Review

Molecular dynamics simulation studies of lipid bilayer systems[★][✿]

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The main structural element of biological membranes is a liquid-crystalline lipid bilayer. Other constituents, i.e. proteins, sterols and peptides, either intercalate into or loosely attach to the bilayer. We applied a molecular dynamics simulation method to study membrane systems at various levels of compositional complexity. The studies were started from simple lipid bilayers containing a single type phosphatidylcholine (PC) and water molecules (PC bilayers). As a next step, cholesterol (Chol) molecules were introduced to the PC bilayers (PC-Chol bilayers). These studies provided detailed information about the structure and dynamics of the membrane/water interface and the hydrocarbon chain region in bilayers built of various types of PCs and Chol. This enabled studies of membrane systems of higher complexity. They included the investigation of an integral membrane protein in its natural environment of a PC bilayer, and the antibacterial activity of magainin-2. The latter study required the construction of a model bacterial membrane which consisted of two types of phospholipids and counter ions. Whenever published experimental data were available, the results of the simulations were compared with them.

Abbreviations: PC, phosphatidylcholine; DMPC, 1,2-dimyristoyl-phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-phosphatidylcholine; PEPC, 1-palmitoyl-2-elaidoyl-phosphatidylcholine; POPE, 1-palmitoyl-2-oleoyl-phosphatidylethanolamine; POPG, 1-palmitoyl-2-oleoyl-phosphatidyl-*rac*-glycerol; Chol, cholesterol; Echol, epicholesterol; MD, molecular dynamics.

Biological membranes enclose every cell (plasma membrane) and numerous intracellular organelles (internal membranes). The main biological functions of membranes involve the maintenance of the cell integrity, control of interactions between the cell and its environment, separation of intracellular compartments, and receiving and transducing signals necessary for the cell functioning.

As was elegantly shown by Gorter & Grendel (1925), the common structural feature of biological membranes is a lipid bilayer with intercalated or loosely attached proteins, sterols, peptides, and other constituents, held together mainly by non-covalent interactions. The most prevalent molecule that forms the bilayer of eukaryotic cell membranes is phosphatidylcholine (PC). PC is an amphipathic molecule with a polar headgroup and two hydrophobic hydrocarbon chains. In aqueous solutions, PCs spontaneously form bilayers; their polar heads are exposed to water and their hydrocarbon chains make up a hydrophobic core of the bilayer. In biological membranes, the hydrophobic core is "fluid", which is assured by the liquid-crystalline state of the bilayer.

The aim of the studies described in this paper is to better understand the basic molecular mechanisms of membrane functioning. These mechanisms are difficult to reveal by experimental methods due to their limited spatial and time resolutions. Since a molecular dynamics simulation gives atomic resolution and spans the observation time (on the time scale of 10^{-8} s) by steps of a few femtosecond, this method was employed in these studies.

Due to the great structural and dynamic complexity of membranes, the studies were started from the simplest membrane systems, i.e. hydrated PC bilayers, which are, nevertheless, the most important structural element of the biological membrane. Then, the complexity of the membrane systems was gradually increased by successive addition to the bilayer of other natural membrane components like cholesterol, peptides and protein. The struc-ture and dynamics of a membrane system of higher complexity were compared with those of the lower one.

HYDRATED PC BILAYERS

In the first stage of our studies, three computer models of hydrated PC bilayers were used: 1) dimyristoylphosphatidylcholine (DMPC), 2) palmitoyloleoylphosphatidylcholine (POPC), and 3) palmitoylelaidoylphosphatidylcholine (PEPC). PCs forming these bilayers have identical headgroups, but differ in the hydrocarbon chain lenghts and the presence of a double bond in the β-chain (cf. Fig. 1). The β- and γ-chain of DMPC (14 carbon atoms each) and the γ-chain of POPC and PEPC (16 carbon atoms) are fully saturated. The β-chain of POPC and PEPC (18 carbon atoms) is mono-unsaturated, i.e. has one double bond between C9 and C10. In the β-chain of POPC, the double bond is in *cis*, while in the β-chain of PEPC, it is in the *trans* conformation (cf. Fig. 1). Among mono-*cis*-unsaturated PCs, POPC is the most abundant. Phospholipids with *trans*-unsaturated hydrocarbon chains are rare in nature. The reason for this has not been satisfactorily explained yet.

