Environment of a Membrane Protein

Benoît Roux

Université de Montréal, Centre-Ville, Canada

1 Introduction 923
2 The Problem of an Initial Configuration 924
3 Main Results 924
4 Summary 928
5 Related Articles 929
6 References 930

Abbreviations

Ala = alanine; DMPC = dimyristoyl phosphatidylcholine; GA = gramicidin A; Gly = glycine; Leu = leucine; Phe = phenylalanine; PRC = photosynthetic reaction center; SDS = sodium dodecyl sulfate; Trp = tryptophan; Tyr = tyrosine; Val = valine.

1 INTRODUCTION

Molecular dynamics simulation is a powerful theoretical approach to gain insight into the structure and dynamics of complex biological macromolecular systems (see ***Molecular Dynamics: Techniques and Applications to Proteins***).[1] It consists in calculating the position of all the atoms in a molecular system as a function of time, using detailed models of the microscopic forces operating between them, by integrating numerically Newton's classical equation of motion $\boldsymbol{F} = m\boldsymbol{a}$. The calculated classical trajectory, though an approximation to the real world, provides ultimate detailed information about the time course of the atomic motions, which is difficult to access experimentally. In this article, I will describe the main qualitative features observed from computer simulations of a protein molecule in the environment of a phospholipid bilayer membrane.

Biological membranes are complicated extensive bimolecular sheetlike structures, about 60–100 Å thick,[2] mostly formed by lipid molecules held together by many cooperative noncovalent interactions (see ***Molecular Dynamics: Studies of Lipid Bilayers*** and ***Permeation of Lipid Membranes: Molecular Dynamics Simulations***). Lipid molecules possess long nonpolar hydrocarbon chains attached to a polar moiety. The 'hydrophobic' nonpolar chains are expelled by water (see ***Hydrophobic Effect***). In contrast, the 'hydrophilic' polar headgroup of the molecule is strongly associated with water molecules. Molecules possessing both hydrophobic and hydrophilic moieties are said to be 'ampipathic'. Under physiological conditions, lipid molecules spontaneously self-assemble to form an extensive bimolecular sheet called a bilayer. In a bilayer membrane, the polar headgroup of the lipids are exposed to bulk water while the hydrocarbon chains are segregated from it. The cohesion of the bilayer structure arises from its ability to simultaneously satisfy the contrasting solvation requirements of the hydrophobic and hydrophilic moieties of the lipid molecules.

The viability of a living cell depends upon the ability of the membranes to isolate and protect the integrity of its content and to provide regulatory, selective, permeation mechanism for the transport of material in and out of the numerous cellular compartments. Most transport mechanisms that have evolved are mediated by membrane-bound proteins incorporated into the lipid bilayer. For example, ion channels are highly specific membrane-spanning protein structures which serve to facilitate the passage of selected ions across the lipid barrier.[3] An important aim of biophysical sciences is to understand the relationship between the three-dimensional structure of ion channels and their function at the molecular level. However, membrane proteins are difficult to study because they are not soluble in water and are easily denatured. In recent years, powerful tools such as X-ray crystallography,[4] electron microscopy,[5] and nuclear magnetic resonance (NMR)[6,7] have been developed to characterize the three-dimensional structure of membrane proteins at atomic resolution. Despite this progress, many of the factors responsible for the function of biomembranes are still not well understood.

The lipid membrane has a dual role: it is both a permeability barrier, and a solvent for integral membrane proteins. A membrane should not be regarded as a static structure, but rather as a complex dynamical two-dimensional liquid-crystalline fluid mosaic of oriented proteins and lipids.[8] Many different experimental approaches are currently used to investigate and characterize biological membranes. The interested reader will find good descriptions of those methods in Refs. 2, 9, and 10. However, despite the vast amount of data available, the complexity of membranes is such that experimental results remain very difficult to interpret at the microscopic level. For this reason, even qualitative information gained by performing detailed computer simulations of protein–membrane complexes can be valuable. In this article, I will describe the results from molecular dynamics simulations of the gramicidin A (GA) channel, embedded in a dimyristoyl phosphatidylcholine (DMPC) phospholipid bilayer.

