ABSTRACT The mitochondrial membrane undergoes extreme remodeling during fission. While a few membrane-squeezing proteins are recognized as the key drivers of fission, there is a growing body of evidence that strongly suggests that conical lipids play a critical role in regulating mitochondrial morphology and fission. However, the mechanisms by which proteins and lipids cooperate to execute fission have not been quantitatively investigated. Here, we computationally model the squeezing of the largely tubular mitochondrion and show that proteins and conical lipids can act synergistically to trigger buckling instability and achieve extreme constriction. More remarkably, the study reveals that the conical lipids can act with different fission proteins to induce hierarchical instabilities and create increasingly narrow and stable constrictions. We reason that this geometric plasticity imparts significant robustness to the fission reaction by arresting the elastic tendency of the membrane to rebound during protein polymerization and depolymerization cycles. Our in vitro study validates protein–lipid cooperativity in constricting membrane tubules. Overall, our work presents a general mechanism for achieving drastic topological remodeling in cellular membranes.

INTRODUCTION Mitochondria are dynamic organelles that form intricate networks and undergo continuous structural remodeling via the opposing processes of fission and fusion (Labbé et al., 2014; Kraus and Ryan, 2017; Ramachandran, 2018). While balanced mitochondrial dynamics is essential for cellular homeostasis, disruption in such dynamics is linked to various cancers, cardiac dysfunction, and neurogenerative disorders including Alzheimer’s, Parkinson’s, and Huntington’s diseases (Youle and van der Bliek, 2012; Itoh et al., 2013; Labbé et al., 2014). Pioneering experimental studies have provided insights into the molecular machinery of one such key process, mitochondrial fission (Figure 1A). According to the current working model, the fission event is a three-step process (Lee et al., 2016; McBride and Frost, 2016; Lu et al., 2018): in the first step, actin–myosin cytoskeletal networks apply forces at endoplasmic reticulum (ER)-mitochondria contact sites to initiate mitochondrial constriction; in the next step, GTP-regulated dynamin-related protein 1 (Drp1) polymerization enhances the constriction, and in the final step, dynamin 2 facilitates complete membrane fission to create two daughter mitochondria (Figure 1).

While the fission proteins play an inarguably critical role, a growing body of evidence reveals that conical lipids, present in abundance in mitochondria (Figure 1), regulate mitochondrial morphology and fission. For example, two conical lipids, phosphatidylethanolamine (PE) and cardiolipin (CL) have been found to be essential for mitochondrial function and cell viability (Gohil et al., 2005; Schlame and Ren, 2009). A moderate reduction in the PE concentration has been shown to cause a significant alteration in mitochondrial functions and morphology (Steenbergen et al., 2005; Chan and McQuibban, 2012; Joshi et al., 2012; Tasseva et al., 2013; Van Der Veen et al., 2014). Recently, CL has been shown to promote Drp1 self-assembly and stimulate Drp1 GTPase activity (Macdonald et al., 2014). CL degradation to PA, on the other hand, has been shown to restrict Drp1 activity in fission (Adachi et al., 2016). Interestingly, while CL facilitates Drp1 polymerization, Drp1 also promotes local CL clustering and non-bilayer phase transition (Stepanyants et al., 2015). Another...
Lipids catalyze mitochondrial fission

How are the superconstrictions, observed during in vivo and in vitro studies (Stepanyants et al., 2015; Lee et al., 2016), stabilized through dynamic protein polymerization and depolymerization cycles?

The lack of experimental techniques to image membrane dynamics at the length and time scales relevant for mitochondrial fission makes it difficult to parse the roles of proteins and lipids. Therefore, to address the above issues, we resort to computational modeling and investigate this fission puzzle. We employ membrane physics and differential geometry to investigate the shape evolution of an idealized tubular mitochondrion leading to “membrane necking” in the presence of the established membrane remodeling effects of fission proteins and of conical shaped lipids (Figure 1, b–e). The study reveals that proteins and lipids can act synergistically to trigger buckling instability to generate extreme constriction. More remarkably, the study shows that the conical lipids can collaborate with multiple fission protein partners to trigger hierarchical instabilities that lead to stepwise constriction of the tubular structure. These sequential instabilities, in addition to promoting constriction, stabilize constricted geometries by arresting the elastic tendency to revert to the undeformed geometry during protein cycles. Data from in vitro studies (previously published and new) validate the core modeling proposal and show that conical lipids are critical to achieving and maintaining superconstrictions in membrane tubules. Because the estimated lipid concentrations required for inducing instability are well within the physiologically known concentrations in mitochondria, our findings may be of relevance to mitochondrial fission events in living cells. Furthermore, because curvature-inducing proteins and lipids are ubiquitous in cells, the proposed mechanisms might be at play in other topological remodeling events in cellular membranes.