An important element of molecular modelling studies is the verification of the model against the available experimental data. Although DMPC is not the most representative phospholipid for biological membranes, the studies described here were commenced from a DMPC bilayer, because there is a large body of experimental results for this bilayer. The results of MD simulation were exhaustively compared with those of experimental studies, which showed that the computer model faithfully reproduces most features of the experimental system.

PC bilayers used in these studies consisted of 72 ($6 \times 6 \times 2$) PC molecules. The bilayers contained 1622 (DMPC) and 1922 (POPC and PEPC) water molecules, thus water constituted about 39% by weight in each system. The
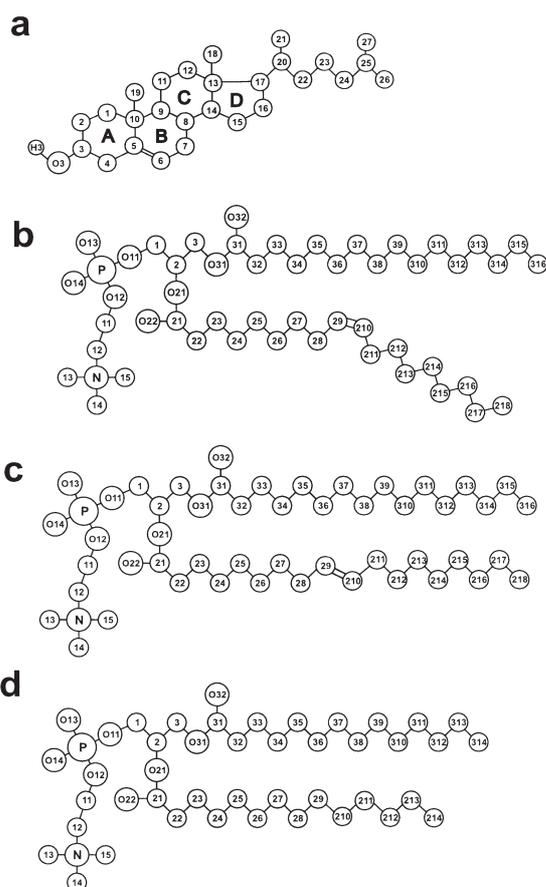
Figure 1. Molecular structure of (a) Chol, (b) POPC, (c) PEPC, and (d) DMPC with numbering of atoms (the chemical symbol for carbon atoms, C, is omitted).

The Chol rings are labelled A, B, C, and D.

bilayers were simulated for at least 5 ns. The equilibration times were 1 (DMPC and PEPC bilayer) and 3 ns (POPC bilayer). For the analyses 2-ns trajectories were used. Details concerning systems construction and equilibration are given in (Pasenkiewicz-Gierula *et al.*, 1997; 1999; Róg, 2000).

Structural and dynamic studies of lipid bilayers focus either on "global" membrane properties or on the properties of one of the two distinct regions of the bilayer, i.e. the hydrophobic core and the membrane/water interface. The global parameters are the surface area per PC molecule, bilayer thickness, and electron density profile across the bilayer. The hydrophobic core of the bilayer is character-

ised by disorder of the hydrocarbon chains and low permeability to polar molecules. Parameters which describe the disorder and low permeability are the number of *gauche* conformers/chain, the molecular order parameter profile, the lifetimes of *gauche* and *trans* conformations, and the hydrophobic profile of the membrane. The membrane/water interface is a polar, multi-component region where specific PC–PC and PC–water interactions take place. This region is described by giving the number of water molecules strongly interacting with PCs, water bridges formed by water molecules simultaneously hydrogen (H) bonded to two PCs (Fig. 2a), charge pairs formed between positively charged choline groups and negatively charged phosphate or carbonyl oxygen atoms of different PCs (Fig. 2b), and interlipid links per PC molecule (see Table 1). The average values of the parameters obtained in the simulations and in experiments for the three PC bilayers are compared in Tables 1 and 2. Similarities between corresponding simulated and experimental values indicate that the simulated PC bilayers verify positively against experimental data for the three membrane regions.

The most important result of this part of our studies is the demonstration that at the membrane/water interface there is an extended network of interactions among the PC headgroups. Because of a smaller average PC–PC distance, this network is more branched in the DMPC bilayer than in the POPC and PEPC bilayers. Nevertheless, in each bilayer, about 98% of all PC molecules are linked at any instant. The organisation of the membrane/water interface is similar in the POPC and PEPC bilayers, thus it is not influenced by the conformation of the double bond in the β-chain.

