Bending-mediated superstructural organizations in phase-separated lipid membranes

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Abstract. Lipid bilayers consisting of natural lipids and cholesterol can phase-separate into two immiscible fluid phases. These phases can further get organized into elaborated patterned superstructures, hexagonal arrays and stripes, of about micron periodicity. These periodic patterns must be maintained by a macroscopic inter-domain repulsion that competes with interfacial tension and they are not predicted for systems with pair-wise molecular interactions. Herein, we present simultaneous topography and fluorescence imaging of two-phase membranes that reveal the role of membrane bending mechanics in superstructural organizations. We observe that two-phase membranes are all curved. Real-time imaging demonstrates that these curved domains repel each other by bending the intervening region to the opposite direction. This type of macroscopic mechanical interaction may contribute to spatial organization in live cell membranes that cannot be explained solely by microscopic intermolecular interactions and phase separations, such as spatial organization of signaling molecules and their coupling to topography observed in endocytotic pits or intercellular junctions.

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The cell membrane is extremely complex and heterogeneous, containing two-dimensional (2D) (molecular) and 3D (topographical) heterogeneous organization on multiple length scales [1]–[7]. 2D structures include microclusters enriched with signal proteins, whereas microvilli and endocytotic pits are examples of 3D heterogeneity [8, 9]. The distribution of these structures is highly regulated within cells and they are involved in many biological functions, including signal transduction [1]–[6]. Therefore, how the heterogeneity of cell membranes is generated and maintained is an important question to understand cellular functions. Here, our interest is directed towards physical interactions that generally contribute to such structuring.

For a physical understanding of cell membrane organization, synthetic lipid bilayers have been studied extensively as model systems [8, 10]. Recent fluorescence microscopy studies have revealed a wealth of information about 2D (molecular) organization in lipid bilayers, by imaging the fluorescent molecules incorporated into bilayers [11]–[13]. Such studies include the imaging of phase separation in lipid bilayers containing natural lipids and cholesterol [11, 14], which might contribute to partitioning of cell membrane proteins [15, 16]. More recent studies showed that the immiscible membrane phases can even couple to membrane curvature [17]–[19]. The spontaneous curvature of homogeneous membranes is determined largely by molecular shapes of components [10]. However, the flexibility of membranes allows phase-separated membrane domains (zero spontaneous curvature) to bend to minimize the interface of phases with minor elastic energy cost for the bending [17]. Conversely, it has also been shown that phase-separated domains with different bending rigidity can be sorted to differently curved substrates to minimize the total elastic energy [18, 19]. Phase separation and its coupling to curvature provides a means of driving macroscopic molecular organizations, which explains how chemical and topographical heterogeneity in the cell membrane is generated.

To investigate macroscopic bending-mediated organization in membranes, periodically patterned domains in immiscible lipid bilayers [17, 20] might be good tools. There must be a repulsive interaction between aligned domains to maintain the periodic patterns [21]. Observed patterns are hexagonal arrays and parallel stripes in about micron periodicity [20]. Such superstructures are common in nature and, in general, the repulsive inter-domain force maintains patterns and competes with the attractive intermolecular force that leads to phase separation [21]. Theoretical and recent experimental studies suggest that membrane curvature might have a role in such inter-domain forces [21]–[23]. To directly investigate the role of membrane curvature in the generation of superstructures, we resolved the topography of the superstructures formed in quasi-planar lipid bilayers by simultaneous fluorescence and interferometry microscopy. We found coupling of membrane bending and patterns (all domains in patterns are curved) and, importantly, bending of intervening regions of patterns that effectively generates repulsive interactions between approaching domains. Such macroscopic mechanical interaction might have roles in the higher order organization of cell membranes as well.

