Docosahexaenoic and Eicosapentaenoic Acids Segregate Differently Between Raft and Non-Raft Domains

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Supporting Material

Detergent extraction of cells

Cells

EL4 cells were maintained at 37 °C in a 5% CO_2 incubator. The cells were grown in RPMI 1640 1X with 10% heat inactivated fetal bovine serum (HyClone, Logan, UT) supplemented with 2 mM L-glutamine and 1% penicillin/streptomycin. 10-20 x 10^6 cells were treated with fatty acids complexed to BSA as previously described (1). Briefly, EPA and DHA (Nu-Chek Prep, Elysian, MN) free fatty acid stocks were stored with BHT (Sigma) in amber vials to prevent oxidation and were complexed to fatty acid free BSA (Roche, Indianapolis, IN) at a 1.5:1 ratio. Cells were incubated with 25 μM fatty acids in serum-free RPMI overnight (~15.5 hours) to maximize uptake of fatty acids. BSA treatment in the absence of EPA or DHA served as control (1). We have previously reported that treatment of cells under these conditions results in uptake of the fatty acids into polar lipids (~80-85%) (1,2).

Biochemical analysis of DRM

Fatty acid-treated cells were washed with cold PBS and dissolved in cold MBS buffer (150 mM NaCl, 2 mM EDTA, 25 mM Mes at pH 6.5) containing 1% Triton X-100 and a protease inhibitor cocktail (Sigma). Cells were broken with a 23g needle and placed on ice for 30 minutes. Broken cells (2.5 mL) were then mixed with an equal volume of 90% sucrose in MBS. This solution (4 mL) was overlaid with 4 mL of 35% sucrose followed by 4mL of 5% sucrose. The three component solution was centrifuged at 40,000 rpm in a SW41Ti Beckman rotor (Brea, CA) for 20 hours at 4 °C. Sucrose gradient fractions were then collected from the top to bottom of the tubes on ice. Radiolabeled cholesterol (^{14}C or ^{3}H labeled, Perkin Elmer, Waltham, MA) controls were acquired to determine DRM (raft) and detergent soluble membrane (DSM, non-raft) fractions. Generally, DRM and DSM were defined as fractions 3-6 and 9-12, respectively.

Lipids were extracted from DRM and DSM fractions using methods previously described (1). Extracted fatty acids from DRM and DSM were methylated using boron trifluoride (Sigma) and analyzed using a Shimadzu gas chromatograph (GC-2010, Columbia, MD). Identified peaks were based on standards (Nu-Chek Prep). DRM and DSM data were obtained from 3-4 independent experiments. Statistical significance was established with a one-way ANOVA followed by a Bonferroni multiple comparison t test using GraphPad Prism.

Table S1 Average order parameters $\overline{S}_{CD}$ derived from ^{2}H NMR spectra for PEPC-d_{31}, PDPC-d_{31} and POPC-d_{31} in 1:1 mol mixtures with SM, and in 1:1:1 mol mixtures with SM and cholesterol at 37 ºC. There is a correspondence of the average order parameter to the bilayer thickness (3).

Membrane composition	$\overline{S}_{CD}$		$\Delta\overline{S}_{CD}$
	No cholesterol	With cholesterol	
PEPC-d_{31}/SM	0.100	0.156	0.056
PDPC-d_{31}/SM	0.122	0.192	0.070
POPC-d_{31}/SM	0.137	0.231	0.094

