Journal Name

ARTICLE TYPE

Cite this: DOI: 00.0000/xxxxxxxxxx

This is the title†

Full Name,*[a] Full Name,[b‡] and Full Name[a]

Received Date
Accepted Date

DOI: 00.0000/xxxxxxxxxx

The abstract should be a single paragraph which summarises the content of the article. Any references in the abstract should be written out in full *e.g.* [Surname *et al., Journal Title*, 2000, **35**, 3523].

[a] *Address, Address, Town, Country. Fax: XX XXXX XXXX; Tel: XX XXXX XXXX; E-mail: xxxx@aaa.bbb.ccc*

[b] *Address, Address, Town, Country.*

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 00.0000/00000000.

‡ Additional footnotes to the title and authors can be included *e.g.* 'Present address:' or 'These authors contributed equally to this work' as above using the symbols: ‡, §, and ¶. Please place the appropriate symbol next to the author's name and include a `\footnotetext` entry in the the correct place in the list.

1 Introduction

The cell is a highly complex unit present in all living organisms: it continues the building block of life. Essentially, consists of a closed domain containing smaller organelles in a highly complex and crowded aqueous solution, all enclosed by a bilayer made of mainly pospholipids and containing fatty acids, sugars, cholesterol and proteins, among others. This bilayer is called cell membrane or cytoplasmic membrane. Membranes itself are very complex molecular organizations with variable and inhomogeneous composition, and its atomic level understanding is a very difficult task. For this reason, the employment o9f membrane mimetics and models has become common practice. Cita 1

Membrane proteins play a significant role in human pathologies . About 30% of human genes code por membrane proteins and Cita 2 y they are targeted by more than 50% of drugs . Therefore, most drugs have to cross membrane interfaces to reach their active site, and cita 4 consequently, the activity of these drugs depends, among other factors, on their ability to perform this task. This is particularly true for cita 5 y local anesthetics (LA). For more than hundred years it has been observed that the effectiveness of many LA correlates positively with lipophilicity , showing the importance of becoming incorporated into the bilayer. It is widely accepted that inhibition of voltage gated cita 7-1 Na^+ channels are directly involved in the mechanism of LA and three possible pictures have been proposed: (a) LA directly binds the cita 11 pore of the channel blocking the transit of ions, (b) LA reaches the active site by one of the lateral cavities filled with hydrophobic membrane components, and (c) the presence of LA near the interface modifies the structure and dynamics of the bilayer itself, perturbing the channel conformational dynamics and functioning. Despite which mechanisms are actually taking place, crossing membrane interfaces cita de to become incorporated into the hydrophobic domain appears to be a crucial step for most drugs.

Benzocaine is a well known LA for topical use. It has been widely employed anesthetizing the oropharynx for trans-esophageal echocardiography, bonchoscopy, esophagogastroduodenoscopy, in cold sores, mouth ulcers, toothache, sore gums and denture ache among others. A significant number of cases of BZ induced cyanosis (methemoglobinemia) have been reported along the years, how- cita 22 ever it still remain in use. cita 23

Benzocaine has been subject of a significant number of studies, including free energy transfer from water to the interior of different membrane mimetics , estimations about location and orientation in different bilayers and monolayers, interactions with a variety of cita 24- solvents, encapsulation in different structures for controlled delivery purposes among others. All the evidence confirms that Benzocaine cita 28- is able to cross the interface of membrane mimetics to become incorporated into the hydrophobic bilayer to finally be located at the cita 33- inner interface. cita 38-

