

t alterations, excepting sodium sulphate which was oven dried 24 hours before use. Deuterated 1-decanol ( $\text{DeOH}-\alpha-\text{d}_2$ ) was synthesized by reducing ethyl decanoate ( $\text{C}_{12}\text{H}_{24}\text{O}_2$ ) with lithium aluminum deuteride ( $\text{LiAlD}_4$ ) and purified by vacuum fractional distillation.

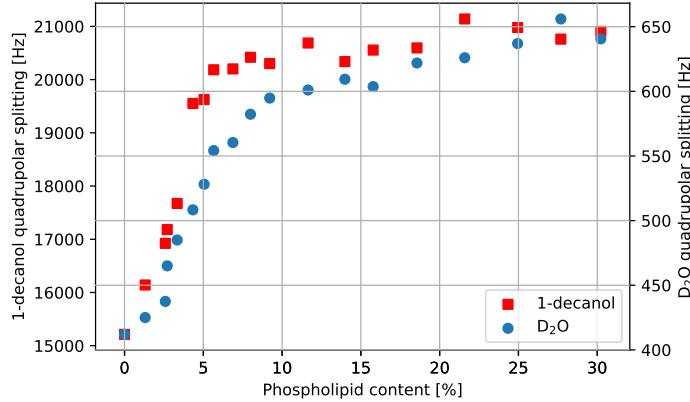


Figure 1 Quadrupolar splittings of 1-decanol- $\alpha$ - $d_2$  and  $\text{D}_2\text{O}$  in  $^2\text{H}$ -RMN as a function of the phospholipid content in the mimetic.

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??.

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  $^2\text{H}$ -RMN spectrum, which will be used to assess the mobility, location and orientation of each deuterium labeled component.

Both these requirements were fulfilled by employing the membrane mimetic developed by Bahamonde *et al*<sup>7</sup> as starting point, then introducing and maximizing the 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 ??),  $^2\text{H}$ -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  $^2\text{H}$ -NMR spectrum characteristic of a nematic phase (see figure ??).

During this step of maximization, we noticed a phase transition upon reaching 6%-10%, this phase transition was also evidenced by polarized light microscopy (see figure ??) where a transition from a *Schlieren* pattern (fig. ??) to an *oily streak* pattern (fig. ??) was observed. While both patterns are characteristic of lyotropic nematic phases, further experiments are required to assess the exact structure of each phase.

The second maximization step was performed by reducing the amount of SDS used to prepare each mimetic and subsequently adding phospholipid until a nematic phase was no longer obtained.

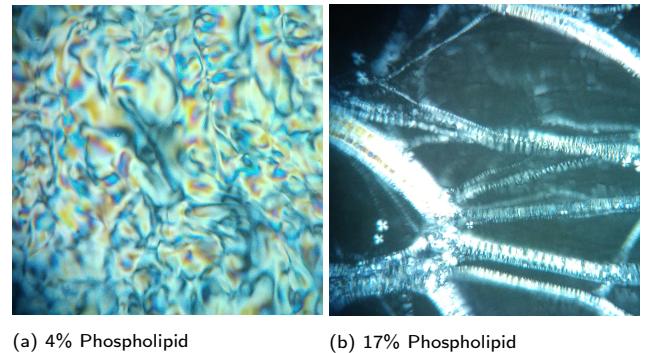


Figure 2 Polarized light microscopy pictures of membrane mimetics at different phospholipid concentration. Both pictures taken at 100x zoom.

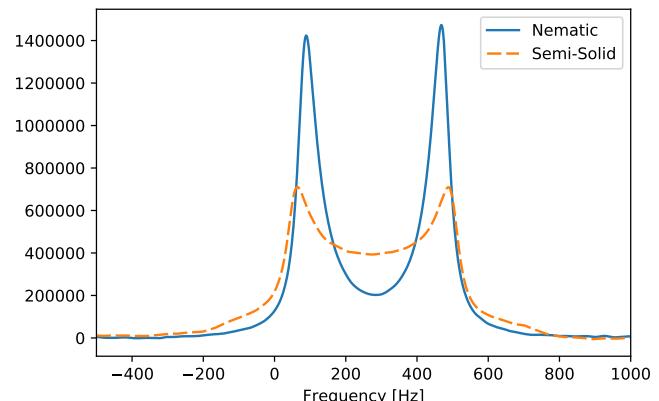


Figure 3 Comparison between  $^2\text{H}$ -NMR spectra from HDO in nematic and semi-solid phases

The results from the membrane mimetic candidates prepared in this step are summarized in figure ???. From these preparations, again, the composition with the highest phospholipid content that retained a nematic phase was chosen for further experimentation. The exact composition of the membrane mimetic is detailed in table ??

