size in MD simulations

Since the goal of our construction method is to provide an optimal initial configuration with the best surface area, the results obtained from the MD simulations can be used to examine the validity of our method.

Prior to any other analysis, the convergence behavior of all MD simulations is verified. The root mean square differences (RMSD) of the atomic position with respect to a reference structure is a useful value to examine the intermolecular conformational variation of a protein. The RMSD of C_α and all atoms of GA with respect to its initial structure are plotted as a function of simulation time in Fig. 3. Since the atoms of GA and the P atoms of DMPC are restrained during the initial 40 ps simulation and the C_α atoms of GA are restrained until 60 ps, the RMSD values are very small during this simulation period. When the restraints are removed, the RMSD values increase quickly and reach a relatively stable plateau after 150 ps MD simulation. It is clear that the RMSD values for both C_α and all the atoms of GA in the medium size system are less than that in the other two systems. In addition, the convergence behavior of the medium size system is better than that of the other two systems. This result indicates that an inappropriate surface area may cause the deviation of the GA channel from its correct conformation in the DMPC bilayer.

Since MD simulations are performed in the constant pressure regime, changes of the system size with simulation time should be a useful value to appraise the reliability of the method. Figure 4 shows changes of the surface area of all three systems with simulation time. It is clear that after 100 ps the surface area of the medium size system recovers to its initial value and equilibrates at 7.87 nm^2. However, for the large size system (9.41 nm^2) it deceases and equilibrates at 8.88 nm^2, and for the small size system (6.55 nm^2) it increases and equilibrates at 6.94 nm^2. In addition, the changes of the *Z*-axis length for all three systems with simulation time are presented in Fig. 5. The situation is similar to that presented in Fig. 4. After 100 ps, the medium size system remains at its *Z*-axis length of 7.61 nm, while for the small size system it increases to 7.82 nm, and for the large size system it decreases to 7.38 nm. These results suggest that the configuration of the medium size system is more stable than the other two systems used in our

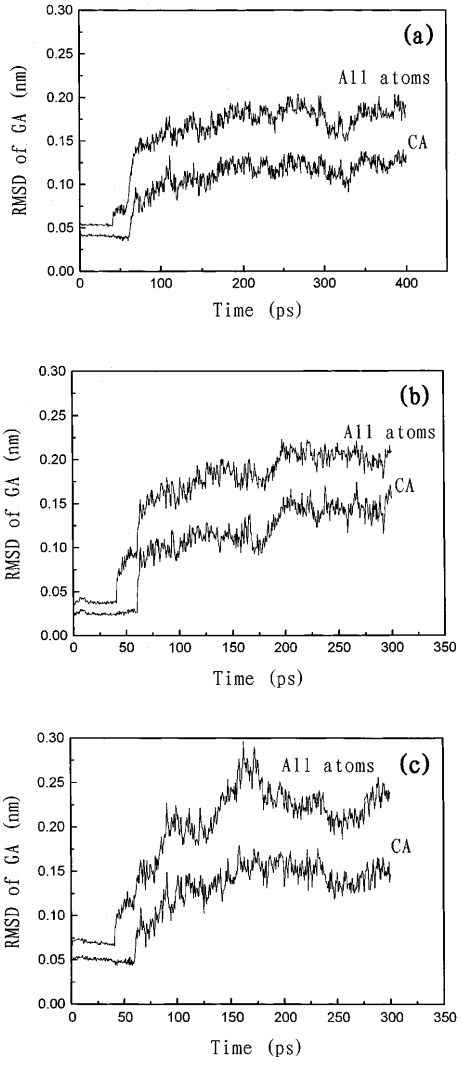


Fig. 3 The RMSD of C_α and all atoms of GA relative to the initial structure versus the MD simulation time: **a** for 400 ps MD simulation of the medium size system; **b** for 300 ps MD simulation of the small size system; **c** for 300 ps MD simulation of the large size system

