1. INTRODUCTION

An important stage of the mechanism of action of any drug molecule is the diffusion through cellular membranes. The favored positions of these drug molecules within the target membranes also affect their transport and metabolism. The most important membrane encountered by drug molecules is the plasma membrane into which these pharmaceuticals are inserted into two different fully hydrated phospholipid bilayers, namely, dioleoylphosphatidylcholine (DOPC) and dipalmitoylphosphatidylcholine (DPPC), to investigate the effects that these drugs have on the bilayer. We determined the role of the lipid chain length and saturation on the behavior of the two drugs. Pure, fully hydrated DOPC and DPPC bilayers were also simulated, and the results were in excellent agreement with the experimental values. Various structural and mechanical properties of each system, such as the area per lipid, area compressibility modulus, order parameter, lateral lipid diffusion, hydrogen bonds, and radial distribution functions, have been calculated to assess how the drug molecules affect the different bilayers. From the calculated results, we show that fentanyl and propofol generally follow similar trends in each bilayer but adopt different favorable positions close to the headgroup/chain interface at the carbonyl groups. Propofol was shown to selectively form hydrogen bonds at the carbonyl carbon in each bilayer, whereas fentanyl interacts with water molecules at the headgroup interface. From the calculated free-energy profiles, we determined that both molecules show a preference for the low-density, low-order acyl chain region of the bilayers and both significantly preferred the DOPC bilayer with propofol and fentanyl having energy minima at ~6.66 and ~43.07 kcal mol$^{-1}$, respectively. This study suggests that different chain lengths and levels of saturation directly affect the properties of these two important molecules, which are seen to work together to control anesthesia in surgical applications.
have also been studies that suggest that propofol acts at G protein-coupled receptors by inhibiting the function of M1 muscarinic acetylcholine receptors.9 These studies suggest possible binding sites of propofol, but a complete mechanism for the action of anesthesia still remains unknown. Due to its properties, fentanyl is used in general surgery as the analgesic for the action of anesthesia still remains unknown. Due to its anesthetic action.14 #Gloebacter violaceus work showed that fentanyl also modulates the ability to potentiate propofol during anesthesia. Our previous simulations of propofol and fentanyl in the center of each bilayer also shown inTable 1. Here, we see an increase in the area per lipid calculated for each pure bilayer simulation are shown in Table 1. The results obtained were within around 3% of the experimental values from the literature, which suggests that our simulated pure bilayers are in the correct phase and have equilibrated sufficiently to be used for the drug molecule simulations. The results for bilayers containing propofol are also shown in Table 1. Here, we see an increase in the area per lipid when propofol is simulated in the center of each bilayer (0.25 Å in DOPC and 0.27 Å in DPPC) due to the drug molecule interacting in the upper chain region around the carbonyl groups at the headgroup–chain interface. When propofol was simulated in the water phase, we observed natural diffusion into both types of lipid bilayers, leading to a larger

Table 1. Averaged Structural Properties of Pure and Drug-Containing Lipid Bilayer Systems, Where Area per Lipid = (A_L), Volume per Lipid = (V_L), Isothermal Area Compressibility Modulus = (K_A), Bilayer Thickness = (D_{HH}), and Lateral Lipid Diffusion Coefficient = (D_{xy})

| lipid         | drug     | A_L (Å²) | V_L (Å³) | K_A (m Nm⁻¹) | D_{HH} (Å) | D_{xy} (10⁻⁵ cm² s⁻¹) |
|---------------|----------|----------|----------|-------------|------------|---------------------|
| DOPC experimental | none     | 67.4, 72.5 | 1303     | 265, 318  | 35.3, 37.1 | 11.5, 17.1         |
| DOPC calculated | none     | 70.1 ± 0.2 | 1274.2 ± 1.1 | 285.2 ± 19.7 | 37 ± 0.2 | 6.30                |
| DPPC experimental | none     | 63.1, 64.3 | 1232     | 231 ± 21 | 38, 38.17 | 12.5, 15.2         |
| DPPC calculated | none     | 62.9 ± 0.3 | 1175 ± 0.2 | 231.9 ± 22.7 | 37.8 ± 0.2 | 8.8                |
| DOPC          | propofol center | 70.3 ± 0.3 | 1275 ± 1.5 | 419.5 ± 15.6 | 36.25 ± 1.1 | 12.75              |
| propofol water | 70.94 ± 0.2 | 1279.7 ± 1.0  | 333 ± 30.1 | 36.75 ± 0.5 | 11.25                  |
| fentanyl center | 70.33 ± 0.2 | 1261.7 ± 1.3 | 227.6 ± 16.0 | 36.5 ± 1.0 | 7.9                    |
| fentanyl water | 70.2 ± 0.4 | 1262.3 ± 1.3 | 274 ± 20.5 | 37.25 ± 0.2 | 6.4                    |
| DPPC          | propofol center | 63.17 ± 0.4 | 1176.8 ± 1.5 | 319.5 ± 22.7 | 37 ± 0.7 | 17.5                |
| propofol water | 63.50 ± 0.5 | 1177.8 ± 0.9 | 243.1 ± 16.0 | 37.25 ± 0.9 | 14                    |
| fentanyl center | 63.58 ± 0.4 | 1179 ± 1.1  | 236.2 ± 25.0 | 37.5 ± 0.8 | 8.3                    |
| fentanyl water | 62.86 ± 0.6 | 1179 ± 1.0  | 331.2 ± 13.0 | 37.25 ± 0.70 | 6.4                    |

“Water and center refer to the starting positions of the drug molecule.”

Figure 1. Molecular structures of (a) propofol, (b) fentanyl, (c) dipalmitoylphosphatidylcholine, and (d) dioleoylphosphatidylcholine. The first and second carbons of the glycerol fragment are denoted as the sn-1 and sn-2 positions, respectively.

