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Interactions between Model Cell Membranes and the Neuroactive Drug Propofol

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ABSTRACT. Vibrational sum frequency spectroscopy (VSFS) complemented by surface pressure isotherm and neutron reflectometry (NR) experiments were employed to investigate the interactions between propofol, a small amphiphilic molecule that currently is the most common general anaesthetic drug, and phospholipid monolayers. A series of biologically relevant saturated phospholipids of varying chain length from C₁₈ to C₁₄ were spread on either pure water or propofol (2,6-bis(1-methylethyl)phenol) solution in a Langmuir trough, and the change in the molecular structure of the film, induced by the interaction with propofol, was studied with respect to the surface pressure. The results from the surface pressure isotherm experiments revealed that propofol, as long as it remains at the interface, enhances the fluidity of the phospholipid monolayer. The VSF spectra demonstrate that for each phospholipid the amount of propofol in the monolayer region decreases with increasing surface pressure. Such squeeze out is in contrast to the enhanced interactions that can be exhibited by more complex amphiphilic molecules such as peptides. At surface pressures of 22–25 mN m⁻¹, which are relevant for biological cell membranes, most of the propofol has been expelled from the monolayer, especially in the case of the C₁₆ and C₁₈ phospholipids that adopt a liquid condensed phase packing of its alkyl tails. At lower surface pressures of 5 mN m⁻¹, the effect of propofol on the structure of the alkyl tails is enhanced when the phospholipids are present in a liquid expanded phase. Specifically, for the C₁₆ phospholipid, NR data reveal that propofol is located exclusively in the head group region, which is rationalized in the context of previous studies. The results imply a non-homogeneous distribution of propofol in the plane of real cell membranes, which is an inference that requires urgent testing and may help to explain why such low concentration of the drug are required to induce general anaesthesia.

1. Introduction

Both phospholipids and synthetic surfactants are amphiphilic molecules with distinct polar and non-polar regions, which cause them to self-assemble in aqueous solution. Many different self-assembled structures can form depending on the molecular shape and the interactions, as commonly described using the critical packing parameter¹. Double chained phospholipids typically have a packing parameter close to one, meaning that they preferentially self-assemble into planar structures, such as lamellar phases and biological membranes. In contrast, single chain surfactants in excess water tend to self-assemble into structures such as spherical or rod-like micelles. When such surfactants encounter a phospholipid membrane, they can be incorporated and as a result change the curvature of the membrane². In the presence of sufficient surfactant(s), this often leads to the complete disruption of the biological membrane. Much is known about this important topic, as described in the excellent review by Heerklotz³.

Many low molecular weight drugs, like classical surfactants, contain both hydrophobic and hydrophilic groups. However, due to their lower molecular weight and less distinct separation into hydrophobic and hydrophilic regions, the self-assembly of such drugs in aqueous solutions is less easy to describe in general terms. It is commonly observed that this type of drug interacts with phospholipid membranes. This is most often not the cause of the wanted effect of the drug, but it has rather been suggested to cause some of the unwanted toxic effects⁴. To gain more understanding of the interaction between such amphiphilic drugs with phospholipid membranes, we focus the present study on one particular drug molecule, propofol, which is predominantly hydrophobic but contains a hydrophilic OH-group located in the middle of the molecule (see Figure 1). Propofol (2,6-bis(1-methylethyl)phenol) is a useful intravenous agent with several important applications⁵. It is probably most well-known for inducing general anaesthesia. Even

though it is more than 150 years since general anaesthesia was introduced in medical surgery practice⁶, the molecular mechanism by which drugs can produce this state is still under debate⁷⁻⁹, and occasions remain when standard dosing of such agents fails to induce a reliable amnesic state^{10, 11}.

In the case of propofol, several mechanisms of action^{12, 13} have been proposed, where potentiating GABA_A (gamma aminobutyric acid type A) receptors^{7, 14, 15} is the most acknowledged one. The binding site of propofol to the GABA_A protein is in the transmembrane region¹⁶, suggesting that the amphiphilic nature of propofol is of importance for its action, and the presence of propofol can possibly lead to perturbations of the molecular order of the lipid membrane¹⁷. For instance, Tsuchiya¹⁸ studied a series of structural analogues to propofol and found that propofol is the most effective in fluidizing model membranes.

Propofol has also been shown to have antioxidant, anxiolytic, analgesic, immunomodulatory and anticancer effects^{5, 19}. Thus, being widely used as an intravenous agent, it is of importance to have a thorough knowledge about how propofol interacts with cell membranes, and it has been suggested that it penetrates the lipid bilayer forming the plasma membrane²⁰. However, it remains unclear how these interactions occur on a molecular level.

The location of a drug in a monolayer represents another important clue in its mode of interaction. A previous study on pre-mixed sample of propofol with a saturated phosphatidylcholine found that the drug lowers the compressibility of the monolayer, especially at low surface pressures, that it is located close to the head group of the phospholipid due to hydrogen bonding with the phosphate, and that at the same time it increases the order in the alkyl tail region¹⁷.

The aim of our study is to obtain a more extensive molecular picture of interactions between propofol and different phospholipid membranes with respect to their fluidity. To this end, we have chosen to use model phospholipid monolayers at the air-water interface as a first proxy for biological membranes. While a monolayer can behave differently to a bilayer as the flexibility and the hydrophobicity of the air differs from that of the headgroups of the lipid, this approach minimizes substrate interactions²¹ associated with supported lipid bilayers, and is more straightforward to increase complexity of the system in terms of mixtures of phospholipids as well as additional components such as cholesterol and sphingomyelin in future studies. We have used the surface specific laser technique vibrational sum frequency spectroscopy (VSFS) to investigate propofol-induced changes in the molecular order in both the hydrophilic head group region and the hydrophobic tail region of three saturated phosphatidylcholines of different tail lengths (C₁₄, C₁₆, and C₁₈). Moreover, the propofol itself has been monitored in addition to the water molecules hydrating the lipids and the propofol. The studies have been performed at a surface pressure of 22–25 mN m⁻¹, to mimic a biological membrane²², as well as at 5 mN m⁻¹, in order to gain understanding of how the surface pressure affects the ability of propofol to interact with phospholipids. To learn about the specific location of propofol in a phospholipid monolayer we also performed measurements on the C₁₆-phospholipid using neutron reflectometry (NR) to resolve the location of the drug in the model monolayer.

