Switchable positioning of plate-like inclusions in lipid membranes: Elastically mediated interactions of planar colloids in 2D fluids

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We demonstrate how manipulating curvature in an elastic fluid lamella enables the reversible relative positioning of flat, rigid, plate-like micrometer-scale inclusions, with spacings from about a micrometer to tens of micrometers. In an experimental model comprising giant unilamellar vesicles containing solid domain pairs coexisting in a fluid membrane, we adjusted vesicle inflation to manipulate membrane curvature and mapped the interdomain separation. A two-dimensional model of the pair potential predicts the salient experimental observations and reveals both attractions and repulsions, producing a potential minimum entirely a result of the solid domain rigidity and bending energy in the fluid membrane. The impact of vesicle inflation on domain separation in vesicles containing two solid domains was qualitatively consistent with observations in vesicles containing many domains. The behavior differs qualitatively from the pure repulsions between fluid membrane domains or interactions between nanoscopic inclusions whose repulsive or attractive character is not switchable.

INTRODUCTION

Efforts to understand cell signaling and trafficking have highlighted the importance of membrane curvature in controlling the interactions of membrane species (1, 2). The manipulation of nano- and microscale objects on surfaces, however, transcends biology: Understanding the principles of interfacial assembly will enable the creation of sophisticated devices, for instance, sensors and actuators with materials-integrated function. Motivated by biology and transferrable to interfacial materials are findings that (i) curvature within coexisting fluid domains produces interdomain repulsions that stabilize domains that would otherwise coalesce (3–5) and (ii) inclusions, nanoparticles, or proteins are seen in experiments to bend a membrane to produce interactions that are exclusively attractive (6–8). Models predict that interactions of wedge-shaped inclusions (9), anisotropic domains (10), and bound Janus particles (11) can contain distinct minima that depend on the shape of inclusions or particles, their relativeorientations, and their interactions with the membrane. Here, we examine how, as a result of the bending energy of the fluid membrane, flat, rigid, plate-like microscale membrane inclusions experience further distinctive interactions combining both attractions and repulsions in response to the curvature of a fluid membrane, and producing stable pairwise configurations over a large range in length scales. Unlike the situation where interactions are either purely attractive or repulsive, or where the material character of the inclusion determines the interactive minimum, interactions that have tunable local minima are needed to accomplish positioning and patterning on surfaces, especially those of nonzero Gaussian curvature as in this study. The distinctive features of membranes containing plate-like domains are that (i) attractions and repulsions combine to produce a minimum that sets domain spacing and (ii) the interactions respond to mechanical perturbations, reversibly shifting the minimum so that domains reposition between stable configurations, achieving different arrangements with the same material system.

In some multicomponent phospholipid membranes, solid membrane domains coexist with fluid domains, integral within a single bilayer (12–15), constituting plate-like inclusions. Solid membrane domains have molecular order and symmetry across the bilayer (16–19), rendering them flat and rigid to bending and shear (20, 21). These features are an important distinction from the character of fluid membrane domains that, as a result of their inability to sustain shear loads, assume dimples, caps, and other shapes of nonzero Gaussian curvature (3–5). Far more than just an enhanced bending stiffness, the two-dimensional (2D) plate-like shear elasticity of the solid domains leads to an energetic preference to suppress the positive Gaussian curvature required by the global spherical shape of the vesicle. Unlike strictly fluid domains, the resistance of solid domains to spherical deformations grows larger with increased domain diameter (22).

The persistent flat character of a solid domain is exemplified in Fig. 1 for a giant unilamellar vesicle (GUV) containing a single solid domain that comprises about 16% of the membrane area. Here, the fluid portion of the membrane is forced, by the shear rigidity of the solid domain, to take on all the curvature required by the topology of the GUV. Also ultimately important to patterning lamellar materials, solid domains often take on varied shapes as a result of their molecular order. The persistence of complex solid domain shapes, stripes, facets, dendrites, and flowers (12, 14, 23, 24) suggests that interdomain line energies are held constant, a distinction from coexisting fluid domains that are driven to be rounded, coalesce, and ripen, by line tension (4, 25, 26).

The current paper demonstrates how the flat, stiff nature of solid plate-like domains, forcing curvature into the fluid membranes of vesicles, produces unique features in solid domain interactions that are distinct from the interactions between coexisting fluid domains in well-studied lipid mixtures (3–5), or colloid and protein-scale membrane inclusions (6–8, 27). These differences include solid domain interactions that are both attractive and repulsive, and the ability to toggle interactions by osmotic adjustments or mechanical...

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manipulation of the fluid portion of the membrane. The presence of a distinct minimum in the pair potential between solid domains, its range that varies over the full length scale of vesicles, and its strength relative to \( kT \) facilitate rapid reversible domain positioning on length scales from about a micrometer to tens of micrometers. We demonstrate that interactions depend on the solid domain size relative to the vesicle size and on the ratio of membrane area to vesicle volume, quantified via an index termed “excess area.”

