COMPARISON OF NVE AND NPγT ENSEMBLES AND THE EFFECT OF γ VALUES IN THE SIMULATION OF A PEPTIDE IMBEDDED IN DMPC BILAYERS .

The endogenous opioid peptide dynorphin A(1-17) and its smaller fragment Dyn A(1-13) have been shown to behave similarly in DMPC bilayers with MD simulations in NVE ensembles (*Biophys. J.* 79 (2000) 2331; *J. Phys. Chem. B* (2001)). Specific interactions of the aromatic and basic residues with the bilayer membrane determine the orientation of these peptides within the bilayers. The influence of flexible box lengths on the properties of the membrane-imbedded peptides can be probed in simulations with NPγT ensembles, but the precise value of γ for such heterogeneous systems is not known. MD simulations on Dyn A(1-13) in DMPC bilayers for a period of 2 to 4 ns were done using NPγT ensemble with γ values 0, 45, 65 and 85 dyn/cm. When γ = 0, a dramatic contraction of the area is observed as shown also by others in pure bilayer simulation (*J. Chem. Phys.* 111 (1999) 1281). This contraction severely restricts the peptide so that the peptide orientation is close to the starting point even after 2 ns and the individual residue interactions are different from those in the NVE ensemble. In contrast, with γ = 45 or 65 the individual residue interactions are similar to those in NVE ensembles. These results indicate the nature and magnitude of the effect produced by the choice of γ values for simulations of complicated systems.

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Comparison of NVE and NPT ensembles and the effect of γ values in the simulation of a peptide imbedded in DMPC b

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Introduction

● Issues of methodology in membrane simulations include force field, calculation of non-bonded forces and the choice of boundary conditions [1].

● Force fields like CHARMM and GROMACS are being continuously improved to reproduce the many experimentally observed membrane properties. The use of Ewald sums effectively takes all non-bonded interactions into account.

● Periodic Boundary Conditions (PBC) with the following ensembles have been used in many of the membrane simulations [2]:
NVE (constant number of atoms, volume and energy)
NVT (constant number of atoms, volume and temperature)
NPAT (constant number of atoms, pressure, area and temperature)
NPγT (constant number of atoms, pressure, surface tension and temperature)

● In NVE, NVT and NPAT ensembles, knowledge of the correct surface area of the lipid is required to produce meaningful simulations. The addition of peptides, proteins and other compounds is expected to influence the area of the lipid.

● In the NPγT ensemble, the dimensions of the cell are fully flexible and they adjust dynamically to the appropriate surface area per lipid and lamellar spacing.

●The value of surface tension γ must be specified in the NPγT ensemble. Surface tension is a macroscopic property and it is difficult to estimate the value of γ for the microscopic patch typically studied in the simulations. Addition of peptides and other substituents presents further challenges. The use of non-zero γ value is the subject of debate in the literature [1,3].

● In the present study, molecular dynamics simulations of neat bilayers consisting of 90 lipids have been carried out using NPγT and NPAT ensembles. Non-bonded interactions have been calculated using both Particle-Mesh Ewald method and a spherical cut-off of 18 Å. Different γ values are tested in the simulations.

● Simulations also include membranes containing the opioid peptide dynorphin A(1-13) (Sequence: YGGFLRRIRPKLK). The influence of different γ values on the structure and orientation of the peptide is studied. The results are compared with our earlier simulations of the same peptide using NVE ensemble [4,5].

Methods

Neat Bilayers

● The protocol developed by Woolf and Roux [6] was used to construct the hydrated lipid bilayer system. The program CHARMM was used with PARM 22 all atom parameter set [7].
Z = 0 Å was the center of the bilayer and the Z-axis was the bilayer normal. Each leaflet in the bilayer is composed of 45 DMPC lipids. The system contains ~ 30 water molecules per lipid, which amounts to full hydration. The total number of atoms is ~ 18,300. Temperature of the system is 330K and the time step used is 0.002 ps

● Two sets of simulations were carried out and they differed in the treatment of non-bonded interactions; a spherical cut-off of 18 Å was used in one and a Particle-Mesh Ewald (PME) method was used in the other.

