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Similarities and differences of serotonin and its precursors in their interactions with model membranes studied by molecular dynamics simulation

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HIGHLIGHTS

- We simulate four NPT molecular dynamics simulations of serotonin and related molecules on lipid bilayers.
- Main interactions were studied for all simulation systems.
- Trp, 5-hydroxy-tryptophan and serotonin show similar localization on lipid bilayer-water interphase.
- No crossing events were observed through hydrophobic lipid region.

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ABSTRACT

In this work, we report a molecular dynamics (MD) simulations study of relevant biological molecules as serotonin (neutral and protonated) and its precursors, tryptophan and 5-hydroxy-tryptophan, in a fully hydrated bilayer of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidyl-choline (POPC). The simulations were carried out at the fluid lamellar phase of POPC at constant pressure and temperature conditions. Two guest molecules of each type were initially placed at the water phase. We have analyzed, the main localization, preferential orientation and specific interactions of the guest molecules within the bilayer. During the simulation run, the four molecules were preferentially found at the water–lipid interphase. We found that the interactions that stabilized the systems are essentially hydrogen bonds, salt bridges and cation-π. None of the guest molecules have access to the hydrophobic region of the bilayer. Besides, zwitterionic molecules have access to the water phase, while protonated serotonin is anchored in the interphase. Even taking into account that these simulations were done using a model membrane, our results suggest that the studied molecules could not cross the blood brain barrier by diffusion. These results are in good agreement with works that show that serotonin and Trp do not cross the BBB by simple diffusion.

1. Introduction

Serotonin (5-hydroxy-tryptamine, 5-HT) is a monoamine neurotransmitter and hormone implicated in several physiological processes by activating multiple receptors. Abnormalities of the serotonergic system have been implicated in many psychiatric disorders including anxiety, depression, psychosis, migraine, and others [1]. Serotonin is physiologically synthesized by a two-step reaction: a first and limiting step where the essential amino acid L-tryptophan (Trp) is converted into 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase and a second step where the 5-HTP is decarboxylated by the aromatic L-amino acid decarboxylase into 5-HT [2] (see Fig. 1).

During several years 5-HTP was extensively proposed for the treatment of migraine and others syndromes affecting serotonergic system [3,4]. This serotonin precursor could be extracted from *Griffonia simplicifolia* seeds [3] and orally administered to enhance serotonin transmission or improve several symptoms. The administration of 5-HTP, instead of Trp, is a good and more effective choice because it by-passes the first limiting step of 5-HT biosynthesis and it is entirely converted to 5-HT [5]. However, the clinical use of 5-HTP still under discussion because its contraindications and side effects.

The blood–brain barrier (BBB) acts like an interphase that restricts and regulates exchange of substances – ions, nutrients, ami-

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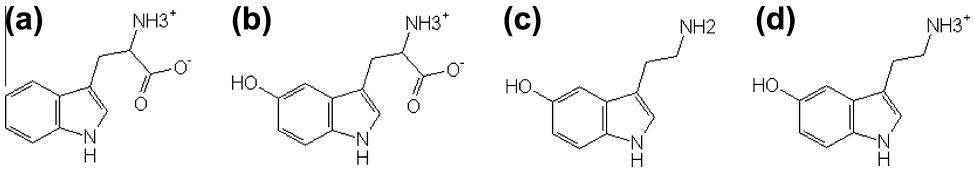

Fig. 1. Structures of four guest molecules studied: (a) L-Tryptophan (Trp), (b) 5-hydroxy-tryptophan (5-HTP), (c) neutral serotonin (n5-HT), (d) charged serotonin (p5-HT). The four guest molecules studied share a 3 substituted indolic moiety. Just Trp and 5-HTP are present as zwitterions because they bear a carboxylate group at 2-aminoethyl substituent, in addition to the NH_3^+ group. The molecules n5-HT and p5-HT bear a NH_2 and NH_3^+ group respectively. Trp is the unique molecule which is not bearing OH group at position 5 of indole.

