Membrane Insertion of MoS₂ Nanosheets: Fresh vs. Aged

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Abstract

Fresh two-dimensional (2D) molybdenum disulfide (MoS₂) can absorb the hydrocarbon contamination from the ambient air and cause surface aging. Thus, understanding how the surface aging process of MoS₂ affects the interaction with biomolecules is crucial for its applications in the biomedical field. Here, we employed atomistic molecular dynamics simulations to investigate the interactions of fresh and aged MoS₂ nanosheets with POPE lipid membranes. Our results show that even though both the fresh and aged MoS₂ nanosheets are capable of spontaneous insertion into the POPE bilayer membrane, the fresh MoS₂ nanosheet displays significantly more robust interaction than the aged one. The potential mean force (PMF) calculations further confirm that the fresh MoS₂ nanosheet is more energetically favorable than the aged one in penetrating into the POPE lipid membranes, with the former having ~17 kJ/mol stronger binding affinity than the later. This work provides a deeper understanding of the surface-aging-dependent interaction of MoS₂ nanosheet with biomolecules, which might help the design of better MoS₂-based nanodevices with appropriate surface properties.

Introduction

Molybdenum disulfide (MoS_2) is a member of the so-called transition-metal dichalcogenide (TMD) family, which has received great attention lately due to their unique physical and chemical properties. Previous studies have shown wide applications of MoS_2 nanosheets in optoelectronics,¹ field emission transistors,² gas sensors³⁻⁴ and hydrogen storage.⁵ More recently, MoS_2 nanosheets have also shown increasing interest in the biomedical field. For example, it has been reported that their strong near-infrared (NIR) absorption feature enables them to be used for photothermal therapy in cancer treatment.⁶⁻⁷ Also, the moderate direct bandgap of MoS_2 nanosheet has been explored for protein and DNA detections.⁸⁻⁹ While the strong absorbance of X-ray by Mo atoms makes it an appealing contrast agent in X-ray computed tomography imaging.⁷

Recent studies also demonstrate that the functionalized MoS_2 nanosheets have high inhibitory and bactericidal activities against ESKAPE pathogens by destructing their cell membranes.¹⁰ On the other hand, Yang *et al.* proposed that the antibacterial effects of MoS_2 nanosheets to the *Escherichia coli* (*E. coli*) is attributed by the chemical oxidation and membrane stress mechanisms.¹¹ In addition, MoS_2 nanosheets can also disrupt the integrity of the cell membranes and extract the phospholipids from the membrane bilayers.¹² These investigations have shown that the MoS_2 nanosheets hold strong interactions with cell membranes and other biomolecules, which makes them appealing to studies of nanotoxicity (and potential nanomedicine as antibacterial agents).

It is worth noting that the freshly prepared MoS_2 nanosheets (fresh MoS_2) can absorb hydrocarbons when they are exposed to air and induce surface aging (named as aged MoS_2), which thereby affects the surface hydrophobicity and topology and causes changes in the water contact angles (WCA) on the MoS_2 surface as evidenced by a series of variable values measured by different experiments.¹³⁻¹⁶ More importantly, the surface aging process of a MoS_2 nanosheet may result in a potential

effect on its interaction with biomolecules at the bio-nano interface, which is yet unknown to a large extent.

In this paper, we employ molecular dynamics (MD) simulations to investigate the underlying mechanism of the interactions between the MoS₂ nanosheets (with fresh vs. aged) and the POPE lipid membrane, aiming to understand how the surface aging process of MoS₂ affects interactions with biomolecules. Based on MD simulations, together with free energy calculations, we found that the fresh MoS₂ nanosheet presents a stronger binding affinity to the POPE lipid membranes than the aged one. The results suggest that the surface aging process of MoS₂ could affect the interaction with biomolecules.

Results and Discussion

Here, we applied the MD simulations to investigate the interactions of fresh and aged MoS₂ nanosheets with POPE lipid membrane. In order to efficiently model both fresh and aged MoS₂ surfaces, we have developed a simplified model by only adjusting the Lennard-Jones parameter, ϵ_s , (the depth of the potential well of a sulfur atom)¹⁷, which demonstrates a surprisingly linear relationship between the ϵ_s and water contact angle (WCA). In this study, the surface-aging dependent interaction of a MoS₂ nanosheet with membranes can thus be “achieved” using this simple MoS₂ force field model.¹⁷⁻¹⁸ **Table 1** lists two sets of force field parameters for the fresh and aged MoS₂ nanosheets, respectively.