2. Experimental and Theoretical Methods

2.1 Materials

Propofol (European Pharmacopoeia) was bought from Sigma Aldrich with a purity higher than 99%. 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-distearoyl-

d70-*sn*-glycero-3-phosphocholine-1,1,2,2-d₄-N,N,N-trimethyl-d₉ d-DSPC, 1,2-dipalmitoyl-d₆₂-*sn*-glycero-3-phosphocholine-1,1,2,2-d₄-N,N,N-trimethyl-d₉ d-DPPC, 1,2-dipalmitoyl-d₆₂-*sn*-glycero-3-phosphocholine d₆₂-DPPC, and 1,2-dimyristoyl-d₅₄-*sn*-glycero-3-phosphocholine-1,1,2,2-d₄-N,N,N-trimethyl-d₉ d-DMPC were purchased from Avanti Polar Lipids with purity higher than 99%, and were used as received. Their respective chemical structures are seen in Figure 1.

Figure 1. The chemical structures of the studied phospholipids and propofol.

Biological cell membranes contain both well-ordered and more fluid domains, and three lipids have been studied in order to elucidate the effect of propofol on both ordered and disordered monolayers, with DSPC (C₁₈) forming highly ordered structures, while DMPC (C₁₄) forms disordered layers. DPPC (C₁₆) is in between the two. The short tail saturated phospholipid DMPC was used rather than phospholipids with unsaturated fatty acid chains (which are common in cell membranes) to avoid oxidation of the double bond in contact with air, as it has

been noticed in our previous work²³⁻²⁴. However, the short chain DMPC still possesses a highly disordered structure, similarly to the unsaturated phospholipids (e.g. POPC) in cell membranes, and serves, thus, as a good substitute.

2.2 Experimental Protocols

In order to investigate phospholipid monolayers at various surface pressures a KSV MiniMicro Langmuir trough made of TeflonTM (with Derlin® hydrophilic barriers) was used in all experiments except NR, in which a Nima trough was used. Spreading solutions were prepared using chloroform (> 99.8%, Sigma Aldrich), and at least 5 min were allowed for chloroform evaporation before the experiments started. After spreading the monolayer either on pure water or 0.89 mM propofol solution, the Delrin (polyoxymethylene) barriers were compressed at a constant rate of 6 mm/min. Compression measurements for DSPC, d-DSPC, DPPC, and d-DPPC were carried out at 21°C, while for DMPC and d-DMPC the temperature was 26°C to ensure that the experiments were performed above the transition temperature occurring slightly above 20°C^{25, 26}. The surface pressures reported for mixed propofol/phospholipid systems are relative to a surface pressure of zero for the pure propofol solution, which itself has a surface pressure of 15 mN m⁻¹. The concentration of 0.89 mM propofol was chosen because the slope of the surface tension isotherm is constant, which indicates maximum surface coverage, yet the plateau has not been reached thus solubility issues resulting in beads of propofol sitting on the liquid surface are circumvented.

2.3 Vibrational Sum Frequency spectroscopy (VSFS)

Our VSFS instrument has been described in detail before^{24, 27}, and only a brief description is provided here. A Nd:YAG laser (PL-2251A-20, Ekspla) generates a 1064 nm wavelength beam at 20Hz, which is sent to an optical parametric generator/optical parametric amplifier (OPG/OPA

– LaserVision). Here two beams are generated: a visible beam at a fixed wavelength of 532 nm and an IR beam tunable over the wavenumber region 1 000–4 000 cm⁻¹ (approx. 20–400 μJ). The beams are subsequently overlapped on the sample surface (approx. 1 mm in diameter) to produce the sum frequency (SF) beam bearing the signature of the interfacial molecules. The SF beam passes through a monochromator and detected by a photomultiplier tube before the signal is processed using a computer where the SF intensity is normalized to the product of the intensities of the incoming visible and IR beams²⁸ in order to account for beam intensity fluctuations.

The recorded sum frequency intensity, I_{VSF} , is proportional to the square of the molecular number density multiplied with the orientationally averaged molecular hyperpolarizability $\langle\beta^{(2)}\rangle$ of the probed moieties. For a molecule to be VSFS active it has to be both IR and Raman active, according to equation 1:

$$\beta_{abc}^{(2)} = \frac{\alpha_{ab}\mu_c}{\omega_n - \omega_{\text{IR}} - i\Gamma_n} \quad (1)$$

where a , b and c are the molecular coordinates, α_{ab} the Raman polarizability tensor, μ_c the transition IR dipole moment, ω_n the peak position frequency, ω_{IR} the irradiating infrared frequency, Γ_n the damping constant of the n^{th} resonant mode and i the imaginary unit.

In order to access orientational information about the probed molecular species spectra with different polarizations of the three beams are needed, where S and P denote the polarizations perpendicular to and parallel with the plane of incidence, respectively. In the present case the spectra were acquired in the SSP polarization combination, where the repeat letters refer to the polarization of the SF, visible and IR beams, respectively.

2.4 Neutron Reflectometry (NR)

NR measurements were performed using the horizontal neutron reflectometer FIGARO at the Institut Laue-Langevin (Grenoble, France)^{29, 30}. The technique provides a measure of the ratio of

the number of neutrons in the specular reflection to those in the incident beam with respect to the momentum transfer, q_z ,

$$q_z = \frac{4\pi\sin\theta}{\lambda} \quad (2)$$

where θ is the angle of incidence and λ is the neutron wavelength³¹. Data were recorded at $\theta = 0.62^\circ$ and 3.78° with chopper pulses of 7 % $\delta\lambda/\lambda$, and wavelengths with a range of 3–22 Å were used to generate the reduced data. The background was subtracted from these data as a result of use of the area detector. The data analysis was carried out using Motofit³². More information about the modeling approach and the parameters used is described in the Supporting Information, and an extended description of the application of the technique to systems at fluid interfaces can be found in a recent review of the subject area³⁰.

The interfacial structure of the adsorbed molecules at the air-water interface was resolved by comparing different structural models to data recorded in 4 isotopic contrasts: d_{62} -DPPC/ D_2O , d_{62} -DPPC/ACMW, DPPC/ D_2O and DPPC/ACMW, as in the approach used in ref.³³, where ACMW is air contrast matched water, i.e. 8.1% v/v D_2O in H_2O . Data were recorded first on pure lipid monolayers at 5 mN m⁻¹, and were then recorded on lipid monolayers spread on 0.89 mM propofol solution until the surface pressure was raised by 5 mN m⁻¹.

