

Hybrid QM/MM Molecular Dynamics Study of Benzocaine in a Membrane Environment: How Does a Quantum Mechanical Treatment of Both Anesthetic and Lipids Affect Their Interaction

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ABSTRACT: Biomolecular dynamics studies using a QM/MM approach have been largely used especially to study enzymatic reactions. However, to the best of our knowledge, the very same approach has not been used to study the water/membrane interface using a quantum mechanical treatment for the lipids. Since a plethora of biochemical processes take place in this region, we believe that it is of primary importance to understand, at the level of molecular orbitals, the behavior of a drug in such an odd environment. In this work, we take advantage of an integration of the CPMD and the GROMACS code, using the Car–Parrinello method, to treat the benzocaine local anesthetic as well as two of the membrane lipids and the GROMOS force field to treat the remaining lipids and the water molecules.

■ INTRODUCTION

From very simple models, such as dielectric interfaces in the 1980s and 1990s, to all atoms simulations with thousands of lipids, going through several methods and models developed, biological membranes as well as their proteins have become one of the most studied systems by molecular dynamics (MD) simulations, especially because of the huge advances in the computer capability.^{1–11} Recent works, especially those using coarse-grained approximations, have shown MD simulations in membrane systems with sizes and time scales that were inconceivable in the 1990s.¹

Since a plethora of biochemical phenomena take place in the water/membrane interface, it has always been the aim of both experimental and theoretical studies.^{12–18} This region is also of paramount importance to the drug development, since several membrane proteins are targets for such molecules. Moreover, the membrane is a remarkable barrier to a majority of the drugs that act inside the cell. For this reason, several studies have been trying to develop new methods to drug delivery, such as the use of new nanomaterials¹⁹ or liposomes.²⁰ Numerous studies attempt to understand how could we better use computational methods to study such regions, for instance, using more accurate methods of molecular modeling, such as quantum mechanical (QM) calculations.^{21,22} Meanwhile, because this interface has electrostatic properties that, at first, could not be studied by classical molecular modeling, the application of QM methods seems to be a more accurate approach. Furthermore, the behavior of the charge distribution over a drug surface, especially those containing aromatic groups, will adapt to the media when crossing a membrane interface, opposite of the classical MD approach, where it remains constant.

Hence, to better investigate the water/membrane interface, it will be necessary to use QM calculations, where both the drug and the membrane lipids have their electronic orbitals explicitly

simulated. However, a pure QM approach is impossible since the minimum size of a fully solvated membrane patch is greater than our current computational power, especially due to the memory demand of an ab initio calculation.

To elucidate it, we adopted in this work a hybrid MD simulation, using both quantum and molecular mechanics (QM/MM),²³ adopting the ab initio Car–Parrinello molecular dynamics (CPMD) to the quantum part²⁴ and the GROMOS force field,²⁵ implemented in the GROMACS, to the classical one.^{26,27} We believe that this is the first work using such approach since to our knowledge no other work has tried to adopt a QM/MM method to study the dynamics of lipid membranes. A QM/MM approach was used in some other works to study lipid membranes, as in the study of the solvation of the dipalmitoylphosphatidylcholine (DPPC) headgroup²² and in the study of the transmembrane potential in the same lipid,²¹ however neither of them included a drug nor carried out a QM/MM dynamics.

Our interest in this problem emerged during our previous studies with local anesthetics (LA), where we carried out MD simulations to investigate the hydration shells around these molecules²⁸ and the LA/membrane interaction.²⁹ The LA are drugs that cause reversible interruption on the signal transmission through the neuronal cells, inducing absence of pain sensation and paralysis. The action mechanism of these drugs, as well as its toxicity, has been the object of several studies, and it is largely accepted that by controlling ion flux through transmembrane channels, LA reversibly decrease the rate of depolarization and repolarization of excitable membranes.^{30,31}

Despite several studies, the action mechanism of LA at molecular level on the nervous system remains controversial as well as how the lipid membrane contributes to this

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process.^{31–33} Regarding their structures, LA are tertiary amines (see the illustration made with VMD³⁴ in the Figure 1),

