Two-Dimensional Al Hydroxide Interaction with Cancerous Cell Membrane Building Units: Complexed Free Energy and Orientation Analysis

A. A. Tsukanov[1,2,a)] and S. G. Psakhie[1,2,b)]

[1]*National Research Tomsk Polytechnic University, Tomsk, 634050 Russia*
[2]*Institute of Strength Physics and Materials Science SB RAS, Tomsk, 634055 Russia*

[a)] Corresponding author: a.a.tsukanov@yandex.ru
[b)] sp@ispms.tsc.ru

Abstract. The application of hierarchical nanoparticles based on metal hydroxides in biomedicine, including anticancer therapy and medical imaging, is a rapidly developing field. Low-dimensional aluminum oxyhydroxide nanomaterials (AlOOH-NM) are quite promising base to develop hybrid theranostic nano-agents with core-shell architecture, which is determined by AlOOH-NMs physicochemical properties such as: large specific surface area, pH-dependent charge, amphoteric behavior, high surface density of polar groups capable to form non-covalent bonds, low or null cytotoxicity and biocompatibility. Characterization of the system behavior within interface between NM and plasmatic membrane is crucial for the understanding of nano-agent—cell interaction. In the present work the complex *in silico* study including the free energy estimation and orientation analysis of phosphatidylcholine (POPC) and phosphatidylethanolamine (POPE) lipids interacting with AlOOH nanosheet was conducted to understand the effect of such nanomaterial on cancerous cell plasmatic membrane.

INTRODUCTION

Modern chemistry and nanotechnologies provide the unprecedented opportunities for the development of multifunctional hierarchical nanoparticles for biomedical and oncological purposes. Multifunctional nanoparticles, nano-agents allow targeted delivery, medical imaging and controlled release of the therapeutics, increasing efficiency of such treatments as anticancer chemotherapy, hyperthermal and photodynamic therapies, radiotherapy etc. Two-dimensional nanomaterials based on layered hydroxides such as anionic clays (layered double hydroxides) [1–4] and cationic clays (e.g. montmorillonite) [5–8] are quite suitable base for hierarchical anticancer nanoparticles development.

Recently it has been experimentally found that the low-dimensional aluminum oxyhydroxide is prospective nanomaterial (AlOOH-NM), at least, to be adjuvant in anticancer therapy [9, 10]. The mechanism of AlOOH-NM antitumor activity may be provided by the oxyhydroxide influence on pH of extracellular media [11, 12] or/and by probable violations in the cell membrane structure accompanied by membrane protein dysfunction, as well as by facilitating cellular uptake of the therapeutic agents, or something fourth. In any case, it is under-explored so far. The present *in silico* study indirectly investigates the AlOOH nanosheet interactions with the cancerous cell membrane, in order to understand whether AlOOH effect is associated with the plasmatic membrane disruption.

RESULTS AND DISCUSSION

Complexation of the Free Energy Change Profile with Adsorbate Orientation

The thermodynamics on an interface between the nanomaterial and the phospholipid bilayer plays a decisive role in nanoparticle–cell interaction, determining the mechanism of either the cell's uptaking the nanoparticle (NP) or the nanoparticle's disrupting the cell membrane.

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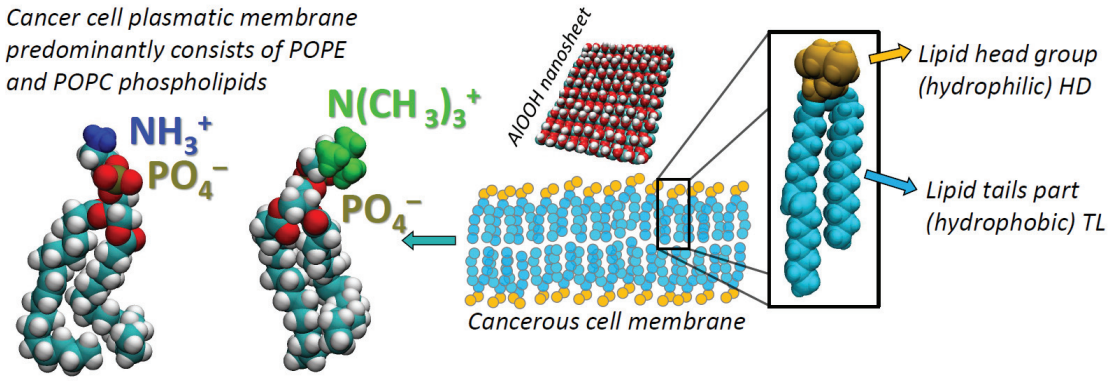

FIGURE 1. The POPE and POPC lipids are typical building blocks of mammalian cell membranes, in particular, of cancerous cell. Lipid consist of two parts—polar hydrophilic head group and aliphatic tails, which are hydrophobic. To understand the interaction behavior of two-dimensional nanomaterial and the cell membrane, it's proposed to estimate the single lipid affinity to the nanomaterial, using free energy analysis combined with an analysis of the lipid orientation during steered adsorption (red—oxygen, white—hydrogen, cyan—carbon, golden—phosphorus, blue/green—amine and choline groups, respectively)

Mammalian cells membranes, including those of the cancerous cells, are bilayers of phospholipids (Fig. 1) with diverse type of functional membrane proteins and peptides embedded. Lipids palmytoil-oleoyl-glycero-phosphatidyl-ethanolamine (POPE) and palmytoil-oleoyl-glycero-phosphatidyl-choline (POPC) are the most common building blocks of such type of cells.