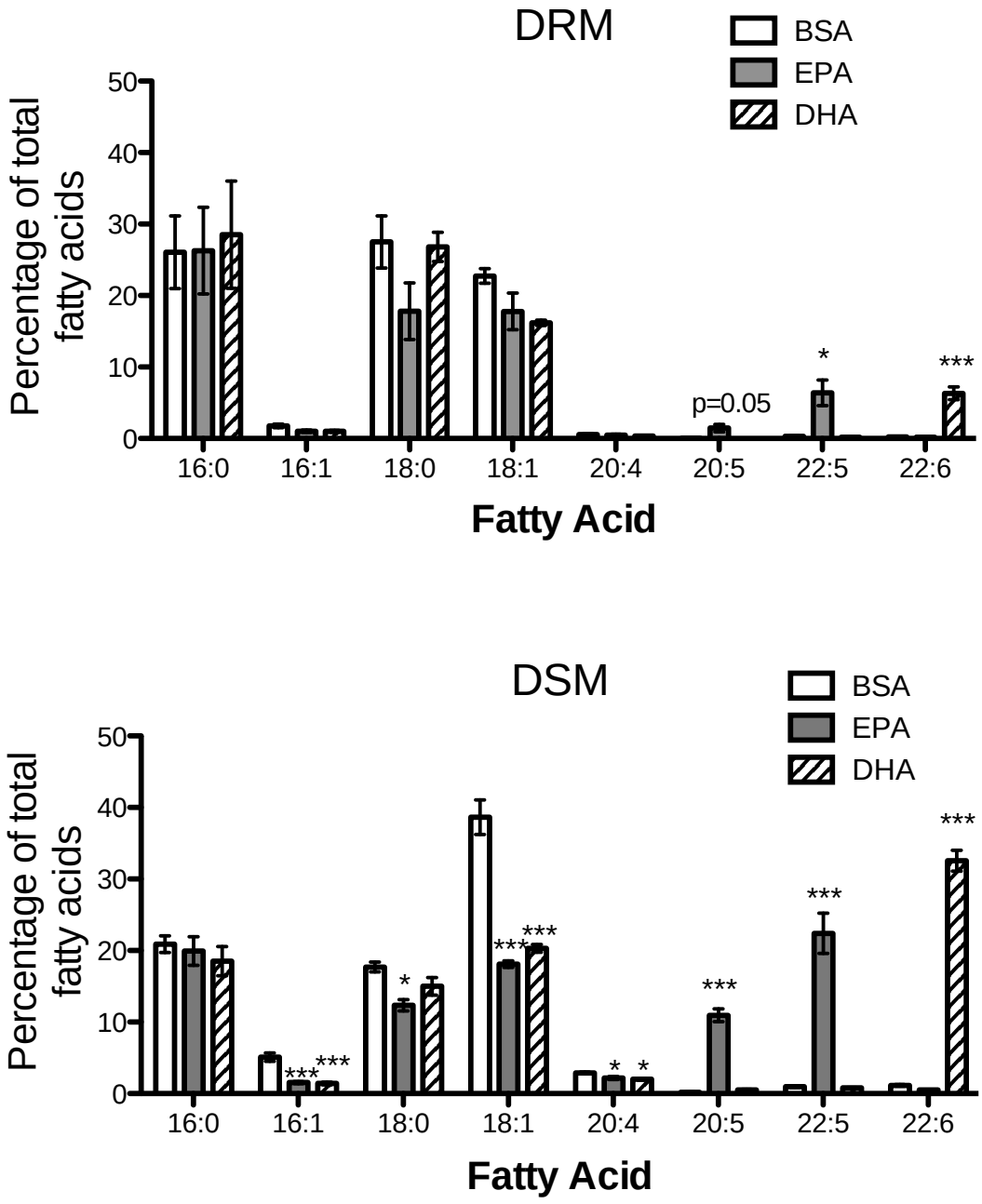

Figure S1 Fatty acid analysis of the DRM (upper panel) and DSM (lower panel) fractions of EL4 T cells. EL4 T cells were treated overnight with 25μM BSA (control), EPA or DHA in serum free conditions and subjected to cold detergent extraction. Fatty acids were then extracted from DRM and DSM, methylated and analyzed with gas chromatography. Asterisks denote significance from BSA (*$p<0.05$ and ***$p<0.001$).