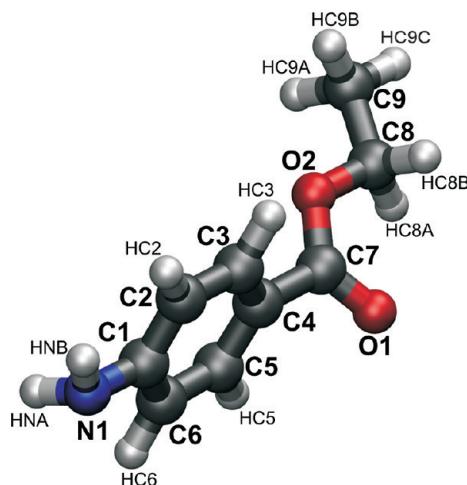


Figure 1. Illustration of the BZC molecule and its atom identification. In the classical MM simulations all hydrogen atoms are grouped to the carbon atoms in which they are bonded (creating CH, CH₂, and CH₃ groups), except those in the amine terminal HNA and HNB that are always explicit simulated.

typically amphiphilic, and despite their different structures, they share chemical features that are relevant to their biological function: an aromatic ring, a polar group, and an ionizable amine with relatively high pK_a values, commonly around 7.5–9.0.³⁵ The exception of this rule is the ethyl 4-aminobenzoate, also known as benzocaine (BZC), a LA that presents some particular differences. Its pK_a is near 3.5, thus there is virtually no presence of the charged type in physiological media, but its activity has the same general properties of the other LA.³²

In our previous work, we observe that BZC, despite its neutral charge, behaves as the charged LAs in the water/membrane interface.²⁹ This result provides evidence that the charged form of the LA is of paramount importance to the LA effect. This result is not completely unexpected since BZC is an amphiphilic molecule and such molecules normally have their free energy minimum, a stable position, in this portion of the membrane. However, especially due to its aromatic ring, BZC can be polarized depending on the environment it is inserted. Because our results suggested that BZC was stable in a position where the media is completely different from the media where the drug was parametrized, our conclusions could be a simulation artifact. To elucidate this question in this work, we are suggesting that this equilibrium position could be used to a most complete calculation using QM/MM methods, where BZC and the nearest lipids could be simulated using quantum mechanics, and the rest of the system is simulated using classical molecular mechanics (Figure 2A). Since the time scale of this ab initio QM/MM dynamics is thousands of times smaller than a purely classical MD, we aim to observe if the equilibrium position of the BZC is advantageous and sustained or if it is quickly repelled, in a time scale that we believe to be adequate.

METHODS

Classical MD. As discussed in our previous work,²⁹ the molecular model for BZC was constructed in accordance with Pub Chem (<http://pubchem.ncbi.nlm.nih.gov>) register: 2337.

