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Computer Simulation Studies on Passive Recruitment Dynamics of Lipids Induced by the Adsorption of Charged Nanoparticles

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1 Abstract

The recruitment dynamics of lipids on the biomembrane is believed to play an important role on a variety of cellular processes. In this work, we investigate the nanoparticle-induced recruitment dynamics of lipids in the heterogeneous phospholipid bilayers of distearoyl-phosphatidylcholine (DSPC) and dioleoyl-phosphatidylglycerol (DOPG) via coarse-grained molecular dynamics simulations. Three dynamic modes of individual charged DOPG lipid molecules in the recruitment process have been taken into account: lateral diffusion, protrusions, and flip-flops. Based on analysis of the mobility pattern of lipids, structural variations of the membrane as well as activation energy of the structure of lipid eyelids characterized as potential of mean force, we have concluded that the electrostatic attraction of nanoparticle plays a crucial role in the recruitment process of lipids in phospholipid bilayers. These studies are consistent with experimental observations and to some extent give insight into the origin of some cellular processes such as signaling, formation of lipid rafts, and endocytosis.

2 Introduction

The recruitment dynamics of lipids on the biomembrane is believed to play an important role on a variety of cellular processes such as signaling, formation of lipid rafts, and endocytosis[1, 2]. Knowledge of the mobility of lipids in the recruitment process is an issue of biological and technological importance. Lipid membranes are not static, homogeneous structures, but present a great deal of movement modes for the individual lipid molecules in the bilayer, including (i) in-plane translational motion of lipids (diffusion)[3, 4], (ii) out-of-plane translational motion of lipids (protrusions)[5, 6], and (iii) transverse motion

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of lipids from one leaflet of the bilayer to the other (flip-flops)[7]. The biomembrane is in its fluid state in the physiological environment, and therefore the dynamics of lipids can be mediated by the some external stimulation such as the adsorption of charged nanoparticles. As a result, the electrostatic attraction will naturally have a significant impact on the mobility of lipids in the membrane and the structure of the bilayer itself as well, which would contribute to the lipids recruitment[8] and surface reconstruction of the biomembranes[4]. Despite the vast amount of research effort in the last decade, it is still difficult to assess the charge effects on the dynamics of lipids and the cell uptake of charged nanoparticles in most of the existing studies. The underlying organizing principles of the lipids recruitment dynamics also awaits consensus.

Lateral motions of lipids in the membrane are believed to have an effect on the structure and function of membrane by contributing to the assembly of lipid-proteins complexes, and affect the endocytosis process of cells[9, 10]. It is well known that the lateral flow patterns of lipids would be strongly affected by the interactions of charged objects such as binding proteins and nanoparticles. Such electrostatic attraction would result in the lipid organization, the domain formation and the control of membrane binding[11, 12, 13]. Previous researches have reported that charged nanoparticles could induce local lipids clustering in lipid bilayers. The local surface reconstruction of the lipid bilayer has been observed upon the adsorption of charged nanoparticles on biomembranes in the experiment[4] and computational simulations respectively[14]. However, It is merely a local phenomenon which is due to the changes of tilt ordering and conformation of lipids mediated by the adsorption of nanoparticles. This behavior of lipids clustering doesn't refer to the diffusion of lipids. Recently, some research evidences based on the finite element method have suggested that the interaction of charged nanoparticles affect the mobility patterns of lipids in the membrane. Lateral diffusion of charged lipids was found to be mediated by the nonspecific electrostatic interactions, which could induce the local aggregation of lipids in the membrane[8, 15]. Nevertheless, the microscopic lipids dynamics in the lateral diffusion is still far from well understood.

Besides the effect on the lateral diffusion of lipids, the electrostatic interaction would lead to the protrusions of lipids out of the membrane plane, which is regarded as a precursor of membrane fusion and fission[16]. When nanoparticles adsorbs on the membrane, the electrostatic attraction stimulates the lipids at sites of nanoparticles localization to be protrusive towards the nanoparticles[6]. The effect of protrusions of lipids results in two kinds of changes of the membrane. On the one hand, the collective motions of individual lipids brings about the bending of the membrane to wrap the nanoparticles, which is related to the cell behavior of endocytosis[17, 14]. On the other, the effect due to the lipids protrusions also reflects on the formation of transient defects in lipid membranes. The adsorbed nanoparticle disturbs the membrane, indicating that the effect changes the equilibrium state of the biomembrane. Consequently, the protrusions of lipids increase

the probabilities of defects formation in the outer leaflet of the lipid bilayer and of entry of water molecules into the interior of the membrane[18]. Such perturbation of membrane due to the adsorption of charged nanoparticles is a common phenomenon, which could evolve to a water pore in the membrane and thereby compromise the membrane integrity[19].