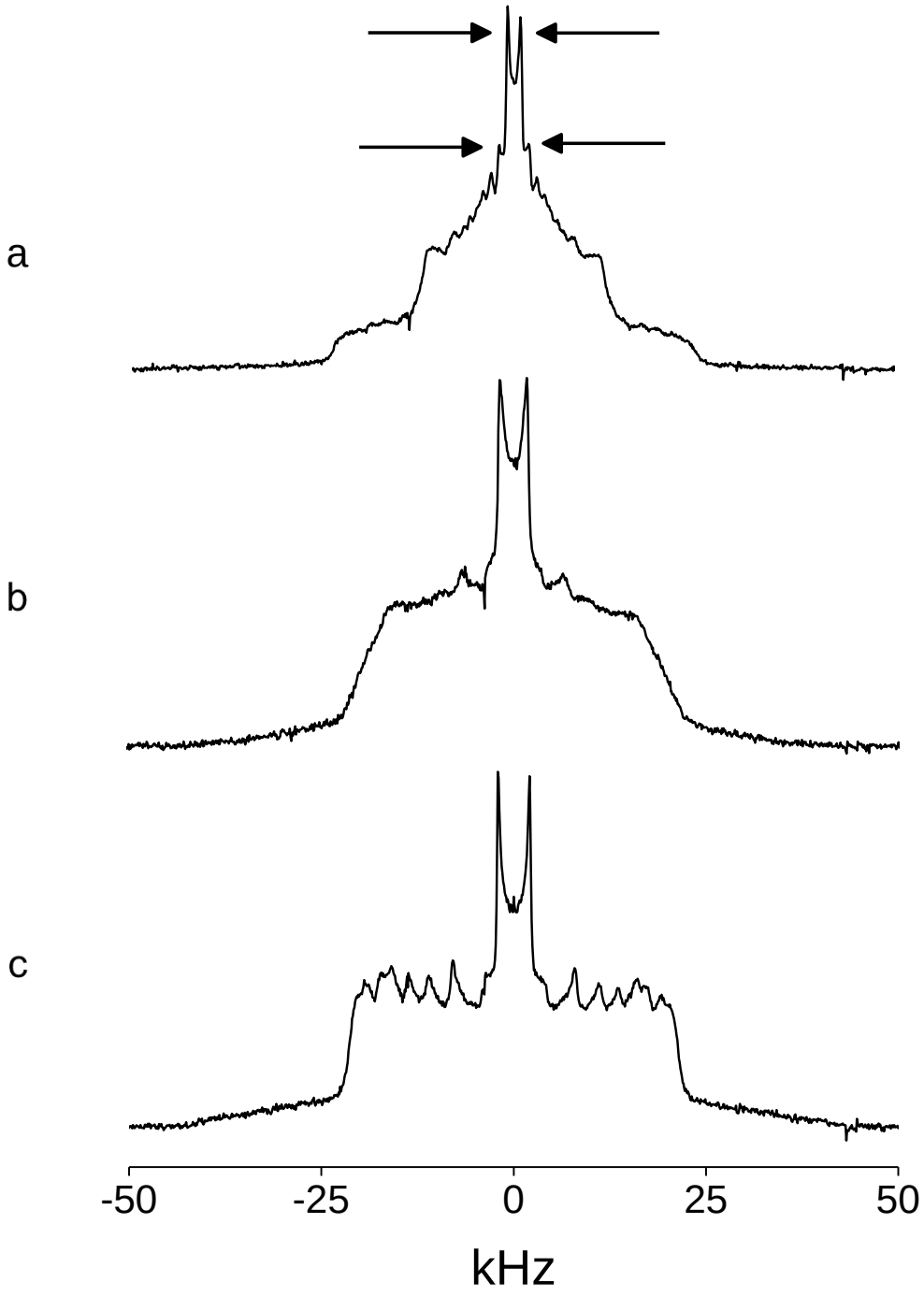

Figure S2 ^{2}H NMR spectra for 50 wt% aqueous dispersions in 50 mM Tris buffer (pH 7.5) of PEPC-d_{31} (a), PDPC-d_{31} (b), and POPC-d_{31} (c) in 1:1:1 mol mixtures with SM and chol at 37 °C. Arrows highlight that the signal for the terminal methyl group is split in two in the spectrum for PEPC-d_{31} but not in the spectra for PDPC-d_{31} and POPC-d_{31}.