RESULTS

Excess area controls interdomain morphology

Vesicles containing multiple solid domains, such as those in the fluorescence micrographs of Fig. 2, present different solid domain positionings that suggest that vesicle inflation is a key variable that can be manipulated to direct domain assembly. These vesicles were formed from lamella containing 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), and upon cooling from the one-phase region to the two-phase region, flat solid stable domains, nearly pure in DPPC, were formed (28, 29). The fluid membrane is made visible by a small amount [0.1 mole percent (mol %)] of a fluorescent lipid, while the solid domains, which exclude tracers, are dark. In Fig. 1 and throughout this study, the solid domains are fixed in shape, flatness, and area within detectable limits. With this study limited to low tensions, there was no measurable impact of tension or membrane manipulation on the solid area, shape, or the phase transition temperature, as would be expected (24, 29, 30).

Each panel of Fig. 2 features two equatorial views and bottom and top views of a vesicle. The second version of the equatorial view highlights local curvature. In Fig. 2A, the local radii of curvature in the fluid regions are seen to be smaller than the overall vesicle radius, a reflection that this vesicle is somewhat deflated, and therefore has “excess area”—more membrane than is needed to encapsulate the vesicle contents. Here, the solid domains are arranged in a roughly evenly spaced pseudohexagonal lattice pattern over the surface of the vesicle. The nearest neighbor edge spacings of the solid domains exceed the domain size by up to a factor of 2 or more, and the domain organization is forgiving of domain polydispersity, suggesting long-range interdomain repulsions. In Fig. 2B, the radii of curvature of the fluid membrane regions are larger than in Fig. 2A and more closely approach the vesicle radius, implying an inflated vesicle with a more nearly spherical shape and lower excess area. Here, some of the domains are seen to be more closely spaced than in Fig. 2A. In Fig. 2C, where the local and global vesicle curvatures are most closely matched and this vesicle is more inflated than the vesicles in Fig. 2 (A and B), an interdomain attraction is evident: Solid domains are localized in one area of the vesicle, while large areas of fluid membrane, empty of solid domains, are found on the other side. Neighboring domains do not touch but are separated by roughly order micrometer-wide regions of fluid membrane, suggestive for interdomain interactions that (i) are attractive at large separations and (ii) have an equilibrium spacing at a mesoscopic multi-micrometer length scale. The vesicles in Fig. 2 (A to C) are similar in overall size (30 ± 3 μm diameter), solid area fraction, domain size, and approximate domain number, with differences in local versus global curvature being the important difference between the three vesicles.

The ability of vesicles to exhibit smaller local radii of curvature in their fluid membranes depends on the extent to which the vesicles are filled with aqueous solution. Even slight deflation of a spherical vesicle, for instance, made from an entirely fluid membrane without solid domains, facilitates local membrane curvature. This motivates a formal definition of excess area \( A_{xs} \) as the actual vesicle membrane area normalized on the area of a sphere of equal volume.

Excess area can be quantified for a given vesicle by measuring the vesicle surface area and volume. In the current study, these measurements were facilitated by aspirating vesicles into micropipettes at low suction so that each vesicle assumed a regular shape: a combination of spherical caps and a cylindrical portion that were easily quantified in micrographs.

\[ A_{xs} \text{ grows from unity for a fully inflated spherical vesicle (but one in which the membrane is not stretched due to over-inflation) to larger values, for instance, as internal fluid is osmotically removed.} \]

\[ A_{xs} \text{ is related to the “reduced volume” used in some works (31–33) to describe deflated vesicles, with } V_{\text{red}} = A_{xs}^{-3/2}, \text{ but } A_{xs} \text{ is a more intuitive quantity for the current focus on membrane curvature. For instance, when } A_{xs} = 1.10, V_{\text{red}} = 0.87. \]

When the fluid vesicle membrane contains some solid domains, the solid remains flat, exemplified in Fig. 1A, producing flat facets in an otherwise globally spherical vesicle shape. Thus, even with full inflation, vesicles having solid domains have an excess area slightly greater than unity.

Reconfiguring pairwise domain separations with osmotic inflation

The observations in Fig. 2 and the hypothesis that excess area, through its influence on local membrane curvature, is a key variable affecting domain arrangement motivated an examination of pairwise interactions between solid domains. Vesicles containing two domains were produced using lipid mixtures of 40% DPPC and 60% DOPC. Vesicles were first electroformed at elevated temperatures in the one-phase region to ensure uniform composition. Later, in preparation for the study itself, aliquots were reheated to the one-phase region and subject to controlled cooling to produce only two domains. In the case of 40% DPPC, the overall solid area fraction was 15 ± 3%, based on measured domain sizes, consistent with the thermodynamic phase diagram (28, 29). With the solid distributed evenly between two domains in a vesicle, the effective domain diameter, normalized on the diameter of an equal-area sphere for each vesicle, \( D_{\text{vs}} \), is ~0.53 for this condition. We note that the diameter of an equal-area sphere provides a measure of vesicle’s size, a length scale to which other lengths can be compared. While vesicles with different degrees of inflation have different excess areas and shapes, the sphere of equal-area presents a well-defined “size”
corresponding conceptually to the fully inflated spherical state of a given vesicle: $D_r \equiv \sqrt{A/\pi}$, where any solid domains conform to the sphere. When membrane area $A$ is measured using micropipettes, $D_r$ is known. When micropipette aspiration is not possible, $D_r$ can be estimated from micrographs of vesicles as long as they are not substantially deflated.

