GROMPALA: a membrane-implicit modelling method to screen lipid-interacting molecules

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Abstract

In this chapter, we describe an improved version of our previously published Monte Carlo method IMPALA, based on an implicit description of the membrane. The implementation of the implicit water-membrane forcefield IMPALA into GROMACS molecular dynamics software suite is called GROMPALA. The method aims to decrease computational costs compared to explicit environment representation in MD simulation. We attend to gain a more accurate description as compared to IMPALA by taking advantage of the all-atom molecular dynamics algorithms. GROMPALA is designed to get insight into molecule-membrane interactions taking place on reasonable time scales, notably to screen large sets of peptides, than can serve as primary selection tool for further atomistic molecular dynamics simulations.

Keywords: molecular modelling; amphipathic peptides; implicit membrane; hydrophobicity

Abbreviations: MD: molecular dynamics, MC: Monte Carlo, ASA: accessible surface area, VdW: Van der Waals, MCe: mass centre; GB: generalized Born; MAG: magainin; DDK: Dermadistinctin K; MLT: mellitin; TM: transmembrane

Introduction

Biological life sciences such as pharmacology, toxicology, bioindustry or cosmetology depend on knowledge about how membrane-related metabolism, transport and disruption processes take place. Proteins interacting with the membrane are essential in those phenomena, are present in all cells and represent more than one third of the genome. Understanding protein-membrane interactions is hence of fundamental importance.

Over the last decade, molecular dynamics (MD) has gained attractiveness in that domain. MD is a valuable tool to study interactions between proteins or peptides and membrane because it gives access to the atomistic details of the interaction as well as energetics and dynamics of the observed processes [1]. MD is based on the use of the motion equations of Newton and on a forcefield to simulate how an ensemble of atoms moves relative to each other. Forcefields include potential equations and parameters to reproduce stretching, bending and rotations of covalent bonds, to maintain planarity and chirality of several groups as well as to simulate Van der Waals and electrostatic interactions. The parameters which depend on defining atom types have been calibrated to reproduce a wide range of experimental values. MD studies have proven to be able to reproduce biophysical and biological processes, for solutes in uniform solvent, as well as for membrane environments. However, due to the high computational cost of molecular mechanics simulations using explicit membrane, there is a growing interest for implicit representation of the lipid bilayer.

A wide range of models for the interaction with implicit membranes have been developed and are the object of several reviews [2,3]. These models can be classified as knowledge-based [4–10] or physics-based [11–14]. The former are usually based on experimental data for the free energy of transfer of small molecules, typically amino acids, from water to apolar media, e.g. octanol [5,6,9]. Atomic solvation parameters are derived from these data and they are then often used with atomic solvent accessible surface area and tuned according to the atomic position in a membrane slab to compute the solvation energy of bigger molecules, such as peptides or proteins. The methods give optimal positions for the molecule inside the membrane which will be compared with experimentally known orientation for parametrization. Knowledge-based methods can also be combined with forcefields to include Van der Waals, electrostatic and torsion energy in the energy function and sample the protein conformational space [4,9]. The method presented in this paper is part of these methods, as described further below.

For the physics-based methods, the membrane protein interactions are decomposed into electrostatic and nonpolar contributions. For the electrostatics, a membrane can be approximated as a region with low dielectric constant, in contrast to water which has a high dielectric constant. This can be described with the Poisson-Boltzmann (PB) theory [15]. While several groups have used this model [10,12], it is computationally expensive and difficult to use for MD simulations [16]. Several faster methods have hence been developed [17,18] that mainly use the Generalized Born (GB) approach, which has been first introduced by Still et al [19]. The GB equation is derived from the Born model [20], a solution of the PB equation for a charged spherical solute in a solvent with different dielectric constant. The GB approximation expresses the electrostatic solvation energy for

a set of charged spheres, representing the biomolecule, and accounts for the effect of the dielectric medium on the pairwise interactions of charged particles [19]. The key point is the calculation of the sphere radius, named Born radius, because it depends on the position and volume of all the other atoms in the solute [21]. The Coulomb field approximation is used to compute Born radii [3]. However, as the membrane is modelled as a layer with different dielectric constants, the GB approach needs to be adapted. In the first membrane model based on GB, proposed by Spassov et al [22], the dielectric constant is the same within the membrane and for the protein. A procedure to handle multiple dielectric environments with GB was then proposed by Feig et al.[16]. For the nonpolar contribution, it mainly corresponds to the cost of cavity formation and is usually approximated by a term proportional to the solvent accessible area [2].