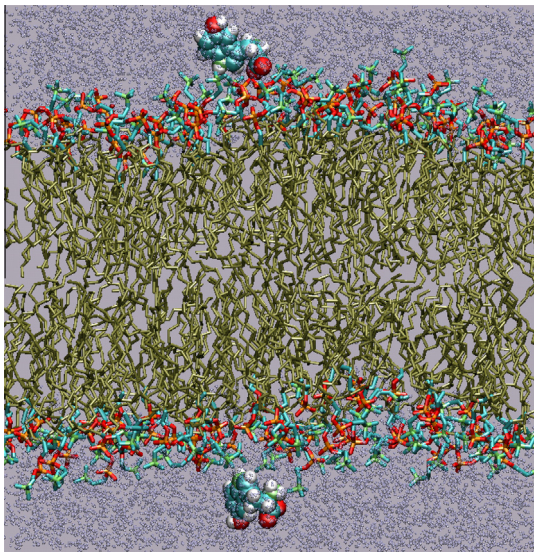

Fig. 2. Snapshot of initial configuration for 5-HTP in water region, surrounding a POPC bilayer, visualized by VMD 1.8.7 software package [48].

no acids, peptides and neurotransmitters – between central nervous system (CNS) and circulating blood. It is well known that both, 5-HT and Trp enter to the CNS through the BBB, during a process facilitated by transporters. 5-HT is transported by serotonin transporter SERT, located both at luminal (blood) and abluminal (brain) faces of brain capillary endothelial cells membrane [6]. The SERT carrier is involved in serotonin transport both from and to CNS. Furthermore, the large transporter LAT-1 is responsible for the transport of most neutral amino acids through BBB [7]. In this way Trp should compete with other amino acid, such as phenylalanine and tyrosine, for binding to LAT-1 [8,9]. However, in our knowledge, there is not experimental data supporting that the intermediate 5-HTP cross BBB by a specific carrier. Some authors such Birdsall [5] suggest that it could cross BBB by passive diffusion. In relation to this fact, recent studies demonstrate that the intravenous administration of 5-HTP increase levels of 5-HT in brain extracellular fluid and then in whole blood [10]. This indicates that 5-HT could cross from brain to blood through BBB, and that 5-HTP could cross from blood to brain through an unknown mechanism.

Despite its low occurrence in nature [11], the amino acid Trp was extensively studied. Trp residues in membrane proteins are related with several functions including protein organization, anchoring and orientation within the bilayer. Besides, it is common to find Trp at the water–lipid interphase in membrane proteins [12,13]. There are several works that addressed the interaction of molecules such indole with lipid bilayers through MD simulations [13,14]. Comparing with other amino acids analogues studied by simulation [15], molecules such indole, have interfacial preference due to a combination of weak electrostatic factors and the hydrophobic nature of the indolic ring. Furthermore, Johansson and Lindahl [16] studied the solvation of several amino acids in transmembrane segments of membrane proteins by MDs. They found that Trp, like tyrosine (Tyr), acts as membrane helix anchors. Physicochemical studies of Trp analogues and small peptides containing Trp suggest that this behavior arises from enhanced stability due to distinct interfacial interactions such as hydrogen bonds, dipolar and cation-π interactions [17].

In this work, we study by molecular dynamics (MD) simulations the interactions of serotonin, 5-HTP and Trp with model membranes of 1-palmitoil-2-oleoil-*sn*-glicero-3-phosphatidyl-choline (POPC). Model membranes of POPC were chosen since phosphatidyl choline lipid types are the most abundant in mammal endothelial cell membranes [18] such as those of BBB. Furthermore, model bilayers of POPC are used to study permeation and diffusion of several molecules and drugs as simplified model membranes of the BBB [19]. Trp and 5-HTP are amino acid, so they are found at zwitterionic form. Serotonin is essentially found protonated at physiological pH [20]. Nevertheless, in this study we consider both ionization states: protonated (p5-HT) and neutral (n5-HT). We focus our attention on the localization, orientation and specific interactions of the guest molecules within the bilayer. Since, they are very similar molecules, with different physiological properties, we aim to understand the main similarities and differences between them in their interaction with model membranes.

2. Methodology

Here, we study the interaction between Trp, n5-HT, p5-HT or 5-HTP (Fig. 1) and a bilayer of POPC. All simulated systems consist in a lipid bilayer containing 150 POPC molecules (75 in each leaflet) fully hydrated with 4200 water molecules and two identical molecules (5-HT and precursors), under periodic boundary conditions (PBC). The initial structures were built using Packmol package [21]. The guest molecules were originally placed into the water phase (as shown in Fig. 2). In order to assure the electroneutrality, we have added chloride counter ions when protonated molecules are present.

Simulations were performed using the NAMD2 program [22] with the PARAM27 CHARMM parameter set [23] and the water molecules were described by the TIP3P model [24]. The amino acid Trp is already parameterized into CHARMM force field [25].