Despite the dynamic nature of the fluid membrane, which allows long-range motion of solid membrane domains, distinct preferred separations between pairs of solid domains were apparent for vesicles containing two domains. These were quantified in video micrographs, reporting the edge-edge domain separation at the region where the edges of two domains were closest, and using corrections for vesicle curvature in the Supplementary Materials. We note that while we tend to conceive domains as circular when sometimes they are hexagonal or slightly irregular, the measure of the closest edge-edge separation does not rely on assumption of a particular domain shape. This study focuses on compact domains, with the examples in Fig. 3 being typical. In particular, we excluded from the study vesicles whose domains contained substantial projections or attached nubs or satellites.

In the examples of Fig. 3A, four vesicles of differing excess areas exhibit different edge-edge domain separations that are well established and long lived in time, varying only by fluctuations on the order of micrometers or less. The images in Fig. 3A were taken at focal planes that show the vesicle shape and micropipette aspiration for different excess areas. The accompanying images in Fig. 3B focus on the domains themselves and illustrate how edge-edge separation was determined. The solid markings show where we chose the closest edge separations, and the alternatives along the dashed lines illustrate edge-edge measurement paths that were not chosen because of the appearance of larger edge-edge separations. Analysis along these alternate measurement paths nonetheless produced distances that were less than a micrometer different from that long the chosen path. Thus, we claim precision in the edge-edge domain separations of about a micrometer, resulting from two factors: (i) optical resolution and video quality of a moving object and (ii) the correction in the Supplementary Materials for vesicle curvature (34), where the vesicle was approximated as a sphere to translate the measured projected separation to an in-membrane domain-domain distance. When domains were on opposite sides of a vesicle, which occurred only for large excess areas, there was a need to use two images obtained at slightly different times, decreasing the precision.

With greater spatial resolution of the domain separations, it would be possible to analyze these data to determine the shapes of inter-domain potentials. The average domain separation for a given vesicle at a given condition, however, is an indicator of the position of a minimum in the potential between the two solid domains. The preferred positions, reflective of this minimum, typically persist, although motion in the fluid vesicle membrane allows both diffusion and convective motion of solid domains. Domain pairs were seen to translate together at their preferred separation as the vesicle rotated and tumbled. However, the domains did deviate from the preferred separation when disturbed, for instance, when a vesicle brushed the bottom of the chamber. Ultimately, though, the domains did return to the preferred separation when force was removed.

An important feature in Fig. 3A is that the domain separations are fixed in time within about a micrometer. The small fluctuations in separation that appear with the thermal fluctuations of the membrane itself were typical and did not increase substantially with increased domain separation over the full range studied. The fact that the domain pairs always exhibited well-defined separations and coordinated motion suggests that for the range of conditions studied, a potential minimum in their interaction was always large relative to $kT$. This further suggests that both attractions and repulsions were present in the interaction over the full range of conditions studied and that attractions did not grow in an otherwise repulsive potential as variables were tuned.

While several variables, such as domain and vesicle size, may be among those determining the relative preferred domain positions, Fig. 3 (C and D) establishes how changes in vesicle inflation, and hence in excess area, produce changes in domain separation. By altering osmotic conditions to drive water diffusion into or out of a vesicle, the curvature in the fluid membrane contour. Solid domains appear dark due to exclusion of a tracer. The three different vesicles, in (A) to (C), are increasingly inflated.

**Fig. 2. Images of vesicles composed of DOPC and DPPC imaged at different focal planes: Equatorial, top, and bottom.** The second equatorial view is annotated to highlight the curvature in the fluid membrane contour. Solid domains appear dark due to exclusion of a tracer. The three different vesicles, in (A) to (C), are increasingly inflated.
Dependence of domain separation on excess area

To determine a quantitative relationship between excess area and preferred or equilibrated domain separation in vesicles containing two solid domains, we used micropipette aspiration to quantify vesicle area and volume after recording domain positions in equilibrated vesicles. To sample a range of excess areas, vesicles originally containing 0.15 M sucrose solution from electroformation were re-equilibrated in glucose solutions of different osmolarities from 0.15 to 0.20 M.