In our lab, we have developed a Monte Carlo method using an implicit description of the membrane, called IMPALA [6]. The forcefield was parameterized for mimicking a membrane in aqueous environment by considering 1) the hydrophobic effect between the membrane and a solute and 2) the perturbation effect of the solute on the lipid acyl chain organization. Both energy restraint terms depend on the solvent accessible surface area and a membrane potential which mimics the hydrophilic profile of the lipid bilayer. While being very simplistic, this method notably allowed to accurately study and classify different lipid-interacting peptides [23] and to predict entire membrane protein orientation into lipid bilayers [24,25]. The main limitation of the method resides in the fact that the conformation of the peptide is not modified following its insertion into the implicit membrane.

In this chapter, we describe the implementation of the implicit water-membrane forcefield IMPALA into GROMACS molecular dynamics software suite. We call the resulting hybrid method GROMPALA. The method aims to decrease computational costs compared to explicit environment representation in MD simulation. We attend to gain a more accurate description as compared to IMPALA by taking advantage of the all-atom molecular dynamics algorithms. GROMPALA is designed to get insight into molecule-membrane interactions taking place on reasonable time scales, notably to screen large sets of peptides, than can serve as primary selection tool for further atomistic molecular dynamics simulations.

Description of the GROMPALA methodology

We have implemented the algorithm from Ducarme et al [6] into Gromacs software [26,27]. In the Impala membrane model, two pseudo energy terms are considered: a) the hydrophobic energy restraint (eq.2) and b) the lipid perturbation energy restraint (eq.3). Both terms depend on atomic transfer energy and accessible surface area (ASA). A Monte Carlo (MC) procedure is used to explore optimal insertion configurations. The conformation of the lipid-interacting molecule is considered to be rigid and the membrane hydrophobicity is modelled by an empirically parameterized symmetric sigmoidal curve, C(z) (eq.1):

$$C(z) = 1 - \frac{1}{1 + e^{\acute{a}(z - z_0)}}$$ *(equation 1)*

where α is a constant equal to 1.99; z_0 corresponds to the middle of polar heads and z is the position in the membrane.

The hydrophobicity of the membrane for the interaction is simulated by E_{pho} :

$$E_{pho} = -\sum_{i=1}^{N} S_{(i)} E_{tr(i)} C_{(zi)} \quad \textit{(equation 2)}$$

Where N is the total number of atoms, $S_{(i)}$ the accessible surface to solvent of the i atom, $E_{tr(i)}$ its transfer energy per unit of accessible surface area and $C_{(zi)}$ the $_{zi}$ position of atom i.

The perturbation of the bilayer by insertion of the molecule was simulated by the lipid perturbation restraint (E_{lip}):

$$E_{lip} = Klip * a_{lip} \sum_{i=1}^{N} S_{(i)} \left(1 - C_{(zi)}\right) \quad \textit{(equation 3)}$$

where a_{lip} is an empirical factor fixed at 7.53624 Kj.mol^{-1}Å^{-2} and Klip, a weight factor comprised between 0.1 and 1.

In our Impala implementation to Gromacs (Grompala), the MD routine replaces the original MC approach. We have adapted the values of accessible surface area and VdW radii for each atom type of Grompala. A performance optimized program routine for the calculation of solvent accessible surface areas has been implemented to the Gromacs core program by modifying the Gromacs tool g_sas, based on the DCLM method [27]. After calibration, ASA probe radius was set to 0.115. Both parameters (ASA and VdW radius) were associated to the corresponding atom type definition of the OPLS-AA forcefield [28].The transfer energy values were taken from [29].

Preliminary energy minimization has been carried out in absence of Impala forcefield. The MD part of the simulations was done without periodic boundary conditions at 323 K for 10 ns by steps of 2 fs. A dielectric constant of 1 was used and Coulomb and Van der Waals have been computed without cutoff, since the systems studied are small. Each calculation is repeated 7 times.

