

Review

Simulation studies of the interaction of antimicrobial peptides and lipid bilayers

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Abstract

Experimental studies of a number of antimicrobial peptides are sufficiently detailed to allow computer simulations to make a significant contribution to understanding their mechanisms of action at an atomic level. In this review we focus on simulation studies of alamethicin, melittin, dermaseptin and related antimicrobial, membrane-active peptides. All of these peptides form amphipathic α -helices. Simulations allow us to explore the interactions of such peptides with lipid bilayers, and to understand the effects of such interactions on the conformational dynamics of the peptides. Mean field methods employ an empirical energy function, such as a simple hydrophobicity potential, to provide an approximation to the membrane. Mean field approaches allow us to predict the optimal orientation of a peptide helix relative to a bilayer. Molecular dynamics simulations that include an atomistic model of the bilayer and surrounding solvent provide a more detailed insight into peptide–bilayer interactions. In the case of alamethicin, all-atom simulations have allowed us to explore several steps along the route from binding to the membrane surface to formation of transbilayer ion channels. For those antimicrobial peptides such as dermaseptin which prefer to remain at the surface of a bilayer, molecular dynamics simulations allow us to explore the favourable interactions between the peptide helix sidechains and the phospholipid headgroups. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Peptide; Bilayer; Simulation; Molecular dynamics; Mean field

Contents

1. Introduction	186
2. Simulation methods	186
2.1. Mean field simulations	187
3. All-atom simulations	195
3.1. Alamethicin	197

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3.2. Melittin	198
3.3. Dermaseptin	198
4. Conclusions and the future	199
Acknowledgements	199
References	199

1. Introduction

Small α -helical peptides mediate simple antimicrobial defence mechanisms in a number of organisms [1]. Although there are abundant experimental data for antimicrobial peptides, their mechanism of action remains somewhat uncertain [2–5] (see also other articles in this issue). Many antimicrobial peptides appear to associate with the lipid headgroups of bacterial membranes. This has led to the proposal of the ‘carpet effect’ [6], whereby peptides adsorbed onto a bilayer surface are suggested to disrupt the packing of lipid molecules (possibly via formation of toroidal pores; see below) such that the membrane becomes leaky. This is supported by solid state NMR experiments on magainin (an anti-microbial peptide found in frog skin [7]), which indicate that its helix adopts an orientation parallel to the bilayer surface, a view which is supported by fluorescence experiments [8]. The early stages of the mechanism of membrane perturbation by antimicrobial peptides may also involve a conformational equilibrium between surface-bound and inserted α -helices (Fig. 1). Thus, at higher concentrations, anti-microbial peptides such as magainin and alamethicin may re-orient to become *perpendicular* to the bilayer surface [9,10], i.e., they adopt a transmembrane (TM) orientation. TM peptide helices may then associate to form water-filled cavities within bilayers [11]. Electrophysiological experiments reveal stepwise increases in ionic current across lipid bilayers on exposure to antimicrobial peptides. These have been interpreted as evidence for discrete ion channels formed by bundles of parallel α -helices spanning the lipid bilayer [2,12]. However, for those often basic peptides that appear to interact strongly with lipid headgroups, an alternative mechanism is possible. In order to explain both the formation of water-filled pores and the retention of specific interactions of peptides with lipid headgroups, the forma-

tion of toroidal pores has been proposed [10]. In this mechanism an α -helical peptide adopts a perpendicular orientation with respect to the bilayer surface but, due to local reorganisation of lipid packing, maintains close interactions with lipid headgroups along its entire length. The result is an aqueous pore, through which ions can flow, leading to cell permeabilisation and eventual death. However, its molecular structure is rather different from that of a bundle of α -helices inserted in an otherwise intact lipid bilayer. Thus, one may distinguish between channel-forming peptides, such as alamethicin, and basic antimicrobial peptides, such as magainin and dermaseptin. The former generates bundles of α -helices. The latter form a carpet of surface-bound helices which, at a sufficiently high peptide concentration, may result in toroidal pore formation via local reorganisation of the bilayer structure [5].

In this review we are largely concerned with simulation studies of the early stages of the interactions of antimicrobial peptides with bilayers. In particular the nature of interactions between the bilayer and peptides that adopt a surface helix orientation is explored, and different levels of simulation are compared. Much of the work to be discussed has focused on a relatively small number of peptides, whose sequences are given in Table 1. These include several genuine anti-microbial peptides, and also a series of synthetic peptides derived from the pore-forming domain of α -endotoxin CryIIIa from *Bacillus thuringiensis* [13]. A more general review of experimental studies of peptides which bind to membrane interfaces is provided by [14].

2. Simulation methods

Central to any simulation is the potential function used. This describes the potential energy of a system

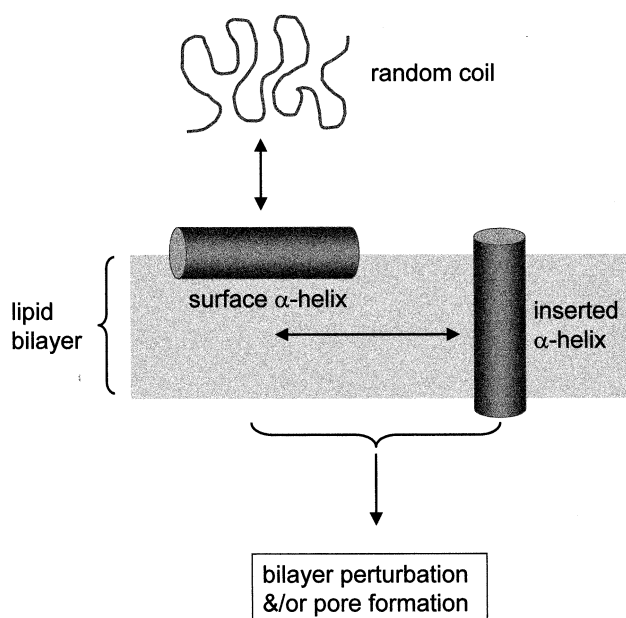


Fig. 1. Interaction of an antimicrobial peptide with a lipid bilayer. In aqueous solution, the peptide may exist either as a random coil or as an α -helix, with the coil being favoured relative to the helix. On binding to the membrane surface, the α -helical form is stabilised. The helix may then adopt an inserted, i.e., transbilayer orientation. Following binding/insertion of the peptide, the bilayer may be perturbed (e.g., a change in lipid phase may be facilitated) or helices and lipid may co-assemble to form a transbilayer pore.

as a function of the coordinates of its constituent atoms, taking into account both covalent (bond lengths, bond angles, torsion angles) and non-covalent (van der Waals and electrostatic) interactions [15]. There are two broad classes of simulations of peptide–bilayer interactions: (i) ‘mean field’ simulations in which the bilayer and surrounding solvent are not included explicitly, but are modelled via an empirical potential which mimics preferential partitioning of hydrophobic sidechains into the bilayer core; and (ii) ‘all-atom’ simulations, in which water and phospholipid molecules, in addition to the peptide per se, are modelled explicitly in terms of their constituent atoms. Clearly all-atom simulations offer a more detailed and realistic model of peptide–bilayer interactions. However, they are significantly more costly (approx. 100-fold) in terms of computer time, and thus only allow one to explore relatively short (1–10 ns) timescale events. Two reviews of earlier simulation studies of membranes are [16,17].

There are two alternative approaches to investigat-

ing how a peptide and a bilayer may interact. In a Monte Carlo (MC) simulation [18], random changes in the position/orientation of, e.g., a peptide helix are made relative to a (mean field) bilayer, and then accepted/rejected on the basis of the change in the overall energy of the system. This method has the advantage of enabling an efficient search of a large number of peptide orientations, but does not provide a temporal sequence of events, i.e., there is no time axis. MC simulations may use either a rigid helix model, in which case the random steps are the position/orientation of the helix relative to a bilayer, or may employ a simplified model of the peptide such that the random moves correspond to (local) changes in peptide conformation in the presence of a bilayer. The latter approach has been pioneered by Skolnick and colleagues [19,20].

