

Membrane bending energy selects for symmetric growth of protein assemblies

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Membrane remodeling into vesicles is essential for processes such as receptor transport and viral budding. In many cases, this remodeling is driven by the assembly of multivalent protein lattices that stochastically grow while coupled to bending the membrane. Here, we ask how membrane mechanics can bias the structural pathways sampled during lattice growth. Using a continuum membrane model coupled to growing human immunodeficiency virus Gag lattice intermediates, we show that radially symmetric lattice growth is energetically favored over eccentric growth because it induces membrane bending at lower cost per protein. For rigid lattices, the penalty for asymmetric growth is controlled primarily by the shape of the lattice perimeter rather than by the density of protein-membrane links, with all membrane deformations retaining strong radial symmetry. Reevaluating the energy relative to the circumscribing radius of the lattice thus collapses all growth pathways to a similar curve. When the lattice is flexible, asymmetric growth introduces another cost, as these intermediates also generate weaker curvature. Analytical spherical-cap estimates support the trends in our numerical energy minimization calculations, and our results remain robust across a range of tested modeling assumptions including membrane spontaneous curvature. Although kinetic barriers along growth pathways are not assessed, our results show that membrane bending can strongly select for symmetric assembly pathways, providing a mechanical bias against irregular lattice growth during membrane remodeling.

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I. INTRODUCTION

Membrane remodeling driven by multiprotein assemblies is essential for diverse cellular processes such as cell division [1], endocytosis [2], and viral budding [3]. Reshaping the two-leaflet membrane bilayer requires work to overcome the preferred flat equilibrium for symmetric leaflets. Leaflet asymmetry and thus curvature can be induced by individual proteins that insert amphipathic helices into a single leaflet [4,5], by changes in lipid composition, as occurs during budding of the human immunodeficiency virus (HIV) virion [6,7], and by proteins that locally reduce membrane surface tension [8]. Multivalent protein structures are particularly effective at large-scale bending [1,2] because they impart relatively rigid and spatially organized reshaping forces [9,10]. A prominent example is the immature HIV-1 Gag lattice, which assembles at the plasma membrane into an incomplete spherical shell necessary for viral budding [11–16]. Importantly, in cells, the Gag proteins arrive at the membrane as monomers and small oligomers, and thus the lattice grows at the membrane over time [17,18]. This coupling between assembly and membrane remodeling means that the pathways sampled during growth will depend on which intermediates are most energetically favorable. In particular, membrane bending may favor some

lattice growth geometries over others by penalizing intermediates that deform the membrane inefficiently. Although previous studies have used computation to interrogate how multiprotein assemblies induce membrane remodeling via clathrin cages [19], colloids [20], and filaments [21], our focus here is on the inverse cause and effect: How does the mechanical cost of membrane bending bias the intermediate structures sampled along self-assembly pathways?

The HIV Gag immature lattice is an excellent system for quantifying the role of membrane mechanics on self-assembly pathways. Gag monomers can assemble in solution into structures that closely resemble the membrane-bound lattices [22,23], as recapitulated by stochastic reaction-diffusion simulations [24]. However, the formation of complete structures [25] and their remodeling dynamics [26] is quite sensitive to the assembly conditions, producing a wide array of intermediate structures along assembly pathways in solution with irregular or eccentric perimeters. Further, it is well known due to Euler's polyhedron formula that a hexagonal lattice cannot perfectly tile the spherical surface. Because the immature HIV lattice does not form pentamers, it instead must incorporate defects [27]. The frequency of defects can vary depending on assembly conditions [26], and Gag cofactors control assembly kinetics [28] and help minimize unproductive, kinetically trapped intermediates [25]. Because kinetically trapped states can persist for hours or more [29], we reasoned that membrane localization and bending could provide another selection mechanism to ensure robust lattice growth during viral budding.

To address this question, we perform computer simulations where coarse-grained, particle-based protein lattices representing HIV Gag assemblies are coupled to a continuum

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membrane model [30,31]. A computational approach like this allows us to evaluate growing intermediates along assembly pathways and quantitatively compare how structural features and lattice rigidity determine energetics along distinct pathways [25,28,32–37]. These approaches exceed the spatial or temporal resolution of imaging or *in vitro* experiments [22,38], and with our models we can tightly control lattice eccentricity, which measures deviation from radial symmetry. Assessing structures during membrane remodeling is important because of their central role in controlling the membrane shape, with curvature of the membrane often matched to the assembly's radius [19,39] or cylindrical shape [40,41]. For the immature HIV lattice, the size and curvature of the budded, membrane-enveloped virions appear to be largely encoded by the intrinsic curvature of the Gag lattice, based on its size when assembled purely in solution [22,23,42]. Given that a curved lattice can assemble when decoupled from membrane bending, we ask here how the energetic cost of membrane bending might substantially narrow assembly pathways under these more physiologically relevant conditions, eliminating structures accessible in three dimensions (3D) [25]. Eliminating parallel assembly pathways can be beneficial, as it can prevent kinetic traps that result from multiple incompatible intermediates [29].

Because the nucleation and growth of the Gag lattice on the membrane spans several hundred nanometers and 5–25 min [43,44], with $>10^3$ Gag monomers and $>10^5$ lipids, any start-to-finish simulations of the coupled dynamics of stochastic self-assembly with dynamic membrane remodeling are at the limit of current tools [45]. In our approach, we have therefore decoupled the dynamics of reaction-diffusion simulations of Gag recruitment and lattice self-assembly [25] from the membrane remodeling dynamics. We instead use energy minimization of the continuum membrane coupled to lattices along snapshots of lattice growth with increasing Gag subunits. An advantage of abstracting the lipid membrane to an elastic curved surface is that it provides substantial speedups compared to coarse-grained molecular dynamics (CG-MD) [34], which models lipid particles interacting with pair potentials. The material properties of the membrane, specifically the bending modulus and the membrane tension, are also readily controlled in the continuum models, albeit at the cost of capturing local lipid heterogeneity [45]. We previously showed that this model can recapitulate curvature sensing by amphipathic helices inserted to the bilayer with both one [31] and two leaflets (including lipid tilt) [46], with excellent agreement to multiple experimental studies [46–48]. Although we have sacrificed the dynamics accessible in CG-MD simulations, which have demonstrated how collections of proteins on the membrane can induce curvature of varying depths and shapes [34,39,40,49], including budding steps for other viruses [37,39] and membrane scission events [50,51], our approach supports quantitative shape and energy comparison across multiple possible assembly pathways. We further validate that Brownian dynamics simulations [52] of our rigid lattices coupled to the membrane [53] retain the membrane shape deformations of our energy-minimized structures. Previous studies using particle-based protein models coupled to continuum membranes have studied membrane deformations induced by flexible monomers [41,54], polymers [55], fila-

ments [56,57], or lattice structures [19,58–60], but have not systematically compared the energetic cost of physically accessible but structurally distinct assembly pathways driving membrane remodeling.

In this study, we first quantify the energetics of rigid Gag lattices of increasing size when each snapshot is coupled to membrane remodeling, thus testing the energetic cost of a possible assembly pathway coupled to remodeling. By comparing to theoretical estimates of bending driven by spherical caps [20], our results indicate a substantial energetic penalty per protein for irregular/eccentric structures. We thus predict energetic selection pressure against asymmetric growth of structures, despite these structures being accessible for purely 3D assembly. To more systematically confirm this observation and explain with theory, we generated highly controlled lattices with tunable eccentricity, rigidity, and varying density of membrane attachment sites. We show that the membrane bending energy for rigid lattices is largely controlled by the overall shape and eccentricity of the lattice perimeter rather than by Gag attachment density. By rescaling our plots of membrane bending energy versus Gag monomer numbers to control for variations of eccentricity, our results collapse onto a relatively universal curve. This result is maintained even if the Gag monomers can locally change the spontaneous curvature of the membrane, and the membrane deformation is retained when the membrane and lattice are both dynamically propagated using Brownian dynamics. For flexible lattices, the eccentric lattices also produce another cost, as they induce a significantly weaker curvature. We discuss extensions to this work and possible implications for viruses like severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as well as the potential design benefits of using rigid eccentric lattices to create large membrane deformations with a small number of proteins.

II. METHODS AND MODELS

Our model systems combine a continuum surface model of a membrane with a rigid or flexible protein lattice, coupled together via stiff harmonic bond potentials. We assume here that when the protein lattice is rigid, its energy is fixed and unchanging. Our lattices also have a fixed preferred curvature of $2(50\text{ nm})^{-1}$, even as the size or eccentricity varies, matching the intrinsic curvature of the observed spherical lattice. The membrane will reshape to minimize the energy imposed by the harmonic constraints coupling it to the protein lattice, the energy of the lattice, and its own mechanics. Our continuum surface model has mechanics controlled by the Helfrich-Canham-Evans, or HCE [61–63] Hamiltonian. To evaluate the shape and energy of our continuum membrane as it adopts arbitrary topologies, we numerically represent it in 3D space using a finite element method (FEM) that applies the limit-surface method to smoothly parametrize the surface [30]. All FEM code is available open source in Ref. [64].

A. Theory on the membrane and membrane-protein energetics

1. Background on the energy function describing the isolated membrane mechanics

Our continuum membrane model uses the HCE Hamiltonian $E_{\text{mem}}(\mathbf{p}, A)$, consisting of a membrane bending energy

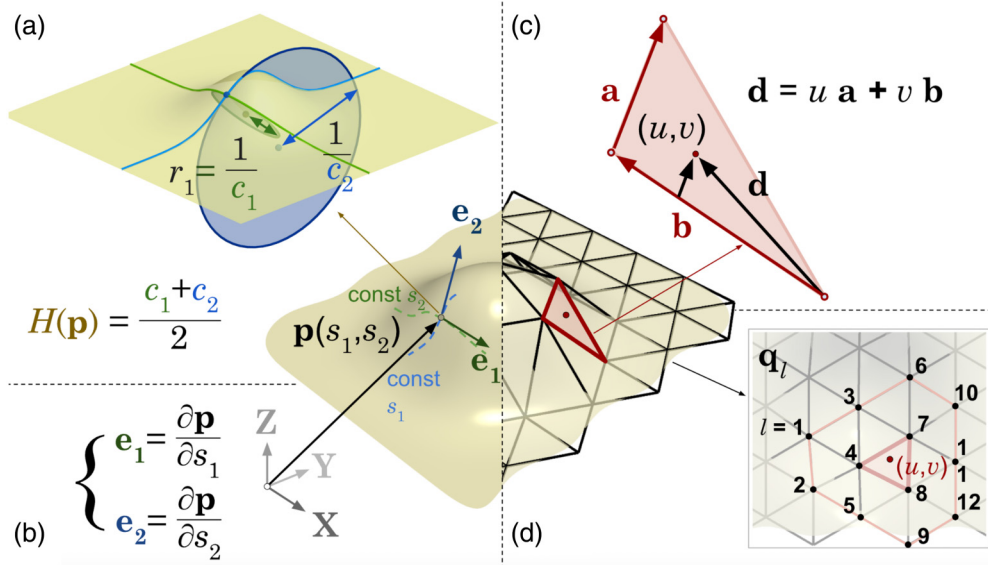


FIG. 1. The continuum membrane model quantifies the membrane energy as it depends on shape due to variations in local curvature and total area, numerically represented using finite element methods. (a) Mean curvature H at some point $\mathbf{p}$ on the limit surface is defined as the average of curvatures c_1 , c_2 along two orthogonal tangent basis vectors at this point (see Fig. S1 of the Supplemental Material [81]). (b) Definition of tangent basis vectors $\mathbf{e}_1$, $\mathbf{e}_2$ at point $\mathbf{p}$ on the limit surface via curvilinear coordinates (s_1, s_2) . (c) Barycentric coordinate (u, v) defined inside an element triangle via linear combination of the edge vectors. (d) Any point on the continual limit surface can be calculated from the coordinator vectors of the 12 neighboring vertices $\mathbf{q}_l$ (l enumerates the neighboring vertices) and barycentric coordinate (u, v) of the point on the triangle.