RESULTS

Actin–lipid cooperativity

We follow the chronological sequence of protein activity revealed by the experimental studies. First, we model the constriction due to actin filaments and conical lipids. We increase the magnitude of the actin force in the center of the spherocylinder (Figure 2b-1, magenta domain) and the lipid concentration in the adjacent domains (Figure 2b-2, blue domains). This local increase in the lipid concentration could be a consequence of curvature-mediated lipid dynamics, as has been revealed in several experimental and theoretical studies (Mukhopadhyay et al., 2008; Kamal et al., 2009; Renner and Weibel, 2011; Sakuma et al., 2011; Koldsø et al., 2014; Boyd et al., 2017). The results here are presented for $\alpha = 45^\circ$. The results for the radial force are presented in the Supplemental Material (Supplemental Figure S4).
FIGURE 2: Predicted role of actin–lipid cooperativity during membrane constriction. (a) The force-deformation curve shows a linear response in the initial phase but then undergoes a drastic snap-through transition around 30% deformation (from stage 2 to stage 3), indicating a classic buckling instability. The actin force domain is shown in magenta and the domains with higher lipid concentrations are shown in blue. After instability, the force deformation proceeds along a nonlinear curve with increasing stiffness (magenta arrow). Upon unloading (decreasing the actin force), the shape takes a new path (blue arrow), maintaining 68% deformation upon complete removal of the actin force (stage 4). This shows that the lipids arrest the constricted state preventing the spherocylinder from reverting to the undeformed configuration, thereby inducing a geometric plasticity. (b) The computed shapes at stages 1, 2, 3, and 4 during the force-deformation response shown in a.

The force-deformation response and the shape evolution are shown in Figure 2 (Supplemental Movie S1). The force-deformation curve (Figure 2a) presents the first key result that reveals a classic buckling instability. The constriction increases linearly up to ~30% (Figure 2b-2), at which point it undergoes a rapid snap-through transition reaching nearly ~58% constriction (Figure 2b-3). A further increase in the force and lipid concentration resumes constriction along a smooth curve (magenta arrow) with increasing slope indicating strain hardening. If we reduce the applied actin force, to simulate the cyclic nature of actin polymerization and depolymerization (Li et al., 2015; Moore et al., 2016), the geometry evolves along a curve different from the loading curve (blue arrow). Despite a complete removal of the actin force, there is a nearly ~68% residual constriction in the spherocylinder (Figure 2b-4). This geometric plasticity arises from lipid localization and is critical to arrest the elastic tendency of the spherocylinder to expand back to its undeformed geometry and stabilizes the constricted geometry. In the absence of lipid aggregation, the spherocylinder reverts to the original undeformed shape upon actin depolymerization (Supplemental Figure S5). The details of the geometries in Figure 2 and the prescribed lipid curvatures are presented in Supplemental Figure S6.

Drp1–lipid cooperativity

Next, we investigate the constriction induced by Drp1 and its synergy with the conical lipids. We continue from the ~68% constricted geometry (diameter ~160 nm) obtained in Figure 2 after actin depolymerization and gradually increase the circumferential curvature, stiffness, and the Drp1-coated area in the center of the spherocylinder to simulate Drp1-mediated squeezing (Figure 3b). As before, increased constriction is accompanied by an increase in lipid concentration in the adjacent (blue) domains. Because Drp1 polymerization is known to sequester CL (Stepanyants et al., 2015), we prescribe CL-induced curvature underneath the Drp1 coat. We simulate the polymerization phase until the geometry achieves ~80% constriction reaching a diameter ~94 nm, a value in the established range for Drp1 (Francy et al., 2015). We then split the Drp1 coat and move them apart. This splitting and moving apart of the Drp1 coat has been recently revealed in an in vivo study (Lee et al., 2016).

The deformation response shown in Figure 3 (Supplemental Movie S2) presents our second key finding. Drp1 polymerization, as per expectations, leads to a more constricted and elongated neck (Figure 3b). In contrast, the structure first expands mildly upon splitting and increased separation between the two Drp1 domains (Figure 3c). But at a critical separation, the system undergoes an unexpected second instability that significantly enhances the constriction leading to an extreme necking (Figure 3d; diameter ~32 nm). This superconstricted geometry is a consequence of lipid enrichment in the blue domain, which has a natural propensity to close the tubule to form hemispherical geometry as on the poles. These results again reinforce the core notion that conical lipids can cooperatively act with another fission protein to induce instability and generate stable superconstrictions.