Molecular dynamics (MD) simulations employ numerical methods to integrate classical equations of motion (which need a potential function in order to calculate the force and hence acceleration of each atom), yielding the coordinates of all atoms in a system as a function of time. Thus, in principle, MD simulations may be used to propose a sequence of events during the interaction of a peptide and bilayer. However, the times accessible to such simulations remain short (up to approx. 10 ns) and so one remains uncertain whether an optimal peptide–bilayer interaction has been reached. Over the past few years considerable experience has been accumulated in MD simulations of lipid bilayers [17], and such methods are now becoming widely applied to examine peptide–bilayer interactions [21].

2.1. Mean field simulations

2.1.1. Theory

A simple mean field simulation approach uses a ‘hydrophobic slab’ to mimic the presence of a lipid bilayer. Thus, the simulations are performed essentially in vacuo but a region of space is included which represents the bilayer (Fig. 2). Each amino acid sidechain is assigned a hydrophobicity, h , which is either negative (for a hydrophobic sidechain) or positive (for a hydrophilic sidechain). It should be noted that there is some discussion as to which is the most appropriate hydrophobicity scale to use [22]. Having decided upon a suitable scale, the stand-

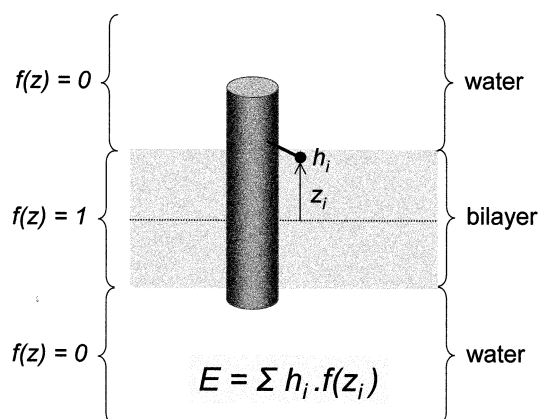


Fig. 2. Mean field model of the interaction of a sidechain i in an α -helix with a lipid bilayer. The sidechain is assigned a hydrophobic energy h_i (positive for hydrophilic sidechains; negative for hydrophobic), which is multiplied by a function, f , which depends on the position of the sidechain relative to the centre of the bilayer (z). The overall peptide–bilayer interaction energy is obtained by summing $h_i f(z_i)$ over all residues within the helix.

ard potential energy function is incremented by a hydrophobic interaction energy term:

$$E = E_{\text{COVALENT}} + E_{\text{NON-COVALENT}} + E_{\text{HYDROPHOBIC}} \quad (1)$$

Typically, the hydrophobic term is calculated on a sidechain by sidechain basis and depends on the z coordinate (see Fig. 2) of the centre of the sidechain. However, more complex atom-based hydrophobicities have also been devised [23]. A mean field approach was first used by [24] in studies of glycyphorin. More recently, Biggin and Sansom [25,26] have shown how a term may be added to the potential function which mimics the presence of a transbilayer voltage difference:

$$E = E_{\text{COVALENT}} + E_{\text{NON-COVALENT}} + E_{\text{HYDROPHOBIC}} + E_{\Delta V} \quad (2)$$

This may be used to simulate, e.g., voltage-induced reorientation of a helix from a surface-bound to a transmembrane orientation.

A limitation of the mean field approach as outlined above is the treatment of the lipid bilayer as a simple hydrophobic slab, i.e., the omission of any representation of the polar headgroups of the lipid molecules. Analysis of all-atom MD simulations of

pure bilayers [17] has revealed that the ‘interfacial’ region created by the intermingling of headgroups and water provides a rather complex environment, in terms of electrostatic properties, and so any mean field model is inevitably a gross simplification. However, a relatively straightforward approach to inclusion of a headgroup term has yielded some promising results. The mean field potential becomes

Table 1
Sequences of peptides discussed in this review

Peptide toxins	
Alamethicin	Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol
Caerin 1.1	Gly-Leu-Leu-Ser-Val-Leu-Gly-Ser-Val-Ala-Lys-His-Val-Leu-Pro-His-Val-Val-Pro-Val-Ile-Ala-Glu-His-Leu-NH ₂
Melittin	Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH ₂
Magainin	Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Gln-Ser
Dermaseptin-B	Ala-Met-Trp-Lys-Asp-Val-Leu-Lys-Lys-Ile-Gly-Thr-Val-Ala-Leu-His-Ala-Gly-Lys-Ala-Ala-Leu-Gly-Ala-Val-Ala-Asp-Thr-Ile-Ser-Gln
Cecropin-P1	Ser-Trp-Leu-Ser-Lys-Thr-Ala-Lys-Lys-Leu-Glu-Asn-Ser-Ala-Lys-Lys-Arg-Ile-Ser-Glu-Gly-Ile-Ala-Ile-Ala-Ile-Gln-Gly-Gly-Pro-Arg
Peptide fragments of δ -endotoxin (a multiple helix toxin)	
$\alpha 2$	Gly-Gly-Ala-Leu-Val-Ser-Phe-Tyr-Thr-Asn-Phe-Leu-Asn-Thr-Ile-Trp-Pro-Ser-Glu-Asp-Pro-Trp-Lys-Ala-Phe-Met-Glu-Asn-Val-Glu-Ala-Leu-Met
$\alpha 3$	Asp-Tyr-Ala-Lys-Asn-Lys-Ala-Leu-Ala-Glu-Leu-Gln-Gly-Leu-Gln-Asn-Asn-Val-Glu-Asp-Tyr-Val-Ser-Ala-Leu-Ser-Ser-Trp-Gln-Lys
$\alpha 4$	His-Ser-Gln-Gly-Arg-Ile-Arg-Glu-Leu-Phe-Ser-Gln-Ala-Glu-Ser-His-Phe-Arg-Asn-Ser-Met-Pro-Ser-Phe-Ala
$\alpha 5$	Phe-Leu-Thr-Thr-Tyr-Ala-Gln-Ala-Ala-Asn-Thr-His-Leu-Phe-Leu-Leu-Lys-Asp-Ala-Gln-Ile-Tyr-Gly
$\alpha 6$	Lys-Glu-Asp-Ile-Ala-Glu-Phe-Tyr-Lys-Arg-Gln-Leu-Lys-Leu-Thr-Gln-Glu-Tyr-Thr-Asp-His-Cys-Val-Lys-Trp-Tyr-Asn-Val-Gly-Leu-Asp-Lys-Leu
$\alpha 7$	Tyr-Glu-Ser-Trp-Val-Asn-Phe-Asn-Arg-Tyr-Arg-Arg-Glu-Met-Thr-Leu-Thr-Val-Leu-Asp-Leu-Ile-Ala-Leu-Phe

Aib, α -aminoisobutyric acid.

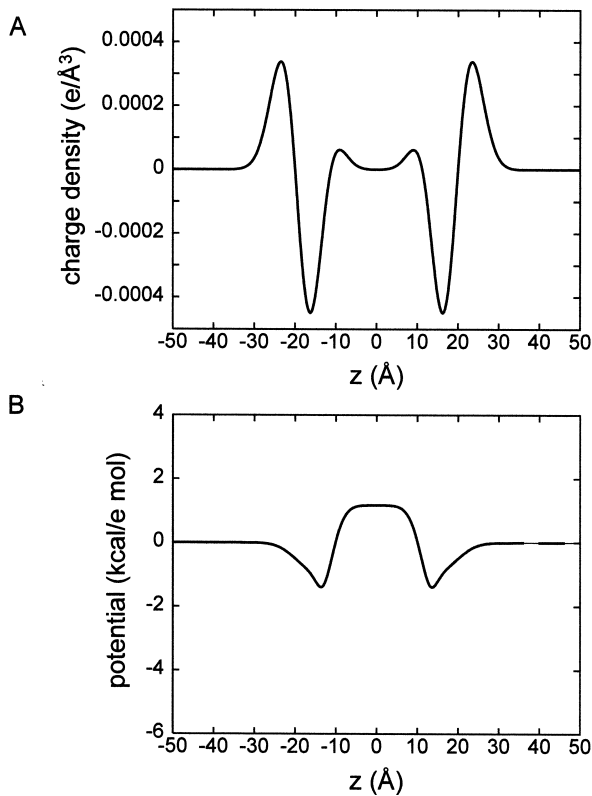


Fig. 3. Origin of the 'headgroup' term in the refined mean field potential. (A) Charge density profile along the bilayer perpendicular (z); (B) electrostatic potential profile.