To test and calibrate Grompala, we investigated four amphipathic peptides and one transmembrane domain that have been described in the literature for their interaction with lipids, notably by NMR. Concerning the amphipathic helical peptides, Magainin2 (MAG) (PDB ID: 2MAG) and Dermadistinctin K (DDK) (PDB ID: 2JX6) are antimicrobial peptides that have been shown to form amphipathic α-helices oriented parallel to the membrane surface [30,31]; Magainin has been previously used as test peptide for IMPALA [6]. The Influenza hemagglutinin HA2 fusion peptide (PDB ID: 2XKA) has been reported to be a helical hairpin at the lipid/water interface [32]. Mellitin (PDB ID: 2MLT) is a highly hemolytic helical peptide from Bee venom. It has been shown to be able to adopt a wide range of orientations in the membrane, from parallel to the membrane surface to a transmembrane configuration [33–35]. This peculiar behavior has also been observed with IMPALA [6] and is hence a good check for Grompala. To compare those amphipathic helices to transmembrane domains, the M3

segment of the alpha subunit of the nicotic acetylcholine receptor from Torpedo californica has been chosen (PDB ID: 3MRA) [36].

Calibration of Klip

Previously, we observed that the lipid perturbation restraint had to be weighted for some molecules, since in some cases, molecules interacting experimentally with the membrane were unable to insert into the IMPALA bilayer. The weighting factor Klip allows restoring lipid insertion with values varying between 0.1 and 0.8 (unpublished data). For Grompala, we tested Klip values between 0.1 and 0.8 for the different peptides. The best results were obtained with values between 0.45 and 0.65 for all the molecules (data not shown) and we show the results obtained for Klip=0.5 for all the peptides.

Peptide behavior in the Grompala membrane

Different parameters have been analyzed for the five peptides: the conservation of the helical conformation along the simulations, the position of the mass centre (MCe) and the orientation (tilt) of the peptide into the implicit membrane. For the helical conformation calculation, the DSSP method is used [37]; the orientation of the peptide is defined by the axis between the MCe of the 3 first and 3 last Cα of the helical part of the peptide only. For DDK peptide, the tilt was calculated using the 7 first and 7 last atoms, whatever the conformation is (the peptide is mainly destructured-see below).

For the antimicrobial peptides DDK and MAG that have been found experimentally to orient on the surface of the membrane, they are both oriented mostly parallel to the lipid plane with Grompala (Fig. 1A and 2A respectively), with insertion angles around 90° and around 60-70° towards the membrane normal, for DDK (Fig.1D) and MAG respectively (Fig.2D). Both peptides have their mass centre located in the hydrophilic interface, at 11-12 Å from the bilayer centre (Fig. 1C, 2C). This is in very good agreement with the experimental data [30] and with IMPALA calculations (not shown).

The peptide conformation was also followed during the simulation. Figures 1A and C shows that DDK is partly helical (residues 15-21 and 29-32), but the N-terminal part is destructured. For MAG (fig.2A,B), a helical structure is observed at residues 5-11 in few simulations; in the other calculations, the MAG peptide is even more destructured (data not shown).

For mellitin, the results are presented on Fig.3. We can clearly see that the helical structure is pretty well conserved, with the N-terminal part being in turn (fig. 3A,B). As previously observed by Impala [6] and also experimentally [33–35], the insertion angle can adopt a larger array of values, from 100 to 150° (fig.3D), the mass centre being located at the interface between the hydrophobic tails and the hydrophilic lipid headgroups (around 10-11 Å) (fig. 3C).

The HA2 peptide that shows a helical hairpin structure experimentally [32], is oriented parallel to the membrane interface (Fig. 4A) with its mass centre around 12 Å (Fig. 4C), the tilt staying at 90° during the whole simulation

(Fig.4D). The helical structure is preserved for the second helix (residues 16 to 22) (fig. 4B), and the overall hairpin configuration is observed (fig. 4A).

For 3MRA (Fig. 5), the results are in very good agreement with the experimental behavior, i.e. a transmembrane insertion (Fig.5A) that appears in the few first nanoseconds of the simulation (MCe position around 10 Å-fig. 5C and an angle around 180°-Fig. 5D). The helical conformation is preserved along the whole peptide for the whole simulation (fig. 5B).

Conclusions and perspectives

The results of Grompala for the four amphipathic peptides are encouraging, since an interfacial position is predicted for all of them. For mellitin, our results are in agreement with the fact that this peculiar peptide can adopt a wider variety of positions into the membrane, suggesting that our method is adapted to distinguish between strictly interfacial peptides and those having more specific features. It also clearly distinguishes between amphipathic and transmembrane peptides, as shown for 3MRA.