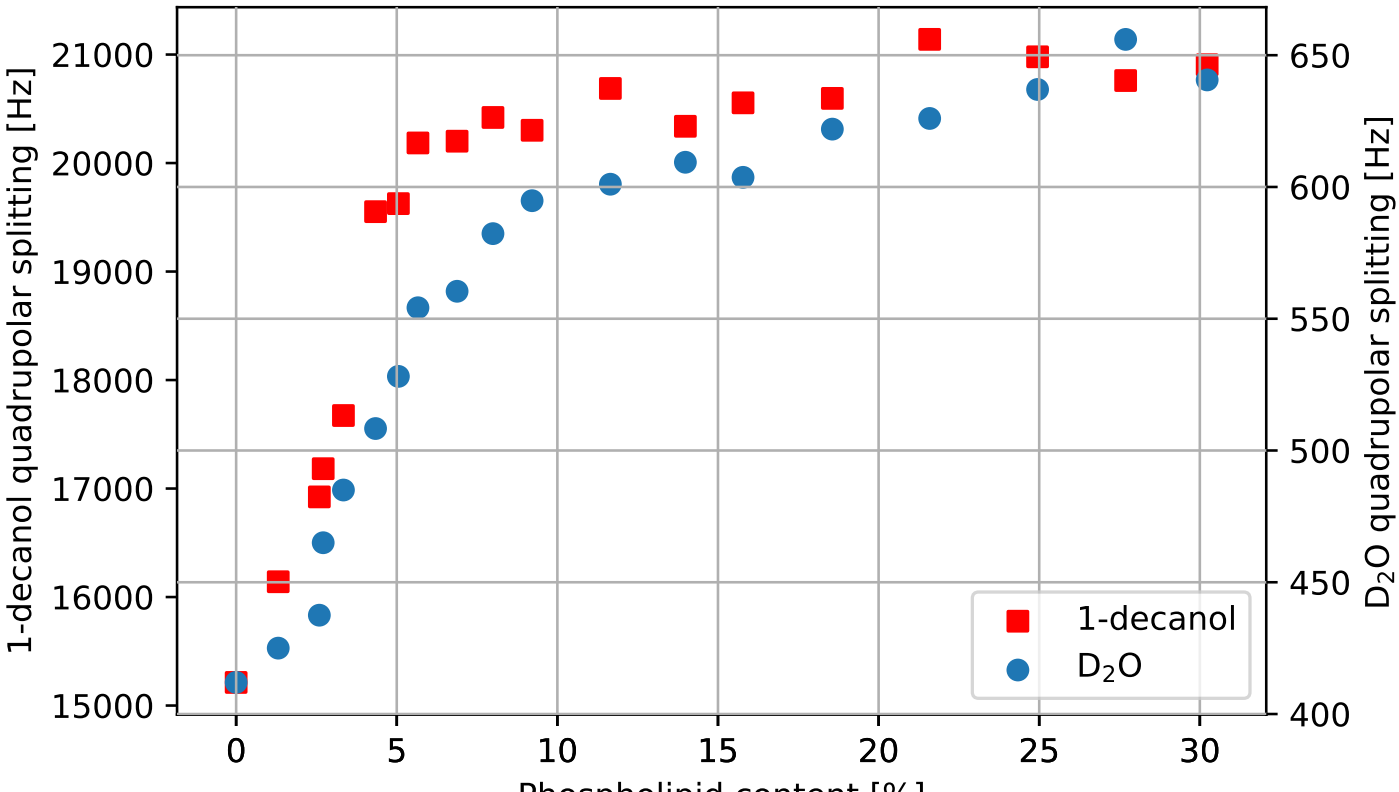
Contrary to most LA, Levodopa (or L-DOPA), the precursor of the neurotransmitter dopamine, commonly used in treatment of Parkinson's disease, is able to cross membrane interfaces only via active processes. Theres is evidence that neurotransmitters may cita 43 function as anesthetics and it is postulated that they share the passive mechanism (c) mentioned above for LA. Therefore, in the absence cita 44 of appropriate specific receptors, Levodopa should remain attached to the outer interface and not reach the hydrophobic bilayer. cita 45

In this article, we developed a new nematic lyotropic liquid crystal, with bilayer structure, susceptible to be used as membrane mimetic.