Effect of dynamin 2

Finally, we model the effect of the dynamin 2 on mitochondrion squeezing. We assume that dynamin 2 constrists the tubule further, in a manner similar to Drp1. We start from the buckled geometry in Figure 3d. An increase in the cylindrical curvature brings the spherocylinder to the fission state with a tubule diameter ~5 nm (Figure 4a and Supplemental Movie S3). Notably, the decrease in the radius results in a dramatic increase in the in-plane stress in the membrane to ~8 mN/m (Figure 4b). For a membrane with a stretch modulus of 250 mN/m and an area extensibility of 3% (Rawicz et al., 2000), the rupture tension is nearly 7.5 mN/m. Thus, the squeezing by dynamin 2 brings the geometry very close to the rupture point, potentially resulting in the culmination of the fission reaction. It is important to note that the constriction of 5 nm diameter can also be reached in the absence of dynamin 2 with a higher lipid concentration. However,
In vitro validation of protein–lipid cooperativity

We now compare the numerically computed shapes with the shapes observed in in vitro experiments previously published by Ramachandran and coworkers (Stepanyants et al., 2015) to test the validity of the modeling predictions (Figure 5). The procedure is described in detail elsewhere (Stepanyants et al., 2015); briefly, liposomes composed of 35% PE, 25% CL, and 40% PC were preincubated with soluble Drp1 to allow for the formation of helical Drp1-decorated membrane spherocylinders. GTP hydrolysis was later initiated by the addition of GTP to these membranes tubules, and samples were obtained and negatively stained at defined time points for electron microscopy (EM) visualization. Because the in vitro setup lacks actin and dynamin 2 proteins, we subjected the membrane tubule (150 nm in diameter to match experimental geometry) to squeezing effect from Drp1 proteins and conical lipids only.

Figure 5a shows the initial geometry and Figure 5, c, and e, shows the geometries before and after the splitting of the Drp1 coat, respectively. As before, Figure 5e shows the extreme constriction upon reaching a critical separation between the split Drp1 domains leading to a stable superconstriction. The computed shapes in Figure 5, a, c, and e, show excellent agreement with in vitro shapes (Figure 5, b, d, and f). Not only are the superconstricted geometries in Figure 5, e and f, in excellent agreement, the irregular geometries with local bumps in Figure 5, c and d, show good resemblance.

Our computational predictions validate previous EM observations of Drp1-induced local membrane constrictions in mixed lipid bilayers containing conical lipids (Stepanyants et al., 2015), and strongly suggest that proteins and lipids act synergistically to create superconstrictions conducive for fission. It was also previously noted that in the absence of PE, no membrane constrictions were observed despite the presence of CL to bind Drp1 (Stepanyants et al., 2015). Guided by these combined predictions, we further investigated the role of conical lipids experimentally in membrane squeezing and stabilization. Consistent with our numerical assessment, under identical experimental conditions as earlier, but with a reduced concentration of PE (22.5%) in the membrane, no superconstrictions were observed despite the presence of a Drp1 helical coat enwrapping the tubulated membrane (Figure 6). The observed experimental shape, in fact, closely resembles the computed shape in the absence of lipid localization (Figure 6b). The formation of Drp1-dependent membrane necks at 35% PE, but not at 22.5% PE, further strongly suggests that adequate spherical curvature from conical lipids is essential for extreme membrane remodeling.

FIGURE 3: Predicted role of Drp1-lipid cooperativity during the constriction process. (a) The deformation response due to Drp1 and lipid generated curvatures from the 68% constricted state obtained in Figure 2b-4. (b) During the first phase, the curvature and stiffness of the Drp1 coat and the lipid localization is increased, causing the constriction to undergo a monotonic increase. (c) In the second phase, the coat is split and the two domains are moved apart. (d) Upon reaching a critical separation, the shape undergoes a second buckling instability, which yields a highly constricted state. The insets show the zoomed-in view of the constrictions. The red domain indicates the Drp1-coated region, and the adjacent blue domains show the regions of lipid localization.

FIGURE 4: Simulation of the final stage leading to membrane fission. (a) Dynamin 2–induced squeezing from the postbuckling shape presented in Figure 3. (b) Increased squeezing leads to extreme tubulation with near-rupture in-plane stress in the membrane.
Furthermore, what our calculations suggest is that the lipid concentrations discussed here are the concentration differences between the inner and the outer leaflets of the membrane and not their absolute concentrations in the leaflets.