On the other hand, the parameters for 5-HT and 5-HTP were mostly taken from the CHARMM Trp, because of their structural similarities, and estimated from quantum chemical calculations. In this way, we carried out quantum chemical calculation, using Gaussian03W package [26], in order to obtain the optimized geometry of these molecules. The optimization was done at the density functional theory (DFT) [27] level using the B3LYP [28] functional and 6-311G** [29] basis set. In this way we obtained the equilibrium bond lengths and angles of the structures of interest. The partial atomic charges were obtained from a single point HF/6-31G* [30] calculation within the Merz–Singh–Kollman protocol [31]. The calculations were performed using the polarized continuum

model (PCM) [32] with solvent dielectric constant of 80, corresponding to liquid water at environment conditions. PCM was the chosen methodology in order to obtain the zwitterionic charge separation of the amino acids.

Classical atomistic MDs were performed at the NPT conditions. The temperature was maintained constant at 310 K, through a Langevin thermostat by applying friction and random force [22]. The Langevin thermostat was used without coupling to hydrogens and with a damping coefficient of 1/ps. Besides, pressure was maintained at 1 atm by using a Langevin barostat [33] with a piston period of 5 ps and a damping time of 5 ps. The simulation box was fully flexible. A multiple-time step algorithm, RESPA [34], was used with the shortest time step of 2 fs. The short-range forces were computed using a cut-off of 10 Å and the long-range forces were taken into account by means of the particle mesh Ewald (PME) technique [35].

The initial structures were energy-minimized for 2000 steps using the Steepest Descent algorithm to remove bad contacts. The simulation consisted of an equilibration period of about 10 ns, within each of the interesting molecules migrated to their preferential localization relatively to the membrane, followed by 50 ns production run.

3. Results and discussion

Here, we summarized the main results of the analysis of our simulations, performed over the 50 ns of the simulations run of the 4 systems.

3.1. Localization of guest molecules in the bilayer

In order to have an idea of the overall organization of the systems under study, we have calculated the electron density profile (EDP), by time averaging the net charges per 0.1 Å thick slabs, as function of the z coordinate (normal to the lipid bilayer). The $z = 0$ corresponds to the bilayer center. In Fig. 3 we show the EDPs of the different systems under study (from top to bottom): (a) Trp, (b) 5-HTP, (c) n5-HT and (d) p5-HT. Besides, the EDPs corresponding to the different molecule types are shown in the following colors: POPC in black, water in blue and guest molecules in red. As we can see from the water EDP there are three well defined regions: a bulk aqueous phase, an intermediate complex water–lipid interphase in which the polar headgroups are hydrated, and a poor hydrated hydrophobic region. The POPC electron density profiles reveal a distribution characteristic of a lipid bilayer organization. Superimposing the POPC distributions of the four studied cases (results not shown) we can see that the bilayer organization was not significantly affected.

Furthermore, guest molecules are essentially found at water–lipid interphase, with access to the water phase. In some cases, this access leads to both molecules to be in the same monolayer, and this is the reason for the observed asymmetric distributions. The 5-HTP molecule shows higher access to the water phase than the other molecules. By the other hand, the p5-HT, even bearing a net charge, shows a more compacted distribution than the other molecules at the interphase.

In order to find more details on the water–lipid interphase localization of the guest molecules, we have investigated the orientation of separated groups of the guest molecules, as well as their main specific interactions with both, POPC lipids and water molecules. In this way, as a first step, we performed a detailed EDP of the different groups of the guest molecule EDP, shown in Fig. 4A–D. The selected groups are: the OH lateral group in purple (10,000-fold for visualization purposes), the amino lateral group in black, and benzene and pyrrole rings in red and green, respectively. In order to facilitate the comparison, we have included the total guest molecule (in turquoise) and the phosphates POPC EDPs (in dotted black). A representative scheme of the preferential orientation of the molecules is shown in the onset of Fig. 4A, B and D. Since no preferential orientation was found for the n5-HT (Fig. 4C) case - the maximum of the different groups distributions are located at the same position in z axis – the onset scheme show a random chosen orientation of the molecule. Besides, the area per lipid of the bilayer slightly increases in presence of the n5-HT (61.5 Å^2) respect to the other guest molecules ~60 Å^2. This effect can be explained by the less restricted rotations – and consequently higher effective volume – of the n5-HT compared to the other molecules. The area per lipid of a plain bilayer using the same model and simulation conditions was reported as 58 Å^2 [36].