Since the electrostatic attraction is a long-range force, the attraction of charged nanoparticles not only promotes the protrusions of lipids in the outer leaflet of the lipid bilayer, but also increases the probability of transverses of lipids towards the nanoparticles in the inner leaflet of the membrane. The slow rate of flip-flop in the lipid bilayer makes it hardly being investigated via molecular dynamics simulations[7]. Yet the participation of nanoparticles would facilitate this process. The flip-flop of lipids have been found in the solid nanoparticle permeation[20] and in the interaction between the dendrimer nanoparticle with the membrane[21], implying the possibility of a flip-flop would also exist in the lipid recruitment due to the electrostatic attraction of charged nanoparticle.

Motivated by the above problems, the specific objective in this paper is to investigate the mobility of charged lipids in the nanoparticle-induced recruitment process including the dynamics modes of lateral motion, protrusion, and flip-flops. A positive charged nanoparticle and a heterogeneous lipid bilayer were modeled, and several coarse-grained molecular dynamics simulations were performed. As a result, some trajectories analysis of the mobility of individual lipids, structure variations of lipid bilayers as well as the free energy profiles of the lipids protrusion were obtained to shed light on the mechanism of a nanoparticle-induced passive recruitment dynamics of lipids on the membrane.

3 Method

Molecular dynamics simulations techniques provide some intuitionistic information for these microscale studies. In consideration of a relatively large length and time scales of the biological phenomenon in the simulations, the MARTINI coarse-grained (CG) lipid model was employed for its ability to reproduce experimental properties of various lipid assemblies[22, 23]. The CG model includes four main types of interaction sites: polar (P), nonpolar (N), apolar (C), and charged (Q). For particles of type N and Q four subtypes (0, d, a, and da) are further distinguished to allow fine-tuning of the Lennard-Jones (LJ) interactions, reflecting hydrogen-bonding capabilities.

A solid, nearly spherical nanoparticle of $\sim$6.8 nm was built as a hydrophilic, positively charged one, which consists of two kinds of beads, charged (Qd) beads for the charged surface part of the nanoparticle and non-polar (N0) beads for the inner part. The beads comprising a single nanoparticle are constrained to move as a rigid body. The discrete surface charges of nanoparticles were modeled in terms of the nature of biomolecules and

those nanoparticles of surface functionalization. And a positively charged nanoparticle of the surface charges density ($\rho_e = 1.8\,\mathrm{e/nm^2}$) was considered in the simulations.

A cubic periodic box with cell dimensions of $51.2 \times 48.8 \times 22.1$ nm^3 was constructed at the origin, containing ∼400000 CG water molecules and a patch of CG biomembrane. Here the standard MARTINI CG water model in the MARTINI force field was used in the simulation. The MARTINI CG water beads were modeled as one neutral bead representing four all-atom water molecules. Therefore the polarity of water molecules and their electrostatic interaction with the charged nanoparticles wasn't considered in the simulation. The biomembrane model in the simulations consists of two kinds of lipid species: distearoyl-phosphatidylcholine (DSPC), which is a neutral-charged phospholipid; and dioleoyl-phosphatidylglycerol (DOPG), a negatively charged phospholipid. The lipid bilayer contains 7200 lipids with a 5:1 molar mixture of DSPC and DOPG which were uniformly mixed together. After energy minimization, equilibration runs have been performed for 40 ns with a time step of 40 fs. The final equilibrium configuration was used as the starting state for the next simulation with nanoparticles participation (see Supplementary Information, Section 1).

A positively charged nanoparticle was then added into the system. The final equilibrated configuration included a patch of heterogeneous biomembrane, a charged nanoparticle and CG water beads in a box with dimensions of $51.1 \times 48.9 \times 22.1$ nm^3. Some Na^+ ions as counterion were added to neutralize the system. To facilitate the following discussion, we defined the midplane of the lipid bilayer as the x–y plane and introduced the z–axis which is perpendicular to the bilayer surface. The leaflet of membrane near to the nanoparticle was defined as outer leaflet and the other leaflet far from the nanoparticle as inner leaflet. A Berendsen thermostat/barostat was used in the simulations[24] to maintain a constant temperature (T) of 325 K and a constant pressure (p) of 1 atm in the NpT ensemble for mimicing biological conditions. Electrostatic interactions were calculated using the particlemesh Ewald (PME) summation method, with a real-space cutoff of 1.2 nm, a grid spacing of 0.12 nm, and fourth-order interpolation. A cutoff of 1.2 nm was used for van der Waals interactions, and the Lennard-Jones potential was smoothly shifted to zero between 0.9 and 1.2 nm to reduce the cutoff noise. A timestep of 30 fs was used for integrating the equations of motion and the simulation time was $\sim 1\ \mu$s. All the simulations were performed with the GROMACS 4.0.7 simulation package[25].