E_B and area constraint energy E_A [Eq. (1)], which we redefine here for background. The membrane bending energy E_B quantifies the cost of bending dependent on a material parameter κ , the bending modulus, and the preferred or spontaneous curvature along the surface C_0 . It is defined as an integration over the whole membrane surface S of a quadratic penalty of local mean curvature $H(\mathbf{p})$ measured at each point $\mathbf{p}$ on the curvilinear surface as it deviates from $C_0(\mathbf{p})$ [Fig. 1(a)]. Here, we set the spontaneous curvature to 0 nm^{-1} or flat ($\forall \mathbf{p} \in S, C_0(\mathbf{p}) = 0$), which is true for bilayer membranes with symmetric leaflets. For Sec. III F, we relax this assumption to quantify the effect of nonzero C_0 . The local mean curvature $H(\mathbf{p}) = \frac{c_1 + c_2}{2}$, where $c_{1,2}$ measure the principal surface curvatures, is therefore zero for a flat membrane. The bending modulus κ accounts for the free-energy cost as the membrane deformation increases and is set to 83.4 pN nm consistent with experimental measurements [65,66]. Because κ is an emergent material property of the lipid membrane, unless in a system with phase separation [67–69], κ is constant throughout the membrane surface. The area constraint energy E_A is defined in terms of total membrane area A and equilibrium membrane area A_0 , which is the total membrane area if under zero tension. Below, we discuss how A_0 is determined numerically to maintain low tension. E_A is parametrized by the constant μ_A set to 250.0 pN/nm [70,71]. In total, we therefore have

$$E_{\text{mem}}(\mathbf{p}, A) = E_B + E_A = \int_S \frac{1}{2} \kappa (2H(\mathbf{p}) - C_0(\mathbf{p}))^2 \sqrt{g} ds_1 ds_2 + \frac{1}{2} \mu_A \frac{(A - A_0)^2}{A_0}, \quad (1)$$

where $\sqrt{g}$ is the curvilinear factor required for area integration on a curved surface defined as the magnitude of the cross product of two tangent basis vector $\sqrt{g} = \|\mathbf{e}_1 \times \mathbf{e}_2\|$ [see Fig. 1(b)]. We note that we treat κ and μ_A as independent variables to allow for flexibility in our simulations, although they are generally coupled through bilayer properties and lipid composition [72]. When the spontaneous curvature is set to zero for the symmetric membrane, the bending energy simplifies to

$$E_B = 2\kappa \int_S (H(\mathbf{p}))^2 \sqrt{g} ds_1 ds_2. \quad (2)$$

Tension τ in this study is reported as the partial derivative of area constraint energy with respect to global area. Therefore, we can calculate tension based on area constraint energy from our simulations according to the following formula:

$$\tau = \frac{\partial E_A}{\partial A} = \mu_A \frac{A - A_0}{A_0} = \sqrt{\frac{2\mu_A E_A}{A_0}}. \quad (3)$$

All parameters are defined along with the values used in Table S1 of the Supplemental Material [81], and for completeness, we also define all other variables of the theory/model in Table S2 of the Supplemental Material [81].

2. Energy function coupling the protein lattice to the continuum membrane

The particle-based protein lattice is assembled from rigid subunits with bonds between them. We treat proteins with particle-resolved structures, not as density fields [73–76], to assess structures that are stochastically sampled during self-assembly with either rigid or flexible bonds between subunits.

We assume that the bond between the lattice subunits and the membrane can be simplified to a harmonic bond connecting the center of mass (COM) of a protein monomer (here, the Gag protein) and the corresponding point on the limit surface of the control mesh vertex [Eq. (4)], which is initially set to the closest mesh vertex to the protein's center of mass and periodically updated every fixed number of steps. The harmonic bond potential and force used in rigid body interaction model are defined as

$$\begin{aligned} E_{\text{hb}} &= \frac{1}{2} k_{\text{hb}} (r_b - l_b)^2, \\ \mathbf{F}_{\text{hb}} &= -k_{\text{hb}} (r_b - l_b) \hat{\mathbf{r}}_b, \end{aligned} \quad (4)$$

where E_{hb} and $\mathbf{F}_{\text{hb}}$ are the energy and force exerted on the membrane due to the harmonic bond with parameters k_{hb} (the force constant) and l_b (the equilibrium bond length). r_b is the distance between the protein and the membrane, and $\hat{\mathbf{r}}_b$ is the unit vector of the displacement vector pointing from scaffolding protein to the lipid. Although the actual interaction between HIV Gag protein and lipid membrane involves a series of hydrophobic and electrostatic interactions between Gag's retroviral matrix (MA) domain and PI(4, 5)P₂ [77,78]; the bond length here describes a theoretical equilibrium distance between the center of mass of Gag protein and the membrane midplane. In the simulation, l_b is set to 9 nm.

The total membrane energy of the coupled membrane-lattice system will be the sum of the continuum membrane terms along with the contributions from the harmonic bonds connecting the surface to the lattice, and the lattice itself

$$E_{\text{tot}}(\mathbf{p}, A, r_b) = E_B + E_A + \sum E_{\text{hb}} + E_{\text{lattice}} + E_{\text{reg}}, \quad (5)$$

where here we note the addition of the purely numerical (not physical) regularization energy E_{reg} , which serves to protect the triangular mesh from deforming during gradient descent to maintain relatively uniform sizes and shapes of triangles for stable numerical integration [30] (Fig. 1). E_{reg} should converge to near zero as the numerical minimization of the system energy reaches its minimum and the forces also converge to zero. For analytical calculations, we therefore set $E_{\text{reg}} = 0$.

In most of our results, we assume that the membrane is much softer than the rigid protein lattice, since the estimated bending moduli of viral lattices is ~ 10 -fold higher than membranes [9,79,80], and thus $E_{\text{lattice}} = \text{constant}$. We describe in the next section the flexible lattice that relaxes this assumption. We also assume the links connecting the lattice to the membrane are effectively rigid to isolate the remodeling to the membrane surface. We therefore set k_{hb} to the very large value of 10^3 pN/nm, to keep these bonds effectively rigid. We do not expect softer links to the membrane to qualitatively change our results, as in recent work we found that their energetic cost is relatively small compared to the membrane and lattice energies [53].

3. Energy function for the flexible protein lattice

In Sec. III G, we relax the assumption of a rigid protein lattice. The lattice energy term is now dependent on the deformable structure of the protein lattice, $E_{\text{lattice}} \neq \text{constant}$. This energy function E_{lattice} characterizes the cost of each protein-protein bond length (σ_{ij}), angles (θ_{ij} , θ_{ji}), and tor-

sion (ω_{ij} , ϕ_{ij} , ϕ_{ji}) deviating from equilibrium values (such as $\sigma_{ij,0}$) using harmonic potentials. The corresponding force constants are k_σ , k_θ , k_ϕ , k_ω , and the energy function is

$$\begin{aligned} E_{\text{lattice}} &= \frac{1}{2} \sum_{\substack{i, j \in \text{bound} \\ \text{pairs}}} (k_\sigma (\sigma_{ij} - \sigma_{ij,0})^2 + k_\theta (\theta_{ij} - \theta_{ij,0})^2 \\ &\quad + k_\theta (\theta_{ji} - \theta_{ji,0})^2 + k_\phi (\phi_{ij} - \phi_{ij,0})^2 \\ &\quad + k_\phi (\phi_{ji} - \phi_{ji,0})^2 + k_\omega (\omega_{ij} - \omega_{ij,0})^2). \end{aligned} \quad (6)$$

For the Gag lattices assembled using nonequilibrium reaction-diffusion self-assembly simulator (NERDSS) simulations, each monomer can form up to five bonds with neighbors, one via its dimer interface, two that stabilize the hexameric rings, and two that stabilize a trimeric ring. Each of these bonds is parametrized by the above force constants and equilibrium bond and angle values. The automatic construction of this energy function from rigid lattice snapshots has been detailed in a recent paper [53]. We list the parameters used here in the Supplemental Material [81]. The force constants are chosen to have the same value across each harmonic potential to minimize the range of parameters, and the effects of varying individual force constants on the emergent rigidity of the lattice were characterized previously [53].

For the ideal lattices generated using Monte Carlo optimization (specified in Sec. II B 8), we added bonds between each protein center of mass to adjacent protein centers of mass by converting the lattice into a triangular mesh. We do so by projecting the cap to the XY plane and applying Delaunay triangulation, while the original 3D coordinates are retained as molecule COM. For each edge within the triangular mesh, paired binding interfaces are placed along the direct COM-COM connection line at a fixed distance of 1.5 nm from each molecule's COM. We can then use the same energy function as above, including both bond and angle terms, where we note that the ϕ angles are not defined due to collinearity of the defined vectors, and following the NERDSS definitions, we adapt the ω angle definition accordingly [24]. For additional details on the angle definitions, see Sec. 3.5 of the Supplemental Material [81]. The energy of the lattice coupled to the membrane is minimized using the same stochastic gradient descent algorithm as with the rigid lattice.