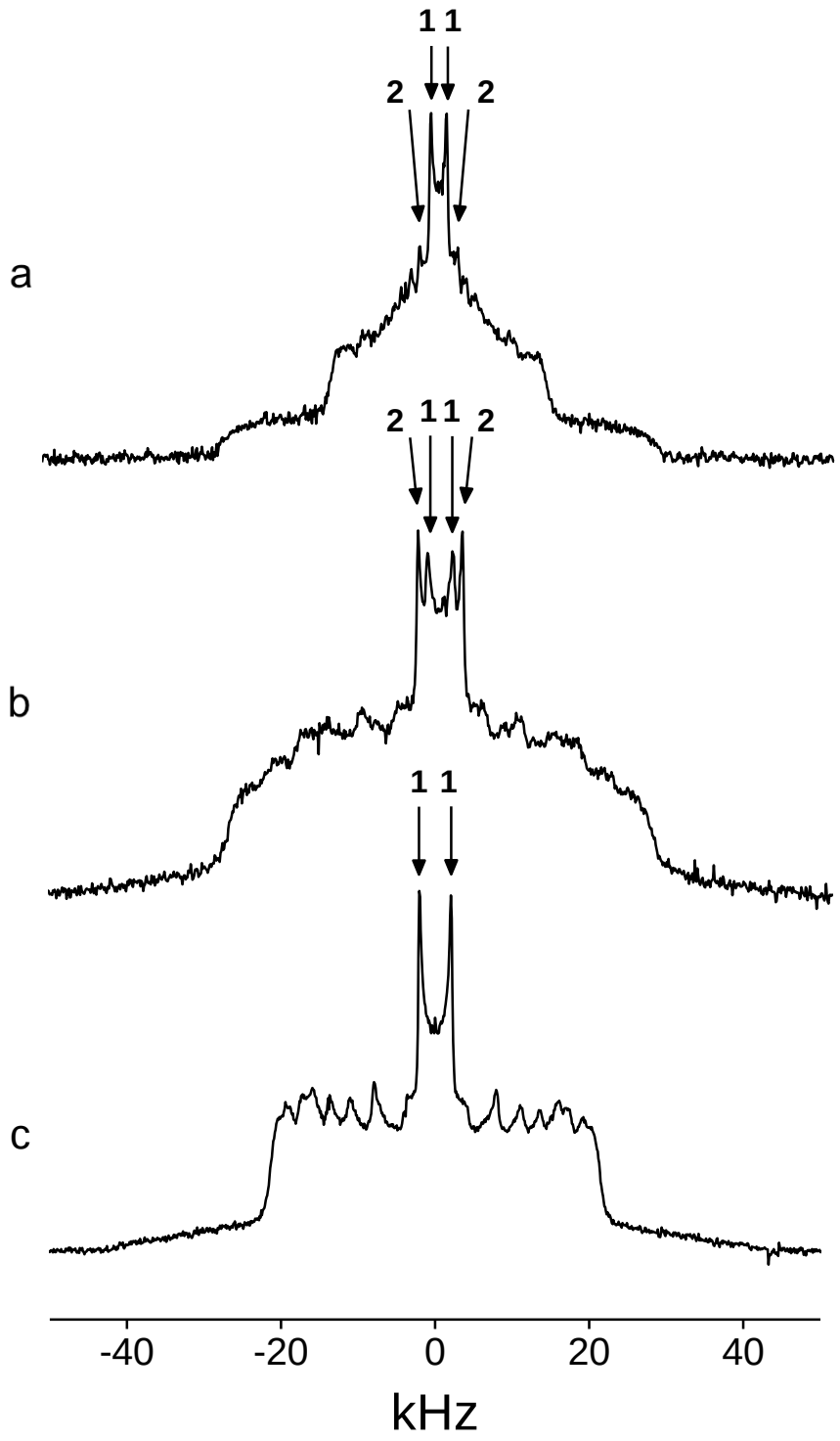

Figure S3 ^{2}H NMR spectra for 50 wt% aqueous dispersions in 50 mM Tris buffer (pH 7.5) of PEPC-d_{31} (a), PDPC-d_{31} (b) and POPC-d_{31} (c) in 1:1:1 mol mixtures with SM and chol at 30 °C. Arrows highlight that the signal for the terminal methyl is split into a pair of inner (1) and outer (2) peaks in the spectra for PEPC-d_{31} (a) and PDPC-d_{31} (b), whereas in the spectrum for POPC-d_{31} (c) there is only a single pair (1) of peaks.

