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How reliable are molecular dynamics simulations of membrane active antimicrobial peptides?☆

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ABSTRACT

Membrane-active antimicrobial peptides (AMPs) are challenging to study experimentally, but relatively easy to investigate using molecular dynamics (MD) computer simulations. For this reason, a large number of MD studies of AMPs have been reported over recent years. Yet relatively little effort has focused on the validity of such simulations. Are these results reliable, and do they agree with what is known experimentally? And how much meaningful information can be obtained? To answer these questions, we demonstrate here some of the requirements and limitations of running MD simulations for several common AMPs: PGLa, melittin, maculatin and BP100. The two most important findings are: (a) simulation results depend strongly on force field parameters, making experimental verification of the simulations obligatory, and (b) slow orientational and conformational fluctuations mean that much longer sampling timescales (multi-μs) are needed if quantitative agreement between simulation averages and experimental data is to be achieved. This article is part of a Special Issue entitled: Interfacially Active Peptides and Proteins. Guest Editors: William C. Wimley and Kalina Hristova.

1. Introduction

Membrane-active antimicrobial peptides (AMPs) are found in many organisms and are currently of major pharmaceutical interest as a potential source of new antibiotics against increasingly common multiresistant pathogens [1,2]. Most of these peptides kill bacteria by physically interacting with and disrupting their cell membranes. The exact molecular mechanism concerned is not fully understood at present, with a large number of models proposed over the last decades [3]. Because it is experimentally challenging to study these highly mobile peptides in membranes, molecular dynamics (MD) simulations have been proposed as an alternative. Over the last years, countless such simulations have been presented on a large number of AMPs and related cell-penetrating peptides [4,5]. Because the results of these simulations have been almost as diverse as the mechanisms proposed, the question arises as to how accurate MD actually is and whether these results can be trusted. Interestingly, there is relatively little information on this issue to date. Most MD studies of membrane-active peptides lack any but the most basic verification versus experimental data, so the results have to be taken 'as is'. Previously, in the absence of any quantitative experimental measurements, this situation may have been acceptable. However, considerable progress has been made in recent years to obtain highly accurate information on AMPs and other membrane-active peptides from methods such as oriented circular dichroism (OCD) [6] and solid state NMR [7,8]. Many of these experiments unfortunately say little about the transition state for pore formation and only give information on the more populous ground states. However, they nevertheless provide a valuable benchmark for comparison to MD simulations.

In this short report, we highlight some of the problems of MD simulations of AMPs and how to avoid them. In particular, we show that the results depend vitally on a correct force field parameter balance and on achieving long enough sampling times. Otherwise, MD can yield results that contradict what has been measured experimentally. We do not perform a generic force field comparison here, but focus exclusively on the subject of peptides in membranes. Ultimately, only a combined experimental/computational approach will allow identifying the specific physicochemical properties that lead to antimicrobial function, and thus allow to predict and to improve the therapeutic impact of new AMP sequences.

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2. Methods

All simulations were performed and analyzed using GROMACS version 4.6.3 (www.gromacs.org) [9] and HIPPO beta (www.biowerkzeug.com), using the CHARMM27 force field [10], the OPLS-AA force field [11], the GROMOS96-53a6 force field [12], and the TIP3P water [13]. CHARMM36 all-atom lipid parameters were used [14], and united atom lipid parameters were taken from Ulmschneider and Ulmschneider [15], and Berger et al. [16]. Electrostatic interactions were computed using particle-mesh-Ewald (PME), and a cut-off of 10 Å was used for the van der Waals interactions. Bonds involving hydrogen atoms were restrained using LINCS [17]. Simulations were run with a 2 fs time-step, and neighbor lists were updated every 5 steps. All simulations were performed in the NPT ensemble, with water, lipids, and the protein coupled separately to a heat bath with T = 35 °C and a time constant $\tau_T = 0.1$ ps using weak temperature coupling [18]. Atmospheric pressure of 1 bar was maintained using weak semi-isotropic pressure coupling with compressibility $\kappa_z = \kappa_{xy} = 4.6 \cdot 10^{-5}$ bar^{-1} and time constant $\tau_P = 1$ ps [19]. All peptides were constructed as ideal α-helices and inserted into a preformed lipid bilayer made up of 58 DMPC lipids and ~30 water molecules per lipids, as described previously [20]. The 1 μs PGLa dimer simulation at 60 °C was performed on the Anton machine at Pittsburg Supercomputing Center, all other simulations (cumulative time of 37 μs) were performed on conventional clusters. For all quantities, the standard deviation of the mean was calculated by block averaging over 10 blocks, and these are plotted as error bars.

