

Supporting Information for: **Pathological levels of Glucosylceramide change the biophysical properties of artificial and cell membranes**

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Table 1. Composition of the studied mixtures. The molar ratio of POPC, C16-SM and Chol in mixtures without or with 5 or 10 mol% of GlcCer is shown. The fraction of the I_o phase, X_{I_o} , of the ternary mixtures, is also shown.

X_{I_o}	Without GlcCer			5 mol% GlcCer			10 mol% GlcCer		
	X_{POPC}	X_{C16-SM}	X_{Chol}	X_{POPC}	X_{C16-SM}	X_{Chol}	X_{POPC}	X_{C16-SM}	X_{Chol}
0	0.72	0.23	0.05	0.68	0.22	0.05	0.64	0.21	0.05
0.26	0.60	0.26	0.14	0.60	0.25	0.13	0.54	0.24	0.13
0.51	0.45	0.30	0.25	0.43	0.28	0.24	0.41	0.27	0.23
0.84	0.34	0.33	0.33	0.32	0.31	0.32	0.31	0.29	0.30
0.98	0.25	0.35	0.40	0.24	0.33	0.38	0.23	0.31	0.36
1.00	0.15	0.37	0.48	0.14	0.36	0.45	0.14	0.34	0.43

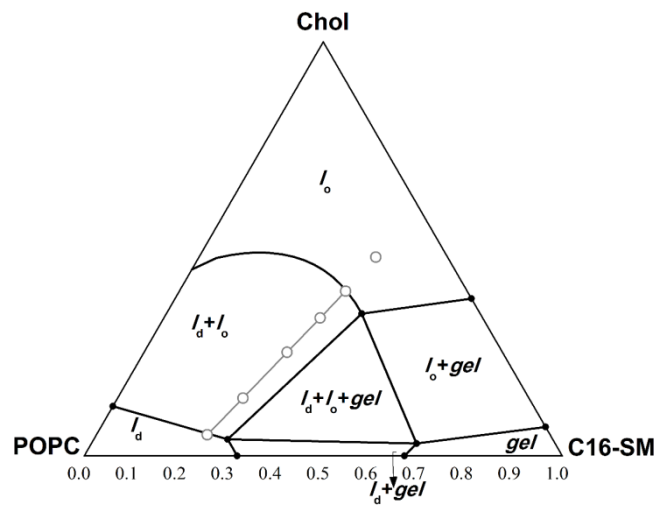


Figure S1 - POPC/C16-SM/Chol ternary phase diagram

The grey line is a tie-line that contains a 1:1:1 POPC/C16-SM/Chol mixture. The grey dots correspond to the mixtures used in this study. (adapted from de Almeida *et al.*(1)).