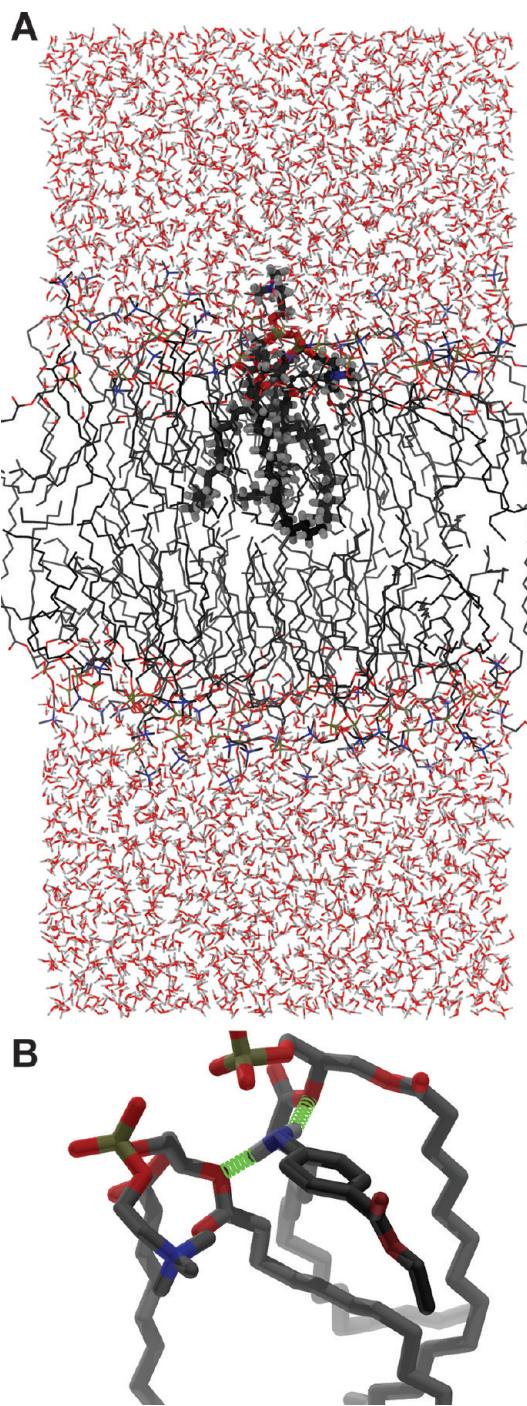


Figure 2. (A) Illustration of the LA in a fully solvated membrane patch. The system is constituted by 3827 water molecules, 1 BZC LA molecule, and 64 DPPC lipid molecules. Highlighted is the BZC and two of the DPPC lipids, which were the part of the system treated with QM methods during the QM/MM MD. (B) Illustration of the BZC/DPPC interaction showing two hydrogen bonds that holds BZC close to the DPPC polar region. No hydrogen atoms are shown to simplify the observation.

The partial charges were obtained using the Gaussian 03 package,³⁶ employing a DFT method.^{37,38} We used a combination of the Becke exchange³⁹ three-parameter method⁴⁰ with a Lee–Yang–Parr correlation functional⁴¹ in the popular B3LYP formulation.⁴² The 6-31G(d,p) basis set^{43,44} was employed, and we fitted the molecular electrostatic

potential through the ChelpG scheme⁴⁵ to obtain the partial charges. The atomic distances, bond angles, dihedrals, and charges from the lowest energy conformation were used to estimate the force field parameters for these drugs, according to GROMOS force field.²⁵ The BZC molecule was solvated in a box with SCP water model,⁴⁶ with approximately 3800 water molecules and 64 phospholipids, with periodic boundary conditions and simulated in a semi-isotropic NpT ensemble.^{3,47}

The BZC was placed randomly in water, at approximately 1.2 nm away from the membrane surface. The classical MD studies were carried out using the GROMACS package.^{26,27} The parameters for the membrane assembled with DPPC lipids were achieved from Tieleman's work.^{47,48} The system was thermodynamically coupled at 320 K using a Berendsen thermostat⁴⁹ for equilibration and a Nose–Hoover thermostat for production dynamics.^{50,51} The semi-isotropic pressure coupling was employed following Tieleman's model,³ using Parrinello–Rahman barostat.⁵² Electrostatic interactions were represented by particle mesh Ewald (PME)⁵³ with cutoff of 0.9 nm, while van der Waals interactions were considered to a cutoff of 1.0 nm. All bonds were constrained using the LINCS algorithm,⁵⁴ the geometry of SCP water molecules was constrained using the SETTLE algorithm,⁵⁵ and the time step for integration was set to 2.0 fs.

Before the simulations, a water relaxation dynamics was carried out with 500 ps, with position restraints for the membrane and the LA, followed by an unrestrained run of 2 ns for system equilibration and by a production dynamics for 50 ns. The most significant stable position of the BZC in the membrane was used in another MD simulation, as a control for the QM/MM simulation. This control simulation was carried out for 18 ps, and its time-step integration was set to 0.5 fs. The major difference in this simulation was the writing frequency, which was set to maximum.

QM/MM MD. Since a fully ab initio quantum dynamics simulation of a membrane system still remains inaccessible, a possible strategy to treat such a system is a QM/MM approach. The so-called hybrid methods have been developed to elucidate problems where only a part of a large system needs to be treated at the QM level.^{56,57} In this work, we used an integration of the CPMD and the GROMACS codes²³ that use the Born–Oppenheimer approximation. The BZC molecule and two of the DPPC lipids were treated using QM methods by the CPMD package.^{24,58} The rest of the lipids and the water were treated using the same GROMOS force field used in the MM work, as shown in the Figure 2A.

The QM atoms were treated with the Becke exchange³⁹ and Lee–Yang–Parr correlation functional,⁴¹ so-called BLYP, and the plane wave basis set of the Car–Parrinello method. The MD was carried out using the same parameters, described in the Classical MD Section, for both QM/MM and MM simulations, except the time step that was reduced to 0.5 fs as well as in the control MD simulation. The initial step for the QM/MM calculation was selected from the most relevant position at the end of the MM simulation. A hybrid Born–Oppenheimer MD was carried out during 18 ps using the Vanderbilt ultrasoft PP with plane wave cutoff of 30 Ry.⁵⁹ In both MM and QM/MM simulations, the use of counterions was not necessary once the total charge of the system is zero. Also, the QM region in the QM/MM calculation has neutral charge.

