Effects of Low-level Lipid Peroxidation on the Permeability of Nitroaromatic Molecules across a Membrane: A Computational Study

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Abstract: Lipid peroxidation (LPO) in cellular membranes can cause severe membrane damage and potential cell death. Although oxidized phospholipids have been proved to lead to great changes in the structures and properties of membranes, effects of low-level LPO on membrane permeability have not yet been fully understood. Here, we explored the molecular mechanism of low-level LPO changing the permeability of nitroaromatic molecules across a lipid bilayer by all-atom molecular dynamics simulations. The results reveal that the enhanced passive transport of nitroaromatic molecules lies in the size of defects (i.e., water “finger” and “cone”), which is further dependent on the extent of LPO and the structural feature of solutes. In detail, if the solute can form more hydrogen bonds with water, which stabilizes the water into a large-size cone, there is a greater permeability coefficient (P). Otherwise, a small-size finger only results in a small increase of P. For example, the presence of 15% oxidized lipids could result in an increase of 2,4,6-trinitrotoluene (TNT’s) P by more than 2 orders of magnitude (from 1.7 × 10−2 to 2.39 cm·s−1). The result suggests that the membrane permeability can be greatly promoted in the physiologically relevant environment with low-level LPO, and more importantly, clarifies the contributions of both the hydrophobicity of the membrane interior and the structural feature of solutes to such enhanced permeability. This work may provide significant insight into the toxic effects of nitroaromatic molecules and the pharmaceutical characteristics of tissues with oxidative damage.

Introduction

Lipid peroxidation (LPO), which results from an oxidative attack on the unsaturated acyl chains of lipids by free radicals,1,2 is involved in a number of diseases such as atherosclerosis,3 cancer,4 and neurodegenerative disorders.5 LPO can strongly modify membrane structures. In detail, oxidized lipid chains can readily bend toward the membrane-water interface,7,8 leading to the variation of membrane properties, for example, an increase of the area per lipid, a decrease of the membrane thickness, and a disorder of lipid tails.9–12 In addition, Siani et al. found that the presence of hydroperoxized phospholipids could facilitate the lateral diffusion of lipids and significantly reduced the stretching modules of membranes.13 Boonnoy et al. observed a stable water pore in the 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine (PLPC) bilayer with 50% aldehyde product and further formation of micelle at higher concentrations of oxidized lipids in the simulations up to 1 μs.9,14 These structural variations can further affect membrane functions, such as resulting in an increase of permeability.7,13,15,16 Yusupov and co-workers reported that the activation free energy of four reactive oxygen species generally decreased in 50% oxidized lipid bilayers.17 The flip-flop rates of α-

Tocopherols in the oxidized bilayers are proved to be up to an order of magnitude larger than that in the pure lipid bilayer.15 Recently, Su et al. also found that the translocation rate of small nanoparticles was strongly enhanced upon LPO, without the formation of water pores.19

In the physiological or pathological environment, the extent of LPO is relatively low due to the presence of numerous antioxidants and saturated fatty acids that are not susceptible to peroxidation. Davis et al.18 found that the ratio of truncated oxidation products to the parent phosphatidylcholine was only 0.1–2% during in vitro oxidation of purified human low-density lipoprotein. Besides, the results of mass-spectrometric analysis also showed that about 0.1–10% lipids were oxidized in several types of cells triggered to apoptosis.21 Under such conditions, the integrity of lipid bilayers can be well maintained and the membrane will not undergo large deformation such as the formation of pores or micelles.22 Accordingly, Wong-Ekkabut et al.7 found that the water permeability did not show a great change when the LPO ratio...
(i.e., the concentration of oxidized lipids) of 11.1 mol % was introduced. However, Runas et al.22 found that the LPO ratio of 2.5–10 mol % could result in increased passive permeability of fluorescent molecules by 1 order of magnitude. In addition, Volinsky et al.23 also reported a notably enhanced flip-flop rate (3–4 orders of magnitude) of lipid molecules in the presence of 20 mol % oxidized lipids, without the formation of water pores. Recent work focusing on plasma species has reported that the oxidation of the lipid bilayer (without pore formation) did not strongly affect the free-energy barriers of NO, NO2, N2O4, O3, and O4 whereas it reduced the barriers of OH, HO2, and H2O2, and therefore increasing their translocation probability across the membrane.24 These results suggested how the low-level LPO (<20%) affects the passive diffusion of solute molecules across a membrane still remain controversial and has yet been fully understood. To the best of our knowledge, although the changes of membrane permeability induced by LPO have been extensively studied, the cause of enhanced permeation remains to be fully identified. A lot of literature studies explained this enhancement with respect to the lipid membrane, for example, increased disorder of lipids, spontaneous formation of water pores,25,26 and decreased hydrophobicity of the membrane interior,32 whereas neglected the contribution of the structural feature of solutes, which may result in the different influence of LPO on the permeation, especially passive permeation without the water pore of different solute molecules.