Table 1

Compound	Content [%]
Sodium dodecyl sulphate	18.65%
Sodium sulphate	1.55%
1-decanol	3.92%
Phospholipid mixture	26.76%
Water	49.12%

## 1 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 tested force-fields were:

- CHARMM36?

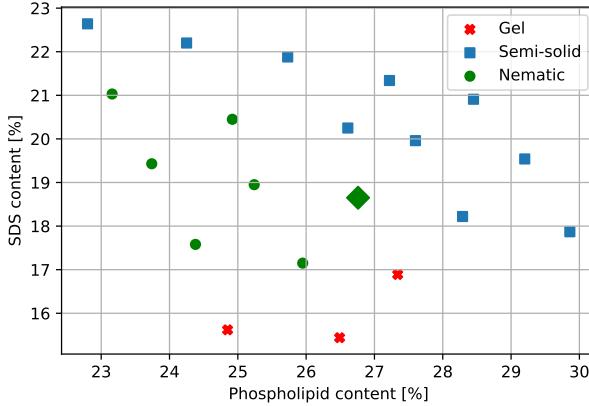


Figure 4 Partial phase diagram of the membrane mimetic when varying SDS and phospholipid content. The selected composition is marked as a green diamond.

- Berger<sup>?</sup> with partial charge distribution according to Merz-Kollman method?
- Berger with partial charge distribution according to Gromos53A6<sup>?</sup> parameters.
- Gromos53A6 with partial charge distribution according to Merz-Kollman method. And
- Gromos53A6 with partial charge distribution according to its own parameters.

All simulations were performed using the GROMACS-2016<sup>?</sup> 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<sup>?</sup>, while pressure was equilibrated with a semi-isotropic Berendsen barostat<sup>?</sup>. 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<sup>?</sup> arranging a bilayer with composition in the same proportion as the membrane mimetic in table ?? 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, deuterium order parameters ( $S_{CD}$ ) were calculated for the SDS aliphatic chain, which were used to calculate a predicted quadrupolar splitting employing equation ?? which depends on a coupling constant ( $e^2qQ/h$ ) and the angle ( $\theta$ ) between the normal to the bilayer and the applied magnetic field.

$$\Delta v = \frac{3}{4} \frac{e^2 q Q}{h} (3 \cos^2 \theta - 1) S_{CD} \quad (1)$$

These predicted quadrupolar splittings were compared with experimental results, as shown in figure ??, concluding that employing the Gromos53A6 force-field yields the results that are closer to experimental ones (see figure ??), obtaining a good correlation on the carbons near to the interface and diverging towards the interior of the bilayer.

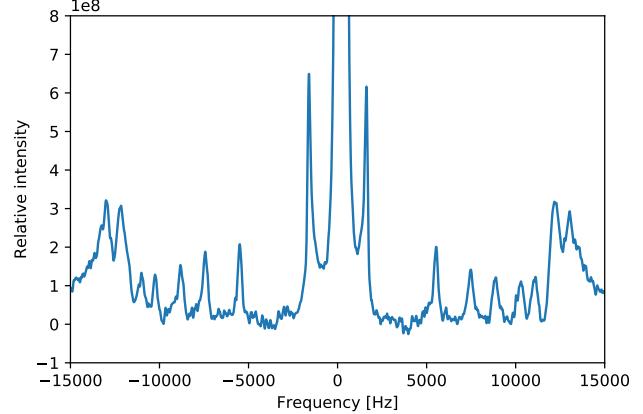


Figure 5  $^2\text{H}$ -NMR spectrum of membrane mimetic enriched with SDS-d<sub>25</sub>. Central peak (not shown completely) belongs to HDO present in the sample, while the rest of the signals are split SDS-d<sub>25</sub> signals which were used as reference to calibrate a computational model.

