commentary

Membrane domains beyond the reach of microscopy[1]

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The concept of lipid rafts describes the lateral compartmentalization of cellular membranes into domains of different compositions and physical properties (1). Raft themselves are relatively tightly packed domains enriched in sterols, sphingolipids, saturated lipids, and specialized raft-preferring membrane proteins (2). These patches in living cells are hypothesized to be nanoscopic and dynamic, serving key roles in biological processes including signal transduction, lipid and protein sorting, and viral entry during host cell infection. The physical chemistry underpinning the raft concept is that biomimetic lipid mixtures containing sterols and various phospholipids spontaneously separate into large, stable coexisting liquid membrane phases. One of these phases, the liquid ordered phase (Lo), is enriched in sterols and saturated lipids, forming the synthetic model of lipid rafts (3–5). However, the relevance of such synthetic membrane insights to the behavior of living cell membranes has been questioned. A major mismatch between model membrane systems and living cells is that synthetic membranes often produce micron-sized lipid phases, whereas macroscopic domains are generally not observable in living cell membranes, with the notable exception of yeast vacuoles (6, 7). Many features of living membranes are not adequately represented in model membrane experiments, including *a*) diversity of lipid and protein species, *b*) interaction with the cellular cytoskeleton and other cytoplasmic features, *c*) the energy-dependent out-of-equilibrium nature of living cells, and *d*) lipid asymmetry of the bilayer, although asymmetry in model membrane vesicles has been addressed in recent studies (8). These limitations prohibit simple inferences of the physical behavior of living membranes from studies of synthetic systems.

In that context, a major development has been the discovery of coexisting liquid phases in giant plasma membrane vesicles (GPMVs) obtained through vesicle budding from mammalian plasma membranes (PMs) (9). These vesicles preserve the lipid and protein diversity of the living membrane yet are still capable of macroscopic phase separation, allowing direct measurements of ordered phases in cell-derived membranes (10). However, micron-sized large domains only appear in GPMVs far below physiological temperature, typically in the range of 10–20°C. Thus, the relationship between thermotropic phase separation in GPMVs and the organization of the PM in living cells persists as a major open question in membrane biology. One intriguing proposal draws on observations of critical fluctuations in GPMVs near their miscibility transition temperature to extrapolate that nanoscopic fluctuations should persist well below microscopically observable length scales (11). Although this hypothesis has been supported by some experiments (12) and simulations (13), the presence of nanoscopic domains in GPMVs above the phase transition temperature had not been directly tested until the recent studies of Li et al. published in this issue of the *Journal of Lipid Research*.

The London group has developed a robust experimental framework that relies on measurements of Förster resonance energy transfer (FRET) between two lipidic fluorophores to probe for membrane nanodomains (**Fig. 1**) (14). The principle behind the method is probes that are carefully chosen to partition differentially between coexisting Lo and liquid disordered (Ld) phases. If one of these probes, the FRET acceptor (Fig. 1, pink hexagons), for example, is excluded from Lo domains, then FRET efficiency will be reduced by its spatial separation from the donor. This is true regardless of domain size, as long as domains are sufficiently larger than the Forster distance for efficient energy transfer. In this article, the donor fluorophore was diphenylhexatriene (DPH), which distributes approximately equally between Lo and Ld phases, while the acceptor was octydecylrhodamine B (ODRB), which partitions strongly to the Ld phase. This choice of fluorophores allows detection of Lo domains down to ~10 nm in radius.

The central result of the paper is that while macroscopic domains in GPMVs were no longer observable above room temperature, membrane nanodomains were detectable by FRET even up to physiological temperature. Importantly, the authors also investigated numerous changes to membrane lipid composition that affected the stability of both macroscopic domains and nanodomains.