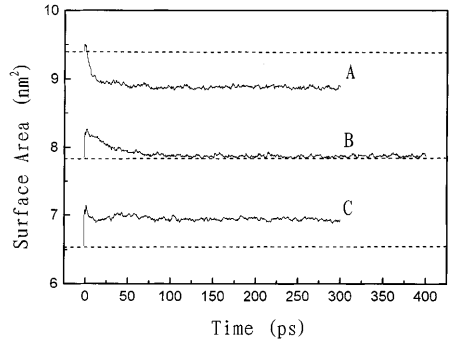


Fig. 4 The changes of the GA-DMPC system surface area with simulation time for the three systems: the large size system (9.41 nm^2) for 300 ps MD simulation (*A*); the medium size system (7.84 nm^2) for 400 ps MD simulation (*B*); and the small size system (6.55 nm^2) for 300 ps MD simulation (*C*). The initial surface areas of the systems are drawn with *dashed lines* as a reference

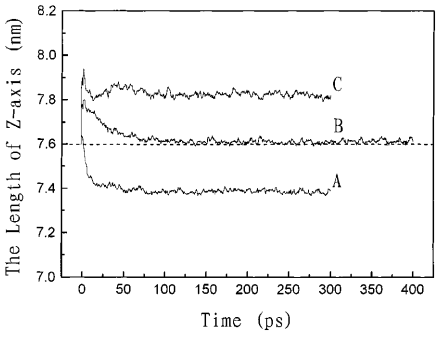


Fig. 5 The changes of *Z*-axis length with simulation time for the three systems: the large size system (*A*); the medium size system (*B*); and the small size system (*C*). The initial *Z*-axis length of the systems (7.60 nm) is drawn with the *dashed line* as a reference

simulations and demonstrates again that the initial surface area has the dominant influence on the final state of the system. On the other hand, the fact that all simulations are equilibrated well in our work indicates that the GROMOS 96 force field is suitable for molecular modeling of lipid bilayer and protein-membrane systems.

However, what factor causes the changes of the small and large size systems? It is worth noticing that both the surface area and the length of the *Z*-axis for the small size system increase, while those for the large system decrease. Since the density of bulk water is constant and the conformation of GA is maintained well during the simulations, we may conclude that the changes of the surface area and the length of the *Z*-axis are mainly contributed from the DMPC molecules. Figure 6 shows the average density distributions of the P atoms of DMPC along the *Z*-axis for the three systems. As shown in this figure, the distributions of the P atoms for the three systems are very broad and display a distinct shift in their peaks, but only the distribution of the medium size system has a maximal value at $Z = \pm 1.75$ nm, which is in good accord with the experimental data (1.8 nm) (Engelman and Lewis 1983). We could point out that only the medium size system is more likely in the L_α phase, while the final state of the small size system tends to the gel-like phase and that of the large size

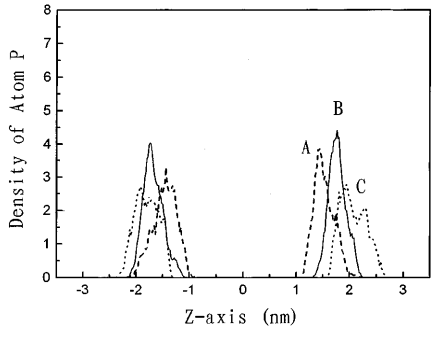


Fig. 6 The average distribution density of P atoms along the *Z*-axis for the three systems with different surface areas: the large size system (*A*); the medium size system (*B*); the small size system (*C*)

system tends to a liquid-like disorder phase in our simulations. According to these results, we suggest that 7.84 nm^2 is the best surface area of our model system. Also based on these results, we should emphasize again that the incorrect surface area would lead the protein-membrane system to an incorrect state in the MD simulations.