For the initial geometry, we require 1.2 and 0.6% PE concentrations in the spherical cap and the cylindrical domains, respectively (Figure 8a). For the states just before instabilities in the actin–lipid and Drp1–lipid constriction phases, the concentration in the spherical domains remains unchanged but the concentration in the cylindrical domains reduces to 0.5 and 0.19%, respectively, and the concentration in the regions adjacent to the protein domain (blue domains next to the red domain) increases to 0.80 and 1.56%, respectively (Figure 8, b and c). This suggests that in comparison to the initial concentration of 0.6%, only 0.20 and 0.96% change in concentrations are required to trigger instabilities and achieve extreme necking. The analysis for CL lipids shows a similar qualitative behavior and the instability is achieved at lower values of lipid sorting (Supplemental Figure S8).

These numbers show that the interleaflet concentration difference required for achieving instability is minimal. Although we do not explicitly model the dynamics of lipids and their lipid distribution, the sorting of conical lipids has been revealed in both the experimental and the modeling studies. For example, coarse-grained molecular dynamics studies have revealed membrane-curvature–dependent lipid clustering (Koldø et al., 2014; Boyd et al., 2017). In addition, experimental studies have shown cardiolipin localization in high curvature domains of bacterial spheroplasts (Renner and Weibel, 2011) and strong asymmetric distribution of conical 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) lipids (Sakuma et al., 2011). Furthermore, what our calculations suggest is that even if the curvature-based lipid sorting is weak, as was revealed in Kamal et al. (2009), lipids could still actively contribute to mitochondrial shape transitions. In fact, it is remarkable that the experimental study predicts a 4% asymmetry in 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) concentration in a vesicle of 100 nm radius (Kamal et al., 2009), which nearly coincides with the required asymmetric PE concentration to trigger instability in a similarly sized spherocylinder investigated in our in vitro study (data shown in Supplemental Figure S9).
Is the proposed mechanism robust?

We consider three extensions of the simulations presented earlier in the Results section. First, because mitochondria exhibit diverse shapes, we simulate the squeezing of a spherocylinder with varied aspect ratios ($L/2R_0$). The shape transitions for aspect ratios of 1.5 and 4 are shown in Supplemental Figures S10, S11, S12, and S13, respectively. These results again show the proposed sequential instabilities and the superconstriction as predicted for the case of a perfect spherocylinder. Second, we model a local increase in the concentration of conical lipids near the protein domain without any change of lipid concentration in the cylindrical domain. Such a scenario can potentially occur in two cases: 1) conical lipids are recruited from the ER at the constriction sites as proposed in the literature (Osman et al., 2011; Mesmin, 2016), and 2) proteins and membrane curvature either catalyze flipping of lipids between the leaflets or cluster lipids due to direct electrostatic interactions to give rise to an increased lipid asymmetry. The corresponding shape transitions during the actin–lipid and Drp1–lipid phases are shown in Supplemental Figures S14 and S15. As seen in Figures 2 and 3, the spherocylinder still undergoes sequential instabilities during the step-by-step constriction process. Third, we test the impact of the sizes of the protein-active area (actin and Drp1) and the lipid domains (adjacent blue domains) on shape transition. The results for the cases with 50% increase and 25% decrease in the observed in the experiments is not capable of triggering instability. This is a key prediction made by the computational model. The instability is a result of energetics, where the system prefers to undergo a large deformation in order to reach an energetically optimal state. This is driven by the conical lipids in the inner leaflet in the blue domains, which want the spherocylinder to close up. It is also important to note that the superconstricted shape is ideal for the conical lipid clusters and is a natural shape for the formation of daughter mitochondria.

As far as direct evidence is concerned, it is extremely difficult to identify an instability during a constriction process either in vitro or in vivo. To track instability, a continuous high-resolution time-dependent observation has to be made. However, remarkably, the study by Moore et al. (2016) shows constricted shapes of mitochondria in postactin phase in DRP1-deficient cells. This suggests that these constricted shapes are potentially stabilized via buckling instabilities mediated by lipids. The constricted tubules should otherwise rebound back to their original shapes due to the high elastic energy stored in the superconstricted domain. In addition, the in vitro results of Stepanyants et al. (2015) also clearly show constricted tubules after Drp1 depolymerization. Because there is no active force acting on the tubules in this phase, instability-induced equilibrium shape is likely the explanation for the observed geometries.