2. RESULTS AND DISCUSSION

2.1. Area per Lipid. The area per lipid is considered to be one of the most important properties to describe the behavior of the bilayer and whether it is in the correct, biologically relevant L₅ phase. This area can be calculated easily from our MD trajectories. The calculation involves dividing the area of the bilayer by the number of lipids.10,11 The areas per lipid calculated for each pure bilayer simulation are shown in Table 1. The results obtained were within around 3% of the experimental values from the literature, which suggests that our simulated pure bilayers are in the correct phase and have equilibrated sufficiently to be used for the drug molecule simulations. The results for bilayers containing propofol are also shown in Table 1. Here, we see an increase in the area per lipid when propofol is simulated in the center of each bilayer (0.25 Å in DOPC and 0.27 Å in DPPC) due to the drug molecule interacting in the upper chain region around the carbonyl groups at the headgroup–chain interface. When propofol was simulated in the water phase, we observed natural diffusion into both types of lipid bilayers, leading to a larger
increase in area due to disruption in the headgroup regions. This expansion of $A_l$ can be rationalized in terms of the lone-pair repulsions as propofol diffuses through the headgroup region. A similar pattern is observed when fentanyl is in the center of the bilayer as we observe further increases in the area, especially for DPPC. Our simulations of DPPC indicate that fentanyl does not lie as close to the headgroup region as propofol does but rather lies parallel and slightly below the carbonyl groups. We only observed spontaneous diffusion into the DOPC system for fentanyl, which caused a small 0.1 Å increase in the area per lipid. This observation can be explained by fentanyl diffusing in a linear conformation, which causes less disruption, whereas propofol is much more dynamic in its diffusion process, adopting more conformations. The calculated volume per lipid followed the same trend as the area per lipid as they are directly proportional.

### 2.2. Isothermal Area Compressibility Modulus

The isothermal area compressibility modulus ($K_A$) is the stress required to induce an isotropic expansion in volume, which can be calculated from MD simulations as

$$K_A = \frac{2k_BT\langle A_l \rangle}{n_{lipid}\sigma^2_A}$$

where $k_B$ is Boltzmann’s constant, $T$ is the simulation temperature, $\langle A_l \rangle$ is the average area per lipid, $\sigma_A^2$ is the variance in the area per lipid over the simulation, and $n_{lipid}$ is the number of lipids in the simulation box. $K_A$ is a standard descriptor of the membrane phase, with large values being characteristic of the $L_n$ gel phase, as the chains would be fully extended and their van der Waals interactions would strengthen and lead to tighter, more ordered lipid packing.

Our results for the $K_A$ of the pure bilayers are in excellent agreement with the experimentally obtained values. When propofol starts in a position in the center of either membrane and diffuses toward the carbonyl region, we found an increase in $K_A$, which induces a closer packing of the lipid chains. The conformational changes of the membrane chains increase the aforementioned interactions between them, causing the bilayer to stiffen (see Figures 4 and 13). The largest increase in $K_A$ is observed for DOPC, owing to the presence of the double bond in the chain, in which the drug molecule inhibits the fluidity by binding in the upper chain region. During the diffusion process from the water phase into the membrane, we observe a small increase in $K_A$ due to the fast diffusion through the headgroup region. This observation suggests that the process of stiffening is not instantaneous, and the direction of diffusion plays a key role. In the DOPC bilayer, we calculate a lower $K_A$ for fentanyl compared to that for propofol, which indicates that fentanyl is causing more fluctuation within the headgroup region of the bilayer (see Figure 2). For the natural diffusion of fentanyl into the DOPC bilayer, we observe that $K_A$ decreases by a small amount (11.2 m N m$^{-2}$) compared to that of the pure membrane, which shows that the diffusion does not allow the chains to pack particularly tightly together. For fentanyl in the DPPC bilayer, we calculate a small 4.3 m N m$^{-2}$ increase compared to that of the pure membrane, even though the drug molecule positions itself parallel to the headgroups at the interface, as it does in DOPC. As such, the difference in the chain structure plays a significant role in the way that the structural properties of lipid bilayers are affected by drug molecules. The most unexpected result found for fentanyl is that fentanyl is the simulation with the drug molecule in the water phase where we saw no diffusion, even though the resulting $K_A$ shows an increase of 99.3 m N m$^{-2}$ over the pure bilayer. This result is interesting because fentanyl has no direct contact with the lipid chains and spends most of its time during the simulation at the water–headgroup interface. During the course of the simulation, we observe several partial diffusions, where fentanyl enters the headgroup region for 1–4 ns before returning into the water phase. These partial diffusions suggest that fentanyl induces a conformational change in the headgroups, which allows the chains to pack closer together and stiffen the bilayer. To confirm whether there was a change in the conformation of the headgroup environment when fentanyl partially diffuses into the headgroup region of the DPPC bilayer, the angles between the P–N vector and the normal of the bilayer were calculated using the MEMBPLUGIN tool$^{23}$ for VMD.$^{24}$ For the pure DPPC bilayer, an angle of 69.0 ± 2.4° was calculated, which is almost identical in the fentanyl-containing system. To study the specific conformation of the headgroups, which fentanyl causes disruptions when it partially diffuses, headgroup molecules were selected, which were 15 Å from the fentanyl molecule. The P–N vectors were calculated as 73.0 ± 2.0°, which shows that fentanyl causes a conformational change with the headgroup region. Results for propofol are not shown as there was no change in the P–N vector due to the

![Figure 2. Starting positions for fentanyl (A) and propofol (B) in the bilayers. End positions after the production simulation of fentanyl (C) and propofol (D); DOPCs shown as positions were similar in both systems. Headgroups are shown as yellow sticks, headgroup phosphate is shown as an orange sphere, and lipid chains are shown as gray sticks.](image-url)
fast diffusion through the headgroup region into the membrane interior.