On the other hand, for Trp (A), 5-HTP (B) and p5-HT (D) we can see that the indole moiety enters deeper in the bilayer than the lateral chain. Besides, we can see a superposition between the pyrrole ring and the phosphate groups of the POPC lipids. In order to elucidate if this superposition could be related to one specific interaction, we have calculated the radial distribution functions between the H atom of indolic NH (guest molecules) and O phosphate group of POPC for the four guest molecules. The results are shown in

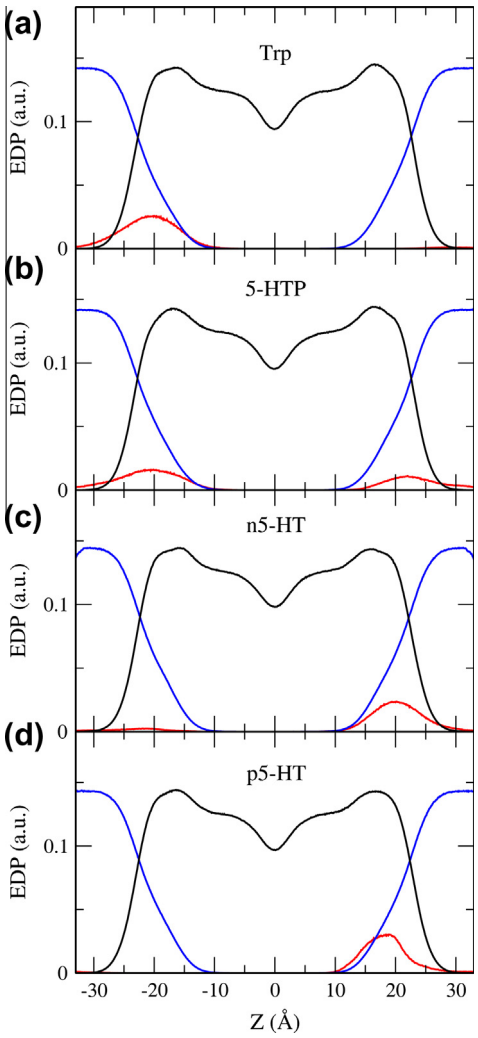

Fig. 3. Electron density profiles for all systems: Trp-POPC, a; 5-HTP-POPC, b; n5-HT-POPC, c; p5-HT-POPC, d. POPC electron density is depicted in black, water in blue and guest molecules in red (15-fold to easiest visualization). $z = 0$ correspond to bilayer center. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

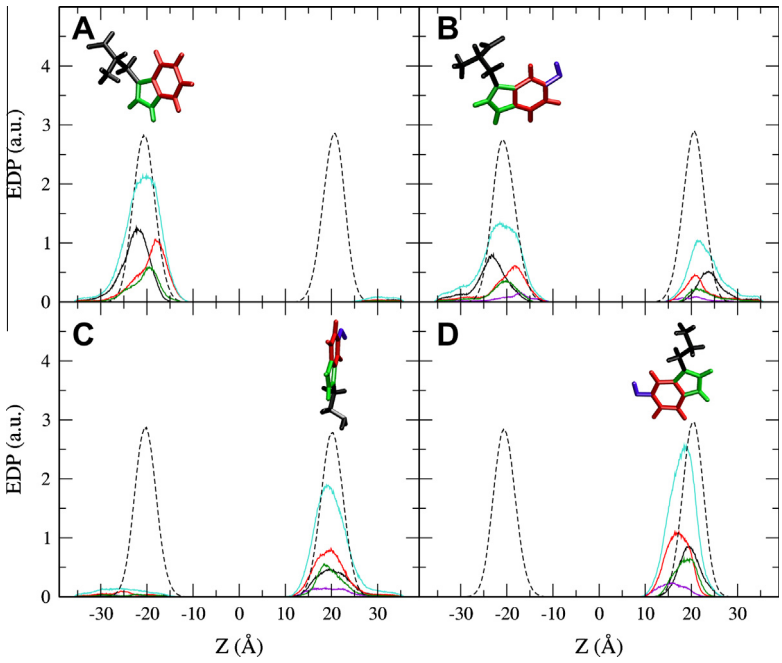

Fig. 4. Detailed electron density profiles for all systems and possible guest molecule orientation: Trp, A; 5-HTP, B; n5-HT, C; p5-HT, D. Total electron density for any guest molecule is depicted in turquoise, amino lateral group in black, benzene ring in red, pyrrole ring in green, OH in purple (10,000-fold to easiest visualization) and Phosphate of the POPC in black dotted line and scaled to serve as reference location. Guest molecules orientations are schematized in the same color scale than detailed EDP for the functional groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