The initial configuration of a MoS₂ nanosheet and POPE lipid membrane is illustrated in **Fig. 1** (details can be seen in the Methods section). **Fig. 2** shows the final conformations of the fresh and aged MoS₂ nanosheets inserting into the lipid membrane from three independent trajectories. As can be clearly seen from **Fig. 2** that both the fresh and aged MoS₂ nanosheets finally bury themselves into the lipid

membranes with their orientations perpendicular to the membrane surfaces. In addition, both MoS₂ nanosheets (with fresh vs. aged) are mostly in direct contact with the hydrophobic tail of the lipid molecules and parallel to the tail chains. The results reveal that both the fresh and aged MoS₂ nanosheets can penetrate into the POPE lipid bilayer membrane, independent of their surface properties.

To better understand the interaction process, we further analyzed the time evolution of the atom contact number between the MoS₂ nanosheets (fresh vs. aged) and lipid membrane (**Fig. 3a and c**). Here, the atom contact number was recorded when the distance between a MoS₂ nanosheet and any heavy atom of lipid molecules is less than 0.5 nm. In addition, the binding conformations of the fresh and aged MoS₂ with the POPE membrane at four key snapshots were also illustrated in **Fig. 3b and d**. Initially, in the case of the fresh MoS₂ nanosheet, it moved freely in the water and has intermittent contacts with the lipid membrane, yielding an atom contact number less than 20 (0-5 ns). At $t = 5$ ns, fresh MoS₂ started to contact the membrane with a point-to-face orientation pointing to the target membrane. At $t = 18$ ns, the fresh MoS₂ has changed its binding conformation into a face-to-face orientation with an increase in atom contact number close to 100. At $t = 22$ ns, the fresh MoS₂ nanosheet has changed its conformation and is to tilt itself with one of the vertexes inserting into the membrane, along with a transient decrease in contact number compared to the value at 18 ns. Starting from $t = 22$ ns, the fresh MoS₂ continued to penetrate into the membrane with a dramatic increase in the atom contact number. After 65 ns, the interactions between the fresh MoS₂ and POPE membrane reached an equilibrium state with limited fluctuation in contact number (~275). As for the aged MoS₂, the general trend in the change of atom contact numbers and binding conformations is similar to the aforementioned fresh nanosheet as evidenced by the illustrations in **Fig. 3c and d**. These results suggest that both the MoS₂ nanosheets (fresh vs. aged) are capable of penetrating into the POPE lipid membranes.

We then analyzed the time evolution of the interaction energy between the MoS₂ nanosheets (fresh vs. aged) and lipid membrane to further illustrate the insertion processes. As can be seen from **Fig. 4a** and **b**, the van der Waals (vdW) force dominates the interaction energies in both fresh and aged MoS₂ nanosheets with POPE lipid membrane. Moreover, the vdW interaction energy of fresh MoS₂ is lower than that of aged MoS₂ in interacting with the POPE lipid membrane, whereas the Coulombic (Coul) interaction energy of fresh MoS₂ is slightly higher than that of aged MoS₂ (**Fig. S1**). Further analysis showed that the atom contact number between the fresh MoS₂ and head group is lower than that between the aged MoS₂ and head group. Conversely, the opposite trend is observed for the fresh and aged MoS₂ with the tail groups (**Fig. 4c**). In addition, **Fig. 4d** showed that the distance between the center of mass (COM) of the fresh MoS₂ and lipid membrane is shorter than that of the aged MoS₂ with the membrane, indicating that the fresh MoS₂ might have more damage to the cell membrane.

Additionally, we also calculated the binding free energies between the MoS₂ nanosheets (fresh vs. aged) and POPE lipid membranes by monitoring the relative free energy when pulling the MoS₂ nanosheets along the Z-direction perpendicular to the membrane, using the potential of mean force (PMF) method (details can be found in the Methods section). As seen in **Fig. 5** that the free energy of the fresh MoS₂ is ~17 kJ/mol stronger than the aged one, which indicates that the fresh MoS₂ nanosheet is more energetically favorable to penetrate into the cell membrane than the aged one, though both the fresh and aged MoS₂ nanosheets exhibit spontaneous penetrating behaviors to the POPE membrane. Besides, the minimum of the PMF curves corresponds to the COM distance of 0.8 nm and 1.1 nm for the fresh and aged MoS₂, respectively, in agreement with **Fig. 4d** and consistent with the indication that the fresh MoS₂ might have more damage to the cell membrane.

Conclusion

In this work, we employed atomistic molecular dynamics simulations to investigate the interactions between MoS₂ nanosheets (with fresh vs. aged) and the POPE lipid membrane, aiming to understand how the surface aging process of a MoS₂ nanosheet affects its interaction with cell membranes. Our results show that even though both the fresh and aged MoS₂ nanosheets are able to insert into POPE bilayers, the fresh MoS₂ nanosheet presents significantly more robust interaction with the membrane than the aged one. Free energy calculations computed by the potential mean force (PMF) further verify that the membrane insertion process of the fresh MoS₂ nanosheet is more energetically favorable than the aged one, with the former showing ~17 kJ/mol more binding affinity than the latter. Our findings reveal that MoS₂ has a surface-aging-dependent interaction with cell membranes, which may be beneficial to future applications in biomedicine.

