Thesis advisors
Prof. Prabal K. Maiti and Prof. K. Ganapathy Ayappa

Author
Subbarao Kanchi

Abstract

Dendrimers are hyper-branched polymeric nanoparticles with a central core molecule connected by successive dendritic branching layers. The structure controlled parameters of dendrimers such as size, shape, surface chemistry, flexibility and architecture facilitate a wide range of applications in medicine, sensing, catalysis and light harvesting. The understanding of dendrimer interactions with cell membranes and membrane inserted pores formed by pore forming toxins (PFTs) has great importance because of its drug delivery and therapeutic applications respectively. Most drugs are hydrophobic in nature. Thus, the extent of hydrophobicity of the dendrimer core is directly related to its drug encapsulation and retention efficacy. In the first part of this thesis, we carry out fully atomistic molecular dynamics (MD) simulations to characterize the structure of the amine terminated poly (propyl ether imine) (PETIM) dendrimers in solution as a function of dendrimer generation ($G2-G6$) at different protonation levels. Our calculations prove that PETIM dendrimers have relatively greater hydrophobicity and flexibility when compared with their extensively investigated Polyamido-amine (PAMAM) counterparts. Hence, we hypothesize that the PETIM dendrimers have stronger interactions with cell membranes.

In order to understand the microscopic interaction mechanism of the dendrimers with the lipid bilayers we have carried out MD simulations of PAMAM dendrimer-dimyristoylphosphatidylcholine (DMPC) lipid bilayer complex using three different force fields (CHARMM, GAFF and GROMOS) in presence of explicit water. CHARMM and GAFF dendrimers initially interact with the lipid head groups and move away from the lipid bilayer in the course of simulation but in case of GROMOS, the dendrimer is adsorbed on the surface of lipid bilayer and strongly bound to the lipid head group atoms. Potential of the mean force (PMF) profiles of the dendrimer along the bilayer normal shows that there is a repulsive barrier (~ 20 kcal mol^{-1}) between dendrimer and lipid bilayer in case of CHARMM and GAFF force fields but in case of GROMOS force field, the nature of interaction is attractive (~ 40 kcal mol^{-1}). The different behavior of GROMOS dendrimer is attributed to the strong dendrimer-lipid interaction and less surface hydration of dendrimer. We have also studied the effect of solvent models on dendrimer-membrane inter-

actions. We find that the CHARMM dendrimer is strongly bound to the lipid bilayer in presence of implicit solvent (GB), whereas binding is not observed in the presence of an explicit solvent (TIP3P). The opposite nature of dendrimer behavior in the presence of explicit and implicit solvents demonstrate that hydration effects are very important in capturing the dendrimer-lipid interaction and make a case for refinement of the existing dendrimer/lipid FF.

Designing effective nanoscale blockers for membrane inserted pores formed by pore forming toxins, which are expressed by several virulent bacterial strains, on a target cell membrane is a challenging and active area of research. Here we demonstrate that PAMAM dendrimers can act as effective pH controlled gating devices, once the pore has been formed. We have used fully atomistic MD simulations to characterize the cytolysin A (ClyA) protein pores modified with fifth generation (G5) PAMAM dendrimers. Our results show that the PAMAM dendrimer, in either its protonated (P) or non-protonated (NP) states can spontaneously enter the protein lumen. Protonated dendrimers interact strongly with the negatively charged protein pore lumen. As a consequence, P dendrimers assume a more expanded configuration efficiently blocking the pore when compared with the more compact configuration adopted by the neutral NP dendrimers creating a greater void space for the passage of water and ions. To quantify the effective blockage of the protein pore, we have calculated the pore conductance as well as the residence times by applying a weak force on the ions/water. Ionic currents are reduced by 91% for the P dendrimers and 31% for the NP dendrimers. The preferential binding of Cl^- counter ions to the P dendrimer creates a zone of high Cl^- concentration in the vicinity of the internalized dendrimer and a high concentration of K^+ ions in the transmembrane region of the pore lumen. In addition to steric effects, this induced charge segregation for the P dendrimer effectively blocks ionic transport through the pore. The binding free energies of NP dendrimers ($\sim$ 200 kcal mol^{-1}) are found to be almost double when compared with P dendrimers ($\sim$ 100 kcal mol^{-1}). Our investigation shows that the bio-compatible PAMAM dendrimers can potentially be used to develop therapeutic protocols based on the pH sensitive gating of pores formed by pore forming toxins to mitigate bacterial infections.