4. Analytical solutions to minimum energy structure of a membrane adhered to a spherical cap

For comparison with our numerical simulation results, we establish a series of theoretical approximations to the membrane bending energy induced by a perfectly spherical cap with a spherical radius of r_M . Previous work has analytically derived the membrane shape function that minimizes the energy of the lipid membrane upon attaching to a sphere, with and without membrane tension [20]. Specifically, the attachment region is assumed to be the same as the spherical surface. We follow the same approach as previous work by Deserno [20] but make one change, as we do not assume an infinite boundary condition; instead, we assume a finite boundary condition where the relaxation region becomes flat ($\frac{dh}{d\rho} = 0$) at a fixed distance $\rho = \rho_R$ and the rest of the membrane with

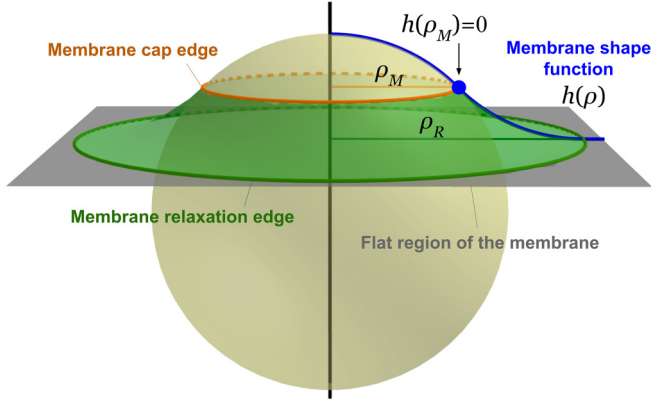


FIG. 2. Theory can predict the bending energy of a membrane deformed by a sphere, including a continuous curvature relaxation. We illustrate here how the shape function $h(\rho)$ that measures the membrane height at a radius of ρ from the z axis (black vertical line) is defined assuming a perfectly spherical indentation with azimuthally symmetric relaxation. The scaffolded spherical cap is delimited by the orange line at a radius of ρ_M . The membrane at large distances is assumed to be flat (gray surface). The relaxation region in green captures the continuous change in curvature from the cap to the flat membrane at the radius ρ_R . The membrane shape function captures these changes in curvature, as the theory assumes radial symmetry.

$\rho > \rho_R$ is completely flat, as a more accurate representation of the finite simulated system size (see Fig. 2).

The simplest cap model assumes that only the attachment region matters and the energy of the rest of the membrane is negligible. The bending energy of the attachment cap region $E_{B,\text{cap}}$ is then

$$E_{B,\text{cap}} = 4\pi\kappa(1 - \cos\theta), \quad (7)$$

where θ is the polar angle from the z axis to the radii pointing to the end of the spherical cap. Therefore, $\sin\theta = \frac{\rho_M}{r_M}$ and ρ_M is the radial distances from the z axis to the start of the relaxation function (see Fig. 2 and Fig. S2 of the Supplemental Material [81]). With no relaxation region, ρ_M is the radius where the sphere intercepts the flat plane. For this simple cap, we can equivalently note that $h = r_M(1 - \cos\theta)$, with h the height of the spherical cap. The area of this spherical cap is given by $A_{\text{cap}} = 2\pi r_M h$. The energy per unit area is thus independent of cap size, as it is dependent only on the sphere radius: $E_{B,\text{cap}}/A_{\text{cap}} = 2\kappa/r_M^2$. However, if we consider a patch of the membrane where at the boundary the membrane becomes flat ($\frac{dh}{d\rho} = 0$), there is a continuous curvature change region between the cap and the boundary, which we call the “relaxation region” in the following derivation (Fig. 2). The shape function of this relaxation region that detaches from the sphere will determine its bending energy. To briefly describe the solution, consider the shape function $h(\rho)$, where h measures the height of the membrane and ρ measures the azimuthal distance from the z axis at the origin, given a membrane bending modulus κ . We make three simplifying assumptions: (1) Azimuthal symmetry: For any real function F , $\frac{\partial F}{\partial \phi} = 0$. (2) Negligible spontaneous curvature: $C_0(\mathbf{p}) \approx 0$. (3) Negligible membrane tension: $E_A \ll E_B$, and therefore

$E \approx E_B$. We solve for the shape function under the following three boundary conditions: (1) Membrane is continuous at transition: $h(\rho = \rho_M) = 0$. (2) Membrane is differentiable at transition: $\frac{dh}{d\rho}|_{\rho=\rho_M} = -\tan\theta$. (3) Membrane becomes flat at end of relaxation: $\frac{dh}{d\rho}|_{\rho=\rho_R} = 0$, where ρ_R is the radial distance from the z axis to the end of the relaxation function.

The bending energy of the relaxation region $E_{B,\text{relax}}$ then turns into

$$\begin{aligned} E_{B,\text{relax}} &= \int_{\rho_M}^{\rho_R} \int_{\phi=0}^{2\pi} \frac{1}{2} \kappa \frac{(h'' + \frac{h'}{\rho} + \frac{h'^3}{\rho^3})^2}{(1 + h'^2)^3} \rho d\phi d\rho \\ &= \pi \kappa \int_{\rho_M}^{\rho_R} \frac{(h'' + \frac{h'}{\rho} + \frac{h'^3}{\rho^3})^2}{(1 + h'^2)^3} \rho d\rho. \end{aligned} \quad (8)$$

The shape function $h(\rho)$ of the relaxation region that minimizes $E_{B,\text{relax}}$ is then solved numerically (see detailed result and background [82] in Sec. 3.3 of the Supplemental Material [81]). To compare the prediction of the cap and relaxation model,

$$E_{B,\text{cap+relax}} = E_{B,\text{cap}} + E_{B,\text{relax}}, \quad (9)$$

with our analytical results, we use $r_M = r_G + l_b$ to include the membrane displacement by the lattice (r_G) and the harmonic bonds (l_b). We set $\rho_R = 135$ nm, which is half of the boundary box size of the simulation.

This analytical function assumes that the tension is zero, and therefore the area energy does not contribute to the total energy, which is distinct from the energy function used to minimize the continuum membrane. To assess the impact of the additional area energy term on the predicted shape function of the relaxation region and its energy, we therefore also evaluated the minimum energy solution with the addition of the area energy (see Sec. 3.3.3 of the Supplemental Material [81]). The solution for $h(\rho)$ is no longer analytic due to the addition of the boundary conditions on the domain size. We solve for this iteratively (see the Supplemental Material [81]), finding that this more accurate model predicts only relatively small changes in $h(\rho)$ and a $<2\%$ change in the bending energy. We therefore conclude that any deviations between theory and our continuum membrane simulations are not due to inconsistencies in the membrane tension assumptions.

B. Numerical methods

1. Background on the finite-element model for defining surface geometry

Numerical implementations of continuum surface models use the FEM. Here, we use the subdivision limit-surface technique [83,84] as described in previous research [30] and implemented in our previous work [31], as it allows for continuous and differentiable measurements of membrane curvature along the surface [30] (Fig. 1). The advantage of this method is that instead of measuring energy along the discrete and discontinuous triangular mesh, here referred to as the “control mesh,” the energy is evaluated on the smooth limit surface, which has continuous first and second (excepting irregular vertices) derivatives needed for calculating forces and curvatures. Mathematically, any arbitrary point $\mathbf{p}$

on the limit surface S is determined by a weighted average of the surrounding backbone vertices $\mathbf{q}_l$ of the control mesh [Figs. 1(c) and 1(d)]. The point $\mathbf{p}$ can be represented by barycentric coordinates (u, v) on its local element triangle, facilitating a spline fit using shape functions [84]. We provide further implementation details in Secs. 3.1 and 3.2 of the Supplemental Material [81].

The energy function in Eq. (1) is widely used in continuum models of membranes, and we verified that our numerical implementation correctly evaluates the energy defined above (Fig. 1). In previous work, we showed that our numerical implementation reproduces shape changes including with the addition of volume constraints for enclosed membranes (e.g., vesicles) [31]. We further quantified shape and energy variations with local changes to spontaneous curvature as produced by helix-inserting proteins [31] and by scaffolding of the membrane by rigid clathrin lattices [19]. We tested that the addition of the regularization energy E_{reg} to ensure the reshaping of the triangular mesh does not disrupt convergence to a low-energy structure [31,46]. We note that we have not implemented edge flipping or dynamic remeshing of our triangular mesh [85,86]. As a result, we limited our lattices to a radius of up to 40 nm (max azimuthal radius of protein lattice is 50 nm, which is the spherical radius of the Gag lattice used in the simulation). With larger lattices, the numerical reshaping of the membrane topology leads to large changes in mesh triangles that limit numerical convergence.

2. Boundary conditions for the continuum membrane model

All simulations are run under periodic boundary condition in $\mathbf{X}$ and $\mathbf{Y}$ but not in the $\mathbf{Z}$ direction, to mimic modeling a patch of a much larger membrane surface. We use a box of 270 nm $\times$ 270 nm in $\mathbf{X}$ and $\mathbf{Y}$ directions (unlimited in $\mathbf{Z}$ direction) as a compromise to balance finite size with numerical speed of calculations. We also verified for a subset of smaller lattice simulations that varying the box size did not significantly affect the results. For the periodic boundary, we create three rows of temporary or “ghost” vertices outside the edge of the mesh with positions determined by translation of the mesh vertices at the opposite edge. Three rows are needed because with the limit-surface setup, each vertex requires 12 surrounding vertices to compute a position $\mathbf{p}$ on the limit surface (see Sec. 3.2.4 of the Supplemental Material [81] for implementation details on the periodic boundary condition).

3. Numerical integration of the continuum energy function across the surface

We use the second-order Gaussian quadrature scheme to numerically estimate the integral in our energy function. In our previous research, we demonstrated that the second-order Gaussian quadrature produces membrane structures with adequately accurate and converged estimations of the energy, while having a considerably lower computation time cost compared to integral methods with higher precision [31].

4. Self-assembled lattices from NERDSS simulations

We previously ran stochastic, particle-based reaction-diffusion simulations of Gag lattice self-assembly with the NERDSS software [24]. The highly coarse-grained rigid

structure of each Gag monomer and its interactions with one another were designed from the experimental Cryo-ET structure of the immature Gag lattice [12]. The model and methods were previously published [25], so we briefly summarize here. Each Gag monomer has five interaction sites for another Gag monomer: one homodimer site, two hexamer sites that bind to form a hexameric ring, and two trimer sites that form a trimeric ring. The protein monomers from the NERDSS model have a surface area of $\sim 8.5 \text{ nm}^2$ by assembling into spherical shells with a trihexagonal lattice containing up to $N_{\text{sphere}} = 3700$ monomers when the radius $r_G = 50 \text{ nm}$, consistent with the Cryo-ET structure [27]. The curvature of our lattice is constant at r_G^{-1} . The enveloped membrane is offset from the lattice by the harmonic bonds, giving the membrane the radius of 59 nm and an average membrane area per monomer of 11.8 nm^2 . Because a rigid hexagonal lattice does not perfectly tile a sphere, the lattice also contains defects, which is consistent with the experimental structures [25,26]. Depending on the initial conditions and strength of interactions (controlled by rates k_{on} and k_{off}), the Gag lattices do not always form complete lattices but instead become kinetically trapped in intermediates with varying sizes and morphologies [25]. We sampled from these Gag lattice structures that are stochastically assembled during our 3D simulations as a function of monomer numbers and morphology. The morphology of lattices can be quantified by a regularity index that measures deviations from ideal spherical growth. Note that these solution simulations did not include any membrane. The relationship between the number of Gag monomers n_{Gag} in the NERDSS lattices and azimuthal radius ρ_G of a compact or ideally spherical Gag lattice cap is given by $\rho_G = 2r_G \sqrt{(\frac{n_{\text{Gag}}}{N_{\text{sphere}}})(1 - \frac{n_{\text{Gag}}}{N_{\text{sphere}}})}$. This is used to compare with the theoretical predictions.