4 Results and Discussion

The recruitment process of lipids on the membrane induced by the adsorbed nanoparticle is revealed by a series of trajectory snapshots during the simulation, shown in Figure 1. In the initial configuration, the charged nanoparticle suspends in the water. Driven by the electrostatic attraction, the nanoparticle adsorbs on the membrane. The adsorption of a

nanoparticle deforms the membrane, recruits the counter-charged lipids clustering at the site of its localization, and results in membrane bending to wrap the nanoparticle partly. Stimulated by the adsorption of nanoparticles, lipids in the edge of the wrap package become more protrusive; and a lipid "eyelid" comes into being and spreads across to seal the nanoparticle entirely into the lipid package. Even though the adsorption of the nanoparticle intensifies lipids fluctuation and enhances the membrane undulations, no water pore in the membrane is found in the simulation, indicating that the adsorption of nanoparticle does not compromise the membrane integrity.

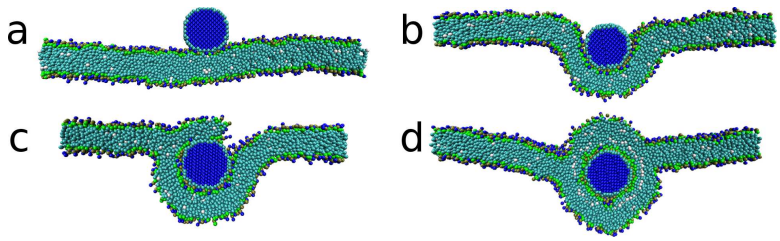

Figure 1: Trajectory snapshots of the lipids recruitment simulation in stereo views: (a) the nanoparticle adsorbs on the membrane, (b) the nanoparticle is partly wrapped by the membrane, (c) a lipid "eyelid" appears and spreads to cover the nanoparticle, and (d) the nanoparticle is fully wrapped by the membrane. A blue bead cluster represents the nanoparticle; cyan beads and green beads represent the lipid bilayer. The explicit water molecules are omitted for clarity. The images are created with VMD[26].

The dynamics of the biomembrane depends significantly on the response of lipids in the membrane to the interaction of charged nanoparticles. In the following, we consider three typical dynamic modes of individual lipid molecules: lateral translational motion in the bilayer, protrusions out of the bilayer, and transverse from one leaflet of the bilayer to the other. Several dynamics properties have been analyzed including the movement pattern of lipids, structural variations of the membrane as well as activation energy of the lipid "eyelid" characterized by free energy changes profiles.

4.1 Lateral Diffusion

The random lateral motion of lipids can be affected by the electrostatic interactions, which would have a influence on the local aggregation and organization of lipids in the membrane[8]. To gain insight into the induced diffusion of charged lipids on the membrane, we inspected the lateral motion trajectories of individual charged lipids DOPGs in the simulation. Here, the normal projected position of center of mass (COM) of DOPGs in the x–y plane can be calculated to reflect the lateral movement of DOPGs. The positions of DOPGs in the starting configuration are regarded as the reference. And the changes of the projected position of DOPGs relative to the reference positions over time are shown as vectors in Figure 2, where the direction of the vector denotes the direc-

tion of the motion of a corresponding DOPG molecule, and the length of the vector, the displacement of the motion of a DOPG.

Recent researches reported that the long-range diffusion of lipid molecules were collective in nature which could be characterized as a flow-like behavior[9]. For evaluating the effectiveness of the MARTINI coarse-grained force field, the flow patterns of DOPGs in absence of charged nanoparticles have been obtained as a negative control case via coarse-grained molecular dynamics simulations. It is found that the in-plane motion trajectory of charged lipids yields a type of collective dynamics in spite of some local disorder flows in the plots (Details can be found in Supplementary Information, Section 2). With the sampling time intervals increase, the trajectories of DOPGs gradually present a 2D streaming pattern or flow field, illustrating the presence of concerted motions of lipids at least over tens of nanometers. These results are consistent with the recent findings of a previous molecular dynamics simulations of flow-like motions of lipids as the means of long-range collective dynamics at a nanometer scale in the membranes[27].

Then, the plots of flow pattern of lipids in presence of the nanoparticle have been obtained shown in Figure 2. Insets in Figure 2 show the lateral displacements of DOPG lipids in the outer leaflet at different time points of 5, 25, 40, and 60 ns with respect to the reference state of DOPGs. The similar collective motion of DOPGs can also be found in the presents of charged nanoparticles, where the movement of charged lipids yields a flow-like pattern in some regions far from the localization of the nanoparticle, shown in Figure 2.