2.3. Lipid Lateral Diffusion. The lateral diffusion of a lipid bilayer is an important dynamical property, as it can affect many membrane parameters. For example, one study reported a connection between the lateral lipid diffusion and the viscosities in different parts of the membrane. In this work, we have calculated the lateral diffusion coefficient of the lipids without (for reference) and with the drug molecules, using the mean-squared displacement (MSD) of the membrane, in the \( xy \) direction, over 20 ns window lengths and averaged over time origins separated by 200 ps (Figure 3). This property is related to the Einstein equation in two dimensions:

\[
D = \lim_{t \to \infty} \frac{1}{4t} \langle (|\Delta r(t)|^2) \rangle
\]

where \( \langle (|\Delta r(t)|^2) \rangle \) is the MSD in the \( XY \) plane in time \( t \), and \( D \) represents the lateral diffusion coefficient. The diffusion coefficient can be obtained from the gradient of the linear portion of the MSD plot. Table 1 lists the calculated lateral diffusion coefficients for pure DOPC and DPPC, which are comparable to those calculated in the Lipid14 paper. Our production runs were performed in the NPT ensemble using Langevin dynamics to control the temperature, which randomizes particle velocities and can therefore affect dynamic membrane properties such as lateral diffusion. Slightly more accurate results can be obtained using the microcanonical (NVE) ensemble, which was applied in the original lipid14 publication. However, as our results for the pure bilayers were within the experimentally determined values, we decided to continue the drug molecule simulations in the NPT ensemble. With the addition of propofol within the bilayer, we observe a slight increase in the lateral diffusion coefficient for both lipid systems (Table 1). This finding is unexpected since the drug molecules can penetrate the lipid bilayer where they occupy a portion of the free volume within the membrane and reduce the lateral diffusion.27 This hypothesis is indeed observed when we have a spontaneous diffusion of propofol from the water phase as it occupies the free volume space within the headgroup region, whereas propofol starting within the bilayer does not penetrate fully into the headgroup space. For fentanyl, we observe a significant decrease in the lateral diffusion constant in both systems with respect to propofol. This difference in behavior can be explained by the larger size of the fentanyl molecule compared to that of propofol, which therefore occupies more free volume within the bilayer, hence limiting the fluidity of the membrane.

2.4. Lipid Tail Order Parameters. The hydrocarbon tails that make up the hydrophobic interior of the bilayer are highly dynamic, which accounts for the overall fluidity of the membrane.28 The mobility of these chains at individual carbon positions can be evaluated by measuring the order parameter. A value of \( S_{CD} = 1 \) implies a position parallel to the bilayer normal, and a value of \( S_{CD} = 0 \) implies total random motion. Experimentally, this property is determined by using deuterium NMR quadrupole splitting, which entails substituting the hydrogen atoms at each carbon position with deuterium and measuring their dynamic variations by \(^1H\) NMR. A detailed description of the procedure can be found here. This property can also be calculated from our simulations, where the carbon–deuterium order parameter is determined by the tensor \( S \) in the equation:

\[
S = \frac{1}{2} (3 \cos^2 \theta - 1)
\]

where \( \theta \) represents the angle formed between the \( Z \) direction and the bilayer normal. Simulated results for the pure bilayers are shown in Figure 4, with comparison to literature values. Our results obtained for the pure bilayer simulations are in good agreement with the trends observed experimentally. Figure 5 shows the results obtained for the bilayers containing the drug molecules. For the DOPC sn-1 chain, we observe very little change when the drug molecules are present compared to the pure bilayer. A very small increase is observed between carbons 3 and 6, with respect to the pure bilayer, which suggests a slightly restricted motion of the chains at this position due to the presence of the two drug molecules. A small decrease in the order parameter for fentanyl between carbons 10 and 11 suggests more mobility at these positions.
This behavior is also seen in the sn-2 chain for both anesthetic molecules, although note the lower values in carbons 12–14, which could account for the reduced area compressibility calculated for fentanyl due to the chains becoming more flexible and hence requiring less force to induce an isotropic expansion.

In the DPPC system, we see clearer differences between the drug-loaded bilayers compared to that between the pure bilayer reference. In the sn-1 chain, we observe that propofol increases the order parameter from carbons 5 to 12, reducing the chain mobility and hence requiring more force to induce an isotropic expansion within the bilayer, in agreement with \( K_A \). The introduction of fentanyl causes only a negligible change in the order parameter of the pure DPPC bilayer. The sn-2 chain shows a clear difference in the 6–13 region compared to the pure membrane. Propofol causes a higher-order parameter for these carbons, as seen in the sn-2 chain, whereas fentanyl shows lower values at this point, which would suggest a more flexible chain and hence a lower \( K_A \). Table 1 shows that there is no correlation between the lipid chain mobility and \( K_A \), which indicates that the flexibility in the headgroup region could be the link between them. We should note here that a similar study conducted on the local anesthetic articaine in a DMPC bilayer found higher-order parameter values for the neutral form of the molecule, similar to propofol in the DPPC bilayer. The differences observed in that study are larger, which is most likely due to the higher concentration of drug molecules used in the simulations.

### 2.5. Hydrogen Bonds

Hydrogen bonds were observed between the drug molecules and water, and propofol was also observed to form hydrogen bonds with the headgroups of both lipid systems. The conditions used to determine if a hydrogen bond was formed were set by a bond distance of 2.5 Å between the donor hydrogen and the acceptor atom with a maximum angle of 30° between the donor hydrogen and acceptor hydrogen vectors. Figure 6a shows that the pattern of hydrogen bonding to water is similar in both bilayer systems. Hydrogen bonds were mostly formed between the hydrogen of the propofol hydroxyl group and the oxygen of the water molecules. A few times during the simulations, the conformation allowed the hydroxyl group to coordinate with the propofol hydroxyl oxygen. The average number of hydrogen bonds formed per frame was similar for DOPC (0.120) and DPPC (0.119). Figure 6b shows the hydrogen-bonding pattern for fentanyl in both bilayers. Hydrogen bonds are formed faster within the DPPC bilayer as this is the most fluid membrane (Table 1, \( D_{xy} \)), which allows the drug molecule to move faster toward the interface with water and hence form hydrogen bonds. Fentanyl has two extra possible hydrogen-bonding sites, i.e., carbonyl oxygen and both nitrogens, compared to propofol that has only one (hydroxyl oxygen) site, which explains the different number of hydrogen bonds formed. Fentanyl forms bonds between both the carbonyl oxygen and the neighboring nitrogen to water hydrogen. No bonds are formed to the...
piperidine nitrogen due to steric hindrance and the conformation adopted by fentanyl. Again, we calculate very similar hydrogen bonds in each bilayer, i.e., 0.758 and 0.715 for DOPC and DPPC, respectively. From Figure 6c, we can see that propofol forms hydrogen bonds to the lipid headgroups in both DOPC and DPPC, which fentanyl is unable to do. The carbonyl oxygen of the sn-1 chain is favored in both lipids over the sn-2, due to the conformation that the headgroup adopts throughout the simulations, which makes it better accessible for bonding. Figure 6d shows a snapshot from the simulation of DOPC, where we noted a number of water molecules entering the hydrophobic phase and forming “anchoring” hydrogen bonds to fentanyl. This behavior was observed in both bilayers but to a greater extent in the DOPC membrane. This phenomenon was not seen with propofol, which could account for its higher mobility within the bilayer compared to that of fentanyl. Table 2 shows the average number of hydrogen bonds per frame between lipid headgroups and water molecules, which is used as a means to assess the effect of the drug molecules on the ability of the headgroups to form hydrogen bonds to the surrounding media. In the DPPC bilayer containing either of the two drug molecules, we observe very little change in the number of hydrogen bonds between the headgroups and water compared to the pure bilayer. The results for the DOPC system show a clear increase in the number of hydrogen bonds formed in the presence of fentanyl. This observation can be explained by the disruptions caused by fentanyl in the headgroup region (Figure 7), which can accommodate more water molecules at the interface, thereby increasing the potential for hydrogen bonding. These results highlight the different behavior observed in the two different PC lipid bilayers despite their close structural similarity.