(in the absence of a transbilayer voltage, for simplicity):

$$E = E_{\text{COVALENT}} + E_{\text{NON-COVALENT}} + E_{\text{HYDROPHOBIC}} + E_{\text{HEADGROUP}} \quad (3)$$

The headgroup term may be modelled as an electrostatic potential operating on charged groups within a peptide. For example, one may calculate the charge density along the normal of a pure lipid bilayer that has been generated by an all-atom MD simulation (Fig. 3A). Combining this with a simple model of the change in dielectric constant from high (in water) to low (in the hydrophobic core of the bilayer) yields an electrostatic potential profile across a bilayer (Fig. 3B) (see [27] for details). For a PC bilayer this profile corresponds to a 'dipole' potential [28] such that the interior of the bilayer is positive relative to the surrounding water, which correlates with the observed higher rate of membrane crossing by negative com-

pared to positive hydrophobic ions. In calculating the $E_{\text{HEADGROUP}}$ term, a further refinement which may be employed is to scale the partial atomic charges on ionizable sidechains, such that they are assumed to be fully charged when outside the bilayer, but electrically neutral when in the bilayer core [29].

2.1.2. Alamethicin

Alamethicin (Alm) is a 20-residue peptide, rich in the helix-promoting amino acid α -amino isobutyric acid (Aib). It has been intensively studied, both as a membrane-active peptide and as a model ion channel [30–32]. Alm forms channels in response to a *cis*-positive transbilayer voltage. Channel activation is suggested to correspond to a voltage-induced switch of Alm helices from a surface-associated to a bilayer-inserted orientation. Alm forms multi-conductance channels in lipid bilayers. These channels are formed by parallel bundles of transmembrane helices surrounding a central pore. A change in the number of helices per bundle alters the single-channel conductance level.

The potential function in Eq. 2 has been used in simulations of the interaction of Alm with a lipid bilayer [26]. Alm was assumed to be initially bound to the surface of a bilayer, in approximately its crystallographic conformation [33], with its polar sidechains pointing out away from the bilayer (Fig. 4). Within a few tens of ps, the Alm helix spontaneously inserted its N-terminus into the bilayer, adopting a transmembrane (TM) orientation, which was then retained for the remainder of the simulation. Interestingly, the time to insertion decreased as the transbilayer voltage was increased from 0 to 200 mV, thus providing a parallel with experimental studies (reviewed in [12,30–32,34]), which indicate that channel formation by Alm is strongly voltage dependent. However, it should be noted that because the rather simple mean field model used in these simulations does not include any representation of the viscosity of the bilayer or solvent, the relationship between the time in the simulation and the real (i.e., experimental) timescale is uncertain.

The modified mean field potential in Eq. 3 has been used in a longer (5 ns) simulation of Alm [29]. An initial MC simulation, using the method outlined in [13] and a rigid Alm molecule, suggested an approximately TM orientation as the lowest en-

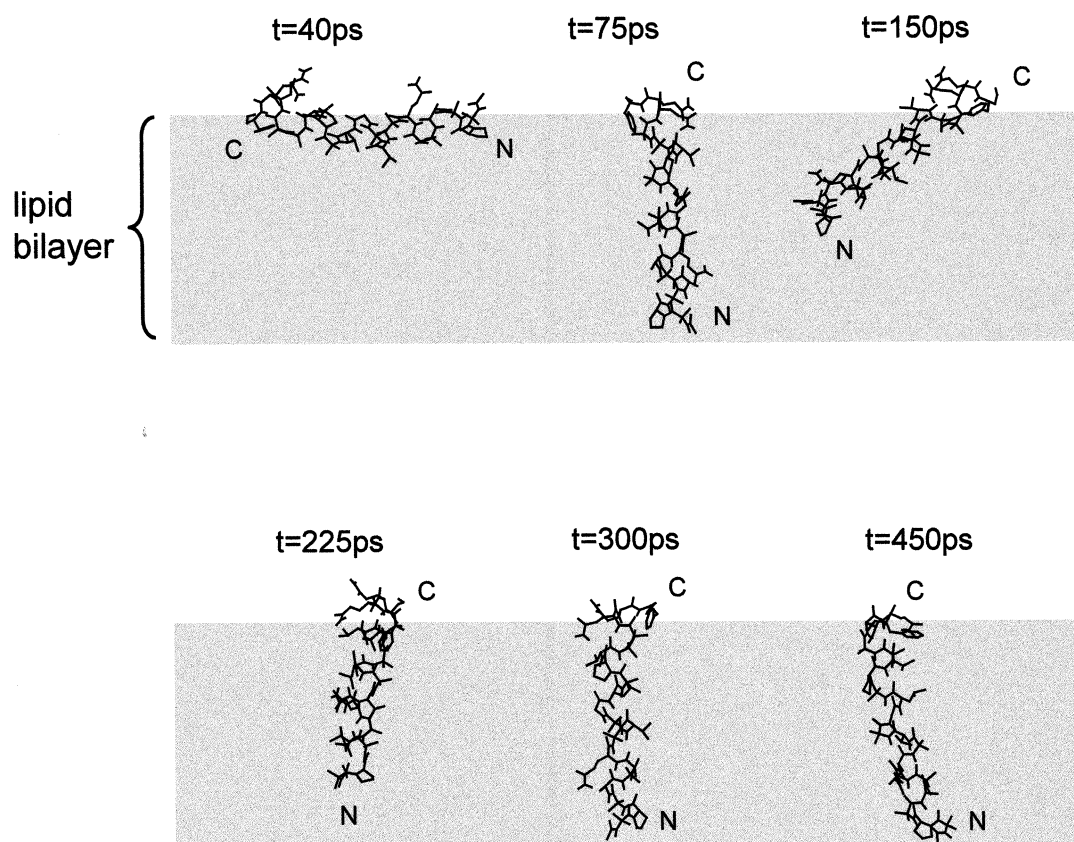


Fig. 4. Snapshots of an Alm-bilayer mean field simulation with a transbilayer voltage of $\Delta V = +200$ mV (see text and [26] for details). The hydrophobic 'slab' which represents the bilayer is shown in grey. The Alm helix initially has a surface-associated orientation ($t = 40$ ps), which fluctuates in orientation subsequent to insertion ($t = 75, 150$ and 225 ps) and then relaxes to a membrane-spanning orientation ($t = 300$ and 450 ps).

ergy configuration of the system. This was used as the starting point for a 5-ns MD simulation, during which all atoms of the Alm molecule were allowed to move freely (i.e., no restraints were applied). A transbilayer voltage difference was not included. The results (see Fig. 5) indicate that the Alm helix is able to switch dynamically between an inserted (i.e., TM) and a surface-bound location. Analysis of the energetics reveals that these two orientations differ by only a few kcal/mol in their interaction energies, with the surface-bound form being associated with a more favourable $E_{\text{HEADGROUP}}$. Furthermore, the surface-bound form seems to be associated with a pronounced kink in the Alm helix. This simulation suggests that the 'balance' between an inserted and a surface-bound orientation may be sensitive to peptide-headgroup interactions. However, it is unlikely that the very rapid switching of Alm between the two

orientations is physically plausible. Rather it is an artefact of the mean field simulation procedure, which does not yet take into account the viscosity of the lipid bilayer. This system would seem to merit further investigation, both experimentally and computationally.

