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# Simulation and analysis of cellular internalization pathways and membrane perturbation for graphene nanosheets

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ABSTRACT

Clarifying the mechanisms of cellular interactions of graphene family nanomaterials is an urgent issue to the development of guidelines for safer biomedical applications and to the evaluation of health and environment impacts. By combining large-scale computer simulations, theoretical analysis, and experimental discussions, here we present a systematic study on the interactions of graphene nanosheets having various oxidization degrees with a model lipid bilayer membrane. In the mesoscopic simulations, we investigate the detailed translocation pathways of these materials across a 56 × 56 $nm^2$ membrane patch which allows us to fully consider the role of membrane perturbation during this process. A phase diagram regarding the transmembrane translocation mechanisms of graphene nanosheets is thereby obtained in the space of oxidization degree and particle size. Then, we propose a theoretical approach to analyze the effects of various initial equilibrium states of graphene nanosheets with membrane on their following cellular uptake process. Finally, we demonstrate that the simulation and theoretical results reproduce some important experimental findings towards the mechanisms of cytotoxicity and antibacterial activity of graphene materials. These results not only provide new insight into the cellular internalization mechanism of graphene-based nanomaterials but also offer fundamental understanding on their physicochemical properties which can be precisely tailored for safer biomedical and environment applications.



## 1. Introduction

Graphene is a single atomic sheet of graphite with $sp^2$-bonded carbon atoms in a closely packed honeycomb two-dimensional (2D) lattice [1,2]. This new carbon nanoform and its derived materials exhibit great promises in the fields of electronics [3], photonics [4], composite materials [5], sensors and metrology [6], and biomedicine [7–11]. To realize their potentials, health and environmental impacts of graphene-based materials should be thoroughly evaluated [9–11]. Moreover, resolving the safety and toxicity issues associated with these emerging nanomaterials will be not only beneficial to their integration into new composites, nanoelectronics, etc. [3–6] but also in the case of possible biomedical applications such as photothermal ablation of tumors [12] and nanotechnology-based drug delivery [9,11]. Understanding how graphene nanomaterials interact with cell membrane is related to how they cause cytotoxicity and is therefore critical to the development of safer graphene-based biomedical technologies and to the management of graphene health and environmental issues. However, compared to other synthetic carbon material, e.g., fullerenes [13,14] and carbon nanotubes [15–17], much less is known about the fundamental mechanisms of cell membrane interactions with graphene materials that is a main challenge in current graphene materials development.

Recent studies have shown that pristine graphene (PG) and graphene oxide (GO) exhibit strong antibacterial activity [18–21]. The physicochemical characteristics of graphene materials seem to play a key role in the efficiency of the bacterial killing and thereby can be tailored to reduce the hazard impact on human health and the environment. For instance, experimental studies demonstrate that sharp edges of graphene nanosheets may result in physical damages on cell membranes of *Escherichia coli* (*E. coli*), leading to the loss of bacterial membrane integrity and the leakage of RNA [18–20]. However, the results are questioned by another study, wherein *E. coli* grow faster by forming dense biofilms around the suspended GO after it is added to the bacteria [22]. By this token, the exploration for the mechanism of antibacterial activity of graphene-based materials is challenging and will certainly stimulate further studies.

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The main thrusts to the thorough understanding and controlling of the interactions of graphene-based materials with cell membranes may be attributable to the intrinsically higher complexity of such interactions. The unique physicochemical characteristics, e.g., 2D geometry and various oxidization degrees, can lead to unconventional nano-bio interfaces when these nanomaterials encounter cell membrane [23]. Their cellular interactions as well as transmembrane translocation pathways should thereby be evidently different with uniform nanoparticles [24]. However, the directly experimental visualization for the detailed dynamical process of a graphene sheet in a single cell is not yet possible because it occurs on a length scale of tens of nanometers and a timescale that is submillisecond [8–11]. Tailored computer simulations are of the ability to identify and separate individual contributions to the phenomenon or process of interest, and thereby offer an alternative approach to address these issues [25–31]. However, few simulation models have been constructed to study the process. For instance, atomistic molecular dynamics (MD) simulation shows that the graphene nanosheet with one corner carbon atom restrained can insert through cell membrane and vigorously extract large amounts of lipid molecules, leading to degradation of the membrane [21]. Coarse-grained (CG) MD simulation demonstrates that direct membrane penetration of graphene particles begins with localized piercing at sharp corners or at protrusions along their edges [29].

Despite these previous studies, a comprehensive understanding of the interaction of graphene-based materials with cell membranes still presents a challenging task to the physical chemistry and biomedicine communities. Specifically, these previous simulations have limitations because of the reduced dimensionality of the system or the shortage of simulation time. In this case, the membrane perturbation or fluctuation may be significantly alleviated, impeding some possible transmembrane translocation pathways. On the other hand, very little has been known about the dependence of detailed dynamical process on the oxidization degree and size of graphene. This has a tight relation to the membrane integrity and, fundamentally, the cytotoxicity or antibacterial activity because experiment results have demonstrated that GO dispersion shows a higher antibacterial activity than PG [20,32]. From this perspective, extensive simulation and, particularly, theoretical analysis should be used to obtain a thorough understanding of the detailed transmembrane translocation pathway and its dependence on the specific physicochemical parameters. These issues are central to exploring the mechanisms of cytotoxicity and antibacterial activity of graphene-based materials.