Average structural properties

In this section we focus on the detailed analysis of the medium system since it is the best system in our simulations. As shown in Fig. 7, the average distributions along the bilayer normal direction (Z-axis) for the different components of the system are plotted from MD trajectories of the medium size system. The values of those average distributions could help us obtain insight on information about the organization of our model system (Egberts et al. 1994; Damodaran et al. 1995). The resulting profiles are consistent with those determined by an X-ray experiment (Engelman and Lewis 1983), a neutron scatting experiment for an oriented multibilayer system (Tournois et al. 1989), and MD simulations (Woolf and Roux 1996; Shen et al. 1997). The profiles in Fig. 7 indicate the following features:

(1) The atoms of the GA channel are mainly located within a region of 2.6 nm in the bilayer interior, and the distribution is very symmetrical to the bilayer center. This illustrates again that the channel conformation is very stable even though the channel is entirely free in our MD simulation.
(2) The distributions of the alkyl chains of DMPC are reduced near the center of the bilayer, which are in agreement with the previous simulation studies (Damodaran et al. 1995; Woolf and Roux 1996).
(3) The water distribution falls rapidly in the interface of the hydrophilic and the hydrophobic regions, which spans about 1.3 nm. In the region from −0.9 nm to 0.9 nm the water distribution is very small since it is mainly contributed by the single file of hydrogen-bonded waters inside the GA channel.

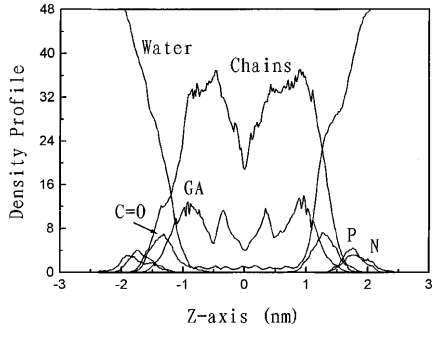


Fig. 7 The average distribution density profiles for various components of the simulation system along the Z-axis (the bilayer normal). The profiles are plotted with the time-averaged data from 400 ps MD trajectories for the medium size system

(4) The distribution of nitrogen atoms (N atoms) of DMPC is similar to that of the P atoms but even more broad.

All above features indicate that the structure of the lipid bilayer and GA channel in the medium size system in our MD simulations is very stable and fairly consistent with the experimental or previous simulation data.

Order parameter

Beside the bilayer normal length, the order parameter is another important criterion for lipid conformation and dynamics (Loof et al. 1991; Egberts et al. 1994). The formal definition of the order parameter tensor S is given by

$$S_{ij} = \tfrac{1}{2}\langle 3\cos\theta_i \cos\theta_j - \delta_{ij}\rangle \quad (1)$$

where θ_i represents the angle between the ith molecular axis and the bilayer normal (Z-axis in this paper), δ_{ij} is the delta function, and the brackets denote an ensemble average (Egberts et al. 1994). In order to compare with experimental data, the deuterium quadrupolar splittings (DQS) order parameter (S^i_{CD}) has been calculated from the MD trajectories. As shown in Fig. 8, the order parameters for the three systems have a variation in quantity. It seems that the larger is the surface area of the system, the smaller will be the order parameter. This

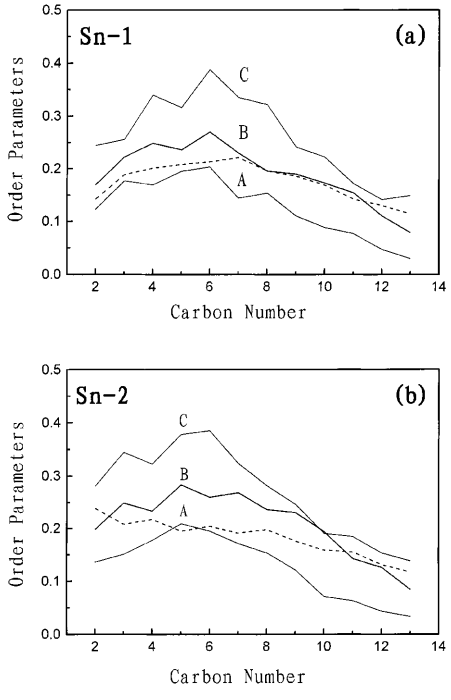


Fig. 8 Order parameters as a function of the carbon atom number for two alkyl tails of DMPC: **a** for the Sn-1 tail; **b** for the Sn-2 tail. The values of the order parameters for all three systems are plotted: the large size system (A); the medium size system (B); and the small size system (C). The experimental data for a pure DMPC bilayer in the L_α phase is plotted with the *dashed line* as a reference