Lipid bilayers containing three or more components (e.g. saturated and unsaturated lipids and cholesterol) can phase-separate into immiscible liquid-disordered (Ld) and liquid-ordered (Lo) phases [11, 14]. Ld and Lo phases have distinct properties (e.g. molecular mobility) at the physiological temperature [11, 14]. Complex intermolecular interactions that lead to immiscibility are well understood [24]. Instead, we use immiscible domains for the investigation of long-range forces in membranes by examining the interactions between...
separated domains. We are particularly interested in periodic superstructural patterns of domains, such as hexagonal arrays and parallel stripes, which were previously observed in giant unilamellar vesicles (GUVs; \( \sim 20 \mu \text{m} \) or larger) [17, 20]. As widely studied in other physical systems, such periodic superstructures are indicative of long-range forces that compete with preferential intermolecular attraction, which gives rise to phase separation in the first place [21]. We speculated that membrane curvature could contribute to long-range repulsion, as also suggested in past theoretical studies [20]–[22]. Therefore, we seek to directly resolve the topography of membrane superstructures and analyze the effects of curvature on superstructure formation.

We have already measured the potential of the mean force to stabilize these patterned superstructures [20]. The measurement was enabled with dynamic epi-fluorescence imaging of superstructures in vesicles. However, in vesicles, subtle topographical variations were hard to resolve. Therefore, we wished to form superstructures in planar membranes, because in planar geometry, various interferometry techniques can be applied to resolve nanometer-scale membrane topography [25]. We found that the supported intermembrane junction system can meet our goal of generating planar superstructures [26]–[28]. In this system, a support-free planar lipid bilayer can be stably fixed at a \( \sim 50 \text{nm} \) spacing distance from a solid-supported bilayer and extending over a continuous area of more than \( 0.01 \text{mm}^2 \) (figure 1(a)).

To make the supported intermembrane junctions, we ruptured GUVs of Ld/Lo phase-separating lipid bilayers (containing DOPC (dioleoylphosphocoline, unsaturated tail lipid), sphingomyelin (saturated tail lipid), cholesterol and Texas Red-DPPE) on another lipid bilayer directly supported on a glass coverslip (the detailed method is provided in the Supporting information available from stacks.iop.org/NJP/12/095001/mmedia). Ruptured GUVs form support-free bilayers. These support-free bilayers also phase-separate into Ld and Lo phases, which are distinguished by differential partitioning of Texas Red-DPPE (Ld phase contains more Texas red DPPE and is shown in red throughout this paper). However, we rarely (but occasionally) observed spontaneously occurring patterned superstructures in these bilayers. Therefore, we introduced two more steps in the procedure to yield superstructures (figure 1(c)). In the new procedure, after the formation of the junctions, we increased the temperature of the system above the miscibility temperature and then molecules in the support-free bilayers (lipids and cholesterols) became miscible. On top of the miscible bilayers, we added cholera toxin subunit B (CTxB), which binds and oligomerizes natural lipids GM\(_1\) (ganglioside) doped by 1% in the bilayers. After we lowered the system temperature below the miscibility temperature again, phase-separated domains were reformed. When bound with CTxBs, bilayers spontaneously formed superstructures, reproductively (figure 1(b) and (d)). Note that mixing–demixing of phase-separated domains without CTxB binding typically did not generate superstructures (superstructure formation without CTxB binding occurs only in less than 1% of our observations) and that CTxB-GM\(_1\) is preferentially partitioned into Lo phases\(^{14}\) (figure 1(e)). This result might suggest that a Lo phase containing CTxB-GM\(_1\) becomes more rigid and stable for superstructure formations or that CTxB binding alters the spontaneous curvature of the system (more CTxB binds to the upper leaflet of the bilayer) and somehow circumvents a kinetic barrier to form metastable superstructures.

Interference of reflection and/or emission light from substrates and membranes in close proximity can provide excellent topographical information down to nanometer resolution [25]. Thus, superstructures in the planar bilayer junction systems are well suited to high-resolution topographical imaging. Among various interferometric techniques, we used reflection
Figure 1. (a) Schematic drawing of membrane domain superstructures in planar bilayers. The intermembrane junction was formed by the rupture of GUVs on a glass-supported bilayer. After the rupture, GUVs formed support-free planar bilayers, stationarily fixed at a ~50 nm spacing distance from the glass-supported bilayer. GUVs contained a phase-separating mixture (DOPC, sphingomyelin and cholesterol, doped with GM1 and Texas Red-DPPE). Binding of cholera toxin subunit B (CTxB) improved the yield of superstructures (see text for details). (b) Fluorescence imaging of membrane domain superstructures in the support-free bilayers of the junction system. Doped Texas Red-DPPE preferentially partitions to the liquid-disordered (Ld) phase (red in the image), which can be distinguished from the liquid-ordered (Lo) phase (black in the image). (c) A strategy to form superstructures in higher yield. The junction system containing phase-separated support-free bilayers was heated and then the domains became miscible. CTxB was added and it bound to GM1, and then the system temperature was cooled down. CTxB-GM1 was preferentially partitioned to the reformed Lo phase, and superstructures were reproductively formed in these CTxB-bound bilayers. (d, e) Fluorescence images of superstructural domains for Texas Red-DPPE (d) and FITC-labeled CTxB (e).

