DHA Disorders Raft-like Domains as Revealed by Solid State ^{2}H NMR

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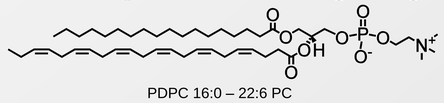
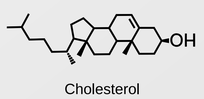
Introduction

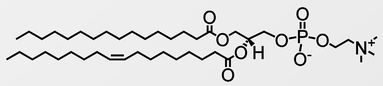
Research continues on the dietary health benefits of omega-3 polyunsaturated fatty acids (n-3 PUFA). One of the major bioactive components of n-3 PUFA is docosahexaenoic acid (DHA, 22:6), with 22 carbons and 6 double bonds. However, its molecular mode of action remains unclear. A hypothesis is that these fatty acids are incorporated into the cell membrane and modify the structure of lipid rafts, thus affecting cell signaling.[1]



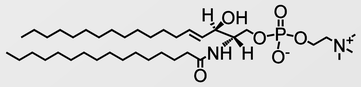
PDPC 16:0 – 22:6 PC



Cholesterol



POPC 16:0 – 18:1 PC

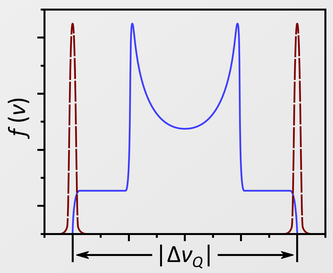


PSM

We used solid-state ^{2}H NMR spectroscopy to compare molecular organization of PDPC-d_{31}[2] and PSM-d_{31} in PDPC:SM:cholesterol (1:1:1 mol) mixtures. For each phospholipid the palmitoyl chains (at *sn*-1 position for PDPC and amide linked for SM) were perdeuterated. We compared this system to a POPC:PSM:cholesterol (1:1:1 mol) control.

^{2}H NMR Spectroscopy

NMR observes the interaction of nuclear spins (as spectra) with an applied magnetic field. The quadrupolar moment of ^{2}H interacts with the electric field gradient of the carbon-deuterium bond, which results in a broad spectrum (solid blue). Angular motion results in averaging and thus narrowing of the observed spectrum, which is quantified as an order parameter (S_{CD}).



$$|\Delta\nu_Q| = \frac{3}{2}\chi_Q|S_{CD}|$$

$$\chi_Q = 168\ \text{kHz}$$

We observe a superposition of spectra for each ^{2}H in a perdeuterated acyl chain. By integrating the measured spectrum against the frequency the first moment (M_1) is found. This is related to the averaged order parameter. We can perform a transformation called depaking (dashed red) to enhance the edges of the spectrum. This is used to quantify multiple components within spectra.

$$M_1 = \frac{\int_{-\infty}^{+\infty}|\omega|f(\omega)d\omega}{\int_{-\infty}^{+\infty}f(\omega)d\omega}$$

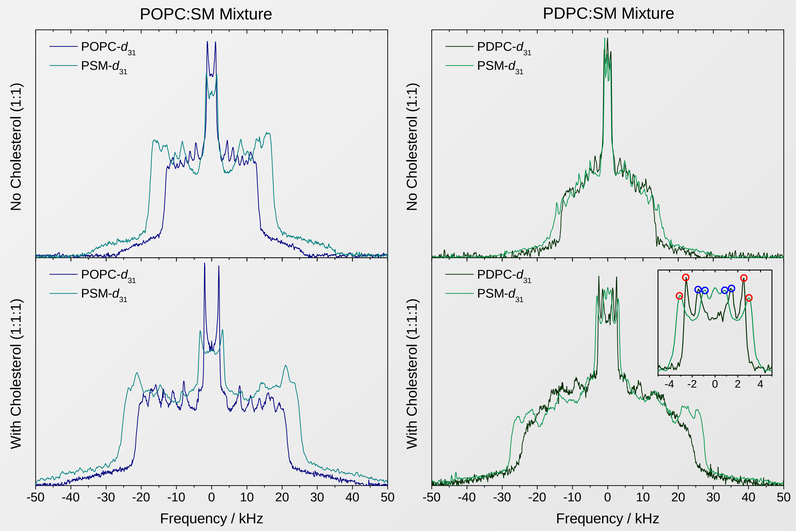
$$M_1 = \frac{\pi}{\sqrt{3}}\chi_Q\langle|S_{CD}|\rangle$$