is consistent with the experimental data in which large order parameters were found for the gel phase and a small one for the L_α phase (Horvath et al. 1980; Meier et al. 1986). Compared with experimental order parameters for a pure DMPC lipid bilayer in the L_α phase (Seelig and Seelig 1974, 1980), we find that the DMPC lipid of the medium size system seems to be more likely in the L_α phase than the other two systems. However, the difference between our simulation and the experimental data in some carbon segments of DMPC (see Fig. 8) indicates that the final state of the medium size system deviates somewhat from the purely L_α phase. Those deviations could be produced by the high concentration of the GA channel in the lipid bilayer. Therefore, it should be noted that both the protein and the lipid in the protein-membrane system have effects on the final state of the system so that more attention should be paid in computer simulations to the protein-membrane system.

Interaction energies between main components of the system

Table 1 lists the average L-J and electrostatic (E-S) interaction energies among the three main components of our system (GA, DMPC, and water). The values in Table 1 may help us to understand the characteristics of the interactions between membrane-protein and between membrane-water. The following features can be extracted from Table 1. The total non-bonded interaction energy between GA and DMPC for the medium size system (−548.6 kJ/mol) is lower than that for the other two systems (−468.4 kJ/mol and −454.2 kJ/mol). This means that the DMPC molecules are in a good match with the GA protein in the medium size system during the MD simulation, as mentioned above in the analysis of the RMSD of GA. The E-S interaction energy between GA and DMPC is mainly contributed from both the head group of DMPC and the polar aromatic side chains on the two mouths of the GA channel, where these groups are quite flexible. Therefore, the E-S interaction energy of GA-DMPC is smaller than the L-J energy for the three simulation systems and the corresponding standard deviation is very large compared with its average value. This view has also appeared in previous work (Woolf and Roux 1996). The total L-J interaction energy between the solute molecules for the medium size system is lower than those obtained from the other two systems, which is consistent with their energy order in the contraction process. Thus, this demonstrates again that it is reasonable for us to choose this value as the criterion for determining the best surface area of the system in the contraction process. The total interaction energies between GA and water for the three systems are similar in quantity. This means that the GA channels in the three systems have been completely hydrated in our MD simulations.

Conclusions

In this paper, we have proposed a method to construct the suitable initial configuration of the membrane-protein system for the MD simulation. This method can provide us with a series of optimal initial configurations with different surface areas and help us to choose the best surface area for the model system simulated in a certain bilayer phase. The surface area has a very important influence on the results of the MD simulation for the protein-membrane system, but it is very difficult to select this value from the experimental data that are within a broad range. The contraction process presented in this paper can be used to overcome this problem and change the surface area from a very large value to a small one. The best surface area can be determined by comparing the final EM energy of the systems associated with different areas.

To examine the validity of this method, the GA-DMPC system has been used as a model owing to its simplicity and an abundance of experimental data. The 10 configurations were chosen from hundreds of starting configurations and then used for the contraction process. During contraction, we have found that energy profiles of all 10 configurations versus the surface area show a similar shape and their surface areas with the lowest energy are located in the same region. This result

Table 1 Interaction energies between the main components of the three systems with different surface areas in the MD simulations[a]

