Mechanical properties of drug loaded diblock copolymer bilayers: A molecular dynamics study

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In this work, we present results of coarse-grained simulations to study the encapsulation of prilocaine (PLC), both neutral and protonated, on copolymer bilayers through molecular dynamics simulations. Using a previously validated membrane model, we have simulated loaded bilayers at different drug concentrations and at low (protonated PLC) and high (neutral PLC) pH levels. We have characterized key structural parameters of the loaded bilayers in order to understand the effects of encapsulation of PLC on the bilayer structure and mechanical properties. Neutral PLC was encapsulated in the hydrophobic region leading to a thickness increase, while the protonated species partitioned between the water phase and the poly(ethylene oxide)-poly(butadiene) (PBD) interface, relaxing the PBD region and leading to a decrease in the thickness. The tangential pressures of the studied systems were calculated, and their components were decomposed in order to gain insights on their compensation. In all cases, it is observed that the loading of the membrane does not significantly decrease the stability of the bilayer, indicating that the system could be used for drug delivery. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5028377>

I. INTRODUCTION

Polymersomes are vesicles composed of amphiphilic block copolymers that in the presence of water self-assemble in a bilayer structure.^{1–5} The intrinsic characteristics of polymersomes are closely related to the structural properties of their membranes and thus on the nature of the chosen copolymers.^{5–9} They usually present high stability,^{10,11} and their chemical versatility allows the fine tuning of their properties—such as thickness, polarity, size, and elasticity—for a specific application.⁵ Among them, biocompatible polymersomes based on poly(ethylene oxide) (PEO) and poly(butadiene) (PBD) have been used effectively in drug delivery, targeting, and imaging applications.^{12,13}

Computer simulations can provide a unique tool for a better understanding of these systems to guide the rational design of nanocarriers.¹⁴ In particular, Molecular Dynamics (MD) simulations¹⁵ are suitable for studying polymersomes.¹⁶ Nevertheless, the polymersome sizes often range from 100 nm up to the micron diameter size,¹⁷ which precludes their fully atomistic treatment.^{18,19} To address this issue, coarse-grained (CG) models can provide sufficient simplification to achieve

reasonable computing times while retaining sufficient details to accurately reproduce cooperative and mesoscopic-scale phenomena.^{20–23}

A key question when using a drug carrier loaded with an active biological ingredient is to understand how the loading of the carrier by the active ingredient affects its stability. This can be conveniently studied using MD simulations to explore the mechanical properties of the loaded bilayers.²⁴ These kinds of simulations allow us to calculate the pressure profiles that yield important information on stability. Examples of these studies have been presented by Fábíán *et al.* who have performed lateral pressure studies to explore the behavior of lipid bilayers containing general anesthetics;²⁵ Orsi *et al.* have successfully employed a CG approach to obtain curvature elastic parameters of lipid bilayers from lateral pressure profiles which are in agreement with atomistic and experimental data.^{26,27} In this direction, the calculation of curvature elastic properties on bilayers may help in the design of stable drug loaded systems with special features, such as increase of flexibility and compressibility.

In this work, we explore the encapsulation of prilocaine (PLC) on PEO-PBD bilayers by means of MD simulations using a CG approach within the MARTINI force field.^{22,23} The CG model for PEO-PBD systems has been presented in our previous publication,²⁸ showing that the

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model is stable and can reproduce basic experimental properties of the simulated systems. The anesthetic prilocaine (PLC) is widely used in dentistry; it has a pK_a of 7.89 leading to two different ionization states, neutral (nPLC) and protonated (pPLC), depending on the pH.^{29,30} Due to these different protonation states, it is a good candidate to study the encapsulation of both hydrophobic and hydrophilic compounds on copolymer bilayers. In this direction, we simulated loaded bilayers at different drug concentrations for each nPLC (high pH) and pPLC (low pH). We also characterize key structural and mechanical parameters in order to understand the effects of encapsulation of PLC on the stability of the bilayers.

II. METHODS

A. Molecular dynamics simulations

We modeled our systems using a CG approach based on the MARTINI force field.^{22,23} The CG model for PEO-PBD copolymers was taken from our previous work.²⁸ PLC species were modeled using the parametrization developed by Pickholz and Giupponi.²⁹ Explicit solvation was included in the simulations using the polarizable water (PW) model.³¹ The cross terms for nonbonded interactions between PLC species and the remaining compounds were transferred from the MARTINI force field according to the equivalent MARTINI types associated with the interacting beads.