Passive diffusion, while not the only pathway for small molecules to cross a membrane, is also a generic mechanism by which drugs and environmental toxins can enter a cell.27,28 2,4,6-trinitrotoluene (TNT) is one of the most widely used explosives in civil and military fields. As a class C carcinogen rated by the Environmental Protection Agency, TNT can cause cataracts, hemolytic crisis, urinary tract infection, reproductive toxicity, and kidney and liver tumors.29 Moreover, a number of studies showed that oxidative stress (or LPO) in the metabolic process of nitro groups was one of the main factors of TNT to result in diseases.30,31 Therefore, the full understanding of the interactions between TNT (as well as its metabolites) and the lipid bilayers containing oxidized lipids is crucial to clarify its toxic effects. In the present work, we focused on the effect of low-level LPO on the passive diffusion of TNT and its metabolized products, 2-amino-4,6-dinitrotoluene (2A) and 2,4-diamino-6-nitrotoluene (24DA), across a membrane composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipids (Figure 1). In addition, this work has universal significance. We revealed the essence of low-level LPO promoting the permeation of small (amphiphilic) molecules across a membrane, which may qualitatively explain the above-mentioned contradictory results.

### RESULTS AND DISCUSSION

**Effects of Oxidized Lipids on Structural Properties.**

During our long-time unbiased molecular dynamics (MD) simulations (300 ns), no water pore was observed for both oxidized and nonoxidized membranes, which indicated that the integrity of lipid bilayers was well preserved in the presence of 5–15 mol % oxidized phospholipids (Figure S2). This result accorded with previous observations, in which the POPC membrane was still stable even after 20 mol % POxnoPC was introduced.23 Meanwhile, the membrane structures have undergone slight changes (Table 1). For example, the area per lipid (APL) increased from 63.0 (pure membrane) to 66.9 Å² (the membrane with the oxidation ratio of 15%, 15% system), and the corresponding thickness of the bilayer decreased by 7.6% from 3.94 to 3.64 nm. The order parameter of lipid tails had a similar change trend (Table 1). We also noted that the average order parameters of the sn-2 chain of alOX were around −0.04 to −0.03, in a very good agreement with previous simulations.7 All of the above results also indicated that our all-atom force field parameters could well describe the reorientation of the oxidized lipid chain in the membrane and are reliable.

![Figure 1. POPC molecule, its oxidation product, and the solutes evaluated in this study.](Image)

The mass densities of POPC lipids (oxidized and non-oxidized), water, and aldehyde groups along the bilayer normal (z-direction) are displayed in Figure 2. After alOXs were introduced into the lipid bilayer, their hydrophilic aldehyde groups would leave the hydrophobic region and reach the membrane–water interface (Figure 2c), which would further lead to a decrease of membrane thickness (Figure 2a). We also found that the mass density of lipids in the central region slightly increased, which could be attributed to the interfacial of lipids in different leaflets. To observe the integrity of oxidized lipid bilayers, we also calculated the mass density profiles of water molecules (Figure 2b). The results showed that the water molecules could permeate deeper into the bilayer, which suggested the increased water permeability, but the mass density of water in the hydrophobic core was still zero, indicating the well-preserved integrity of the membrane and the passive transport of nitroaromatic molecules at the corresponding LPO levels.

**Potential of Mean Force (PMF) Profiles.** Although the bilayer was intact, the structural variations would still affect the membrane permeability. To quantitatively describe this affection, we calculated the potential of mean force (PMF) profiles (Figure 3) of TNT and its two metabolized products (2A and 24DA) across the oxidized bilayer. For comparison, the PMF profiles of these molecules across the nonoxidized POPC bilayer were also taken from our previous study.32 PMF profiles showed that these solutes (TNT, 2A, and 24DA) could spontaneously enter into the membrane interior from the water phase; however, there was an obvious barrier in the membrane center. The results clearly demonstrated that the pure barrier (ΔGpure, equal to the free-energy difference from the water phase to the position of the free-energy maximum) is markedly decreased upon the low-level LPO, implying the weakened barrier function of the membrane and the increased permeability of solute molecules.23 In detail, ΔGpure of TNT across the pure membrane is about 15.9 kJ·mol⁻¹, and this
value decreases to 5.3 kJ·mol⁻¹ by 10.6 kJ·mol⁻¹ when alOXs of 15% were introduced into the natural bilayer. It should be stressed that in the system with a lower oxidation ratio (10 and 5%), the value of $\Delta G_{PB}$ for TNT also drops by 5.3 and 8 kJ·mol⁻¹, respectively. The similar results were also observed for 2A and 24DA; in these systems, the decrease of 6–8 kJ·mol⁻¹ was found. We noted that in the case of 24DA, the most significant variation of $\Delta G_{PB}$ occurred in the system with 10% alOXs (10% oxidized membrane), which may be rationalized by the close contact of 24DA with aldehyde groups in the oxidized lipid tails in this system (Figure S3, see later analysis). The calculation of free energy clearly showed that the permeability of nitroaromatic exogenous compounds can be changed even if the degree of lipid peroxidation is low.

