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Digestion of lipid micelles leads to increased membrane permeability†

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Lipid-based drug carriers are an attractive option to solubilise poorly water soluble therapeutics. Previously, we reported that the digestion of a short tail PC lipid (2C6PC) by the PLA2 enzyme has a significant effect on the structure and stability of the micelles it forms. Here, we studied the interactions of micelles of varying composition representing various degrees of digestion with a model ordered (70 mol% DPPC & 30 mol% cholesterol) and disordered (100% DOPC) lipid membrane. Micelles of all compositions disassociated when interacting with the two different membranes. As the percentage of digestion products (C6FA and C6LYSO) in the micelle increased, the disassociation occurred more rapidly. The C6FA inserts preferentially into both membranes. We find that all micelle components increase the area per lipid, increase the disorder and decrease the thickness of the membranes, and the 2C6PC lipid molecules have the most significant impact. Additionally, there is an increase in permeation of water into the membrane that accompanies the insertion of C6FA into the DOPC membranes. We show that the natural digestion of lipid micelles result in molecular species that can enhance the permeability of lipid membranes that in turn result in an enhanced delivery of drugs.

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1 Introduction

Nanoparticle formulations consisting of a range of materials are being developed for a range of applications including use as drug-delivery vehicles (DDVs).^{1–14} Lipid-based nanoformulations are of particular importance due to their biocompatibility,^{15–17} and they have been of increasing interest over the past few years as a result of the success of the various lipid nanoparticles (LNPs) used to deliver the COVID vaccines.¹⁸

Phosphatidylcholine (PC) lipids are the major components of cell membranes. Due to their amphiphilic nature, PC lipids are able to self-assemble into a variety of nanostructures

including bilayers and micelles. The molecular architecture of the lipids can be tailored in order to generate a variety of different self-assembled structures. For example, small changes in the lipid tail length can alter the concentration at which the lipid self-assembles (called the critical micelle concentration (CMC)) as well as the structure of the aggregates formed, with the size of the aggregates formed increasing with the length of the hydrophobic tails of the lipid molecules?

Within the body, PC molecules are degraded *via* the hydrolysis of the PC sn-2 ester bond, a process which is mediated by the calcium-dependent secreted phospholipase A2 (sPLA₂) enzyme.¹⁹ This hydrolysis results in the production of lysophosphocholine and fatty acid molecules.^{20,21} Previously we have combined static and time-resolved small angle neutron scattering with all-atom molecular dynamics simulations to characterise how the structure of micelles containing dihexanoylphosphatidylcholine (2C6PC) changes as the molecule is digested by sPLA₂ into 1-hexanoyl-lysophosphocholine (C6LYSO) and hexanoic acid (C6FA) (Fig. S1†).² Our results show that as the degradation progresses the cmc of the resulting micelles decreases and therefore potentially affect the release of any payload that they are carrying.²

The concentration of sPLA₂ is elevated in various inflammatory diseases, atherosclerosis and cancers, specifically prostate, breast and pancreatic cancer.^{22–30} As a result, the release of anti-cancer agents contained in PC lipid-based DDVs can be triggered by an enzyme that is upregulated in the targeted

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†Electronic supplementary information (ESI) available: (i) A detailed description of the analysis carried out for the various simulations, (ii) plots of the R_G and eccentricity of the micelles as a function of time (iii) the contacts between the EO monomers and the hydration of the EO monomers on the polymers within each micelle (iv) the intrinsic density of the various components within the simulations for each micelle and (v) the outputs of the dimensionality reduction and clustering of the molecules within each micelle. See DOI: <https://doi.org/10.1039/d3nr05083a>



In this manuscript, we present the results of all-atom molecular dynamics simulations that were used to investigate the interactions of 2C6PC micelles at various stages of digestion with two model lipid membranes: an ordered membrane (70 : 30 mole ratio 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC):Cholesterol (CHOL)) and a disordered membrane (pure 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)). Our findings provide insight into how the natural digestion of the 2C6PC molecules in the micelles lead to their enhanced permeability and in turn may increase the delivery of any encapsulated drugs to the cell. In particular, we demonstrate the unique effects that each of the different components of the micelles has on the interfacial and structural properties of the two different membranes. Additionally, we show the different dynamical properties of the various molecules comprising the micelles have once they have inserted into each of the membranes.

