Title

Mind the Line Tension: New Criteria for Nanodomains in Biological Membranes

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Article:

Line tension controls liquid-disordered + liquid-ordered domain size transition in bilayers

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New and Notable:

Mind the line tension – A new criteria for nanodomains in biological membranes

A. T. Dang and T. L. Kuhl

The compositionally diverse lipid bilayer that forms the two-dimensional fluid matrix of the cell plasma membrane is no longer considered to be a passive boundary but to have functionality associated with cholesterol-related heterogeneity [1]. A key question is whether this heterogeneity of the order of 10 nanometers is phase separation into coexisting liquid-ordered (L_o) and liquid-disordered (L_d) regions [2-4]. However, there is little experimental evidence of stable L_o domains (i.e. lipid rafts) in live cells [2, 5]. The work by Usery et al. establishes that there exists an abrupt transition from nanoscopic to microscopic domains in model lipid bilayers in response to minor changes in composition. Line tension was found to have a ubiquitous association with this phenomenon across a variety of lipid mixtures, presenting exciting context for why only nanoscopic heterogeneity is detected in complex biological systems.

L_o domains, which form as a result of favorable enthalpic interactions between high melting temperature lipids and sterols, are thought to occur transiently and on the order of tens of nanometers in size in biological membranes [2, 3]. Early studies on detergent-resistant membranes suggested certain proteins partition preferentially into L_o compartments within the plasma membrane [2, 4], leading to pronounced interest in the properties and functional roles of lipid domains in nature. Now at 20 years since the inception of the lipid raft model [2] while the underlying basis of phase separation is clear, how this is translated in a cellular context remains obscure. The abiding mystery is sustained in large part by the inherent obstacles faced in the characterization of dynamic, nanoscopic domains *in vivo* [4-6]. Much

information has instead been gathered through investigation of biomimetic model systems which permit exquisite compositional control and access to a wide array of characterization techniques **[3, 7]**. The question now is linking these mimetic, well-defined systems to cellular membranes that have a virtual zoo of different constituent molecules.

Usery et al. shed light on the correlation between lipid phase morphology and line tension by examining GUVs produced from a sweeping collection of compositionally varied quaternary phospholipid mixtures. Mixtures were adjusted to contain different ratios of cholesterol, high melting point lipid, low melting point lipid known to induce nanoscopic domains, and low melting point lipid known to induce microscopic domains. A key compositional quantity, ρ, defined as the replacement ratio of the latter two components [8], was introduced as an analytical parameter for the purposes of this study. Multilamellar vesicles (MUVs) and large unilamellar vesicles (LUVs) were also employed, although size and curvature effects were found to have negligible impacts on phase behavior.

By gradually replacing low melting point lipid known to induce nanoscopic domains with low melting point lipid known to induce microscopic domains (increasing ρ value), Usery et al. showed that a stark transition from nanoscopic to micron-sized domains occurred upon reaching some critical value, ρ*. Although ρ* exhibited dependence on the types of lipid incorporated in the immediate mixture, the corresponding line tension was always ~ 0.3 pN. Through meticulous characterization, Usery et al. probed several domain properties using flicker spectroscopy [9], fluorescence measurements, small-angle neutron scattering (SANS), molecular dynamics (MD) simulations, and electron spin resonance (ESR) spectroscopy to arrive at this simple, yet cogent result. ESR spectroscopy in particular revealed that in spite of the dramatic changes observed in domain size, there was actually little variation in other characteristic properties of L_o and L_d phases (e.g. partitioning coefficient, rotational diffusion coefficient, order parameter) upon transition from nanoscopic to micron-sized domains.

In discussion, Usery et al. proposed that competing interactions arising from line tension and dipole-dipole repulsion were principle determinants of domain size. Indeed, the critical observed line

tension (~ 0.3 pN) was on the order of measurements reported in separate studies on L_o-L_d phase boundaries [3, 7]. The balancing role of dipole-dipole interactions, while intuitive and also discussed in independent literature [3], was less well-defined. Another possible explanation may be that entropic fluctuations are sufficient at such low line tensions to prevent domain growth. Still, the overall body of work presented by Usery et al. should be regarded as a meaningful and detailed study on the significance of line tension in regulating the size of domains in fluid phase-separated lipid bilayers. The observation of a recurring line tension value associated with the sudden, astonishing increase in domain size across multiple lipid mixtures inspires an intriguing framework for continued research beyond biomimetic model systems. In environments as complex as native plasma membranes, do line tension-dominated mechanisms of phase separation lead to the widespread formation of compositionally distinct, but similarly sized L_o nanodomains? Is the critical line tension altered by the presence of proteins which partition preferentially into L_o phases [10]? If so, could biology make use of modulations in line tension to coordinate cell processes? There is ample opportunity to build upon this extensive and compelling work by Usery et al.

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