HYDRATED LIPID BILAYERS CONTAINING CHOLESTEROL

Cholesterol (Chol) constitutes up to 50% of the lipids in the plasma membranes of
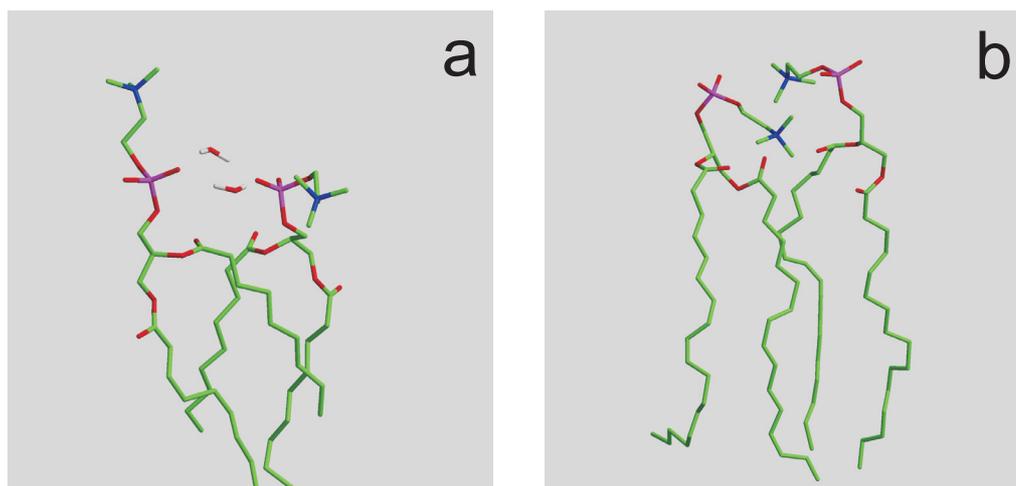
Figure 2. Examples of DMPC–DMPC pairs linked by short-distance interactions in the bilayer.

(a) Two DMPC molecules are linked by two water bridges. (b) Two DMPC molecules are linked by multiple charge pairs. The image was produced with MolScript (Kraulis, 1991) and Raster3D (Merritt & Bacon, 1997).

eukaryotic cells (Sackmann, 1995). The biological roles of Chol involve the maintenance of proper fluidity (e.g., Kusumi et al., 1983), reduction of passive permeability (e.g., Subczynski et al., 1994), and increasing the mechanical strength and elasticity (e.g.,

Table 1. Average values (ranges) of parameters that characterise liquid-crystalline PC bilayers.

The surface area per PC molecule, membrane thickness (the distance between the points at which the electron density of lipids becomes half of its peak value), number of *gauche* conformers per γ-chain, lifetime of the *trans* conformation averaged over all *trans* conformers in the γ-chain, lifetime of the *gauche* conformation averaged over all *gauche* conformers in the γ-chain, obtained experimentally (EXP) and in simulations (SYM) of DMPC, POPC, PEPC, and DMPC–Chol bilayers.

Bilayer		Area/PC (Å^2)	Thickness (Å^2)	Number *gauche*/chain	Lifetime *trans* (ps)	Lifetime *gauche* (ps)
DMPC	SYM	60.2±1	36±1	2.8±0.1	190 (125–245)	54 (39–76)
	EXP	60[a]	35[b]	2.6±0.3[c]	ND	ND
POPC	SYM	64±1	38±1	3.3±0.1	180 (115–250)	60 (38–68)
	EXP	63–66[d,e]	35–41[b]	2.4–3.6[c,f]	ND	ND
PEPC	SYM	64±1	38±1	3.4±0.1	180 (120–220)	61 (38–75)
	EXP	ND	ND	2.4–3.6[c,f]	ND	ND
DMPC–Chol	SYM	58.4±0.6	36±1	2.7±0.1	190 (130–250)	55 (38–69)
	EXP	ND	ND	ND	ND	ND

ND, not determined. [a]Nagle, 1993; [b]Rand & Parsegian, 1989; [c]Tuchtenhagen et al., 1994; [d]Smaby et al., 1997; [e]Hyslop et al., 1990; [f]Meraldi & Schlitter, 1981.