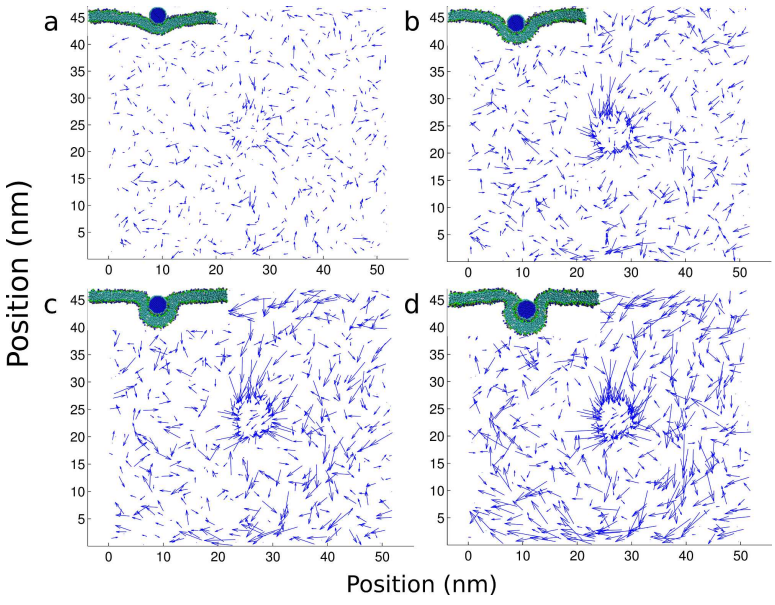

Figure 2: Lateral displacements of DOPGs in the outer leaflet at different times in the trajectory. Panels (a-d) show the data at time of 5, 20, 40, 60 ns with respect to the reference time respectively.

Besides the free flow-like collective dynamics of lipids, the oriented diffusion of DOPG

molecules towards the localization of the nanoparticle is also revealed by tracking motion of DOPGs in the simulation. In contrast with the free lateral diffusion of lipids far from the nanoparticle, the motion behavior of lipids around the nanoparticle presents more directionality under the external stimulations of the adsorption of nanoparticles. The electrostatics attraction of a charged nanoparticle not only promotes the clustering of DOPGs around the nanoparticle (shown in Figure 3), but also recruits the aggregation of DOPGs around the rim of the wrap package (shown in Figure 4). Different from the local clustering of lipids due to the changes of tilt ordering reported in previous works[4, 14], here the aggregation of lipids around the nanoparticle results from the lateral diffusion of lipids in the membrane.

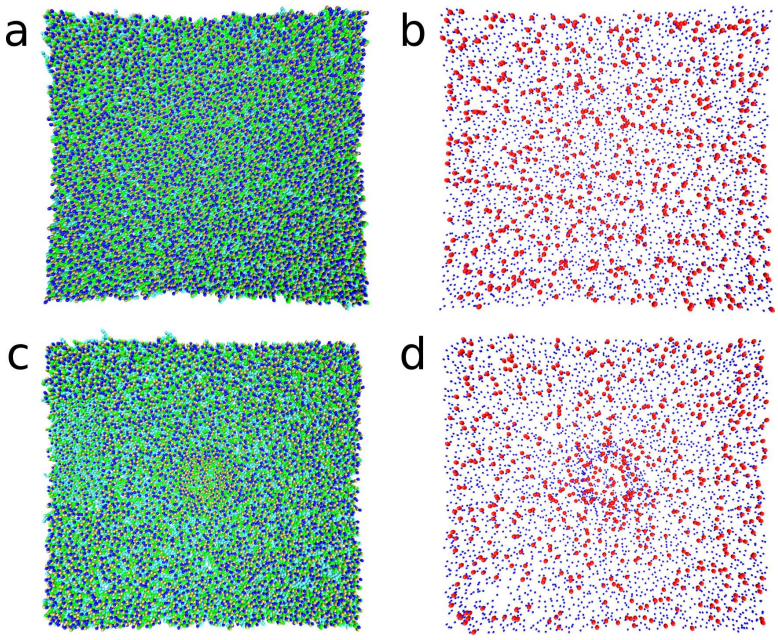

Figure 3: The clustering of DOPGs around the nanoparticle. Inset (a) and (c) represent the configure of the lipid bilayer before and after the recruitment of lipids respectively. Insets (b) and (d) depict the distribution of lipids' headgroups corresponding to the inset (a) and (c), where red dots stand for the position of DOPGs and blue dots, DSPCs.

The co-existence of two kinds of lateral motion of DOPGs in the membrane attributes to the electrostatic attraction of charged nanoparticles. The electrostatic attraction between the positively charged nanoparticle and the negatively charged DOPGs is a long-range force which certainly recruits the DOPGs diffuse orientedly towards the nanoparticle. Nevertheless, it appears that charged lipids around the nanoparticle does not move strictly along the direction from their original position to the nanoparticle. These phenomena could result from the correlated mobility of lipids on the membrane. The fact of collective lipids motions in nature suggests that the movement of individual DOPG molecule is strongly correlated with those of its neighbors' of DSPCs. The electrostatic attraction between the neutral-charged DSPC molecules and the nanoparticle is weaker than that between the negatively charged DOPGs and the nanoparticle, implicating that

the adsorption of the nanoparticle have an weaker influence on the flow motion of the DSPCs than that of DOPGs. Therefore the flow-like behavior of lipids affects the oriented diffusion of DOPGs to the nanoparticle. Concerning two motion modes of DOPGs coexisting in the simulation, here two factors might help us explain the difference between flow-like motions of DOPGs far from the nanoparticle and oriented motion of DOPGs near the nanoparticle: (i) the electrostatic attraction decreases with the increasing distance between the nanoparticle and lipids; (ii) those bending lipid bilayer, which are tightly bound to the surface of NPs, screen the range of electrostatic interactions and weaken the attraction of nanoparticle to the DOPGs in the distance. These factors would contribute to oriented movement of DOPGs that near the nanoparticle whereas free lateral diffusion of those DOPGs that far from the nanoparticles.