In this paper, we report an extensive investigation of the cellular interaction of graphene nanosheets with various oxidization degrees by combining large-scale computer simulations, theoretical analysis, and experimental discussions. The advantage of the present computer simulations is that a large membrane with $56 \times 56$ nm$^2$ is constructed through the self-assembly of lipids and is used as a model membrane to study the translocation pathway of graphene materials, allowing the exploration of detailed membrane perturbation during the dynamical process. By varying the oxidization degree and size of graphene nanosheets, the transmembrane translocation pathways and corresponding membrane perturbations are systematically examined, allowing us to construct a phase diagram in the two-parameter space. In the theoretical analysis, a theoretical approach is proposed to calculate the bending energy during the wrapping of a graphene nanosheet by membrane, and thereby we can analyze the effects of various initial equilibrium states of graphene nanosheets with membrane on their following cellular uptake process. The relationships between existing experimental observations and the results of simulations and theoretical analysis are finally discussed, where we point out the implications of these findings for the mechanisms of cytotoxicity and antibacterial activity of graphene-based materials.

## 2. Model and methods

Full technical details on the simulation method and the models of membrane and graphene nanosheet can be found in Supporting Information. Briefly, the coarse-grained (CG) simulations in this paper are on the basis of dissipative particle dynamics (DPD) [33], which is a Lagrangian method derived from coarse-graining of molecular dynamics widely used as a mesoscopic simulation method for biomembrane systems [26,27,31,34,35]. In our system, the interaction forces between beads include the conservative force, the dissipative force, the random force, the spring force and the angle force. The last two forces are additional forces and introduced to represent the interactions between bonded beads. Details about the interaction parameters between different types of beads can be found in Supporting Information. To simulate the system with a tensionless membrane, we use *N*-varied DPD method [36], facilitating the control of membrane tension through offering sufficient excess area to release the extra tension induced by the large membrane deformation due to the graphene nanosheet.

In our DPD model of a CG graphene nanosheet, six carbons of a benzene ring in graphene are modeled as a CG bead [37], as displayed in Fig. S1. We calibrate the Young's modulus of our CG graphene nanosheet model to the experimentally found elasticity of graphene [38–40]. Here we select the constants of the modulus of the spring and the modulus of the angle bend as 700 kcal·mol$^{-1}$·Å$^{-2}$ and 700 kcal·mol$^{-1}$·rad$^{-2}$ respectively. Each amphiphilic lipid consists of a head group and two tails (see Fig. 1). The head group contains three connected hydrophilic beads. Each tail includes three connected hydrophobic beads. At the beginning of our simulations, a stable, tensionless square bilayer membrane with $56 \times 56$ nm$^2$ is obtained firstly through the self-assembly of lipids. We set $\Delta t = 0.02\tau$ as the time step. In the present simulation, we use a modified velocity-Verlet algorithm due to Groot and Warren to solve the motion equation [33]. We can get $\tau = 7.7$ ns and the total physical time of each calculation is at least 45 μs. More details regarding the methods can be found in Supporting Information.

## 3. Results and discussions

### 3.1. Computer simulations

In the current simulation study, we focus on the detailed dynamical process and membrane perturbation when a graphene nanosheet with a defined oxidization degree and size interacts with a lipid bilayer membrane. Fig. 1 shows the CG models for the representative entities used in the DPD simulations. A lipid is represented in our model by three linearly connected hydrophilic beads, representing the polar headgroup, to which two tails of three hydrophobic beads are jointed. Solvent particles are represented by a single bead. Self-assembly of 10,000 lipids yields a bilayer membrane with $56 \times 56$ nm$^2$, which allows us to fully consider the perturbation and fluctuation of the membrane during its interaction with graphene nanosheet. A DPD model of CG graphene nanosheet, which has proved its effectiveness in the simulation of graphene–surfactant interaction, is applied in the present simulations (for more details see Supporting Information) [37]. To investigate the role of oxidization degree, we simulate the graphene oxide nanosheets with various oxidization degrees, i.e., edge oxidized graphene (eGO), sparsely oxidized graphene (sGO, with an oxidization degree of 20% for basal carbon atoms), and densely oxidized graphene (dGO, with an oxidization degree of 40% for basal carbon atoms) [41,42]. The oxidized beads are arranged randomly on the edge and plane of each square graphene nanosheet, but localized with high correlations between oxidization loci resulted from the fact that a carbon atom on some broken $\pi$-bond may be oxidized with much higher probability than its counterpart with an intact $\pi$-bond [41]. For each type of graphene nanosheet, its edge length, $l_g$, is increased from 3.5 nm to 10.5 nm to systematically examine the effect of size on its interaction with membrane. The initial position of the graphene nanosheet has just a trivial effect on the following dynamic process [29]. Therefore, a graphene nanosheet with parallel orientation is positioned above the membrane in our simulations. Each simulation runs at least 45 μs while the structure and specific parameters are monitored. Compared to