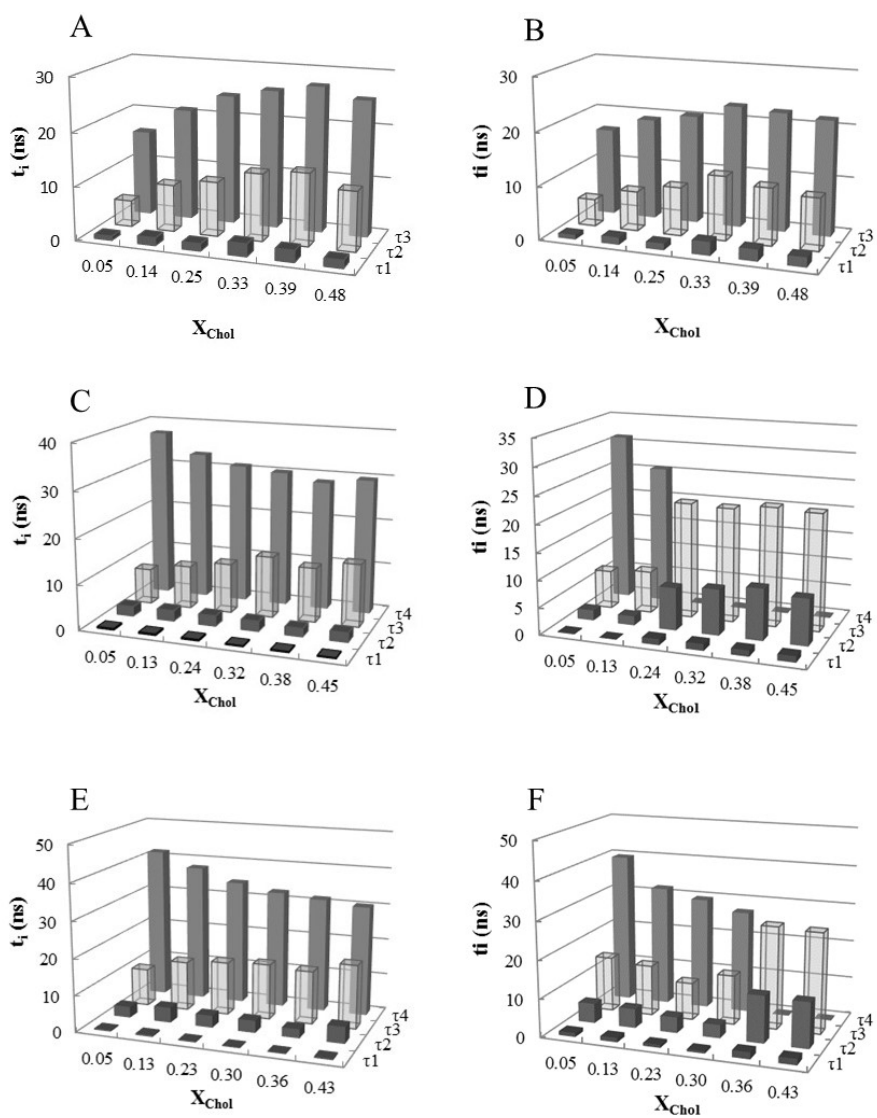


Figure S2 - Analysis of t-PnA fluorescence intensity decay components in POPC/C16-SM/Chol and POPC/C16-SM/Chol/C16-GlcCer mixtures.

Variation of the lifetime components of t-PnA intensity decay, in POPC/C16-SM/Chol mixtures containing (A, B) 0, (C, D) 5 and (E, F) 10 mol% of C16-GlcCer. Measurements were performed at pH 7.4 (A, C, E) or at pH 5.5 (B, D, F). Values are means \pm SD of at least 3 independent experiments.

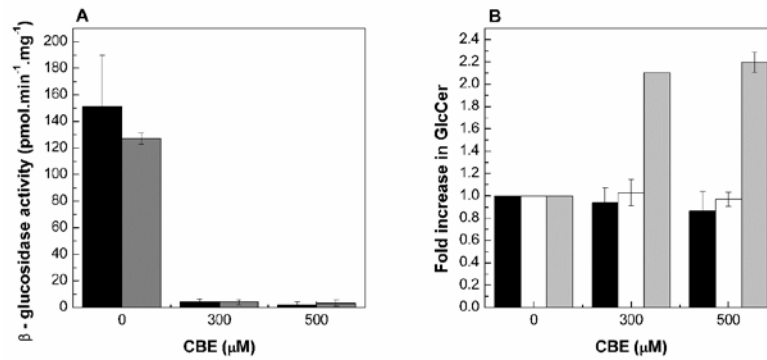


Figure S3 – CBE-induced glucosidase inhibition and GlcCer accumulation.

(A) Activity of β -glucosidase in control cells and cells treated with different concentrations of CBE after 1 (black bars) and 2 (gray bars) days of incubation. Inhibition of the enzyme results in (B) time (1 day – black bars; 3 days – white bars; 6 days – light gray bars) and CBE concentration-dependent increase in GlcCer levels.

References

1. de Almeida RFM, Fedorov A, Prieto M (2003) Sphingomyelin/Phosphatidylcholine/Cholesterol Phase Diagram: Boundaries and Composition of Lipid Rafts. *Biophys J* 85(4):2406–2416.