The pure lipid monolayers were modeled using 2 interfacial layers: alkyl tails with a volume fraction of 1, and hydrated head groups with a volume fraction calculated in such a way as to preserve an equal number of tails and head groups. Inter-layer roughness values of 3.0 Å from capillary waves were included in the model. The monolayers with propofol were modeled by comparing 3 different locations of the drug: in the same layer as the lipid alkyl tails, in the same layer as the lipid head groups, and in a third layer under the lipid head groups. These 3 models were selected as being physically realistic possibilities as a result of the different driving forces

associated with the interaction. More information about the modeling approach and the parameters used is described in the Supporting Information.

3. Results and discussion

3.1 Surface pressure isotherm experiments

The surface pressure isotherms for DSPC, DPPC, and DMPC monolayers on an aqueous subphase in the absence and presence of 0.89 mM propofol are shown in Figure 2. The recorded surface pressure isotherms of these phospholipids in the absence of propofol are in agreement with data reported in the literature³⁴⁻³⁷. Briefly, upon compression DSPC goes directly from the so-called gaseous phase, where patches of ordered molecules are present in the monolayer²⁴, to a condensed phase where the molecules are packed closely together over the entire surface.

On the contrary, the surface pressure isotherm for DPPC shows the presence of a liquid expanded phase, before a transition to a mixed liquid expanded/liquid condensed co-existence, and then a condensed phase at high surface pressures. Furthermore, the surface pressure isotherm for DMPC, due to its shorter hydrocarbon tail, does not show any condensed phase as the monolayer collapses directly from the liquid expanded phase at approximately 40 mN m⁻¹. In the presence of propofol the mean molecular area for a given low surface pressure is larger for all three phospholipids. This is a clear indication that there is an interaction between propofol and the phospholipid monolayer. The isotherm of DSPC is strongly expanded at low surface pressures. The lift off point is shifted from about 57 Å². On water subphase containing 0.89 mM propofol the surface pressure starts rising at about 130 Å².

The surface pressure increases gradually upon compression until about 10 mN m⁻¹, at which point a condensed phase is achieved. Under this condition the area/molecule is similar to that found in the absence of propofol.

Figure 2. Comparison of A) DSPC, B) DPPC and C) DMPC surface pressure isotherms on a pure water subphase and on a 0.89 mM propofol solution. The surface pressure is defined as the surface tension decrease compared to that of the respective subphase prior to spreading the phospholipid, i.e., without propofol pure water defines the surface pressure 0 mN m⁻¹ and with propofol the pure propofol solution defines 0 mN m⁻¹.

This is the case due to the steep nature of the isotherm in the condensed phase, despite the different definition of zero pressure in the two experiments. This is a strong indication that propofol is removed from the interface upon compression.

In the case of DPPC and DMPC, the presence of propofol in the subphase also leads to an expansion of the monolayer at low surface pressures and a continuous increase in surface pressure without clear phase transitions. Therefore we may infer that the drug interactions invoke a fluidizing effect on the morphology of the monolayer.

All three systems (DSPC, DPPC, and DMPC) collapse at the same absolute surface pressure on both water and propofol solution, respectively. The apparent differences observed in Figure 2 are caused by the difference in surface tension reading before the experiment: 72 mN m^{-1} for pure water and 57 mN m^{-1} for propofol solution (which by our definition are both zero surface pressure). More precisely, at high areas per lipid molecule the relevant reference state is the propofol solution and this reference state is utilized in the figures. We note that the surface pressure of 0.89 mM propofol solution relative to pure water is about 15 mN m^{-1} . The collapse pressure of the phospholipids on the propofol solution is lower than that observed for the phospholipids relative to water. However, if we should compare these values against the same reference (pure water) we need to add 15 mN m^{-1} to the collapse pressure obtained on 0.89 mM propofol solution. If this is done, the collapse pressure and thus the stability is the same on the pure water and the 0.89 mM propofol subphase. This comparison is appropriate since, as we show below, propofol completely leaves the interface at high surface pressures. Therefore, we conclude that the stability of the monolayer is not significantly influenced by the presence of propofol in the subphase.

It is interesting to note that qualitatively the changes to the surface pressure isotherms are reduced at higher surface pressures, which may mean that the drug is squeezed out of the monolayer with increasing surface pressure. However, a more direct probe of the interface such as VSFS is required in order to confirm this inference. In the following, directly connected to the isotherms, the vibrational features of the system involving water, propofol and phospholipid will be explored using VSFS, focusing mainly on propofol-induced changes in the terminal methyl, tail and head group regions of the phospholipids as well as on the water of hydration.

3.2 VSFS experiments: terminal methyl groups of propofol

In order to be able to observe propofol at different surface pressures from the VSF spectra, we have used phospholipids with deuterated tails and partially deuterated head groups to form monolayers on top of the 0.89 mM propofol solution. Thus, the symmetric stretch of propofol at $2\,873\text{ cm}^{-1}$ is clearly distinguished from the phospholipid CD stretches and CH peaks in the region $2\,900\text{ cm}^{-1}$ to $2\,980\text{ cm}^{-1}$, originating from non-deuterated parts of the head group³⁸ (see Figure 1 and Figure 3D).

Propofol, with a preferred orientational order, is present at the interface at low surface pressures for all three lipid systems. This is shown by two prominent peaks: the symmetric methyl stretch ($s\text{CH}_3$) stretch at $2\,873\text{ cm}^{-1}$ and the asymmetric CH_3 ($a\text{CH}_3$) at $2\,965\text{ cm}^{-1}$,^{19, 39} (see Figure 4A). In the following we focus on the $s\text{CH}_3$ around $2\,870\text{ cm}^{-1}$, since the $a\text{CH}_3$ stretch at $2\,965\text{ cm}^{-1}$ overlaps with the non-deuterated CH_2 peak at around $2\,950\text{ cm}^{-1}$ from the phospholipid. Further, the aromatic CH stretches of propofol appear above $3\,000\text{ cm}^{-1}$. Note that the VSF spectrum of propofol on water is shown in the inset in Figure 4A. Unfortunately, the spectra at low propofol concentrations have low intensities and we decided not to fit any of these spectra.

Figure 3. Drop of the VSFS intensity of the sCH₃ peak of propofol as a function of phospholipid surface pressure. A – dDSPC, B – dDPPC, C – dDMPC all on propofol solution and D – dDSPC on pure water.