The dependence of the preferred domain separation on excess area is shown in Fig. 4. Because vesicle size varies in the range of 15 to 40 μm, the domain separation on the y axis is normalized on $D_v$, the diameter of an equal area vesicle, as described above. For these data, the separation varied from about a micrometer up to ~20 μm, measured in the membrane contour. The data points spanning up to $A_{xs} \approx 1.3$ in the main part of the plot were obtained from domain separations observed before vesicles were aspirated in micropipettes to quantify excess area. These vesicles had uniform overall compositions corresponding to a solid area fraction of 15 ± 3% for the 60% DOPC/40% DPPC lipid composition. Because vesicles contain only two domains, the solid area fraction from the lipid composition sets the domain to vesicle size ratio, which was essentially fixed, excepting the slight variations, highlighted by the blue and red colors for the data in the main curve. Thus, data suggest that even slight variations in the domain to vesicle size ratio have a small but measurable effect on domain separation, especially when the excess area is small.

The main feature of Fig. 4 is that the domain spacing increases from the micrometer scale, for nearly spherical vesicles with small excess areas, to an antipodal configuration with separations on the order of tens of micrometers for somewhat deflated vesicles with large excess areas. An excess area ratio of unity is not possible for vesicles with flat domains because these vesicles cannot assume a spherical shape even when fully inflated. The domain separation, when normalized on the effective vesicle diameter, approaches a maximum possible value that depends on the domain size or solid

Fig. 3. Domain separations are fundamentally stable in time but can be increased or decreased through changes in the excess area. (A) Domain separations for four example vesicles with different excess areas. Images for the free vesicles are presented along with images for each vesicle after aspiration, showing differences in excess area and positioning in micropipette. $D_v$ for each vesicle was determined on the basis of the membrane area measured during micropipette aspiration. (B) Example images of the four vesicles from (A), focusing on the domain edges, with a solid line denoting the chosen direction of closest edge-edge separation and dashed lines highlighting alternatives that appear further separated but still measured within no more than a micrometer of the path along the solid line. (C) Normalized and dimensional domain separation in time, as a result of the injection of a drop of DI water near 220 s. Near 60 s, the disturbance in domain separation results from introduction of micropipette nearby but soon recovers. $D_v$ (estimate) = 18.8 μm. (D) Normalized and dimensional domain separation in time as a result of injecting a drop of concentrated glucose solution solution near 105 s. $V_s$ (estimate) = 10.9 μm. Error bars are the size of the points themselves, with sample error bars on select points at 35, 199, and 350 s in (C) and at 55 and 250 s in (D). Domain separations, when normalized, are divided by $D_v$. vesicle, the excess area was varied through changes in vesicle volume that do not affect the domain size or the membrane area. In Fig. 3C, a vesicle, initially equilibrated in a glucose solution, is disturbed by the insertion of a micropipette nearby, but the preferred domain position is quickly reestablished. When a drop of water is introduced from the pipette, the glucose solution outside the vesicle is diluted. Water diffuses across the membrane (sucrose and glucose cannot pass) to inflate and reequilibrate the vesicle. As this happens, the domains approach more closely to establish a new equilibrium position. In a different experiment, in Fig. 3D, a vesicle initially equilibrated in a 0.15 M glucose solution is exposed to a nearby drop of concentrated glucose solution. As the solutions mix outside the vesicle, water is drawn out of the already somewhat deflated vesicle and the domains reequilibrate by further separating. Thus, Fig. 3 establishes that for times on the orders of seconds and minutes, vesicles can be made to equilibrate from one degree of inflation to another and solid domains move into new preferred positions reflective of the degree of inflation of the vesicle. Inflating the vesicle and reducing excess area tend to drive domains toward each other. In the osmotic experiments of Fig. 3 (C and D), the membrane area could not be quantified and so $D_v$ was estimated from the more inflated images of these two vesicles.
area fraction. For the case here of an area fraction of 15%, that maximum is 1.02 calculated in the antipodal configuration, in good agreement with the data.

While the domain separations in the main part of Fig. 4 were measured for free vesicles where the solid domain size was fixed by the lipid composition, additional data were obtained for vesicles in the state of being aspirated in the micropipette. In this state, only the part of the vesicle outside of the pipette was counted toward the excess area. Here, the effective vesicle diameter was based on “bulb-shaped” vesicle portion outside the micropipette. This strategy allowed access to effectively larger domains relative to the diameter of the portion of the vesicle outside the pipette. Measured domain separations are meaningful when the solid domains are outside the pipette and at least one domain is away from the micropipette so that the domain separation was representative of equilibrium. These additional data points are magnified in the inset of Fig. 4. Measurements on aspirated vesicles reveal that in addition to the influence of excess area, the domain size relative to the vesicle size is an important variable. Large domains, relative to the vesicle size, approach more closely for a given value of an excess area. Thus, the data in the inset suggest a family of curves where the different domain sizes lie on slightly different curves. Together with the full range of data, the effect of domain size is most clear for low excess area values, approaching 1.