Concerning the structure of the peptide along the simulations, the helical conformation is not as well conserved as it would by with atomistic molecular dynamics approach, especially in the case of DDK and MAG. The former is defined by NMR as a 33-residues helical structure in the presence of DPC micelles, with the 5 N-terminal amino acids being coiled, and with a distorsion around residues 10-16 [30]. In water, the peptide is destructured, as shown by CD measurements [30]. In the Grompala simulations, the 1-14 N-terminal domain is not structured as an helix, and the 22-28 residues are configured as a kink. In the same way, Magainin is relatively destructured as compared to NMR results [31]. This should be due to the fact that the peptide lays in the vacuum when not inserted into the implicit membrane. For some peptides, this could induce a destabilization of the hydrogen bonding maintaining the helical conformation, since both peptides are indeed destructured in water (i.e. not in a hydrophobic medium). When inserted at the interface, the duration of the simulation might not be enough to allow reappearance of helical structure. Future investigation in that direction should help to improve the structural stability of the peptides in the Grompala methodology.

In conclusion, by using the gold standard molecular dynamics approach combined to the implicit membrane representation of our home-designed IMPALA methodology, we built up an original and sufficiently fast method that should help to predict and screen peptide membrane behavior. This could be the starting point to subsequent MD simulations that are more time-consuming.

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Legend of the figures

Figure 1: Grompala simulation of DDK peptide

A. Final position of the peptide into the implicit membrane. The yellow plane represents the centre of the bilayer, the orange plane, the interface between the lipid polar headgroups and the hydrophobic tails and the green plane, the interface between water and lipid polar heads.
B. Evolution of the secondary structure of each residue of the peptide along the simulation (10 ns). Blue: α helix; green: bend, yellow: β structure; white: coil; mauve: 5-helix; grey: 3-helix.
C. Evolution of the mass centre position of the peptide along the simulation. The planes are the same as in A. The bilayer is symmetric and the tichkness is ±18 Å.
D. Evolution of the angle of insertion (°) of the peptide (as defined in the text) along the simulation.

Figure 2: Grompala simulation of MAG peptide-same representation as for Figure 1.

Figure 3: Grompala simulation of MLT peptide-same representation as for Figure 1.

Figure 4: Grompala simulation of HA2 fusion peptide-same representation as for Figure 1.

Figure 5: Grompala simulation of M3 TM segment-same representation as for Figure 1.

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The software GROMPALA is available on request.