4.2 Protrusion and Wrap

The adsorption of charged nanoparticles induces the membrane bending. The membrane deforms itself in order to increase the amount of its surface area that adheres to the particle, which would result in the wrap of the membrane[28, 17]. A series of images for the wrap process of the nanoparticle are shown in Figure 1. The wrap of the membrane is driven by the increase in adhesion energy with the nanoparticle, and is opposed by the increase in bending energy of membrane[29, 17]. At the beginning of the adsorption of the nanoparticle, the regime of membrane wrap driven by the adhesion energy relies on a small-gradient expansion of the elastic energy. When the nanoparticle is almost completely covered, there is a noticeable sharp curvature change of the membrane around the unwrapped portion of the nanoparticle surface, indicating the severely changes of two principle curvatures form in the neck region of the lipid bilayer. As a result, the bending stiffness prevents any "kink" in the membrane profile at the line of contact, which might suppress the wrap further[17, 30]. These phenomenon are line with our previous work of the homogeneous membrane system that a similar narrow neck of the membrane due to the adhesion energy of the nanoparticle was stable to hinder the membrane wrap in a long simulation of the interaction between the neutral-charged lipids and the charged nanoparticle[14].

Besides being fluid, membranes are also soft structures. When the neck is formed, the membrane cannot bend further to wrap the nanoparticle in the simulations. Instead, the membrane forms a close hairpin structure like a lipid "eyelid" to cover the remaining surface of the particle shown in Figure 4. The similar structure of lipid eyelid has been reported in the work of Smith and coauthors[29]. The lipid eyelid is still a bilayer structure where the lipids come from the outer leaflet of the membrane, and it is due to protrusions of the thermally excited lipids out of the bilayer stimulated by the adhesion energy of the nanoparticle. Shown in Figure 4, the protrusion of the DOPGs cluster can be found in the lipids eyelid spreading from the side of the neck of the wrap package. The lipid

eyelid spreads across the remaining portion of the particle and fuses with the other side of the neck to complete the wrap of the nanoparticle.

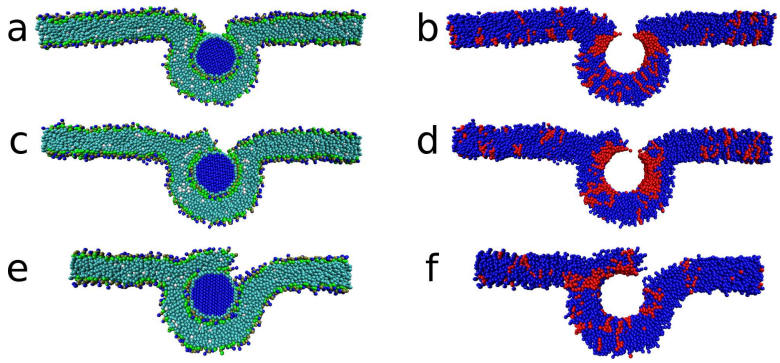

Figure 4: The evolution of a lipid eyelid from the protrusion of DOPGs, (a) the partially wrap of the nanoparticle, (c) the protrusion of lipids budding from the rim of the wrap package, and (e) a lipid eyelid spreading to cover the nanoparticle. Inset (b), (d) and (f) represents side view of inset (a), (c) and (f) respectively, in which red lipids representing DOPGs and blue lipids, DSPCs.

The emergence of the lipid eyelid changes the original structure of the lipid bilayer, which plays a vital role for the fully wrap of the nanoparticle. Since the formation of a lipid eyelid depends on the protrusion of lipids, subsequent energy gain or loss due to the protrusion of DOPGs should be taken into account as explanations. However, the nanoparticle-induced deformation is so complicated involving lipids clustering and protrusions and membrane bending etc, that it is difficult to render a clear extraction of underlying physical principles directly in normal simulations. For simplifying the problems, the "activation" energy of a lipid eyelid can be introduced to reflect the energetic cost of DOPGs clusters transiting from the state of protrusions to a structure of a lipid eyelid. Here the free energy changes of the lipid clusters pulled artificially from the membrane to the water phase have been calculated to investigate the contribution of lipids protrusion by excluding mentioned complications arising from the high complexity of the morphology changes of membranes due to nanoparticles adsorption. Umbrella sampling approach was employed to obtain the potential of mean force (PMF)[31] as a energetic function of the distance between the pulled lipids and the center of the membrane. Because the headgroups of lipids are defined as the pulled group which lie at the surface the membrane, the initial position of PMFs begins at the 2.37 nm in the reaction coordinates which is the distance from the headgroups of restrained lipids to the center of the lipid bilayer. A series of separate biased simulations were performed for 6 ns per simulation window in which the headgroups of lipids were restrained to a given distance from the center of the bilayer by a harmonic restraint on the z-coordinate only. A force constant of 2500 kJ mol^{-1} nm^{-2} was used with a spacing of 0.1 nm between the centers of the biasing potentials to ensure the overlapping of the z histograms between adjacent umbrella sampling windows (see Supplementary Information, Section 3). After the

restrained simulations were completed, the unbiased PMF was obtained by using the weighted histogram analysis method[32].