2.1 Interaction of micelles with membranes

As each of the micelles begin to disassociate while interacting with the DOPC bilayer, the different molecular species approach the interface of the lipid bilayer and then penetrate into the membranes (see Fig. 1). The snapshots show that the C6FA, C6LYSO and 2C6PC molecules penetrate the pure DOPC lipid bilayer in differing amounts and to different depths. During this penetration, the C6FA molecules were the first

The behaviour of the various molecules that comprise the micelles observed in our simulations are consistent with the critical micelle concentrations (cmc) that have been measured

(a)

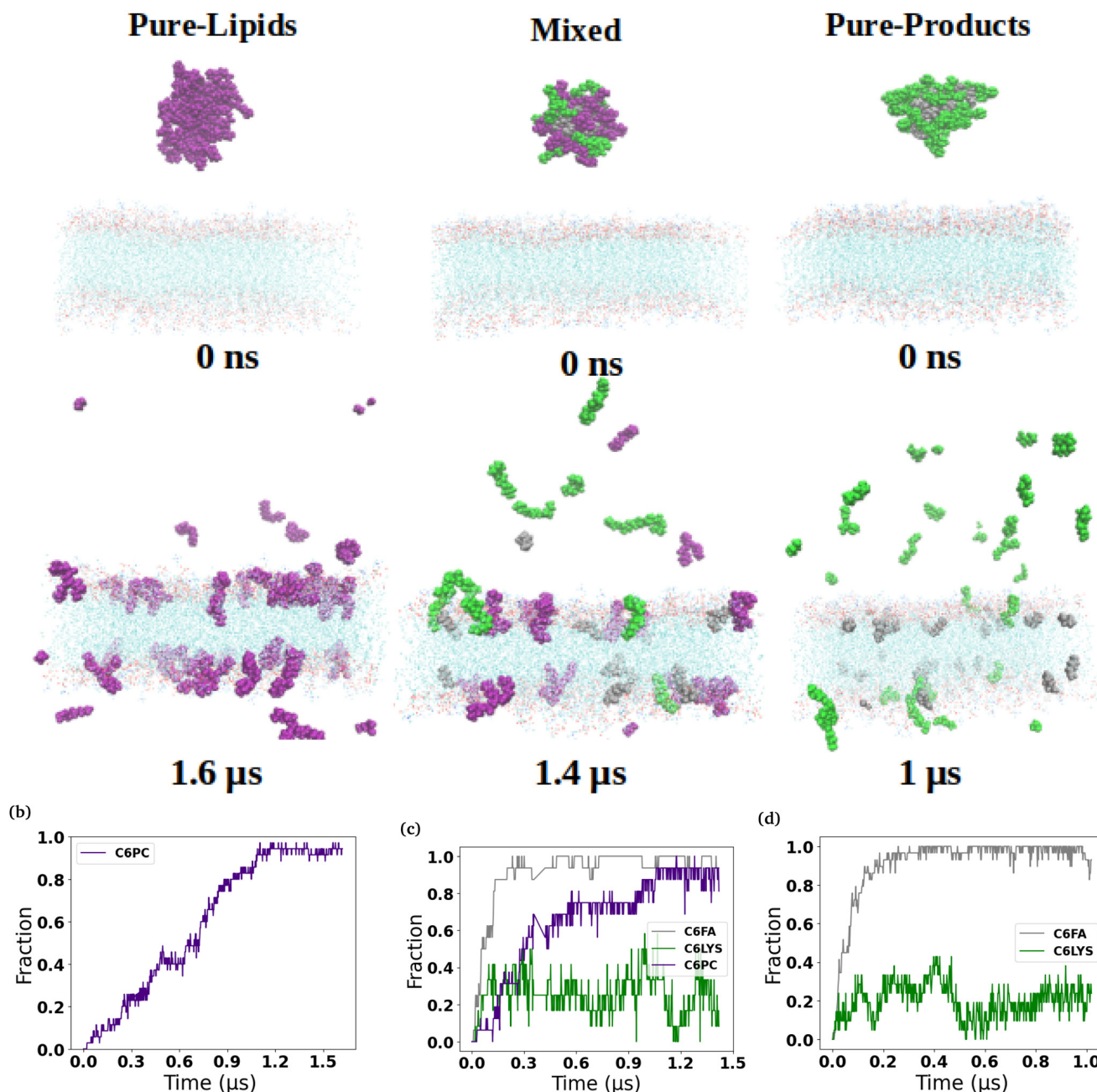


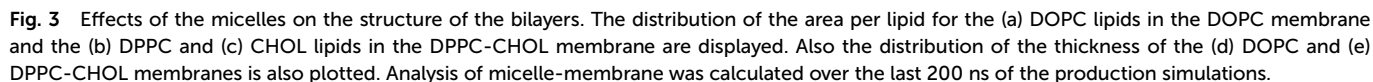
Fig. 1 Interactions of micelles with the DOPC membrane. (a) Representative snapshots of three different lipid-based micelles (C6PC purple, C6FA sliver, C6LYS green) interacting with DOPC (cyan) membrane over time. From left to right, they are the Pure-Lipids, Mixed and Pure-Product systems, respectively. Water molecules and ions were removed for clarity. The fraction of the micelle molecules that have inserted into the membranes are shown in the bottom row for each of the three micelles (b–d).