Methods

Molecular dynamics simulation

In this work, the triangular MoS₂ nanosheet (side length of 2.89 nm) was used due to its small size and was also proved to have the capacity in penetrating into the bilayer.¹⁹ The membrane with surface dimensions of 6.25 × 6.25 nm² was constructed by using CHARMM-Gui (<http://www.charmm-gui.org>), yielding 168 palmitoylcholinephosphatidylethanolamine (POPE) lipids.²⁰ Two types of MoS₂ nanosheets (with fresh vs. aged) were placed above the lipid membrane with a minimum distance of about 1.2 nm (as shown in **Fig. 1**). Two systems were placed in the same box (6.25 × 6.25 × 12.73 nm), containing 10473 water molecules, 48 Na ions and 48 Cl ions.

All MD simulations are carried out with the software package GROMACS (version 5.1.4).²¹ VMD software was utilized to visualize MD trajectories and draw snapshots.²² The force field parameters of the fresh and aged MoS₂ nanosheets were employed based on our previous works.¹⁷⁻¹⁸ The CHARMM 36 force field²³⁻²⁵ and

TIP3P water model²⁶ were adopted for POPE molecules and water molecules, respectively. Following similar protocols in our previous studies²⁷⁻³³, periodic boundary conditions of these systems were treated in all directions (x y and z). The temperature and pressure were fixed at 300 K and 1 atm using v-rescale thermostat³⁴ and semi-isotropic Parrinello-Rahman algorithm (x+y, z).³⁵ The long-range electrostatic interactions were treated with the PME method,³⁶ and the van der Waals (vdW) interactions were calculated with a cutoff distance of 1.2 nm. The geometrical properties of solute bonds associated with hydrogen were kept constant at their equilibrium values with the LINCS algorithm,³⁷ and water geometry was also constrained using the SETTLE algorithm.³⁸ All simulations were performed for 200 ns and data were collected every 10 ps.

Potential of mean force (PMF)

The PMF profiles of the fresh and aged MoS₂ nanosheets along the z-direction perpendicular to the membrane surface were calculated using umbrella sampling simulations.³⁹⁻⁴¹ The distance (d) to the center of the membrane was restrained at a reference distance (d₀) with a harmonic force

$$F = k \times (d - d_0)$$

where k is the force constant (2000 kJ mol⁻¹ nm⁻²). The spacing of the sampling windows was 0.1 nm. For each d₀, the system was equilibrated for 2 ns, followed by a 10-ns productive run. The free energy profiles were obtained by the Weighted Histogram Analysis Method.⁴²⁻⁴⁴

ACKNOWLEDGEMENT

We thank Mei Feng and Jianxiang Huang for help with the manuscript. This work is partially supported by the National Natural Science Foundation of China (Grants 11574224 and U1967217) and China Postdoctoral Science Foundation (Grant 2019M652069 and 2019T120506). R.Z. also acknowledges the financial support from W. M. Keck Foundation (Grant award 2019-2022).

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Figures

Table 1 . Force field parameters for the fresh and aged MoS₂ nanosheets, respectively. (Derived

			$\sigma(\text{nm})$	$\varepsilon \text{ (kJ/mol)}$	Charge
Fresh	MoS ₂	Mo	0.2551	0.5441	0.76
		S	0.3550	1.6744	-0.38
Aged	MoS ₂	Mo	0.2551	0.5441	0.76
		S	0.3550	1.0450	-0.38

from our previous work¹⁷⁻¹⁸)

Figure 1. The simulation model of MoS₂ nanosheet on the POPE lipid membrane in a water box. Molybdenum and sulfur atoms are displayed by pink and yellow spheres, respectively. The

phospholipids are represented in blue lines with P atoms as red lines. Sodium and chlorine ions are shown as green and purple spheres, respectively.

Figure 2. Last snapshots of the fresh MoS₂ nanosheet (a) and aged MoS₂ nanosheet (b) on the POPE lipid membranes from three independent trajectories at 200 ns.

Figure 3. The number of contact between the fresh (a)/aged (c) MoS₂ nanosheets and POPE lipid membranes along 100 ns simulations. The binding conformations of the fresh (b) and aged (d) MoS₂ nanosheets to POPE lipid membranes at four key time points.

Figure 4. The interaction energies (including vdW and Coul energies) between the fresh (a) and aged (b) MoS₂ nanosheets and lipid membrane during simulations. (c) The number of contact between fresh/aged MoS₂ nanosheets and head/tail groups of lipid membranes. (d) The distance between the center of mass (COM) of the MoS₂ nanosheets (fresh vs. aged) and lipid membranes at equilibrium.

Figure 5. Potential of mean force (PMF) curve showing the free energy as the MoS₂ nanosheet moving along the Z-axis direction, normal to the membrane surface.