^{2}H-NMR quadrupole splittings. To test the capability of the new mimetic to reproduce cellular membrane behavior, Benzocaine and Levodopa were dissolved in the mesophase solution. According to previous evidence, Benzocaine should spontaneously be incorporated inside the bilayer and become located around the inner interface, whereas Levodopa should remain attached to the outer interface. To observe experimentally the bilayer dynamical behavior and interactions, ^{2}H-NMR quadrupole splittings from HDO, DeOH-α-d_2 and SDS-d_{25} were measured. Besides, to test for Benzocaine and Levodopa location and dynamics, splittings from Benzocaine-d_4 and Levodopa-d_3 were also measured. To obtain a more detailed characterization of the bilayer interface and information about location, orientation, dynamics and interactions of Benzocaine and Levodopa with the bilayer components, the experimental measurements were complemented with classical molecular dynamics (MD) simulations. The results show that prevous observations on Benzocaine and Levodopa dissolved in different membrane mimetics are reproduced in this case, reaffirming the membrane mimetic character of the new mesophase.

2 Preparation of the membrane mimetic

The reagents SDS, SDS-d_{25}, sodium sulphate and the phospholipid mixture were purchased from *Sigma Aldrich*. DeOH, deuterium oxide and HPLC-grade water were purchased from *Merck*. All these reagents were employed without alterations, excepting sodium sulphate which was oven dried 24 hours before use. Deuterated 1-decanol (DeOH-α-d_2) was synthesized by reducing ethyl decanoate ($C_{12}G_{24}O_2$) with lithium aluminum deuteride ($LiAlD_4$) and purified by vacuum fractional distillation.



In order to achieve a membrane mimetic that behaves as similar as possible to the actual cellular membrane, it is necessary to maximize its phospholipid concentration. This is important considering that multiple researchers have concluded that there are specific interactions between the phosphate from the multiple phospholipids and certain amino-acids that modify the membrane permeability where this interaction occurs. citar es

Also, the membrane mimetic must orient itself in presence of an external magnetic field. This is so the deuterated probes in the mimetic can produce a *quadrupole splitting* in its ^{2}H-RMN spectrum, which will be used to assess the mobility and orientation of each deuterium labeled component.

Both these requirements were fulfilled by employing the membrane mimetic developed by Bahamondes *et al* as starting point, then citar al introducing and maximizing a concentration of phospholipid onto this composition. The maximization was performed in two steps. In a first step, a batch of membrane mimetics was prepared, each one with an increasing amount of phospholipid from 0% up to 30% (see figure 1), ^{2}H-RMN spectra and polarized light microscopy pictures were taken to each prepared membrane mimetic. From these, the composition with 22% of phospholipid was chosen to continue with the next maximization step, as this was the composition with highest phospholipid content that retained a ^{2}H-NMR spectrum characteristic of a nematic phase (see figure 2).