**Permeability Coefficient.** To quantitatively estimate the permeability variation of TNT, 2A, and 24DA in the presence of different concentrations of alOXs, the inhomogeneous solubility-diffusion model was utilized to calculate the permeability coefficient ($P$). $P$ describes the ratio between the flux through the membrane ($J$), and the solute concentration difference ($\Delta C$) across it,33

$$P = \frac{J}{\Delta C},$$

and represents the permeation speed through the membrane. The results are presented in Figure 4. We found that the permeability coefficients of these nitroaromatic compounds were generally enhanced by around 1–2 orders of magnitude, suggesting the notable promotion of their capability to transfer across the lipid membrane and enter cells, even if only 5% POPC lipids were oxidized. Interestingly, the variations of $P$ ($\Delta P$) between different solutes also showed a remarkable discrepancy. For example, the value of $P$ of TNT increased by more than 2 orders of magnitude, from $1.7 \times 10^{-2}$ cm·s⁻¹ (pure POPC bilayer) to $2.39$ cm·s⁻¹ (15% oxidized system). The result of 24DA also showed an increase of about 1–2 orders of magnitude, from $6.9 \times 10^{-2}$ cm·s⁻¹ (pure POPC bilayer) to 1.11 cm·s⁻¹ (10% oxidized system). However, the

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**Table 1. APL, Thickness, and $S_{CD}$ for sn-1 and sn-2 Chains of POPC and alOX in the Systems at Different alOX Concentrations**

| system | APL (Å²) | thickness (nm) | $S_{CD}$ |
|--------|----------|--------------|----------|
|        | sn-1-POPC | sn-2-POPC | sn-1-alOX | sn-2-alOX |
| 0      | 63.0 ± 1.2 | 3.94 ± 0.06 | 0.185     | 0.140     |
| 5%     | 64.3 ± 1.2 | 3.84 ± 0.06 | 0.172     | 0.133     |
| 10%    | 65.5 ± 1.2 | 3.74 ± 0.06 | 0.161     | 0.122     |
| 15%    | 66.9 ± 1.3 | 3.64 ± 0.06 | 0.154     | 0.114     |

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**Figure 2.** Mass density profiles of lipid (a), water (b), and the terminated aldehyde groups (c) as a function of the distance along the bilayer normal ($z$-direction) from the center of bilayers. The black line in (c) suggests the density of unsaturated C=C double bonds in the pure POPC bilayer. The arrows indicate the changing trend. The snapshot is the conformation of oxidized POPC lipids. The water molecules and lipid tails are not shown for clarity. P and N atoms in lipid heads are shown in blue. The tails of the selected oxidized lipids are colored in white. The aldehyde groups are represented by red spheres.

**Figure 3.** PMF profiles along the normal of the membrane ($z$-direction) as a function of the distance from the bilayer center for (a) TNT, (b) 2A, and (c) 24DA in the presence of different concentrations of alOXs.
change of 2A was relatively small, with only an increase of less than 1 order of magnitude (from 0.99 to 6.75 cm s$^{-1}$) even in the presence of 15% oxidized lipids. To the best of our knowledge, the permeability coefficients of TNT or its metabolites have not yet been reported, but some simulations and experimental works have investigated the effects of low-level LPO on the permeability of water molecules, lipids, and fluorescent molecules.$^{7,22,23}$ Although Wong-Ekkabut et al.$^7$ observed that the permeability of water molecules increased by 1–2 orders of magnitude at high concentrations of oxidized lipids (50 mol %), the results in the presence of lower concentrations of oxidized lipids (11.1 mol %) did not show a great change of water permeability (only 2–4 times larger), regardless of the type of oxidized molecules (with truncated or nontruncated chains). Moreover, experiments of giant unilamellar vesicles (GUV) demonstrated that the presence of hydroperoxy-POPC (POPC–OOH) did not show any apparent change in the water permeability during the photo-oxidation process.$^{24}$ However, a remarkably promoted lipid penetration has been demonstrated by the fluorescence and MD simulations, in which the free-energy difference of a single lipid from the aqueous phase to the membrane center decreased by about 5 kJ mol$^{-1}$, corresponding to an increased permeability coefficient of 3–4 orders of magnitude.$^{25}$ In addition, GUV experiments also showed that the permeability coefficients of fluorescent molecules through the POPC membrane would increase by about 1 order of magnitude in the presence of 10 mol % of POxnoPC.$^{22}$ These theoretical and experimental results suggest that the effect of LPO on the permeability of the membrane might be related to not only the changes of membrane properties but also the feature of solutes. The results presented in our present simulations also showed that the variations of permeability of different solute molecules were different.

Water Defects in the Membrane Interior. Here, we did not observe the significant changes in the structural parameters of the membrane (e.g., thickness, APL), so it was deduced that structural changes (e.g., looser packing) were unlikely to be the main cause of the great enhancement of membrane permeability. Therefore, we further explored the factors leading to the increased permeability, mainly focusing on the water defects (i.e., the water molecules enter into the hydrophobic tail region of the membrane, including the water pore, water “finger”, water “cone”, and so on). Water pores have been considered an important factor contributing to the enhancement of permeability for lipids and water molecules.$^{23,25}$ As shown in Figure 5, the radial distribution function (RDF)

Figure 5. Radial distributions of water molecules around the aldehyde groups in the oxidized lipid tails for the systems with different concentrations of alOXs.

provided clear evidence for the favorable interactions of water molecules with the aldehyde groups in the oxidized lipid tails. Due to the high mobility of aldehyde groups,$^6$ water molecules could easily enter into the membrane interior by following the aldehyde groups in the oxidized lipid tails and formed defects.