General principles for membrane fission

Going beyond mitochondrial fission, the findings of this study reveal some general physical principles that may contribute to our understanding of membrane fission at-large. A significant number of fundamental studies have provided mechanistic insights into the constriction–fission mystery (see the recent review article Frolov et al., 2015, and the references therein). Although several of these studies have recognized the role of lipid properties in membrane fission (Hutner and Zimmerberg, 2001; Kazlo, 2001; Chernomordik and Kozlov, 2003; Allain et al., 2004; Roux et al., 2005; Frolov et al., 2011), the active role of spatially segregated conical lipids in inducing buckling instability to drive extreme membrane constriction has not been demonstrated before. In this regard, this work reveals three key principles. First, it is critical to invoke the correct sequence of curvatures. The protein-mediated squeezing has to precede the lipid localization in order to achieve constriction. The reverse sequence would lead to radial expansion of the membrane tubule. Second, it is favorable to have spatial segregation in the protein and lipid domains. This avoids “curvature conflict” in the same region,
allowing proteins and lipids to impose their complementary curvatures more strongly. Third, disintegration of the protein coat is vital for triggering instability. After proteins supply the initial energy (by squeezing the membrane tubule) and prime the system (by modulating lipid distribution), they hinder the snap-through transition of the membrane due to their stiffening effect. Thus, it is desirable that coat-forming proteins either move apart or leave, allowing the lipids to undergo instability-driven extreme remodeling. These features are a consequence of the interplay between geometry and elasticity, and hence are of a generic nature.

Limitations
As is true for all models, our mathematical framework has some limitations. First, the analytical work presented in this study is based on an idealized mitochondrion that is modeled as a spherocylindrical shell. Similar modeling idealizations restricted to axisymmetric shapes have been routinely and successfully used in the literature before to gain mechanistic insights into the membrane physics literature (please see Kozlovsky and Kozlov, 2003, for example). However, it would be valuable to investigate the mechanics of more realistic nonaxisymmetric shapes in a larger phase space. Such generalization is likely to lead to quantitative changes in the predictions altering the numerical values of actin forces and lipid curvatures required to trigger shape transitions. Second, the model does not explicitly account for the physical forces that may arise from the architectural remodeling of the inner membrane during mitochondrial fission. The recent study from Higgs labs shows that the inner membrane undergoes fission even before Drp1 is recruited to the mitochondria (Chakrabarti et al., 2018). Thus, our predictions should be valuable to understand the subsequent constriction of the outer membrane. However, even if the inner membrane were to apply some internal resistance, the effect would lead to some internal pressure that would oppose constriction. This again, would only lead to quantitative changes in the predictions. Third, our model does not explicitly account for protein–lipid dynamics and membrane fission. As a result, we prescribe force and curvature fields and stop at a shape with a highly constricted neck, which should serve as a precursor to fission. Although the assumptions invoked to bypass these limitations have been well established and employed in several studies (cited in the text), computational models to investigate the dynamics of specific proteins and lipids of interest in the future would provide additional insights. In particular, a stochastic analysis of lipid–protein dynamics would be insightful to understand the interplay between protein kinetics and lipid dynamics giving more in-depth knowledge of energetic barriers in undergoing instabilities and shape transitions. Fourth, in this work, we have prescribed the aggregation of conical lipids at fission sites, and predicted its consequence on the squeezing transition. Although we have analyzed and presented a two-step process, it awaits rigorous in vivo validation. It is possible that the proposed curvature-based lipid aggregation mechanism is triggered only beyond a certain threshold curvature during the squeezing process. In such a scenario, if the actin-induced squeezing does not exceed the threshold curvature, lipid cooperativity and the induced first buckling during the actin phase might not be observed. However, due to the substantially greater curvature at mitochondrial fission sites in the Drp1 phase, we expect the second instability to occur with much higher probability.

In summary, our study reveals a hierarchical instability-based mechanism of membrane squeezing triggered jointly by proteins and conical lipids. Despite various complexities, our findings might be of value to understanding mitochondrial fission in vivo. The in
vitro studies lend support to our modeling predictions. We hope that our work will stimulate more biophysical studies to explore the role of conical lipids and their interactions with mitochondrial proteins during shape transformations observed in apoptosis and mitochondria-associated diseases. In a general context, our work might give new insights into other topological events during cellular transport and remodeling of cellular organelles driven by curvature-inducing proteins and lipids (McMahon and Gallop, 2005; Zimmerberg and Kozlov, 2006).

MATERIALS AND METHODS

We resort to continuum-scale modeling as it possesses the unique ability to quantify the roles of proteins and lipids without getting overwhelmed with the molecular details. The continuum approach has been successfully used to study actin-induced mitochondrial constriction (Manor et al., 2015) and the intricate architecture of the mitochondrial inner membrane and endoplasmic reticulum (Frey et al., 2002; Renken et al., 2002; Ponnuswamy et al., 2005; Terasaki et al., 2013). We model a mitochondrion as a hollow spherocylinder (a cylinder capped with hemispheres at the two ends; see Figure 1b) made of lipid membrane, which undergoes shape transition in the presence of known shape remodeling effects of fission proteins and conical lipids. This assumption is supported by the recent study from Higgs labs that reveals that the inner membrane undergoes fission even before Drp1 is recruited to the mitochondria (Chakrabarti et al., 2018).