● γ values (in dyn/cm) considered in the NPγT ensembles are:
0, 10, 20, 45, 65, 85 and 625 (cut-off 18 Å); 0, 20, 30, 45 (PME)

● For simulations using the NPAT ensemble, the initial surface area per lipid is

Results and Discussion

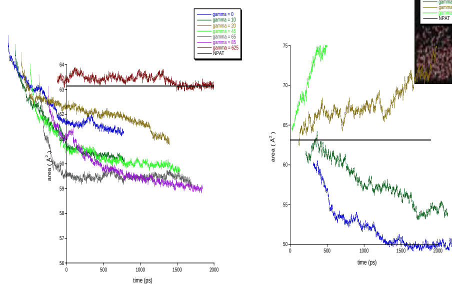


Figure 1: Molecular dynamics trajectories of surface area per molecule as a function of time for each NPγT simulations. Data for cut-off 18 Å (left) and PME (right) simulations are shown. For cut-off 18 Å, even for γ = 85 dyn/cm, the surface area is close to 58.5 Å^2, about 5 Å^2 less than the experimentally estimated value at the temperature of study [8]. To attain the value of 63.1 Å^2, the value of γ has to be increased to 625 dyn/cm (calculated from NPAT simulations). For PME, γ value that will keep the area close to the experimental value is estimated between 20 and 30 dyn/cm.

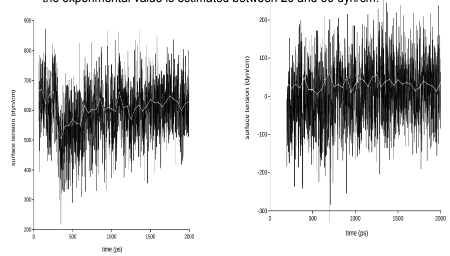
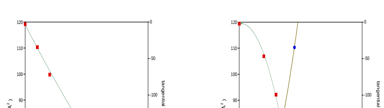
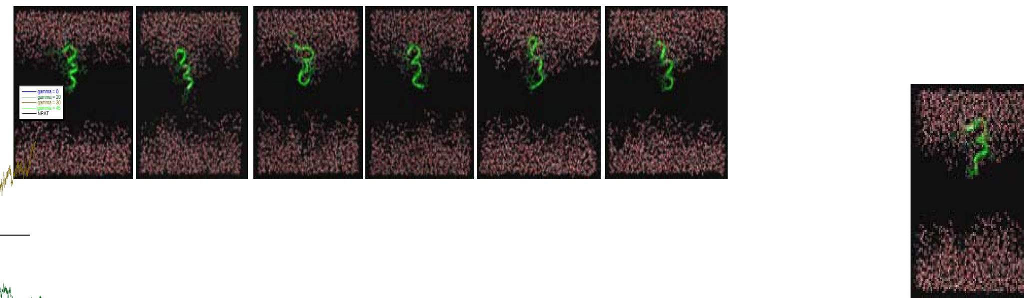


Figure 2: Calculated surface tensions (per interface) as a function of time from the NPAT simulations with constant surface area 63.1 Å^2, a value close to the experimental estimate [8]. The 1 ps (black) and 50 ps (white) block averages are plotted for cut-off 18 Å (left) and PME (right) simulations. The calculated γ value from the simulation using 18 Å cut-off is more than an order of magnitude higher than that of PME simulation. The γ value calculated from PME simulation is close to the estimated value from experiments and other MD simulations [9].





Initial structure | γ = 0 dyn/cm | γ = 45 dyn/cm | γ = 65 dyn/cm | γ = 625 dyn/cm | NPAT | γ = 30 dyn/cm (PM

Figure 4: Structures of dynorphin A(1-13) in DMPC bilayers plotted at the end 2 ns production runs (for clarity, only waters and peptide are shown). The γ values use each panel. For the PME simulation, the structure was saved at the end of 600 ps. The structure from the NVE simulation is from our previous simulation studies [5]. Wit of box, the first tyrosine residue extends to the other half of the bilayer and as a result water molecules penetrates from that side of the bilayer. In all other simulati membrane-water interface and the Phe prefers to be close to the membrane interior. The difference in the behavior of these two aromatic residues is noted in our earlier

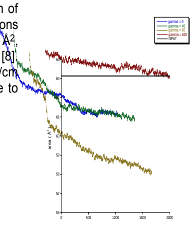


Figure 5: Trajectories of surface area per lipid plotted for peptide – lipid simulations. As observed in the neat bilayer simulations, the γ values (dyn/cm) 0, 45 and 65 resulted in smaller box sizes when cut-off of 18 Å is used. The γ value has to be increased to more than 600 dyn/cm to keep the area close to the experimental estimate for neat bilayers.