Bloom & Mouritsen, 1995) of the membrane. Due to experimental difficulties in the elucidation of the molecular mechanisms of Chol activity in the membrane, a molecular dynamics simulation method was used to study interactions between PC and Chol molecules in the bilayer. In this study, the bilayers (DMPC, POPC, and PEPC) described in the previous section were used, in which 16 PC molecules were replaced with 16 Chol molecules (eight in each leaflet). The three PC–Chol bilayers consisted of 56 PC and 16 Chol molecules, thus the molar content of Chol was about 22 mol% (Fig. 3). Also, a bilayer consisting of 56 DMPC and 16 α-OH-cholesterol (epicholesterol, Echol)

that water penetration of the hydrophobic bilayer core is reduced in the presence of Chol. Because the membrane/water interface is not easily accessible to experimental studies, in analysing molecular dynamics simulation results we pay special attention to this membrane region. In the DMPC–Chol bilayer, the Chol OH group participates in the formation of an interlipid network of interactions observed in pure PC bilayers (cf. previous section). However, like in the POPC and PEPC bilayers, the network is less branched than in the pure DMPC bilayer. The main reasons for this are larger average distance between the PC headgroups and a smaller number of
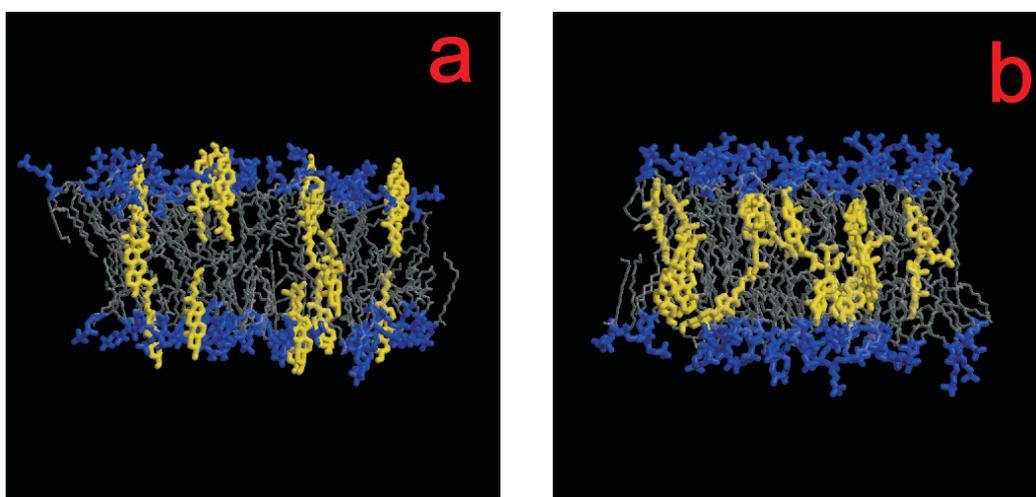

Figure 3. Snapshots of the DMPC–Chol bilayer membrane.

(a) The initial structure. (b) The equilibrated structure at 5 ns of MD simulation. Water is removed to show locations of DMPC and Chol polar groups. Chol molecules are shown as yellow sticks. DMPC headgroups are blue and hydrocarbon chains are grey. The image was produced with MolScript (Kraulis, 1991) and Raster3D (Merritt & Bacon, 1997).

molecules was used. Echol is a less biologically active isomeric form of β-OH-cholesterol (Chol). Studying the DMPC–Echol bilayer was thought to be helpful in elucidating the role of the Chol hydroxyl group in the Pc–Chol interaction. The details of the DMPC–Chol construction and equilibration are given in (Pasenkiewicz-Gierula *et al.*, 2000) and those of other systems in (Róg, 2000).

The results obtained for the simulated systems are in good agreement with most published experimental data (Pasenkiewicz-Gierula *et al.*, 2000). They confirm, among others,

interlipid links per Chol than per PC molecule; because of this, Chol is often an "end" molecule in the network. The latter effect is more pronounced in the DMPC–Echol bilayer. At the interface, the OH group of Chol interacts with the PC headgroup *via* direct H bonds (16% of Chol), water bridges (33% of Chol), and charge pairs (34% of Chol). In the DMPC–Echol bilayer the number of direct H bonds between Echol and DMPC is twice as large and the number of charge pairs is one half of the corresponding numbers in the DMPC–Chol bilayer.