for these molecules experimentally. While there is not any published cmc values for C6LYSO, there are cmc values published for C8LYSO (57 mM), C10LYSO (5.7 mM) and C12LYSO (0.6 mM),⁴⁰ so the cmc for C6LYSO would be greater than 57 mM. The cmc for 2C6PC was found to be ≈ 14 mM.^{41,42} As we have studied the free hexanoic acid (C6FA), there are no experimentally obtained cmc values, but a coarse-grain mole-

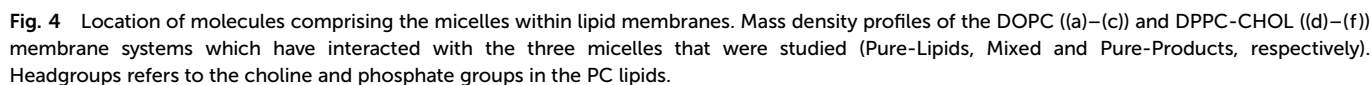
cular dynamics simulation study reported a cmc of 9.26 mM at 50 °C, which is an order of magnitude larger than the cmc found for the sodium salt of hexanoic acid.⁴³ Therefore the fact that we find that the C6LYSO is the least likely of the molecules to remain in the micelle or insert into the membrane, and that C6FA is the most likely to be in either environment agrees with the rank order of these cmc values.



	DOPC				DPPC-CHOL			
	Bilayer	PL	Mixed	PP	Bilayer	PL	Mixed	PP
Thickness	3.89 (0.04)	3.73 (0.05)	3.76 (0.04)	3.83 (0.04)	4.87 (0.06)	4.60 (0.07)	4.94 (0.03)	4.85 (0.06)
APL _{PC}	0.68 (0.02)	0.74 (0.02)	0.72 (0.01)	0.71 (0.01)	0.58 (0.02)	0.57 (0.01)	0.59 (0.04)	0.58 (0.03)
APL _{CHOL}	—	—	—	—	0.40 (0.03)	0.41 (0.13)	0.40 (0.07)	0.40 (0.04)
f_{2C6PC}	—	0.95	0.95	—	—	0.03	0.04	—
f_{C6FA}	—	—	0.98	0.98	0.57	0.55	—	—
f_{C6LYSO}	—	—	0.35	0.23	—	—	0.07	0.08
$\langle -S_{CH,sn1} \rangle$	0.122	0.115	0.115	0.117	0.425	0.422	0.421	0.422
$\langle -S_{CH,sn2} \rangle$	0.121	0.105	0.106	0.107	0.412	0.398	0.398	0.399
n_{H_2O}	14.7 (0.3)	17.3 (0.6)	15.9 (0.7)	16.6 (0.4)	7.8 (0.1)	6.5 (0.1)	7.2 (0.1)	6.9 (0.1)



To understand the orientation of the C6FA molecules that have inserted into the DOPC membrane, we measured the angle formed by the vector which connects the terminal carbon (C2) in the hydrocarbon chain of the fatty acid to the double-bonded oxygen (O2) on the other end of the molecule and the z-axis that is normal to the membrane's interface



The radial distribution functions (RDFs) were used to determine the distribution of water molecules around the PC headgroups of the DOPC and DPPC lipid molecules and around the oxygen in the hydroxyl group of the cholesterol molecules in our membranes. By calculating the integral of the RDF from 0 to a distance corresponding to the first peak minimum, the