The interaction between the BZC and the DPPC was analyzed using the radial distribution function (RDF). This

function, also called $g(r)$ function, is defined as the average radial density of a certain observable to a distance r from an origin, which provides an insight regarding the local structure of the surrounding media along the time. For r larger than the correlation distance, the RDF decays to the media density, usually normalized to one. This analysis provides us with the potential of mean force (PMF), since $W(r) = -kT \ln g(r)$. If $g(r) > 1$, then $\ln g(r) > 0$ and the potential is attractive, otherwise for $g(r) < 1$, the potential is repulsive.

To simplify the discussion, we will denote that as nDPPC_{QM}, the quantum DPPC lipids are near to the BZC (two lipids) in the QM/MM simulation, as nDPPC_{MM}, the classical DPPC lipids are near to the BZC (the same two lipids as in the nDPPC_{QM}) in the MM simulation, and as fDPPC_{MM}, the classical DPPC lipids are far from BZC (62 lipids) in both MM and QM/MM simulations.

RESULTS AND DISCUSSION

Choosing the QM region in a hybrid QM/MM calculation is not simple task, especially when we are investigating biologically relevant systems, which are usually large.⁶⁰ In our system, we picked up all molecules that were up to 3.5 Å away from any atom of the BZC molecule. The distance was selected to avoid those molecules that were not interacting with the anesthetic directly, keeping just the molecules that we could have charge transference. We were especially interested in the electron transfer between the amine group of the anesthetic and the lipids, thus farther molecules are not of our interest. This knowledge is important since the size of the QM region is determinant for both result and simulation time.

Hybrid QM/MM MD studies of membrane lipids are novel but certainly necessary to understand drug behavior at water/membrane interfaces. Since QM/MM is an unusual approach for such a system, the consistency of the membrane lipids behavior had to be tested. To do that, after a successfully visual evaluation, we analyzed the root-mean-square deviation (rmsd) of the nDPPC_{QM} lipids as well as the radial distribution function (RDF) of fDPPC_{MM} lipids around the nDPPC_{QM} lipids and the hydration of these molecules using the same RDF method. All these graphics are presented in the Figure 3 comparing the results for the nDPPC_{QM} molecule to those to the nDPPC_{MM}.

The graphics show that there are no major differences between the lipids behavior in both QM/MM and classical simulations, keeping the membrane properties intact. Despite the small time scale, and perhaps because of that, we believe that there is no evidence that a QM/MM simulation is creating artifacts in this water/membrane interface that could interfere in our results. The rmsd analysis of the nDPPC_{QM} lipids compared to the nDPPC_{MM} (Figure 3A) shows that there is a tendency for the same behavior, enforcing a stability of the system. Figure 3B shows that the distribution of the fDPPC_{MM} lipids around the nDPPC_{QM} lipids keeps the same behavior as the one found in the pure MM simulation. Figure 3C shows that the hydration of the PO₄ region of both nDPPC_{QM} and nDPPC_{MM} lipids did not display substantial changes, keeping its overall behavior.

After establishing that the membrane behavior remains normal when after the introduction of quantum lipids, we started to study the behavior of the anesthetic in this interface region. As discussed, the equilibrium position of the BZC was raising some doubts, however, our new findings, using a QM/MM simulation, showed that this position was remarkably