The rapid development of the computational technologies and simulation techniques, such as constrained or steered molecular dynamics (SMD) and thermodynamic integration [13] for the modeled system of more than hundreds of thousands interacting particles, provides the performance of *in silico* experiments of the diverse NP–cell membrane [14, 15] and NP–protein [16, 17] interactions, etc. However, direct MD modeling of two-dimensional nanomaterial interaction with the cell membrane may be a time-consuming process, depending on the size of the representative nanosheet fragment and, as a consequence, on the modeled cell membrane dimensions, which must be at least twice as large as nanosheet. From a computer modeling point of view the most suitable way in this case would be the consideration of single phospholipid interaction with the nanosheet fragment in the water solution. Furthermore, the most efficient *in silico* way to characterize interaction of the single molecule with the nanosheet or substrate is the estimation of the Gibbs free energy of this molecule adsorption, including calculation of the free energy change profile versus distance between adsorbate and adsorbent centers of mass.

The AlOOH nanosheet, being a single nanolayer of the delaminated boehmite, consists of irregular octahedra of AlO_6, where two inequivalent types of oxygen atoms are presented: O_b—bridging oxygen bonded to four Al atoms and O_h—hydroxyl oxygen bonded to two Al and one H atoms (see, for example, [18] and references therein). Both surfaces of the AlOOH nanosheet have hydroxyl groups exposed to the solvent. That is why nanosheet is capable of participating in hydrogen bonding.

The phospholipid molecule consists of two long hydrophobic tails and polar (or even negatively charged) head group, which brings local positive charge on its amine NH_3^+ or choline $N(CH_3)_3^+$ group and local negative charge on phosphate group PO_4^- (Fig. 1). To understand the behavior of such a long and "non-uniform" molecule, interacting with the nanosheet, not only free energy of adsorption obtained from potential of mean force (PMF) analysis is necessary but also the analysis of functional groups position or molecule orientation could be very important.

According to the idea formulated above, the series of constant velocity SMD simulations of POPE and POPC lipids forced adsorption/desorption on AlOOH nanosheet was conducted. To estimate free energy of adsorption as well as to reconstruct free energy change profiles the combined process free energy estimation (COPFEE) procedure [19] was used. To analyze the orientation of lipids during pulling process the head group center of mass (CoM) position (Fig.2, yellow circles) and the position of tails CoM (Fig. 2, blue circles) were calculated every 1 ps as a function of distance between whole lipid CoM and a central plane of the nanosheet, making it possible to merge them on the same plot with COPFEE profile. Results of complexed analysis for POPE and POPC lipids are represented on diagrams Fig. 2a, b and Fig. 2c, d, respectively.

