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Imaging of protein partitioning in plasma membranes with coexisting fluid phases

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The membrane raft hypothesis postulates the existence of lipid bilayer membrane heterogeneities, or domains, important for cellular membrane functioning, including lateral sorting, signaling and trafficking. The *in vivo* characterization of lipid membrane heterogeneities thus far has been challenging. Lipid membrane rafts have been suggested to be enriched in lipids that confer fluid ordered phase like character to these compositional heterogeneities. Lipid model membranes, on the other hand, allow fluorescence imaging of lipid domain coexistence, but so far micron-size coexisting fluid phases have been demonstrated only in simple, including ternary, lipid mixtures. We found that giant *plasma membrane vesicles* (PMVs) obtained from cultured rat basophilic leukemia cells can phase segregate into optically resolvable micron-size phases that are identified as both being fluid. We examined the partitioning of fluorescent lipid analogs and hydrophobic membrane markers and found them to be similar to the partitioning behavior in model membrane systems that show coexisting fluid ordered and fluid disordered phases. Significant temperature dependence of the tendency of PMVs to phase separate was found and analyzed. An advantage of these PMVs is that they contain, at least a large subfraction, of proteins associated with native plasma membranes. We are therefore able to study the partitioning of membrane proteins redistributing between coexisting fluid membrane phases. Trans-membrane proteins, outer leaflet associated peripheral and cytosolic peripheral membrane proteins were examined for their fluid phase partitioning. We suggest our method as a new approach for studying aspects of biological membrane heterogeneity.