2.6. Radial Distribution Function. To gain further insight into the distribution of water and the drug molecules around the bilayer, we have carried out radial distribution function (RDF) analyses. Figure 8 shows the RDF plots for the oxygen atom of water around the phosphorous of the headgroup. From the insets of each graph, we can see that when fentanyl is added to the membrane there is a higher density of water molecules around the phosphate group. This difference is slightly larger in the DOPC system, which agrees with the observations that fentanyl causes more water molecules to penetrate into the headgroup region. RDFs were also computed for the atoms in each drug molecule, which exhibited hydrogen bonding to water and, in the case of propofol, the lipid headgroup. Figure 9a displays that water is more concentrated around the carbonyl oxygen of fentanyl in DOPC, with an average hydrogen-bond distance to water of 1.75 Å. The peak for the oxygen-to-oxygen density is a further 1 Å away, which indicates the length of the O−H bond in the water molecule. Figure 9b shows the same trend for DPPC as for DOPC, although the densities for N to H and N to O are closer, with the O-to-O density slightly higher by 0.05. Figure 9c shows the dominance of hydrogen bonding between propofol and the headgroup region. The conformation of the headgroup in DOPC increases the sn-1 lipid chain in such a way that it is better accessible to form hydrogen bonds to propofol. We found a higher density for sn-1 and sn-2 in DPPC due to the differences in chain mobility between the two systems; see Figure 9d. A sharp shoulder peak is observed at 1.95 Å in the propofol DPPC system, which is due to hydrogen bonds forming at slightly different distances.

Table 2. Number of Hydrogen Bonds Formed between Lipid Headgroups and Water

| lipid | drug   | average bond per frame |
|-------|--------|------------------------|
| DOPC  | none   | 464.880                |
|       | fentanyl | 481.300               |
|       | propofol | 467.752               |
| DPPC  | none   | 396.409                |
|       | fentanyl | 397.453               |
|       | propofol | 399.372               |

Figure 7. Multiple water molecules moving through the DOPC lipid headgroup into the hydrophobic phase of the bilayer. Lipid headgroup is shown as yellow sticks, lipid tails are shown as gray sticks, phosphorous atom of the phosphate group is shown as an orange sphere, water molecules within 5 Å of fentanyl are shown as blue spheres, and fentanyl is shown as sticks.
2.7. Clinical Concentration Simulations. By conducting these simulations at clinical concentrations, we can observe how these drug molecules perturb the membrane structure in a way that we cannot observe in the single-molecule simulations. Initially, the molecules were added to the water phase outside of the membrane, and over the course of the simulations, they diffuse into the membrane interior. Figure 10 shows the final positions of the drugs within the membrane, which were very similar in DOPC and DPPC, so only one is shown. The average positions of the drug molecules over the course of the simulations can be calculated with decomposed electron density profiles computed using CPPTRAJ. These profiles (Figure 11) were constructed for each drug molecule in each bilayer using an average over all replicates. Both drug molecules are located predominantly under the lipid headgroups in the membrane interior. This observation is consistent with other molecules that possess anesthetic properties, such as alcohols, benzocaine, and halothane. The two drug molecules prefer slightly different depths within the bilayers, with propofol having a density maximum at approximately 10.3 Å from the bilayer center in both DOPC and DPPC, while fentanyl has a density maximum at...
which allows more space in the headgroup region that can be filled with additional water molecules, thereby increasing the hydration at the headgroup/extracellular region.

To assess how these drug molecules at clinical concentrations alter the membrane ordering and dynamics, we have calculated headgroup tilt angles, $S_{CD}$ order parameters (Figure 13), and lipid lateral diffusions (Table 3). Order parameter plots show little or no changes when single drug molecules were incorporated into the bilayers (Figure 5), but when clinical concentrations are used, we observe more significant changes in the membrane dynamics. The tilt angle of a phospholipid headgroup is an important property because of the dipole moment associated with the zwitterionic headgroup, which is involved in long-range electrostatics, which can affect many of the bilayer properties.33 Drug molecules, which are located in or close to the headgroup region, may cause disruption of this angle, which was observed for lidocaine40 and articaine.33 Table 4 shows the average calculated angles between the P–N headgroup vector and the bilayer normal for two pure reference systems and the systems containing clinical concentrations of the drug molecules. Results are obtained using the MEMBPLUG tool33 for VMD. For these calculations, all lipids were included as opposed to lipids located close to each drug molecule due to the higher number of drug molecules in the systems. The data presented in Table 4 show small influences from the drug molecules, more so for propofol due to the increased number of molecules in the system compared to fentanyl. Results for single-molecule simulations were identical to those for the pure systems, so they are not shown here. More significant results were obtained for the previously mentioned lidocaine and articaine as these molecules are charged, which caused a decrease in the angle by around 20° due to the charged molecules located within the lipid headgroup area, so the positive charge on these molecules causes a repulsion of the choline groups outwards toward the water phase. Both propofol and fentanyl in our simulations are neutral, and they therefore reside in the upper chain region near the ester group and slightly deeper toward the membrane center for fentanyl (Figure 11), so there is no charge–charge interactions to significantly alter the P–N vector. The increased hydration of the headgroups (Figure 12) can therefore be explained by the drug molecules causing separation in the headgroup region, which is seen as an increase in the area per lipid, shown in Table 3, which allows water molecules to penetrate deeper into the headgroup region where hydrogen bonds are formed to the drug molecules.