To further describe the permeation of water molecules into the membrane in the presence of oxidized lipids, we calculated the number of water molecules residing in the low-density tail region ($|z| < 0.5$ nm) as a function of simulation time in different systems (Figure 6), based on the 300 ns unbiased simulations. The results showed that the capability of water molecules entering into the hydrophobic core of the membrane increased with increasing concentration of alOXs. To give a quantitative insight, we defined a parameter, occupancy ratio of water molecules ($R_w$), which was given by

$$R_w = \frac{t_0}{t}$$

where $t_0$ is the time in which the low-density tail region was occupied by more than or equal to one water molecules, $t$ refers to the total simulation time (i.e., the last 100 ns of total simulation time, the first 200 ns was treated as system equilibrium). Surprisingly, the results (Figure 6) showed that even the concentration of oxidized lipids (alOXs) as low as 5% could lead to a great increase of $R_w$ by a factor of around 10 (from 0.42 to 4.9%), suggesting the notably increased probability of the formation of defects in the hydrophobic core of the membrane. It should be noted that the tail region was the main barrier for the transmembrane process of TNT, 2A, and 24DA (see also Figure 3). These defects would decrease the hydrophobicity of the tail region and further resulted in increased permeability. The result could well explain the generally enhanced permeability of solutes, even though only 5% of oxidized lipids were introduced and the structures of the membranes were not significantly altered.

Moreover, to search the origin of different permeability variations of TNT, 2A, and 24DA, the behaviors of these solutes at the bilayer center (with free-energy maximum) were also explored. In the pure POPC membrane without oxidized lipids, no stable water molecules were found in the central region for all species due to the hydrophobicity of the bilayer.
interior (data not shown). However, in the binary mixed bilayers with alOXs, we observed that there were some water molecules stably residing in the neighboring region of solutes at the bilayer center (last for tens of nanoseconds). Moreover, these stable water molecules were also distinct, which could be classified as a single water molecule (Figure 7i), water finger (a string includes 2–4 water molecules which connect the solute and membrane–water interface, Figure 7e), and water cone (a cone consists of water molecules, which connected the solutes and membrane–water interface, usually including more than 5 water molecules, Figure 7c).

In the case of TNT in the membrane with 5% of alOXs (TNT-5% system), we did not observe the formation of any stable water molecules (Figure 7a). However, when the concentration of alOXs increased to 10%, the single water molecule was observed to be captured by the neighboring solutes (Figure 7b), and a stable water cone was found in the TNT-15% system (Figure 7c). For compound 2A, the water finger was found for all of the three systems in spite of various concentrations of alOXs (Figures 7d–f). In addition, the results of 24DA also demonstrated the formation of a water finger in the 24DA-10% system (Figure 7h) and the single water molecule in the 15%-24DA system (Figure 7i). Furthermore, the results also indicated that the size of stable defects (i.e., the number of water molecules contained in the defects) might be relevant to the extent of permeability enhancement of solutes. For example, in the TNT-15% system (i.e., the formation of water cone), the $P$ of TNT increased by 2 orders of magnitude (Figure 4a), whereas only an increase of around 1 order of magnitude was obtained for 2A and 24DA (Figure 4b,c), in these cases the water finger or a single water molecule was observed. This result could be well rationalized by the further decline of local hydrophobicity of the membrane interior induced by the water cone with a larger size. One point worth emphasizing was that in the 10% oxidized system, 24DA induced a larger stable defect (i.e., a water finger) than the 5 or 15% systems. The snapshots showed that 24DA closely contacted with an aldehyde group in the oxidized lipid tails in the 10% oxidized system (see Figure S3). This close contact, which was not observed in 24DA-5 or 24DA-15% system, could promote the penetration of water molecules into membranes and further result in a larger decrease of the free-energy barrier, as presented in Figure 3c. The RDF analysis of solutes at the bilayer center with water molecules quantitatively described the different capabilities of solutes to stabilize the surrounding water molecules (see Figure S4).

In the biased simulations, the constrained force on the solutes may have a significant effect on the interactions between nitroaromatic molecules and water defects. To confirm the above results, we used the result of biased simulations, in which the solutes are constrained at the center of the bilayer as the initial structures to perform additional 120 ns unbiased MD simulations. We only consider the 15% systems and conclude that other systems have similar results. As plotted in Figure S5, the solutes (TNT, 2A, and 24DA) do not easily reach the center of the bilayer in 120 ns of unbiased MD simulations. The snapshots corresponding to the closest distance suggest that TNT can also lead to the formation of water cone without a constrained force, which is not observed for other molecules, in excellent agreement with the results from biased simulations.

Thus, it was inferred that the effect of oxidized lipids on the passive membrane permeation of nitroaromatic molecules had two aspects: (1) the oxidized lipid chain reversal could increase the permeation of water molecules into the hydrophobic core of membranes, which would lead to the generally enhanced

Figure 7. Typical snapshots of TNT (a–c), 2A (d–f), and 24DA (g–i) at the center of the membrane with 5–15 mol % of alOXs. The N and P atoms in lipid heads are colored in yellow, and the water molecules are represented by cyan lines or cyan spheres.
permeability of solutes; (2) after the solutes were introduced into the hydrophobic region of membranes (e.g., the bilayer center), the strong, attractive interactions between solutes and water molecules could bring the neighboring transient water molecules to be stable. The stable water molecules would further reduce the local hydrophobicity of the membrane interior and were conductive for solutes to overcome the corresponding free-energy barrier. Recently, Razzokov et al.\textsuperscript{24} observed that LPO did not strongly affect the passive permeability of NO, NO\textsubscript{2}, N\textsubscript{2}O\textsubscript{4}, O\textsubscript{2}, and O\textsubscript{3}, while significantly increased the permeation of more hydrophilic OH, HO\textsubscript{2}, and H\textsubscript{2}O\textsubscript{2}, which, based on our conclusions, can be well explained by the stronger hydrogen bonding ability of the latter molecules. We deduced that the above results were nonspecific and might also be applicable to other amphiphilic molecules, at least the stable water molecules have been observed in the passive transport of amphiphilic phospholipids through the oxidized POPC membrane by Volinsky and co-workers.\textsuperscript{23}

