# Long-chain unsaturated sphingolipids: a glimpse into lipid complexity through AFM, DSC and fluorescence

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The effects of C24:1 sphingolipids have been tested in phospholipid bilayers containing cholesterol. Confocal microscopy, differential scanning calorimetry (DSC), and atomic force microscopy (AFM) techniques have been used. More precisely, the effects of C24:1 ceramide (nCer) were evaluated and compared to those of C16:0 ceramide (pCer) in bilayers composed basically of dioleoyl phosphatidylcholine, sphingomyelin (either C24:1, nSM or C16:0, pSM) and cholesterol. Combination of equimolecular amounts of C24:1 and C16:0 sphingolipids were also studied. Results show that both pCer and nCer are capable of forming segregated gel domains. Force spectroscopy data point to nCer having a lower stiffening effect than pCer, while the presence of nSM reduces the stiffness. DSC reveals Tm reduction by nSM in every case. Furthermore, pSM seems to better accommodate both ceramides in a single phase of intermediate properties, while nSM partial accommodation of ceramides generates different gel phases with higher stiffnesses caused by inter-ceramide cooperation (Figure 1). If both pSM and nSM are present, a clear preference of both ceramides towards pSM is observed. These findings show the sharp increase in complexity when membranes exhibit different sphingolipids of varying N-acyl chains, which should be a common issue in an actual cell membrane environment [1,2].

## References

[1] García-Arribas A.B., González-Ramírez E.J., Sot J., Areso I., Alonso A. & Goñi F.M., Langmuir 33 (2017) 5545-5554
[2] García-Arribas A.B., Alonso A. & Goñi F.M., Chem Phys Lipids 199 (2016) 26-34

## Figures

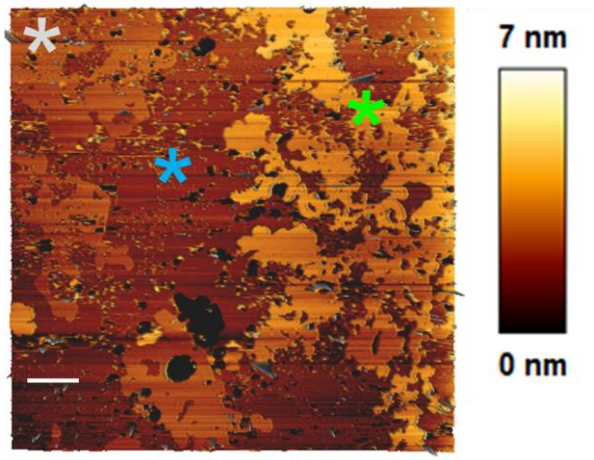


**Figure 1:** DOPC:nSM:Chol (2:1:1) + 15% nCer + 15% pCer bilayer (AFM height image). Each ceramide appears to segregate into different domains that can be distinguished by height (asterisks in the image) or by nanomechanical resistance but not by RhoPE fluorescence (see Poster).