We have built a fully hydrated neat PEO-PBD bilayer using 100 PEO₁₄-PBD₂₂ chains per monolayer (subscripts represent the lengths of the corresponding blocks) and 9000 PW. To investigate the effect of encapsulation of PLC on the bilayer, different number of PLC molecules, denoted as N_{nPLC} for the neutral case and N_{pPLC} for the protonated one, were incorporated into the neat PEO-PBD bilayer. We have used $N_{nPLC} = 104, 208, 312, 416, 520$, and 624 for nPLC-loaded systems and $N_{pPLC} = 26, 52, 78, 104, 130$, and 156 for pPLC-loaded ones. We have explored different initial conditions for both nPLC and pPLC, and we have found that nPLC has high affinity for the bilayer core (formed by the hydrophobic PBD), while pPLC partitions between the

PEO-PBD interface and water (see Figs. S1 and S2 of the [supplementary material](#)). Therefore, the systems used for our study were initially built by inserting nPLC molecules into the bilayer core and the pPLC ones between the PEO-PBD interface and water. For the latter case, one chloride counterion (Cl^-) per pPLC molecule was also added in the water region in order to balance the net charge of the system. A schematic representation of the different components used in the systems' construction and their corresponding CG models are depicted in Fig. 1.

MD simulations were performed using GROMACS 5.0.7.³² All simulations were carried out using the NPT ensemble, periodic boundary conditions in all directions, shifted Lennard-Jones (LJ) potential (a cutoff radius of 1.2 nm), shifted Coulombic potential (a cutoff radius of 1.2 nm) with a relative dielectric constant $\epsilon_{rf} = 2.5$, and time steps of 20 fs. Temperature was kept constant at 300 K using the Nosé-Hoover thermostat³³ with a coupling constant of 6.0 ps. Pressure was equilibrated at 1 bar using the Parrinello-Rahman barostat³⁴ with a coupling constant of 6.0 ps and compressibility of $4.5 \times 10^{-5} \text{ bar}^{-1}$. The geometry of water molecules was kept fixed by means of the LINear Constraint Solver (LINCS) algorithm.³⁵ Neat and nPLC-loaded systems were simulated for $2 \mu s$, while pPLC-loaded cases were extended up to $2.5 \mu s$. For all systems, the equilibrium trajectories were obtained at the last $1 \mu s$. To illustrate the systems' organization for each PLC type, snapshots of equilibrated bilayers loaded with $N_{nPLC} = 204$ and $N_{pPLC} = 78$ are shown as representative examples in Fig. 2. Details of the simulated systems used in this work, corresponding sizes, and simulation times are shown in Table I.

B. Bilayer characterization

All the bilayers simulated in this work were characterized by their different structural and mechanical properties as follows. Mass density profiles (MDPs) and tangential pressure (P_T) profiles were calculated by time averaging of the net mass and tangential pressures in 100 slabs along the plane of the bilayer over the equilibrium trajectory (for a detailed description of tangential pressure calculation, see Sec. II C). All profiles were symmetrized with respect to the center of

FIG. 1. Sketch of the systems' construction and CG models corresponding to each component. Bead names and equivalent MARTINI types (in parentheses) are indicated. For the neutral case (nPLC), molecules are incorporated into the PBD region. For the protonated case (pPLC + Cl^-), molecules are distributed between the PEO-PBD interface and water (PW).

FIG. 2. Equilibrium snapshots of two representative cases illustrating the system organization for each PLC type: (a) $N_{nPLC} = 204$ and (b) $N_{pPLC} = 78$. Components identified by color: PBD (pink), PEO (purple), water (cyan), nPLC (green), pPLC (red), Cl^- (blue).

the bilayer ($z = 0$). The first integral moment of the lateral pressure profile (τ) was obtained by splitting the equilibrium trajectories every 200 ns, computing the average lateral pressure profile on each interval, and calculating the integral in Eq. (6) (see Sec. II C). The choice of 200 ns was based on a statistical analysis of τ fluctuations on different interval lengths (see Figs. S3 and S4 of the [supplementary material](#)). The membrane thickness (d) represents the thickness of the hydrophobic core, formed by the PBD component. The d values were computed by splitting the equilibrium trajectories every 1 ns and calculating the width at half height of the PBD MDPs averaged on each interval. The area per chain (A_c) is the area of the simulation box on the plane of the membrane divided by the number of copolymer chains per monolayer. The A_c values were determined by splitting the equilibrium trajectories every 1 ns and calculating the average values on each interval. The hydrophobic core volume (V_h) is defined as the volume occupied by the hydrophobic core along the simulation box. The V_h values were calculated every 1 ns as the membrane thickness d multiplied by the area of the simulation box on the plane of the membrane averaged on the corresponding interval.