5. Controlling membrane tension by specifying the equilibrium area and assigning confidence intervals

The total membrane energy depends on the area constraint energy E_A , which is parametrized by μ_A and the equilibrium area A_0 . Experimentally, most living cells have a relatively low tension, with typical values in the range 0.01–0.04 pN/nm [87] and in higher tension cellular membranes such as cytoskeleton-scaffolded membrane in moving cells and membranes of epithelial cells under osmotic stress are 0.15–0.30 pN/nm [88,89]. To keep our remodeled membranes at nonzero tension defined in Eq. (3) while minimizing E_{tot} , we optimize the values of A_0 (see Fig. S3 of the Supplemental Material [81]). Note that this approach is different from assuming negligible tension in the system, i.e., $\mu_A = 0$ and thus $E_A = 0$. However, the minimized surfaces do tend to have a quite low, but nonzero, E_A . We do not use a tensionless membrane ($\mu_A = 0$), as this is nonphysiological and supports membrane buds that are indefinitely tall with gradual curvature changes in the relaxation region that eliminate bending energy costs in this region (not at the cap). We verify that we can achieve a reasonable tension as well as we vary A_0 (Fig. S4 of the Supplemental Material [81]). Given that the minimum energy and tension depend on the specific choice of A_0 (Figs. S3 and S4 of the Supplemental Material [81]), we generated samples of 8–16 minimum energy

measurements due to variations in A_0 around our numerically determined energy minimum. We report in our results the minimum energy values we find along with error bars from the sampled range of energy values. Our reported confidence intervals are calculated with bootstrap resampling tests (Sec. 3.6 of the Supplemental Material [81]).

6. Definition of initial membrane shape under the protein lattice

Most simply, the membrane would be initialized in a flat state, which works well when the equilibrium configuration remains close to flat [31], resulting in small forces. In our case, starting from a flat membrane configuration attached to the Gag lattice is challenging. It leads to large forces in the cap region and minimal forces near the end of and beyond the relaxation region. This uneven distribution of force together with a large maximum force complicates the energy minimization process: If we use the step size according to the empirical step-size rule, it becomes very small and slows convergence. In contrast, using a large time step results in mesh distortion, ultimately causing the FEM solver to fail. Interestingly, despite the slow convergence, we could push these systems into local minima, but they had a significantly higher energy and distinct structure from our best solutions reported below (Fig. S5 of the Supplemental Material [81]). To resolve the difficulties with converging these structures and test the dependence on initial conditions, we initialized the system with a precurved configuration (see Sec. 3.2.5 of the Supplemental Material [81]). The membrane is initially put in a near-to-equilibrium state that is based on the analytical solution to the membrane shape solved with no tension and the small-gradient approximation. However, in this analytical solution, the membrane bending energy in the relaxation region converges to zero at infinite distance and results in infinite membrane bulge height, which is not feasible (or physically realistic) within a finite bounded simulation. Therefore, we give the model a relaxation radius (ρ_R) and calculate the lowest energy state analytical solution with that relaxation radius.

7. Computational optimization and energy minimization of the coupled system

We use nonlinear conjugate gradients with the Wolfe condition [90] to propagate the membrane shape and minimize the total energy, and at times we switch to steepest descent when minimization proceeds too slowly (see Fig. S6 of the Supplemental Material [81]). The direction of propagation of the Gag lattice is based on the force exerted by the membrane on the Gag lattice. The forces and their numerical implementation following from the bending and area energies have been defined in previous work [31,46], and the forces from the harmonic coupling to the protein lattice are defined in Eq. (4). The step size is controlled as a small value satisfying the empirical rule, $\max(\|\text{force}\|) \times \text{stepSize} \ll \text{IFace}$, where IFace is a constant hyperparameter (Table S3 of the Supplemental Material [81]). The number of propagations of the Gag lattice per propagation step of the lipid membrane is a free parameter (see Table S3 of the Supplemental Material [81]). In the simulation, k_{hb} is initially set to a small value 1.5 pN/nm and gradually increased to a final value of 10^3 pN/nm, which

makes sure that the finite element mesh does not deform too rapidly from large forces.

8. Theoretical lattices from Monte Carlo optimization

To establish a theoretical baseline to compare with the self-assembled lattices from stochastic NERDSS simulations [24], a Monte Carlo optimization program is used to generate theoretical lattices with a given lattice size (maximal radius) and eccentricity, e . For an ellipse defined as

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} = 1 \quad (b \geq a > 0), \quad (10)$$

where b is the major axis and a is the minor axis. The eccentricity e is then defined as

$$e(a, b) = \sqrt{1 - \frac{a^2}{b^2}}. \quad (11)$$

Here, the Gag “monomers” are simplified to nodes lying on the spherical surface, and instead of placing the monomers at the position of every Gag monomer, we place the mesh attachments at the centers of a hexagonal lattice. Specifically, the Monte Carlo optimization starts with a set of randomly placed nodes representing these attachment sites on the Gag spherical surface within the boundary defined by the major and minor axes. To generate an “ideal” mesh with evenly placed nodes at a separation of l_{gg} apart, we used a Monte Carlo simulation subject to a Lennard-Jones potential [91] between the nodes restricted to the surface and the boundary. The minimum of the Lennard-Jones energy is placed at the separation l_{gg} , which is by default = 4.7 nm, and is increased to test the role of attachment density in Sec. III. This density of attachments is less than if every Gag monomer from the NERDSS lattices were to link to the membrane by threefold ($11.8\text{--}31.4\text{ nm}^2$ per attachment). We nonetheless refer to these attachment sites as enumerating the number of Gag “monomers,” despite the lower density. To accelerate the simulation, the Lennard-Jones potential only applies between each node and its closest neighbor using KDTree. The method is further elaborated in Sec. 3.4 of the Supplemental Material [81].

9. Nonzero spontaneous curvature of the membrane

In Sec. III F, we relax our assumption that the membrane spontaneous curvature is zero. Here, we altered the spontaneous curvature to a fixed, nonzero value ($C_{0,a}$) at the element triangles of which at least one vertex is attached to the protein lattice. We did not change the global spontaneous curvature due to two reasons. First, changing the spontaneous curvature only at the attachment more accurately captures how the protein can locally induce changes to the adjacent leaflet by, for example, inserting a helix or lipidation group into this leaflet [46,92]. Moreover, under small-gradient approximation, we can prove that the minimum bending energy shape of the membrane when attached to a spherical shell is insensitive to the change in global spontaneous curvature (see Sec. 3.3.5 of the Supplemental Material [81]). This only leads to a global shift in bending energy but does not change the minimal energy shape function. We note that asserting a nonzero spontaneous curvature to a single-layer continuum model is somewhat ambiguous, because while each leaflet can have a

nonzero value, a symmetric bilayer will maintain a preferred curvature of zero. Nonetheless, recent numerical simulations on a double-layer continuum model have demonstrated that it is the spontaneous curvature of the adjacent leaflet, and not the bilayer that dominates the bending energy cost of the membrane deformation [46].

10. Quantifying the cylindrical asymmetry e_{cyl} of membrane deformations

We measured cylindrical asymmetry of the membrane surface after fitting/centering the cap region while excluding boundary-dominated regions. The cylindrical asymmetry e_{cyl} is defined as the approximated eccentricity averaged by areal weight over 64 contour planes, and has a value between 0 (perfectly spherical) and 1 (collapsed to a line). The eccentricity is calculated from a complex value q_k (usually called polarization or quadrupole ellipticity) that is used in weak gravitational-lensing research [93,94], which applies second-moment or Minkowski tensors to evaluate contour-dependent shape anisotropy (see Sec. 3.5.3 of the Supplemental Material [81] for details).

11. Fourier-space dynamics simulation of the membrane deformation by lattices at physiologic temperatures

To verify the shape changes of the membrane induced by a rigid or flexible lattice at a physiologic temperature, we also performed dynamic simulations of a continuum membrane coupled to our lattices using Fourier-space Brownian dynamics (FSBD) with periodic boundary condition [52] implemented via *hoomd-blue* [95]. Compared to our energy minimization approach in all other results, here we introduce stochastic dynamics at a finite temperature of 310 K. Otherwise, the energy function is preserved: The membrane is represented as a continuum following the Helfrich energy function, the lattices are again attached to the membrane using harmonic links, and the lattice is either simulated as a fully rigid structure or following the flexible energy function [Eq. (6)]. Briefly, in FSBD, the membrane height field is represented by the Fourier amplitudes, and the simulation state is updated directly in Fourier space rather than by moving real-space mesh vertices or converting back and forth. Each mode was advanced directly in Fourier space according to a Brownian dynamics equation containing the deterministic forces from the energy function and stochastic thermal fluctuations that incorporate hydrodynamics [52]. The real-space trajectories are then reconstructed via inverse Fourier transform. The canonical ensemble sampled during the trajectories is evaluated on the second half of the trajectory to capture equilibrium. This simulation software is available open source in Ref. [96]; further validation and applications of this method are described in a recent publication [53].

III. RESULTS

A. Stochastically assembled lattices vary in their sizes and regularities, leading to varied membrane energy

As we show in Fig. 3, when we bend the membrane via rigid lattices that were stochastically assembled, the total membrane energy cost is higher when the lattice contains

a larger number of Gag monomers. This increase in membrane energy cost is consistent with theoretical predictions for spherical caps of increasing size [Eq. (6)], primarily driven by the bending energy [Fig. 3(b)]. However, the value of the membrane energy from the stochastic lattices is frequently much higher than that predicted by theory, even with the finite-size boundary correction [Fig. 3(b)]. The simple cap theory assumes the relaxation area energy is zero and thus disregards any boundary size effect. The cap and relaxation theory accounts for the boundary size effect [Eq. (8) and Sec. II], but this is still insufficient to explain the discrepancy with the simulations. Visual inspection of the Gag lattice structures suggests that more irregular or noncompact lattices result in larger membrane energy and thus larger deviations from the theoretical predictions. For example, the 227-Gag configuration is highly irregular relative to an ideal spherical cap and shows a significantly higher energy than predicted, whereas the 157-Gag case is more regular and only deviates moderately. We note that even for irregular lattices, it is the perimeter that is irregular and not the curvature, which remains constant at r_G^{-1} throughout the lattice.

Interestingly, despite the irregularity of the lattices, the membrane also prefers to bend into a symmetric caplike deformation, with minimal “wrapping” around the irregular perimeter [Fig. 3(c)]. By performing the optimization from an initially flat membrane, we were able to sample structures with a wrapped, irregular membrane shape (Fig. S5 of the Supplemental Material [81]). However, these metastable minima have significantly higher energy than the caplike deformations, meaning they are less favorable and would be escaped with sufficient thermal energy (the energy minimization is effectively zero temperature). Although the symmetric caplike deformations reshape a larger area of the membrane than the contour-hugging deformation, the curvature of these caplike surfaces is lower, integrating to a lower total bending energy. Hence, in this case, a larger bending area of lower curvature is more energetically favorable than a smaller bending area but of higher curvature. As we show below, we can systematically vary the regularity of the lattice by using idealized meshes that deviate from perfect sphericity, as quantified by their eccentricity, e , and reproduce this same trend of high energy, but still caplike deformations induced by irregular lattices.