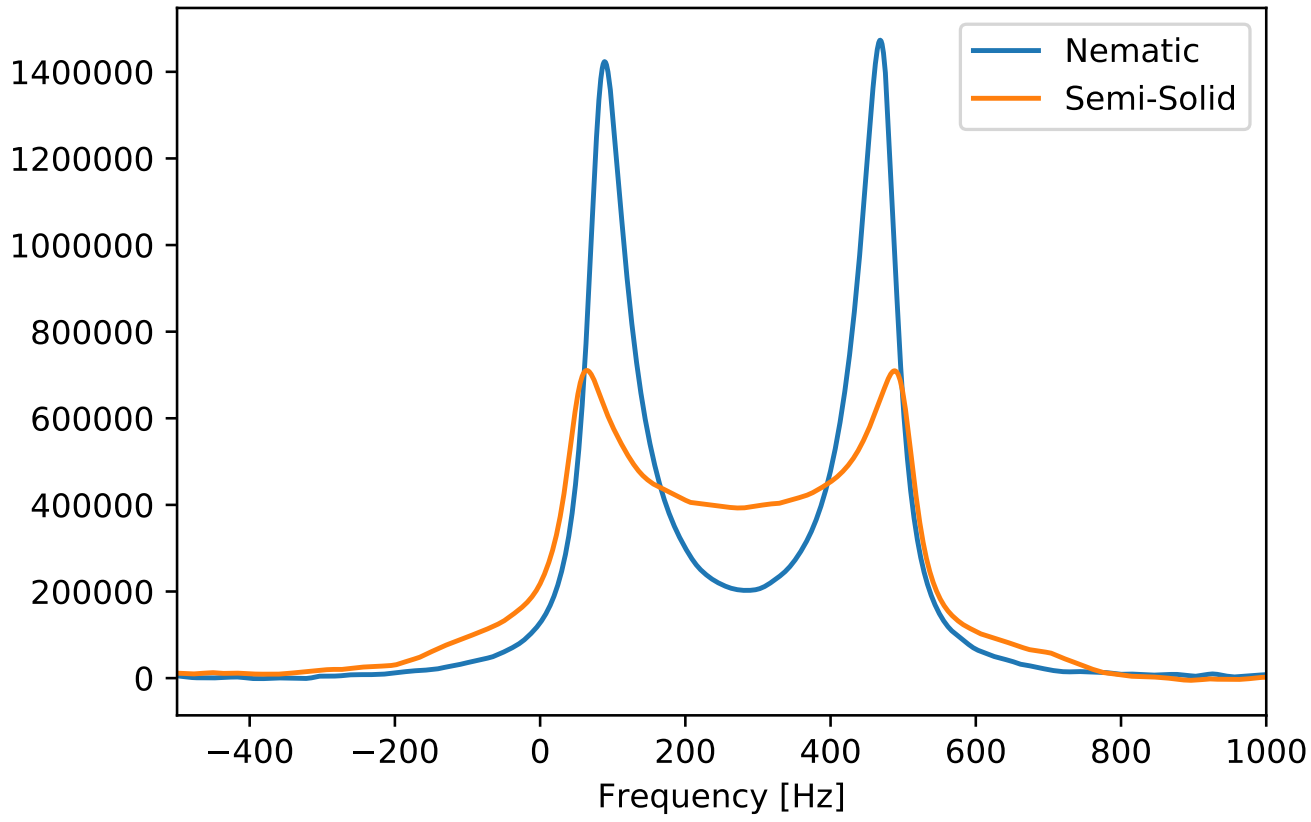


Fig. 2 Comparison between ^{2}H-NMR spectra from nematic and semi-solid phases

The second maximization step was performed by reducing the amount of SDS used to prepare each mimetic and subsequently adding

nematic phase was chosen for further experimentation. The exact composition of the membrane mimetic are detailed in table 1