The choice of a molecular system for computer simulations is critical. For meaningful theoretical studies it is essential to investigate model systems that have been the object of extensive experimental studies (structural and functional) to have a broad basis of structural and functional data for comparison. Comparison with experimental data is crucial to make sure that meaningful information can be obtained from the computer simulations. The GA:DMPC simulated system represents an ideal prototypical molecular model of a protein embedded in a phospholipid bilayer. GA is a simple pore-forming polypeptide able to form ion-conducting channels in lipid membranes. Over the years, it has proved to be an extremely useful model system to study the principles governing ion transport across lipid membranes.[11,12] In particular, it is structurally well characterized: the three-dimensional conformation of the conducting state of the channel has been determined to atomic resolution in sodium dodecyl sulfate (SDS) micelles,[6] and in DMPC bilayers[7,13] using nuclear magnetic resonance (NMR) techniques. In the next sections, the problems for setting up the simulations and the main results will be briefly exposed. The article is concluded with a discussion of computer simulations of biomembranes and the importance and their growing importance.

2 THE PROBLEM OF AN INITIAL CONFIGURATION

In principle, the application of molecular dynamics simulations to complex protein–membrane systems is straightforward. In practice, the extension of current computational methodologies to simulate a protein in a fully solvated explicit phospholipid bilayer represents a major challenge.[10] The very slow configurational relaxation present in such an environment implies that a very long simulation time is needed for equilibration of the long hydrocarbon chains of the lipids (see Refs. 10, 14, and 15 for a critical discussion of current problems). This suggests that available trajectories, which are typically on the order of one nanosecond or less, can mostly explore the neighborhood of the starting configuration. Therefore, it is desirable to start a simulation from a configuration that corresponds as closely as possible to the equilibrated state of the membrane system to reduce the computer time needed for equilibration.

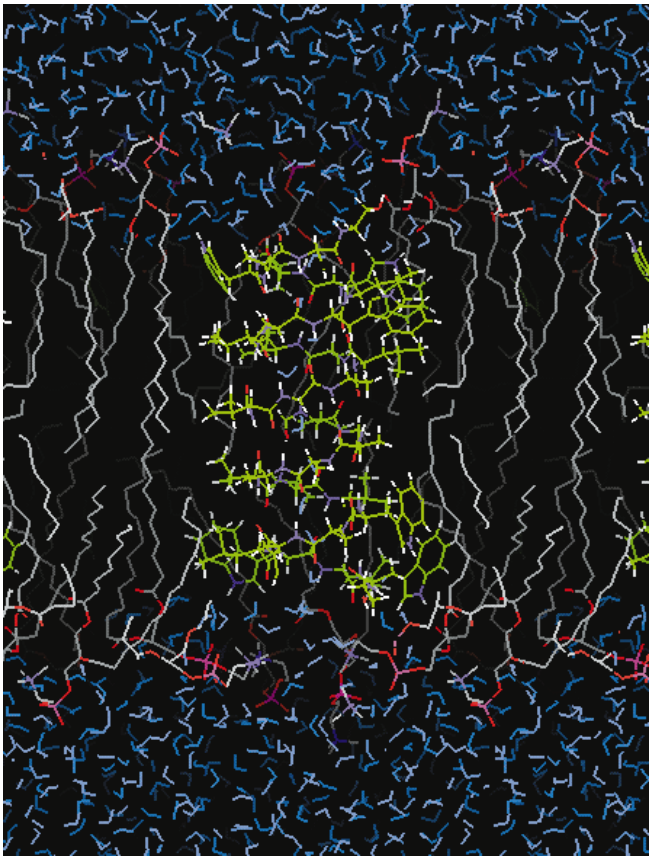

Figure 1 Molecular graphics representation of the GA:DMPC system. The ratio DMPC:GA is 8:1 with 45 wt% water. This corresponds to 16 DMPC molecules, one GA dimer channel, and about 650 water molecules. The simulation systems represent models for the oriented samples utilized in solid-state NMR experiments.[7,18,19,20] The center of the bilayer membrane is located at $Z = 0$ and the channel axis is oriented along the Z direction. Periodic boundary conditions are applied along the Z direction to simulate a multilayer system. Hexagonal periodic boundary conditions in the XY direction are used to simulate an infinite system within the plane of the bilayer. More details about the simulation may be found in Ref. 22. The force field PARAM22 of the program CHARMM was used (see ***CHARMM: The Energy Function and Its Parameterization***)