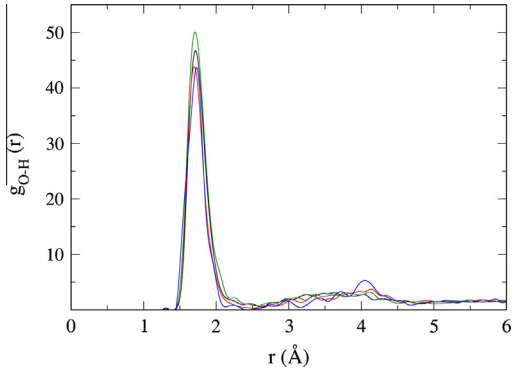

Fig. 5. Radial distribution functions for all systems. The atom pair considered is non-ester Op (phosphate group, POPC) and H (indolic NH group, guest molecule). Trp is depicted in black, 5-HTP in red, n5-HT in green and p5-HT in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. We can see a well defined peak ~1.7 Å in all the cases, characteristic of hydrogen bond kind of interactions.

3.2. Salt bridge Interactions

Salt bridge (SB) is a kind of non-covalent electrostatic interaction established between charged pairs [37–40]. In membrane proteins, that kind of interaction is involved in transmembrane segments binding and orientation processes [41,42].

In our case, the salt bridges between the center of mass of lateral amino group (guest molecule) and center of mass of phosphate group (POPC) was investigated through the radial distribution function analysis. In Fig. 6 we show the $g_{SB}(r)$ for the three studied cases. From this analysis, we found that charged and zwitterionic guest molecules form SB, as evidenced by the first peak at ~4 Å. However, the intensity of these interaction is higher for the protonated molecule (p5-HT), as expected, followed by the zwitterionic ones (Trp and 5-HTP). Furthermore, in order to see the stability of SB for each case, we have calculated the minimum distance between lateral amino groups of guest molecules and phosphate of POPC during time. The results are shown in the onset of Fig. 6.

3.3. Cation-π Interactions

Cation-π interaction is a noncovalent molecular interaction between the face of an electron-rich π system (e.g. benzene, ethyl-

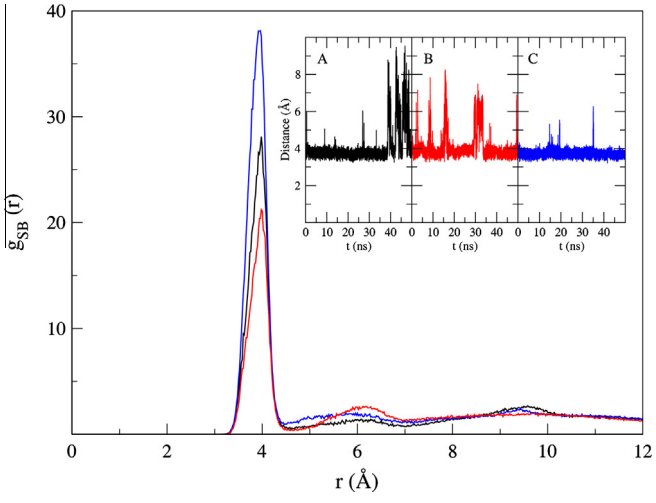

Fig. 6. Radial distribution functions for the three charged systems. The atom pair considered is lateral amino group (guest molecules) and phosphate groups (POPC). Trp is depicted in black, 5-HTP in red and p5-HT in blue. Onset: minimum distance between lateral amino groups of guest molecules and phosphate of POPC during time. Trp, A; 5-HTP, B; p5-HT, C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

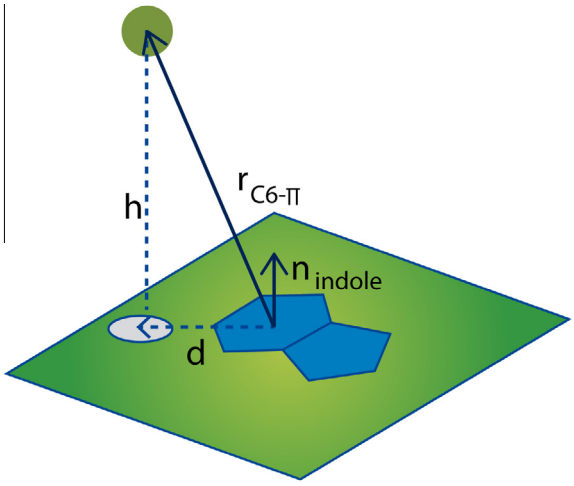

Fig. 7. Schematization of distance (r) requirements for cation-π interaction candidates from the center of the six-member ring of indole. The rigid indole ring is considered as a plane, in which n is the normal of this plane. Distance out-of-plane h and in-plane d determine the angular distance dC6-π requirement to find a cation donor for cation-π interaction.

ene, acetylene) and an adjacent cation. An important implication of the cation-π interaction and the related "polar-π" interactions is that phenylalanine (Phe), tyrosine (Tyr), and Trp should not be considered simply "hydrophobic" amino acids. They are in fact distinct from the conventional hydrophobic residues such as valine (Val), leucine (Leu), and isoleucine (Ile). Because of the dual nature of benzene hydrophobic yet quadrupolar aromatic amino acids are expected to play a unique role in protein structure and function [43].