2.1.3. Caerin

The caerins are a family of antimicrobial peptides from the Australian green tree frog *Litoria splendida*. Caerin 1.1 (Table 1) contains a central PXXXXP motif as well as several basic amino acids and has been shown by solution NMR to form a highly kinked α -helix [35]. NMR structures (obtained from peptide dissolved in TFE/water) indicate that the PXXXXP motif provides a flexible hinge between two α -helical regions. A combination of MC and MD simulations similar to that described for Alm (i.e., Eq. 3) has

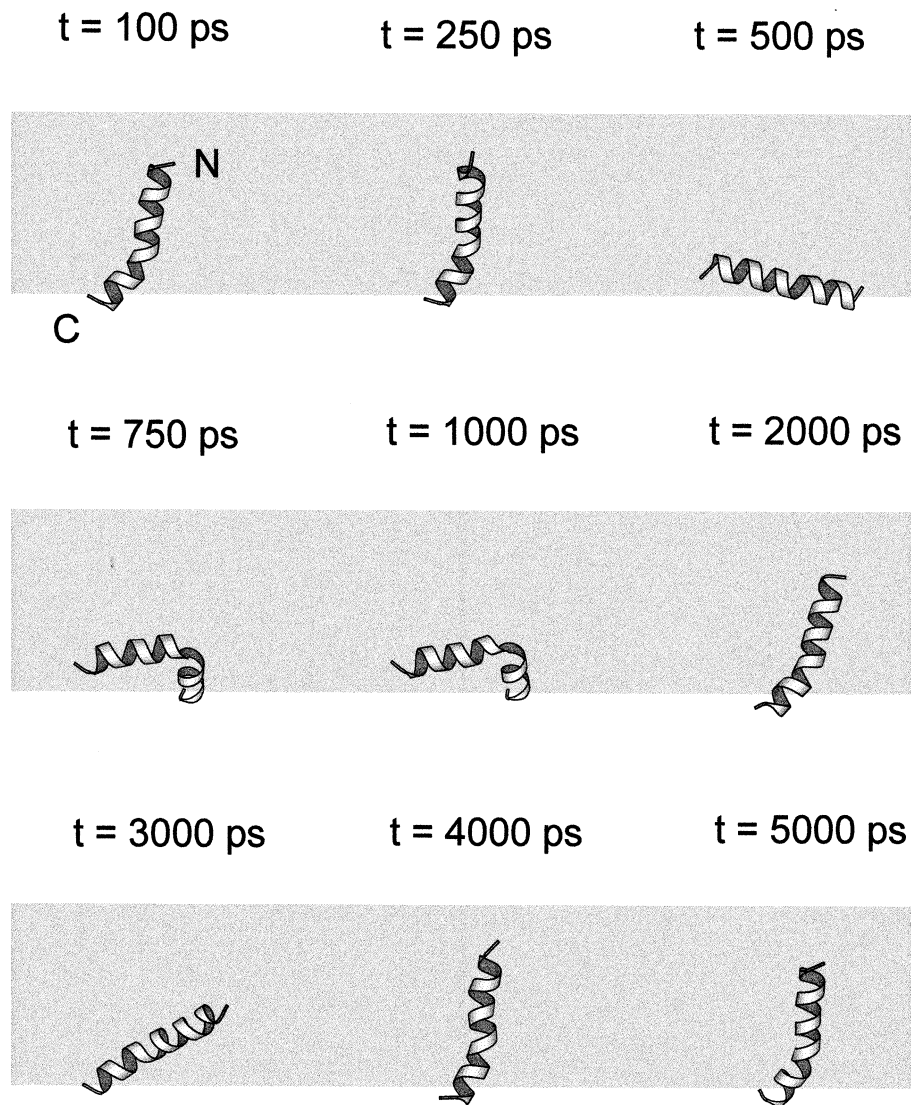


Fig. 5. Snapshots from a 5-ns mean field (using Eq. 3) simulation of Alm. The helix is shown in 'ribbons' format and the bilayer in grey.

been applied to this peptide [29]. MC simulations were used to select an initial peptide conformation and orientation for a subsequent MD simulation. Thus, each of the 20 structures in the NMR ensemble was used in an MC simulation to find the orientation corresponding to the most favourable peptide–bilayer interaction. Note that the peptide was treated as a rigid body in these simulations. The lowest energy configuration, i.e., the conformation of the peptide that yielded the most favourable interaction with the bilayer, was then used as the starting point for two 5-ns MD simulations. In each of these simulations (Fig. 6) distance restraints were employed to

maintain the two α -helical regions seen in the NMR structure. The starting structure (from the MC simulation) had its N-terminal helix at the surface and parallel to the bilayer plane, whilst the C-terminal helix was inserted into the bilayer core. Early on in both simulations the C-terminal helix de-inserted. Subsequently both helices lay on the surface of the bilayer, but changed their orientation relative to one another as consequence of the flexible hinge provided by the PXXXP motif. Thus, the simulation results suggest that caerin acts via a modified 'carpet' mechanism, rather than via Alm-like pore formation. This is in agreement with the proposal

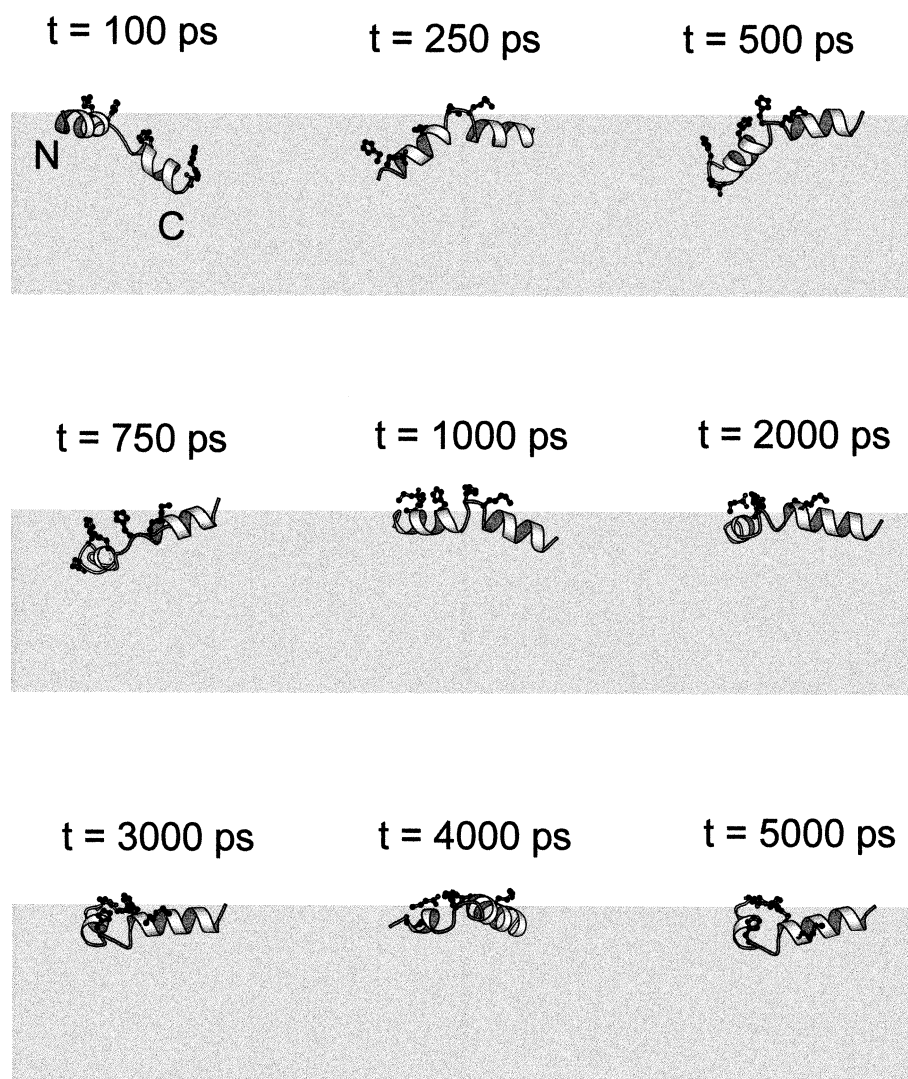


Fig. 6. Snapshots from a 5-ns mean field (using Eq. 3) simulation of caerin 1.1. The helix is shown in 'ribbons' format and the bilayer in grey. Charged sidechains are shown in 'ball-and-stick' format.

of Wong et al. [35] for the mode of action of this peptide.