B. Membrane bending energy per protein is higher for more eccentric lattices

To systematically test the role of lattice asymmetry on the energetics of pathways of lattice growth coupled to membrane bending, we constructed artificial hexagonal lattice meshes using Monte Carlo sampling (Sec. II). For each lattice size, we systematically varied the eccentricity of these lattice caps, keeping the lattice curvature constant at $r_G^{-1} = (50 \text{ nm})^{-1}$, meaning that where the lattice attaches to the membrane, the preferred curvature would match a spherical cap [Fig. 4(a)]. Higher eccentricity reflects an increasingly elongated arrangement of the lattice “monomers.” In the ideal cap, the monomers form a lattice with radius r , whereas in the eccentric lattice, the major axis has a radius $b > r$, and minor axis $a < r$. Our simulation results show that the higher the eccentricity of the lattice, given the same number of Gag monomers

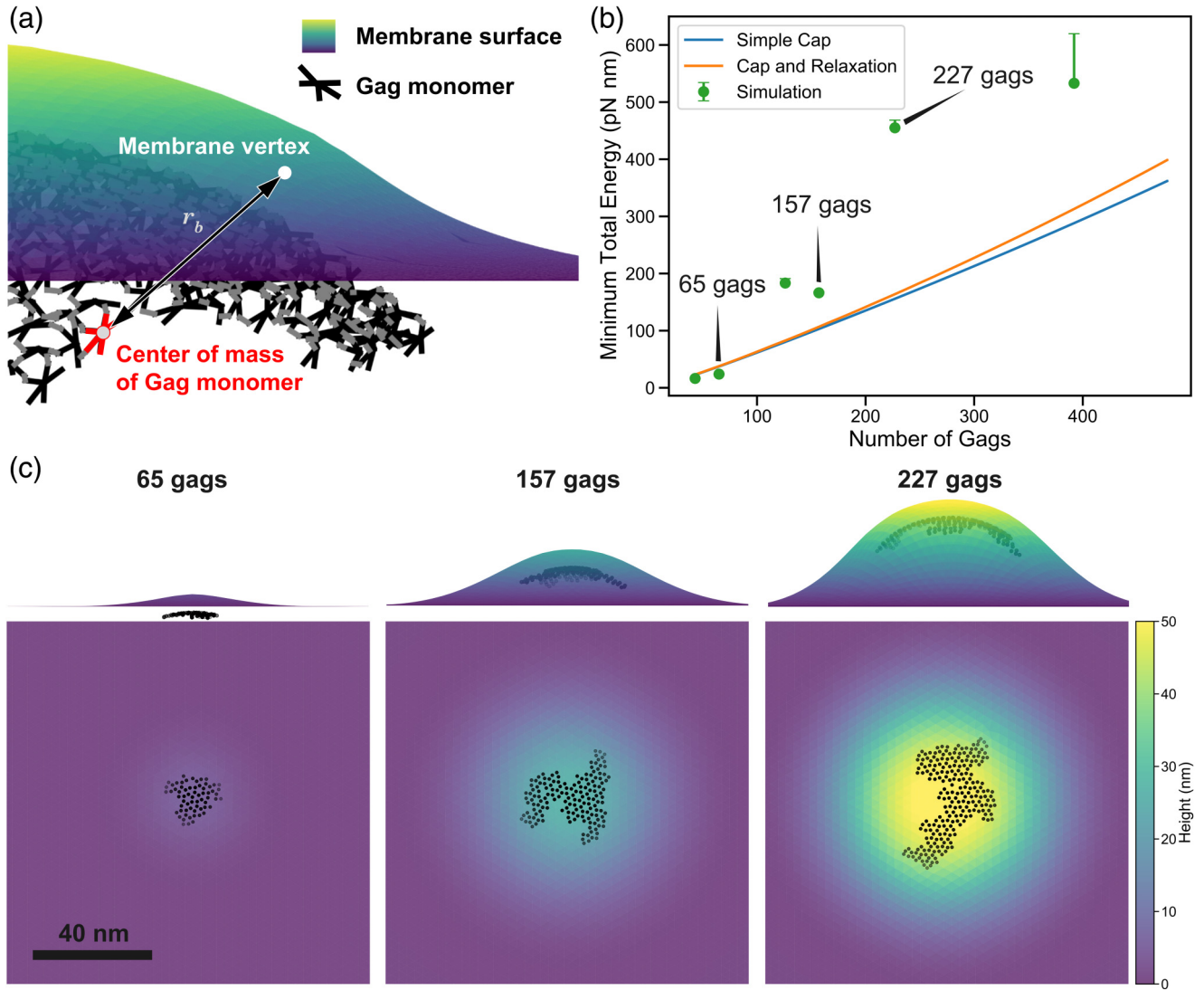


FIG. 3. Energy increases with the size and irregularity of the Gag lattices. (a) Rigid Gag lattices stochastically assembled from NERDSS simulations are connected to the membrane surface via harmonic bonds. Gag monomers are in black; surface is contour in colors by height. The average membrane area per attached Gag is 11.82 nm^2 based on a harmonic bond length of 9 nm . (b) The minimized total membrane energy measured for the continuum membrane surface plus the harmonic bonds (Sec. II). Theoretical curves are calculated based on a simple (discontinuous) cap model blue curve [Eq. (6)] and accounting for a continuous curvature relaxation with the boundary size effect orange curve [Eq. (8)]. Error bar represents 95% confidence interval from bootstrap resampling based on a single Gag lattice structure, but across variations in the values of A_0 (see the Supplemental Material [81]). See Sec. II for converting number of Gags to radius used in theory. (c) Increasing the sizes of the Gag lattices on the membrane acts as a proxy for the time-evolved pathways of coupled self-assembly and remodeling. Energy-minimized structures of Gag lattices with the continuum membrane surface show increased membrane remodeling around larger lattices. Each black point is the center of mass of a Gag monomer, revealing the trihexagonal lattice tiling. A membrane energy minimization is run with bending modulus of $\kappa = 83.4 \text{ pN nm}$, area stretching constant of $\mu_A = 250.0 \text{ pN/nm}$, and $270 \text{ nm} \times 270 \text{ nm}$ boundary, which is maintained in all simulations.

(lattice nodes), the higher the total membrane energy, $>99\%$ of which arises from the bending energy (Fig. 4(b) and Fig. S7 of the Supplemental Material [81]). The minimal contribution of the area stretching energy to the total energy is expected, as we are focused on a regime of low membrane tension. The more eccentric lattices clearly drive a larger invagination of the membrane given the same number of monomers (Fig. 4(c) and Fig. S8 of the Supplemental Material [81]). Thus, we see a deformation with a similar height when 137 monomers bend the membrane with $e =$

0.87 versus 302 monomers at $e = 0$ (Fig. 4). The height of the deformation, measured simply as the difference between the maximum and minimum z coordinate of our control mesh vertices [Fig. 4(c)], correlates well with the energy [Fig. 4(b)], as expected from the theory of spherical caps. Here, again we see that despite the noncircular perimeter of the eccentric lattices, the membrane still bends into a relatively circular or radially symmetric invagination with a radius of $\sim b$.

The penalty associated with bending the membrane with growing lattices that are more eccentric is not linear in the