A lipid membrane is treated as a 2D elastic fluid surface. The strain energy for such a surface depends on the local curvatures of the surface. In regions where the membrane possesses isotropic properties, strain energy depends on the two curvature invariants: the mean curvature (H) and the Gaussian curvature (K) (Canham, 1970; Helfrich, 1973; Jenkins, 1977; Lipowsky, 1991; Steigmann, 1999). Because conical lipids such as CL, PE, and DAG generate spherical curvatures (Figure 1c) that remain invariant in all the directions, the strain energy in CL/PE-rich domains is given by the well-known Helfrich–Canham energy \( W = \kappa (H - H_0)^2 + \kappa K \), where \( H_0 = 1/R_0 \) is the spontaneous curvature imposed by the lipids and \( \kappa, \kappa' \) are the bending moduli. A detailed explanation for why conical lipids will generate spherical curvatures as opposed to cylindrical curvatures is provided in the Supplemental Material (Supplemental Figure S1). In the current model, the spontaneous curvature generated by lipids is assumed to be proportional to the difference in their concentrations between the two leaflets (Supplemental Figure S3) and compute the shape evolution of the idealized spherocylinder (a cylinder capped with hemispheres at the two ends; see Figure 1b) made of lipid membrane, which undergoes shape transition in the presence of known shape remodeling effects of fission proteins and conical lipids. This assumption is supported by the recent study from Higgs labs that reveals that the inner membrane undergoes fission even before Drp1 is recruited to the mitochondria (Chakrabarti et al., 2018).

One of the key features of our mathematical framework is that it allows spontaneous curvatures and bending moduli to depend on the surface coordinates, thus allowing seamless modeling of membrane heterogeneity, a feature critical to assessing the nuances of extreme localized remodeling. Because lipid membranes can undergo only 2–3% areal dilation before rupture, we impose areal incompressibility. We construct the system free energy and minimize it to obtain the Euler–Lagrange equations. We simplify the equations for the axisymmetric setting (Supplemental Figure S3) and compute the shape evolution of the idealized spherocylindrical mitochondrion. The simulations are quasistatic and deterministic in nature. The initial spherocylindrical geometry is obtained by prescribing two distinct conical lipid concentrations in the hemispherical and cylindrical domains. Here, we assume that a higher areal density of conical lipids leads to higher effective spontaneous curvatures. Further details of the model are presented in the Supplemental Material.

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REFERENCES

Adachi Y, Ioh K, Yamada T, Cerveny KL, Suzuki TL, Maconald P, Frohman MA, Ramachandran R, Iijima M, Sesaki H (2016). Coincident phosphatidic acid interaction Restraints Drp1 in mitochondrial division. Mol Cell 63, 1034–1043.

Allain J-M, Storm C, Roux A, Amar MB, Joanny J-F (2004). Fission of a multiasphere membrane tube. Phys Rev Lett 93, 158104.

Boyd KJ, Alder NN, May ER (2017). Buckling under pressure: curvature-based lipid segregation and stability modulation in cardiolipin-containing bilayers. Langmuir 33, 6937–6946.

Canham PB (1970). The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. J Theor Biol 26, 61–81.

Chakrabarti R, Ji W-K, Stan RV, de Juan Sanz J, Ryan TA, Higgs HN (2018). INF2-mediated actin polymerization at the ER stimulates mitochondrial calcium uptake, inner membrane constriction, and division. J Cell Biol 217, 251–268.

Chan EYL, McQuibban GA (2012). Phosphatidylserine decarboxylase 1 (Psdc1) promotes mitochondrial fusion by regulating the biophysical properties of the mitochondrial membrane and alternative topogenesis of mitochondrial genome maintenance protein 1 (Mgm1). J Biol Chem 287, 40131–40139.

Chernomordik LV, Kozlov MM (2003). Protein-lipid interplay in fusion and fission of biological membranes. Annu Rev Biochem 72, 175–207.

Choi S-Y, Huang F, Jenkins GM, Chan DC, Schiller J, Frohman MA (2006). A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. Nat Cell Biol 8, 1255.

Frohman MA, Bohinc K, Gauger DR, Iglic A, Kraij-Iglic V, May S (2005). The Influence of anisotropic membrane inclusions on curvature elastic properties of lipid membranes. J Chem Inf Model 45, 1652–1661.

Francy CA, Alvarez FJD, Zhou L, Ramachandran R, Mears JA (2015). The mecanoenzymatic core of dynamin-related protein 1 comprises the minimal machinery required for membrane constriction. J Biol Chem 290, 11792–11793.