In particular, the indole ring is an electron rich aromatic system [44]. It has hydrogen bond donor character, due to its indolic NH group, and ability to form cation-π interactions, due to its π electron system. Since aromatic Trp, and related molecules like 5-HTP and 5-HT, are electrically characterized by its quadrupolar moment, acts like a π-donor interacting with the positively charged amino group of POPC choline. This kind of interaction was studied from the radial distribution function between the centroid of benzene ring of indole, of the guest molecules, and charged nitrogen (N) atom of choline, in POPC (as shown in Fig. 7) such in the work of Petersen et al. [17]. The calculated $g_{c\text{-}\pi}(r)$ functions for

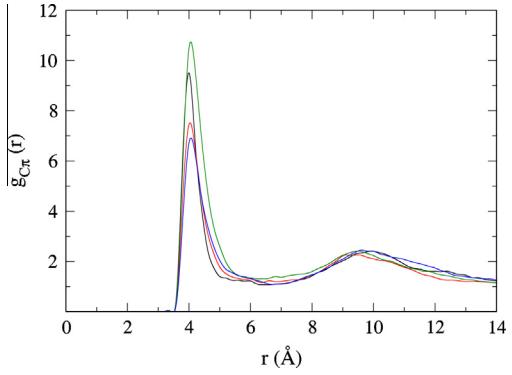

Fig. 8. Radial distribution functions for all systems. The atom pair considered is centroid of benzene ring of indole (guest molecule) and charged N atom of choline groups (POPC). Trp is depicted in black, 5-HTP in red, n5-HT in green and p5-HT in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Average number of hydrogen bonds per molecule between lateral amino, NH indolic and OH (guest molecules) and non-ester oxygen atoms of phosphate and carbonyl groups, Op and Oc (POPC). The statistical errors are also shown.

		Op	Oc
Trp	NH_3^+ lateral	1.118(4)	0.107(2)
	NH indole	0.354(2)	0.077(2)
5-HTP	NH_3^+ lateral	0.803(3)	0.023(1)
	NH indole	0.352(2)	0.006(1)
	OH substituent	0.329(2)	0.100(2)
n5-HT	NH_2 lateral	0.125(2)	0.072(2)
	NH indole	0.349(2)	0.028(1)
	OH substituent	0.367(2)	0.098(2)
p5-HT	NH_3^+ lateral	1.568(4)	0.043(1)
	NH indole	0.507(2)	0.050(1)
	OH substituent	0.122(1)	0.168(2)

the four cases under study are shown in Fig. 8. We can see a well-defined peak at ~4.0 Å for all guest molecules.

3.4. Hydrogen bonds

We adopted the following geometric criteria for the hydrogen bond (HB): the distance between the acceptor and the hydrogen is shorter than or equal to 2.0 Å, and the angle between donor-hydrogen-acceptor should be smaller than 30°.

The number of HB made by a selected pair was determined by counting the number of pairs that satisfy these geometric criteria and by averaging over time (50 ns).

Taken into account that POPC just can act as acceptor through the non-ester oxygen atoms of phosphate (Op) and carbonyl (Oc) groups – oxygen atoms that are just bonded to one heteroatom – we have calculated number of HB between them and the 3 donor groups (amino lateral, NH indole and OH lateral) of guest molecules. In Table 1, we summarized the average number of HB between these groups.

Amino lateral: For the charged and zwitterionic guest molecules we found a strong interaction between the hydrogens of this group with Op oxygens. The stronger interaction was found for the p5-HT (with an average of 1.56 HBs per guest molecule). This strong interaction could be one of the factors of the anchoring of this molecule, as discussed above. Furthermore, for n5-HT, as expected since this group is neutral and has just two hydrogens atoms, the number of HB is one order of magnitude lower (0.13 HB per molecule). By the other hand, the HB formation with the Oc is considerable low for the four molecules.

