Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

Cholesterol sequestration by xenon nano bubbles leads to lipid raft destabilization

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1. Backmapping

The backmapping technique employed here is well-established and has been implemented by numerous authors in different contexts . The way we used here is particular convenient for getting the bilayer structure - a particular strength of Martini. The more problematic issue - the reduced entropy that may become important in free energy comparisons between Martini and atomistic models - is not of concern here. Backmapping is discussed in detail by Wassenaar et al. [1]. Martini's greatest success has been in simulations of lipid-based systems, the reviews by Marrink et al. provide excellent discussions of the different aspects of Martini, see for example Refs. [2, 3].

The number density of cholesterol plotted in Fig. 1b (0 Xe/lipid), at both 295 and 323 K, shows that the raft is stable. Also, in Fig. 3a we observe that the number of hydrogen bonds between DPPC and Cholesterol remains constant at 295 K (at 0 Xe/lipid) indicating raft stability. The raft starts to melt at 323 K (the number of hydrogen bonds DPPC-Cholesterol drops) but this effect is normal and expected (and not product of incompatibility between CG and atomistic mapping), since such temperature is higher than the miscibility temperature.

We have also used the same approach in related (and more complex) systems before, for example in studies of amyloid peptides in lipid bilayers [4].

2. Transition temperature

Experimental results, as cited in the manuscript, have determined the transition temperature to be between 295 and 323 K. Our aim was not to determine the transition temperature as it is a very demanding task, see for example the discussion in the recent study by Sun et al. [5]. In addition, Pluchakova et al. [6] have assessed different force fields for studies of the main phase transition. A Gromos force field was able to reproduce the temperature within 6.7 % of the experimental value. Only the Slipids force field did slightly better (4.8%) and the CHARMM36, that is often considered as the best lipid force field, was slightly worse (8.6%). The problems with GROMOS were in reproducing lipid diffusion, but such properties are not of concern here. We would also like to mention that our approach to lipid raft systems is similar to those (in terms of force fields and methods) that have been successfully taken in a number of previous studies, see for example [7, 8].

We did not perform simulations at different temperatures to determine the miscibility transition temperature. Nevertheless, the evolution of the number of hydrogen bonds between DPPC and Cholesterol at both temperatures, indicate that the transition temperature is safely above 295 and below 323 K consistent with previous studies as cited above.

3. Comparison with real doses

How well the concentrations used in our simulations match those in a real situation is beyond the scope of our paper. Nevertheless, let us discuss an estimate (below). We would like to emphasize, however, that the estimate below is a back-of-an-envelope calculation.

Let us first consider the number of neurons in our brain. According to Azevedo et al. [9] this number is around 86 billions. Next, consider the number of proteins per neuron to be about 50 billions [10] (see also the discussion in https://brain.mpg.de/news-events/news/news/archive/2019/may/article/protein-supply-in-long-nerves-how-do-neurons-do-it.html). Multiplying these two numbers gives $\sim 4.3 \times 10^{21}$. Since the number of proteins and lipids in a cell are roughly the same, this also provides an estimate for the number of lipids in our brain.

Let us take the concentration of 2 Xe/lipid we used in our simulations. Thus, we must bring to the brain $\sim 9 \times 10^{21}$ atoms. Such number accounts for 15 mmol, which is equivalent to 2 g of xenon (since the molecular weight of Xe is 131 g).

Now consider that the Tidal Volume (the volume of air taken in each inspiration) is 500 ml. Multiplying this volume by 0.7 (because the Minimum Alveolar Concentration MAC of Xe is 70 % [11]), we get in the lungs 350 ml of xenon in each breath. Taking into account that the blood/xenon partition coefficient is 0.115 [12], 40.25 ml of xenon enters into the blood each time. Since the density of xenon is 0.00589 g/cc, in each breath 0.24 g of xenon enters in our blood. In conclusion, in about 8.33 respirations (around a minute) a patient starts to be anesthetized. We would like to stress, however, that this number is just a coarse estimate.

4. Ion channels and lipid rafts

One of the current paradigms states that general anesthesia is due to the blockage of ion channels, specifically Ca and Cl channels, claiming that xenon (or other gaseous anesthetic molecules such as halothane or nitrous oxide) act as antagonists of glycine, which is needed to open the channels. Since the neurotransmitter glycine cannot bind due to the presence of the anesthetics, the channels remain closed.

However, in our view, as discussed in the manuscript, we argue that the hydrophobic xenon atom cannot antagonize with the hydrophilic glycine. Rather, xenon destabilizes the rafts where the channels are immersed. In doing so, it is very plausible that the channels stop functioning normally. That this is indeed a possible mechanism is supported by the calculations of the inter-membrane lateral pressure profile – the pressure that pushes on the membrane proteins, see for example Refs. [13, 14]. Importantly, as these studies show, the lateral pressure profile is very sensitive to the membrane structure and composition, and that the forces on the proteins are such that the can directly influence the conformational state. Such behaviour has also been reported in experiments by some of us as well as others [15, 16].