Frey TG, Renken CW, Perkins GA (2002). Insight into mitochondrial structure and function from electron tomography. Biochim Biophys Acta 1555, 196–203.

Frohman MA (2015). Role of mitochondrial lipids in guiding fission and fusion. J Mol Med 93, 263–269.

Frolov VA, Escalada A, Aikmov SA, Shnyrova AV (2015). Geometry of membrane fission. Chem Phys Lipids 185, 129–140.

Frolov VA, Shnyrova AV, Zimmerberg J (2011). Lipid polymorphisms and membrane shape. Cold Spring Harb Perspect Biol 3, a004747.

Gohil VM, Thompson MN, Greenberg ML (2005). Synthetic lethal interactions of the mitochondrial phosphatidylethanolamine and cardiolipin

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biosynthetic pathways in Saccharomyces cerevisiae. J Biol Chem 280, 35410–35416.
Guo T, Gregg C, Boukh-Viner T, Kryakov P, Goldberg A, Bourque S, Banu F, Haile S, Miljevic S, San KH, et al. (2007). A signal from inside the peroxisome initiates its division by promoting the remodeling of the peroxisomal membrane. J Cell Biol 177, 289–303.
Helfrich W (1973). Elastic properties of lipid bilayers: theory and possible experiments. Z Naturforsch C 28, 693–703.
Huang H, Gao Q, Peng X, Choi S-Y, Sarma K, Ren H, Morris AJ, Frohman MA (2011). P-RNA-associated germline nuance formation and spermatogenesis require MitolPLD profusogenic mitochondrial-surface lipid signaling. Dev Cell 20, 376–387.
Huttner WB, Zimmerberg J (2001). Implications of lipid microdomains for membrane curvature, budding and fission: commentary. Curr Opin Cell Biol 13, 478–484.
Itoh K, Nakamura K, Iijima M, Sesaki H (2013). Mitochondrial dynamics in neurodegeneration. Trends Cell Biol 23, 64–71.
Jenkins JT (1977). The equations of mechanical equilibrium of a model membrane. SIAM J Appl Math 32, 755–764.
Joshi AS, Thompson MN, Fei N, Hüttemann M, Greenberg ML (2012). Cardiolipin and mitochondrial phosphatidylethanolamine have overlapping functions in mitochondrial fusion in Saccharomyces cerevisiae. J Biol Chem 287, 17589–17597.
Kamal MM, Mills D, Grzybek M, Howard J (2009). Measurement of the membrane curvature preference of phospholipids reveals only weak coupling between lipid shape and leaflet curvature. Proc Natl Acad Sci USA 106, 22245–22250.
Koldzic H, Shorthouse D, Hélie J, Sansom MSP (2014). Lipid clustering correlates with membrane curvature as revealed by molecular simulations of complex lipid bilayers. PLoS Comput Biol 10, e1003911.
Kozlov MM (2001). Fission of biological membranes: interplay between dynamin and lipids. Traffic 2, 51–65.
Kozlovsky V, Kozlov MM (2003). Membrane fission: model for intermediate structures. Biophys J 85, 85–96.
Kraj-Iglívá V, Heinrich V, Svetina S, Žekš B (1999). Free energy of closed lipid membranes. Eur Phys J B 10, 75–79.
Lee JE, Westrate LM, Wu H, Page C, Voeltz GK (2016). Multiple dynamin family members collaborate to drive mitochondrial division. Nature 540, 139–143.
Li S, Xu S, Roelofs BA, Boylan M, Lederer WJ, Sesaki H, Karbowski M (2015). Transient assembly of F-actin on the outer mitochondrial membrane contributes to mitochondrial fission. J Cell Biol 208, 109–123.
Lipowsky R (1991). The conformation of membranes. Nature 349, 475–481.
Lu B, Kennedy B, Clinton RW, Wang EJ, McHugh D, Stepansyants N, Macdonald PJ, Mears JA, Qi X, Ramachandran R (2018). Steric interference from disordered regions controls dynamin-related protein 1 self-assembly during mitochondrial fission. Sci Rep 8, 10879.
Macdonald PJ, Stepansyants N, Melvrotta N, Mears JA, Qi X, Sesaki H, Ramachandran R (2014). A dimeric equilibrium intermediate nucleates Drp1 reassembly on mitochondrial membranes for fission. Mol Biol Cell 25, 1905–1915.
Manor U, Bartholomew S, Golani G, Christenson E, Kozlov M, Higgs H, Spudich J, Lippincott-Schwartz J (2015). A mitochondria-anchored isoform of the actin-nucleating spire protein regulates mitochondrial division. Elife 4, e08828.