**Orientation of Solutes at the Center of Membrane.**

The above results showed that the different capabilities of solutes to interact with water molecules in the hydrophobic core of the membrane (or the capability to stabilize the neighboring water molecules) might be the crucial factor to result in the discrepancy for the permeability of different solutes. Then, we also explored the reason why the water cone was only observed in the case of the 15%-TNT system. The distributions of tilt angle $\beta$ ($\beta$ was defined as the angle between the normal direction of the benzene ring plane of solute molecules and the $z$-direction of the system) were calculated for all of the solutes as displayed in Figure 8. It was suggested that the normal direction of the benzene ring plane of TNT preferred to be perpendicular to the $z$-direction at the center of the nonoxidized membrane. Whereas in the system containing 15\% alOXs, the normal direction of the benzene ring plane of TNT readily became parallel to the $z$-direction, which ensured that all three nitro groups of TNT could be in close contact with water molecules. We deduced that the ability of TNT to reorient inside the membrane caused the larger capability to stabilize the surrounding water molecules, and so resulted in the formation of a water cone. This reorientation was not found for the systems containing 2A or 24DA. Instead, the perpendicular orientation was maintained in these cases, which could be attributed to the different abilities of functional groups ($-\text{NO}_2$ and $-\text{NH}_2$) to bind water molecules.\textsuperscript{32} These observations were also supported by the snapshots presented in Figure 7. It should be noted that the reorientation of solutes is unlikely to be a necessary factor for the significant promotion of passive permeability under low-level lipid peroxidation. Based on our present result, we inferred that if the interaction between solutes and water molecules (mainly H-bonds) is stronger enough to induce the formation of water cone or even water pore, a remarkably enhanced permeability is also expected.

**CONCLUSIONS**

In this work, we performed all-atom simulations to investigate the effect of low-level lipid peroxidation (LPO, 5−15 mol \%) on the passive permeability of three nitroaromatic molecules (TNT and its two metabolites) through lipid bilayers and the corresponding mechanisms at the molecular level. The polar moiety of oxidized lipids (the aldehyde group in this work) at
their terminals can strongly modify membrane structures and their biological functions due to their reversal. Consequences similar to previous studies included the increased area per lipid, bilayer thinning, and decrease of lipid tail order parameter. Additionally, we focused on the permeability changes of nitroaromatic molecules and their nature.

PMF profiles reveal the different-level decrease of the free-energy barrier (5.3–10.6 kJ·mol⁻¹) in the hydrophobic core of bilayers for these nitroaromatic compounds (TNT, 2A, and 24DA), which corresponds an increase of permeability coefficients (P) by 1–2 orders of magnitude. Meanwhile, the variation degree of P shows a remarkable discrepancy for different solutes. For example, the presence of 15 mol % oxidized lipids can lead to an improvement of more than 2 orders of magnitude in the permeability of TNT, while only an increase of less than 1 order of magnitude was observed for 2A permeability even at the same concentration of oxidized lipids.

The introduction of oxidized lipids is followed by the deeper penetration of water molecules into the hydrophobic core of the membrane and the formation of defects, and further leading to an increase of the permeability of nitroaromatic molecules. More importantly, the role of structural features in the enhanced permeability of solutes induced by LPO has been qualitatively estimated, and we reveal that the degree of permeability enhancement depends not only on the concentration of oxidized lipids but also on the structural characteristics of solutes. Specifically, at the same concentration of oxidized lipids, the increase in permeability of solutes capable of forming a larger-size water cone or water pore is greater than that of solutes which can only form a smaller-size water cone or water pore is greater than oxidized lipids, the increase in permeability of solutes capable of even a single water molecule, which is dependent on the characteristics of solutes. Specifi-

### METHODS

**System Preparation.** In this work, 128 phospholipid molecules of POPC (Figure 1) were used to build the model of pure lipid bilayers by the CHARMM-GUI bilayer builder, which was hydrated by around 13 000 TIP3P water molecules. Lipid bilayers were oriented perpendicular to the z-axis of the simulation box. The final size of the box was about 6.4 × 6.4 × 12.7 nm³, and the periodic boundary conditions were applied in all directions. The ionic concentration was set to 0.15 M NaCl.

To introduce the LPO, the binary mixture systems were constructed by replacing various numbers of POPC lipids with one of its principal oxidative products (aLOX, with an aldehyde functional group, Figure 1) in the model of lipid bilayers. The concentrations of aLOX were chosen as 5, 10, and 15 mol % to represent the low-level LPO. These systems containing oxidized lipids were built using the Packmol package. The all-atom CHARMM36 force field was utilized to describe POPC molecules. The parameters of nitroaromatic molecules were obtained from the CHARMM General force field (CGenFF). The missing force field parameters of aLOX were obtained from quantum chemistry calculations by the Gaussian program, and the detailed process and parameters are provided in the Supporting Information.