The lipid cluster has been pulled form the membrane to the water in the biased simulation. The tails of lipids are hydrophobic so that lipids prefer remaining in the hydrophobic inner layer of the membrane to being pulled into the water. The pulling of a lipids cluster enhances the protrusion of its neighboring lipids out of the membrane to preserve the hydrophobic microenvironment, which leads to an obvious energy barrier in the pulling process. Shown in Figure 5, the level of energy barrier seems relevant to the number of pulled lipids. Two kinds of trends of PMF profiles have been obtained for different number of retrained lipids, implying that the number of protrusive lipids could be a key factor for the formation of a lipid eyelid. In the case of 1 and 4 lipids in the pulled group in the Figure 5 (a) and (b), the PMF profiles rise smoothly at the beginning of the pulling process because most of the hydrophobic parts of lipid tails are still in the membrane. And then, the PMF profiles show a steep upward trend. Such the rising energy barrier originates partly from the repulsion between the hydrophobic tails of lipids and the water, and partly from the elasticity energy of the membrane deformation due to the protrusion of the lipid cluster. The more the restrained lipids are pulled, the more neighboring lipids are stimulated; consequently the higher the energy barrier is. Yet, the hydrophobic interaction between the pulled lipids and those neighboring lipids is too weak to affect the membrane morphology significantly. As being pulled in a certain position, the retrained lipids are exposed in the water. Those protrusive neighboring lipids draw back into the bilayer and the lipid bilayer recovers its equilibrium state of a flat configuration again. Hence the PMF profiles drop steeply when moving lipids from the bilayer into the water phase.

The other tendency of PMF profiles has been found in the case of 10 and 20 lipids in the pulled group in the Figure 5 (c) and (d). There are two stages of the PMF profile increases in the process of pulling lipids. (i) In the first stage, the changing trend of PMFs at the beginning of the pulling process is roughly the same as the case of 1 and 4 lipids, but the changing extent is quite different. With the increasing number of pulled lipids, more and more neighboring lipids are involved protruding to preserve the hydrophobic microenvironment of those pulled lipids, which deform the topology of membrane severely. As a result, the PMF profiles rise steeply due to the deformation of the membrane morphology. Instead of being exposed in the water, the pulling of restrained lipids compels the membrane bending and enhances the protrusion of neighboring lipids. Therefore a lipid tether of a closed loop structure comes into being at last. There is another pulled lipid tether structure reported in the work of Baoukina and his coauthors, in which their lipid tether presents a high curvature form of the lipid bilayer[33]. Different from their pulled lipid tether, the lipid eyelid here is a close loop budding structure in the lipid bilayer, where most of lipids come from one leaflet of the membrane. The lipid eyelid of

a growing budding structure prevents the exposure of hydrophobic tails of pulled lipids in the water[29]. (ii) In the second stage, the growth of PMFs becomes slow when the restrained lipids are pulled to a certain position. The inflection point in the PMF profiles can be identified as the point of activation energy which represents the formation of a lipid eyelid from the biomembrane. Although the values of activation energy of a lipid eyelid vary in the case of 10 or 20 lipids, the PMFs show that the inflection point of PMF profiles always appears at a relatively constant distance of ∼7.5 nm, which should be the critical distance of the structural transition from the protrusion of lipids to a closed loop budding structure in the biomembrane. The upward tendency of PMFs becomes slow once a lipid eyelid forming from the biomembrane, suggesting that the formation of lipids eyelids could relieve the elasticity energy cost of the membrane deformation to some extent.

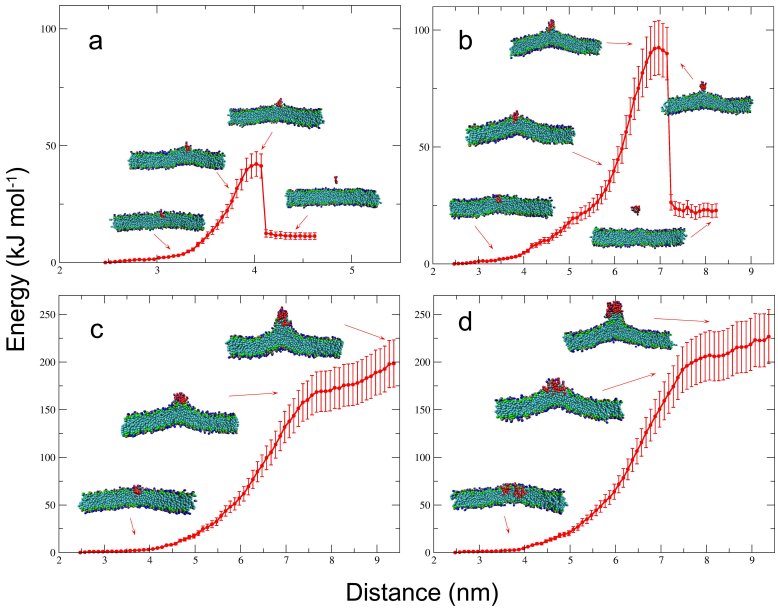

Figure 5: PMFs of a lipids cluster as a function of its distance from the midplane of the DSPC bilayer, estimated at a force constant of 2500 kJ mol^{-1} nm^{-2}. Inset images (a-d) are snapshots of the protrusion of the lipids cluster consisting of 1, 4, 10, and 20 lipids respectively, in which red lipids representing a pulled lipids cluster.