2.1.4. Dermaseptin, cecropin P, magainin

The dermaseptins, also isolated from frog skin, are a family of membrane-perturbing antimicrobial peptides [6,36–38]. They are 27–34 residues in length and inhibit the growth of bacteria, protozoa and fungi. They adopt a largely α -helical conformation when in a membrane-mimetic environment and bind to phospholipid bilayers. Synthetic dermaseptin B (DS-B) has been shown to bind tightly to both zwitterionic and negatively charged phospholipid vesicles in a

non-cooperative manner [39], supporting a surface location rather than formation of an oligomeric bundle of bilayer-inserted helices. Modelling the sequence of this 31-residue peptide as an idealised α -helix reveals it to be highly amphipathic. It was thus of interest to investigate in more detail the nature of the interaction of DS-B with lipid bilayers. In particular, one would like to be able to predict whether DS-B prefers to associate with the surface of a bilayer (as do some other basic antimicrobial peptides, e.g., cecropin P1 [3]; see below), or whether it inserts into lipid bilayers to form a transmembrane helix in a similar manner to Alm.

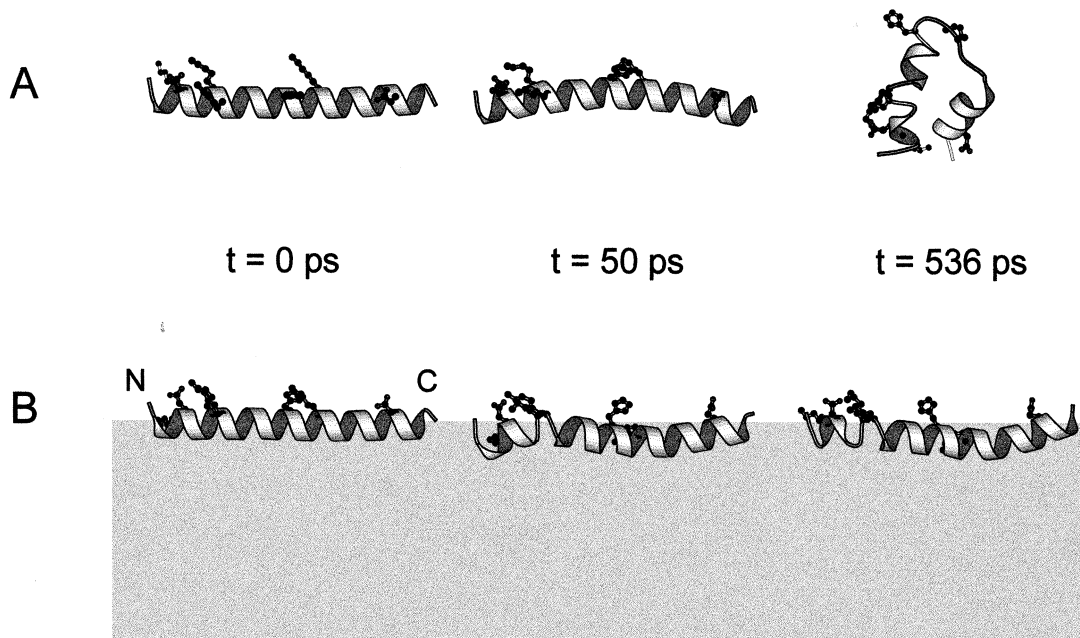


Fig. 7. Comparison of (A) an in vacuo simulation and (B) a mean field simulation of dermaseptin B at the surface of a bilayer. The regions which adopt an α -helical conformation are shown in 'ribbons' format and charged sidechains are shown in 'ball-and-stick' format.

The extended mean field model (i.e., Eq. 3) has been used to investigate the effect of the lipid bilayer on the stabilisation of the α -helical structure of DS-B [27]. MD simulations of DS-B revealed that the lipid bilayer stabilises the α -helical conformation of the peptide (Fig. 7) relative to simulations in the absence of the mean field bilayer potential (i.e., in vacuo). This is in agreement with FTIR data [27] and with all-atom simulation results (see below). Furthermore, omission of the headgroup term from the potential function (i.e., use of Eq. 1 rather than Eq. 3) resulted in a lesser degree of stabilisation of the surface bound helix. There was no indication of a propensity of DS-B to insert into the bilayer. Thus, a surface-associated orientation appears to be the most stable arrangement of this peptide, again in agreement with the 'carpet' model of permeabilisation.

The simpler mean field approach (Eq. 2) has also been used for a porcine antimicrobial peptide cecropin P [3], which is also rich in basic amino acids (Table 1). This peptide, modelled as an extended α -helix, remained at the bilayer surface even when a transbilayer voltage difference was applied. This agrees with FTIR-ATR data in support of a surface location [3].

The mean field approach (Eq. 3, with scaling of titratable charges) has been used to explore the interaction of magainin (Table 1) with both neutral (PC) and anionic (PS) bilayers [29]. The MC search method was employed to identify minimum energy orientations of a magainin α -helix relative to PC and

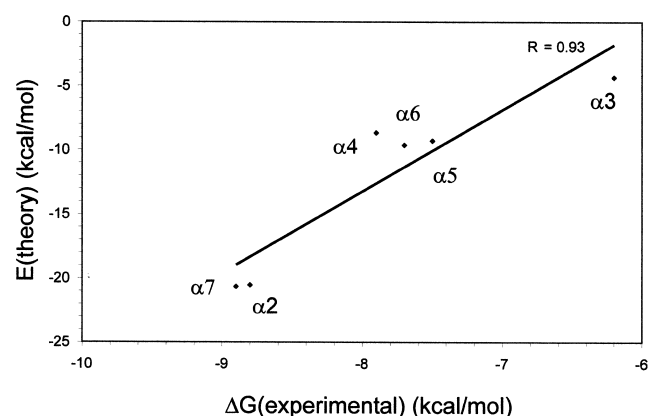


Fig. 8. MC simulations of peptide-bilayer interactions, applied to peptides $\alpha 2$ to $\alpha 7$ of δ -endotoxin. The minimum potential energy of interaction, E , for each peptide helix is plotted against the experimental estimate of its free energy of interaction with a phospholipid bilayer, ΔG . The best-fit line ($r = 0.93$) is superimposed on the data points.