**MD Simulations.** To remove the unreasonable contacts, the steepest-descent algorithm was first utilized for energy minimization. After 1 ns pre-equilibrium, 300 ns NPT simulations were performed for all model membranes, and the last 100 ns was used to calculate the properties of the membrane. All MD simulations were performed using GROMACS software (version 5.1.2). The simulations were carried out with time steps of 2 fs in conjunction with constraints applied to all bonds involving hydrogen by the LINCS algorithm. The SETTLE algorithm was chosen to constrain the bond length and angle of water. Electrostatic interactions were solved by the particle mesh Ewald (PME) algorithm, and van der Waals interactions were switched to zero smoothly between 1.0 and 1.2 nm. A velocity rescale thermostat was used to maintain the temperature at 300 K with a coupling constant of 0.2 ps. Semi-isotropic pressure at 1 bar was controlled by the Parrinello–Rahman barostat with a coupling constant of 2 ps and compressibility of 4.5 × 10⁻⁵ bar⁻¹. The trajectory was stored at every 20 ps. The snapshots were produced by the VMD 1.9.2.

**Potential of Mean Force (PMF) and Permeability Coefficient.** The calculation of free-energy (ΔG) profiles (or potential of mean force, PMF) was performed using the umbrella sampling method. Due to the symmetry of lipid bilayers, we only calculated the free-energy differences of solute molecules transferring from the aqueous phase (z = 4 nm) to the bilayer center (z = 0 nm). A total of 32 windows were divided, with the space of 0.2 nm in the aqueous phase (from 4 to 2.2 nm) and 0.1 nm inside the membrane (from 2.1 to 0 nm). The initial structures were obtained from steered molecular dynamics (SMD). Each window was run for 50 ns in the NPT ensemble, and the first 30 ns was treated as equilibrium.

Considering that the membrane was an inhomogeneous environment, the inhomogeneous solubility-diffusion model, which has been proved to capture the permeation of small molecules, was utilized to calculate the permeability coefficient (P) of all of the compounds. In this model, the permeability coefficient can be calculated by

\[ P = \left( \int_{-4 \text{nm}}^{4 \text{nm}} \frac{\Delta G(z)/RT}{D(z)}dz \right)^{-1} \]  

where \( \Delta G(z) \) and \( D(z) \) are the free energy and diffusion coefficient at a given z-position from the bilayer center, \( \Delta G(z) \) is obtained from umbrella sampling calculations, and \( D(z) \) can be calculated according to the force autocorrelation method using the following equations

\[ D(z) = \frac{(RT)^2}{\int_0^\infty \langle \Delta F(z, t)\Delta F(z, 0) \rangle dt} \]

\[ \Delta F(z, t) = F(z, t) - F(z, 0) \]

where R is the gas constant, T is the temperature (K), \( F(z,t) \) is the constrained force on the solute at a given z-position along the z-direction.

**Key Parameter Calculations.** The parameters of the area per lipid (APL) and the deuterium order parameter (\( S_{CD} \)) of hydrocarbon tails were computed to explore the effect of the
LPO ratio on the structural properties of the membrane as follows

$$A_{PL} = 2 \frac{L_x L_y}{N_{lipid}}$$

$$S_{CD} = \frac{1}{2} (3 \cos^2 \theta - 1)$$

$L_x$ and $L_y$ is the box length in the $x$- and $y$-direction, respectively, and $N_{lipid}$ is the total number of lipids (including the alox) in the bilayer. $\theta$ is the angle between the C$-$$D$ (C$-$$H$ in the present simulations) vector and the bilayer normal. The brackets indicate the ensemble average. The thickness of the membrane is considered as the distance of the center of the mass of phosphorus atoms in the two leaflets.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03462.

2. Notes

The authors declare no competing financial interest.