References

1) S.R. Shaikh, *et al.* 2015. *BBA* **1848**, 211-219

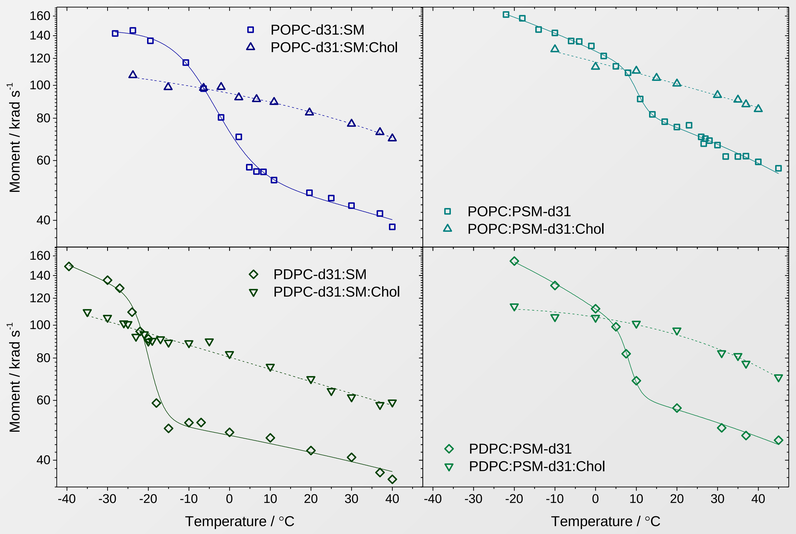
2) J. A. Williams, *et al.* 2012. *Biophys. J.* **103**, 228-237.

Sample Spectra (30 °C)



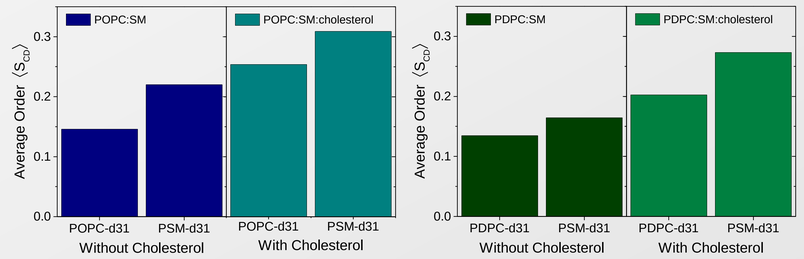
Sample spectra for POPC:SM (1:1 left) and PDPC:SM (1:1 right) mixtures without (top) and with cholesterol (bottom) at 30 °C. Terminal methyl groups that we attribute to separate domains are indicated by small circles in the inset for the PDPC:SM:cholesterol mixture.

First Moments



The moment plots show the mixtures with (dashed lines) cholesterol (1:1:1) and without cholesterol (solid lines). The top plots show the POPC:SM mixture (blues) with POPC-d_{31} on the left and PSM-d_{31} on the right. The bottom plots show the PDPC:SM mixture (greens) with PDPC-d_{31} on the left and PSM-d_{31} on the right.

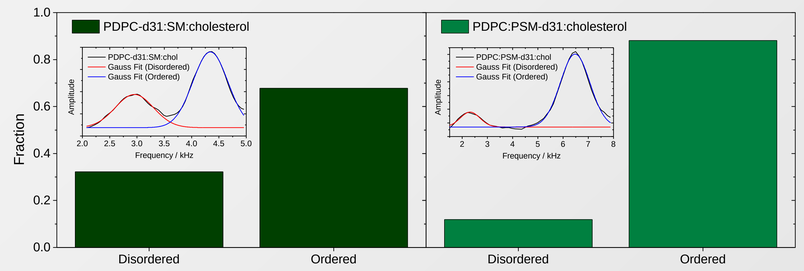
Average Order Parameters



The average order parameters calculated from the moments at 30 °C for each component in the POPC:SM (left) and PDPC:SM (right) mixtures.

Analysis and Discussion

We interpret the difference in spectra for each component in POPC:SM and PDPC:SM in terms of segregation into PC-rich (less ordered) and SM-rich (more ordered) domains. With the addition of cholesterol the domains become larger in the PDPC:SM mixture as indicated by the resolution of a pair of methyl components in the spectrum.



By analyzing the terminal methyl components in the depaked spectra for PDPC:PSM:cholesterol at 30 °C (PDPC-d_{31} left inset and PSM-d_{31} right inset), the percentage of each lipid in each domain was determined (green bars). The percentages reveal that a large amount of PDPC infiltrates the ordered raft-like domain while little SM infiltrates the disordered non-raft-like domain.

Domain Analysis

	Disord.	Ordered
PDPC-d_{31}	32 %	68 %
PSM-d_{31}	12 %	88 %

Conclusion

We found significant interaction of PDPC with SM domains. With the addition of cholesterol, there is indication of increased domain separation, although PDPC still mixes with the majority of the SM. From the average order parameter we see that these domains are more-disordered compared to the POPC:SM mixture. The modified structure of the raft-like domains, due to PDPC, could potentially alter the environment and thereby function of signaling proteins in these domains.