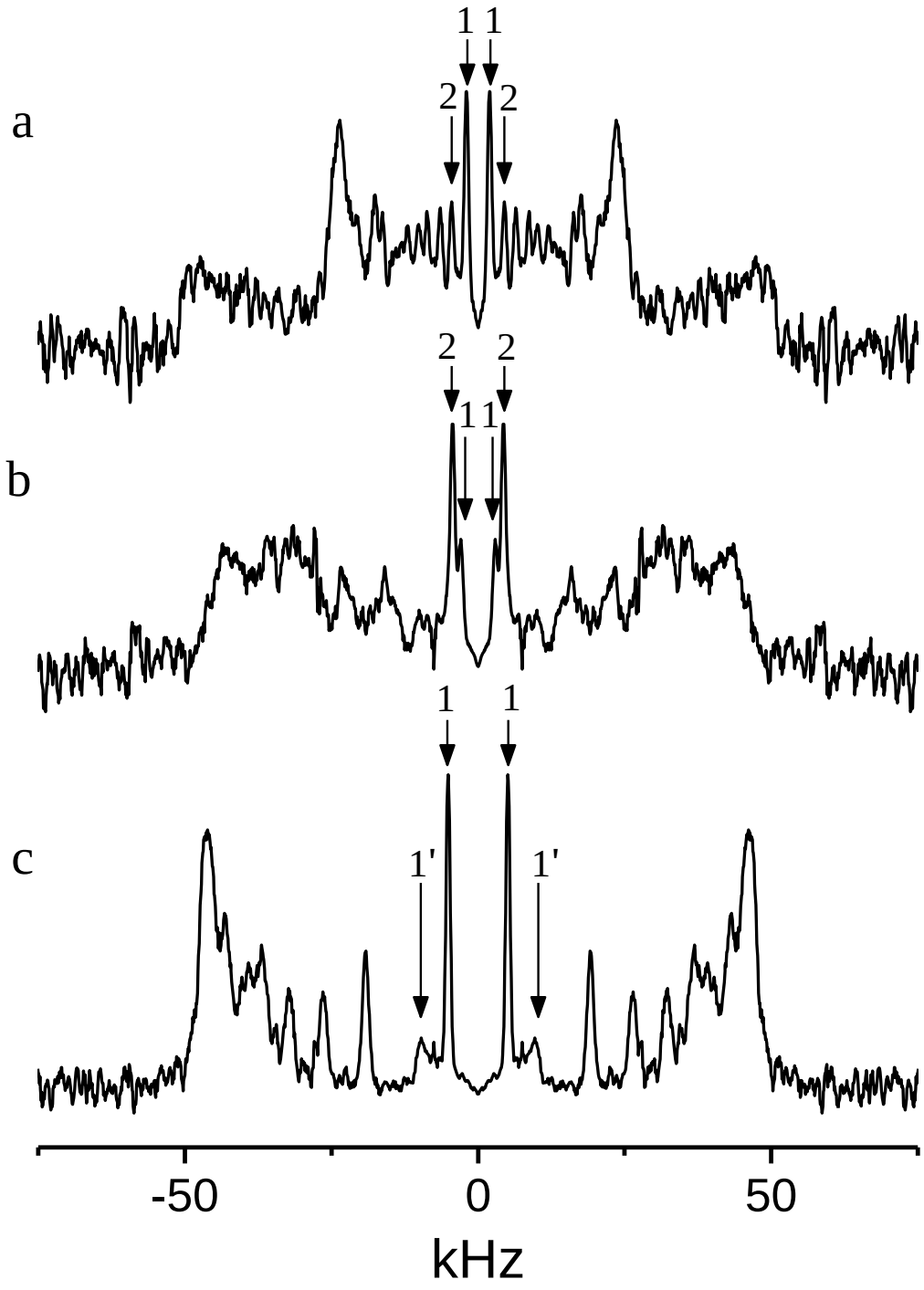

Figure S4 FFT depaked ^{2}H NMR spectra for 50 wt% aqueous dispersions in 50 mM Tris buffer (pH 7.5) of PEPC-d_{31} (a), PDPC-d_{31} (b) and POPC-d_{31} (c) in 1:1:1 mol mixtures with SM and chol at 30 °C. Arrows highlight that the signal for the terminal methyl is split into a pair of inner (1) and outer (2) peaks in the spectra for PEPC-d_{31} (a) and PDPC-d_{31} (b), whereas in the spectrum for POPC-d_{31} (c) there is only a single pair of peaks (1) with a pair of satellite peaks (1′) that are artifacts produced by the FFT depaking algorithm.

Supporting References

1. Rockett, B. D., M. Salameh, K. Carraway, K. Morrison, and S. R. Shaikh. 2010. n-3 PUFA improves fatty acid composition, prevents palmitate-induced apoptosis, and differentially modifies B cell cytokine secretion in vitro and ex vivo. *J. Lipid Res.* 51:1284–1297.

2. Rockett, B. D., A. Franklin, M. Harris, H. Teague, A. Rockett, and S. R. Shaikh. 2011. Membrane raft organization is more sensitive to disruption by (n-3) PUFA than nonraft organization in EL4 and B Cells. *J. Nutr.* 141: 1041-1048.

3. Petrache, H. I., S. W. Dodd and M. F. Brown. 2000. Area per lipid and acyl chain length distributions in fluid phosphatidylcholines determined by ^{2}H NMR spectroscopy. *Biophys. J.* 78:3172-3192.