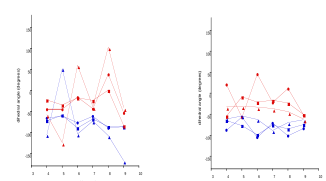


Figure 6: Average ϕ (blue), ψ (red) angles for residues 4 to 9, calculated for the last 1 ns of production runs. The total production runs in these simulations were 2 ns.
Left: The γ values (dyn/cm) used in the simulations are 0 (●) 45 (■) and 65 (▲)
Right: Analysis for simulations with ensembles NPγT (●), NPAT (■) and NVE (▲). The γ value used in this NPγT ensemble is 625 dyn/cm to keep the surface area close to the experimental estimate. Both NPγT and NPAT simulations used a spherical cut-off of 18 Å to calculate the non-bonded interactions. The data for NVE simulation was taken from our previous studies.
It is clear that the shrinkage of box size results in significant distortion of alpha-helical structure (left). When the area is kept close to 63 Å^2 (experimental estimate for neat bilayers [8]), the helical structure is reasonably maintained.

Summary and Conclusions

● Simulations of the membrane patch of size 90 lipids using NPγT ensemble with different γ v different schemes for non-bonded interactions: the spherical cut-off of 18 Å and the Particle M comparison purpose, simulations with NPAT ensembles were also carried out with the area of the

● The opioid peptide dynorphin A(1-13) was simulated in DMPC bilayers to investigate the effe structure and orientation of the peptide. Four different γ values were used for 18 Å cut-off. NP comparison. With PME, a γ value of 30 dyn/cm was used.

● In total, 19 simulations ranging from 1.0 ns to 4.0 ns were used for analysis.

● Results from neat bilayer simulations show that *γ = 0 dyn/cm results in the contraction of the sir* spherical cut-off and PME schemes and also observed by others [9].

● In simulations in which a spherical cut-off of 18 Å was used, only *a slight increase in the surfa ranging from 10 to 85 dyn/cm.* γ has to be increased to 625 dyn/cm (obtained from NPAT simu close to 63 Å^2, the value estimated for DMPC from experiments at 50° C [8].

● In PME simulations, it is estimated that the *γ value that will keep the surface area close to the e 20 and 30 dyn/cm.* This agrees well with the recent MD simulations of membrane patch of size estimated to simulate this smaller membrane patch is in the range of 30 – 40 dyn/cm.

● It has been shown previously that the value of γ calculated from NPAT simulations depen calculating the long-range coulombic interactions [10]. However, the observed difference in that s cut-off of 12 Å and Ewald summation technique were used. In the present study, more than an observed in the calculated γ value between cut-off 18 Å and PME schemes. It should be pointed o simulations are longer and the area used in the NPAT simulations are close to the experimental e

● The contribution from long range forces between various components of lipids (headgroups a may explain the significant difference in the γ values calculated from the two NPAT simulations.

● In the peptide-bilayer simulations, for γ values 0, 45 and 65, the contraction of the simulation b alpha-helical structure in the peptide.

● The behavior of aromatic residues in the peptide is similar to that observed in our previ ensemble for all γ values except for γ = 0 dyn/cm. In these cases, Tyr-1 likes to interact with the m hydrophobic Phe residue (Phe-4) prefers to be close to the membrane interior. When γ = 0 dyn/c of the bilayer and attracts water molecules from that side.

● Although constant pressure simulations with fully flexible box lengths are desirable in the compounds like peptides and proteins, value of the the surface tension must be chosen with car simulations shows that it affects both the orientation and the structure of the peptide.