INTERACTION OF A PEPTIDE WITH ANIMAL AND BACTERIAL MEMBRANES

This part of the studies was aimed at the elucidation of the molecular basis of biological activity and selectivity of magainin-2 (M2). M2 is a natural, 23-residue peptide of antibacterial and antifungal activities. In membrane environments it forms an amphipathic α-helix; the total charge of the peptide is +4 e. The present view on M2 biological activity is that it permeabilises the cell membrane (Saberwal & Nagaraj,1994) by physical interaction with the lipid bilayer. The antibacterial activity of M2 is related to its membrane selectivity. Due to a hydrophobic moment and positive electrostatic charge, M2 preferentially binds to bacterial membranes, which contain negatively charged phospholipids, and punctures the membrane by forming pores. Eukaryotic cell membranes contain mainly neutral PC molecules, thus the electrostatic interaction between M2 and PC is weak, and M2 does not strongly affect these membranes. In the M2 selectivity, also Chol may play a role. Chol is a common constituent of the animal cell membrane but practically is absent from the bacterial cell membrane. However, the molecular mechanisms of M2 activity and selectivity have not been satisfactorily explained yet, mainly because the interpretation of experimental results is difficult.

To verify the existing hypothesis concerning M2 activity and selectivity, we applied molecular modelling methods. Several computer models of bilayers containing M2 were constructed and simulated. As a model of the ani-

Table 2. Average values of parameters characterising the membrane/water interface in liquid-crystalline PC bilayers.

Numbers of H bonded water molecules/PC (H.b.w./PC), clathrated water molecules/PC (clath.w./PC), PC bound water molecules (bound w./PC), water bridges/PC (w.b./PC), charge pairs/PC (c.p./PC), and PC–PC links/PC (link/PC), obtained experimentally (EXP) and in simulations (SYM) of DMPC, POPC, PEPC, and DMPC–Chol bilayers.

Bilayer		H.b.w. /PC	clath.w. /PC	boundw. /PC	w.b. /PC	c.p. /PC	link /PC
DMPC	SYM	4.5	6.1	10.6	0.71	2.0	2.7
	EXP	5[a]	ND	11[b]	ND	ND	ND
POPC	SYM	5.0	6.6	11.6	0.52	1.6	2.4
	EXP	ND	ND	ND	ND	ND	ND
PEPC	SYM	5.0	6.6	11.6	0.53	1.7	2.3
	EXP	ND	ND	ND	ND	ND	ND
DMPC–Chol	SYM	5.2	6.5	11.7	0.59	1.6	2.3
	EXP	ND	ND	ND	ND	ND	ND

ND, not determined. [a]Borle & Seelig, 1983; [b]Volke *et al.*, 1994.

mal membrane, the POPC–Chol bilayer was used (cf. previous section). A model of the bacterial membrane was built of 54 molecules of 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE), 18 molecules of 1-palmitoyl-2-oleoyl-phosphatidyl-*rac*-glycerol (POPG), and 1955 water molecules. As in a bacterial membrane, the proportion of POPE (75%) to POPG (25%) molecules was 3 : 1. To neutralise the electrostatic charge on the POPG molecules, 18 Na^+ ions were added to the bilayer. The details concerning the construction of the bacterial membrane are given in (Murzyn & Pasenkiewicz-Gierula, 1999).

The molecular dynamics simulation method, because of the still limited computational potential of presently available computers, is not suited to simulate the whole process of bacterial membrane perforation by M2 molecules. However, the method can be applied to study the initial stages of M2 interactions with animal and bacterial bilayers, when M2 molecules are oriented parallel to the bilayer surface, and also the final stage of M2 interaction with the bacterial bilayer, when the M2–POPG pore is formed. The pore consists of approximately 5 M2 and 20 POPG molecules, which interact *via* Coulomb attraction. M2 molecules forming the pore are oriented perpendicular to the bilayer surface.

To compare early stages of M2 interactions with animal and bacterial bilayers, two M2 molecules were placed at the membrane/water interface of both bilayers, with their helix axes parallel to each other. The bilayers were MD simulated for 5 ns. Conformational analysis of the peptides showed that the secondary structure of the first and second M2 molecule in the animal bilayer is 30 and 65% α-helix, and in the bacterial membrane it is 50 and 65% α-helix. These results are in good agreement with experimental data (Wieprecht *et al.*, 1999; Wenk & Seelig, 1998). Further analyses of the generated trajectories for the two systems are in progress.