The calculated order parameters (Figure 13) show that these drugs at clinical concentrations have a significant impact on the dynamics of the lipid chains. For DOPC-containing propofol, we calculate an increase in $K_A$, which suggests a rigid/stiff bilayer, an increase in thickness, and a decrease in lipid lateral diffusion. The $S_{CD}$ plot shows a higher-order parameter in the upper chain region for propofol, more apparent in the sn-1 chain, resulting from the propofol occupying this space, which reduces the density of lipid tails here, causing them to straighten. Similar results are seen for fentanyl, in which $K_A$ and $D_{HH}$ also increase but to a smaller extent compared to those for propofol. The diffusion calculated for fentanyl in DOPC is almost identical to that of the pure system, which suggests that the number of molecules present in the system is a crucial factor in lipid lateral diffusion. The $S_{CD}$ order parameters for carbons 9 and 10 in both DOPC systems show a significant decrease; these carbons form the double bond in the DOPC lipid chain, which is a region where the drug...
molecules do not spend any notable time. We suggest that this decrease in order is due to the positioning of the drugs near the ester groups, which disrupts the conventional packing of the lipid chains. For the DPPC systems, we see similar trends in $K_A$, $D_{HH}$, and $D_{xy}$ compared to the DOPC systems. The upper region of the carbon chains in which propofol resides has an increased order parameter, which suggests stiffening of that region, as seen in the $K_A$ value and lower diffusion coefficients. For fentanyl, we see lower-order parameters for carbon 6 onwards in both the sn-1 and sn-2 chains, suggesting higher-chain mobility in this region, but this is not what we see in the diffusion coefficients, which show a decrease in membrane fluidity.

### 2.8. Potentials of Mean Force

The potential of mean force (PMF) is an important part of the overall membrane permeability, which can be obtained from

$$ R = \frac{1}{P} = \int_{z_1}^{z_2} \frac{\exp[\beta W(z)]}{D(z)} dz $$

where $R$ is the resistivity, $P$ represents the permeability, $\beta$ is the thermodynamic $\beta$ ($\beta = 1/k_BT$), $W(z)$ is the PMF, $D(z)$ is the local diffusivity coefficient, and $z$ is the variable, which describes the position of the solute along the transmembrane axis. The PMFs were calculated by pulling the drug molecule from the center of the membrane into the water phase, which has been shown by Filipe et al.\textsuperscript{41} to give faster convergence of the PMF compared to pulling from the water phase into the membrane. The PMFs were then symmetrized, i.e., the profiles were adjusted to be identical on either side of the bilayer center and both start and end at 0.\textsuperscript{42} Our chosen method of computing the PMFs for our systems was the Umbrella sampling.\textsuperscript{43} This approach involves applying a harmonic potential between the center of mass (COM) of the drug molecule and the bilayer, with a harmonic force constant of 2.5 kcal mol$^{-1}$ rad$^2$. Figure 14 shows the PMF profiles for propofol in DPPC and DOPC. We can see that there is a large difference of 5.54 kcal mol$^{-1}$ between the free energy at $z = 0$ in both bilayers. The negative PMF values for both bilayers

### Table 3. Averaged Structural Properties of Pure and Clinical Concentration Systems, Where Area per Lipid $= (A_L)$, Volume per Lipid $= (V_L)$, Isothermal Area Compressibility Modulus $= (K_A)$, Bilayer Thickness $= (D_{HH})$, and Lateral Lipid Diffusion Coefficient $= (D_{xy})$

| system         | $A_L$ (Å$^2$) | $V_L$ (Å$^3$) | $K_A$ (m Nm$^{-1}$) | $D_{HH}$ (Å) | $D_{xy}$ ($10^{-8}$ cm$^2$ s$^{-1}$) |
|----------------|---------------|---------------|---------------------|--------------|-------------------------------------|
| DOPC pure      | 70.1 ± 0.2    | 1274.2 ± 1.1  | 285.2 ± 19.7        | 37 ± 0.2     | 6.3                                 |
| DPPC pure      | 62.9 ± 0.3    | 1175 ± 0.2    | 231.9 ± 22.7        | 37.8 ± 0.2   | 8.8                                 |
| DOPC propofol  | 76.8 ± 0.4    | 1363.8 ± 2.3  | 666.7 ± 31.0        | 37.4 ± 0.3   | 5.8                                 |
| DOPC fentanyl  | 71.0 ± 0.3    | 1283.4 ± 1.6  | 394.7 ± 15.9        | 37.25 ± 0.2  | 6.4                                 |
| DPPC propofol  | 70.3 ± 0.4    | 1280 ± 0.9    | 312.1 ± 26.0        | 37.7 ± 0.4   | 6.0                                 |
| DPPC fentanyl  | 66.0 ± 0.2    | 1242.2 ± 1.4  | 256.2 ± 14.0        | 37.6 ± 0.3   | 6.4                                 |

### Table 4. Average DOPC and DPPC Angles between the P–N Vector and the Normal of the Bilayers for Pure and Drug-Containing Systems

| system                  | P–N vector angle (deg) | standard error (±) |
|-------------------------|------------------------|--------------------|
| pure DOPC               | 68.88                  | 2.30               |
| propofol DOPC           | 70.60                  | 2.31               |
| fentanyl DOPC           | 70.14                  | 2.30               |
| pure DPPC               | 69.00                  | 2.4                |
| propofol DPPC           | 71.1                   | 2.0                |
| fentanyl DPPC           | 70.02                  | 2.4                |