**REFERENCES**

1. Ayala, A.; Muñoz, M. F.; Argéuelles, S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid. Med. Cell. Longevity* **2014**, *No*. 360438.
2. Yin, H.; Xu, L.; Porter, N. A. Free Radical Lipid Peroxidation: Mechanisms and Analysis. *Chem. Rev.* **2011**, *111*, 5944–5972.
3. Berliner, J. A.; Heinecke, J. W. The Role of Oxidized Lipoproteins in Atherosclerosis. *Free Radical Biol. Med.* **1996**, *20*, 707–727.
4. Spitterl, G. The Relation of Lipid Peroxidation Processes with Atherosclerosis: A New Theory on Atherosclerosis. *Mol. Nutr. Food Res.* **2005**, *49*, 999–1013.
5. Wu, R. P.; Hayashi, T.; Cottam, H. B.; Jin, G.; Yao, S.; Wu, C. C. N.; Rosenbach, M. D.; Corr, M.; Schwab, R. B.; Carson, D. A. Nf2 Responses and the Therapeutic Selectivity of Electrophilic Compounds in Chronic Lymphocytic Leukemia. *Proc. Natl. Acad. Sci.* **2010**, *107*, 7479–7484.
6. Simonian, N. A.; Coyle, J. T. Oxidative Stress in Neurodegenerative Diseases. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 83–106.
7. Wong-Ekkabut, J.; Xu, Z.; Triampo, W.; Tang, I. M.; Tielman, D. P.; Monticelli, L. Effect of Lipid Peroxidation on the Properties of Lipid Bilayers: A Molecular Dynamics Study. *Biophys. J.* **2007**, *93*, 4225–4236.
8. Neto, A. J.; Cordeiro, R. M. Molecular Simulations of the Effects of Phospholipid and Cholesterol Peroxidation on Lipid Membrane Properties. *Biochim. Biophys. Acta, Biomembr.* **2016**, *1858*, 2191–2198.
9. Boonnoy, P.; Jarerattanachat, V.; Karttunen, M.; Wong-ekkabut, J. Bilayer Deformation, Pores, and Micellation Induced by Oxidized Lipids. *J. Phys. Chem. Lett.* **2015**, *6*, 4884–4888.
10. Khandelia, H.; Mouritsen, O. G. Lipid Gymnastics: Evidence of Complete Acyl Chain Reversal in Oxidized Phospholipids from Molecular Simulations. *Biophys. J.* **2009**, *96*, 2734–2743.
11. Jurkiewicz, P.; Ołzyńska, A.; Cwiklik, L.; Conte, E.; Jungwirth, P.; Megli, F. M.; Hof, M. Biophysics of Lipid Bilayers Containing Oxidatively Modified Phospholipids: Insights From Fluorescence and EPR Experiments and From MD Simulations. *Biochim. Biophys. Acta, Biomembr.* **2016**, *1858*, 2498–2511.
12. Jarerattanachat, V.; Karttunen, M.; Wong-Ekkabut, J. Molecular Dynamics Study of Oxidized Lipid Bilayers in NaCl Solution. *J. Phys. Chem. B* **2013**, *117*, 8490–8501.
13. Siani, P.; Souza, R. M. D.; Dias, L. G.; Itri, R.; Khandelia, H. An Overview of Molecular Dynamics Simulations of Oxidized Lipid Systems, with a Comparison of Elba and Martini Force Fields for Coarse Grained Lipid Simulations. *Biochim. Biophys. Acta, Biomembr.* **2016**, *1858*, 2498–2511.
14. Boonnoy, P.; Karttunen, M.; Wongekkabut, J. Alpha-Tocopherol Inhibits Pore Formation in Oxidized Bilayers. *Phys. Chem. Chem. Phys.* **2017**, *19*, 5699–5704.
15. Kunimoto, M.; Inoue, K.; Nojima, S. Effect of Ferrous Ion and Ascorbate-Induced Lipid Peroxidation on Liposomal Membranes. *Biochim. Biophys. Acta* **1981**, *646*, 169–178.
16. Chatterjee, S. N.; Agarwal, S. Liposomes as Membrane Model for Study of Lipid Peroxidation. *Free Radical Biol. Med.* **1988**, *4*, 51–72.
17. Yusupov, M.; Van, D. P. J.; Neyt, E. C.; Bogaerts, A. Synergistic Effect of Electric Field and Lipid Oxidation On the Permeability of Cell Membranes. *Biochim. Biophys. Acta, Gen. Subj.* **2017**, *1861*, 839–847.
18. Boonnoy, P.; Karttunen, M.; Wong-ekkabut, J. Does a-Tocopherol Flip-Flop Help to Protect Membranes Against Oxidation? *J. Phys. Chem. B* **2018**, *122*, 10362–10370.
19. Su, C.; Merlitz, H.; Thalmann, F.; Marques, C.; Sommer, J. Coarse-Grained Model of Oxidized Membranes and their Interactions with Nanoparticles of Various Degrees of Hydrophobicity. *J. Phys. Chem. C* **2019**, *123*, 6839–6848.
20. Davis, B.; Koster, G.; Douet, L. J.; Scigelova, M.; Woffendin, G.; Ward, J. M.; Smith, A.; Julia Humphries, K. G. B. C.; et al. Electrospray Ionization Mass Spectrometry Identifies Substrates and Products of Lipoprotein-Associated Phospholipase A in Oxidized Human Low Density Lipoprotein. *J. Biol. Chem.* **2008**, *283*, 6428–6437.
21. Tyurin, V. A.; et al. Mass-Spectrometric Analysis of Hydroperoxy- And Hydroxy-Derivatives of Cardiolipin and Phosphatidylserine in Cells and Tissues Induced by Pro-Apoptotic and Pro-Inflammatory Stimuli. *J. Chromatogr. A* **2009**, *1267*, 2863–2872.
22. Runas, K. A.; Malmstadt, N. Low Levels of Lipid Oxidation Radically Increase the Passive Permeability of Lipid Bilayers. *Soft Matter* **2015**, *11*, 499–505.
23. Volinsky, R.; Cwiklik, L.; Jurkiewicz, P.; Hof, M.; Jungwirth, P.; Kinnunen, P. K. Oxidized Phosphatidylcholines Facilitate Phospholipid Flip-Flop in Liposomes. *Biophys. J.* **2011**, *101*, 1376–1384.
24. Razzkov, J.; Yusupov, M.; Cordeiro, R. M.; Bogaerts, A. Atomic Scale Understanding of the Permeation of Plasma Species across Native and Oxidized Membranes. *J. Phys. D: Appl. Phys.* **2018**, *51*, No. 365203.
25. Lee, H.; Malmstadt, N. Effect of Low Levels of Lipid Oxidation on the Curvature, Dynamics, and Permeability of Lipid Bilayers and
their Interactions with Cationic Nanoparticles. J. Phys. D: Appl. Phys. 2018, 51, No. 164002.

(26) Van der Paal, J.; Neys, E. C.; Verlackt, C. C. W.; Bogaerts, A. Effect of Lipid Peroxidation On Membrane Permeability of Cancer and Normal Cells Subjected to Oxidative Stress. Chem. Sci. 2016, 7, 489–498.