Interaction type	Interaction pairs	Simulation systems		
		Large size (9.41 nm^2)	Medium size (7.84 nm^2)	Small size (6.55 nm^2)
L-J interactions	GA-DMPC	−423.9 ± 21.9	−484.5 ± 26.7	−429.7 ± 27.7
	GA-Water	−713.0 ± 33.0	−734.3 ± 39.3	−687.3 ± 30.9
	DMPC-DMPC	−1212.1 ± 64.8	−1298.1 ± 48.7	−1201.4 ± 58.7
	DMPC-Water	−992.4 ± 58.4	−892.7 ± 55.4	−909.9 ± 48.7
E-S interactions	GA-DMPC	−44.5 ± 31.8	−64.1 ± 28.0	−24.5 ± 24.6
	GA-Water	−459.2 ± 51.6	−386.0 ± 39.4	−430.7 ± 44.3
	DMPC-DMPC	−3128.6 ± 154.3	−2920.3 ± 79.5	−3048.9 ± 69.3
	DMPC-Water	−2631.2 ± 297.5	−3027.0 ± 181.0	−2927.9 ± 169.4

[a] Here the unit of interaction energies is kJ/mol. The standard deviations are also displayed. The initial surface areas of the simulation systems are reported in parentheses

implies that the best surface area decided by the contraction process is independent of the specific configuration and the L-J interaction energy may play a key role in determining the phase of the lipid. This view, however, still needs more examination in pure lipid bilayer systems and other membrane-protein systems.

From our MD simulations, we have found that both the surface area and the *Z*-axis length for the medium size system are not changed, while they are decreased for the large size system and increased for the small size system. Furthermore, the bilayer normal length of the medium size system is most consistent with the experimental data of the DMPC bilayer in the L_α phase, as we expect. Besides, the average distributions of different system components along the *Z*-axis for the medium size system show a stable bilayer structure for the membrane-protein system and have similar results with those obtained from previous work. These results also demonstrate that the right choice of surface area plays an important role in the final state of the lipid bilayer in MD simulations. On the other hand, the RMSD values of GA illustrate that GA matches better with the DMPC bilayer in the medium size system than in the other two systems. This point is also consistent with the analysis of the interaction energies between the main system components.

In addition, according to the analysis of the order parameters of the DMPC, it is found that the transmembrane protein also affects the structure of the DMPC alkyl tails, especially those near the head group. We should point out that both the lipid bilayer and the protein have a significant influence on the conformation and dynamics of each other in our MD simulations. Therefore, more attention should be paid to this point in further MD simulations for the membrane-protein system.

In summary, based on the results of our simulations for the GA-DMPC systems, we believe that our construction method used in this work is of the necessary validity and quality. This method is fairly generic and not limited to the generation of the initial configuration for simple systems like GA-DMPC. With only a few modifications, it can be used for pure lipid bilayers with large amount of lipids and for systems with multiple transmembrane helices in a certain phase lipid bilayer.

Appendix

The potential parameter set for DMPC is reported here. Table 2 lists the values of the force constants and equilibrium bond angles for DMPC. Table 3 lists the potential parameters for the dihedral angles of DMPC. The L-J parameters for DMPC are taken from the GROMOS 96 force field, and the partial atom charges and bond lengths of DMPC are the same as the data reported by Egberts and co-workers (1994).

The Ryckaert-Bellemans potential for the dihedral angles of alkyl chains of DMPC is given by

Table 2 The potential parameters for bond angles of DMPC[a]

Bond types	k_α (kJ mol^{-1})	α_0 (degree)
CH_n—NL—CH_n	450	109.5
NL—CH_2—CH_2	450	109.5
CH_2—CH_n—OS	520	109.5
P—OS—CH_2	530	120.0
OS—P—OM	450	109.6
OS—P—OS	420	103.0
OM—P—OM	780	120.0
OS—CH_2—CH_1	530	111.0
CH_2—CH_2—CH_n	530	111.0
CH_2—CH_1—CH_2	520	109.5
CH_n—OS—C	530	120.0
OS—C=O	730	124.0
OS—C—CH_2	610	115.0
O=C—CH_2	685	121.0
C—CH_2—CH_2	670	120.0