Also worth noting, this study focuses on pairs of compact domains that are roundish but sometimes have a rounded hexagonal shape. In a few instances, domains had a convex edge, such as in Fig. 3Biv. The vesicle of Fig. 3Biv is specifically noted as iv in Fig. 4. In addition, other vesicles having at least one indented domain, like that in Fig. 3Biv, are marked with gray stars in Fig. 4. These data for vesicles containing indented domains did not deviate from the main curve.

Modeling interdomain energetics
The observations summarized in Figs. 3 and 4 imply the existence of an effective pairwise interaction between disc-like solid membrane domains in a fluid membrane that is dependent on the excess area of a vesicle relative to a sphere. For large excess areas, domains repel at large (10 μm and greater) in-membrane separations and maximize their separation, while decreasing excess area leads domains to attract one another from large distances. The equilibrium in-membrane spacing between domains decreases as excess area is further reduced. The need to understand the physical origin of these emergent interactions motivates a model exploring the interplay between the global shape of the two-phase vesicle composed of rigid domains in a flexible fluid layer and the ratio of its bounding membrane to interior contents. To provide an analytical perspective on the energetics, a simplified 2D vesicle geometry was developed. Here, the vesicle was modeled as a closed 1D curve, composed of rigid, solid segments connected by fluid segments, which together bound the vesicle interior (an area in the model). We considered shapes in which the total length of the fluid membrane contour plus solid domains are fixed, along with the fixed interior area, mimicking the fixed excess area of 3D vesicles having constant membrane surface area and aqueous volume. The vesicle energy is computed as $E = \frac{1}{2} f_{ij} d_{s_i} \kappa^2(s_i)$, where $\kappa(s_i)$ is the curvature at arc-position $s_i$ in the fluid membrane contour and $B$ is the bending modulus of the fluid membrane, which is the 1D analog to a simple Helfrich bending of a fluid membrane (35). Because experiments, for instance, Fig. 1, suggest that curvature is expelled from the solid domains, these are modeled fixed-length straight segments (e.g., configurations in Fig. 5). While this 2D treatment cannot provide a quantitative comparison to the balance of surface to volume in 3D vesicles, it does incorporate the essential elements to describe how curvature is expelled and optimally distributed to the fluid membrane as the interior of vesicles becomes progressively more inflated.

As described in detail in the Supplementary Materials, we considered a simplified variational class of curved shapes for the fluid membrane contours composed of three or four circular arcs that connect smoothly (i.e., with continuous tangents) with other fluid segments and with solid domains at their junction points. With this variational class, the model minimizes the vesicle energy with respect to shape while keeping the enclosed area and lengths of fluid and solid domains fixed. In Fig. 5A, we compare the optimal shapes of the multi-arc ansatz to exact equilibria of the constrained bending energy (computed via solutions in section SI.4) for configurations with a single solid domain as a function of variable inflation of the interior. The optimal shapes (and energetics shown in fig. S5) of single-domain vesicles and their qualitative dependence on inflation and solid domain fraction are well captured by the multi-arc ansatz. Notably, the multi-arc ansatz exhibits strongest quantitative agreement with exact equilibria in the high-inflation regime, where bending energy concentrates into high-curvature ‘hinges’ that flank the edges of the solid domain and bridge to the predominantly low curvature portion of the fluid phase. To predict the dependence of total mechanical energy on in-membrane separation (i.e., the arc length of the fluid portion) between domains $\Delta$, illustrated in Fig. 5D, we use a two-domain variant of the multi-arc ansatz. Figure 5B shows plots of the normalized energies of vesicles having a fixed solid fraction of 0.24 as a function of reduced separation $\Delta/D_{\text{eff}}$ where $\pi D_{\text{eff}}^2/4$ is the enclosed area. Curves are shown for various values of excess length of the 2D model, defined as the total arc.
length of fluid and solid membrane domains normalized by $\pi D_{\text{eff}}$, the circumference of a circle enclosing the same area. For high values of the excess length (1.03 and above for this solid fraction), the vesicle is substantially deflated and the optimal vesicle configuration maximizes the separation between solid domains. Here, domains are positioned in an antipodal configuration. For smaller excess lengths below this threshold where the vesicle is less deflated, the energy develops a minimum at an intermediate domain separation, closer than the antipodal separation. The equilibrium separation decreases as the vesicle is further inflated and the excess length is reduced toward 1. The equilibrium spacing $\Delta^*$ as a function of the excess vesicle length is plotted in Fig. 5C for this value of solid fraction and for a range of other values. A series of energy-minimizing configurations of the 2D model corresponding to a solid membrane fraction of 0.24 and various excess lengths are shown in Fig. 5D.