A method was developed for assembling protein–membrane complexes from pre-equilibrated and pre-hydrated phospholipid molecules.[16] The method is an extension of the approach used by Venable et al.[17] to generate pure lipid bilayers. To construct the configuration, the lipid molecules taken in random conformers analogous to those found in a fluid bilayer are disposed around the protein-like elementary building blocks. The construction of the system is consistent with the notion that the membrane constitutes a partly ordered, partly disordered, liquid-crystalline phase. The initial configuration is then refined and equilibrated until no systematic changes are observed. A large number of atomic systems representing the GA channel embedded in a fully hydrated DMPC bilayer were generated with this approach.

The atomic systems, with a DMPC:GA ratio of 8:1 and 45 wt% water, are models of the oriented samples studied by solid-state NMR in the laboratories of Cross,[7] Cornell,[18] Davis,[19] and Koeppe.[20] The results from the simulation were extensively compared with available data in previous publications.[16,21–23] The excellent agreement that was observed with available experimental data strongly supports the validity of the current simulations. A typical configuration of the GA:DMPC system is shown in Figure 1. Details about the simulations are given in the figure caption.

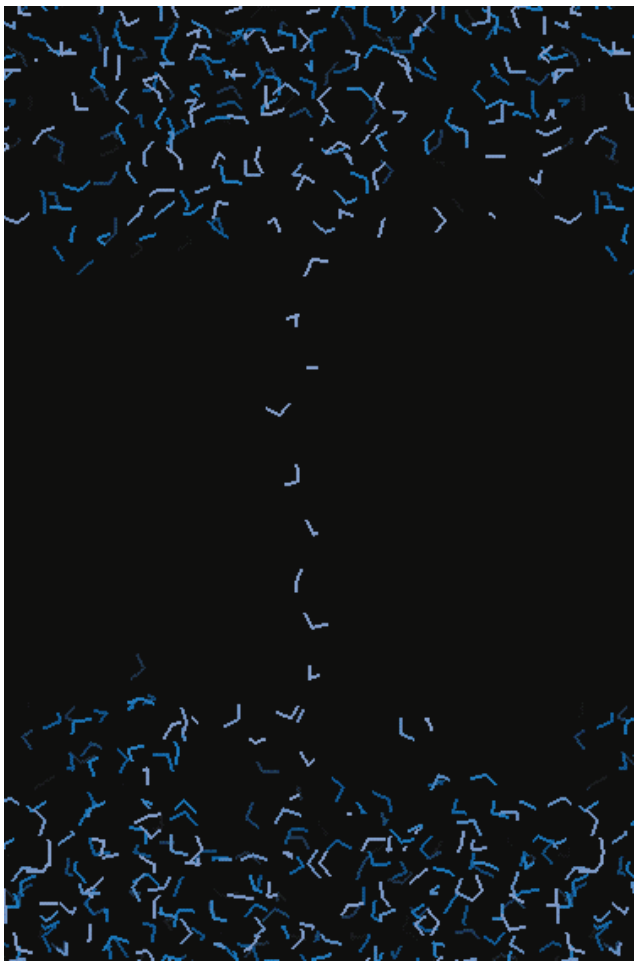

Figure 2 The narrow pore is filled with a single file of eight to nine hydrogen-bonded water molecules. This figure was made from the configuration shown in Figure 1

3 MAIN RESULTS

3.1 The Average Structure of a Protein–Membrane System

Membranes are often pictured as a structureless planar slab of nonpolar material providing a thermodynamic driving force partitioning the amino acids according to their solubility;[24,25] hydrophobic amino acids are more likely to be found within the hydrocarbon core of the membrane whereas charged and polar amino acids are more likely to be found in the bulk solvent. The membrane–solution interface is described as a sharp demarcation between hydrophilic and hydrophobic regions.[26,27] It is clear that such a view oversimplifies the true complexity of protein–membrane complexes. Analysis of the average distribution of various components along the bilayer normal obtained from the current molecular dynamics simulations provides detailed information about the organization of a protein–membrane system.