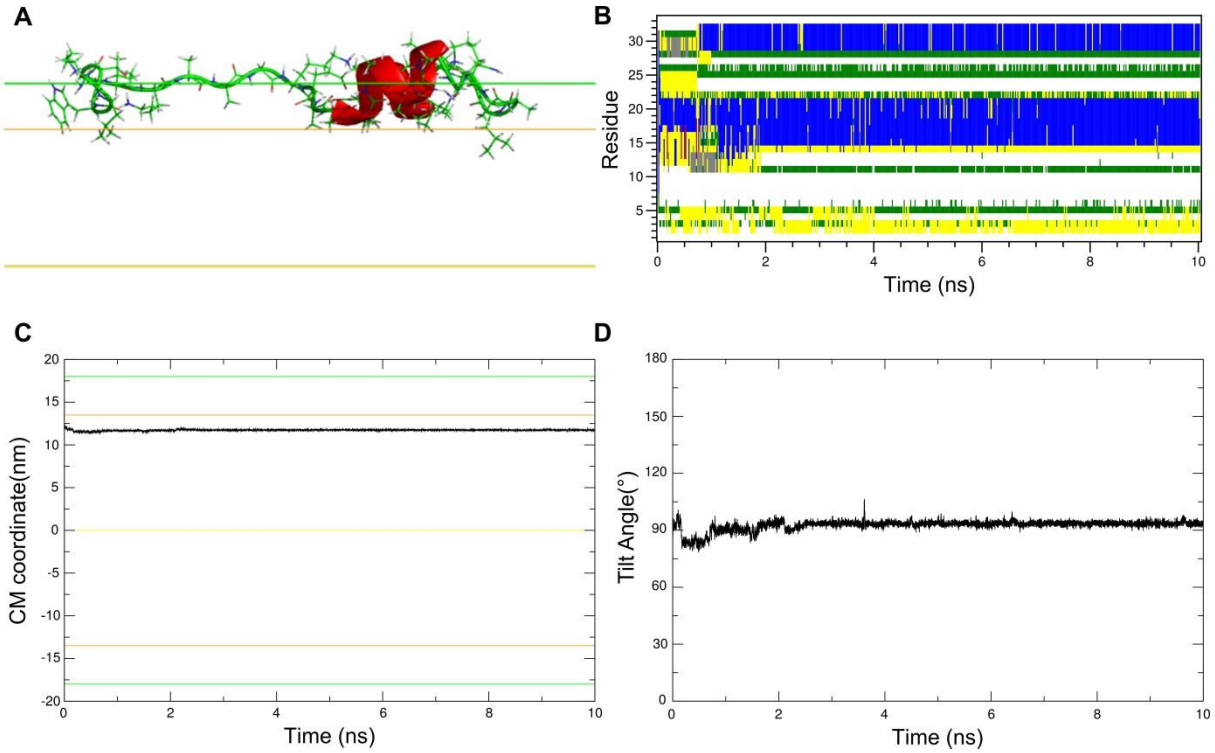

Figure 1

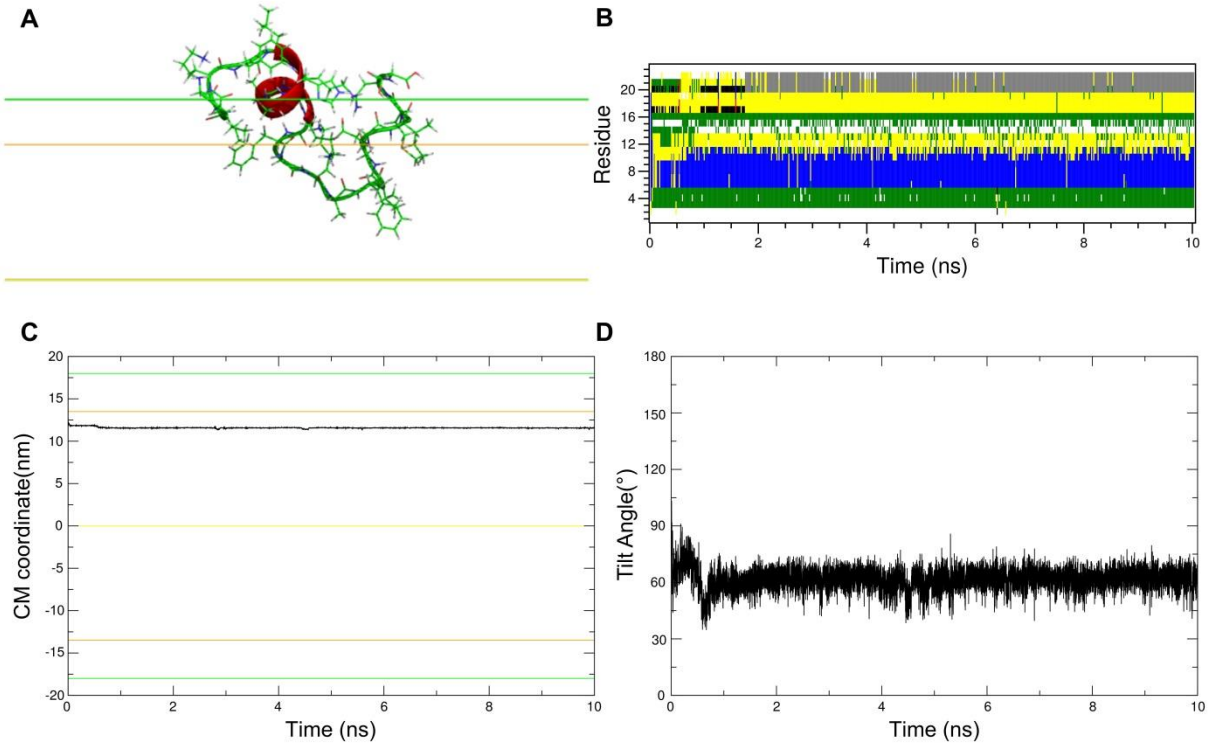

Figure 2

