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Effect of Surface Charge and Hydrophobicity on Phospholipid-Nanoparticle Corona Formation: A Molecular Dynamics Simulation Study

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ABSTRACT

Using coarse-grained molecular dynamics simulations, we find that a combination of nanoparticle (NP) surface charge and hydrophobicity lead to three different kinds of corona structures formed by dipalmitoylphosphatidylcholine and dipalmitoylphosphatidic acid lipids, namely monolayer-coated, bilayer-coated, and attached-bicelles. Within this CG model, a charge neutral NP forms a bilayer-coated corona only when the NP is strongly hydrophilic with contact angle $\theta < 20^{\circ}$. For $\theta > 20^{\circ}$, a positive or negative zeta potential in excess of ~60 mV is needed to form a bilayer corona. Less charged NPs form monolayers with lipid tails completely covering the NP for $\theta > 70^{\circ}$, and bicelles attached only to a small fraction of the NP surface form for more moderately hydrophilic NPs ($20^{\circ} < \theta < 70^{\circ}$). Our results suggest that the NP hydrophobicity rather than surface charge determines the NP-lipid corona structure when the zeta potential of the NP is in the typical experimental range ($\leq$50 mV).

The interactions of nanoparticles (NPs) with biomolecules is critical in biomedical materials such as fluorescent biological labels [1], protein detectors [2], DNA structure probes [3], anti-tumor medications[4] and for materials used in tissue engineering [5], and drug and gene delivery [6]. Upon encountering a NP, biomolecules often rapidly adsorb onto the NP surface to minimize the NP surface energy, thus forming a NP-biomolecule complex, which is usually referred to as the corona structure. Therefore, *in vivo*, it is frequently the corona structure rather than the pristine NP that determines the interactions of the NP with biological tissues [7]. It has been shown that the biomolecule-NP corona, particularly the protein-NP corona, can influence particle biodistribution and biocompatibility [8], and impact NP uptake into cells [9]. Because of their smaller sizes, NPs can directly enter the respiratory tract through inhalation and deposit deep within the lung, eventually encountering the pulmonary surfactant lining of alveoli [10]. The pulmonary surfactant is composed of mainly phospholipids (~90%) with the remaining ~10% being surfactant proteins, which include SP-A (surfactant protein A), SP-B, SP-C, and SP-D [11]. The saturated zwitterionic lipids dipalmitoylphosphatidylcholine (DPPC) are the most abundant phospholipids in the pulmonary surfactants. The hydrophilic head of the DPPC contains atoms with partial charges and the tail is hydrophobic. Thus, it is likely that the charge and hydrophobicity of the NPs would strongly determine the NP-lipid corona structures. Details of the NP-protein corona structures and their biological impact on cellular uptake and their relevance for targeted drug delivery have already been investigated widely [9,12,13]. However, the molecular details of the NP-lipid corona structure are much less explored.

Recent experimental studies have shown that the NP-protein corona structure strongly depends on hydrophobicity and surface charge of the NPs [14,15]. Lundqvist et al. [14] showed that the plasma protein components in the NP-corona structure strongly depend on the surface charge of a polystyrene NP, whose charge was modified by amine and carboxyl functionalization. Gessner et al. [15] showed that the amounts of adsorbed plasma proteins on the NP surface decrease with the NP hydrophobicity. However, to date, little work has been reported on the dependence of lipid corona formation on NP surface charge and hydrophobicity.

Molecular simulations are in principle a good way to examine these dependences and are becoming increasingly common in studies of NP-lipid interactions. For example, in a recent study, Zuo and co-workers investigated the translocation behavior of a cationic, anionic and neutral NP with two extreme hydrophobicities (super hydrophilic and

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super hydrophobic) across pulmonary surfactant monolayers [16]. They observed that the hydrophilic NPs generally translocate quickly across the monolayer irrespective of their charge state but the hydrophobic NPs are usually trapped within the surfactant filament. Hu et al. [17] also recently investigated computationally the pulmonary surfactant coronas of both a super-hydrophobic polystyrene NP and a super-hydrophilic silver NP. However, a systematic study of the surface charge and hydrophobicity-dependent molecular structures of NP-lipid coronas has not yet been carried out. In this study, we therefore employ coarse-grained (CG) molecular dynamics (MD) simulations to unveil the molecular structure of NP-lipid coronas as functions of NP hydrophobicity and surface charge. We choose DPPC as our model lipids as they are the major components of the pulmonary surfactants. To investigate the effects of ionic lipids on the corona structure, we also included anionic dipalmitoylphosphatidic acid (DPPA) lipids in some of our simulations. While DPPA is not present in lung surfactant, phosphatidic acids are present, and DPPA is convenient for our purposes because the tail groups are identical to those of DPPC.

The coarse-grained MD simulations were performed using the MARTINI force field [18], which has been used extensively to simulate biomolecules [18,19]. Although MARTINI was developed mainly for organic molecules, it has been used to model inorganic NPs as well [20,21]. The coarse graining method used here lumps together several atoms (for example, one cerium and two oxygen atoms of CeO_2) into a single bead, but can still capture the characteristics of the actual material by choosing an appropriate MARTINI bead type that gives the best fit to the properties of the actual material, as determined by either experimental or by all-atom simulations. In a recent study [22], we used the MARTINI force field to model the hydrophobicity of the NPs made of different MARTNI bead types. In particular, we showed that the contact angles (θ) of MARTINI water droplets on flat surfaces made of MARTINI beads of types "C_1", "C_2" "C_3" "C_5" and "N_0" are 114°, 88°, 77°, 70°, and 22°, respectively. These contacts angles might be used to choose CG bead types to build NPs that represent the wettability of various NP materials. While specific interactions between NPs and biological materials are also likely to be important, because of the huge range of NP chemistries, a useful starting point for understanding NP interactions is to consider the effect of simple wettability (quantified by flat-surface contact angle) and charge on the structures formed by the most common lipids on "generic" NPs, represented by assemblages of CG beads of the types given above.