interference contrast microscopy (RICM) (figure 2(a)). RICM does not involve fluorescence and thus is suitable for the imaging of fluorescently heterogeneous membranes (figure 1(b)), while fluorescence interference contrast microscopy (FLIC) is most effective for homogeneously fluorescent samples [25, 27]. For RICM in the junction system, we monitored the interference of reflections of 541–551 nm band-passed light from both the supported lipid bilayer on
Figure 2. (a) Schematic drawing of reflection interference contrast microscopy (RICM). We imaged a collection of reflections from both the support-free bilayer and the glass-supported bilayer. Reflection lights interfered and thus provided contrast along with the spacing distance between two bilayers. (b, c) Simultaneous fluorescence (b) and RICM (c) imaging of the superstructures in support-free bilayers. (d, e) Blow-up images of a domain in (b) and (c), respectively (d: fluorescence; e: RICM). (f) RICM intensity diagram along the line drawn in (e) (top), and corresponding schematic diagram of membrane topography (bottom). (g, h) Membrane domains that are not in patterns coalesce at any time (G, by fluorescence) and have no detectable topography (h, by RICM).

the substrate and the support-free lipid bilayer containing superstructures. The magnitude of interference correlates with the distance between two lipid bilayers [25].

Using simultaneous RICM and fluorescence imaging, we observed the topography of membrane domains organized in superstructures (figure 2(a)). We could resolve remarkable variations in the interference (RICM, figure 2(c)) that exactly matches the geometry of the
superstructures imaged by fluorescence microscopy (figure 2(b)). Most importantly, RICM intensities of periodic domains as well as of the intervening area (Lo and Ld phases, respectively, in the case of figures 2(b) and (c)) exhibited gradients. These RICM images clearly indicate that both periodic domains and the intervening area are curved but in different ways (figures 2(d)–(f)). Thus, the curvature and composition of the membrane are coupled in the periodic membrane domains. The direction of the curvatures will be discussed in the next section. We observed such couplings in all superstructures, but never in randomly distributed domains (figures 2(g) and (h)), suggesting an important role for membrane curvature in the formation of superstructures. The result of figure 2(h) (negligible intensity variance between Lo and Ld phases) also confirms that intensity patterns in superstructural Ld/Lo phases (figure 2(c)) are not due to artifacts caused by the differential reflective index of the two phases [10, 18]. Note that molecular compositions and distributions within each phase are homogeneous, confirmed by imaging fluorescent molecules (Texas-Red PE or FITC- labeled CTxB) (figures 1(d) and (e)).

How could the coupling of curvature–composition govern the long-range interaction and subsequent superstructure formation? We have previously shown that domains in arrays are fluctuating around stiff hexagonal lattices (figure 3(a)) [20]. Analysis of these fluctuations provides quantitative information about the real potential of the mean force between domains [20]. Here, we monitored the dynamics of the topography of domains during their lateral fluctuations as well. Dynamic RICM-fluorescence imaging revealed that the topography of fluctuating domains (and their immediate surroundings) was largely fixed. However, the topography and geometry of the intervening region were dynamically altered (figure 3(b) and movie 1 available from stacks.iop.org/NJP/12/095001/mmedia). Therefore, the mean curvature of the intervention of approaching domains was enhanced (figure 3(c)). In contrast, phase-separating domains not in patterned structures did not exhibit any deformation and fused to each other any time, indicating a minimal level of opposing force between flat domains (embedded in flat surroundings) (figures 2(g) and (h)). Altogether, we speculate that curved membrane domains bend the intervening region (other phase) towards the opposite direction and, in such a topography, minimize the total elastic energy of the system. We directly observe approaching domains to bounce off each other by the curvature (figure 3(c)). Bending of the intervening membrane between domains creates repulsive forces between domains. Note that RICM provides information about the degree of curvature but not the direction of the curvature, because interference is periodic in the direction of the optical axis.