NH indole: The ability of indolic structure to form a hydrogen bond could explain differences of behavior between molecules, like Trp, with other hydrophobic and aromatic amino acid like L-Phe [17]. In Phe the benzene group lacks a hydrogen bond donor such indolic NH. Our results show a comparable HB number (~0.36 HB) for the Trp, n5-HT and 5-HTP and grows up to ~0.5 HB for the p5-HT (anchoring). By the other hand, the HB interaction with the Oc is quite low for the four guest molecules.

OH lateral: This group is not present in Trp. By the other hand, the other molecules are doing HB ~30% of the time (5-HTP and n5-HT) and ~10% of the time (p5-HT) with the Op and 10–15% with the Oc.

By the other hand, the four guest molecules have the ability to form hydrogen bonds with water, since they bear donor and acceptor hydrogen bond groups in their structures. In Table 2, we show the results of the number of HB formed, per guest molecule, with water. As water molecules can act both as donor and acceptor we have separated in Table 2 the HB made by the Ow (acceptor) and the total number of HB. It is important to point

Table 2
Average number of HB per molecule between lateral amino, carboxylate, NH indolic and OH (guest molecules) with water oxygen atoms and total water, and are expressed per molecule. The statistical errors are also shown.

		O–H_2O	H_2O
Trp	NH_3^+ lateral	0.658(4)	1.229(4)
	COO^- lateral	–	4.176(4)
	NH indole	0.338(2)	0.312(2)
5-HTP	NH_3^+ lateral	0.602(4)	1.541(4)
	COO^- lateral	–	4.105(4)
	NH indole	0.356(2)	0.410(2)
	OH substituent	0.341(2)	0.779(3)
n5-HT	NH_2 lateral	0.352(2)	0.913(4)
	NH indole	0.321(2)	0.319(2)
	OH substituent	0.329(2)	0.779(3)
p5-HT	NH_3^+ lateral	0.552(3)	1.045(3)
	NH indole	0.303(2)	0.265(2)
	OH substituent	0.353(2)	0.725(3)

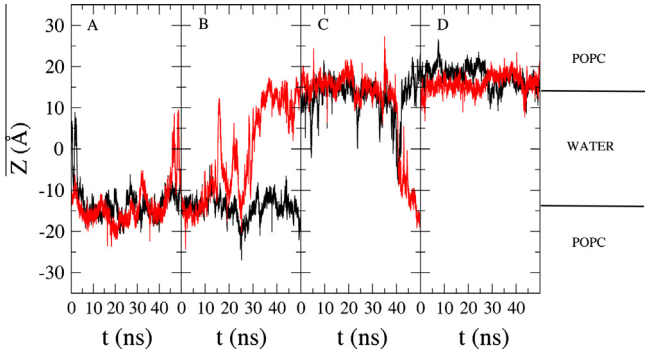

Fig. 9. Water centered trajectories along *z* axes. In red and black are depicted every one of both residues of any guest molecule. Trp, A; 5-HTP, B; n5-HT, C; p5-HT, D. $z = 0$ correspond to center of water region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

out that both, Trp and 5-HTP are zwitterionic and bear an extra carboxylate group (COO^-) that is able to interact with the water molecules.

As an overall view, we can say that 5-HTP is the guest molecules that most interact with water through HB (~6.8). This is due to the fact that this molecule has greater number of polar groups in its structure, and consequently more groups to be solvated. These two molecules, Trp and 5-HTP, have more access to the water phase, as we can see in the EDP and will discuss below. Our results show that the COO^- group strongly interacts with water forming ~4.1 HB in average for both zwitterionic structures.

We can see that serotonin in both ionization states are the molecules that have less HB with water (~1.9). For the n5-HT amino lateral group we can see, if we compare the donor acceptor type of HB, that is the only one that act as an acceptor, as well (0.64), because it is not charged.

The OH substituent has also the capability to act as donor an acceptor. We can see from Table 2 that the interaction of this group as donor is higher for the p5-HT (competing with the Op and Oc) groups. This group is quite polar, and needs to be solvated, acting as a donor. In this way, the three molecules that bear this group are essentially 80% of the time forming HB.

By the other hand, the NH of indole ring acts as donor in the range of 70–85% of the time.

Looking for the performance of the other molecules, the number of HB formed with water are in good agreement with the amount of donor and acceptor groups bearing in the structure and ionization state. In addition, detailed EDP demonstrates preferential orientation of charged groups (NH_3^+ and COO^-) in Trp and 5-HTP and NH_3^+ in p5-HT. The fact of facing these groups to water explains highest number of HB established with water: 5-HTP > Trp > n5-HT > p5-HT, in addition to simulation time spends in water region.