TABLE I. Summary of the simulated systems used in this work.

System ^a	No. PLC molecules	CG sites	Simulation time (μ s)	Total node hours ^b
Neat	...	34 200	2.0	30
N104	104	34 720	2.0	30
N208	208	35 240		
N312	312	35 760		
N416	416	36 280		
N520	520	36 800		
N624	624	37 320	2.5	35
P26	26	34 356		
P52	52	34 512		
P78	78	34 668		
P104	104	34 824		
P130	130	34 980		
P156	156	34 938		

^aNeat system corresponds to the neat PEO-PBD bilayer; N-labeled systems represent the nPLC-loaded bilayers; P-labeled systems identify pPLC-loaded bilayers.

^bTimes estimated using one Intel Xeon E5-2680 v4 processor (14 cores, 2.4 GHz), 256 GB RAM, and one Nvidia Tesla P100 GPU board.

C. Pressure profiles and curvature calculations

The local pressure of a system at position $\mathbf{r}$ is represented by the tensor $P^{\alpha\beta}(\mathbf{r})$, where α, β represent the Cartesian components. For a system consisting of pointwise particles, it can be expressed as the sum of kinetic and configurational contributions,^{25,36–39}

$$P^{\alpha\beta}(\mathbf{r}) = \left\langle \sum_i m_i v_i^\alpha v_i^\beta \delta(\mathbf{r} - \mathbf{r}_i) \right\rangle + P_C^{\alpha\beta}(\mathbf{r}), \quad (1)$$

where m_i , v_i , and $\mathbf{r}_i$ are the mass, velocity, and position of particle i , respectively, the brackets $\langle \cdot \rangle$ denote ensemble averaging, and $P_C^{\alpha\beta}(\mathbf{r})$ represents the configurational contribution of the local pressure tensor. For pairwise additive potentials, $P_C^{\alpha\beta}(\mathbf{r})$ can be written as^{25,38,39}

$$P_C^{\alpha\beta}(\mathbf{r}) = \frac{1}{2} \left\langle \sum_{i \neq j} f_{ij}^\alpha \int_{C_{ij}} ds^\beta \delta(\mathbf{r} - \mathbf{s}) \right\rangle, \quad (2)$$

where f_{ij} is the force on particle i due to particle j and the integration contour C_{ij} is an open path connecting the particles i and j .

Since the integration contour is arbitrary, the local pressure is not uniquely defined.³⁶ Among the different choices of the integration contour that have been used in the literature, the Irving-Kirkwood⁴⁰ and Harasima⁴¹ paths have been shown to provide comparable profiles.³⁸ However, the use of the Harasima contour is computationally more efficient and, as discussed below, is used here.

Hereinafter, the tangential directions are denoted as x, y and the normal one is denoted as z . For systems with planar geometry, the pressure tensor is diagonal and only depends on the z coordinate.⁴² The tangential component of the pressure tensor is defined as $P_T(z) = [P^{xx}(z) + P^{yy}(z)]/2$. Using the Harasima contour, $P_T(z)$ can be decomposed as a sum of contributions coming from each particle in the system (for more details, see Ref. 39). Therefore, it can be expressed as^{25,39}

$$P_T(z) = \frac{V}{A} \left\langle \sum_i P_T^i \delta(z - z_i) \right\rangle, \quad (3)$$

where P_T^i represents the contribution of the particle i to the tangential pressure, z_i is the position of the particle i in the z -direction, A is the area of the system in the xy -plane, and V is its volume. As seen in Eq. (3), the use of the Harasima path makes the calculation of $P_T(z)$ straightforward

and computationally efficient. Moreover, it is possible to obtain the contribution to $P_T(z)$ coming from a given group G of particles i , in the form

$$P_T^G(z) = \frac{V}{A} \left\langle \sum_{i \in G} P_T^i \delta(z - z_i) \right\rangle, \quad (4)$$

which is equivalent to Eq. (3) when G is the total system.