ACS Omega 2020, 5, 14340−14353 https://dx.doi.org/10.1021/acsomega.0c00813

Figure 13. $S_{CD}$ order parameters for DOPC (top) and DPPC (bottom) containing clinical concentrations of propofol and fentanyl.
indicate that \( z = 0 \) is a favorable position for propofol, which is not surprising as this molecule possesses a lone ionizable hydroxyl group with a \( pK_a \) of 11, while the remaining structural components are highly lipophilic. This high lipophilicity gives a poor water miscibility (150 µg L\(^{-1}\)) and a log \( P \) value of 4.16. As such, we would expect that propofol would be very stable within the hydrophobic, low-density, and low-order acyl chain region. A small dip in the minimum is observed at \( z = \pm 1.0 \) Å in the DOPC system, where the PMF drops to \(-6.66 \) kcal mol\(^{-1}\). A similar but more pronounced pattern is seen in the DPPC system, where the PMF drops to a minimum of \(-1.47 \) kcal mol\(^{-1}\) at 2 Å further away than in DOPC. This difference of 5.19 kcal mol\(^{-1}\) between the minima of the two systems occurs because propofol is closer to the high-density acyl chain region. We found that the higher order in DPPC is caused by the shorter chain length and full saturation, which allows closer packing of the chains. Propofol prefers to reside close to carbon 13 in DPPC and carbon 17 in DOPC at the PMF minima, which explains their energy difference. As propofol traverses toward the water phase, we observe a steep increase in free energy until it enters the water phase at 18 Å where the free energy is set to 0. For DPPC, we do observe a slight increase in free energy within the headgroup region to a maximum of 0.06 kcal mol\(^{-1}\). This is not unusual due to the highly structured nature of the headgroup region, characterized by strong specific interactions that restrict their motion, which can hinder the diffusion of hydrophobic solutes. To investigate this behavior further, we have plotted in Figure 15 the \( z \)-dependent diffusion coefficient profile. From the diffusion profile, we can see the decrease in diffusion within the DPPC system at, and close to, the headgroup region compared to that of DOPC, which accounts for the increase in energy observed compared to that obtained for DOPC. Figure 14 shows a small barrier at 10 Å in the DPPC bilayer, which is most likely due to the excess hydration of the drug molecule, which is overcome as the drug penetrates deeper into the membrane interior; we can see this reflected in Figure 15, where propofol has diffusion coefficient slightly higher than in DOPC. These results show that the difference in chain length and saturation can significantly alter the drug diffusion, suggesting that propofol will be more selective toward the longer and unsaturated part of the cellular membrane.

Figure 16 depicts a trend similar to the lower PMF in the DOPC system for fentanyl compared to that for propofol. The minimum energy occurs when fentanyl is at \( z = 0.0 \) Å with a PMF of \(-43.07 \) kcal mol\(^{-1}\). In the DPPC system, the minimum occurs at \( z = \pm 2.0 \) Å but at 35 kcal mol\(^{-1}\) higher than in DOPC. This result is again expected because fentanyl is a large hydrophobic and lipophilic molecule. We can rationalize the difference in the PMF due to fentanyl’s close proximity to the high-density and less mobile acyl chain region in DPPC. In both lipids, we observe a linear increase in the PMF as fentanyl
traverses from the membrane center into the water phase, where the PMF is defined as 0. Unlike propofol, we do not observe the increase in PMF within the headgroup region for fentanyl in the DPPC system, owing to the linear conformation adopted by fentanyl when it diffuses through the headgroups, as observed in our nonbiased simulations. Figure 17 shows the z-dependent diffusion profile for fentanyl in both bilayers. Our umbrella sampling simulations indicate that fentanyl has a significantly lower diffusion and mobility over the range around 20 Å, leading to a higher PMF. However, the free energy is much lower in DOPC, because fentanyl adopts a more favorable conformation within this less ordered environment. As fentanyl moves into the water phase, we see a large increase in diffusion, which almost reaches the same values as it does in the DPPC bilayer. The diffusion profile of the molecules shows the greatest variance between propofol and fentanyl in this study. We should note here that the center of the bilayers are theoretically the most stable region for both drug molecules, as indicated by the PMF plots, but we have to remember that these simulations are carried out with harmonic restraints in each window. In the unrestrained simulations for single drug molecules and clinical concentrations, the drugs do not reside at the bilayer center during the simulations (Figure 11) but are mostly located at the upper chain region/headgroup interface due to the highly dynamic nature of the lipid chains.

3. CONCLUSIONS

In this study, we have utilized fully atomistic molecular dynamics simulations to model the physical and mechanical properties of pure and drug-containing lipid bilayers. Propofol and fentanyl show similar trends in the way they alter the properties of pure and drug-containing lipid bilayers. Propofol and fentanyl show similar trends in the way they alter the properties of pure and drug-containing lipid bilayers. Propofol and fentanyl were generated using Avogadro51 and geometry optimized using the Amber-GAFF forcefield52 with the steepest decent method. Atom types, bond types, and atomic partial charges were assigned using the AM1-BCC semi-empirical quantum mechanical method53 and GAFF52 forcefields. The TIP3P water model58 was used to describe the water molecules. More in-depth details of the general simulation procedure used can be found in the original lipid14 paper.26 All production simulations were carried out in the isothermal—isobaric (NPT) ensemble, which maintains a constant number of particles, pressure, and temperature. The temperatures were maintained at the lipid-relevant temperature of 303 K for DOPC and 323 K for DPPC to be consistent with the experiment, using the Langevin thermostat59 with a collision frequency of γ = 1.0 ps−1. The pressure was maintained at 1 atm using the anisotropic Berendsen method60 with a pressure relaxation time of 1.0 ps. Three-dimensional periodic boundary conditions with the usual minimum image convention were used. The SHAKE algorithm61 was used to constrain covalent bonds to hydrogen, allowing the use of a 2 fs time step. Electrostatic interactions were treated with the PME method.
using a cutoff of 10 Å. The pure bilayer simulations were run for a total of 125 ns, with the first 25 ns discarded from the final analysis. Simulations containing the drug molecules were run from the equilibrated pure bilayer structure for a total of 100 ns each after a short 5 ns equilibration in which the drug molecules were harmonically restrained to allow equilibration of the solvent or bilayer. The biased MD umbrella sampling simulations were run using the GPU version of AmberTools16,62,63