McBride HM, Frost A (2016). Cell biology: double agents for mitochondrial division. Nature 540, 43.
McMahon HT, Gallop JL (2005). Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 438, 590–596.
Mesmin B (2016). Mitochondrial lipid transport and biosynthesis: a complex balance. J Cell Biol 214, 9–11.
Moore AS, Wong YC, Simpson CL, Holzbaur ELF (2016). Dynamic actin cycling through mitochondrial subpopulations locally regulates the fission-fusion balance within mitochondrial networks. Nat Commun 7, 12886.
Mukhopadhyay R, Huang KC, Wingreen NS (2008). Lipid localization in bacterial cells through curvature-mediated microphase separation. Biophys J 95, 1034–1049.
Osman C, Voelker DR, Langer T (2011). Making heads or tails of phospholipids in mitochondria. J Cell Biol 192, 7–16.
Ponnuswamy A, Nulton J, Mahafy JM, Salamon P, Frey TG, Baljon AR (2005). Modeling tubular shapes in the inner mitochondrial membrane. Phys Biol 2, 73.
Ramachandran R (2018). Mitochondrial dynamics: the dynamin superfamily and execution by colliusion. Semin Cell Dev Biol 76, 201–212.
Rawicz W, Olbrich KC, McIntosh T, Needham D, Evans E (2000). Effect of chain length and unsaturation on elasticity of lipid bilayers. Biophys J 79, 328–339.
Renken C, Siragusa G, Perkins G, Washington L, Nulton J, Salamon P, Frey TG (2002). A thermodynamic model describing the nature of the crista junction: a structural motif in the mitochondrion. J Struct Biol 138, 137–144.
Renner LD, Weibel DB (2011). Cardiolipin microdomains localize to negatively curved regions of Escherichia coli membranes. Proc Natl Acad Sci USA 108, 6264–6269.
Roux A, Cuveler D, Nasso P, Prost J, Bassereau P, Goub D (2005). Role of curvature and phase transition in lipid sorting and fission of membrane tubules. EMBO J 24, 1537–1545.
Sakuma Y, Urakami N, Taniguchi T, Imai M (2011). Asymmetric distribution of cone-shaped lipids in a highly curved bilayer revealed by a small angle neutron scattering technique. J Phys: Condens Matter 23, 284104.
Schlame M, Ren M (2009). The role of cardiolipin in the structural organization of mitochondrial membranes. Biochim Biophys Acta 1768, 2080–2083.
Steenbergen R, Nanowski TS, Beigneux A, Kulinski A, Young SG, Vance JE (2005). Disruption of the phosphatidylserine decarboxylase gene in mice causes embryonic lethality and mitochondrial defects. J Biol Chem 280, 40032–40040.
Steigmann DJ (1999). Fluid films with curvature elasticity. Arch Ration Mech Anal 150, 127–152.
Stepansyants N, Macdonald PJ, Francy CA, Mears JA, Qi X, Ramachandran R (2015). Cardiolipin's propensity for phase transition and its reorganization by dynamin-related protein 1 form a basis for mitochondrial membrane fusion. Mol Biol Cell 26, 3104–3116.
Tasseva G, Bai HD, Davidescu M, Haromy A, Michelakis E, Vance JE (2013). Phosphatidylethanolamine deficiency in mammalian mitochondria impairs oxidative phosphorylation and alters mitochondrial morphology. J Biol Chem 288, 4158–4173.
Terasaki M, Shemes T, Kasturi N, Klemm RW, Schalek R, Hayworth KJ, Hand AR, Yankova M, Huber G, Lichtman JW, et al. (2013). Stacked endoplasmic reticulum sheets are connected by helicoidal membrane motifs. Cell 154, 285–296.
van der Veen JN, Lingrell S, da Silva RP, Jacobs RL, Vance DE (2014). The concentration of phosphatidylethanolamine in mitochondria can modulate ATP production and glucose metabolism in mice. Diabetes 63, 2620–2630.
Walani N, Torres J, Agrawal A (2014). Anisotropic spontaneous curvatures in lipid membranes. Phys Rev E 89, 62715.
Walani N, Torres J, Agrawal A (2015). Endocytic proteins drive vesicle growth via instability in high membrane tension environment. Proc Natl Acad Sci USA 112, E1423–E1432.
Yole RJ, van der Bliek AM (2012). Mitochondrial fission, fusion, and stress. Science 337, 1062–1065.
Zimmerberg J, Kozlov MM (2006). How proteins produce cellular membrane curvature. Nat Rev Mol Cell Biol 7, 9–19.