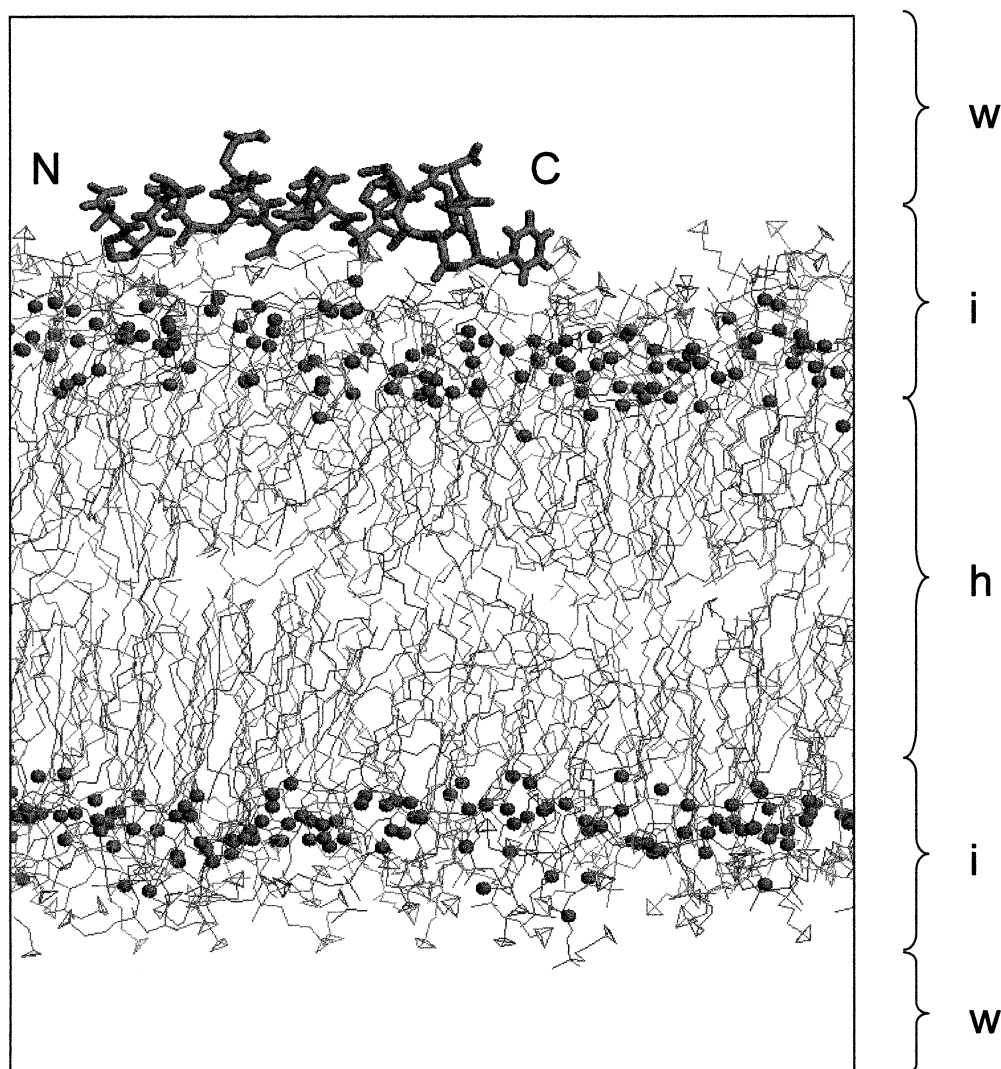


Fig. 9. Snapshot, at $t=0$, of the simulation system for a single Alm helix at the surface of a bilayer. The water molecules on either side of the POPC bilayer are omitted for clarity. The carbonyl atoms of the lipid headgroups, marking the furthest extent of penetration of water molecules into the bilayer, are shown as small grey spheres. The approximate extents of the bulk water (w), interfacial (i) and hydrophobic core (h) regions are indicated.

PS bilayers. With both bilayers the MC search predicted a surface-associated orientation for magainin, with the hydrophilic side of the peptide directed away from the bilayer. This is in agreement with solid state NMR experiments [7] and with earlier mean field MC simulations which employed a simplified representation of the peptide [19]. The calculated peptide–bilayer interaction energies indicated that, as expected, the cationic peptide interacted more strongly with the anionic PS bilayer.

A mean field MC method using atom-by-atom hydrophobicities has been devised by Ducarme et al.

[40]. This has been used in a combined experimental and computational study of mundtacin, a novel antimicrobial peptide from *Enterococcus mundtii* [41]. This basic peptide was predicted to contain an α -helical region which adopted an oblique orientation at the water–bilayer interface. This was suggested to correlate with a membrane-destabilising effect.

Overall, at least for these examples, the mean field simulation procedure seems to be able to discriminate between those peptides that insert into the bilayer (e.g., Alm) and those which remain bound to the surface (e.g., DS-B, magainin, CecP).

Alamethicin - Summary of Simulations

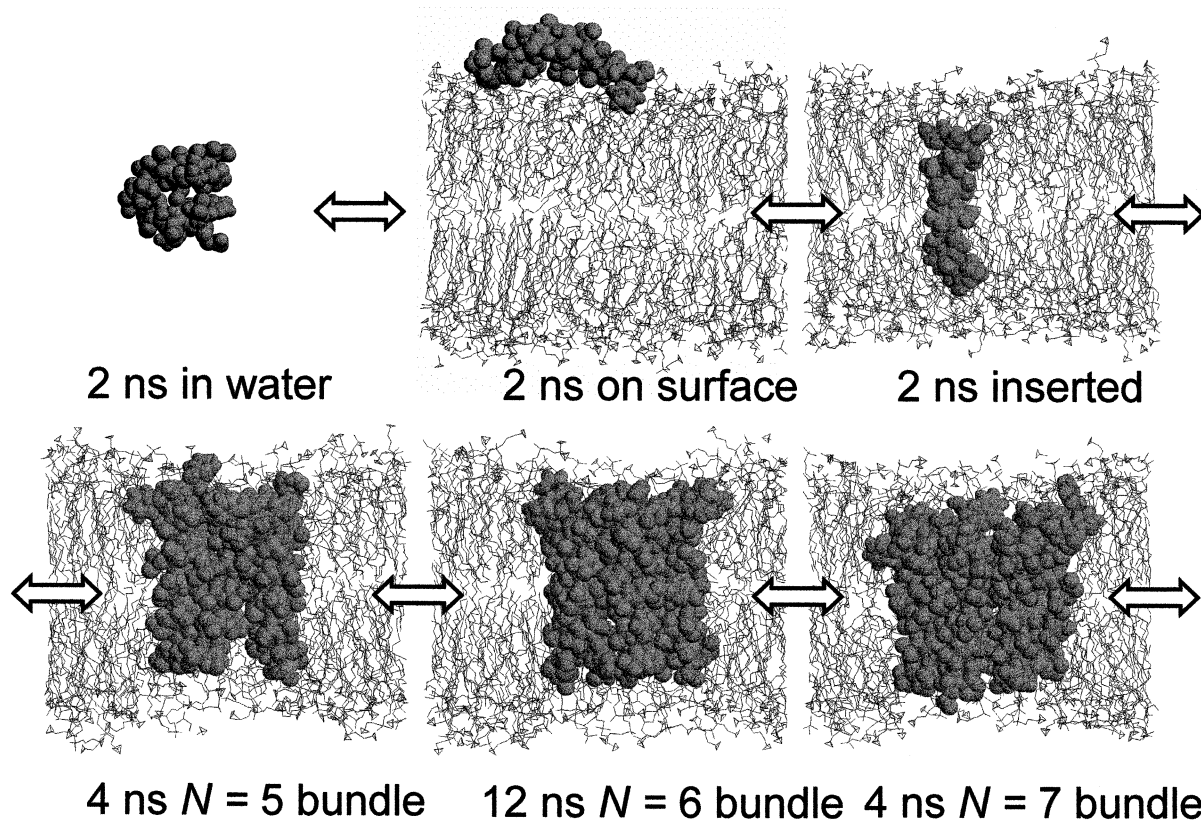


Fig. 10. Summary of the Alm MD simulations. Single Alm molecules in water; at the surface of a POPC bilayer; spanning a POPC bilayer; and Alm helix bundles in a POPC bilayer with $N=5$; $N=6$; and $N=7$ helices bundle. In each case Alm is shown in space-filling format, the POPC molecules are shown in bonds format, and the water molecules are omitted for clarity.

2.1.5. Synthetic δ -endotoxin peptides

In addition to antimicrobial peptides, mean field MC simulations have been used to examine the interaction with a bilayer of a series of synthetic peptides corresponding to the individual helices of the pore-forming domain of CryIII A δ -endotoxin from *B. thuringiensis*. On the basis of experimental spectroscopic data, each of peptides $\alpha 2$ to $\alpha 7$ (see Table 1) was modelled as an α -helix. MC simulations were run for each peptide (using Eq. 3 with scaling of titratable charges), and the calculated optimal energy of interaction between peptide and bilayer was plotted against the corresponding experimental estimate of the free energy of binding of the peptide to a phospholipid bilayer [13]. The resulting correlation (Fig. 8) is encouraging, and suggests that mean field simulation methods may be able to discriminate between different strengths of interaction of pep-

tides with a bilayer. Further work is needed in this area.