The drug molecule was pulled from the center of the bilayer into the water phase along the z-axis by steered molecular dynamics (SMD), for a total of 27 Å with a pulling rate of 1 Å per ns. The force constant for pulling was set at 1.1 kcal mol⁻¹ rad², and the force constant for restraint was set at 2.5 kcal mol⁻¹ rad² for the window simulations. From the SMD simulations, 27 windows were extracted (1 Å apart) along the z-axis from the center of the bilayer to the water phase. Each window was further simulated for 50 ns. The potential of mean force (PMF) profile was constructed using the weighted histogram analysis method (WHAM).64 The analysis in this study was performed using the CPPTRAJ35 program implemented into the AmberTools16,65 package, with visualization through tools supplied in VMD.24 For simulations containing drug molecules, the 5 ns equilibration phase was not included in the analysis. In the single drug molecule simulations, lipids in the leaflet, where the drug molecule interacts, were used for the analysis of lipid properties; in the clinical concentration simulations, all lipids were used.

AUTHOR INFORMATION

Corresponding Authors

Christopher Faulkner – School of Chemistry, Cardiff University, CF10 3AT Cardiff, U.K.; orcid.org/0000-0002-5142-8109; Email: FaulknerC3@cardiff.ac.uk

Nora H. de Leeuw – School of Chemistry, Cardiff University, CF10 3AT Cardiff, U.K.; School of Chemistry, University of Leeds, LS2 9JT, U.K.; orcid.org/0000-0002-8271-0545; Email: n.h.deleeuw@leeds.ac.uk

Authors

David Santos-Carballal – School of Chemistry, Cardiff University, CF10 3AT Cardiff, U.K.; School of Chemistry, University of Leeds, LS2 9JT, U.K.; orcid.org/0000-0002-3199-9588

David F. Plant – AWE, RG7 4PR Reading, U.K.

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c00813

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was undertaken using the Supercomputing Facilities at Cardiff University operated by ARCCA on behalf of CSF and Supercomputing Wales. This equipment is part-funded by the Welsh European Funding Office (WEFO), as well as by the University. The authors acknowledge the Engineering and Physical Sciences Research Council (Grant no. EP/R512503/1) and AWE for funding. Information on the data underpinning the results presented here, including how to access them, can be found in the Cardiff University data catalogue at http://doi.org/10.17035/d.2020.0092362446.

REFERENCES

(1) Berka, K.; Hendrychová, T.; Anzenbacher, P.; Otyepka, M. Membrane position of ibuprofen agrees with suggested access path entrance to cytochrome P450 2C9 active site. J. Phys. Chem. A 2011, 115, 11248–11255.

(2) Palonczyová, M.; Berka, K.; Otyepka, M. Convergence of free energy profile of coumarin in lipid bilayer. J. Chem. Theory Comput. 2012, 8, 1200–1211.

(3) Simons, K.; Toomre, D. Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol. 2000, 1, 31.

(4) Vuyk, M. Pharmacokinetic and pharmacodynamic interactions between opioids and propofol. J. Clin. Anesth. 1997, 9, 235–265.

(5) Robinson, B. J.; Ebert, T.; O’Brien, T.; Colinco, M.; Muzi, M. Mechanisms whereby Propofol Mediates Peripheral Vasodilation in Humans Sympathoinhibition or Direct Vascular Relaxation. Anesthesiology 1997, 86, 64–72.

(6) Langley, M. S.; Heel, R. C. Propofol. Drugs 1988, 35, 334–372.

(7) Trapani, G.; Altmare, C.; Sanna, E.; Biggio, G.; Liso, G. Propofol in anesthesia Mechanism of action, structure-activity relationships, and drug delivery. Curr. Med. Chem. 2000, 7, 249–271.

(8) Nagase, Y.; Kaibara, M.; Uezone, Y.; Izumi, F.; Sumikawa, K.; Taniyama, K. Propofol inhibits muscarinic acetylcholine receptor-mediated signal transduction in Xenopus oocytes expressing the rat M1 receptor. Jpn. J. Pharmacol. 2001, 79, 319–325.

(9) Stanley, T. H. The fentanyl story. J. Pain 2014, 15, 1215–1226.

(10) Phillips, A. S.; McMurray, T.; Mirakhr, R.; Gibson, R.; Elliott, P. Propofol-fentanyl anesthesia: a comparison with isoflurane-fentanyl anesthesia in coronary artery bypass grafting and valve replacement surgery. J. Cardiothorac. Vasc. Anesth. 1994, 8, 289–296.

(11) Bajwa, S. J. S.; Bajwa, S. K.; Kaur, J. Comparison of two drug combinations in total intravenous anesthesia: Propofol-ketamine and propofol-fentanyl. Saudi J. Anaesth. 2010, 4, 72.

(12) Petersen, K. D.; Landsfeldt, U.; Cold, G. E.; Petersen, C. B.; Mau, S.; Hauerberg, J.; Holst, P.; Olsen, K. S. Intracranial Pressure and Cerebral Hemodynamic in Patients with Cerebral TumorsA Randomized Prospective Study of Patients Subjected to Craniotomy in Propofol-Fentanyl, Isoflurane-Fentanyl, or Sevoflurane-Fentanyl Anesthesia. Anesthesiology 2003, 98, 329–336.

(13) Chen, J.-C.; Smith, E. R.; Cahill, M.; Cohen, R.; Fishman, J. B. The opioid receptor binding of dezocine, morphine, fentanyl, butorphanol and nalbuphine. Life Sci. 1993, 52, 389–396.

(14) Faulkner, C.; Plant, D. F.; de Leeuw, N. H. Modulation of the Gloeobacter violaceus Ion Channel by Fentanyl: A Molecular Dynamics Study Biochemistry 2019, 58, 4804 4808 DOI: 10.1021/acs.biochem.9b00881.

(15) Dror, R. O.; Dirks, R. M.; Grossman, J.; Xu, H.; Shaw, D. E. Biomolecular simulation: a computational microscope for molecular biology. Annu. Rev. Biophys. 2012, 41, 429–452.