The existence of an equilibrium domain separation $\Delta^*$ at separations less than the antipodal spacing implies that elastic energy is attractive for domains at larger separations (i.e., force at $\Delta > \Delta^*$ drives domains closer) and correspondingly repulsive for domains closer than $\Delta^*$. The existence of the elastically mediated attractions when domains are beyond their equilibrium spacing is consistent with the experimental observations in Figs. 3 and 4, where the equilibrated domain separations are closer than antipodal. In addition, the tendency of the energetically optimal spacing to increase with excess length in the 2D model is consistent with the observations in Fig. 4 where the preferred domain spacing increases with excess area. Last, we note that, according to the model predictions in Fig. 5C, as the solid domain size (set through the solid membrane fraction) increases relative to the vesicle size, the effect of elastically mediated attractions grows. That is, the onset of attractive interactions shifts to higher values of excess length, meaning that inter-domain attractions persist in effectively “floppier” vesicles, provided that the domain size is sufficiently larger. Put another way, for a fixed excess length, increases in the solid domain size produce closer equilibrium spacings, consistent with the inset in Fig. 4.

DISCUSSION

The observation of distinct spacings between pairs of solid domains in a fluid membrane, from length scales of micrometers to tens of micrometers and spanning separations smaller than the size of a single domain to length scales much greater than individual domains, suggests interaction potentials containing both attractions and repulsions over the full range of conditions studied. The broad agreement between the simplified 2D mechanical model and the experimental observations of solid domain spacings in vesicles confirms that, for a fixed domain size or solid membrane fraction distributed between two equal domains, excess area controls the nature
of interactions, which can be toggled effectively between repulsive or attractive at long range by vesicle inflation alone. In addition, the correspondence argues that the effective interactions are predominately controlled by the elastic energy of the fluid membrane forced to take up curvature that is expelled from predominately flat solid domains. Last, both the experiments and the model provide evidence that the increased domain size or solid area fraction enhance interdomain attractions.

Moreover, the model suggests an insight-building heuristic picture to explain the emergence of both repulsive and attractive interactions and their dependence on excess membrane area or length. Figure 5E presents schematics of 2D vesicle shapes, highlighting the distribution of bending in the fluid membrane. Because the solid remains rigid in every configuration, all curvature is taken up in the fluid. As the interior of the vesicle inflates, the solid faces are pushed out, but as they cannot bend, they lead to high-curvature zones, “hinges,” in the fluid membrane flanking the solid domains (e.g., single-domain shapes in Fig. 5A). For fixed domain positions, the hinges must become sharper and require more mechanical energy as inflation increases, or as the corresponding excess membrane area in 3D and length in 2D decrease. With this in mind, one can think of the effective driving force for domain attraction as a type of “depletion” mechanism, whereby bringing domains together causes their hinges to overlap effectively, releasing some amount of fluid membrane from the high-bending overlap region in exchange for a lower-curvature region away from the hinges, resulting in a net lower energy. Depending on domain size and excess membrane, the length of high-curvature hinge relative to low-curvature fluid changes, with higher inflation and larger domains leading to sharper, narrower hinges. Bringing domains closer together than their natural size of the high-curvature hinged zones leads to overly sharp bending in the shared hinge. This effect leads to repulsion at close distances and, in combination with the attractive “curvature depletion” effect at large separation, sets the equilibrium spacing to a mesoscopic length that is controlled by the elastic range of curvature relaxation away from the solid edge. The dependence of effective hinge size on excess membrane and domain size is captured by the variation of $\Delta^*$ in Fig. 5C.

The interactions between the solid plate-like membrane domains in the current work differ qualitatively from those of fluid domains in previous works, where discrete fluid domains in a second fluid membrane phase favored cap-like shapes with higher curvatures than the surrounding fluid membrane in which they were embedded $(3-5)$. First, the interactions between solid domains are found to be both attractive and repulsive, depending on the inflation of the vesicle. In contrast, previous studies have found that bending-mediated interactions between like-shaped fluid domains, as a result of the mismatch in tangent orientations propagating into the fluid background from the domain edge, are purely repulsive. Second, while we do not consider the effects of thermal fluctuations or thickness mismatch and the associated line tension between fluid and solid membrane domains in our model, we note also that, like the case fluid curvature mismatch, models for these previously studied effects are known to lead only to purely attractive or purely repulsive interactions. In previous models, the attractive or repulsive character of the interactions $(34, 36)$ was set by symmetry or asymmetry $(10)$ between the inclusions themselves (e.g., inclusion thickness or shape) and could not be switched or tuned through reversible osmotic or mechanical manipulation of the vesicles, as established here for membranes containing solid domains.

Last, it must be emphasized that it is the different mechanical properties of solid domains, distinct even from simply stiff to bending or viscous fluid domains, that lead to qualitatively different and switchable domain interactions compared with those of fluid domains. Beyond the effect of solid cohesion to fix the domain shape on the experimental time scale and prevent coalescence or ripening of domains, solid domains have finite shear moduli. By contrast, fluid domains are capable of shear flow at a fixed area. Shearing deformations are required for changes in Gaussian curvature $(37)$. In solids, these shears lead to stresses in the membrane that grow with the domain size $(38)$. Thus, solid domains, which cannot flow, retain a flat, nondimpled shape because of the large stresses required by spherical shape. Introducing these shear-induced stresses either incurs a huge elastic penalty or instead leads to defect or crack formation in the crystalline order $(22)$. By contrast, the process of “dimping” for a fluid domain requires shear flow, along with out-of-plane bending of the thin layer, both of which are readily accommodated by the fluid.