Thus, here we perform coarse-grained molecular dynamic (MD) simulations to determine how the general properties of the NPs, namely wettability and charge, influence NP-phospholipid interactions. A spherical NP of diameter 7 nm containing 1724 beads was built from a bulk FCC crystal. We also carried out limited simulations with particles of cubic shape composed of 1688 beads to investigate the effect of particle shape on corona structure. We also put charges on the surface beads of the NPs to study the effect of NP surface charge on the corona structure. The bead type (hydrophobicity) and surface charge of the NP were changed systematically to map out the role of these two parameters on the NP-lipid corona structure. The pre-built NP was placed in the center of a cubic simulation box of dimension of 20 nm per side. The neighboring beads of the NP were connected by harmonic bonds. The NP was allowed to rotate and translate during the course of the simulation. The lipids were randomly dispersed in the simulation box (Fig. 1). In one set of simulations, we considered DPPC lipids only as they are the predominant component of the pulmonary surfactants. We considered both zwitterionic DPPC and anionic DPPA lipids in another set of simulations to investigate the effect of ionic state of the lipids on the corona structure. The simulation box was then solvated and an appropriate number of sodium and chloride ions were added to achieve charge neutrality, where the NP and the phospholipid were separately neutralized by Na^+ or Cl^- ions, resulting in some Na^+Cl^- in the solution. We then energy-minimized the system using the conjugate gradient method followed by 1000 ns equilibration with a time step of

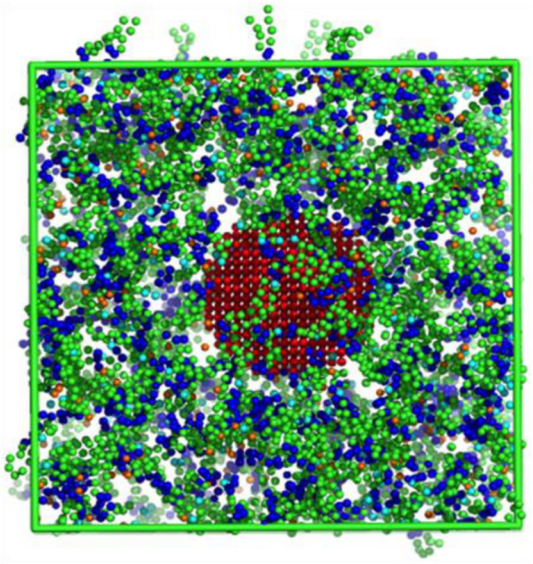

Fig. 1. Initial configuration in a typical simulation box containing an NP, lipids and ions. Water is made invisible for better viewing. [Color code: red represents the NP, green the lipid tails, blue the lipid heads, brown the sodium ions, and cyan the chlorine ions.] (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

20 fs at ambient conditions (e.g., constant pressure (1 bar) and constant temperature (298 K)), which was long enough for essentially all of the randomly dispersed lipids to assemble onto the NP surface into a reproducible structure dependent on NP properties. Temperature and pressure of the system were controlled using a velocity-rescale[23] thermostat and a Parrinello-Rahman barostat [24], respectively. All simulations were performed using the GROMACS-4.6.7 simulation package [25].

We first explore possible corona structures for a wide range of surface charge densities and hydrophobicities of the NP. We will follow this by focusing on NP properties, especially charge densities that more closely match the usual experimental conditions. While for a pure (clean) NP the surface charge density and hydrophobicity may be correlated with each other, in many practical situations, the NP surface contains impurities (such as hydrocarbons) that can significantly alter the inherent hydrophobicity of the NP and expand the range of possible surface properties. Our initial goal here is therefore to explore the effect of the full range of combinations of surface charge and hydrophobicities on the lipid corona structure. Thus, to determine the "phase diagram" of the NP-lipid corona, we simulated the NP-lipid interactions by changing the surface charge density over a very wide range from -5e to +5e (charge of each surface bead of the NP) for each of the "C_1", "C_2" "C_3" "C_5" "N_0" and "P_4" types. Fig. 2(a) shows the resulting "phase diagram" of the corona that self-assembled on the NP in the presence of 600 DPPC lipids. Three different kinds of corona structures were found depending on the hydrophobicity and surface charge of the NP: (i) a monolayer-coated NP (labeled red), (ii) a bilayer-coated NP (green), and (iii) bicelles attached to the NP surface (blue), with all three structures shown in the lower panel of Fig. 2. Note that the phase diagram is symmetric with respect to zero charge since the DPPC lipid is charge-neutral. As can be seen from Fig. 2(a), when the charge density of the NP is very high, only bilayer corona structures are formed irrespective of the NP hydrophobicity. Either a larger single hemispherical bilayer partially coating the NP or two relatively smaller bilayer fragments were found at the NP surface for this category of NP-corona structures. The density of counter ions at the NP surface becomes very large at high charge density, which makes the NP surface effectively super-hydrophilic irrespective of the inherent hydrophobicity of the NP and hence the bilayer structures are formed. However, for relatively