To compare with solid state NMR data of a selectively labeled peptide embedded in a macroscopically oriented membrane sample, the corresponding ^{2}H quadrupolar splitting or ^{19}F dipolar coupling was obtained from the simulations by calculating the local bond order parameter S_{CD}:

$$S_{CD} = \left\langle 1/2 \quad 3\cos^2\theta - 1 \right\rangle.$$

Here, the angle θ is between the C_α and C_β bonds of the labeled residue and the membrane normal, which is parallel to the magnetic field (*z*-direction).

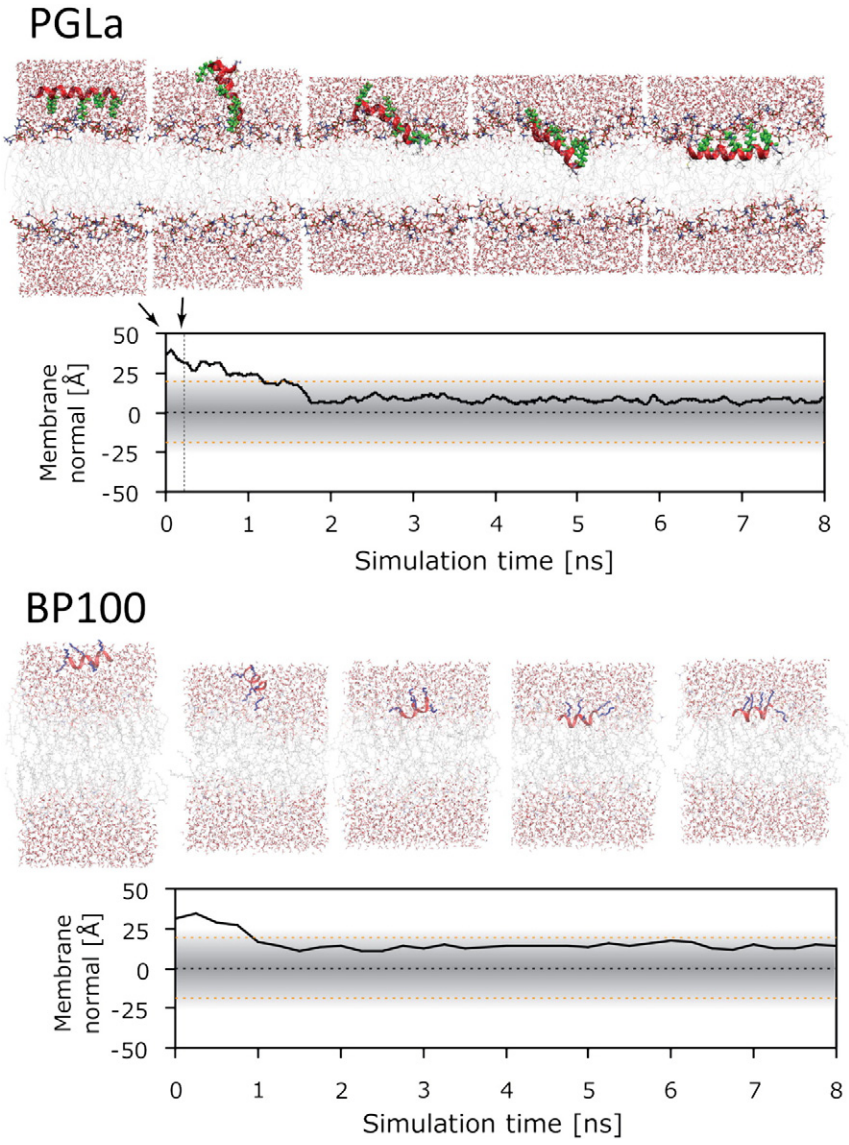

Fig. 1. Two examples of our rapid bilayer insertion simulations, for PGLa (top) and BP100 (bottom). All peptides are initially placed into the solvent, ~40 Å away from the membrane center. The peptides are helically restrained, and the simulation temperature is increased to 90 °C. Rapid insertion is seen during the first 2 ns, and the peptides adopt stable inserted surface (S) states, with the charged sidechains pointing upwards into the bilayer interface. No further transitions occur. The final state of the high-temperature simulations is then used as the starting point for subsequent simulations at room temperature. The approach is completely unbiased, and always yields the same S-state: for example, it plays no role whether the amphipathic peptides initially point their charged sidechains towards the membrane (PGLa simulation, green residues), or away (BP100 simulation, blue residues).