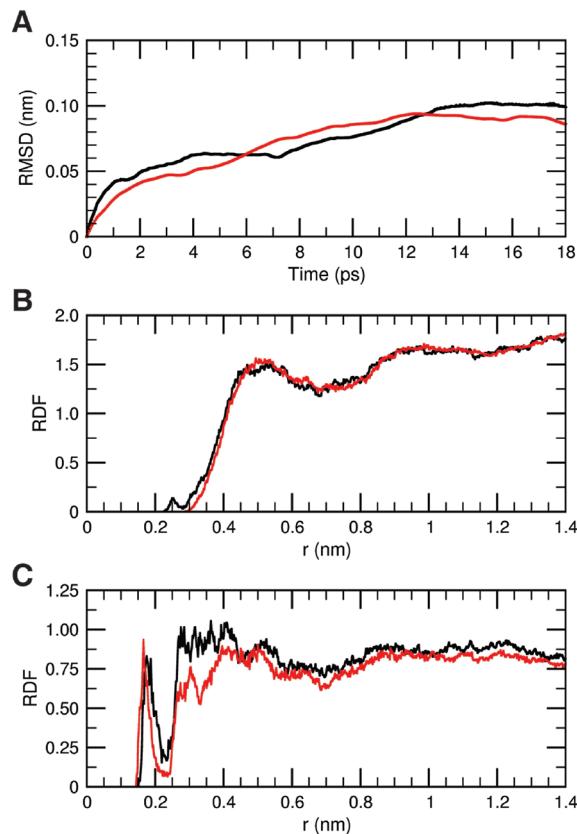


Figure 3. Plots comparing the results obtained for the lipids with both MM and QM/MM methods. (A) Rmsd analysis showing similar behavior for both simulations. (B) RDF analysis of the classical lipids headgroup atoms surrounding the atoms of the headgroup of the two lipids that are near the BZC molecule. (C) RDF analysis of the water molecules atoms surrounding the head groups atoms of the two lipids, which are near the BZC molecule. In red is the QM/MM simulation and in black the classical MD.

stable, and the interaction between DPPC and BZC in the hybrid calculation was even greater than in the pure classical dynamics. The graphic in the Figure 4B shows the distribution of oxygen atoms of the DPPC palmitate group around the BZC molecule. This analysis showed that there is a small difference in the stability position in both simulations and that the BZC–DPPC distance is even smaller in the QM/MM simulation, however it also shows a broader peak in this case, which could indicate that the amplitude of the vibration of the atoms close to the lipid was greater. To better illustrate the stability position, we showed, in the Table 1, the distance between each of the BZC amine hydrogen atoms and the DPPC oxygen atoms that they are interacting, as showed in the Figure 2B. We can observe that all interactions are very stable and that in the QM/MM simulation, the HNA atom holds the same position with a very small deviation.

The BZC molecular dynamical behavior was better analyzed by its rmsd, as shown in the Figure 4A, which shows that the QM treatment of the drug causes a much higher structural deviation. This result does not mean that the BZC becomes unstable in the QM/MM simulation, but the explicit hydrogen atoms are increasing the rmsd. Analysis of the fluctuation of the root-mean-square deviation in each atom (data not shown) shows that the higher deviation is caused by hydrogen atoms, HNA and HNB, in the amine terminal (that is also present in the MM simulation), probably due to the fact that quantum

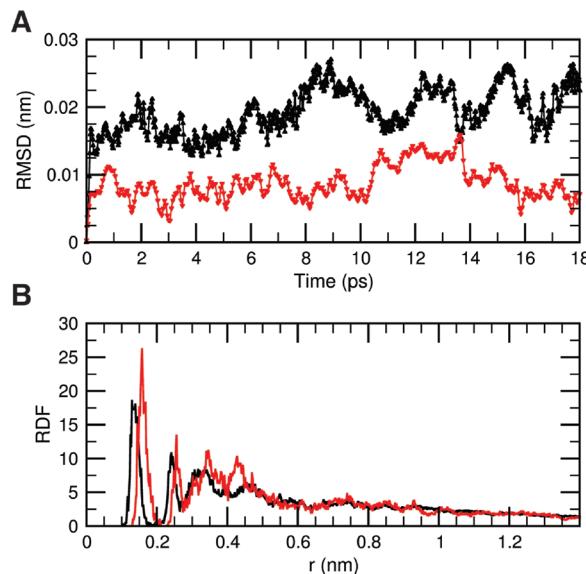


Figure 4. Plots comparing the results obtained for the BZC LA with both MM and QM/MM methods. (A) Rmsd analysis. (B) RDF analysis of the oxygen atoms surrounding the BZC molecule. In black is the QM/MM simulation and in red the classical MD.