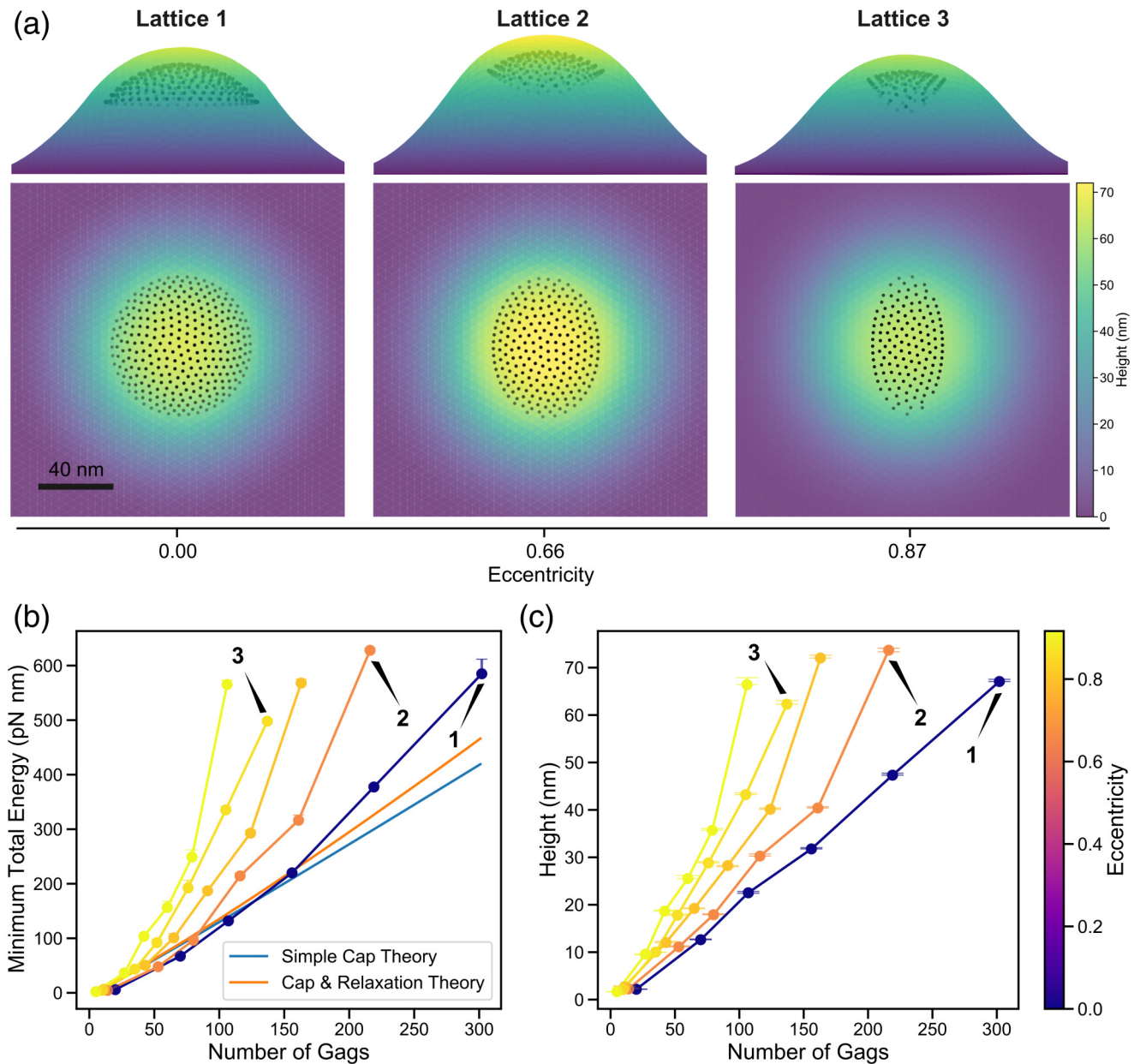


FIG. 4. Lattice growth pathways with high eccentricity induce a higher membrane energy cost. (a) Idealized lattice structures of “Gag” attachments are harmonically linked to the membrane and energy minimized just as done for the NERDSS Gag lattices. From left to right, the size of the major axis remains the same at 40 nm, but the eccentricity increases, causing a reduction in total surface area. Left has 302 Gags, center has 216 Gags, and right has 137 Gags, illustrating how a similar-sized invagination is produced by a small number of Gags when they are more eccentrically arranged. (b) The minimized total membrane energy when attached to lattices of increasing eccentricity (color bar) assembled with Monte Carlo method vs the number of “Gags” or nodes in the lattice. Error bar represents 95% confidence interval from bootstrap resampling across variations in A_0 . Over 99% of the total energy is due to the bending energy (Fig. S7 of the Supplemental Material [81]). Theoretical curves are calculated from Eqs. (6)–(8). For conversion from number of Gags to cap radius, see Sec. 3.3 of the Supplemental Material [81]. The energy per unit area, which is constant for the simple cap theory, is thus increasing for highly eccentric lattices, as they have the same area but significantly higher energy and faster slope of growth with increasing lattice size. (c) Membrane cap height increases with the eccentricity of the lattice with minimum variance. The height of the membrane cap at minimized total membrane energy when attached to Gag lattices of different sizes and eccentricities assembled with Monte Carlo method vs the number of Gags in the lattice. Error bar represents 90% bilateral confidence interval from joint bootstrap resampling. The height of the invagination and the energy are closely correlated.

size of the lattice, as suggested by the energy growth in Fig. 4. For small lattices, the membrane bending energy cost due to eccentric lattices is modest, whereas for large lattices, the bending energy cost grows rapidly (Fig. S9 of the Sup-

plemental Material [81]). The energy of the lattice itself is not considered to contribute here, we note, due to our assumptions of rigidity. These observations further confirm our previous hypothesis: More irregular or eccentric lattice configurations

impose greater energetic penalties on the membrane particularly as they grow larger.

We note that lattices with $e = 0$ form effectively ideal spherical caps, and therefore we expect our numerical simulation results to agree well with the theory in Eqs. (6) and (8) plotted in Fig. 4(b). While the agreement is quite strong, the energy increase with size is somewhat faster for the continuum model. For the small lattices, the continuum membranes have a *lower* energy than theory predicts, whereas for the larger lattices, the continuum membranes have a *higher* energy than theory. At the largest structure, the continuum membrane predicts a higher energy by $\sim 20\%$, which cannot be explained by theoretical assumptions on the role of membrane tension (for example, see Sec. II and the Supplemental Material [81]). By comparing the shape functions of the membrane invaginations predicted by theory with the corresponding cross section of our minimized continuum model solutions, we see they do not overlap perfectly (Fig. S10 of the Supplemental Material [81]). For the large caps, the continuum model predicts a noticeably steeper relaxation region, driving up the bending energy, whereas the deviation is small for the smaller caps (Fig. S10 of the Supplemental Material [81]). These deviations likely stem from the assumptions of the theory, which predicts a complete cap that is then continuously connected to the relaxation region. In contrast, the continuum model can simultaneously modify the shape of both the cap and relaxation regions. Further, the continuum model minimizes the energy of the membrane and the harmonic links to the membrane by moving the lattice, which could bias the final, zero-temperature structure. Because we are performing energy minimization in a very high-dimensional space, our solution could also represent a local minimum, based on the initial height we applied to the membrane and the limits of the periodic boundary conditions. Increasing the membrane size or changing the initial starting configuration to match the theory could result in tighter agreement between both shape and energy.

C. Low global tension in the system

We verified that in our energy-minimized systems of coupled lattices and membranes, the tension did not vary significantly (Fig. S11 of the Supplemental Material [81]). By calculating membrane tension using Eq. (3), we showed that our membrane tension is controlled within physiologic regimes (or lower). Although the tension does increase systematically with the size of the lattice and with its eccentricity, the tension remains below 0.014 pN/nm, with estimated upper bounds on most cell types reaching 0.04 pN/nm [87]. We nonetheless validated that this growth in tension is not the reason for the increasing total energy (Fig. S12 of the Supplemental Material [81]). The area constraint energy, which relates to the membrane tensions [Eq. (3)], contributes minimally to the total energy of the optimal structures ($E_A/E_{\text{tot}} < 10^{-4}$) (Fig. S11 of the Supplemental Material [81]).

D. Density of monomer attachments to the membrane has a minor impact on bending energy

To test whether the density of monomer attachments to the membrane impacted the bending and total energy, we

increased the separation d_{MM} between the monomers while keeping the perimeter size fixed by an azimuthal radius of 30 nm [Fig. 5(a)]. With fewer attachments to the membrane, we do see that the invagination by the lattice becomes slightly higher [Fig. 5(a)]. However, this has negligible impact on the bending energy for the higher densities, where from $d_{\text{MM}} = 3.76$ to 5.64 nm, no significant change occurs in bending energy [Fig. 5(b)] or total energy [Fig. 5(c)]. For reference, we include the simple theoretical cap estimate of the bending energy Eq. (6), which is relatively close to the simulated membrane bending energies (Fig. 5). The simulated values must exceed this theoretical minimum because of the addition of tension and boundary effects. For the lower density lattices, $d_{\text{MM}} = 7.05$ – 9.4 nm, we do see a modest increase in bending energy that is statistically significant, indicating that the very sparse connections do drive higher invaginations which eventually creates a higher bending energy cost. However, the absolute differences are small—all under 14.2 pN nm, which is less than 7% of the total energy. These results show that while the density of attachments between the rigid lattice and the membrane can increase the height of the deformation with lower attachments, its impact on membrane energy is minimal. Hence, the size of the perimeter of the attached lattice is driving the bending energy costs.

E. Circumscribing cylindrical sector estimates the attached membrane energy

From the above results, we find that the shape of the perimeter rather than the density of Gags is the major determinant of the attached membrane energy. Therefore, an assumption is that a Gag lattice with the same circumscribing cylindrical sector that encloses the largest radial dimension of the lattice of $\sim b$ (Fig. 6) leads to the same minimum bending and total energy of the attached membrane. This provides a consistent way to compare pathways of lattice growth of varying shape, regardless of Gag density (Fig. 5) and encompassing all eccentricities. It also clarifies further the origins of the increased energy cost—with the same lattice area, a highly eccentric lattice has a larger maximal radial dimension. We indeed find that the minimized bending energies of membranes of all our lattices agree well with the theoretical approximation when we plot it according to the cylindrical radius (Fig. 6). The theory calculates the bending energy of a spherical invagination with the corresponding radius Eqs. (6)–(8). We observe that the agreement is not perfect, and indeed, the more spherical lattices ($e \rightarrow 0$) tend to have energies that exceed the theoretical estimate. For the higher eccentricities, we see that the energy is typically lower, indicating that the bending energy cost is not quite as bad as one would expect for an eccentric lattice with major axis b versus a fully spherical lattice with radius b . This indicates, as we quantify further below, that there is some radial asymmetry in the bending around the lattice of highly eccentric lattices that does reduce the surface area of the bending membrane. In other words, given a perfectly symmetric lattice of radius b , if some Gag monomers were removed to generate asymmetry where the major axis is still b , the bending energy would decrease slightly, where now the area of the lattice has also decreased. Finally, for the larger invaginations, they all tend to produce larger bending energy

