

Soft Matter

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COMMENT

Comment on “Cholesterol solubility limit in lipid membranes probed by small angle neutron scattering and MD simulations” by S. Garg *et al.*, *Soft Matter*, 2014, 10, 9313

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In a recent article, Garg *et al.* used neutron scattering techniques to determine the limiting amount of cholesterol which vesicles of either POPS or POPC can accommodate. This amount was called “the cholesterol solubility limit”. In light of extensive literature on cholesterol phase separation in phospholipid bilayers, the way in which “solubility limit” is defined in this article and the conclusions derived are misleading and require some clarification.

The nature of the distribution of cholesterol in phospholipid membranes has been the subject of extensive investigation for almost half a century, motivated both by biophysical and medical interests. In the Introduction to their article, Garg *et al.* cite some of the numerous references¹⁻¹¹. Careful reading of each of these reports shows that the solubility limit of cholesterol in lipid membranes is defined as the concentration at which cholesterol phase separation and crystallite formation begin to take place. The presence of crystals is probed by X-ray diffraction, solid state NMR spectroscopy or differential scanning calorimetry. The particular location of the crystals appearing at the very onset of phase separation is not unambiguously identified: in particular, there is no justification for simply assuming that they are disassociated from the membrane, although that may occur with additional increase in cholesterol content. In fact, Ref.15 of Garg *et al.* (present reference #8) was able to demonstrate, using EPR techniques, that non-crystalline cholesterol domains appear in POPS vesicles and that these domains are clearly located within the bilayer.

Yet, the small angle neutron scattering (SANS) technique with contrast matching, which Garg *et al.* employ to study cholesterol solubility in 100 nm POPS or POPC unilamellar vesicles, is only able to identify the concentration above which no additional cholesterol can be accommodated, independent of distribution. These data are new and interesting, but they do not determine a solubility limit as defined in the literature. There is a modification of the SANS measurements employed by Garg *et al.*, which has been reported to detect phospholipid phase separation in vesicles^{9,10}. Yet, the authors only refer to their use of this technique in one sentence in the Discussion; they do not present any data, in spite of the central importance of the existence of domains to the subject of their research. Rather they simply state that they did not observe the SANS signal for domain structure and therefore the membranes must be homogeneous. However as Pencer *et al.* point out¹¹, the characteristic SANS signal is only prominent in

the event of large scale phase separation- as the number of domains grows and the domain size decreases, the characteristic signal is significantly weakened, which may be the case for cholesterol phase separation. This has not yet been demonstrated. As far as their molecular dynamics simulations are concerned, Garg *et al.* comment that such calculations are currently not able to reliably model cholesterol crystallite formation. On the other hand, analytical mean field calculations¹² do show cholesterol phase separation and domain formation in phospholipid membranes.

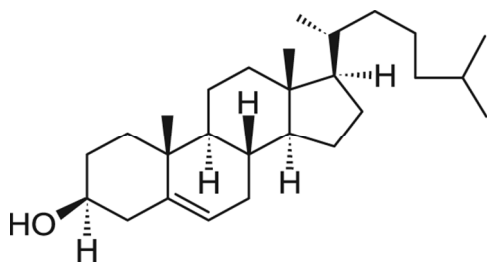
Conclusions

Therefore, in conclusion, the only parameter that the data in the article by Garg *et al.* can convincingly characterize is the maximum concentration of cholesterol which POPS or POPC vesicles can accommodate; there is no information about the solubility limit as consistently defined in the literature by cholesterol phase separation and crystallization. At issue here is not a question of technical error or measurement inaccuracy, but rather one of definition. We request that some clarifying note to this effect be presented in a future issue of *Soft Matter*.

Notes and references

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As consistently described in the literature, the solubility limit of cholesterol in phospholipid bilayers is defined by its phase separation and crystallization.