The main features of the GA:DMPC are obtained from an average over a large number of configurations similar to that shown in Figure 1. The results indicate that the overal thickness of the membrane system (i.e., headgroup to headgroup) is approximately 40 Å. The region covered by the hydrocarbon chains, which corresponds to the hydrophobic core of the bilayer, extends from −12 Å to +12 Å relative to the center of the membrane. The density of the hydrocarbon chains is reduced near the center of the bilayer system, in accord with neutron scattering data of pure bilayers.[28] The channel length is approximately 26 Å, which roughly matches the thickness of the hydrophobic core of the DMPC bilayer. The membrane–solution interface region is much broader than pictured traditionally (see ***Aqueous Interfaces***). The headgroup and glycerol regions span a region of 15 Å in width. The water density decreases smoothly throughout the polar head region, reaching the edge of the hydrocarbon chains region and the carbonyl oxygens of the ester group of the lipids which are found on average about 10 Å to 20 Å on both sides of the bilayer center. Although the experiments have not yet been performed, X-ray and neutron scattering could be used to measure the average density profile of the GA:DMPC system described above.[9,28]

3.2 The Impermeability of the Membrane

In living cells an electrical potential difference exists between the cytoplasm and the extracellular medium owing

Figure 3 Close contact between the GA channel and neighboring lipids. This figure was made from the configuration shown in Figure 1

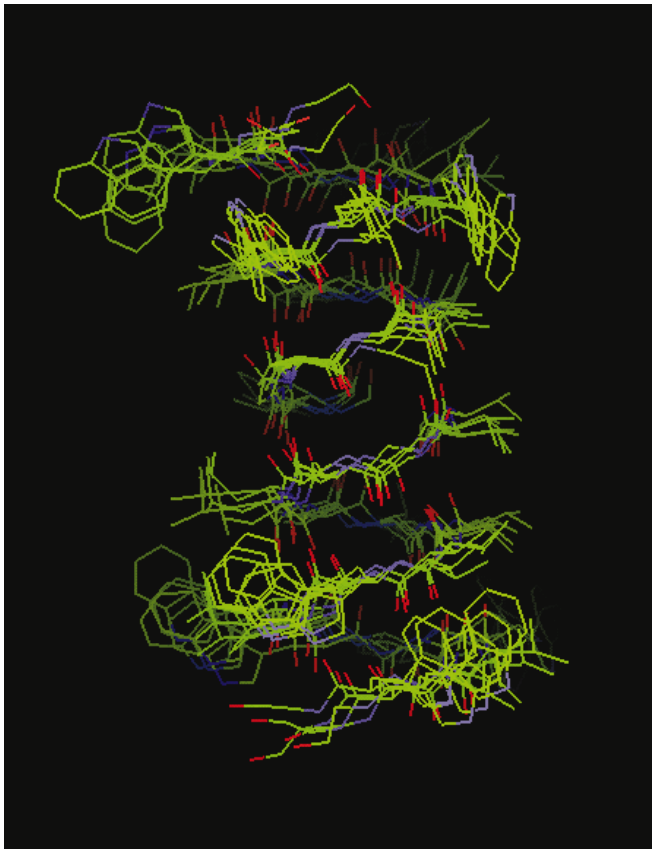

Figure 4 Superposition of five configurations of the GA channel, separated by 50 ps, taken along the trajectory