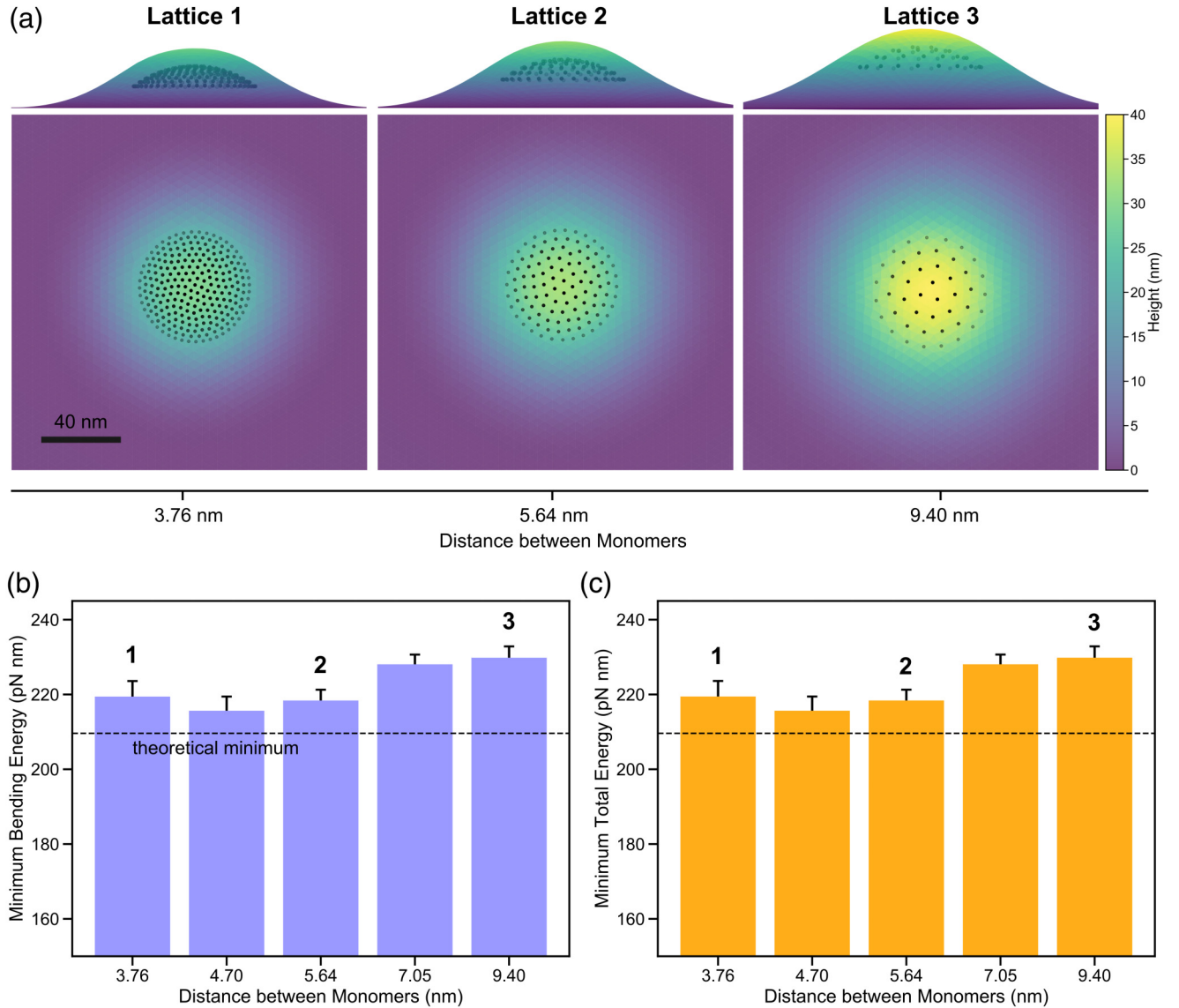


FIG. 5. As density of Gags connecting to membrane is lowered, energy is not substantially changed. (a) After fixing the size (azimuthal radius of 30 nm) and eccentricity ($e = 0$) of the Gag lattice cap, we vary the number of equally spaced monomers/lattice points that attach to the membrane via the distance d_{MM} between monomers, from dense ($d_{MM} = 3.76$ nm) to sparse ($d_{MM} = 9.4$ nm). The heights of the invaginations are similar (colorbar), with lattice 3 slightly higher. (b) The bending energy is relatively constant as d_{MM} increases and fewer monomers attach to the lattice, indicating that it is the perimeter size (azimuthal radius) of the lattice that predominantly determines the bending energy. (c) As expected, the total energy displays the same trends as the bending energy. Error bars show 95% confidence interval of minimum energy from bootstrap resampling. Theoretical minimum bending energy (209.6 pN nm) assumes a simple cap model with no membrane tension [Eq. (6)]. We note there are statistically significant differences between the energy of the smaller lattices ($d_{MM} \leq 5.64$ nm) relative to the $d_{MM} = 7.05$ and 9.4 nm lattices, indicating a somewhat higher bending energy once attachments are sufficiently sparse.

costs than theoretically predicted [Fig. 6(a); $r = 40$ nm], as discussed in Sec. III B, this is likely because of the variations in the minimized shape around the rigid lattice compared to the theoretical predictions.

F. Membrane energy is minimized when spontaneous curvature at the attachment conforms to the lattice

In the above results, we assumed that the spontaneous curvature of the membrane has a flat curvature preference, or $C_0 = 0$. Since both lipid composition or protein insertions

can change this preferred curvature, which for Gag could be driven by its myristoyl insertion [92], we here tested how the bending energy varied with C_0 for a spherical ($e = 0$) and highly eccentric ($e = 0.866$) lattice (Fig. 7). Both membrane bending energy and total energy conform to our theoretical prediction that they minimize around the spontaneous curvature that matches the curvature imposed by the rigid Gag lattice, of -0.034 nm^{-1} . This demonstrates that if the Gag monomers can induce a small negative curvature, the bending energy can be lowered substantially, although within a relatively narrow window (Fig. 7); for negative

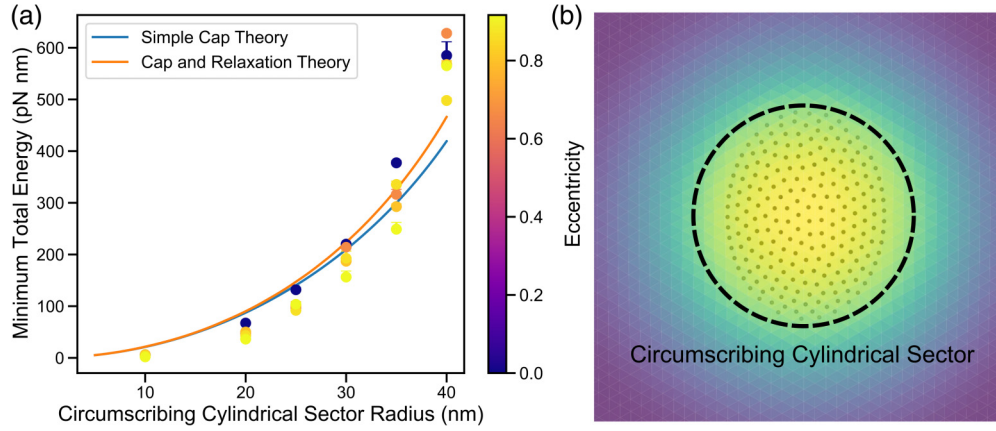


FIG. 6. The membrane energy induced during rigid lattice growth at all eccentricities is largely described by the largest radial extent of the lattice. (a) Our data collapse onto a universal curve with relatively good agreement when plotted vs the radius of the circumscribing cylindrical sector rather than the number of Gags within the lattice. Same data points as previous figure, but with x axis redefined. Simple cap theory from Eq. (6) (blue) and addition of boundary effects and relaxation without the small-gradient approximation from Eq. (8) (orange). (b) We illustrate the circumscribing cylindrical sector here for a representative $e = 0.66$. It is the cylindrical radii that fully enclose the lattice, extending out therefore to the largest radial distance present in the lattice, the major axis b .

$C_0 < -0.06 \text{ nm}^{-1}$, the bending energy cost will grow quadratically worse than even for a membrane with a flat preferred curvature. These results are also consistent with the previous section. A highly eccentric lattice has a similar total bending energy cost only when the circumscribing radius matches the spherical cap (Fig. 7). Hence, even with variations in membrane spontaneous curvature, an eccentric lattice has a higher bending energy cost per protein compared to a symmetric lattice of the same area.

G. For flexible protein lattices, membrane energy and deformation are lowered along all lattice growth pathways, but more strongly for the eccentric lattices

We now relax the assumption that the protein lattice is perfectly rigid and allow the lattice to also reshape to minimize the total energy of the coupled lattice-membrane system. The trend of Fig. 4 that the eccentric lattices have a higher energy per protein is retained with the flexible lattices. To compare the energetics and deformation of coupled lattice growth and

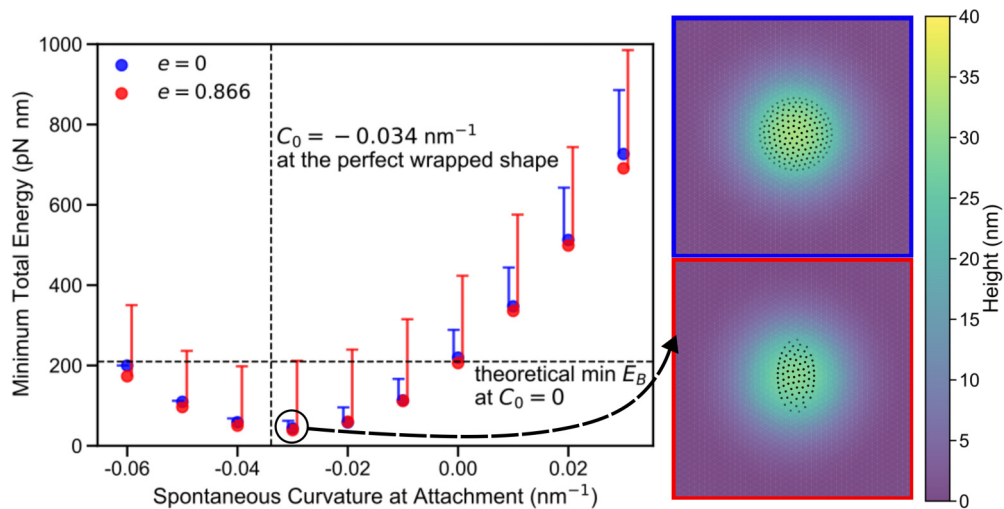


FIG. 7. The membrane bending energy when attached to lattices with two different eccentricities both minimize around the spontaneous curvature that matches the curvature of the rigid Gag lattice. The curvature of the rigid Gag lattice is $-2 / (50 \text{ nm} + 9 \text{ nm}) \approx -0.034 \text{ nm}^{-1}$. (A) The spontaneous curvature of the membrane C_0 is assigned a nonzero value at each Gag-membrane attachment point, ranging here from -0.06 to $+0.04$. The lowest bending energy is achieved when the membrane spontaneous curvature C_0 matches the intrinsic curvature of the Gag lattice. Two cases with different eccentricities (e) are tested here, with $e = 0$ blue points and $e = 0.866$ red points. Importantly, both lattices have the same circumscribing cylindrical radii (30 nm; see Sec. III E for definition), and thus predict comparable energies, although the eccentric (red) lattice has a smaller surface area, or fewer Gag monomers attaching to the membrane. Error bars show 90% confidence interval of minimum energy from bootstrap resampling; error bars are moved sideways to avoid overlapping. Theoretical minimum bending energy (209.6 pN nm) assumes a simple cap model with no membrane tension [Eq. (6)].

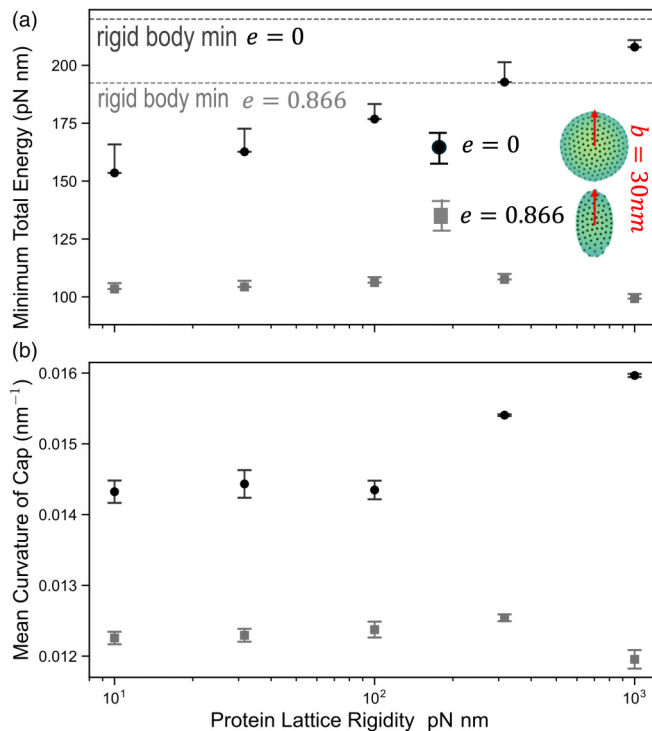


FIG. 8. The total system energy and the deformation of the membrane both decrease with flexible lattices. (a) We vary the rigidity of two lattices, one with $e = 0$ (black circles) and one with $e = 0.866$ (gray squares), where both have the same major axis $b = 30\text{ nm}$ (inset cartoons), hence producing similar *total* energies ($\sim 200\text{ pN nm}$) for perfectly rigid lattices (black and gray dashed lines, respectively). For $e = 0.866$, the minor axis radius is 15 nm and it thus has only ~ 52 Gag monomers and a surface area of 1480 nm^2 , whereas the symmetric lattice has ~ 110 Gags and a larger surface area of 3040 nm^2 . The protein lattice energy E_{lattice} is parametrized by force constants for bond (pN nm) and torsion rigidities (pN/nm) with equal magnitudes varying along the x axis (Sec. II B 10). Total energy defined by Eq. (5) includes the membrane energy and the flexible lattice energy. Relative to perfectly rigid lattices (dashed line), the highly eccentric lattices have reduced their bending energy more strongly compared to the symmetric lattices. We emphasize that a symmetric lattice with the same number ~ 52 Gags as this $e = 0.866$ lattice still has a lower energy (even when rigid) of $\sim 50\text{ pN nm}$, consistent with Fig. 4. (b) The highly eccentric lattices do not induce as significant amount of curvature as their symmetric counterparts. This explains their lower energy in panel (a). Membrane has $C_0 = 0$. Error bars represent 95% confidence interval from bootstrap resampling.