The mechanism of switchable attractive-repulsive interactions relies on the rigidity of large planar inclusions at a high inflation. It is reasonable to consider if similar effects could arise in phase-separated fluid/fluid vesicles due to the bending of fluid domains alone. To better understand how domain solidity promotes switchable interactions, we compare the resistance of domains to deformations into spherical shape for solid versus fluid domains. As a simple heuristic for vesicles containing either fluid or solid domains, consider a finite circular domain of radius $W$ on a vesicle with an interior pressure $P$, which acts to bend the domain outward into a spherical cap of principle curvature $\kappa$. Considering only the bending elasticity of a fluid response characterized by bending modulus $B$, the equilibrium domain curvature is simply proportional to the pressure, $\kappa_{\text{fluid}} \approx \frac{P}{B}$.

The bending of a pressurized fluid domain tends to increase with domain size. In comparison, spherical bending of a 2D solid, according to Föppl–von Kármán plate theory $(37)$, requires an elastic strain that grows as $(xW)^2$, which can be understood geometrically in terms of the length compression of the perimeter needed to project a circular domain onto a sphere without changing its radial length $W$. Balancing elastic cost of solid domain strain against the pressure gives the equilibrium curvature for solids $\kappa_{\text{solid}} \approx \left( \frac{Pc_t^2}{Bt} \right)^{1/3}$, where the 2D Young’s modulus of the solid has been estimated as $B/t^2$, where $t$ is the thickness of the elastic membrane. This not only shows a much weaker dependence of solid bending on pressure but also decreases reciprocally with lateral size as $W^{-2/3}$. That is, fluid domains bend more easily into spherical shapes as their size increases, while solid domains become more resistant to spherical curvature. This generic effect accounts for the fact that solid domains remain flat, even flatten as their lateral size grows. As illustrated by model predictions in Fig. 5, the transition from purely repulsive to long-range attractive interactions only becomes accessible when planar inclusions reach lateral dimensions that are comparable (order of ~10% or larger) to global vesicle radius. This line of reasoning argues that elastically mediated attraction between planar domains may only be accessible for domains with 2D solid elasticity and inaccessible for domains with only fluid bending elasticity.

In summary, this work demonstrated how pairs of solid membrane domains, coexisting in an otherwise fluid bilayer, experience interactions that, as a result of their shear rigidity, can be both attractive and repulsive at the same time. The resulting potential minimum was found sufficiently strong relative to $kT$ over the
range of conditions studied experimentally such that domain pairs remained at a fixed separation and the relative domain position could, for a given material system, be reversibly tuned over a broad range of length scales.

Acting as plate-like inclusions, the solid domains force all the curvature required for the topology of a vesicle into the fluid part of the membrane, where the vesicle shape and solid domain configuration adjust to minimize the fluid bending energy. This situation contrasts the energy distribution and interdomain repulsions in GUVs containing two types of deformable immiscible fluid domains, or the attractions between small membrane inclusions and surface-bound particles that produce membrane bending in experiments. The ability of a simple model to capture the essential features of the current experiments demonstrates that factors such as line tension and thermal membrane fluctuations are not necessary to produce the observed attractions and repulsions between the solid domains.

Interactions were found to be a strong function of the ratio of membrane area to vesicle volume, quantified in the form of excess area and producing domain separations from about a micrometer to tens of micrometers, on opposite sides of the vesicles. This sensitivity imparts the ability to manipulate domain interactions and positions through mechanical means, for instance, using osmolytes and micropipettes. Experimental results, focusing on the regime where the membrane solid, occupying 15 ± 3% of the total membrane area, was divided nearly equally between two domains (producing domains that were in the range of 53% of the vesicle diameter), showed that large domains in this range experience measurably stronger attractions. The 2D variational model suggested that attractions are a strong function of domain size over solid membrane fractions from 12 to 36%, a behavior motivating future experiments including refinements in methods to produce vesicles with regular domains over a greater size range. Additional future work will explore how the geometrical and mechanical coupling captured in this model shapes the quantitative nature of interdomain energetics on fully 3D vesicles with 2D plate-like inclusions.