to the unequal distribution of ions on both sides of the cellular membrane. The existence of the membrane potential largely depends on the ability of a membrane to act as an insulator by presenting a barrier to the passive diffusion of small ions. It plays the role of an ion channel to control and facilitate the passage of selected ions across the membrane by providing an energetically favorable pathway. How is this goal achieved in the case of the GA channel? In the membrane, the dimer channel adopts a β-helical conformation to form a pore of about 4 Å in diameter. The hydrogen-bonded carbonyls line the pore and the amino acid side chains, most of them hydrophobic, extend away into the membrane lipid. A permeating ion is mostly stabilized by interactions with the backbone carbonyl oxygens of the channel. The diameter of the pore is such that the permeation process involves the translocation in single file of the ion and water molecules through the channel interior. The permeation pathway is illustrated in Figure 2 by showing only the water molecules of one configuration of the GA:DMPC system. It is striking that no water molecules are observed in a large region occupied by the hydrocarbon chains of the DMPC. The tight contact between the protein and the hydrocarbon chains prevents any significant penetration of water molecules. This suggests that the plasticity of the hydrocarbon chains is sufficient to preserve the integrity of the hydrophobic core of the membrane and maintain the ability of the membrane to act as an insulator.

A typical configuration illustrating direct contacts between DMPC molecules and the channel are shown in Figure 3. It is observed that the lipid–protein complex is closely packed, leaving no holes in the membrane. Such observations are in accord with the notion that membranes are self-sealing (i.e., holes are energetically unfavorable). However, transient penetration of a small number of water molecules into the hydrophobic core of the membrane is possible. During the simulations, one water molecule entered the bilayer and formed a stable hydrogen bond to the backbone outside the channel. Observation of rare events in a simulation, even though they are not statistically significant, can give useful information. In the observed event, the presence of the protein appears to be directly responsible for the stabilization of this water molecule inside the bilayer. Despite the fact that they are involved in hydrogen bonding in the β-helical GA dimer, the carbonyl groups of the backbone remain accessible for forming hydrogen bonds with water molecules entering the hydrocarbon region. Interestingly, it has been shown using fluorescence lifetime measurements that gramicidin is responsible for increasing the presence of water molecules in membranes.[29]

3.3 Structural Fluctuations

The contact between the membrane lipids and the GA channel is sufficiently tight to maintain the integrity of the hydrophobic core and the impermeability of the membrane. Does this imply that the lipid matrix is very rigid, thereby restricting the protein motions? In fact, the system exhibits a range of dynamical motions. In Figure 4, five configurations of the GA channel taken at a time interval of 50 ps along the trajectory are superimposed. It is observed that the channel undergoes significant structural fluctuations around an average conformation. The nonpolar Val and Leu side chains, pointing into the lipid hydrocarbon, are able to isomerize between various conformers.[21] The Trp side chains fluctuate around a dominant conformation, the polar N–H group of the Trp indole side chains remaining close to the membrane–solution interface.[16,22] It is clear that the channel is not significantly restricted by the lipid matrix.

In contrast to the channel, the DMPC lipids are fluid-like and do not retain a dominant conformation after a long time. Figure 5 shows a superposition of four configurations of a DMPC molecule taken at a time interval of 50 ps along the trajectory. It is observed that there is an ordering of the lipid chains in direct contact with the GA, but the effect is not uniform owing to the irregular surface of the protein. The influence of GA on lipid motion and order is in accord with solid-state NMR[30,31] and FTIR data.[32]

3.4 The Membrane Surface

The phosphate and nitrogen atoms of the lipid headgroup are generally located within the same plane, reflecting the fact that the headgroup dipoles are aligned parallel to the membrane surface on average. The observed orientation of the headgroup is in general accord with ^{31}P solid-state NMR chemical shifts measurements of pure bilayers.[33] However, the polar head region is not static. The P–N vector can fluctuate within a window of $\pm 50^{\circ}$ from the plane of the bilayer. Thus, considerable fluctuations of the polar headgroups at the membrane surface may be expected.