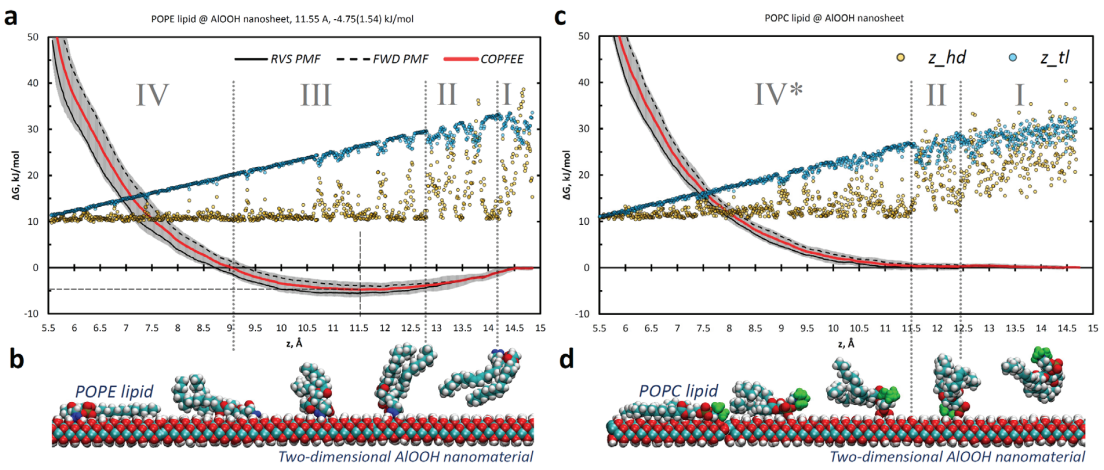

FIGURE 2. Results of complexation the free energy change profiles with the data on lipid molecule orientation during steered molecular dynamics simulations. (a) Complex diagram of POPE lipid adsorption/desorption-orientation near AlOOH nanosheet versus lipid center of mass position (Å): black dashed curve—PMF profile of lipid forced adsorption (forward process), black solid—PMF of lipid desorption (reverse process), red—COPFEE profile (gray—standard deviation error bars), blue circles—lipid tails center of mass position, yellow circles—lipid head group center of mass position; (b) POPE lipid configurations during steered adsorption: red—oxygen, white—hydrogen, cyan (lipid)—carbon, cyan (nanosheet)—aluminum, golden—phosphorus, blue—amino-group; (c) complex diagram for POPC lipid (colors are similar to (a)); (d) POPC molecule configuration in the vicinity of AlOOH nanosheet (green—choline group)

The estimated free energy profile for POPE@AlOOH system has a local minimum $\Delta G = -4.75 \pm 1.54$ kJ/mol at the point corresponding to lipid CoM z-position 11.55 Å (Fig. 2a). It means POPE adsorption by AlOOH nanosheet is energetically favorable, unlike POPC case, free energy profile of which doesn't have local minima in the proximity of nanosheet surface (Fig. 2c). Four phases of lipid—AlOOH nanosheet interaction were defined for POPE case: I—free (spontaneous) rotations of adsorbate molecule, $z > 14.2$ Å; II—singular contacts, $12.8 < z < 14.2$ Å; III—adsorbed state with formation of head-group–nanosheet non-covalent bonds, $z < 12.8$ Å; IV—energy unfavorable configurations. In case of POPC lipid interaction with the nanosheet surface the phase III is absent, since there is no segments in which COPFEE profile has negative values (Fig. 2c). Phase I corresponds to range of distances $z > 12.4$ Å, phase II—11.5 Å $< z < 12.4$ Å, and phase IV—$z < 11.5$ Å. The existence of phase III for POPE case means that both the adsorption of the lipid by AlOOH nanosheet is energetically favorable (since relative free energy level is negative) and adsorption mechanism is associated with the bonding between lipid head and adsorbent surface (the position of head group CoM is closer to nanosheet than one of lipid tails).

The complexed analysis based on *in silico* study data revealed differences in POPC and POPE lipids interaction with two-dimensional AlOOH nanomaterial. Despite the fact that both lipids prefer to form H-bond between head group and AlOOH surface, the adsorption of POPC lipid seems to be unfavorable as compared to POPE lipid case. The obtained results indicate that AlOOH nanosheet would demonstrate affinity to POPE/POPC bilayer head group region and most likely would be adsorbed on the cancerous cell membrane, causing no disruption.

SIMULATION DETAILS

The aluminum oxyhydroxide nanosheet model was built using geometry data from [18]. Lennard-Jones and bond parameters as well as partial atomic charges for AlOOH parameterization were taken from CLAYFF force field [20]. The CHARMM force field parameters were used for POPE and POPC lipids models [21]. The equilibrations of systems were conducted at NPT conditions for 5 ns. After equilibration, the simulation box dimensions were 30×37×33 Å. The cutoff distance for non-bonded pairwise interactions was set to 10 Å. Electrostatic interactions and Lennard-Jones potential were smoothly shifted to zero between 8 and 10 Å. The periodic boundary conditions were applied in all directions. Long-range electrostatics was treated using PPPM (particle-particle particle-mesh) algorithm [22] with a relative accuracy of 10^{-3}. The simulations were performed for the systems at human body

conditions T = 310 K and p = 1 atm. The velocity constant of SMD pulling process was 0.1 Å/ns. The utilization of SHAKE algorithm [23] for all hydrogen atoms allow to increase timestep up to 2 fs.

Visual molecular dynamics (VMD) package [24] (http://www.ks.uiuc.edu/Research/vmd) was utilized for preparation of the models and for visualization of the results. All MD simulations were performed with the LAMMPS package (Sandia National Laboratory) [25] (http://lammps.sandia.gov/index.html).

CONCLUSIONS

The simple computational technique is proposed to characterize the interaction between two-dimensional nanomaterial and the cell membrane. The core of this approach is consideration of a single lipid adsorption/desorption on nanosheet surface during steered MD simulation (instead of direct modeling of nanosheet interaction with the model membrane) followed by a conjoint analysis of free energy change profile and the orientation of lipid, while adsorbing. The proposed approach was applied to indirect study of AlOOH nanosheet interaction with the cell membranes containing POPE and POPC lipids, which is most common building units of mammalian cell membranes, including ones of the cancerous cells. The complex *in silico* adsorption-desorption free energy and orientation analysis revealed that two-dimensional AlOOH nanomaterial do not disrupt POPE/POPC cell membrane and most likely would tend to be adsorbed by cell membrane surface, forming non-covalent bonds with the lipids head groups. Such a behavior may also lead to nanosheet uptake by the cell *via* endosome formation way.

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