MATERIALS AND METHODS
Experimental design
Materials
DOPC and DPPC were purchased from Avanti Polar Lipids (catalog nos. 850375C and 850355C). The tracer lipid Rh-DOPE [1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)] ammonium salt; catalog no. 810150C] was also purchased from Avanti.
Vesicles
GUVs were produced by electroformation on platinum wires, following established procedures (39, 40). Phospholipids in the desired molar proportions were dissolved in chloroform at a concentration near 25 mg/ml. Rh-DOPE tracer was added at a concentration of 0.1 to 0.2 mol % relative to the total phospholipid, sufficiently low to avoid any impact of the tracer concentration on properties like the phase separation temperature or the types of solid domains. As Rh-DOPE is not incorporated into the solid domains, their properties appeared not to have been affected. To produce vesicles, a 10-μl quantity of phospholipid solution was placed droplet-wise on the platinum wire electrodes and the chamber was dried under nitrogen. After sealing between glass coverslips and filling with preheated sucrose solution of 0.15 M, the chamber was maintained at 60° to 65°C, while alternating current was applied to the electrodes at 3 V and 11 Hz for 1 hour. Electroformation at elevated temperatures in the one-phase region of the phase diagram ensures uniformity in the DOPC/DPPC composition of all the vesicles. After electroforming, the vesicle solution was harvested in a syringe, bringing the suspension back to room temperature.

To produce vesicles containing exactly two domains, the DOPC/DPPC ratio was chosen to be 60/40, giving an approximate solid fraction of 15 ± 3% of the total membrane at room temperature, based on established phase diagram (13, 28, 29). After electroforming, vesicle suspensions were diluted in vials containing glucose solutions in the concentration range of 0.15 to 0.20 M. These were placed in a temperature control bath, heated to 50°C in the one-phase region, and cooled at 0.3°C/min to control the nucleation density to about 0.001/μm². This value produces about two stable nuclei on vesicles having equivalent spherical diameters of 25 μm, with greater numbers of domains on larger vesicles. With ~15% of the membrane area occupied by solid phase distributed between two equally sized domains, the domain diameter will be 53% of the vesicle diameter, independent of vesicle size. This was found to be the case in the two-domain vesicles in this study subject to error in quantifying domain size. Sources for error in the experimental control and determination of solid area included slight irregularities in domain shape and temperature, which influences the solid area fraction in the phase diagram. In addition, the presence of a few very small (order 1 μm) domains in a few vesicles causes the measured solid area based on two large domains to fall short of the actual solid area. The vesicles in Fig. 2 (A to C) were produced with a faster cooling rate to produce a greater number of nuclei and a larger number of solid domains.

The solid domain areas in the vesicles in this study, electroformed in sucrose, are in agreement with previously published phase diagrams and nucleation and growth by us (24, 29, 41) and others (28, 42) for the DPPC/DOPC system. Several of these studies were conducted using deionized (DI) water. We find no evidence of impurity-driven transitions or unexpected nucleation.
Micropipettes
Micropipettes were produced on a Kopf Instruments micropipette puller, and their tips were refined on a Technical Products International microforge. Inner diameters were in the range of 3 to 6 μm, and the tip was shaped to have a nearly constant inner diameter in the first few micrometers where the vesicle projects into the pipette.
Measurements
Vesicles were imaged in glucose solutions on a Nikon Diaphot TE-300 inverted fluorescence microscope. In studies using osmotic jumps or micropipettes, an open chamber was used, in which a drop of vesicle-containing solution was held, by surface tension, between two glass coverslips. To increase or decrease the osmotic pressure and alter vesicle inflation, a drop of more or less concentrated sugar solution was added to the chamber and the system was allowed to reequilibrate, allowing water to pass through the vesicle membrane on the time scale of minutes. Measurements of excess area were obtained by aspirating vesicles into the micropipette and then reducing the tension to hold the vesicle in the micropipette without stretching the membrane. The shape of the vesicle at the equatorial focus plane was recorded and analyzed to provide the excess area: The diameter of the vesicle outside the micropipette and the length of the projection inside the pipette were measured.
The areas and volumes of sections of cylinders and spheres were then calculated and combined, as summarized in the Supplementary Materials, using formulas from elementary geometry. Low suction pressure, exerting membrane tension no more than ~1 mN/m and often less than 0.5 mN/m, avoided stretching the fluid membrane areas. With area expansion moduli near 150 mN/m, these low suction pressures produce less than 1% areal strain in the membrane. It is important to note that even when free vesicles appear round in the top or bottom view, they may be deformed and have substantial excess area. In addition, imaging of the top and bottom of the aspirated vesicle enabled further data on domain separation.

Statistical analysis

All data are presented as measured and not averaged. Error bars on measured domain separations and excess areas represent the precision of our microscope. Because vesicle size, the exact degree of inflation, and other features of the vesicles are not amenable to precise control, it was not possible to conduct multiple experiments with vesicles of a prescribed size or degree of inflation. Instead, experiments targeted ranges of variables, and then measured vesicle sizes and excess areas are reported in Fig. 4. Hundreds of vesicles were examined, and those with membrane tethers or irregular solid domains were not included in the analysis.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/14/eabf1943/DC1

View/request a protocol for this paper from Bio-protocol.

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and presentation of 2D vesicle model, including figure design and writing for theory components of the paper. H.W. developed the multi-arc model of 2D vesicles, in collaboration with G.M.G., implemented computations, analyzed model results, and prepared associated figures. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Open access to data is available at https://scholarworks.umass.edu/data/117/. Additional data related to this paper may be requested from the authors.

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