Another event occurred in our simulations is that the lipid eyelid always emerges from one side of the package, and then grows across the remaining particle surface to the other side of the package (shown in Figure 4). No more than one lipid eyelid appears in the simulations. We witness this fact by repeating our simulations several times under the same conditions. The pulling of lipids would induce the deformation of the membrane morphology severely. In the case of adsorption of charged nanoparticles, the protrusions of lipids are driven by the adhesion energy of nanoparticles but suffer from the elasticity energy penalty of the membrane deformation. When the protrusive positions of lipids are restrained to be above a threshold value, the lipid cluster would transit from the status of a protrusion to the closed loop structure of a lipid eyelid. This transition preserves

the hydrophobic microenvironment of biomembranes effectively, which would moderate to some extent the elasticity energy cost of the membrane deformation against the gain of the adhesion energy as well. As a result, the spread of a lipid eyelid would relieve the bending strain of a high curvature form in the neck region and consequently suppress other lipid eyelids' formation as well. However, since the formation of a lipid eyelid is mainly driven by the electrostatic attraction of nanoparticles, we speculate that it is in all probability for the formation of several lipid eyelids stimulated by a nanoparticle of larger surface charge densities to its engulfment; no quantitative explanation is presented at this time.

4.3 Flip-Flops

Another important issue addressed here is the flip-flop behavior of individual lipids induced by the NP interactions. Besides the dynamics modes of the lateral diffusion in the membrane and the protrusion out of the lipid bilayer, there is another motion mode of lipids exists in the membrane, named as flip-flop, where the lipids transverse from one leaflet of the membrane to the other[7]. As mentioned above, the movement of charged lipids could be affected by the electrostatic attraction of charged nanoparticles. We also found the flip-flop behavior of a DOPG lipid in the wrap process induced by the adsorption of a nanoparticle. The trajectories of simulations reveal that the mechanism of flip-flops turns out to be five-steps: (a) the adsorption of nanoparticles makes the bending of the membrane and promotes the formation of high curvatures of membrane form in the neck region of the wrap package. As the electrostatic attraction is a long-range interaction, the adsorption enhances not only the protrusion of lipids in the outer leaflet but also motions as opposed protrusion of lipids in the inner leaflet; (b) the induced reversed protrusions of lipids in the inner leaflet aggravate the membrane's undulation. The lipids are compact with rapidly changing tail orientations, which makes the membrane thinning and bring about defects in the inner leaflet of the membrane consequently; (c) the electrostatic attraction evokes tilts of lipids to the defects, desorption of its headgroup from its native membrane leaflet and entry into the center of the lipid bilayer further. The transient defects in our simulations are quite different from that reported by Song[20]. These defects can recover itself after the desorption of lipids from the its native membrane rather than evolving into a hydrophilic pore; (d) the orientation of lipids' headgroups tilts towards the outer leaflet in the center of the membrane; (e) the inverted lipids transverses from inner leaflet towards the other by reorientation of the tails which are then accommodated in the outer leaflet and takes part in the protrusion of lipids in the neck of the wrap package. Among these five phases, the phase (c) and (d) stay totally for $\sim$3 ns, which doesn't induce the defects evolving into a hydrophilic pore which occurred in the simulations of Tieleman and coauthors[7]. The flip-flop of a DOPG lipid is visualized in Figure 6.