Table 1. Distance between the Atoms in the Hydrogen Bond Indicated in the Figure 2B during Classical and QM/MM MD Simulations^a

interacting atoms	initial distance (nm)	final distance (nm)	average distance (nm)
HNA _{BZC} /O _{DPPC1} (MM)	0.158	0.186	0.192 ± 0.031
HNB _{BZC} /O _{DPPC2} (MM)	0.204	0.195	0.178 ± 0.029
HNA _{BZC} /O _{DPPC1} (QM/MM)	0.158	0.153	0.148 ± 0.009
HNB _{BZC} /O _{DPPC2} (QM/MM)	0.204	0.213	0.221 ± 0.043

^aNote that, at end of the simulation, the QM/MM calculation shows the shortest distance of interaction for the HNA. The index DPPC1 and DPPC2 is used to clarify that each oxygen atom is in a different lipid.

calculation allows the proton transfer between the anesthetic and the lipid. However, we have not found any proton transfer during the simulation, just higher vibration amplitude of these hydrogen atoms (data not shown) and an expressive overlap of the BZC and DPPC wave function (Figure 5). The proton transfer is unlikely to be observed since the reaction barrier is expected to be much larger than $k_B T$. For this transfer to succeed, the BZC should donate a proton to the phosphatidylcholine, however to donate this proton, the BZC should be in a very alkaline media, and to accept the proton, the lipid must be in an acidic region.

To observe the charge behavior over the BZC, we did the same calculation used to parametrize the charges of this LA, a single point DFT B3LYP/6-31G(d,p) with charges fitted from the electrostatic potential using ChelpG scheme. Taking the last position of the QM/MM dynamics, we took the BZC and all of the molecules up to 3.5 Å of the drug, which were now three DPPC lipids and one water molecule. This extra quantum lipid, compared to the QM/MM calculation, was selected to complete all of the first solvation shell of the anesthetic, using the same methodology used to select the QM region in the hybrid calculation, selecting all the molecules that have atoms in a certain distance from any anesthetic atoms. The results

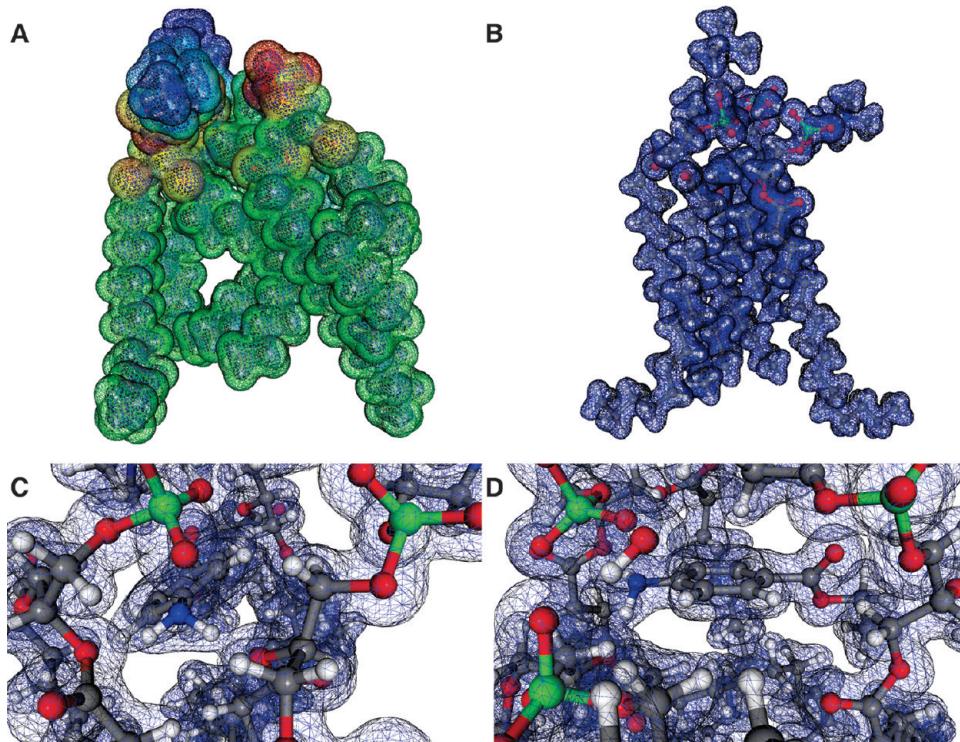


Figure 5. Illustration of the LA interaction with the lipid calculated with the DFT method B3LYP/6-31G(d,p). (A) The electrostatic potential over the solvent accessible surface. Observe in blue, the positively charged potential, in red, the negatively charged potential, and in green, the neutral regions. (B) Electronic surface of the quantum region. (C,D) The detail of the electronic surface in the region of the BZC/lipid interaction. The overlap of both molecules' electronic surfaces can be observed.

presented in the Figure 5 and in the Table 2 support the existence of an interaction between the BZC and the lipid, showing an electronic displacement through the lipids oxygen to the BZC. Figure 5A illustrates the electrostatic potential over the solvent accessible surface of the system, obtained from the DFT calculations, showing the polar headgroup of the lipids and its hydrophobic tail. In Figure 5B–D, we are representing

the electron surface of the molecule with different views detailing electronic surface. These illustrations are showing the point where we found the overlap of the atomic orbital in the BZC/lipid interaction, establishing a strong interaction between these molecules.