[a] Angle potential: $V(\alpha) = 0.5\ k_\alpha\ (\cos\alpha - \cos\alpha_0)^2$

Table 3 The potential parameters for dihedral angles of DMPC

	k_φ (kJ mol^{-1})	m	Cos δ
(a) Proper dihedrals[a]			
CH_3—NL—CH_2—CH_2	3.77	3	1.0
NL—CH_2—CH_2—OS	5.86	3	1.0
CH_2—CH_2—OS—P	3.77	3	1.0
CH_2—OS—P—OS	1.05	3	1.0
CH_2—OS—P—OS	3.14	2	1.0
CH_1—CH_2—OS—P	3.77	3	1.0
OS—CH_2—CH_1—CH_2	5.86	3	1.0
OS—CH_2—CH_1—CH_2	0.42	2	1.0
OS—CH_1—CH_2—OS	2.09	2	1.0
CH_2—CH_1—OS—C	3.77	3	1.0
OS—C—CH_2—CH_2	1.0	6	1.0
CH_1—CH_2—OS—C	3.77	3	1.0
C—CH_2—CH_1—CH_2	5.86	3	1.0
CH_2—OS—C—CH_2	16.7	2	−1.0
	k_ε (kJ mol^{-1} degree^{-2})	ε (degree)	
(b) Improper dihedrals			
CH_1—OS—CH_2—CH_2	0.102		35.264
C—OS—CH_2—O	0.051		0.0

[a] Proper dihedral potential: $V(\varphi) = k_\varphi\ [1 + \cos(\delta)\cos(m\varphi)]$
[b] Improper dihedral potential: $V(\varepsilon) = 0.5\ k_\varepsilon\ (\varepsilon - \varepsilon_0)^2$

$$V(\alpha) = 9.2789 + 12.1554\cos\alpha - 13.1202\cos^2\alpha - 3.0597\cos^3\alpha + 26.24\cos^4\alpha + 31.4946\cos^5\alpha \quad (2)$$

where α is the dihedral angle of the alkyl chains and the unit of $V(\alpha)$ is kJ/mol.

Acknowledgements We thank Prof. van Gansteren for kindly providing us with the GROMOS 96 package and Dr. Marrink for kindly sending us the equilibrium configurations of DPPC lipids and for helpful comments on the initial version of this paper. We also thank Dr. Mark and W. Cheng for helpful discussions. This work was supported in part by the Chinese National Natural Science Foundation No. 39670187 and No. 19774501.

References

Andersen OS (1984) Gramicidin channels. Annu Rev Physiol 46: 531–548

Arseniev AS, Bystrov VF, Ivanov TV, Ovchinnikov YA (1985) ^{1}H-NMR study of gramicidin-A transmembrane ion channel. Head-to-head right-handed, single stranded helices. FEBS Lett 186: 168–174

Bassolino D, Alper HE, Stouch TR (1993) Solute diffusion in lipid bilayer membrane: an atomic level study by molecular dynamics. Biochemistry 32: 12624–12637

Berendsen HJC, Postma JPM, van Gunsteren WF, Dinola A, Haak JR (1984) Molecular dynamics with coupling to an external bath. J Chem Phys 81: 3684–3690

Berendsen HJC, Grigera JR, Straatsma TP (1987) The missing term in effective pair potentials. J Phys Chem 91: 6269–6271

Chiu SW, Nicholson LK, Brenneman MT, Subramanian S, Teng Q, McCammon JA, Cross TA, Jakobsson E (1991) Molecular dynamics computations and solid state nuclear magnetic resonance of the gramicidin cation channel. Biophys J 60: 974–978

Chiu SW, Clark M, Balaji V, Subramanian S, Scott HL, Jakobsson E (1995) Incorporation of surface tension into molecular dynamics simulation of an interface: a fluid phase lipid bilayer membrane. Biophys J 69: 1230–1245

Cross TA, Opella SJ (1994) Solid-state NMR structural studies of peptides and proteins in membranes. Curr Opin Struct Biol 4: 547–581

Damodaran KV, Merz KM Jr (1994) A comparison between DMPC and DLPE based lipid bilayers. Biophys J 66: 1076–1087

Damodaran KV, Merz KM Jr, Gaber BP (1995) Interaction of small pepetides with lipid bilayers. Biophys J 69: 1299–1308