(16) Skjevik, A.; Madej, B. D.; Walker, R. C.; Teigen, K. LIPID11: a modular framework for lipid simulations using amber. J. Phys. Chem. B 2012, 116, 11224–11136.

(17) Kučerka, N.; Nagle, J. F.; Sachs, J. N.; Feller, S. E.; Pencer, J.; Jackson, A.; Katsaras, J. Lipid bilayer structure determined by the simultaneous analysis of neutron and X-ray scattering data. Biophys. J. 2008, 95, 2356–2367.

(18) Evans, E.; Rawicz, W.; Smith, B. Concluding remarks back to the future: mechanics and thermodynamics of lipid biomembranes. Faraday Discuss. 2013, 161, 591–611.

(19) Filippov, A.; Orådd, G.; Lindblom, G. Influence of cholesterol and water content on phospholipid lateral diffusion in bilayers. Langmuir 2003, 19, 6397–6400.

(20) Kučerka, N.; Tristram-Nagle, S.; Nagle, J. F. Closer look at structure of fully hydrated fluid phase DPPC bilayers. Biophys. J. 2006, 90, L83–L85.

(21) Nagle, J. F.; Tristram-Nagle, S. Structure of lipid bilayers. Biochim. Biophys. Acta, Rev. Biomembr. 2000, 1469, 159–195.

(22) Scheidt, H. A.; Huster, D.; Gawrisch, K. Diffusion of cholesterol and its precursors in lipid membranes studied by 1H pulsed field
Simulation-based approaches for determining membrane permeability of small compounds. J. Chem. Inf. Model. 2016, 56, 721–733.

(43) Kästner, J. Umbrella sampling. Wiley Interdiscip. Rev.: Comput. Mol. Sci. 2011, 1, 932–942.

(44) Takita, A.; Masui, K.; Kazama, T. On-line monitoring of end-tidal propofol concentration in anesthetized patients. Anesthesiology 2007, 106, 659–664.

(45) Li, X.; Zhang, Y.; Fan, Y.; Zhou, Y.; Wang, X.; Fan, C.; Liu, Y.; Zhang, Q. Preparation and evaluation of novel mixed micelles as nanocarriers for intravenous delivery of propofol. Nanoscale Res. Lett. 2011, 6, No. 275.

(46) Xiang, T.-X.; Anderson, B. D. Liposomal drug transport: a molecular perspective from molecular dynamics simulations in lipid bilayers. Adv. Drug Deliv. Rev. 2006, 58, 1357–1378.

(47) 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.

(48) Kučerka, N.; Tristram-Nagle, S.; Nagle, J. Structure of fully hydrated fluid phase lipid bilayers with monounsaturated chains. J. Membr. Biol. 2006, 208, 193–202.

(49) Nagle, J.; Wiener, M. Structure of fully hydrated bilayer dispersions. Biochim. Biophys. Acta, Biomembr. 1988, 942, 1–10.

(50) Case, D. A.; Cheatham, T. E.; Darden, T.; Gohlke, H.; Luo, R.; Merz, K. M.; Onufriev, A.; Simmerling, C.; Wang, B.; Woods, R. J. The Amber biomolecular simulation programs. J. Comput. Chem. 2005, 26, 1668–1688.

(51) Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J. Cheminf. 2012, 4, No. 17.

(52) Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. Development and testing of a general amber force field. J. Comput. Chem. 2004, 25, 1157–1174.

(53) Jakalian, A.; Jack, D. B.; Bally, C. I. Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation. J. Comput. Chem. 2002, 23, 1623–1641.

(54) Case, D.; Betz, R.; Cerutti, D.; T.E. Cheatham, I.; Darden, T.; Duke, R.; Giese, T.; Gohlke, H.; Goetz, A.; Homey, N. et al. AMBER. University of California: San Francisco, 2016.

(55) Sall, J. W.; Stratmann, G.; Leong, J.; Woodward, E.; Bickler, P. E. Propofol at clinically relevant concentrations increases neuronal differentiation but is not toxic to hippocampal neural precursor cells in vitro. Anesthesiology 2012, 117, 1080.

(56) Park, K.-E.; Sohn, J.-T.; Jeong, Y. S.; Sung, H.-J.; Shin, I.-W.; Lee, H.-K.; Chung, Y.-K. Inhibitory effect of fentanyl on phenyl-ethynyl-induced contraction of the rat aorta. Yonsei Med. J. 2009, 50, 414–421.

(57) Mai, J. A.; Martínez, C.; Kasavajhala, K.; Wickstrom, L.; Hauser, K. E.; Simmerling, C. ff14SB: improving the accuracy of protein side chain and backbone parameters from ff99SB. J. Chem. Theory Comput. 2015, 11, 3696–3713.

(58) Mark, P.; Nilsson, L. Structure and dynamics of the TIP3P, SPC, and SPC/E water models at 298 K. J. Phys. Chem. A 2001, 105, 9954–9960.

(59) Pastor, R. W.; Brooks, B. R.; Szabo, A. An analysis of the accuracy of Langevin and molecular dynamics algorithms. Mol. Phys. 1988, 65, 1409–1419.

(60) Berendsen, H. J.; Postma, Jr.; van Gunsteren, W. F.; DiNola, A.; Haak, J. Molecular dynamics with coupling to an external bath. J. Chem. Phys. 1984, 81, 3684–3690.

(61) Ryckaert, J.-P.; Ciccotti, G.; Berendsen, H. J. Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J. Comput. Phys. 1977, 23, 327–341.

(62) Salomon-Ferrer, R.; Götz, A. W.; Poole, D.; Le Grand, S.; Walker, B. C. Routine microscopic molecular dynamics simulations with AMBER on GPUs. 2. Explicit solvent particle mesh Ewald. J. Chem. Theory Comput. 2013, 9, 3878–3888.
(63) Le Grand, S.; Götz, A. W.; Walker, R. C. SPFP: Speed without compromise—A mixed precision model for GPU accelerated molecular dynamics simulations. *Comput. Phys. Commun.* 2013, 184, 374−380.

(64) Kumar, S.; Rosenberg, J. M.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A. The weighted histogram analysis method for free-energy calculations on biomolecules. I. The method. *J. Comput. Chem.* 1992, 13, 1011−1021.