To study how propofol penetrates the lipid monolayers, SSP spectra covering the propofol peak at about 2870 cm⁻¹ were acquired at different surface pressures for all three deuterated lipids. As soon as the surface pressure starts to increase, and, accordingly, the mean lipid molecular area decreases, the symmetric CH₃ signature of propofol at 2870 cm⁻¹ decreases in all three cases, demonstrating the squeeze out of propofol from the monolayer with increasing surface pressure. For all lipids, it is obvious that the propofol signal decreases with increasing surface pressure over the whole range of surface pressures studied, displayed for 5, 13, and 24

(22 for d-DMPC) mN m^{-1} in Figure 3, where also the spectrum of a propofol solution without lipid is shown as reference in panels A–C. It is interesting to compare such behavior to that of a short designed antimicrobial peptide interacting with the phospholipids DPPC and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol, where in both cases the strength of the interaction was enhanced rather than diminished with increasing surface pressure⁴⁰.

At the highest surface pressure (lowest area per lipid molecule), when the monolayers become tightly packed the symmetric CH_3 signature of propofol disappears completely in the case of DSPC, and it becomes barely detectable in the monolayers formed by DPPC, while it is slightly more pronounced in the presence of DMPC. Thus, it appears that for the more disordered DMPC (see Figure 4) more propofol remains in the lipid region at higher surface pressures, presumably due to both its shorter alkyl chain and the difference in head group hydration ability. We will discuss this argument later in this paper.

The concentration of the propofol in the interfacial region is gradually changing with the surface pressure of the monolayer. Since the VSFS response depends on both the amount and the average orientation of this species the quantification of the interfacial propofol becomes impractical.

3.3 VSFS experiments: phospholipid tails

The VSFS technique is sensitive to conformational changes in alkyl tails present at an interface^{24, 41}. The order in the monolayer can be estimated from the intensity ratio of the symmetric methyl stretch (2875 cm^{-1}) to the symmetric methylene stretch (2850 cm^{-1})^{42, 43}. Generally, when a monolayer is in an ordered state the backbone bonds adopt an all-trans configuration, and the methylene groups generate little or no signal since there is an inversion center between each pair of methylene groups. As the monolayer turns into a more liquid-like or

disordered state, gauche defects start to occur, breaking the symmetry, which increases the methylene signal. The methyl group will always generate a signal since it resides in a non-centrosymmetric environment (hydrocarbon tails below and air above). Thus, a high CH_3/CH_2 intensity ratio signifies an ordered tail region, whereas a low ratio is observed in disordered systems. Due to the fact that the recorded CH stretches of the phospholipid tail have some contribution from the propofol CH-groups we have chosen not to fit the spectra. The overlap between the two stretches is visible in both Figure 4 and Figure 6.

DSPC

The alkyl tails of DSPC are ordered at high surface pressure both on pure water⁴⁴ and on the aqueous propofol solution. This is clearly shown in Figure 4B by the dominance of sCH_3 (2875 cm^{-1}) and its Fermi resonance sCH_3FR (2937 cm^{-1}) over the significantly less intense symmetric methylene stretch sCH_2 (2850 cm^{-1}). The two spectra recorded on the two subphases are in fact very similar. This is consistent with the data shown in Figure 3, suggesting that essentially no propofol is present in the interfacial region at high compression, in accordance with the Langmuir isotherms in Figure 2A, where the curves below molecular areas $\sim 55\text{ \AA}^2$ essentially overlap.

At lower surface pressure (5 mN m^{-1}) on the water subphase the intensity of the sCH_3 still dominates over the weak sCH_2 , showing that the alkyl tails remain in a highly ordered state. However, when propofol is present in the subphase, the monolayer is more expanded at 5 mN m^{-1} and the sCH_2 peak intensity (at $\sim 2850\text{ cm}^{-1}$) becomes dominant. Thus, the presence of propofol induces disorder in the phospholipid tail region at low surface pressures.

Figure 4. Comparison of VSF spectra of the three phospholipids (DSPC, DPPC, and DMPC) in the CH region at 5 mN m^{-1} and 25 mN m^{-1} (22 mN m^{-1} for DMPC) in the absence and presence of 0.89 mM propofol in the subphase. All peaks are normalized to the intensity of the sCH_3 of DSPC at 25 mN m^{-1} , and their absolute intensities can be directly compared in all plots.

Additionally, a peak at $2\,965\text{ cm}^{-1}$ becomes more distinct, and this peak likely carries contributions from both the antisymmetric methyl stretch of propofol and DSPC, although we believe that propofol is responsible for the main part due to the appearance of the band upon addition of propofol.

DPPC

The tail region of the DPPC monolayer at high surface pressure (25 mN m^{-1}) is in an ordered state both in the absence and presence of propofol (Figure 4D). This is shown by the predominance of the sCH_3 at $2\,875\text{ cm}^{-1}$ over the very weak symmetric methylene stretch at $\sim 2\,850\text{ cm}^{-1}$. The overall peak intensities are slightly weaker in the presence of propofol. This can be explained by a slightly decreased number of probed molecules due to the increased mean molecular area, as seen in Figure 2.

At low surface pressure (5 mN m^{-1}), the intensity of the sCH_2 peak from DPPC is stronger than the intensity of the sCH_3 peak both with and without propofol present in the subphase, demonstrating significant disorder in the tail region. Even though the number of CH_2 oscillators probed in the expanded state in the presence of propofol is significantly lower ($\sim 30\%$) compared to on the pure water subphase, the intensity of the CH_2 peak is stronger due to more gauche defects induced by the interaction with propofol. The presence of ordered propofol in the interfacial region at this low surface pressure is demonstrated by the aCH_3 at $2\,965\text{ cm}^{-1}$ and the aromatic CH peaks at around $3\,030\text{ cm}^{-1}$ and $3\,071\text{ cm}^{-1}$, respectively, which also are seen in Figure 6, although the peak at $2\,965\text{ cm}^{-1}$ probably also carries contributions from the lipid. Additionally, the propofol sCH_3 feature contributes to the overall intensity of the $2\,875\text{ cm}^{-1}$ peak, even though it is difficult to separate it from the sCH_3 emanating from the phospholipid.