To reveal basic interactions holding together the experimentally proposed structure of the M2–POPG pore, a model of the bacterial membrane with a pore was constructed. The bilayer consisted of 138 POPE, 46 POPG, 5 M2, 5909 water molecules, and 26 Na^+ ions. Twenty POPG and 5 M2 molecules formed a supra-molecular structure of the pore. Initially, helix axes of M2 molecules were parallel to one another and to the bilayer normal (perpendicular to the bilayer plane). The system was simulated for 5 ns. Preliminary analyses show that helix axes of M2 molecules change their orientation from parallel to tilted with an average tilt of 30° relative to the normal (Fig. 4). The hydrocarbon chains of POPG molecules display higher conformational disorder than those of POPE molecules. Nevertheless, the pore system is stable. Further analyses are in progress.

AN INTEGRAL MEMBRANE PROTEIN IN A PHOSPHOLIPID BILAYER

The goal of this part of our research was to reveal the molecular mechanism of signal transduction from the ligand to the corresponding G-protein in the vasopressin receptor (V2R). V2R belongs to the G protein-coupled receptor (GPCR) superfamily and as other members of the family, consists of seven antiparallel transmembrane α-helices (7TM) linked by six hydrophilic loops. 3D structure of V2R with atomic resolution has not been determined experimentally. However, from the know amino-acid sequence and homology to rhodopsin (Unger & Schertler, 1995), 3D structure of V2R was obtained by molecular modelling methods (Czaplewski *et al.*, 1998). This structure was the starting configuration in the subsequent MD simulation study.

To study the mechanism of signal transduction in V2R, two DMPC bilayer systems with intercalated V2R were built. In one, a vasopressin (AVP) molecule was docked to V2R, and the other one contained an "empty" receptor, whose vasopressin binding site was
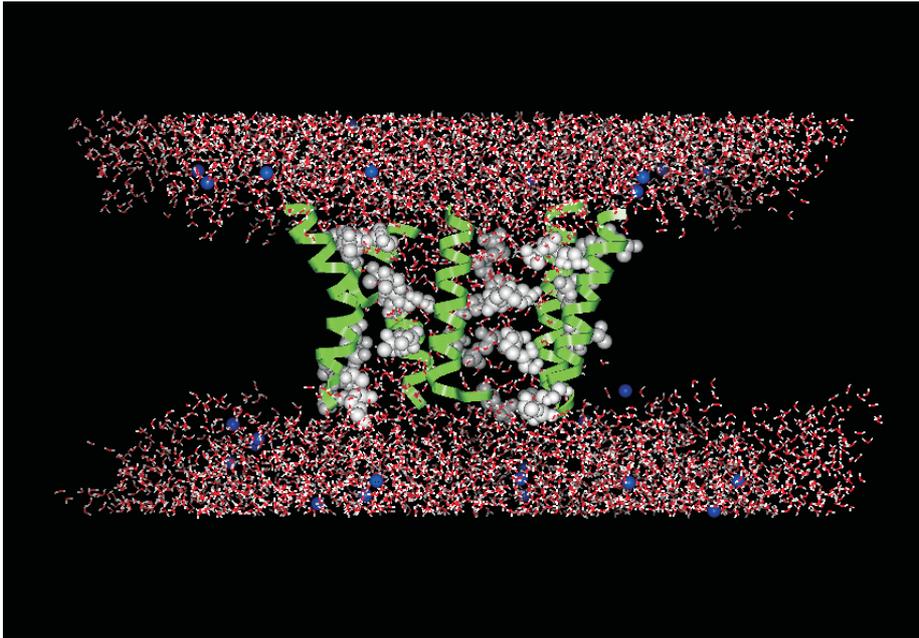
Figure 4. Side view of M2–POPG pore in the bacterial membrane.