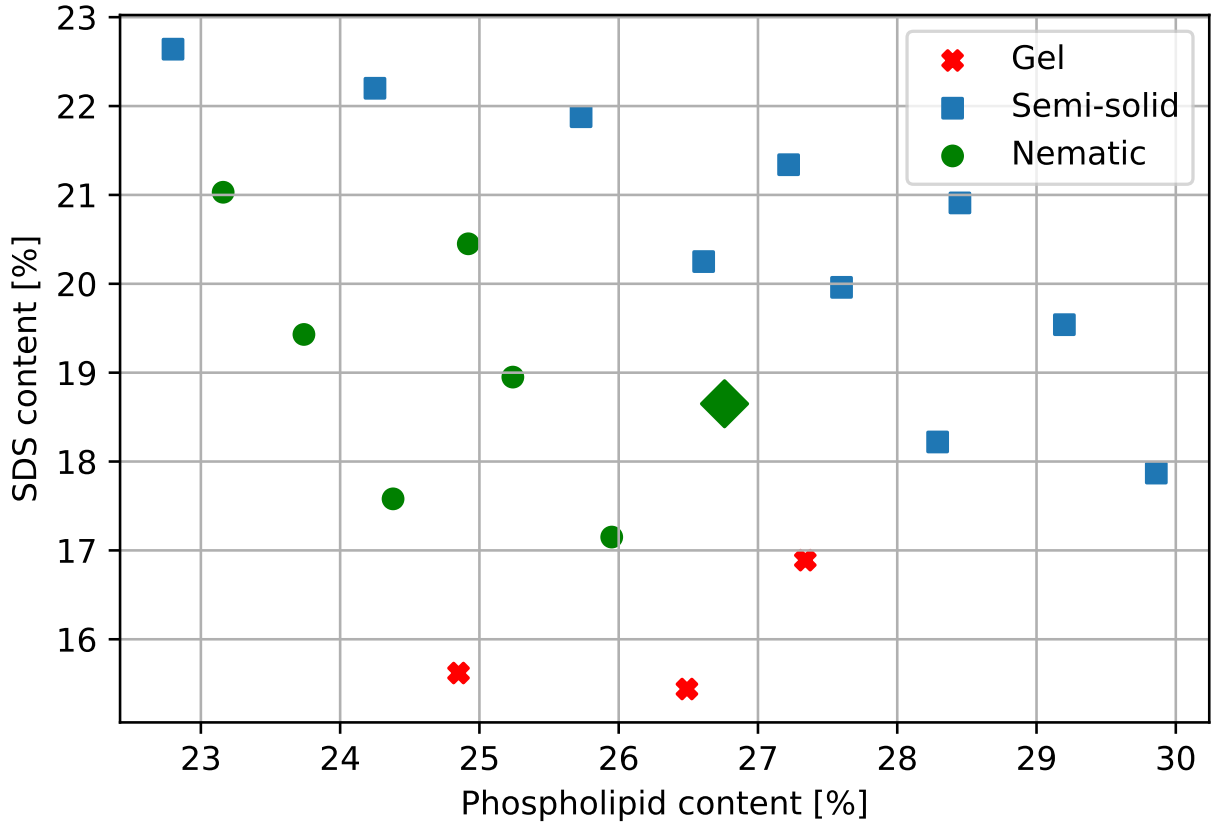


Fig. 3 Phase diagram of the membrane mimetic when varying SDS and phospholipid content. The selected composition is marked as a green diamond.

Table 1

Compound	Content [$\frac{w}{w}$%]
Sodium dodecyl sulphate	18.65%
Sodium sulphate	1.55%
1-decanol	3.92%
Phospholipid mixture	26.76%
Water	49.12%

3 Calibration of a computational model

In order to obtain significant information from a molecular dynamic simulation, it has to be calibrated to reproduce known experimental results.

As a first step, three force-fields with different partial charge distributions were tested. These force-fields were chosen due to their ability to reproduce structures of SDS aggregates.

The force-fields tested were:

- Berger with partial charge distribution according to Gromos53A6 parameters.
- Gromos53A6 with partial charge distribution according to a Merz-Kollman method. And
- Gromos 53A6 with partial charge distribution according to its own parameters.

All simulations were performed using the GROMACS-2016 software bundle. A cutoff scheme was used for non-bonding interactions according to each force-field recommendation values. Temperature control was achieved with a modified Berendsen thermostat, while pressure was equilibrated with a semi-isotropic Berendsen barostat. Both temperature and pressure were adjusted to 310 K and 1 bar respectively.

Before each production simulation, a short equilibration run was performed for a duration of 1 ns with an integration time-step of 1 fs. Afterwards, each production run was calculated to a duration of 40 ns with an integration time-step of 2 fs. TIP3P water model was employed on the simulation with CHARMM36 force-field, while SPC/E was employed on simulations with Berger and Gromos53A6 force-fields. The same initial configuration was employed to test each force-field, and it was generated using the software Packmol arranging a bilayer with composition in the same proportion as the membrane mimetic in table 1 to a total of 9613 molecules. The phospholipid mixture was simulated as a mixture of 39% PLPC, 26% DLPC, 24% DLPE and 11% DOPE. These proportions were chosen in accordance with the composition of the phospholipid mixture employed.

From each resulting simulation run, order parameters (S_{CD}) were calculated from the SDS aliphatic chain, which were used to calculate a predicted qudrupolar splitting employing equation 1 which depends on a coupling constant (e^2qQ/h) and the angle (θ) between the normal to the bilayer and the applied magnetic field.

$$\Delta\nu = \frac{3}{4}\frac{e^2qQ}{h}(3\cos^2\theta - 1)S_{CD} \tag{1}$$

These predicted qudrupolar splittings were compared with experimental results, as shown in figure 4, concluding that employing the Gromos53A6 force-field yields the results that are closer to experimental ones, yielding a good correlation on the carbons closer to the interface and diverging towards the interior of the bilayer.