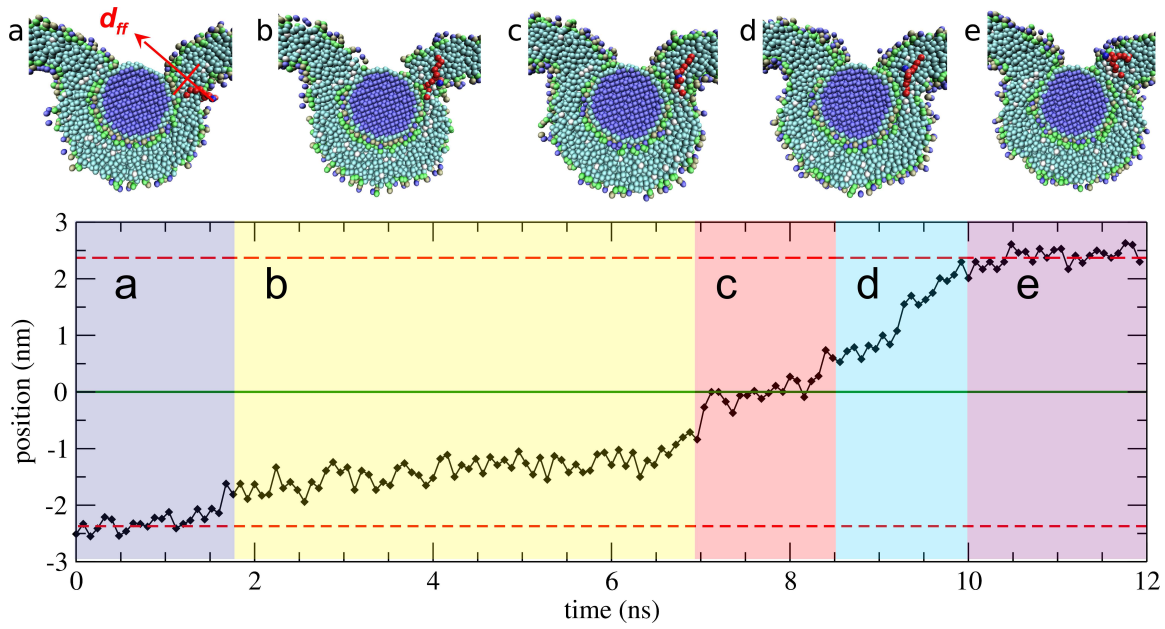

Figure 6: Five phases of the flip-flop of individual lipid induced by the adsorption of a charged nanoparticle on the membrane. Inset images (a-e) show the flip-flop process of a DOPG lipid transversing from the inner leaflet to the outer leaflet, where the corresponding DOPG lipid is represented as a red molecule in the configuration. The transmembrane direction of the corresponding lipid is defined as d_{ff}, denoted as the arrow in the inset image (a).

In general, the behavior of flip-flops in a physiological environment is normally very slow, usually at the order of hours to days[18], which is obviously beyond current computing resource capabilities. Different from this kind of slow process of normal flip-flops in the physiological environment, here the defect-mediated flip-flop of lipids is most likely nanoparticle-promoted. The enhanced flip-flop event in our simulations is a very fast process (time scales of tens of nanoseconds) once a transient water defect has been induced by the adhesion of nanoparticles. These results agree with previous work that flip-flops activity of lipids could also be promoted artificially, e.g., by the translocation of nanoparticles[20] or through electroporation[34]. Nevertheless, the long timescale of flip-flops makes it difficult to observe flip-flops of lipids in an unbiased simulation, even under the conditions of with an electrostatic attraction of charged nanoparticles in our simulations. Not in all the simulations the flip-flops occurs, which reflects the stochastic nature of the flip-flop process. This kind of defect-mediated flip-flop would serves as a suitable pathway for the lipid molecule translocation events in the interactions with the nanoparticles.

5 Conclusion

To summarize, we have investigated the nanoparticle-induced recruitment dynamics of lipids in the heterogeneous phospholipid bilayers consisting of DSPCs and DOPGs. Here three typical dynamic modes of individual lipid molecules have been taken into account as analysis of the movement pattern of DOPGs: lateral translational motion in the bilayer, protrusions out of the bilayer, and transverse from one leaflet of the bilayer to the other. Comparative analysis of in-plane movements of charged lipids reveals the co-existing of the flow-like collective diffusion with neighboring lipids and the oriented motion of lipids in the membrane. The oriented diffusion of DOPGs due to the attraction of nanoparticles conduces to the clustering of DOPGs at the localization of the nanoparticle in the membrane, which would contribute to the bending of membrane to wrap the nanoparticle. In addition, the nanoparticle could be wrapped by membrane driven by the gain in electrostatic attraction at the expense of the elastic energy of the biomembrane. The adsorption of nanoparticles can induce a lipid eyelid to bring out and spread across the nanoparticle to finish the wrap. The activation energy of the lipid eyelid characterized by PMF profiles of lipids cluster protrusion have been calculated, suggesting that the transition from the protrusion of lipids to a closed hairpin structure of a lipid eyelid occurs at the distance of $\sim$7.5 nm. Furthermore, a fast flip-flop behavior of charge lipids, at the order of tens of nanoseconds, has been found in the simulation. We witness that the enhanced flip-flop event is defect-mediated by the adsorption of nanoparticles. Thus we conclude that the adsorption of nanoparticle plays a crucial role in the recruitment process of lipids in phospholipid bilayers. These studies are consistent with experimental observations and to some extent give insight into the origin of the raft-mediated endocytosis of nanoparticles.

6 Acknowledgement

This work is funded by the National Natural Science Foundation of China (Grant No. 31100719), Specialized Research Fund for the Doctoral Program of Higher Education (Grant No. 20111202120017), China Postdoctoral Science Foundation (Grant No. 2012M510756), and Tianjin City High School Science and Technology Fund Planning Project (Grant No. 121111).

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