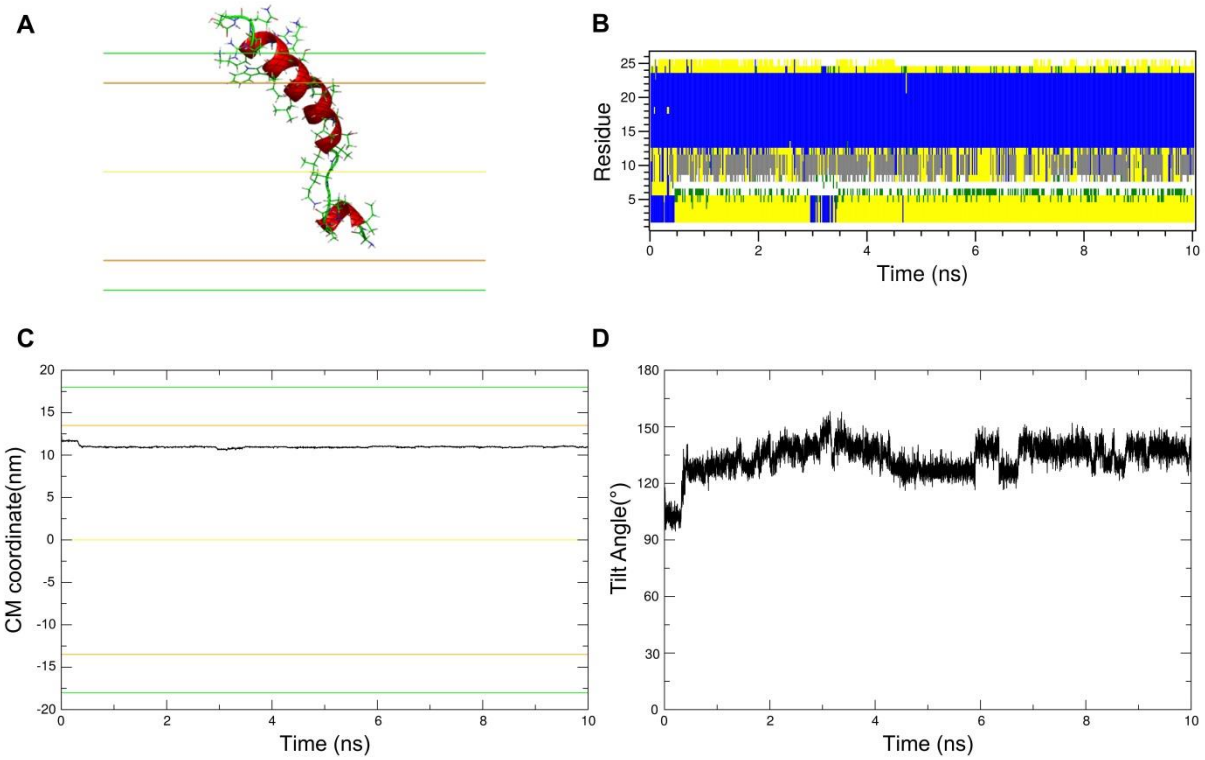

Figure 3

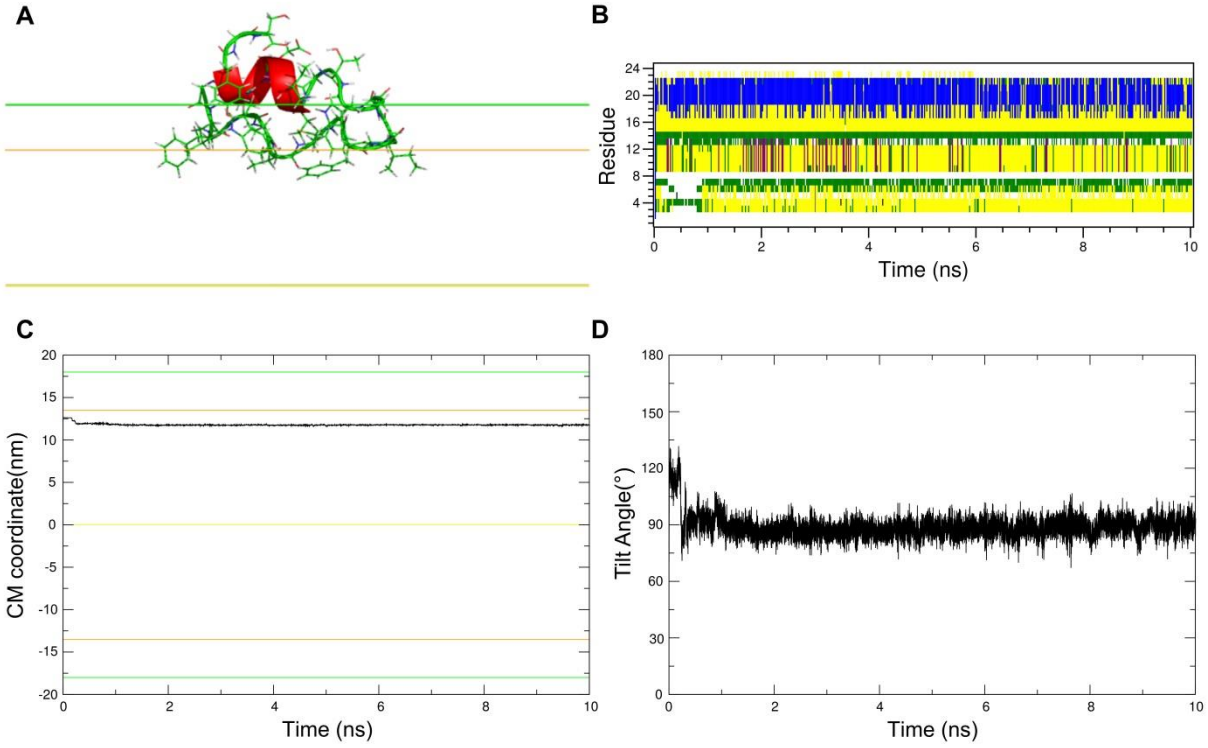

Figure 4