A view of the membrane surface is shown in Figure 6. The channel is almost covered by the polar headgroups. A

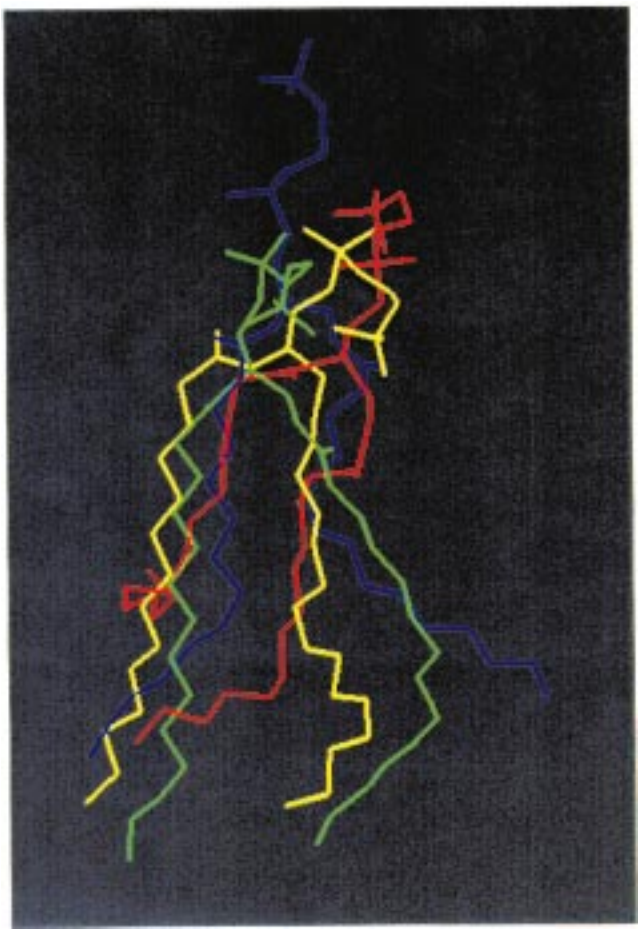

Figure 5 Superposition of four configurations of a DMPC molecule, separated by 50 ps, taken along the trajectory

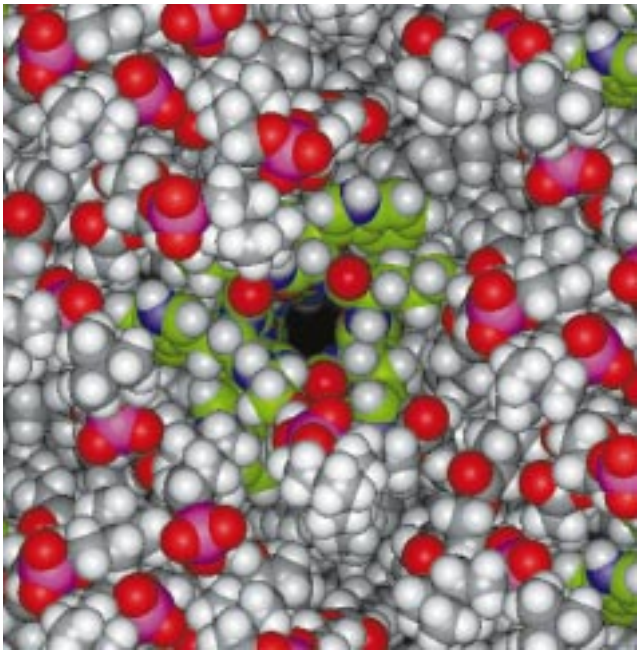

Figure 6 A view of the channel and membrane surface from the bulk water region. This figure was made from the configuration shown in Figure 1

permeating ion must therefore find its way to the channel entry through the polar heads. The diffusion constant of an ion such as K^+ is of 1.96×10^{-5} cm^2 s^{-1}.[3] Since the thickness of the interfacial region is around 20 Å, a permeating ion could require as much as one nanosecond to diffuse from the bulk to the channel entrance.