For clarity, the bilayer lipids and all hydrocarbon chains were removed. M2 molecules are shown as green ribbons, the headgroups of POPG molecules forming the pore are shown in white as CPK models. Water molecules are in standard colours as lines, and Na^+ ions are shown in blue (as the CPK model).

filled with 10 water molecules. A MD simulation was carried out for both systems for 3 ns (Czaplewski *et al.*, 1999a; 1999b). Fig. 5 shows internal polar residues of the V2R/AVP com-
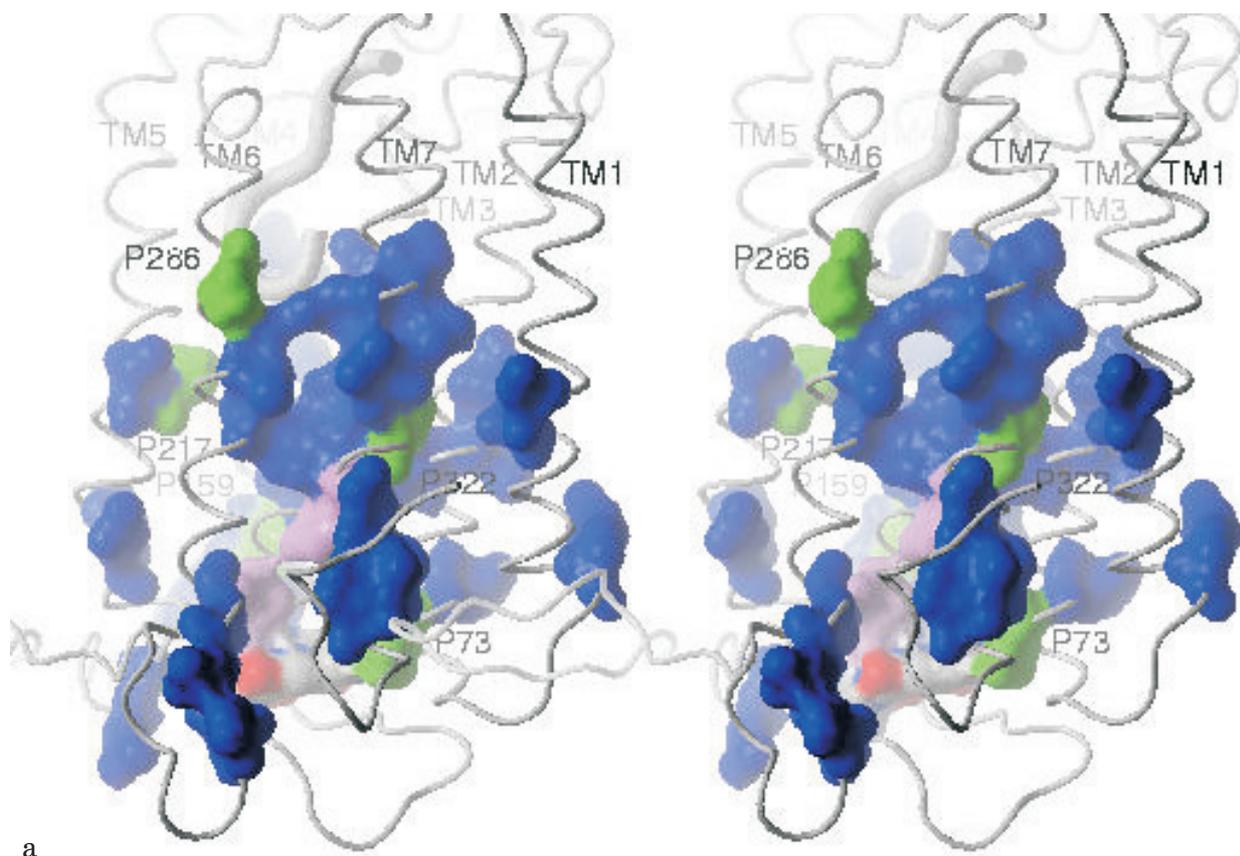

a

Figure 5. Stereo views of networks of internal polar residues (blue) and prolines (green) as CPK models in (a) V2R/AVP complex, and (b) empty V2R.

