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Cytoskeletal pinning prevents large-scale phase separation in model membranes

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During the last decades, artificial lipid bilayers have become an important tool in studies of properties of the plasma membrane of cells. One important feature of cell membranes, which has been difficult to recapitulate in the artificial bilayer systems, is the membrane-associated cytoskeleton. The cytoskeleton is believed to affect diffusion of lipid and protein molecules in the plasma membrane, and is considered to be one of the reasons for the sub-resolution size of membrane domains by preventing large-scale phase separation. Mimicking the eukaryotic actin-based cytoskeleton in vitro is inefficient and complicated, owing to the number of components involved and the nature of membrane binding of the actin-network complex. Here we describe a minimal cytoskeletal network formed by the prokaryotic tubulin homologue, FtsZ. FtsZ has been modified to interact with the membrane through a membrane targeting sequence (MTS) from MinD, another prokaryotic protein. FtsZ-MTS efficiently forms a highly interconnected network on the membrane with a concentration-dependent characteristic mesh size, much similar to the eukaryotic network underlying the plasma membrane. Using giant unilamellar vesicles formed from a quaternary lipid mixture, we demonstrate that, on the one hand, the artificial membrane-associated cytoskeleton suppresses large-scale phase separation below the phase transition temperature, and, on the other hand, preserves phase separation above transition temperature. Our experimental observations support the ideas put forward in our previous simulation study [1]: In particular, the picket-fence effect on phase separation explains why micrometer-scale membrane domains are observed in isolated, cytoskeleton-free giant plasma membrane vesicles, but not in intact cell membranes. The experimentally observed suppression of large-scale phase separation much below the transition temperatures also serves as an argument in favor of the cryoprotective role of the cytoskeleton.

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References

[1] J. Ehrig, E.P. Petrov, P. Schwille: *Near-critical fluctuations and cytoskeleton-assisted phase separation lead to subdiffusion in cell membrane*. Biophys. J. **100**, 80–89 (2011)