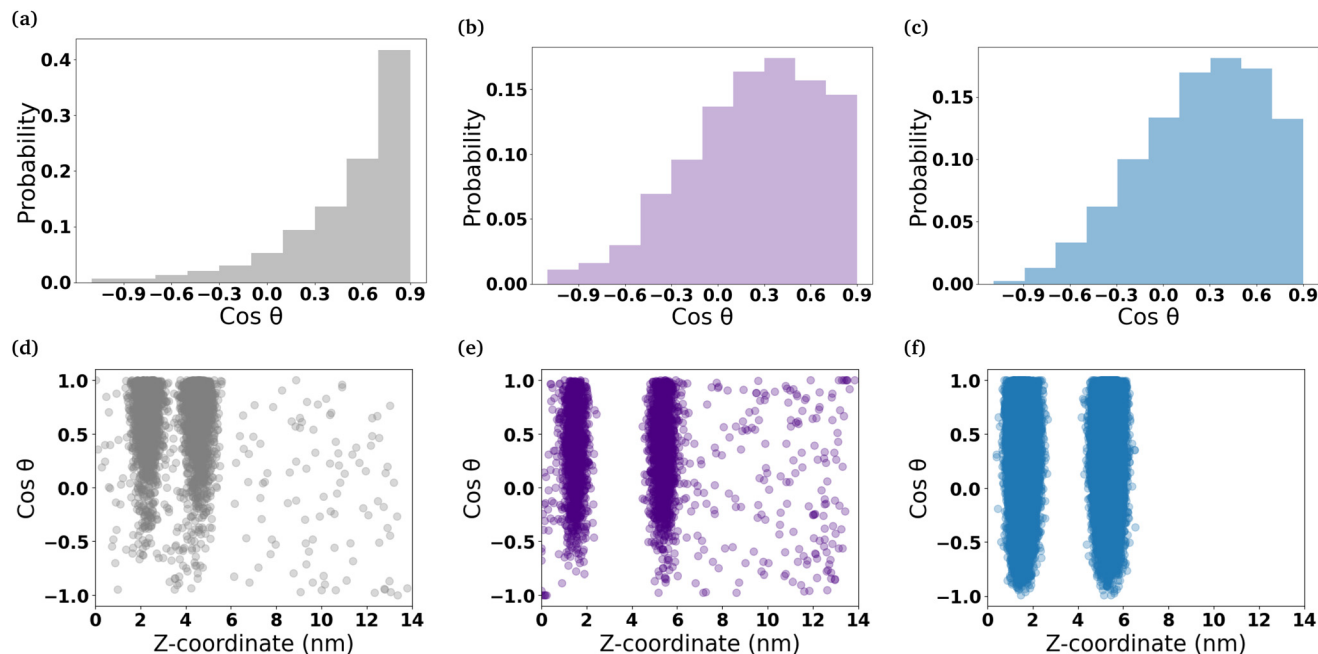


Fig. 5 Orientation of micelle components within DOPC membranes. The histograms show the cosine of the tilt angles of the (a) C6FA molecules that have inserted into the DOPC bilayer. (b) and (c) show the distribution of the cosine of the head group tilt angles of the 2C6PC molecules that have inserted into the DOPC bilayers and the head group tilt angles for DOPC lipids in the membrane, respectively. The scatter plots show how the distribution of the orientation of the (d) C6FA molecules, (e) the 2C6PC lipid head group and (f) the DOPC lipid head group changes with the molecule's location in the z-dimension.

average number of waters in the first hydration shell can be estimated. The RDFs are shown in Fig. S5 and S6.† The calculated coordination numbers for the first shell and the coordination number of waters are reported in Table S3.† The insertion of 2C6PC in the various membrane systems was seen to result in a dehydration of the choline group within the head-group of DOPC. This dehydration of the nitrogen atom in the choline group of the DOPC molecules is likely due to the large steric barrier that exists between the headgroups when more and more molecules were inserted into membranes. Whereas the hydration of the phosphate group is unchanged by the presence of the molecules comprising the micelles within the lipid bilayer. While significantly less 2C6PC inserts into the DPPC-CHOL membrane, there is still a slight dehydration observed of the choline group of the DPPC lipids.

We have also determined the amount of water that is found between the phosphorous atoms in the lipid headgroups in the upper and lower leaflets of the bilayers. The number of water molecules within the bilayer per lipid molecule in the bilayer is reported in Table 1. We see that in the DOPC membrane there is an increased amount of water molecules that permeate into the bilayer when the various components of the micelle are present. While, in contrast, in the case of the DPPC-CHOL membranes, there is a slight decrease in the amount of water present in the bilayer after interaction with the various molecules comprising the micelles. As the penetration of water molecules into lipid bilayers is regularly used to investigate the permeability of a membrane,^{52–54} these

results demonstrate that as the micelle components partition into the membrane they increase the permeability of the disordered (DOPC) membranes.

2.4 Dynamics of micelle components within lipid bilayers

The motion of each molecule that was initially in a micelle was tracked in the z-dimension in each of the simulated systems (Fig. S10 and S11†). This allowed us to see that the C6FA molecules generally move to membranes' interface quickly. 2C6PC molecules were also found to adsorb to the membrane interface, while the C6LYSO molecules, which are more hydrophilic than the other molecules comprising the micelles, remain isolated in solution for longer and when they do come to the membrane interface they only remain there for a relatively short period of time.

Interestingly, we found that all of the micelle components have the ability to flip-flop across the lipid membranes, although we only observed the flip-flop of C6FA molecules across the DPPC-CHOL membrane. Table 2 contains the total number of flip-flops observed and the flip-flop rate for each molecule type in both membranes. The flip-flop rate is unchanged by the presence of the different molecules from the micelles within the membranes. However, as would be expected, we observe significantly less flip-flop of C6FA molecules within the more ordered DPPC-CHOL membrane than within the DOPC membrane.

In order to determine whether the micelle components aggregate with one another within the DOPC membrane, we



In order to investigate if there was any preferential interaction of the various components of the micelles with each

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