In practice, the calculation of $P_T^G(z)$ on planar bilayers is performed by dividing the system in slabs along the z -direction and averaging $P_T^G(z)$ on each slab. For a slab s at position $z = z_s$ with volume V_s , the average $P_T^G(z)$ on the slab s can be obtained by integrating Eq. (4) on V_s and dividing by V_s ,

$$P_T^G(z_s) = \frac{V}{V_s} \left\langle \sum_{i \in G} P_T^i \right\rangle. \quad (5)$$

The calculation is implemented in a modified version of the GROMACS 5.1 package⁴³ which computes P_T^i values, along with the corresponding positions, velocities, and forces for each particle i in the system. Additionally, we have patched this version to enable the Verlet scheme, Single Instruction Multiple Data (SIMD), and Graphics Processing Unit (GPU) acceleration³² for our simulation conditions (shifted cutoff schemes for LJ and Coulombic interactions). For all studied systems, we have taken the equilibrium trajectories obtained in the corresponding simulations (see Sec. II A) and we have performed reruns using our modified GROMACS version in order to obtain the P_T^i values. Then, the contributions $P_T^G(z)$ for each group G in the system were computed by means of Eq. (5).

The lateral pressure profile is defined as $\pi(z) = P_T(z) - P_N(z)$, where $P_N(z) = P^{zz}(z)$ is the normal component of the pressure tensor. Unfortunately, $P_N(z)$ cannot be calculated using the Harasima path.^{25,38,39} However, mechanical stability requires $P_N(z)$ to be constant throughout the system and equal to the external pressure.^{27,44,45} Assuming $P_N(z) = \text{const.} = 1$ bar (the external pressure value used in the simulations, see Sec. II A), we can therefore evaluate $\pi(z)$ for the total system as $\pi(z) = P_T(z) - 1$ bar.

The curvature elasticity of bilayers can be characterized by the first integral moment of $\pi(z)$, defined as

$$\tau = \int_0^{L_z/2} z \pi(z) dz, \quad (6)$$

where $z = 0$ is the bilayer center and $z = L_z/2$ is the upper bound of the simulation box in the z -direction. This parameter is associated with the curvature elastic properties of the monolayers through the relationship $\tau = \kappa^m c_0^m$, where κ^m is the monolayer bending rigidity and c_0^m is the monolayer spontaneous curvature.^{26,27,46,47} The τ parameter can be physically interpreted as the torque stored in each monolayer⁴⁸ and provides a quantitative measurement of the tendency of each monolayer to curve.²⁷

III. RESULTS AND DISCUSSION

A. Neutral prilocaine

In this section, we report the results of the study of the concentration effects of neutral prilocaine on the structural and

mechanical properties of a PEO₁₄-PBD₂₂ bilayer. We observe that nPLC was encapsulated on the hydrophobic core leading to a normal and tangential expansion of the membrane: the membrane thickness d and the area per chain A_c increase with the concentration of nPLC [Figs. 3(a) and 3(b)]. Moreover, the membrane expansion occurs at a constant volume rate as function of N_{nPLC} , as shown by the linear increase of the hydrophobic core volume V_h [Fig. 3(c)]. By linear regression [Fig. 3(c), blue line], we estimate the effective volume occupied by each nPLC molecule (V_{nPLC}), resulting in 0.370 nm³ per nPLC molecule. This value is commensurable with the estimation of V_{nPLC} (0.355 nm³ per nPLC molecule) obtained using its molar mass (220.31 Da) and predicted density (1.03 g/cm³),⁴⁹ indicating that the bilayer can expand linearly to accommodate the nPLC molecules without changing its overall structure.

In the following, we analyze the local organization and mechanical properties of the bilayer structures through the MDPs and P_T profiles for the different components of the studied systems. In Fig. 4, we compare the MDPs and P_T profiles corresponding to the neat bilayer [Figs. 4(a) and 4(b)] with those corresponding to the highest nPLC concentration [N624, Figs. 4(c) and 4(d)]. As seen in the MDPs for the neat case, the PBD, PEO, and water components are organized to form a bilayer structure.²⁸ The PBD is uniformly distributed at the bilayer core. Through visual inspection, we found evidence of strong interdigitation between PBD tails (see Fig. S5 of the [supplementary material](#)). This behavior is

FIG. 3. Membrane thickness (d), area per chain (A_c), and hydrophobic core volume (V_h) as functions of number of nPLC molecules (N_{nPLC}). The blue line in the V_h plot corresponds to the linear regression obtained for the data.