3.5 The Membrane–Solution Interface

As observed in Figures 1 and 4, the Trp side chains are close to the membrane–solution interface, the polar N–H group of the indole ring pointing towards the bulk solution. Hydrogen bonds involving the Trp indole ring were frequently observed during the simulation. A few examples of hydrogen bonding involving the indole side chain of the Trp residues are illustrated in Figures 7, 8, and 9. Figure 7 shows Trp13 forming hydrogen bonds with nearby water molecules. Figure 8 shows Trp11 forming a hydrogen bond with the ester carbonyl group O22 of chain sn(1) of a DMPC molecule. In addition, very complex hydrogen bonding patterns can occur at the membrane–solution interface involving the protein, water molecules, and the polar head groups. Figure 9 illustrates this point by showing a configuration in which hydrogen bonds are present between the ethanolamine tail, a phosphate oxygen, water molecules, and the indole N–H of Trp9.

Functional studies have demonstrated that the interaction of the Trp with the bilayer environment is an important factor in the stability of the β-helical structure of the GA dimer in a

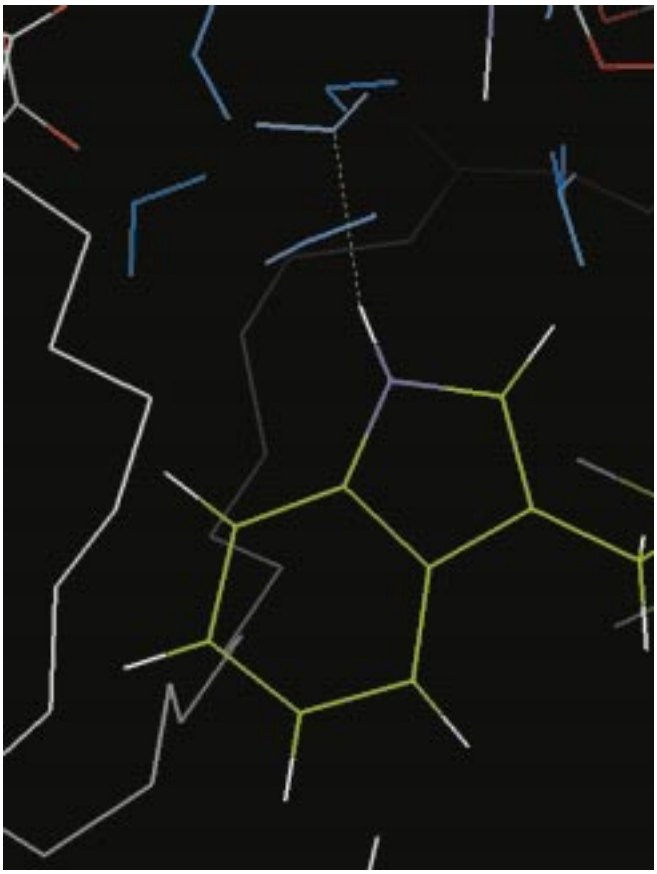

Figure 7 Hydrogen bonds formed by the Trp indole ring of Trp13 with water molecules

Figure 8 Hydrogen bonds formed by the Trp indole ring with the ester carbonyl group of the lipid

membrane.[34] Substitutions of the Trp by Phe have an important influence on the conductivity and on the average life-time of the dimer channel. Such observations are consistent with IR and Raman spectroscopy,[35] and fluorescence spectroscopy experiments,[36] which indicate that the Trp are motionally restricted owing to the formation of hydrogen bonds between the indole ring and the neighboring waters or the lipids.

More generally, the presence of aromatic residues such as Trp, Phe, and Tyr near the membrane–solution interface is a recurrent structural feature of several membrane proteins, e.g., bacterial photosynthetic reaction center (PRC),[4] porins,[37,38] Pf1 and fd viral coat proteins,[39,40] prostaglandin H synthase,[41] the peptide segment of hemmagglutinin responsible for influenza virus fusion,[42] and a large series of α-helical human type I membrane proteins.[43] As an illustration, the GA channel and the PRC are shown in Figure 10. The relative location of the Trp residues in both systems is strikingly similar.