5. Comparison with experimental results and feasibility of the lipid system

We compare our results to experiments to ensure that the results from computer simulations are consistent with the observations and, consequently, that the observed phenomena are indeed real. Moreover, the simulations provide predictions that can be experimentally tested.

Multicomponent membrane simulations habe been reviewed by Marrink et al. [3]. As they point out, the largest simulated lipid system so far consists of about 20,000 lipids and 63 species (using Martini). Although that is a special case, it is generally not easy to include lipid species beyond just a few as the required simulations times become long and, in particular, to achieve a meaningful concentration of any given species the lipid matrix has to be large enough. For example, let us assume a typical all-atom simulation with 256 lipids. If one wants to have about 10 % concentration of, say, cholesterol, one has to add 4 cholesterol molecules (2 in each leaflet; the number should in general be the same in both leaflets). That gives about 15 % concentration. Getting 10% is tricker since 3 cholesterols would give about 11% but one of the leaflets would have 1 and the other 2 cholesterols. The above trivial example demonstrates that in addition to having the concentration in terms of percentage, one must also have a meaningful amount of lipids in terms of the absolute number of any given lipid species. In addition, increasing the total amount of lipids necessitates the addition of water since proper hydration must be achieved (typically around 35 water molecules/lipid). This is the reason why there are very few atomistic simulations above ternary systems and also the reason why we chose a ternary system.

Finally, we would like to mention that simulations have been shown to be useful in this context. In our own research, we have previously used ternary systems to provide predictions of various properties of raft forming systems [17]. We have also combined experiments with simulations using two-component systems. Even there, simulations were able to pinpoint where the differences of different sterols' raft forming abilities arise in multicomponent experimental systems [18, 19]. Recent examples of the usefulness of simulations of ternary systems in the context of rafts are also provided by [20, 21]. Finally, raft simulations have been reviewed, for example, in Refs. [7, 8].

6. Features of the lipid membrane

The bilayers employed in our simulations do not fall into the category of thin bilayers studied by some previous researchers [22]; in thin bilayers such as DLPC and DAPC studied by Marquart et al. [22] cholesterol may undergo significant reorientation and relocation toward the bilayer center. Table I shows the thickness of the bilayers in our simulations.

Table II shows the average tilt angle for the different concentrations. In the absence of Xe, the angle corresponds to 22.0 and 23.6 degrees for T=295 K and T=323 K respectively, which is comparable with the work done, for example, by Khelasvili et al. [23] and to DPPC and DOPC systems with 20% cholesterol by Aittoniemi et al [24] who found cholesterol tilt angles of 19.7 and 24.7 degrees in DPPC and DOPC, respectively. The numbers are also in good agreement with the work performed by Olsen et al. [25], who studied POPC membranes with cholesterol, and found that the tilt angle is around 20 degrees.

Table III shows the number of water molecules in the first coordination shell of cholesterol. For the control case, we have around 1-1.4 waters per cholesterol, which is in good agreement with the result of 1.1 molecules by Pasenkiewicz-Gierula et al. [26] and, about two water molecules found by Chiu et al. [27].

TABLE I. Thickness of lipid membrane

Xe/lipid	295 K		323 K	
	16:0 PC (nm)	18:2 PC (nm)	16:0 PC (nm)	18:2 PC (nm)
0	3.935 (0.030)	3.843 (0.025)	3.856 (0.040)	3.897 (0.022)
1	4.053 (0.030)	3.986 (0.030)	4.016 (0.032)	4.037 (0.034)
2	4.246 (0.037)	4.303 (0.034)	4.353 (0.046)	4.264 (0.027)

TABLE II. Cholesterol's tilt angle

Xe/lipid	295 K	323 K
0	22.0 (0.1)	23.6 (0.1)
1	23.7 (0.1)	24.6 (0.2)
2	22.5 (0.1)	29.0 (0.2)
3	24.1 (0.2)	-

7. Lipid system in the ternary phase diagram

Our lipid concentrations (DLPC 810; DPPC 510 and Chol 238) lie in the fluid ordered phase (Region E) in the ternary phase diagram reported by Feigenson and Buboltz [28], and Chiang et al. [29]. This phase, with a Xchol of 0.18, is indicative of a raft structure at around the temperatures used in our simulations (295-323 K).

TABLE III. Number of water molecules in the first coordination shell

Xe/lipid	295 K	323 K
0	1.353	1.096
1	1.389	0.979
2	1.391	0.818
3	1.318	-

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