DMPC

The hydrocarbon tails of DMPC (Figure 4E and 4F) are, in contrast to those of DSPC and DPPC, in a low ordered state even at high surface pressure (22 mN m^{-1}), both in the absence and presence of propofol in the subphase. This is demonstrated by the methylene peak at 2850 cm^{-1} , which becomes comparable in intensity to that of the methyl peak at 2875 cm^{-1} . In both Figure 4E and F in the presence of propofol the apparently less resolved peaks in the region around $2850\text{--}2875 \text{ cm}^{-1}$ originate from the contribution of the symmetric methyl stretch of both DMPC and propofol. The spectra with and without propofol at 22 mN m^{-1} are quite similar, although the spectrum recorded in the presence of propofol is weaker due to the larger area per molecule and increased tail disorder. Further, the presence of propofol is revealed by the weak shoulder at 2965 cm^{-1} , which in the case of 5 mN m^{-1} dominates over the Fermi resonance at 2937 cm^{-1} . At both surface pressures ordered propofol is also observed at the interface through its aromatic CH (3071 cm^{-1}) peak.

Summary

Our data show that propofol to a large extent leaves the interfacial region as the phospholipid monolayers are compressed, and, therefore, its presence in the subphase has less effect on the tail packing and order at high surface pressure ($22\text{--}25 \text{ mN m}^{-1}$) for the three phosphatidylcholines investigated. This mode of interaction contrasts with that of a recently-studied peptide which enhanced its interaction with both zwitterionic and anionic phospholipid monolayers with increasing surface pressure. At low surface pressure (5 mN m^{-1}), on the other hand, the tail region of all three phospholipids is significantly more disordered in the presence of propofol. It thus appears that propofol promotes disorder, which is consistent with computer simulations

suggesting preferential accumulation of small hydrophobic and amphiphilic molecules at the border between ordered and disordered domains or inside the disordered domains^{45, 46}.

3.4 VSFS experiments: phospholipid headgroups

Above it was shown that phospholipids with the same headgroup but with different length of the hydrocarbon tail respond differently when in contact with propofol. The focus will now be towards the behavior of the headgroup, which is the same for all three phospholipids (Figure 1). The SSP spectra of the phospholipids in the PO and C=O regions are shown in Figure 5. The spectra recorded in the absence and presence of 0.89 mM propofol are compared at low (5 mN m⁻¹) and high (22 or 25 mN m⁻¹) surface pressure. The most prominent peak is the symmetric PO₂ stretch, which is centered at 1 090 cm⁻¹ with a shoulder summing the C-OP and CO-O-C_{ss} stretches at 1 050 cm⁻¹ and 1 075 cm⁻¹, respectively³⁸.

The other three peaks are the antisymmetric PO₂ stretch, the deformation CH₃, and the C=O stretch, which are barely visible at 1 250 cm⁻¹, 1 460 cm⁻¹, and 1 750 cm⁻¹, respectively³⁸. Thus, here we focus on the symmetric PO₂ peak, since it is most intense, and how it is affected by propofol. No signal was observed in the PPP polarization combination in any spectrum, and the acquisition of SPS spectra was not possible since our half-wave plate (MgF₂) is not transparent in the region around 1 100 cm⁻¹. Further, especially at 5mN m⁻¹, it is essentially impossible to distinguish the contributions from the various peaks in the region around the symmetric PO₂ stretch. Hence, drawing conclusions about how the amplitudes change from fitting the spectra would be unreliable, and we only discuss the propofol induced spectral changes qualitatively. Due to the issues mentioned above it was not possible to study orientational changes of the headgroup. It has been shown that the PO₂ peak position is sensitive to its environment. It can be

shifted by 10–15 cm^{-1} due to counterion complexation, hydration state, and conformational changes⁴⁷⁻⁵¹.

Figure 5. VSF spectra of the three phospholipids A) and B) DSPC, C) DPPC, and D) DMPC in the fingerprint region in the absence and presence of 0.89 mM propofol in the subphase at two different surface pressures: 5 and 25 mN m^{-1} (22 mN m^{-1} for DMPC). All features are

normalized to the intensity of the ν_{PO_2} at 25 mN m^{-1} of DSPC hence the absolute intensities can be directly compared between different lipids.

However, the presence of propofol in the subphase does not result in any measurable shift of the ν_{PO_2} peak position for any phospholipid at any surface pressure as observed from Figure 5. Hence, there is no interaction between the phosphate group and propofol that is strong enough to affect the ν_{PO_2} vibration. The intensity of the symmetric ν_{PO_2} stretch depends on the number density and the average orientation of the head group. Its magnitude will decrease when the order in the head group region decreases and when the average orientation of the ν_{PO_2} dipole transition moment changes to become more parallel to the surface (since SSP probes dipole transitions along the surface normal), as well as when the surface number density is reduced.

DSPC

The spectral features of DSPC at high surface pressure (25 mN m^{-1}) are the same in the presence and absence of propofol, as seen in Figure 5A and B. This fact, as well as the spectra recorded in the tail region (Figure 3), demonstrates that there is no significant perturbation of the DSPC monolayer induced by propofol (neither the tails nor the headgroup). This result is consistent with the complete squeeze out of propofol from DSPC monolayers at high surface pressures, as discussed above. At 5 mN m^{-1} (Figure 5B) it is seen that the ν_{PO_2} intensity is significantly weaker in the presence of propofol than without. This is similar to what is observed for the CH stretching region in Figure 4. The reduction of order in the tails is obvious in Figure 4 from the reduced CH_3/CH_2 intensity ratio, but a reduced order in the headgroup cannot be inferred from the spectra since no peak shifts are observed, and the reduction in signal to a large extent depends on the reduced DSPC number density in comparison with the case of pure water

(Figure 2). Hence, we conclude that the main origin of the reduction in peak intensity at 5 mN m^{-1} with propofol is due to a reduced DSPC number density at the water surface.

DPPC

Just as for DSPC, the presence of propofol in the subphase does not affect the spectra for DPPC in the head group region at a surface pressure of 25 mN m^{-1} (Figure 5C). This agrees to a large extent with the DPPC spectra in the CH region, although the intensity is slightly lower in the presence of propofol in the CH region. Moreover, the PO_2 intensity for DPPC is as high as for DSPC, indicating a similar packing and orientation of the headgroups for these lipids, since the molecular area is almost similar (Figure 2). Thus, for both DSPC and DPPC we can draw the conclusion that propofol does not induce any significant changes in the phospholipid headgroup at a biological surface pressure of 25 mN m^{-1} . The PO_2 intensity is significantly lower in the presence compared with the absence of propofol at 5 mN m^{-1} , but no clear peak shifts are observed. Based on the large difference in molecular area in Figure 2 with and without propofol at 5 mN m^{-1} , we attribute the reduction in PO_2 intensity in the presence of propofol mainly due to a reduced number density of DPPC, similar to DSPC.