FIG. 4. MDPs of the total system and each different component (water, PEO, PBD, nPLC, pPLC, and Cl^-) for (a) neat bilayer, (c) highest nPLC concentration (N624), and (e) highest pPLC concentration (P156). System P_T profiles and their contributions coming from each different component (water, PEO, PBD, nPLC, pPLC, and Cl^-) for (b) neat bilayer, (d) highest nPLC concentration (N624), and (f) highest pPLC concentration (P156).

different than the observed one for lipid bilayers, where little or no interdigitation is present.⁵⁰ When nPLC molecules are added into the system, the bilayer structure is essentially conserved, but in order to accommodate the encapsulated molecules, the PBD shows a broader distribution and lower density values in comparison to the neat case. Furthermore, the PBD MDP is no longer uniform around the bilayer center (a drop in the density value is observed here). This change could be associated with a decrease in the overlapping of the PBD tails between the two leaflets when drug concentration increases, leading to a reduction in the amount of interdigitation (see Fig. S6 of the [supplementary material](#)). The nPLC molecules are evenly distributed along the PBD core but show higher densities at the center of the bilayer and near the PEO-PBD interface. This behavior is somewhat different from the one observed on nPLC in lipid systems, where the nPLC is also encapsulated within the lipid bilayer but following a bimodal distribution.^{29,51}

In Fig. 4(b), it is observed that the system P_T values converge to the external pressure (1 bar) in the bulk water phase, as required for bilayers in mechanical equilibrium. The addition of nPLC and pPLC does not change this behavior [Figs. 4(d) and 4(f), respectively], indicating that the membrane is stable under loading of PLC. In the system P_T profile of the neat bilayer, we can recognize three different regions: a positive contribution corresponding to the PEO-water interface, a negative contribution around the PEO-PBD boundary, and a positive contribution in the PBD region. Similar behavior has been observed for P_T profiles on lipid bilayers using a MARTINI CG approach.⁵² With the inclusion of nPLC into the bilayer, the P_T profile of the system exhibits a shape similar to the one observed for the neat case. A detailed description of the balance of the total tangential pressure can be obtained by analyzing the individual contributions of each system component to the P_T profile. For the neat system, the PEO contribution is negative while the PBD and water components are positive. In the PBD region, the main contribution to the total tangential pressure is mostly provided by the PBD component, while in the PBD-PEO boundary, the behavior is dominated by the PEO component. In the PEO-water interface, the positive water contribution is

partially compensated by the negative PEO component. On the other hand, for the nPLC-loaded system, the PEO and water profiles are similar to the former case. The main difference is observed in the PBD region, where nPLC molecules are distributed. The PBD component is clearly increased with respect to the neat case, leading the bilayer area expansion. The nPLC contribution is essentially negative, showing a small positive contribution at the PEO-PBD boundary. A deeper understanding on these systems could be obtained by analyzing the evolution of these properties with the drug concentration.

The nPLC concentration effects on the whole system was assessed by the MDPs and P_T profiles with respect to z , which are shown in Figs. 5(a) and 5(b), respectively. Since bilayers are symmetrical, only the right side of the profiles is shown. In systems with higher amount of nPLC, we observe that the total MDPs are shifted to the right (up to 1 nm), following the membrane thickness expansion. The P_T profiles are also shifted to the same direction following the MDPs behavior, keeping similar shapes to the neat one. To get further insights, we analyze them separately.

The nPLC partial density and pressure contribution are shown in Figs. 5(c) and 5(d), respectively. At the lowest concentration, we observe that the nPLC MDP follows a roughly uniform distribution in the hydrophobic region. As nPLC concentration increases, the nPLC density grows in the bilayer center and also shifts following the bilayer thickness expansion. This will be revisited below. The P_T values are essentially negative and constant around the bilayer center, followed by a minimum at $z \sim 2.5$ nm and a small positive contribution around $z \sim 3.5$ nm.