Considering the localization of guest molecules into lipid bilayers and taking into account all the detailed specific interactions, it should be reasonable to analyze if these molecules cross the membrane through the lipid core. In this way, in the next section we analyze the trajectories of all the studied molecules along the simulation run time.

3.5. Trajectories

The EDP analysis gave us a first picture of the distribution of the guest molecules within the bilayer. In most of cases, we found that the molecules partitioned between the water phase and the water–lipid interphase. In order to obtain more detailed information about this distribution, we have followed the molecules center of mass trajectories for each of the guest molecules over the 50 ns of the simulation run, and the results are shown in Fig. 9. While in Figs. 3 and 4 are centered in the middle of the lipid bilayer, in Fig. 9 $z = 0$ corresponds to the center of the water phase (taking into account the periodic boundary conditions). It is important to point out here that this approximation could be compared against multilamellar vesicle, since the amount of water was estimated for a fully hydrated lipid bilayer [45]. The comparison with the cell diffusion condition is somehow not straightforward. During the 50 ns simulation time we did not find any crossing events of the guest molecules through lipid hydrophobic core. Crossing events was defined as the guest molecule passes from one lipid monolayer to the other one through hydrophobic region. 50 ns is enough time to let small molecules cross the lipid bilayer: for example, local anesthetics (containing 40 atoms) are able to cross a POPC bilayer in a lower time scale of 20 ns [46].

Furthermore, in three of the four cases we observe crossing events through the water region. It is important to point out, that these crossing events occur with a stabilized system. The only system not showing these events is the p5-HT. The reason for that was discussed above and it is related with the stronger interactions that have these molecules with the interphase, which anchor them in the interphase region. By the other hand, n5-HT does not show strong specific interactions, and no preferential orientation. However, even if n5-HT access to the water phase, this is a fast event and then re-enter quickly into the bilayer. By the other hand, the zwitterionic molecules – especially 5-HTP – can remain more time in water.

4. Conclusions

In this work, we carried out molecular dynamics simulations of compounds of physiological and clinical importance as are serotonin and its precursors in a model lipid bilayer, at very low concentration. We aim to recognize similarities and differences of these molecules regarding their localization and main interactions with the lipid bilayer.

Our results show that the four guest molecules are preferentially found at the water–lipid interphase, some of them with access to the water phase. Furthermore, charged and zwitterionic molecules show a preferential orientation within the bilayer: the aromatic ring enters deeper in the lipid region. By the other hand, the neutral serotonin did not show, in average, any preferential orientation. The interactions that stabilize the systems are mainly hydrogen bonds, salt bridges and cation-π.

Hydrogen bond are essentially formed between indolic NH, amino lateral group and OH lateral group are found, with the

non-ester phosphate oxygen atoms of the POPC and, in less extent, with the POPC oxygen atoms of the carbonyl groups. Besides, cation-π interactions were found between the aromatic electronic system of indole and nitrogen atom of choline group. In this way, the protonated serotonin is anchored through all these strong interactions in the water–lipid interphase. However, for the zwitterionic molecules, these interactions are destabilized by the presence of carboxylate group that strongly interacts with water, since the POPC lipids do not have any HB donor group.

During the simulation time length, we did not observed any crossing events through hydrophobic region of the bilayer for any of the molecules. In particular, it is important to remark that even the more hydrophobic of the molecules (neutral serotonin) was unable to cross the bilayer. This is in very good agreement with the existence of a specific carrier (SERT); otherwise, the acid basic balance could shift to form neutral specie at aqueous medium and make the whole serotonin to cross BBB.

Besides, our results show a very similar behavior between the amino acids Trp and 5-HTP. In addition to their structural similarity, these molecules show the same characteristics in their interactions with and preferential orientation within the bilayer (at the interphase) as well as their access to the water phase. As was already mentioned in the introduction it was demonstrated that 5-HTP could cross from blood to brain through an unknown mechanism [10]. Our results suggest that this mechanism is a passive diffusion because 5-HTP (as Trp) was not able to cross the model membrane during the simulation time. At this point it is important to remark the existence of a carrier LAT-1 used by Trp to cross the BBB [7]. This carrier is also used for aminoacids, such as Tyrosine and L-dopa, to reach the CNS. [47]. The same subtle difference between the tyrosine and L-dopa – the presence of an OH substituent in the aromatic ring – is the one between 5-HTP and Trp. In this way, further studies should be carried out in order to reveal if an active mechanism is the responsible for the transport of 5-HTP through the BBB.

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