Further improvement was done to the fitting of predicted results by employing two thermostats in the simulation, one governing the bulk solution with a reference temperature of 310 K and another governing the bilayer components with a reference temperature of 320 K. The reasoning behind this decision was based on the expectation that the increased velocity on the bilayer components would have a further effect on the mobility towards the center on the bilayer rather than in the interface, where Coulombic interactions are stronger and have a restraining effect on the mobility of the bilayer. After this adjustment, the simulation results in predicted quadrupolar splittings in very good agreement with the experimental ones, as shown in figure 5.

4 Membrane mimetic validation

In order to corroborate that the composition achieved (table 1) behaves as a membrane mimetic, we tested the permeation properties of two drugs: Benzocaine, that is known to be able to passively cross the cellular membrane; and Levodopa, which is known to be able to cross the cellular membrane via active mechanisms, therefore it should not be able to permeate a membrane mimetic without specific

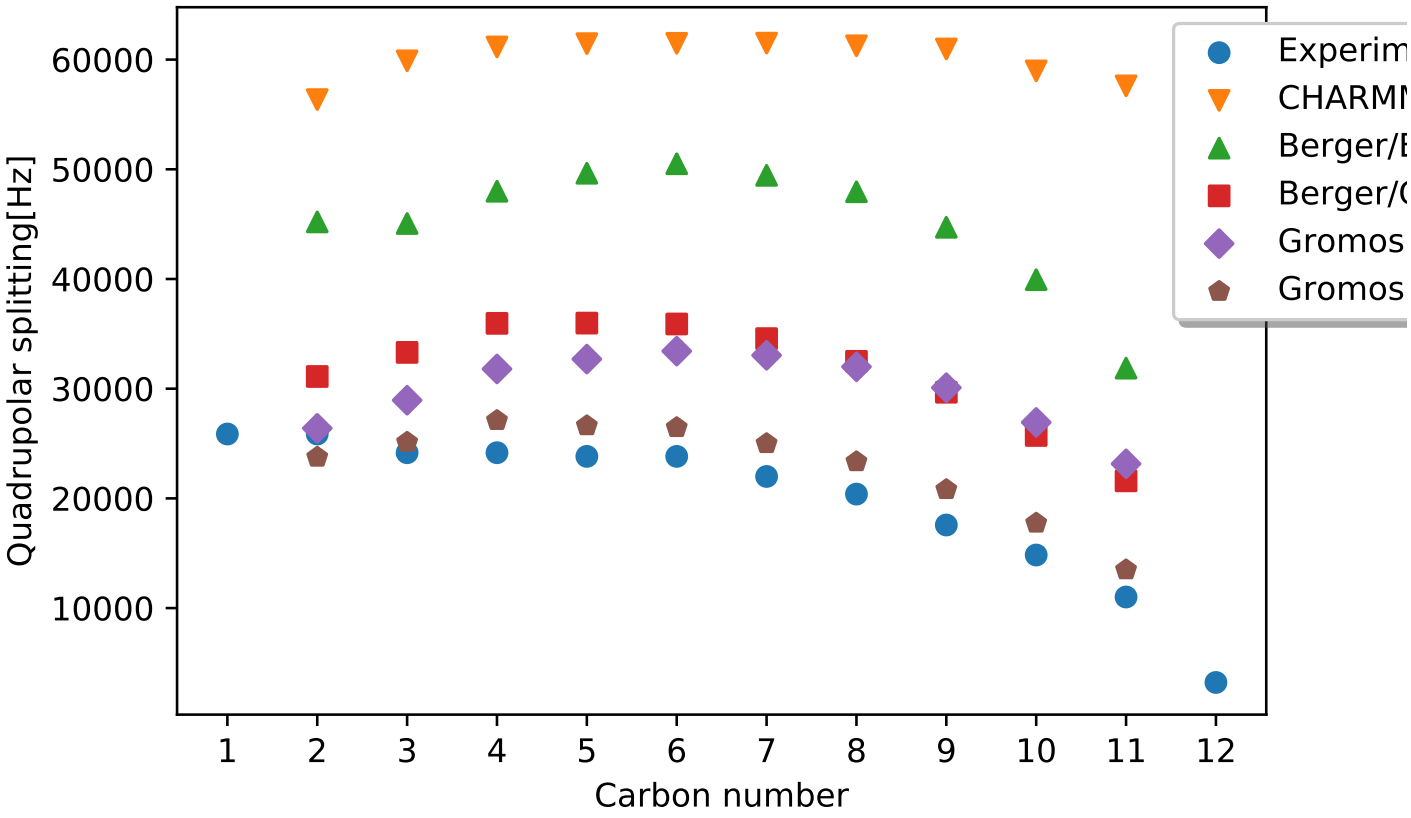


Fig. 4 Quadrupolar splittings of SDS-d_{25} from a ^{2}H-NMR. Comparison between experimental results and predicted by molecular dynamics employing different force-fields

4.1 Benzocaine control

Employing the calibrated model stated before, a potential of mean force (PMF) profile was calculated for the process of Benzocaine permeating the membrane (figure 6) employing the Umbrella Sampling/WHAM method. From this PMF profile it can be deduced that Benzocaine goes though three steps in order to complete its translocation: First, it integrates spontaneously with the outer leaflet of the bilayer, without mayor interactions with the Stern layer. Secondly, Benzocaine "flip-flops" to the inner leaflet of the bilayer with a small activation energy (11.2 kJ). And third and finally, it escapes the bilayer into the bulk at a minor energetic cost (27.1 kJ), showing that Benzocaine permeation through the membrane mimetic is indeed possible.

citar el WHAM