Edholm O, Berger O, Jahnig F (1995) Structure and fluctuations of bacteriorhodpsin in the purple membrane: a molecular dynamics study. J Mol Biol 250: 94–111

Egberts E, Berendsen HJC (1988) Molecular dynamics simulation of a smectic liquid crystal with atomic detail. J Chem Phys 89: 3718–3732

Egberts E, Marrink SJ, Berendsen HJC (1994) Molecular dynamics simulation of a phospholipid membrane. Eur Biophys J 22: 423–436

Engelman DM, Lewis BA (1983) Lipid bilayer thickness varies linearly with acyl chain length in fluid phosphatidylcholine vesicles. J Mol Biol 166: 211–217

Feller SE, Pastor RW (1996) On simulation lipid bilayer with an applied surface tension: periodic boundary conditions and undulations. Biophys J 71: 1350–1355

Feller SE, Pastor RW, Rojnuckarin A, Bogusz S, Brooks BR (1996) Effect of electrostatic force interaction in molecular simulation. J Chem Phys 100: 17011–17020

He K, Ludtke SJ, Wu Y, Huang HW (1993) X-ray scattering with momentum transfer in the plane of membrane. Application to gramicidin organization. Biophys J 64: 157–162

Heller H, Schaefer M, Schulten K (1993) Molecular dynamics simulation of a bilayer of 200 lipids in the gel and in the liquid-crystal phases. J Phys Chem 97: 8343–8360

Horvath LI, Cirak J, Vigh L (1980) Relation of Raman order parameters to spin labeling parameters. Chem Phys Lipids 27: 237–250

Hu W, Lazo D, Cross TA (1995) Tryptophan dynamics and structure refinement in a lipid bilayer environment: solid state NMR of the gramicidin channel. Biochemistry 34: 14138–14146

Hunenberger PH, van Gunsteren WF (1998) Alternative schemes for the inclusion of a reaction-field correction into molecular dynamics simulations: influence on the simulated energetic, structural, and dielectric properties of liquid water. J Chem Phys 108: 6117–6134

Jakobsson E (1997) Computer simulation studies of biological membranes: progress, promise and pitfalls. Trends Biochem Sci 22: 339–344

Jorgensen WL, Impey RW, Chandrasekhar J, Madura JD, Klein ML (1983) Comparison of simple potential function for simulating liquid water. J Chem Phys 79: 926–935

Karplus M, Petsko GA (1990) Molecular dynamics simulation in biology. Nature 347: 631–639

Ketchem RR, Hu W, Cross TA (1993) High-resolution conformation of gramicidin A in a lipid bilayer by solid-state NMR. Science 261: 1457–1460

Koeppe RE II, Andersen OS (1996) Engineering the gramicidin channel. Annu Rev Biophys Biomol Struct 25: 231–258

Loof HD, Harvey SC, Segrest JP, Pastor RW (1991) Mean field stochastic boundary molecular dynamics simulation of a phospholipid in a membrane. Biochemistry 30: 2099–2113

Mackerell AD, Bashford D Jr, Bellot M, Dunbrack RL, Field MJ, Fischer S, Gao J, Guo H, Joseph D, Ha S, Kuchnir L, Kuczera K, Lau FTK, Mattos C, Michnick S, Nguyen DT, Ngo T, Prodhom B, Roux B, Schlenknich B, Smith J, Stote R, Straub J, Wiorkiewicz-Kuczera J, Karplus M (1992) Self-consistent parametrization of biomolecules for molecular modelling and condensed phase simulation. Biophys J 61: A143

Marrink SJ, Berendsen HJC (1994) Simulation of water transport through a lipid membrane. J Phys Chem 98: 4155–4168

Mau NDV, Daumas P, Lelievre D, Trudelle Y, Heitz F (1987) Linear gramicidin at the air-water interface. Biophys J 51: 843–845

Meier P, Ohmes E, Kothe G (1986) Multipulse dynamics nuclear magnetic resonance of phospholipid membranes. J Chem Phys 85: 3598–3614

Merz KM Jr (1997) Molecular dynamics simulation of lipid bilayer. Curr Opin Struct Biol 7: 511–517