DMPC

Already at a high surface pressure (25 mN m^{-1} for DSPC and DPPC, 22 mN m^{-1} for DMPC), the PO_2 signal for DMPC (Figure 5D) spread on pure water is significantly lower (around 50%) than for DPPC and DSPC. This is to some extent due to the higher molecular area for DMPC in comparison with the other lipids (see Figure 2). However, it could in addition be due to a different orientation and an increased disorder of the headgroup. This is due to the fact that DMPC exists in a liquid expanded state and the other lipids in a condensed state at these surface high pressures, meaning that the hydrocarbon chains in DMPC are significantly more disordered

compared with DSPC and DPPC (Figure 3B, 3D and 3F). Addition of propofol induces therefore a reduction in peak intensity, which likely originates from a reduced lipid number density to the largest extent. Similarly, to DSPC and DPPC, the peak intensity is lower at 5 mN m⁻¹ compared with 22 mN m⁻¹, also a result of a reduced number density. A comparison of the intensity of the spectrum in the absence and presence of propofol at 5 mN m⁻¹ shows that there is a notable reduction in intensity in the latter case, which follows given the large reduction in lipid number density (Figure 2).

Summary

The overall picture that emerges from the studies of the headgroup confirms the observation from the CH stretching region, that propofol is excluded from ordered phospholipid regions with increasing surface pressure and, thus, preferentially accumulates in disordered regions or at the border between ordered and disordered regions. This suggests that there is a non-homogeneous distribution of propofol in the lateral direction of phospholipid monolayers consisting of both ordered and disordered regions.

These conclusions appear to be largely consistent with computer simulations of small amphiphilic molecules interacting with phospholipid membranes^{45, 46, 52}. Suggesting that propofol in the normal direction preferentially resides at the border between the non-polar and polar region of the phospholipid monolayer, just as suggested in studies of DPPC and propofol¹⁷ and for ethanol by computer simulations⁵². Since VSFS does not provide irrefutable direct information about the location of the propofol in the lipid monolayer we will return to this issue later when discussing results from NR below.

The result that propofol affects disordered phospholipids to greater extent than when ordered is interesting in view of the fact that cell membranes consist of both ordered and disordered lipid

domains. Hence, these studies show that propofol in the human body potentially interacts with and penetrates cell membranes in the disordered parts more than the highly ordered lipid rafts.

3.5 VSFS experiments: water

We turn our attention now to how the presence of propofol affects the water molecules residing next to the phospholipid monolayer, starting by briefly reviewing the interpretation of the VSF spectra of the pure water interface shown in Figure 6A. It is unambiguously agreed that the feature at $3\,700\text{ cm}^{-1}$ is the free OH bond that vibrates against air, and, as such, it does not participate in any hydrogen bonding. The assignment of the broad band from approximately $2\,800$ to $3\,600\text{ cm}^{-1}$, however, has been for long time been a source of debate⁵³⁻⁶⁰. Nevertheless, it is generally agreed that the hydrogen bond strength varies across the full width of this broad band, so that the OH signal is a signature of a collective vibration of several water molecules. VSF spectra of water hydrating phospholipid monolayers have been extensively studied⁶¹⁻⁶³. Water molecules that are present in the proximity of the zwitterionic phospholipid head group of phosphatidylcholines are oriented by the electric field from these groups, and the orientation of the water molecule thus depends on its specific location with respect to the headgroup^{49, 64}.

Water of hydration will in the following be regarded as water in direct contact with the phospholipid headgroup together with the water oriented in the electric field. The latter contribution is considered to be dominant for a charged phospholipid⁴⁹. A study of a DPPC monolayer on water employing phase sensitive VSFS revealed that the water molecules contributing to the spectra in the wavenumber range $3\,000\text{--}3\,550\text{ cm}^{-1}$ are preferentially oriented with their hydrogen atoms towards the monolayer⁴⁹. Moreover, Mondal *et al.*⁶⁵ have shown that water species having opposite orientation coexist at the interface, the strongly hydrogen bonded water existing in the vicinity of the anionic moiety with net hydrogen up orientation and the

weakly hydrogen bonded water existing in the vicinity of the cationic moiety with net hydrogen down orientation.

Figure 6. Water spectra of A) air-water interface in the absence and presence of 0.89 mM propofol, B) d-DSPC C) d-DPPC, and D) d-DMPC phospholipid monolayers on pure water and on 0.89 mM propofol solutions at selected surface pressures in SSP polarization.

In the present study we use the deuterated form of the phospholipids in order to reduce the spectral interference between the strong methyl peaks of the phospholipids and the weaker water

bands, and to distinguish the propofol sCH₃ peak at 2 873 cm⁻¹ from the corresponding peak of the lipids.

The VSF spectrum of the water surface in the presence of 0.89 mM propofol is shown in Figure 6A where the strength of the free OH vibration at 3 700 cm⁻¹ on pure water is a reference defining one arbitrary unit (a.u.). It is significantly different to that recorded in the absence of propofol. First, we notice a spectral feature centered at about 3 150 cm⁻¹ that is due partly to the OH group⁵⁸ of propofol and partly to strongly hydrogen bonded water interacting with the OH group of propofol present in the interfacial layer. Weakly hydrogen bonded water is also observed by one small band at about 3 660 cm⁻¹, which we assign to O–H vibrations affected by water-hydrocarbon interactions⁶⁶⁻⁶⁸. Moreover, the water region of the VSF spectrum of the propofol solution shows that the free OH vibration at 3 700 cm⁻¹ has vanished, and hence no OH bonds protruding out into the air exist. The hydrocarbon peaks from propofol are observed in the low wavenumber region (< 3100 cm⁻¹).