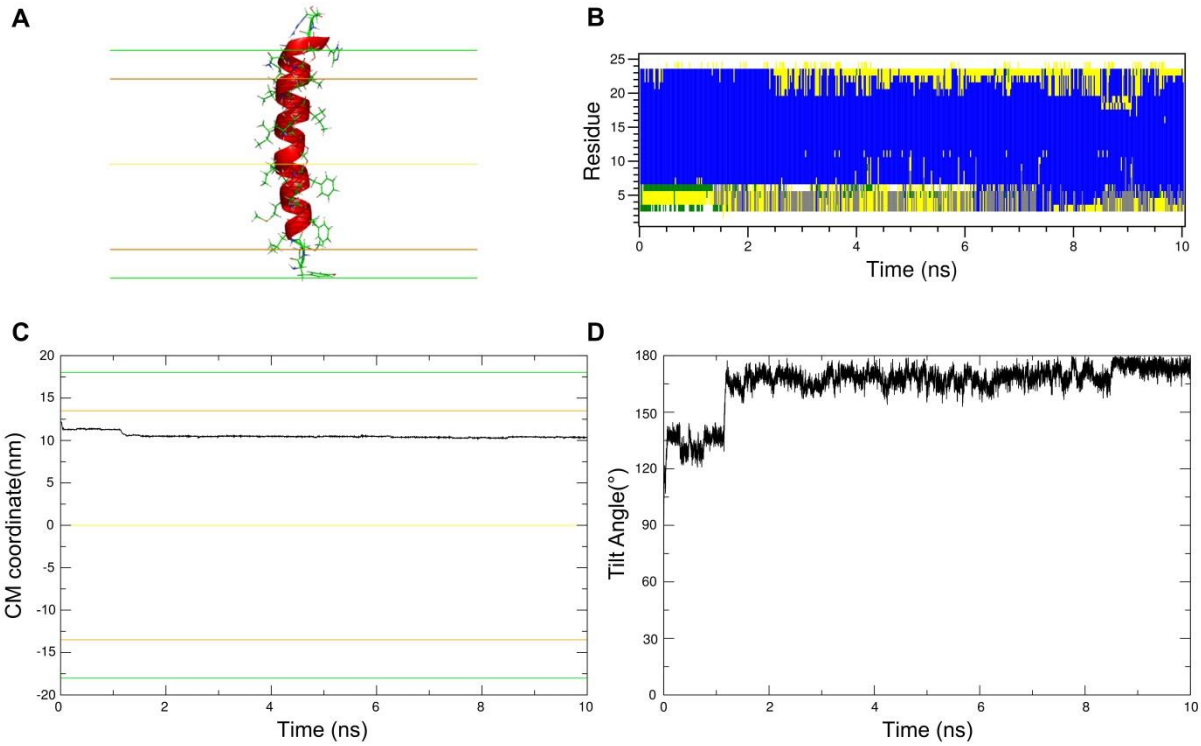
A
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Figure 5