As in other physical systems [21], periodic membrane domains in lipid bilayers exhibited dynamic evolution and instability of shape. We observed two different morphologies of superstructures: stripes and hexagonal lattices (figure 4(a)). RICM-fluorescence imaging showed that periodic stripes were also curved, and are stabilized by bending the surrounding area, as hexagonal domains (figure 4(b)). We also observed reversed superstructure patterns: both Ld and Lo phases were patterned into both stripes and hexagonal arrays. Interestingly, we observed the evolution between stripes and hexagons, in both directions (figures 4(a), (c) and (d)). Another interesting point is that the periodicity of these structures did not change significantly during the evolution of the morphology, as confirmed by the Fourier transform of spatial patterns (figure 4(c)). In fact, shape instabilities (fission, distortion and branching) were not observed, contrary to superstructures in lipid vesicles [20, 21]. In most cases, superstructures grew by simultaneous nucleation of membrane domains, but not by shape instabilities, as observed in lipid vesicle systems [20] (movie 2 available from stacks.iop.org/NJP/12/095001/mmedia).
It is well known from basic physical principles that curvature can induce macroscopic repulsion between domains [21, 22]. The formation of topographic superstructures results from the coupling of phase separation and curvature along with the minimization of elastic energy. Once superstructures are formed, curvature maintains the periodic organization by generating repulsive forces between domains. We have experimentally confirmed this repulsive force by analyzing the topography of membrane domain superstructures. Most previous observations have imaged composition patterns by fluorescence, but without simultaneous real-time tracking of membrane topography [17, 20, 23, 29]. The planar system used here enables rapid imaging of topography with essentially nanometer resolution using RICM, which can easily be performed in parallel with fluorescence. In this way, fundamental assumptions, such as the way in which membrane mechanical properties and composition are influenced by bending stress [18], can be
Figure 4. (a) Evolution of superstructure morphology, from parallel lines to hexagonal arrays, by fluorescence. (b) RICM image for the parallel lines in (a). (c) Fourier transforms of the spatial patterns in images in (a). (d) Elongation of curved domains, from monodisperse domains to an elongated domain as a part of parallel lines (RICM images in the top row and a fluorescence image at the bottom). (e) A fluorescence image for morphological variation of lines and arrays in a single continuous support-free membrane.

studied more directly. Another parameter that might govern 2D superstructures is molecular dipole moments [21], which dominates superstructure formation in lipid monolayers at the air/water interface [30]. However, dipoles in lipid bilayers must be significantly screened in aqueous solution. We have already confirmed that increasing the ionic strength in solution does not affect the superstructures [20], and thus we conclude that an electric dipole has only a minimal contribution. Although this explanation could fit qualitatively to the theoretical
depiction of the curvature–composition coupling in superstructure formation [21, 22], a rigorous theoretical framework will be essential to describe the system quantitatively, and more accurate measurements of membrane structures will be required. Another theoretical study predicts a more complicated inter-domain interaction that includes both repulsion and attraction [31].

We rarely observed topographical fluctuation (by RICM) inside these periodic domains. This contrasts with the fact that we routinely see topographical thermal fluctuations in support-free bilayers without superstructures [27]. Despite the fluidity of individual molecules [32], lipids in these apparently tensioned domains move collectively and thus areas of domains are largely unchanged. In contrast, molecules in the intervening regions move in a less organized manner, and the area and shape of the intervening regions fluctuate (figures 3(b) and (c)). This observation suggests that relatively high curvature and tension in domains are required for generating bending-mediated repulsion. We might speculate that CTxB binding modulates the curvature and tension of domains, but we also observed that binding of CTxB could not bend preformed non-periodic domains (supplementary figure 1 available from stacks.iop.org/NJP/12/095001/mmedia). These observations indicate a complicated balance between various parameters for generating curved domains and superstructures. Such microscopic pictures of lipid and protein interactions and their contributions to macroscopic curvature, tension, mobility and/or bending rigidity are still poorly understood and will be important questions for future studies.