As we already discussed, for the neat system, the PBD MDP presents uniform distribution up to $z \sim 2.5$ nm and drops to zero at $z \sim 4$ nm. This distribution is slightly perturbed by the presence of the drug [as shown in Fig. 5(e)]. A dip in the bilayer center followed by a small peak is observed accounting on the interdigitation decrease. Nevertheless, because of the superposition, these details are difficult to observe. The PBD contribution to the tangential pressure is always positive and shows a maximum at ~ 2.5 nm. The pressure at this maximum increases and its position shifts towards the outside of the membrane as the concentration of the drug increases [see Fig. 5(f)]. These

FIG. 5. MDPs and P_T profiles of the whole system, nPLC, PBD, PEO, and water components for different nPLC concentrations. Only the right side of the profiles is shown.

results could be associated with the hydrophobicity of neutral nPLC.

Indirect effects of the drugs on the hydrophilic distribution of the systems are observed [see Figs. 5(g) and 5(i) for PEO and water, respectively]. For the neat system, we observe that the water density is zero up to $z \sim 2.5$ nm (showing no access to the hydrophobic core). From this point, due to the presence of a complex PEO-water interface, water density starts to increase up to the bulk water phase ($z \geq 7$ nm). On the other hand, in Figs. 5(h) and 5(j), the tangential profiles

of PEO and water are shown. The corresponding P_T PEO contribution is negative with two minima, a deeper one at $z \sim 3.2$ nm (slightly shifted to the left from the density peak) and the second at $z \sim 5$ nm, compensated by water positive contribution with a peak at $z \sim 4.5$ nm. Further analysis reveals that the hydration on the PEO region increases with nPLC concentration, up to ~ 2 water molecules per chain with respect to the neat case (see Figs. S7 and S8 of the [supplementary material](#)).

A fine interplay between the different components leads to stable bilayers, even at high nPLC concentrations. Overall, nPLC is widely distributed along the plane of the membrane in the PBD region, with slightly higher densities in the center of the bilayer and PEO-PBD boundaries. The nPLC effects on the P_T profile are more complex. At the bilayer center, the PBD P_T profile grows up to approximately 75 bars when nPLC concentration increases, which is canceled by the nPLC contribution in each case. This is also observed around the PEO-PBD-water interface. In this way, the system shows good mechanical compliance upon nPLC loading. Furthermore, we explored the first integral moment of the lateral pressure profile (τ) of the whole system for each studied case. The results for different nPLC concentrations are shown in Fig. 6. We observe positive τ values in the range $4\text{--}6$ $k_B T/\text{nm}$, suggesting a tendency of each monolayer to curl.²⁷

B. Protonated prilocaine

We have investigated the effect of pPLC on the bilayer structure following a similar approach to the one used for the neutral case. We observe that d decreases and A_c increases with N_{pPLC} [Figs. 7(a) and 7(b)], but V_h remains essentially constant for all studied pPLC concentrations [Fig. 7(c)]. These results show that pPLC remains present at the interface and in the water, with no access to the hydrophobic core, but the decrease in the thickness of the bilayer reaches a saturation point at $N_{pPLC} \sim 104$, with no significative changes in d and A_c above this value.

More insight into the effect of pPLC on the bilayer structure is obtained by analyzing the MDPs and P_T profiles of the different components in the system. As an example, in

FIG. 6. First integral moment of the lateral pressure profile (τ) as a function of number of nPLC molecules (N_{nPLC}) measured in units of $k_B T/\text{nm}$ (k_B is the Boltzmann constant and $T = 300$ K).

FIG. 7. Membrane thickness (d), area per chain (A_c), and hydrophobic core volume (V_h) as functions of number of pPLC molecules (N_{pPLC}).

Figs. 4(e) and 4(f), we describe the case for the highest pPLC concentration used in this work (P156). Similar to the neutral case, the PBD, PEO, and water components still maintain a bilayer structure. Nevertheless, the distribution of the pPLC molecules is different with respect to the neutral species. In this case, pPLC partition between the PEO-PBD interface and water phase is observed. Counterions (Cl^-) are mostly distributed in the water phase. On the other hand, the P_T profiles corresponding to the whole system and PEO do not present significant changes with respect to the neat case. In the bulk water phase, the water and pPLC positive contributions are compensated by the Cl^- negative component to give overall P_T values in equilibrium to the external pressure (1 bar).

The pPLC concentration effects on the whole system MDPs and P_T profiles with respect to z are shown in Figs. 8(a) and 8(b), respectively. As pPLC concentration increases, the system MDPs are essentially unchanged up to $z \sim 2$ nm, but they are more complex after this position, and then a deeper study is necessary to elucidate the distribution of each component. The system P_T profiles seem to exhibit no significant differences, apart from the shift to the left following the membrane thickness decrease.