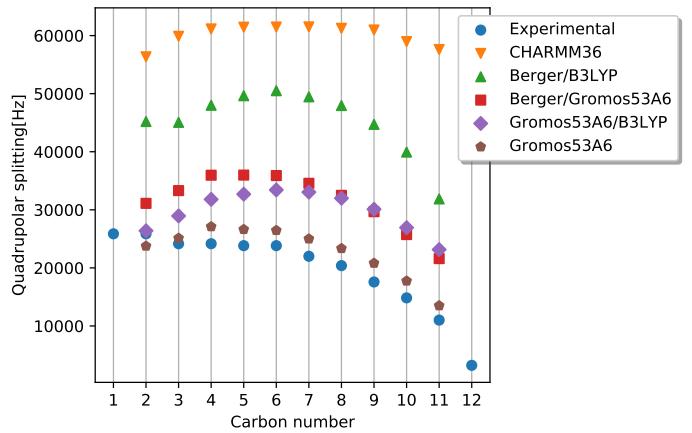


Figure 6 Quadrupolar splittings of SDS-d<sub>25</sub> from a  $^2\text{H}$ -NMR. Comparison between experimental results and predicted by molecular dynamics employing different force-fields

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 ??.

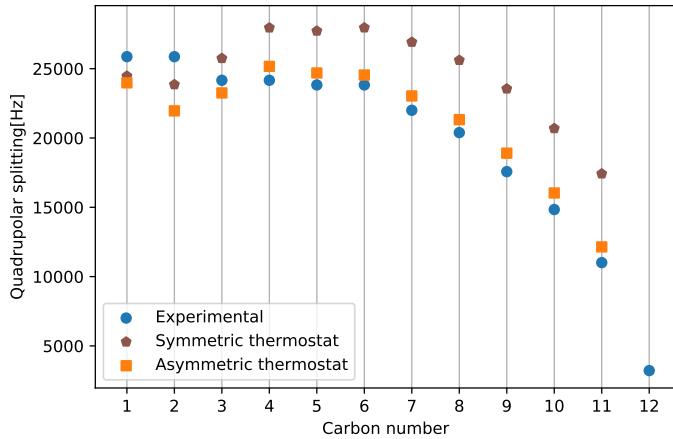


Figure 7 Quadrupolar splittings of SDS-d<sub>25</sub> from a <sup>2</sup>H-NMR. Comparison between experimental results and predicted by molecular dynamics with symmetric and asymmetric thermostat

## 2 Membrane mimetic validation

In order to corroborate that the composition achieved (table ??) 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 receptors.

### 2.1 Benzocaine control

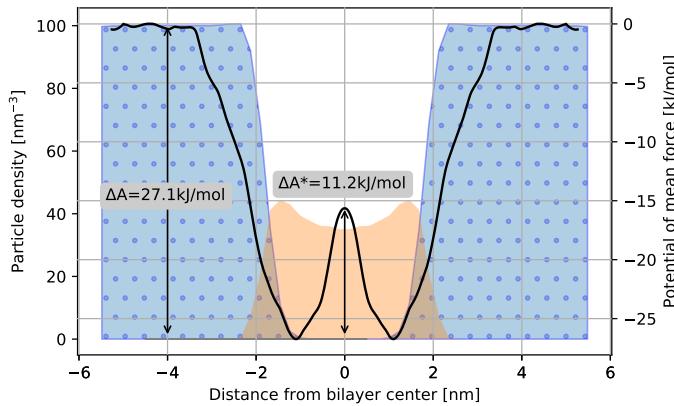


Figure 8 Mean force potential profile for the process of Benzocaine translocating through a membrane mimetic. The blue dotted area represents the water particle density, while the smooth orange area represents the lipid particle density in the simulation.

Employing the calibrated model stated before, a potential of mean force (PMF) profile was calculated for the process of Benzocaine permeating the membrane (figure ??) employing the Umbrella Sampling/WHAM<sup>7</sup> method. From this PMF profile it can be deduced that Benzocaine goes through three steps in order to complete its translocation: First, it integrates spontaneously with the outer leaflet of the bilayer, without major 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.