Due to this strong interaction, the total charge of the BZC is more negative in this environment. It happens as a consequence of the overlap of the DPPC and BZC orbitals, leading to an electronic transference from the lipid to the anesthetic through its amine group. The Figure 6 illustrates the displacement of the electron cloud of BZC in this interface region. Note, in Table 2 and Figure 6, that there are some major variations in the charges over some atoms of the system,

Table 2. Partial Charge Values for Each BZC Atom Group Fitted from the Electrostatic Potential Using the ChelpG Scheme over a B3LYP/6-31G(d,p) Calculation^a

atom name	group	BZC in implicit water model	BZC in membrane
N1	N	-0.80	-0.56
NH1A	H	0.35	0.10
NH1B	H	0.35	0.30
C1	C	0.41	0.41
C2	CH	-0.09	-0.17
C3	CH	-0.24	0.03
C4	C	-0.24	-0.17
C5	CH	0.07	0.04
C6	CH	0.14	-0.05
C7	C	0.68	0.73
C8	CH ₂	0.33	0.49
C9	CH ₃	-0.05	-0.30
O1	O	-0.52	-0.54
O2	O	-0.39	-0.56
total		0.00	-0.25

^aThe partial charges were calculated in an implicit solvent, using the Onsager continuum solvation model to simulate a water media, and at the explicit membrane environment, solvated by a water molecule and three DPPC molecules.

Figure 6. Illustration representing the BZC molecule and the nearest oxygen atom of each quantum lipid showing the difference in the partial charges when in an aqueous environment and in the membrane environment. The charges were calculated with the DFT method B3LYP/6-31G(d,p).

affecting the polarization of this LA. These results are suggesting a very strong LA/membrane interaction, confirming the stability showed in our previous work.²⁹ The extra QM lipid and the water molecule apparently had no major influence in the total charge of the BZC, since there was no electron transference between these molecules.

CONCLUSIONS

Primarily, our results combined show that a QM/MM approach is possible and very efficient to study the behavior of membrane lipids and other molecules in the water/membrane interface. The simulation time scale is still very small but undoubtedly adequate to several studies of drugs and proteins in this region, especially those covering electrostatic properties of such molecules.

In a previous study, we performed a computational analysis of three LAs behavior and stability in the membrane environment by classical MD simulations.²⁹ In this work, we showed, for BZC, lidocaine, and tetracaine, that the charged form of these drugs are oriented at the interface as one of the lipids, while the neutral form can easily cross the interface, entering the membrane in agreement with most experimental results. However, our results suggested that BZC, which exists only in its uncharged form in physiological media, behaves as a charged anesthetic, remaining stabilized and oriented at the interface.

In the present study, we used a QM/MM approach to verify if BZC's odd behavior was merely a consequence of classical MD artifacts. Our hybrid calculations, using a CPMD/GROMACS linkage, show that the stability of the BZC molecule in the water/lipid interface is not an artifact of the previous simulation²⁹ but certainly a promising point of stability for this drug. Pure B3LYP QM calculations showed an overlap of the atomic orbitals of the anesthetic and the lipid, suggesting a strong hydrogen bond between the amine terminal of the drug and the palmitate group of the lipid. This BZC stabilization, at the same place where we found the charged form of some other LAs, lead us to suggest that by reduction of the number of degrees of freedom, the membrane could catalyze the molecular interactions, especially near to the polar head, a unique region for interaction to some ion channels as well as in a variety of membrane receptors. This possibly increases the probability of binding between these drugs and their transmembrane targets, as previously suggested for anesthetics and other molecules.^{29,61}

Hence, evidence from this study, along with the findings from our previous work,²⁹ suggests that the charged forms of the LAs are of paramount importance for the LA effect, since the BZC has the same behavior, spite of its neutral charge. Regarding the methodology, it shows to be very suitable and powerful to treat systems in environments with nonstandard electromagnetic character, such as water/membrane interfaces, presenting new perspectives of our problem and a promising approach for upcoming studies.

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Notes

The authors declare no competing financial interest.

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