For pPLC molecules, a well-defined partition between the PEO-PBD interface and water phase is observed for all cases [Fig. 8(c)]. At the lowest studied concentration, the pPLC MDP exhibit a peak around the PEO-PBD interface and a small positive contribution in the water phase. As pPLC concentration increases, the density grows in both regions, and the observed peak is shifted to the left

FIG. 8. MDPs and P_T profiles of the whole system, pPLC, PBD, PEO, and water components for different pPLC concentrations. Only the right side of the profiles is shown.

according to the membrane thickness decrease. Nevertheless, for $N_{pPLC} \geq 104$, the peak does not show significant changes and the major increases are observed in the water region. The pPLC P_T profiles show a negative contribution around the PBD-PEO boundary [Fig. 8(d)], where minima become deeper as pPLC concentration increases and slightly shifted to the left with respect to the corresponding density peak. A positive contribution in the water phase is also observed following the same behavior as the MDPs in this region.

For all studied pPLC concentrations, the shape of PBD and PEO MDPs is similar to the neat case but shifted to the left following the membrane thickness decrease when pPLC concentration increases [Figs. 8(e) and 8(g)]. The water MDPs also show the left shift as well as a decrease in the density in the bulk water phase (due to the presence of pPLC and CL^-) as pPLC concentration increases [Fig. 8(i)]. Besides, the only noticeable change observed for the PBD P_T profiles is the left shift following the MDPs' behavior [Fig. 8(f)]. This shift is also observed for PEO and water [Figs. 8(h) and 8(j)]. In particular, the PEO P_T profiles showed a difference in magnitude of its minimum with respect to the neat case up to 80 bars approximately.

Particular attention was paid to the interfaces. An increase of the water density is observed in the PEO-water interface. This increase is not linear and seems to converge for $N_{p\text{PLC}} \sim 78$, where ~ 6 extra water molecules per chain are found (see Figs. S9 and S10 of the [supplementary material](#)). With respect to the PEO-PBD interface width, it is stable (near 1.0 nm) for all cases. The lateral expansion was compensated by water penetration. In this sense, the pPLC preferential location within this interface could be attributed to entropic effects.

Similar to the neutral case, all the components in the system are balanced ensuring the stability of the bilayers. Nevertheless, the system behavior is rather different for the protonated case. The pPLC is partially encapsulated in the PEO-PBD interface region, and the amount of encapsulated drug seems to saturate for $N_{p\text{PLC}} \sim 104$, as suggested by the pPLC MDPs. The analysis of the P_T profiles provides insights on the mechanical behavior of the interface: a compensation mechanism on pressures is observed between the PEO and pPLC components. Also, at the water phase and the PBD-PEO-water interface, counterions contributed to the pressure without affecting the system stability. Overall, the system shows good mechanical stability upon pPLC loading.

In order to characterize the pPLC encapsulation in the bilayer, we have estimated the number of pPLC molecules in the PEO-PBD interface (N_i) and in the water phase (N_w) for each pPLC concentration [see Fig. 9(a)]. The evolution of N_i corresponding to the number of pPLC molecules encapsulated into the bilayer along the simulation is plotted in Fig. 9(b). For all cases, we observe that N_i stabilizes after 1.5 μs . As pPLC concentration grows, N_i increases up to $N_{p\text{PLC}} \sim 104$. Beyond this point, no significant changes on N_i are observed. However, for $N_{p\text{PLC}} = 104$, the system presents higher fluctuations than the other cases. More details on the partition behavior could be provided by the free energies of partition between both phases, which are estimated by the equation $\Delta G_{i-w} = -RT \ln(N_i/N_w)$.^{53,54} The ΔG_{i-w} plot is depicted in Fig. 9(c). The change of sign of the free energy with the pPLC concentration shows an affinity change from the PEO-PBD interface ($N_{p\text{PLC}} \leq 104$) to the water phase ($N_{p\text{PLC}} > 104$). The pPLC affinity equilibrium for both phases, estimated by linear regression (blue line), is reached at $N_{p\text{PLC}} \sim 113$. This value is close to $N_{p\text{PLC}} = 104$, which may explain the high fluctuation previously observed for this concentration [Fig. 9(b)]. It is important to highlight that at