<sup>2</sup>H-NMR spectrum was taken from a membrane mimetic sample

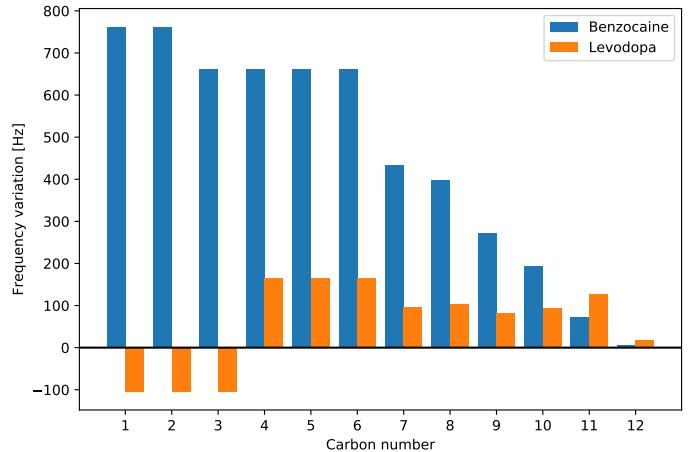


Figure 9 Variation on quadrupolar splittings in <sup>2</sup>H-NMR upon adding Benzocaine or Levodopa to a membrane mimetic sample enriched with SDS-d<sub>25</sub>.

with 8 mg Benzocaine added per gram of mimetic enriched with SDS-d<sub>25</sub>, and compared to a spectrum of the same mimetic without the anesthetic (figure ??). 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 ??), which shows that the most stable position for the Benzocaine to be in, is indeed inside the bilayer and closer to the interface.

### 2.2 Levodopa control

Employing the same method as in Benzocaine, a PMF profile was calculated for the process of Levodopa permeating the membrane mimetic (figure ??). This profile shows that the position of lowest energy for Levodopa to be in, is outside the bilayer, alongside the Stern layer; it also shows that in order to permeate the bilayer, Levodopa requires a rather large activation energy (102.4 kJ mol<sup>-1</sup>) in order to translocate through the membrane, showing that a passive permeation of Levodopa through the membrane mimetic is actually not possible.

This previous result is supported by <sup>2</sup>H-NMR spectra. When studying the effects of adding 7 mg of Levodopa per gram of membrane mimetic enriched with SDS-d<sub>25</sub> the effects are minor to negligible (see figure ??), implying that the Levodopa molecules are unable to enter the bilayer to alter its mobility.

## 3 Conclusions

The high phospholipid content present in this membrane mimetic makes it better at reproducing interactions between proteins (or peptides) and the bilayer, considering that multiple researchers<sup>7,8</sup> have found that there is a specific interaction between arginine and the phosphate from each phospholipid that modify the properties of the membrane where this interaction occurs.

Also, the ability to orient itself in an external magnetic field and its sodium dodecyl sulphate content, which is relatively inexpensive in deuterated form, makes this membrane mimetic ideal for studies of mobility and orientation employing <sup>2</sup>H-NMR.

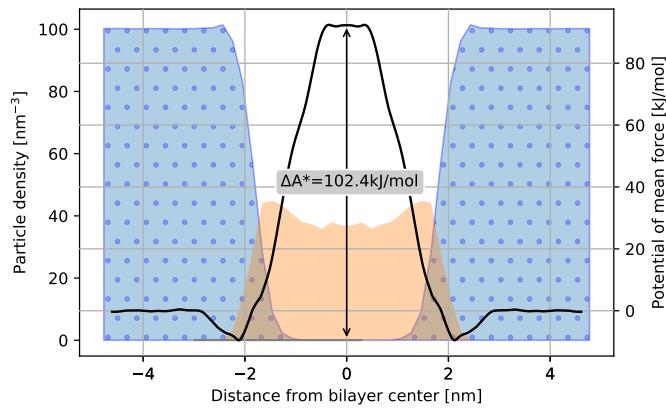


Figure 10 Mean force potential profile for the process of Levodopa translocating through a membrane mimetic. The blue dotted area represents the water particle density, while the smooth orange area represents the lipid particle density in the simulation.

Furthermore, we present a calibrated simulation model employing molecular dynamics, which as shown with the PMF profiles of Benzocaine and Levodopa, it allows to obtain information about the translocating process that is usually unfeasible to obtain via experimental means.

And finally, having proven that Benzocaine, a local anesthetic that permeates the cellular membrane, is able to translocate through the membrane mimetic proposed in this article; and that Levodopa, an amino-acid that cannot permeate passively the cellular membrane, is unable to do so thr