Seeing spots: miscibility transitions in lipid/cholesterol membranes

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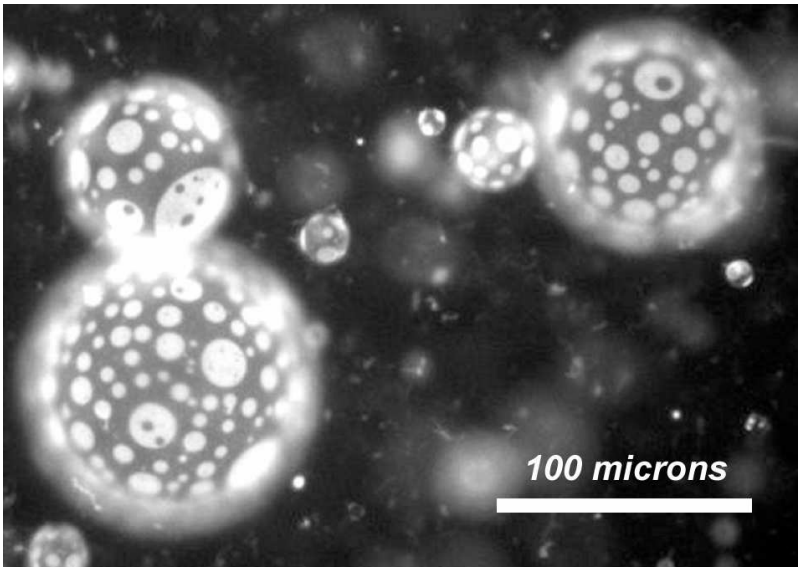
Mammalian cells are surrounded by an outer wall or "plasma membrane" of proteins and lipids arranged in opposing leaflets of a bilayer. There is growing evidence that this membrane is not uniform, but instead laterally phase separates into "raft" domains rich in particular lipids and proteins.

We study a simpler physical model of cell membranes, giant unilamellar vesicles (GUVs). Using fluorescence microscopy, we can directly observe liquid domains in free-floating vesicles containing three components: a lipid with high melting temperature (e.g. a saturated lipid), a lipid with low melting temperature (e.g. an unsaturated lipid), and a “membrane active” sterol (e.g. cholesterol). Liquid domains in vesicles exhibit interesting behavior. They collide and coalesce until only one bright domain and one dark domain remain on each vesicle. Domains also finger into stripes near the critical point, and can bulge out of or into the vesicle (Veatch & Keller, 2003, Veatch & Keller, 2005).



By recording miscibility transition temperatures for many lipid compositions, we have mapped ternary phase diagrams. Our fluorescence microscopy studies give us qualitative tie-lines across the phase diagram. These tie-lines run from a region that is rich in the unsaturated lipid to a region rich in the saturated lipid, with little change in cholesterol. Applying this statement to the figure above, the bright domains are rich in unsaturated lipid, and the dark domains are rich in the saturated lipid, and only to a lesser extent in cholesterol. Using NMR (in collaboration with Klaus Gawrisch’s laboratory at the National Institutes of Health, Bethedsa, MD, USA), we have quantitatively verified the direction of the tie-lines, and have then estimated free energies to transfer lipids between phases, which are at most a few k_BTs (Veatch *et al.*, 2004).

In other studies, we have captured domains in lipid layers on glass substrates, and found that they assume static, noncircular shapes. We have substituted different sterols for cholesterol, and found that those which are structurally similar to cholesterol produce coexisting liquid domains in vesicles, just as cholesterol does (Beattie *et al.*, 2005). Finally, we have compared the phase diagrams of bilayer systems to monolayer systems and found them very different (Stottrup *et al.*, 2005).

Beattie, M.E., Veatch, S.L., Stottrup, B.L. & Keller, S.L. (2005) *Biophysical Journal* in press.
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