FIG. 9. (a) Schematic representation of pPLC partition between the PEO-PBD interface (N_i) and water phase (N_w); pPLC MDP is normalized to the total number of pPLC molecules in the system. (b) Time evolution of the number of pPLC molecules in the PEO-PBD interface (N_i) for different pPLC concentrations. (c) Free energy of partition between the PEO-PBD interface and water (ΔG_{i-w}) as a function of the number of pPLC molecules in the system ($N_{p\text{PLC}}$); the blue line represents the linear regression on the data.

physiological pH conditions, the amount of nPLC molecules corresponding to the pPLC affinity equilibrium is $N_{n\text{PLC}} \sim 70$, estimated through Henderson-Hasselbalch equation considering prilocaine apparent pK_a of 7.6.³⁰ In this direction, it could be possible to increase the amount of loaded drug working at higher pH, shifting the equilibrium in favor of the neutral species.⁵⁵

Finally, we have characterized the τ parameter for the protonated cases, where the values determined for each pPLC concentration are shown in Fig. 10. The τ range is similar to the one observed for the neutral case. However, τ shows no significant changes for $N_{p\text{PLC}} \geq 104$, following interface saturation [Fig. 9(b)].

FIG. 10. First integral moment of the lateral pressure profile (τ) as a function of number of pPLC molecules (N_{pPLC}) measured in units of $k_B T / \text{nm}$ (k_B is the Boltzmann constant and $T = 300$ K).

IV. CONCLUSIONS

Polymersomes are promising drug delivery systems, but their formulation presents many technical difficulties; therefore, computational strategies may allow the optimization of copolymer and drug selection for a specific application.

In this work, we have used an MD CG approach within the MARTINI framework to study the encapsulation of PLC on PEO-PBD bilayers. A bilayer area expansion was observed for both neutral and protonated prilocaine as drug concentration increases, but the bilayer thickness behavior depends on the ionization state: it increases for neutral species and decreases for the protonated ones. Neutral prilocaine is encapsulated in the hydrophobic region, while the protonated species partitioned between the water phase and PEO-PBD interface.

Since the protonated prilocaine seems to be anchored to the PEO-PBD interface, the PEO chain length would not limit the loading capacity of a polymersome composed by this copolymer *per se*. Strong interdigitation between leaflets is observed in all cases. Nevertheless, the overlaps between PBD tails slightly decrease with nPLC concentration. This could affect the stability of the bilayer at high nPLC loading.

The mechanical properties of drug loaded bilayers were evaluated. The presence of the drug has a relative small impact on the tangential pressure of the total systems. Furthermore, we were able to decompose the tangential pressure in their different components and elucidate their spatial interplay.

The first integral moment of the lateral pressure profiles, τ , was calculated for all studied cases. τ is the product between the spontaneous curvature and the bending rigidity constant (always positive^{24,27}). Since bending rigidity constants are system dependent and not straightforward obtainable from calculations, τ comparison between different systems is not possible within the context of this work. Considering that the curvature is zero only for flat structures, this implies that τ values different from zero favor curved structures. In this direction, our results from monolayers (positive τ for all studied cases) suggested a tendency to curve. Besides, periodic boundary conditions limited the formation of curved structures. Further studies could be advisable in order to get information of curved structures,⁵⁶ such as polymersomes.

We have studied concentrations corresponding to high pH (neutral) and low pH (protonated) separately. Because of the prilocaine pKa, a partition between both species comes about at physiological pH. Moreover, pH could be modulated to adjust the balance between neutral and protonated species in order to enhance the drug loading. Our results suggest that under physiological condition, the protonated species will be the limiting factor for drug loading, but cooperative effects between species, not considered here, could modify their loading capacity. Simulations combining them could help elucidating this point.³⁰

Further application of this approach could be used to investigate mechanical properties in order to identify main components on the formulation to make the system stable under the desirable conditions: for instance, the impact of other key structural components such as combination of polymers and complexation with different drugs or active biomolecules (DNA, antibodies, proteins). Moreover, it could be applied to materials that are sensible to a given stimulus or condition. This is the case for pH or temperature sensible DDS or directly linked to mechanical properties: flexible materials such as polymersomes combined with surfactants that can overpass biological barriers.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for snapshots of different initial conditions of nPLC and pPLC loading, τ calculation and statistical analysis on their fluctuations along different subtrajectory lengths, snapshots and MPDs revealing interdigitation between PBD tails, and analysis of hydration effects on the PEO region.

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