DSPC

The water spectra in the presence of a d-DSPC monolayer on a pure water subphase (Figure 6B) show that the free OH vibration is absent at a surface pressure of 5 mN m⁻¹, and instead the peak due to weakly interacting water molecules with the hydrocarbon tails (3 650 cm⁻¹) appears. This peak remains as the surface pressure is increased from 5 mN m⁻¹ to 25 mN m⁻¹. The intensity of the broad water band (~3 000–3 500 cm⁻¹) due to hydration of the phospholipid headgroup increases slightly with increasing surface pressure from 5 mN m⁻¹ to 25 mN m⁻¹ as a result of a higher surface density of headgroups, and this observation is consistent with earlier reports⁴⁴. The overall shape of these adsorption bands is unaltered as the surface pressure is increased; indicating that the hydrogen bond strength distribution is unchanged.

For DSPC monolayers spread on propofol solution at 5 mN m^{-1} , the propofol is present at the interface as revealed by the sCH_3 band at $2\,873 \text{ cm}^{-1}$ and the aCH_3 band at $2\,965 \text{ cm}^{-1}$ (overlapping with the adjacent d-DSPC band) that can be assigned to the drug. At this surface pressure, the intensity of the spectra of the water region is decreased in the presence of propofol in the subphase. One reason is that there are fewer phospholipid head groups per unit area (see Figure 2A) to induce the electric field that orients the water molecules. The other reason is that the presence of propofol at the interface reduces the number of water molecules in this region, since propofol aligns fewer water molecules than the lipid.

The spectra from the whole water region ($\sim 3\,000 - 3\,800 \text{ cm}^{-1}$) taken at a d-DSPC surface pressure of 25 mN m^{-1} overlap perfectly in the absence and the presence of propofol. Thus, the water residing at the interface shows a completely similar structure in the absence and presence of propofol in the bulk solution. Further, at this surface pressure the propofol sCH_3 band at $2\,873 \text{ cm}^{-1}$ has completely disappeared, and in the region around $2\,960 \text{ cm}^{-1}$ the spectral features completely overlap (thus, there are only contributions from the d-DSPC band at $2\,956 \text{ cm}^{-1}$ whereas the propofol band at $2\,965 \text{ cm}^{-1}$ has vanished). All of these observations support the interpretations above that propofol has been fully squeezed out of the monolayer at the high surface pressure and none remains present at the interface.

We note that the maximum intensity of the broad OH band is approximately eight times lower on a subphase containing only propofol (no phospholipid), compared to the case with only d-DSPC on pure water at 25 mN m^{-1} (Figures 6A and 6B have comparable scale). We infer that this mainly is a consequence of the stronger electrical field from the zwitterionic choline group of DSPC compared to that emanating from the dipolar and uncharged OH-group of propofol. This suggests that d-DSPC affects the probed water molecules more than propofol, and as a

consequence the maximum intensity of the broad OH-band is observed at $\sim 3\,250\text{ cm}^{-1}$ for d-DSPC both in the absence and presence of propofol, and not at $3\,150\text{ cm}^{-1}$ where the OH-band recorded on a propofol solution in the absence of phospholipids appears.

DPPC

The water spectra for the DPPC monolayer, shown in Figure 6C, have many similarities to those recorded for the DSPC monolayer. One difference is that, at 5 mN m^{-1} in the absence of propofol, the water band is weaker for the monolayer of DPPC than for that of DSPC. This is mainly due to the larger molecular area in the DPPC monolayer (see Figure 2).

At low surface pressure (5 mN m^{-1}), where the DPPC monolayer is in a liquid expanded state, the broad water band is basically unaffected by the presence of propofol over its entire region, spanning the range from $\sim 3\,100\text{ cm}^{-1}$ to $3\,800\text{ cm}^{-1}$. This is the case in spite of the fact that both d-DPPC and propofol are present in the surface region as revealed by their characteristic peaks. Hence, the surface composition is different on the pure water and on the propofol solution, but the water bands still overlap. This is an intriguing fact that originates from several different parameters, such as the molecular orientation and surface density of propofol and DPPC. Since the lipid results in a stronger water signal than propofol and the lipid number density is lower at 5 mN m^{-1} (figure 2B) in the presence of propofol, there must be another parameter compensating this and hence results in similar band intensities. Such a parameter is the average orientation of the lipid. However, since the phosphate headgroup only was detected in the SSP polarization and the fact that the lipid and propofol bands overlap in figure 4C (CH region), it is difficult to judge how pronounced the effect of orientation is in ordering water layers.

As the DPPC monolayer is compressed to a more ordered state, the VSFS signal of the broad water band increases due to the increased surface density of DPPC. At a surface pressure of 25

mN m^{-1} , the water band is less intense in the presence of propofol in the subphase, and some propofol remains at the interface as evidenced by a trace of the propofol aCH_3 band (2965 cm^{-1}) overlapping the phospholipid non-deuterated CH_2 band (2956 cm^{-1}), as well as a barely visible peak at 2873 cm^{-1} . We attribute the decreased water band intensity to a slightly reduced surface density (figure 2B), whereas the packing order essentially is the same as revealed by the similar spectra in figure 4D.

DMPC

The water bands observed for the DMPC monolayer without propofol, Figure 6D, have obvious similarities to those of the other two phosphatidylcholines. However, the intensities are significantly weaker at both low and high surface pressures due to the lower phospholipid number density (Figure 2) and also due to the more disordered phospholipid structure (high CH_2/CH_3 intensity ratio in figure 4E and F). In the absence of propofol the water band increases with increasing surface pressure as the surface density of DMPC increases, and moreover, the ordering of at least the chains increases, as seen in Figure 4E and F. The water band intensities are lower on the propofol-containing subphase, mainly due to the presence of propofol at the interface that reduces the surface density of DMPC, and, hence, the ordering of water molecules from the charged lipid headgroup. Clear peaks at 2873 cm^{-1} and 2965 cm^{-1} reveal the presence of propofol at both low and high surface pressure, where the partly squeezed out propofol is revealed by the lower peak intensities at the higher surface pressure.

Summary

The intensity of the water bands increases with surface density and phospholipid ordering. Moreover, the hydration water of DSPC, DPPC, and DMPC is affected differently by the presence of propofol, which, since the headgroup is the same, is attributed to the different

propensity of the phospholipids to form ordered conformations, as judged from the VSF spectra of the tail region. Besides the fact that more disorder yields a lower signal, it also results in a lower lipid surface density for a given surface pressure, which further contributes to a reduction in signal. For DSPC, the water hydrating the ordered layer that is present on the water subphase is affected by propofol at low surface pressure (5 mN m^{-1}), but not at high surface pressures when propofol is removed from the interfacial region. At 5 mN/m DPPC is fairly disordered both with and without the presence of propofol, a parameter which leads to a reduction in intensity of the water bands. The intensity of the water band in the presence of DPPC is reduced by propofol at 25 mN m^{-1} due to the presence of small amounts of propofol in the interfacial region. DMPC has an even lower ability to form an ordered phase than DPPC, and this ability is further reduced in the presence of propofol, which shows up as a decrease in the intensity of the water spectrum under all conditions in comparison with DPPC and DSPC, particularly at high surface pressures.