^{2}H-NMR spectum was taken from a membrane mimetic sample with 8 mg Benzocaine added per gram of mimetic enriched with SDS-d_{25}, and compared to a spectrum of the same mimetic without the anesthetic (figure 7). From this result, it can be seen that the presence of Benzocaine alters the quadrupolar splittings of the SDS aliphatic chain from the first up to the sixth carbon, implying that Benzocaine is mostly residing in this area, this is concordant with the results of the PMF profile (figure 6), which shows that the most stable position for the Benzocaine to be in, is indeed inside the bilayer and closer to the interface.

EXAMPLE
IMAGE

Fig. 5 Quadrupolar splittings of SDS-d_{25} from a ^{2}H-NMR. Comparison between experimental results and predicted by molecular dynamics with symmetric and asymmetric thermostat

EXAMPLE
IMAGE

Fig. 6 Mean force potential profile for the process of Benzocaine translocating through a membrane mimetic.

5 Conclusions

The conclusions section should come in this section at the end of the article, before the Conflicts of interest statement.

Conflicts of interest

In accordance with our policy on Conflicts of interest please ensure that a conflicts of interest statement is included in your manuscript here. Please note that this statement is required for all submitted manuscripts. If no conflicts exist, please state that "There are no conflicts to declare".

Acknowledgements

The Acknowledgements come at the end of an article after Conflicts of interest and before the Notes and references.

Notes and references

1 C. D. Abernethy, G. M. Codd, M. D. Spicer and M. K. Taylor, *J. Am. Chem. Soc.*, 2003, **125**, 1128–1129.

2 F. A. Cotton, G. Wilkinson, C. A. Murillio and M. Bochmann, *Advanced Inorganic Chemistry*, Wiley, Chichester, 6th edn, 1999.

3 A. J. Arduengo, III, H. V. R. Dias, R. L. Harlow and M. Kline, *J. Am. Chem. Soc.*, 1992, **114**, 5530–5534.

4 L. N. Appelhans, D. Zuccaccia, A. Kovacevic, A. R. Chianese, J. R. Miecznikowski, A. Macchioni, E. Clot, O. Eisenstein and R. H. Crabtree, *J. Am. Chem. Soc.*, 2005, **127**, 16299–16311.

EXAMPLE
IMAGE

Fig. 7 Variation on quadrupolar splitting upon adding Benzocaine to a membrane mimetic sample.

EXAMPLE
IMAGE

Fig. 8 Mean force potential profile for the process of Levodopa translocating through a membrane mimetic.