We also observed symmetry in superstructure phases: both Ld and Lo phases form superstructures (figures 4(e)). Although there is a slight difference in bending rigidity between Ld and Lo phases [18], other factors, such as local compositional variations, might overcome the subtle difference and generate reverse-phase superstructures. Evolution of morphology occurs symmetrically between stripes and lattices. Periodicity of the patterns was mostly conserved during the transitions (figures 4(c) and (d)). This is contrary to previously observed instabilities of superstructures in lipid vesicles, where we witnessed dynamic evolutions in superstructure periodicity [20]. Another related observation is that the superstructures we observed in support-free bilayers are generated through spontaneous nucleation of multiple domains, whereas in vesicle systems, fissions of monodisperse domains and elongations of parallel stripes are more dominant (movie 2 and [20]). These observations suggest that CTxB-bound domains in support-free lipid bilayers are more stable than spontaneously formed superstructures in retain-free vesicles, in which instabilities were easily introduced by structural fluctuation or variations in hydrostatic or lateral pressures [20, 33].

Coupling of curvature and chemical composition is observed in many sub-cellular structures, including trafficking vesicles, cleavage furrows, and cytoskeleton-mediated membrane ruffles and spikes. Many different types of association and dissociation of proteins with membranes occur, which modulate the curvature, tension and bending rigidity of membranes, and might generate new membrane structures. Our results suggest that these curved structures could regulate the molecular organization of the rest of the cell membranes via curvature-mediated repulsive interactions. Future studies using reconstituted systems will help elucidate molecular organization- and topography-governing principles of cell membranes.

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Appendix. Materials and methods

Texas Red-dipalmitoylphosphatidylethanolamine (Texas Red-DPPE) and Marina Blue DPPE were obtained from Molecular Probes (Eugene, OR). Monosialoganglioside (GM$_1$) was obtained from Matreya (Pleasant Gap, PA). All other lipids were purchased from Avanti Polar Lipids (Alabaster, AL). FITC-labeled cholera toxin subunit B (CTxB) was obtained from Sigma (St. Louis, MO).

Small unilamellar vesicles (SUVs) that contained 93% dimyristoylphosphatidylcholine (DMPC), 5% dioleoyltrimethylammoniumpropane (DOTAP) and 2% Marina Blue DPPE, and GUVs that contained 33% dioleoylphosphocoline (DOPC), 33% sphingomyelin, 32% cholesterol, 1% Texas Red-DPPE and 1% GM$_1$, were produced by the swelling of dried lipid films in water (and subsequent extrusion for SUVs), as previously described [26]. The bottom bilayer of the junction was a conventional supported bilayer and was formed by the fusion and spread of SUVs on glass coverslips etched in piranha solution (a 1 : 3 mixture of hydrogen peroxide and sulfuric acid). GUVs containing an immiscible phase-separating lipid/cholesterol mixture were ruptured on this preformed supported bilayer. Ruptured GUVs and supported bilayers created intermembrane junctions of two planar bilayers with ~50 nm separation. Upper bilayers (ruptured GUVs) were stabilized by occasional pinning of sites between two bilayers [25].

Superstructures of phase-separated membrane domains were formed spontaneously in upper bilayers under certain (unknown) elastic constraints, in either water or buffer (~150 mM salt concentration) at a temperature of 22–30 °C. For protein-binding-induced superstructure formation, the sample chamber was heated to a temperature above the immiscible phase-transition temperature of upper bilayers (~30–35 °C). CTxB of final concentration ~100 µM was added to these homogeneous upper bilayers and the sample was cooled down to achieve immiscible phase separation.