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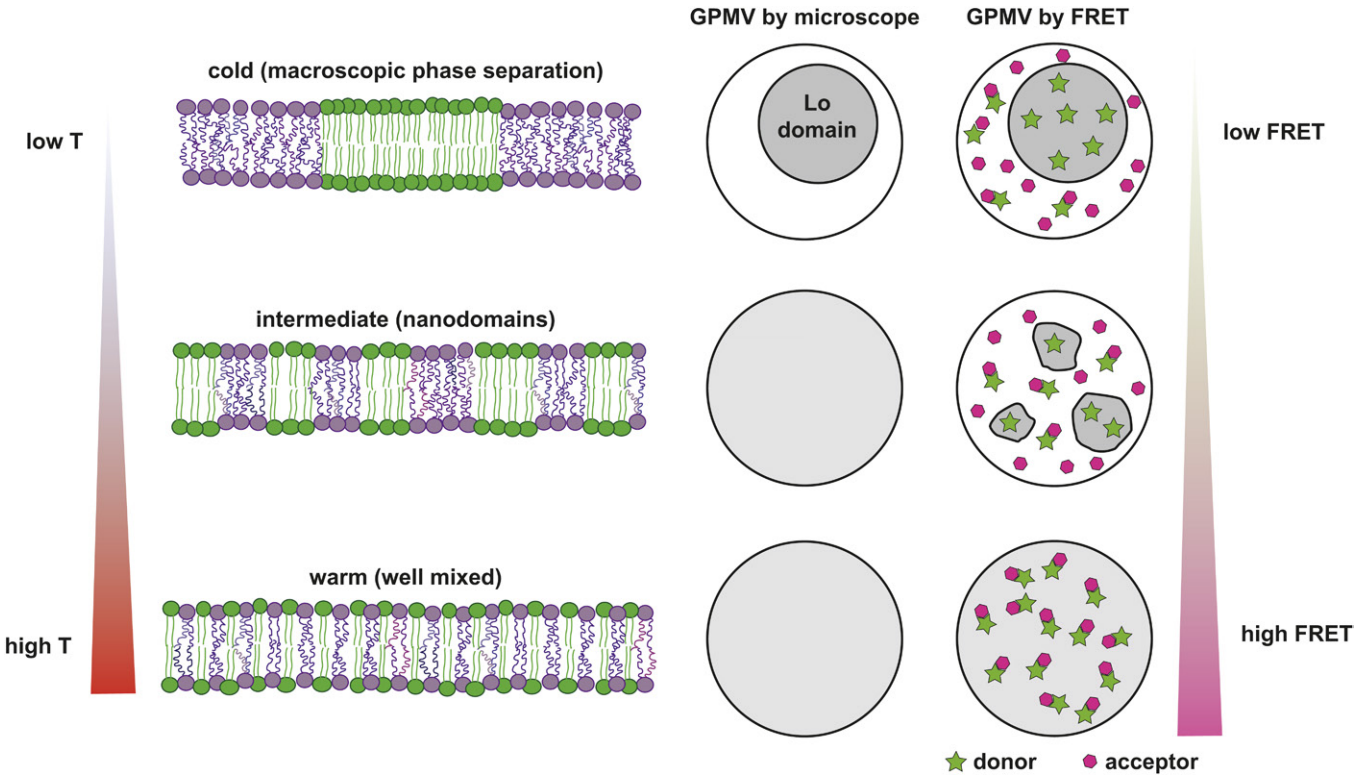

Fig. 1. Application of FRET to detect nanodomains in cell-derived membranes. GPMVs phase separate into macroscopic coexisting Lo and Ld phases below their miscibility transition temperature but appear uniform above ~20°C. Li et al. detect nanodomains up to physiological temperature by measuring FRET between differentially partitioning lipid probes. Nanodomains are represented as irregular shapes because their actual shapes are unknown and the method is sensitive to nano-scale structures irrespective of size and shape. Also, while the red probe is shown being completely excluded from dark domains, the method is sensitive to more subtle probe sorting scenarios.

Across all conditions, there was a strong correlation between these distinct metrics of membrane organization, with nanodomains persisting ~20°C above the macroscopic phase transition regime. While this method does not distinguish between possible mechanisms for generating nanodomains from macroscopic phase separation, this correlation is fully consistent with predictions from criticality (11).

The observations in this article provide strong evidence that the biophysical principles governing Lo/Ld phase separation can be extended to understand the behavior of nanodomains in biological membranes under physiological conditions. While neither synthetic membrane models nor GPMVs completely represent the complexity of the cellular PM, direct confirmation of nanodomains in GPMVs at physiological temperature represents an important step forward. First, it supports previous interpretations of similar spectroscopic measurements in intact cells attributing structure to the presence of ordered phase domains (15, 16). Further, nanodomains recruiting the same components that prefer ordered phases in GPMVs have been observed by super-resolution microscopy (17) and single-particle tracking (18). Finally, observations of nanoscopic domains in GPMVs have recently been reported by cryoEM (19). These observations are pointing the way to the ultimate confirmation of the raft hypothesis by their direct imaging in living cells.

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