4 SUMMARY

The current molecular dynamics simulations of the GA channel in a DMPC bilayer provide detailed information about

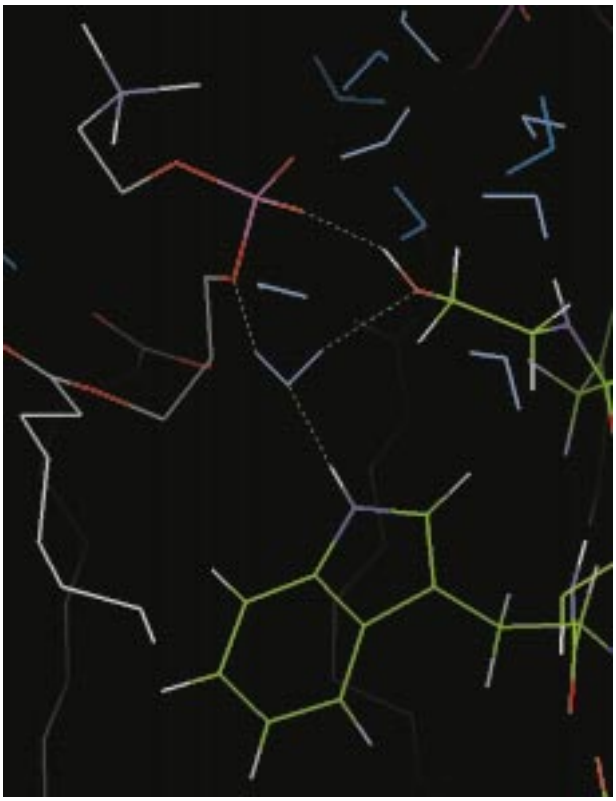

Figure 9 Hydrogen bonding network formed by the Trp indole ring, the ethanolamine tail, the polar head groups, and water molecules

the environment of a membrane protein. The analysis indicate that the membrane–solution interface is much broader and much more complex than pictured traditionally (in fact, the word interface is almost inappropriate). The plasticity of the lipid acyl chains is such that the integrity of the hydrophobic core is maintained in the protein–membrane complex. Several interactions provide the driving forces for the formation and stabilization of biological membranes. Hydrogen bonding plays an important role in the polar headgroup region. According to the current trajectories, the membrane–solution interface is a particularly favorable for the indole side chain of the Trp residues because of their ampipathic character. This may be an important factor in the folding and in the stability of secondary and tertiary structural elements of membrane proteins.

Molecular dynamics simulations of membrane protein systems are currently in progress in several laboratories, e.g., bacteriorhodopsin,[44] Pf1 phage coat protein,[45] an ampipathic helix,[46] and a polyalanine alpha helix.[47] In addition, continued efforts are directed at investigating the properties of pure lipid bilayers (for a review, see Refs. 14 and 15). Those studies show that molecular dynamics simulations can be a powerful tool in exploring the structure and function of biomembranes. Nevertheless, computer studies of membranes are still faced with fundamental issues ranging from basic statistical mechanics,[48] to the development of accurate potential functions.[49] One of the main problems confronting computer simulations of biomembranes is the necessity to increase the simulation time in order to reach sufficient sampling of the configurational space. A typical simulation for a biological

system now consists of 15 000 atoms. Most trajectories to date are on the order of 1 ns or less, although expected progress in algorithms and computer power suggests that simulations of several nanoseconds will soon be routinely possible. In the coming decade it is likely that molecular simulations of biological membrane systems will reach the point where very long timescale issues may be addressed. In conclusion, it can be reasonably expected that molecular simulations will play an increasingly important role in our understanding of biological membranes.

5 RELATED ARTICLES

Aqueous Interfaces; Biomembranes: Modeling; CHARMM: The Energy Function and Its Parameterization; Hydrophobic Effect; Molecular Dynamics: Studies of Lipid Bilayers; Molecular Dynamics: Techniques and Applications to Proteins; Permeation of Lipid Membranes: Molecular Dynamics Simulations; Protein Force Fields.

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