3. All-atom simulations

All-atom simulations of the interactions of antimicrobial peptide with bilayers provide a much more detailed view of the underlying peptide–lipid interactions. In particular, all-atom simulations have the potential to reveal the structural origin of selectivity in interactions between antimicrobial peptides and lipid headgroups. However, all-atom simulations are not without difficulties. As mentioned above, atomistic simulations of pure lipid bilayers are now reasonably well established. Building on these simulations, a number of groups have simulated transmembrane helices (reviewed in [21]) or integral mem-

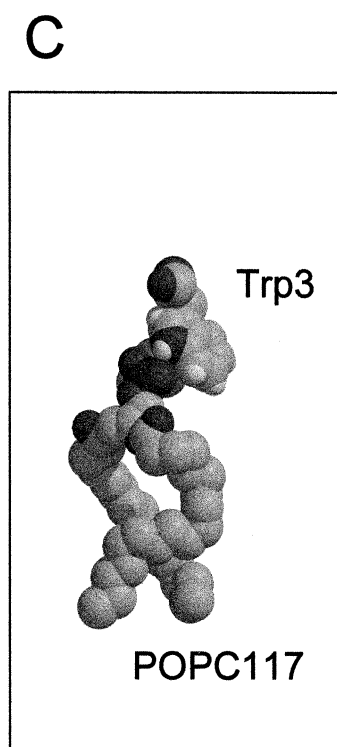
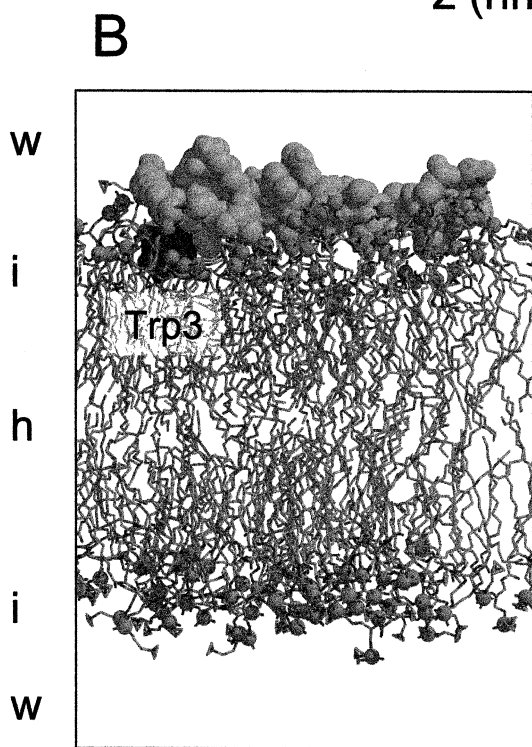
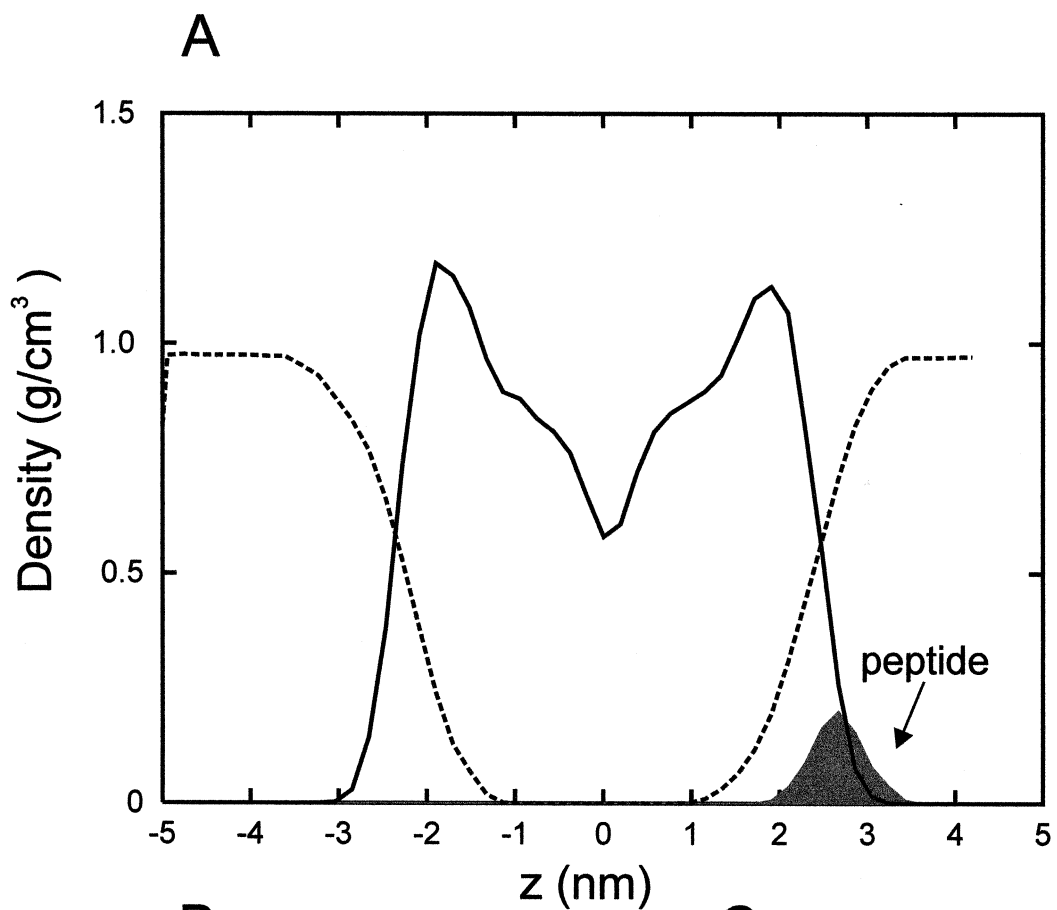


Fig. 11. Simulation of dermaseptin B at the surface of a POPC bilayer. (A) Atomic density profiles along the bilayer normal. The water density is shown as a broken line, the lipid density as a solid line, and the peptide density as a filled grey area. (B) Snapshot of the simulation system at 2 ns. The water molecules are omitted for clarity. The phosphorus atoms are shown as small grey spheres, and the dermaseptin is shown in space-filling format. The Trp3 sidechain is shown in dark grey. Three regions are indicated to the right of the diagram: w, water; i, interfacial region; and h, hydrophobic core. (C) The interaction between the sidechain of residue Trp3 and the phosphate of a POPC molecule. Structure diagrams were drawn using Molscript [59] and Raster3D [60].

brane proteins [42–44] embedded in lipid bilayers or in a bilayer mimetic environment (e.g., a slab of octane immersed in water [45]). A preliminary analysis [46] of such simulations has provided useful insights into protein–lipid interactions.

Although apparently simpler than such complex membrane proteins, membrane-active antimicrobial peptides present some rather difficult challenges to atomistic simulations. The main problem lies in the initial setup of a simulation system. Even if, e.g., a mean field simulation has indicated that a given peptide helix lies on the surface of a bilayer, such a simulation is unlikely to provide sufficiently detailed information as to how deeply into the bilayer a helix penetrates. Thus, one has to assume a starting orientation and position of the helix that almost certainly will have a profound influence on its behaviour in a subsequent atomistic MD simulation. Iteration between successive all-atom and mean field simulations might resolve this difficulty, but this has yet to be explored.

3.1. Alamethicin

Molecular dynamics simulations of Alm in a number of different environments have been used to explore some of the complexities of its mechanism of channel formation. These simulations include: (i) Alm in solution in water or in methanol [47]; (ii) a single Alm helix at the surface of a phosphatidylcholine bilayer [48]; (iii) a single Alm helix spanning a phosphatidylcholine bilayer [47]; and (iv) channels formed by bundles of 5, 6, 7 or 8 Alm helices in a phosphatidylcholine bilayer [49–51]. This set of simulations enables one to explore in atomistic detail the various states during the mechanism of channel formation.