3.6 NR experiments

In order to learn about the location of propofol in DPPC monolayers spread on 0.89 mM propofol solution and compressed to 5 mN m^{-1} , we performed measurements using NR. First, pure lipid monolayers were measured and successfully modelled in four different isotopic contrasts using two stratified layers: alkyl tails with a volume fraction of 1 next to the air and hydrated head groups next to the water. These data and model fits, as well as a table of parameters used in the model, can be found in the Supporting Information. The area per lipid molecule, which can be calculated directly from the applied model, is consistent with that indicated by the corresponding surface pressure isotherm in Figure 2B ($\sim 80 \text{ \AA}^2$).

Three different models were then applied to data from the mixed DPPC/propofol system, where propofol was located: (A) in the alkyl tails layer, (B) in the hydrated headgroups layer, and (C) under the hydrated headgroups in a separate third layer. The applications of these different models are shown in Figure 7. The best model fit to the experimental data, with good agreement in all four measured isotopic contrasts, occurs with propofol located in the hydrated headgroups layer (panel B). Transfer of propofol to the alkyl tails layer (panel A) results in an increase in reflectivity at mid-to-high q_z of the model involving d_{62} -DPPC in D_2O , which is not borne out in the experimental data. This feature evidences the need for an inversion in the scattering length density profile resulting from the propofol (low scattering length density) sitting between a dense layer of deuterated chains and the subphase (both high scattering length density), as highlighted by the arrow in the inset to panel B

Figure 7. Experimental neutron reflectivity data and model fits of DPPC monolayers at 5 mN m^{-1} on 0.89 mM propofol solution: (blue) d_{62} -DPPC/ACMW, (green) d_{62} -DPPC/ D_2O , (purple) DPPC/ACMW and (red) DPPC/ D_2O , where propofol is located A) in the alkyl tails layer, B) in the hydrated headgroups layer and C) under the hydrated headgroups layer. The inset to model B

is the scattering length density (SLD) profiles of the data recorded in the 4 isotopic contrasts, and the green arrow marks the inversion in the profile discussed in the text.

On the other hand, transfer of propofol for inclusion beneath the hydrated headgroups layer (panel C) results in a Kiessig fringe at lower q_z in the reflectivity model than occurs in the data in both contrasts involving D₂O. These features show that the material present at the interface is less extended than the structure of the 3-layer model.

It is interesting to note that the area per lipid molecule, again calculated directly from the applied model, is higher as a result of the propofol interaction than that indicated from the surface pressure isotherms in Figure 2B (156 vs. 114 Å²). This difference indicates an increased solubilization of DPPC as a result of its interaction with the drug, and it may be noted that such a loss of phospholipid from a monolayer at the air/water interface has been observed also in the case of interactions with antimicrobial peptides³⁹. See the Supporting Information. We note also that the location of propofol resolved using NR is consistent with the findings of Hansen¹⁷ *et al.* who demonstrated on pre-mixed propofol-DPPC monolayers that the drug was associated with the headgroups. However, as no PO₂ frequency shift was detected using VSFS in the present work, we may infer that the interaction between propofol and the phosphate group of the lipid is weak.

4. Conclusions

Interactions between phospholipid monolayers and bilayers with surfactants have been extensively investigated during recent years and their destructive effect has been clearly recorded^{1-4,67}. Considerably less attention has been given to interactions between phospholipid layers and small amphiphilic non-surfactants. This is surprising since many important drug molecules fall into this molecular category⁴. In this article we address this general issue by

considering the specific example of saturated phosphatidylcholines interacting with the drug propofol. To this end we utilize vibrational sum frequency spectroscopy (VSFS), neutron reflectometry and surface pressure isotherms to gain insight on where propofol is located and how it affects the molecular structure of the phospholipid monolayers DSPC, DPPC, and DMPC at the air-water interface. VSFS has previously been utilized for gaining insight on molecular order and hydration of phospholipids^{38,61,62} and surfactants⁶⁷ at the air-water interface. This work thus extends these studies to include effects of amphiphilic non-surfactants on order and hydration of phospholipid monolayers.

Our data show that propofol is located in the phospholipid headgroup region at low surface pressures, but it is squeezed out of the monolayer as the phospholipid molecules pack closer together with increasing surface pressure. This effect was inferred from the surface pressure isotherms and then confirmed directly using VSFS and neutron reflectivity. It was demonstrated that propofol has the ability to increase the fluidity of the phospholipid monolayers, and this was enhanced for phospholipid monolayers possessing more disordered tails. Accordingly, DMPC was affected most, followed by DPPC, and DSPC was least affected by propofol. VSF spectra of the tail region show that disordered phospholipid monolayers become even more disordered in presence of propofol. The effect of propofol on the head group region depends on the area per molecule, which is affected by the phospholipid tail length. Propofol does not affect the headgroup of DSPC and DPPC at biological surface pressures, whereas at lower surface pressures as well as for the more disordered DMPC a reduction in signal is observed, which mainly depends on a lower lipid surface number density. The water surrounding the headgroups of a disordered monolayer is affected by the presence of propofol, while the water that is hydrating well-ordered monolayers is not.

Taken together, our data suggest that propofol is incorporated preferentially in disordered phospholipid regions, which is consistent with computer simulations that provide evidence for accumulation of small amphiphilic compounds, like propofol, in such regions or at the border between the ordered and disordered regions^{45,46}. Thus, provided the lipid layer is inhomogeneous with ordered and disordered domains, then one would also expect an inhomogeneous lateral distribution of propofol in the plane of a lipid membrane. Further studies along the lines presented here are needed to clarify the generality of our conclusions. Our findings also suggest that particular attention should be paid to studies devoted to determining the lateral distribution of amphiphilic molecules at the phospholipid-water interface in order to elucidate how lipid domain formations affect interactions with drugs and how drugs affect the phospholipid domains.

SUPPORTING INFORMATION.

Modeling approach and the parameters used in the NR work.

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