In the V2R/AVP complex the network is contiguous and the Thr134–Tyr325 (pink) contact is present, whereas in empty V2R the contact is interrupted. AVP is shown as a thick grey ribbon, and V2R as a thin grey ribbon.
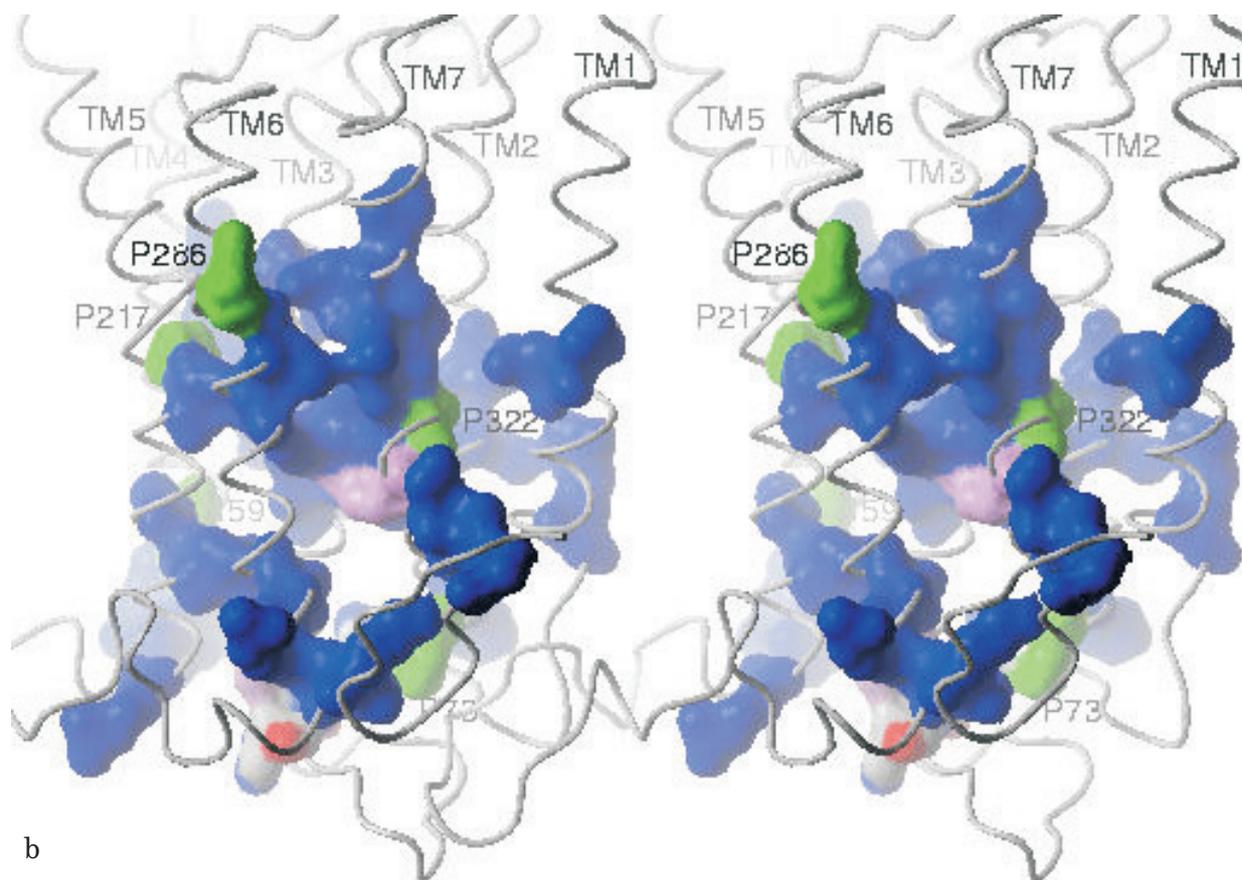
b

Figure 5b

plex (Fig. 5a) and the empty receptor (Fig. 5b). In the V2R/AVP complex, the residues form a contiguous network, extending from the ligand to the cytosolic domain, whereas in empty V2R, the network is interrupted. The main difference between the two networks is the Thr134–Tyr325 contact, present in the V2R/AVP complex but absent in empty V2R. Both Thr134 and Tyr325 are universally conserved over the GPCR superfamily. The contiguous network of internal polar residues might be a pathway of signal transduction in V2R after the binding of the ligand.

PERSPECTIVES

The majority of the studies described in this paper have not been finished, nevertheless, all computer models presented here have been built and MD simulated. In general, a MD simulation provides a plenitude of data, some of which can be obtained by experimental methods, whereas the others are not available experimentally. The former ones are used to verify the computer model, the latter ones provide a deeper insight into the atomic basis of the structure and the dynamics of the studied molecular system. Of all models described in this paper, only for the simple PC and PC–Chol bilayers some firm experimental data exist (cf. Table 1 and 2). For other models, mainly experimental hypotheses are available. Therefore, a positive verification of simpler models is a prerequisite of MD simulation studies of multi-component bilayer systems. Only then, conclusions drawn on the basis of MD simulation results are believed to be, in a rational approximation, applicable to real systems.

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