Intemembrane junctions and membrane domain superstructures were viewed by a Nikon TE300 inverted microscope with regular fluorescence filter sets and an RICM filter (541–551 nm bandpass filter in the excitation slot and blank in the emitter). Light from an arc lamp through a 100× oil immersion lens was used with an illumination numerical aperture of 0.42 and an objective (collective) numerical aperture of 1.3. Images were captured by a Roper CoolSnap CCD camera. Membrane topography was estimated semi-quantitatively from RICM images, or the RICM intensity variance between two minima (interference fringes) was roughly in the scale of the height of the half-wavelength (~273 nm).

References

[1] Douglass A D and Vale R D 2005 Cell 121 937–50
[2] Hu K, Ji L, Applegate K T, Danuser G and Waterman-Storer C M 2007 Science 315 111–5
[3] Tian T, Harding A, Inder K, Plowman S, Parton R G and Hancock J F 2007 Nature Cell Biol. 9 905–14
[4] Kaksonen M, Sun Y and Drubin D G 2003 Cell 115 475–87
[5] Kim N, Stiegler A L, Cameron T O, Hallock P T, Gomez A M, Huang J H, Hubbard S R, Dustin M L and Burden S J 2008 Cell 135 334–42
[6] Kaizuka Y, Douglass A D, Vardhana S, Dustin M L and Vale R D 2009 J. Cell Biol. 185 521–34
[7] Lingwood D and Simons K 2010 Science 327 46–50
[8] Groves J T 2007 Annu. Rev. Phys. Chem. 58 697–717
[9] Mogilner A and Rubinstein B 2005 Biophys. J. 89 782–95

New Journal of Physics 12 (2010) 095001 (http://www.njp.org/)
[10] Lipowsky R and Sackmann E 1995 *Handbook of Biological Physics* (Amsterdam: Elsevier)
[11] Veatch S L and Keller S L 2002 *Phys. Rev. Lett.* **89** 268101
[12] Crane J M and Tamm L K 2007 *Methods Mol. Biol.* **400** 481–8
[13] McConnell H M and Vrljic M 2003 *Annu. Rev. Biophys. Biomol. Struct.* **32** 469–92
[14] Dietrich C, Bagatolli L A, Volovyk Z N, Thompson N L, Levi M, Jacobson K and Gratton E 2001 *Biophys. J.* **80** 1417–28
[15] Harder T 2004 *Curr. Opin. Immunol.* **16** 353–9
[16] Shaw A S 2006 *Nat. Immunol.* **7** 1139–42
[17] Baumgart T, Hess S T and Webb W W 2003 *Nature* **425** 821–4
[18] Parthasarathy R, Yu C and Groves J T 2006 *Langmuir* **22** 5095–9
[19] Yoon T, Jeong C, Lee S, Kim J H, Choi M C, Kim S, Kim M W and Lee S 2006 *Nature Mater.* **5** 281–5
[20] Rozovsky S, Kaizuka Y and Groves J T *J. Am. Chem. Soc.* **127** 36–7
[21] Seul M and Andelman D 1995 *Science* **267** 476–83
[22] Leibler S and Andelman D 1987 *J. Physique* **48** 2013–8
[23] Semrau S, Idema T, Schmidt T and Storm C 2009 *Biophys. J.* **96** 4906–15
[24] Honerkamp-Smith A R, Veatch S L and Keller S L 2009 *Biochim. Biophys. Acta* **1788** 53–63
[25] Parthasarathy R and Groves J T 2004 *Cell Biochem. Biophys.* **41** 391–414
[26] Kaizuka Y and Groves J T 2004 *Biophys. J.* **86** 905–12
[27] Kaizuka Y and Groves J T 2006 *Phys. Rev. Lett.* **96** 118101
[28] Wong A P and Groves J T 2001 *J. Am. Chem. Soc.* **123** 12414–5
[29] Ursell T S, Klug W S and Phillips R 2009 *Proc. Natl Acad. Sci. USA* **106** 13301–6
[30] McConnell H M 1991 *Annu. Rev. Phys. Chem.* **42** 171–95
[31] Korolev K S and Nelson D R 2008 *Phys. Rev. E* **77** 051702
[32] Ariola F, Li Z, Cornejo C, Bitman R and Heikal A 2009 *Biophys. J.* **96** 2696–708
[33] Komura S, Shimokawa N and Andelman D 2006 *Langmuir* **22** 6771–4

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