Binding of alamethicin to the surface of a lipid bilayer is believed to be an early event in channel formation. All-atom MD simulations have been used to compare the structural and dynamic proper-

ties of alamethicin in water and alamethicin bound to the surface of a palmitoyl–oleoyl phosphatidylcholine (POPC) bilayer. The bilayer surface simulation corresponded to a loosely bound alamethicin molecule that interacted with lipid headgroups but did not penetrate the hydrophobic core of the bilayer. Both simulations started with the peptide molecule in an α -helical conformation and lasted 2 ns. In water, the helix started to unfold after approx. 300 ps and by the end of the simulation only the N-terminal region of the peptide remained α -helical and the molecule had collapsed into a more compact form. At the surface of the bilayer, loss of helicity was restricted to the C-terminal third of the molecule and the rod-shaped structure of the peptide was retained. In the surface simulation about 10% of the peptide–water H-bonds were replaced by peptide–lipid H-bonds. These simulations suggested that some degree of stabilisation of an amphipathic α -helix occurred at a bilayer surface (Fig. 9) even without interactions between hydrophobic sidechains and the acyl chain core of the bilayer [48].

Nanosecond MD simulations have also been used to compare the conformation and dynamics of Alm in a non-aqueous solvent (i.e., methanol) with Alm inserted into a POPC bilayer to form a transmembrane helix. In both of these environments there was little change ($C\alpha$ RMSD approx. 0.2 nm over 2 ns and 1 ns, respectively) from the initial helical conformation of the peptide, in marked contrast with the behaviour in water (see above) where there were substantial changes ($C\alpha$ RMSD approx. 0.8 nm over 2 ns). In the bilayer and in methanol, the alamethicin molecule underwent small hinge-bending motions about its central GXXP sequence motif. Analysis of H-bonding interactions revealed that the polar C-terminal sidechains of alamethicin provided an ‘anchor’ to the bilayer–water interface via formation of multiple H-bonds which persisted throughout the simulation. This may explain why the preferred mode of helix insertion into the bilayer is N-terminal,

which is believed to underlie the asymmetry of voltage activation of alamethicin channels.

Two 2-ns MD simulations of a hexameric bundle of Alm helices in a POPC bilayer have been performed in order to explore the dynamic properties of a model of a channel formed by a helix bundle in a fully solvated phospholipid bilayer [49]. The effect on bundle stability of the ionisation state of the ring of Glu18 sidechains was investigated. If all of the Glu18 sidechains were ionised, the bundle was unstable; if none of the Glu18 sidechains was ionised, the bundle was stable. pK_a calculations suggest that either zero or one ionised Glu18 is likely to be present at neutral pH, correlating with the stable form of the helix bundle. The structural and dynamic properties of water in this model channel were examined. As in earlier *in vacuo* simulations [52], the dipole moments of water molecules within the pore were aligned antiparallel to the helix dipoles. This contributes to the stability of the helix bundle.

Different channel conductance levels correspond to different numbers of helices per bundle, ranging from $N=5$ to $N>8$. The results of MD simulations, corresponding to a total simulation time of 24 ns, of $N=5$, 6, 7 and 8 bundles of Alm helices in a POPC bilayer have been compared [50]. All four helix bundles were stable on a multi-nanosecond timescale, correlating with the experimentally observed multiplicity of Alm single channel conductance levels. Simulations of an $N=4$ helix bundle suggested it to be incapable of forming a stable transbilayer pore, again in agreement with the interpretation of single channel studies of Alm.

Overall, these studies illustrate how nanosecond MD simulations may be used to characterise the different states in the process of channel formation by Alm (Fig. 10), and provide a benchmark for simulation studies of other peptides. What they do not allow us to study are the longer timescale transitions between these states. This remains a major challenge to simulation studies of antimicrobial peptides (see below).

3.2. Melittin

The 26-residue peptide melittin, isolated from bee venom, is not strictly speaking an antimicrobial, it has been intensively investigated as a model of pep-

tide–membrane interactions [53]. It has channel-forming properties resembling those of Alm, but also exhibits potent membrane lytic and fusogenic effects. In an all-atom MD simulation of melittin [54] interacting with a DMPC bilayer, the initial configuration of the system was such that the overall helix axis was approximately parallel to the bilayer plane. The melittin helix was oriented such that the helix kink (induced by the GXP motif) resulted in the N-terminus of the peptide penetrating deep into the core of the bilayer. This produced a significant local perturbation of the bilayer structure and allowed penetration of water through the leaflet of the bilayer opposite to that to which melittin was bound. This was suggested to be part of the mechanism of membrane lysis by this peptide. However, further simulations of different helix orientations are needed to probe this in more detail before a definitive mechanism can be established. The α -helical conformation of melittin remained stable throughout the 1-ns simulation. Thus, this simulation corresponded to a ‘tight’ complex between peptide and bilayer in which the peptide was largely buried in the hydrophobic core of the bilayer. A systematic series of MD simulations of melittin have also been run by Lin and Baumgärtner (personal communication), which may provide more detailed insights into its mode of action.

3.3. Dermaseptin

In contrast to Alm and melittin, both of which can insert to form channels, dermaseptin seems to act entirely via surface effects and so provides a good test system for developing an atomistic simulation approach applicable to the majority of antimicrobial peptides. Atomistic simulations on dermaseptin B at the surface of a POPC bilayer [55] complement the mean field studies discussed above. In particular, MD simulations were used to compare the conformational dynamics of a DS-B α -helix in water (total simulation time 4 ns) and bound to the surface of a POPC bilayer (total simulation time 6 ns). In water, the α -helix unfolded on a nanosecond timescale, with loss of helicity prominent at the termini and close to a glycine residue. When loosely bound to the lipid bilayer surface, the α -helical conformation of DS-B was stabilised. This stabilisation was somewhat

greater when the helix was oriented such that its polar surface was directed towards the water and its apolar surface towards the bilayer. In the presence of a bilayer there was only limited loss of α -helicity, mainly at the C-terminus. The peptide helix resided in the bilayer–water interfacial region (Fig. 11A,B). Interestingly, given the proposed roles of tryptophan sidechains in integral membrane proteins [56,57], the sidechain of residue Trp3 formed H-bonds to the phosphate of a lipid headgroup (Fig. 11C) in all three of the peptide–bilayer–water simulations. It is encouraging to observe that stabilisation of the DS-B helix by binding to a bilayer surface is seen in both mean field (see above) and all-atom simulations. This is in good agreement with the experimental studies of White and colleagues [58], who estimate a stabilisation (relative to bulk solvent) of approx. -0.4 kcal/mol/H-bond for α -helix formation by an amphipathic peptide in the interfacial region.

4. Conclusions and the future

From the above it can be seen that simulation studies are beginning to yield valuable insights into how antimicrobial peptides interact with membranes. In particular, mean field methods seem to be of sufficient accuracy to distinguish between those peptides which insert into bilayers and those which bind to the surface. However, current simulations are in an early stage of development, and need to be improved in several areas before they can provide a complete atomistic description of the mechanisms of a range of antimicrobial peptides.

(1) More complex all-atom simulations are needed, which provide a better description of real experimental situations. Particular areas to be addressed include the use of bilayers that contain mixtures of lipids, and calculation of free energy profiles (i.e., potentials of mean force) for the interaction of a peptide and a bilayer as a function of the location and orientation of the peptide relative to the bilayer.

(2) There is considerable scope for improvement in mean field methods. In particular, some sort of representation of the local viscosity of the environment provided by the lipid bilayer would make such simulations more realistic, and a better model of lipid headgroups is needed. Furthermore, simulations to

date have tended to focus on single peptide helices interacting with the surface of a bilayer. However, e.g., the carpet model proposes that multiple helices interact at the bilayer surface. It will be important to use mean field methods to explore such interactions, and to provide systems of multiple helices as starting configurations for all-atom simulations.

(3) The major computational challenge remains one of how to model or simulate membrane perturbation by peptides. This will require advances in, e.g., MD simulations of non-bilayer lipid phases, in order to understand, e.g., the formation of toroidal pores. At present, one can only sketch out how simulations of perturbations might proceed. A hybrid approach might be to use a combination of experimental data and mean field simulations to define different stages in the mode of action of an antimicrobial peptide, followed by atomistic MD simulations to explore each stage in more detail.

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