(27) Shinoda, W. Permeability across Lipid Membranes. Biochim. Biophys. Acta, Biomembr. 2016, 1858, 2254–2265.

(28) Dickson, C. J.; Hornak, V.; Pearlstein, R. A.; Duca, J. S. Structure-Kinetic Relationships of Passive Membrane Permeation from Multiscale Modeling. J. Am. Chem. Soc. 2017, 139, 442–452.

(29) U.S. Agency for Toxic Substances and Disease Registry, Toxicological profile for 2,4,6-trinitrotoluene (TNT), ATSDR publication CAS 118-96-7 (ATSDR, Atlanta, 1995), www.atsdr.cdc.gov/toxprofiles/tp.asp?id=677&tid=125.

(30) Zitting, A.; Szumańska, G.; Nickels, J.; Savolainen, H. Acute Toxic Effects of Trinitrotoluene On Rat Brain, Liver and Kidney: Role of Radical Production. Arch. Toxicol. 1982, 51, 53–64.

(31) Kunimoto, M.; Neuronal Nitric Oxide Synthase (nNOS) Catalyzes One-Electron Reduction of 2,4,6-Trinitrotoluene, Resulting in Decreased Nitric Oxide Production and Increased nNOS Gene Expression: Implication for Oxidative Stress. Free Radical Biol. Med. 2004, 37, 350–357.

(32) Yang, H.; Li, H.; Liu, L.; Zhou, Y.; Long, X. Molecular Simulation Studies On the Interactions of 2,4,6-Trinitrotoluene and its Metabolites with Lipid Membranes. J. Phys. Chem. B 2019, 123, 6481–6491.

(33) Ghaemi, Z.; Alberga, D.; Carloni, P.; Laio, A.; Tartanzi, G. Permeability Coefficients of Lipophilic Compounds Estimated by Multiscale Molecular Simulations. J. Chem. Theory Comput. 2016, 12, 4093–4099.

(34) Weber, G.; Kissell, T.; Baptista, M. S.; Uchoa, A. F.; Pavani, C.; Junqueira, H. C.; Guo, Y.; Baulin, V. A.; Itri, R.; Marques, C. M.; Schroder, A. P. Lipid Oxidation Induces Structural Changes in Biomimetic Membranes. Soft Matter 2014, 10, 4241–4247.

(35) Lis, M.; Wizert, A.; Przybylo, M.; Langner, M.; Swiatek, J.; Jungwirth, P.; Cwiklik, L. The Effect of Lipid Oxidation on the Water Permeability of Phospholipids Bilayers. Phys. Chem. Chem. Phys. 2011, 13, 17555–17563.

(36) Jo, S.; Kim, T.; Iyer, V. G.; Im, W. CHARMM-GUI: A Web-Based Graphical User Interface for CHARMM. J. Comput. Chem. 2008, 29, 1859–1865.

(37) Reis, A.; Domingues, M. R.; Amado, F. M.; Ferrer-Correia, A. J.; Domingues, P. Separation of Peroxidation Products of Diacyl-Phosphatidylcholines by Reversed-Phase Liquid Chromatography-Mass Spectrometry. Biomed. Chromatogr. 2005, 19, 129–137.

(38) Martinez, L.; Andrade, R.; Birgin, E. G.; Martinez, J. M. Packmol: A Package for Building Initial Configurations for Molecular Dynamics Simulations. J. Comput. Chem. 2009, 30, 2157–2164.

(39) Feller, S. E.; MacKerell, A. D. An Improved Empirical Potential Energy Function for Molecular Simulations of Phospholipids. J. Phys. Chem. B 2000, 104, 7510–7515.

(40) Vanommeslaeghe, K.; Hatcher, E.; Acharya, C.; Kundu, S.; Zhong, S.; Shim, J.; Darian, E.; Guvench, O.; Lopes, P.; Vorobyov, I. CHARMM General Force Field (CGenFF): A Force Field for Drug-Like Molecules Compatible with the CHARMM All-Atom Additive Biological Force Fields. J. Comput. Chem. 2010, 31, 671–690.

(41) Zenzie, G.; Saam, J.; Schulten, K.; Tajkhorschid, E.; Gumbart, J. C. Rapid Parameterization of Small Molecules Using the Force Field Toolkit. J. Comput. Chem. 2013, 34, 2757–2770.

(42) Frisch, M. J. et al. Gaussian 09; Revision D.01. Gaussian Inc.: Wallingford CT, 2009.

(43) Van Der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. GROMACS: Fast, Flexible, and Free. J. Comput. Chem. 2005, 26, 1701–1718.

(44) Hess, B. P.-LINCS: a Parallel Linear Constraint Solver for Molecular Simulation. J. Chem. Theory Comput. 2008, 4, 116–122.

(45) Miyamoto, S.; Kollman, P. A. Settle: An Analytical Version of the Shake and Rattle Algorithm for Rigid Water Models. J. Comput. Chem. 1992, 13, 952–962.

(46) Darden, T.; York, D.; Pedersen, L. Particle Mesh Ewald: An N- log(N) Method for Ewald Sums in Large Systems. J. Chem. Phys. 1993, 10, 1059–1062.

(47) Darden, T.; York, D.; Pedersen, L. Particle Mesh Ewald: An N- log(N) Method for Ewald Sums in Large Systems. J. Chem. Phys. 1993, 10, 1059–1062.