Nagle JF (1993) Area/lipid of bilayer from nmr. Biophys J 64: 1476–1481

Nagle JF, Zhang R, Tristram-Nagle S, Sun W, Petrache HI, Suter RM (1996) X-ray structure determination of fully hydrated L-α phase dipalmitoylphosphatidylcholine bilayers. Biophys J 70: 1419–1431

Pastor RW (1994) Molecular dynamics and Monte Carlo simulation of lipid bilayer. Curr Opin Struct Biol 4: 486–492

Pastor RW, Venable RM, Karplus M (1991) Model for the structure of the lipid bilayer. Proc Natl Acad Sci USA 88: 892–896

Roux B, Karplus M (1994) Molecular dynamics simulation of the gramicidin channel. Annu Rev Biophys Biomol Struct 23: 731–761

Ryckaert JP, Bellemans A (1975) Molecular dynamics of liquid n-butane near boiling point. Chem Phys Lett 30: 123–125

Ryckaert JP, Ciccotti G, Berendsen HJC (1977) Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J Comput Phys 23: 327–341

Seelig A, Seelig J (1974) The dynamic structure of fatty acyl chains in a phospholipid bilayer measured by deuterium magnetic resonance. Biochemistry 13: 4839–4845

Seelig J, Seelig A (1980) Lipid conformation in model membranes and biological membranes. Q Rev Biophys 13: 19–61

Shen L, Bassolino D, Stouch T (1997) Transmembrane helix structure, dynamics, and interaction: multi-nanosecond molecular dynamics simulation. Biophys J 73: 3–20

Tieleman DP, Berendsen HJC (1996) Molecular dynamics simulation of a fully hydrated dipalmitoylphosphatidylcholine bilayer with different macroscopic boundary conditions and parameters. J Chem Phys 105: 4871–3880

Tournois H, Gieles P, Demel R, Gier J de, Krujiff B de (1989) Interfacial properties of gramicidin and gramicidin-lipid mixtures measured with static and dynamics monolayer techniques. Biophys J 55: 557–569

Tu K, Tobia DJ, Blaise JK, Klein ML (1996) Molecular dynamics investigation of the structure of a fully hydrated gel phase DPPC bilayer. Biophys J 70: 595–680

Urry DW (1971) The gramicidin A transmembrane channel: a proposed pi(l,d) helix. Proc Natl Acad Sci USA 68: 672–676

van Gunsteren WF, Berendsen HJC (1977) Algorithms for macromolecular dynamics and constraint dynamics. Mol Phys 34: 1311–1327

van Gunsteren WF (1988) The role of computer simulation techniques in protein engineering. Protein Eng 2: 5–13

van Gunsteren WF, Billeter SR, Eising AA, Hunenberger PH, Kruger P, Mark AE, Scott WRP, Tironi IG (1996) Biomolecular simulation: the GROMOS 96 manual and user guide. Vdf Hochschuverlag an der ETH Zurich, Zurich, Switzerland

Venable RM, Zhang Y, Hardy BJ, Pastor RW (1993) Molecular dynamics simulation of a lipid bilayer and hexadecane: an investigation of membrane fluidity. Science 262: 223–226

Wallace BA (1990) Gramicidin channels and pores. Annu Rev Biophys Chem 19: 127–157

Watnick PI, Chan SI, Dea P (1990) Hydrophobic mismatch in gramicidin A/lecithin systems. Biochemistry 29: 6215–6221

Woolf TB, Roux B (1994) Molecular dynamics simulation of the gramicidin channel in a phospholipid bilayer. Proc Natl Acad Sci USA 91: 11631–11635

Woolf TB, Roux B (1996) Structure, energetics, and dynamics of lipid-protein interaction: a molecular dynamics study of the gramicidin A channel in a DMPC bilayer. Proteins Struct Funct Genet 24: 92–114

Xing J, Scott HL (1992) Monte Carlo studies of a model for lipid-gramicidin A bilayer. Biochem Biophys Acta 1106: 227–232