remodeling pathways as eccentricity varies, we assess an $e = 0.866$ and $e = 0$ lattice that have the same major axis and thus comparable total energies when rigid (but $e = 0$ retains the lower per-protein energy cost). In both cases, the energy and deformation are lowered with more flexible lattices (Fig. 8). As expected, the membrane bending energy decreased, as with a flexible lattice it can better resist deformation and retain its preferred flatter curvature. The total energy also decreases, as the cost in energy due to the lattice deforming away from its preferred curvature does not exceed the benefit of less membrane bending [Fig. 8(a)]. To quantify membrane shape in this case, height is not the ideal metric, since most of

the energy decrease arises from deformation of membrane and lattice in the cap region instead of the relaxation region (see Fig. S12 of the Supplemental Material [81]). We instead measure the mean curvature of the membrane region where the protein lattice cap is attached to quantify the deformation of the membrane, showing that a lower curvature is achieved compared to a perfectly rigid lattice [Fig. 8(b)].

The drop in energy and particularly the loss of curvature are more significant for the eccentric lattice compared to their symmetric counterparts [Fig. 8(b)]. As we decrease the rigidity of the lattice, we see that the energy drops rapidly for the eccentric lattice, but relatively slower for the symmetric lattice. The reason is that the flexible symmetric lattice is still quite effective at inducing membrane bending due to its larger surface area and circular perimeter. In contrast, the eccentric lattice is rapidly outcompeted by the membrane, inducing significantly weaker curvature even for relatively stiffer lattices [Fig. 8(b)]. Hence, for flexible lattices, the eccentric growth has come with another cost: They are significantly worse at inducing curvature [Fig. 8(b)]. For Gag lattice assembly and budding, this means that the flexible lattice would produce a larger-radius virion to complete the vesicle budding process, should it follow the eccentric growth pathway.

H. Membrane deformations are not perfectly symmetric for flexible eccentric lattices

To further quantify the membrane shape deformation across growth pathways with varying eccentricity, we introduced a metric for cylindrical asymmetry, e_{cyl} , that varies from 0 for perfectly symmetric deformations to 1 for a linear ridge (see Sec. II). For perfectly rigid lattices, both the symmetric ($e = 0$) and eccentric ($e = 0.866$) structures produce quite symmetric deformations as predicted above by Fig. 6, with the eccentric lattice slightly worse at $e_{\text{cyl}} = 0.13$ [Fig. 9(a), dashed lines]. For flexible protein lattices, the symmetric lattice induces membrane deformations that largely retain its symmetry with a constant value of $e_{\text{cyl}} \sim 0.15$ as rigidity increases, which is a bit higher than the rigid lattice (black dashed line) (Fig. 9). In contrast, the highly eccentric flexible lattice does induce higher e_{cyl} that increases as the lattice stiffens, from ~ 0.2 to 0.32 , despite the fact that these eccentric lattices have good symmetry for perfectly rigid lattices (gray dashed line) [Fig. 9(a)]. The heatmap and the side view of the deformation illustrate how the curvature relaxes more rapidly along one axis in the flexible eccentric lattice [Figs. 9(b) and 9(c)]. However, despite the increase in e_{cyl} for eccentric lattices, these values are still far lower than the asymmetry in the lattice itself, where $e = 0.866$. Hence, despite a reduction in radial symmetry in these highly eccentric flexible lattices, the membrane deformation still generates the lowest energy when its shape has significantly higher symmetry than the lattice cap itself. The overall flatter and more asymmetric geometries observed for these flexible eccentric lattices in Figs. 8 and 9 suggest that highly deformable and asymmetric lattices permit the system to escape curvature frustration by deforming into an energetically cheaper but morphologically unproductive state.

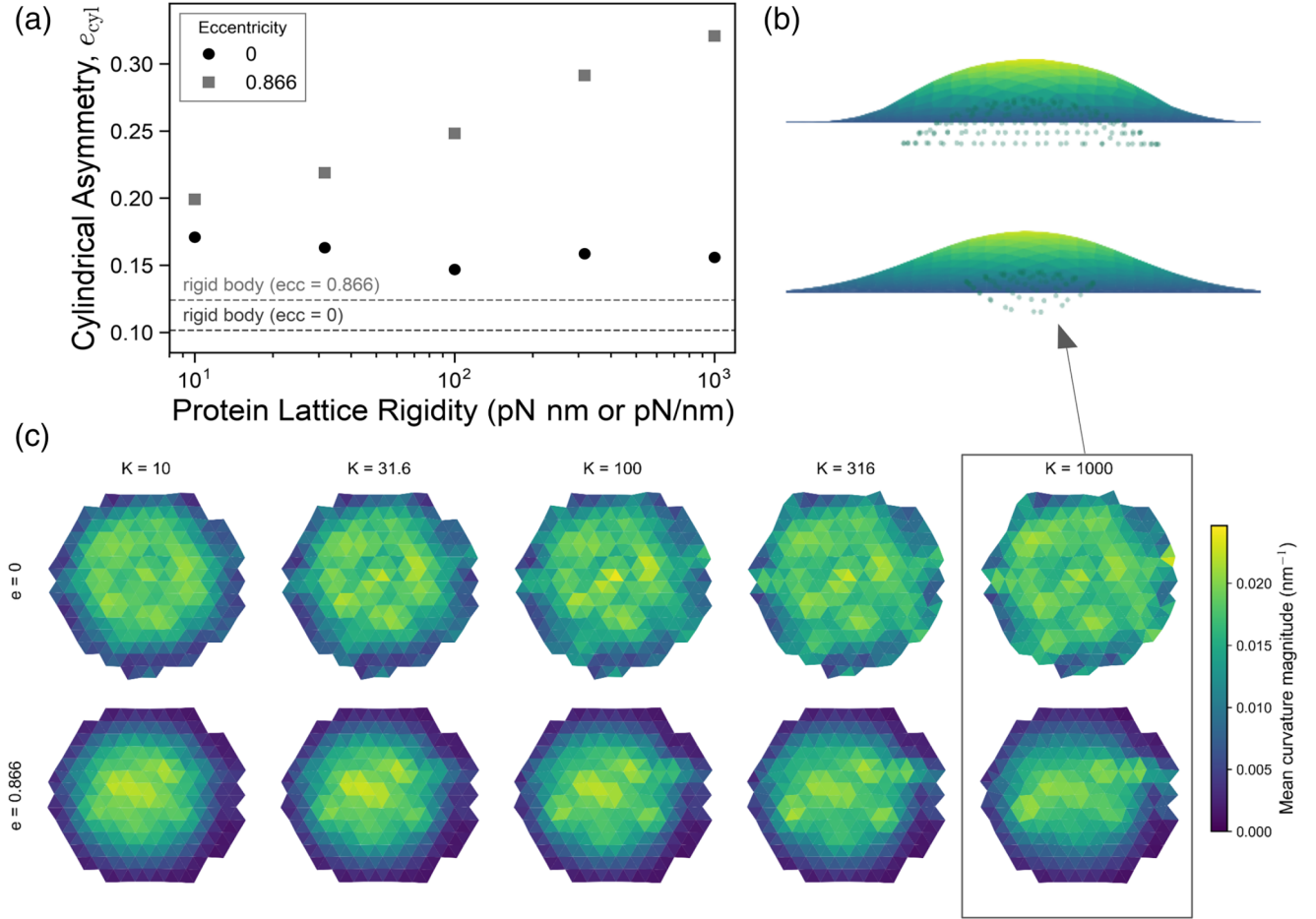


FIG. 9. Membrane deformation becomes more cylindrically asymmetric for flexible, eccentric protein lattices. (a) Cylindrical symmetry vs protein lattice rigidity for the same symmetric (black circles) and eccentric (gray squares) lattices studied in Fig. 8. Dashed lines indicate the low asymmetry measured in the corresponding perfectly rigid lattices. (b) Side view of membrane deformation with flexible lattices (protein lattice rigidity K of 1000 pN nm or pN/nm) of two different eccentricities. (c) Mean curvature heatmap shows increased cylindrical asymmetry in the membrane when flexible eccentric lattices are attached (bottom row, $e = 0.866$). The cylindrical asymmetry is defined as the approximated eccentricity averaged by areal weight over 64 contour planes (see Sec. 3.5.3 of the Supplemental Material [81]). Membrane has $C_0 = 0$.

I. Membrane reshaping is preserved for rigid lattices coupled to a dynamic membrane model

Finally, we verified that the largely symmetric membrane invaginations induced by our lattices were retained at physiologic temperature and with stochastic fluctuations of the membrane surface. We performed Fourier-space Brownian dynamics simulations of the continuum membrane coupled to the Gag lattices assembled previously from NERDSS simulations (see Sec. II), as in Fig. 3. By quantifying the curvature induced by the lattice on this continuum membrane, we find very good agreement with our energy-minimized structures when using the rigid lattices (Fig. 10). This is despite the asymmetry or irregularity inherent to these lattices, supporting our hypothesis that these lattices induce close to radially symmetric invaginations in the membrane because it produces the lowest bending energy. Surprisingly, we found that the flexible lattices coupled to the dynamic membrane were quite poor at inducing curvature, despite reasonably strong force constants that could induce curvature in the ideal eccentric lattices under energy minimization. Compared to the ideal lattices and alter-

nate spherical lattices assembled by the clathrin trimer [53], these Gag lattices have a larger number of bonds due to the connectivity within the trihexagonal lattice, and some of them are disconnected due to defects in the lattice. The emergent rigidity of these Gag lattices therefore appears to be quite soft. Since, physiologically, these assemblies are clearly capable of budding out of living cells, this indicates that the bond forces are either much stronger than we have parametrized and are closer to the perfectly rigid limit (Fig. 10), or possibly the nucleic acids that are packaged in the growing virion might significantly increase lattice stiffness.

IV. DISCUSSION

Our results clearly demonstrate that the membrane energy due to bending around a lattice scaffold increases not only along pathways of lattice size growth, as expected, but along pathways with higher eccentricity of the lattices (with the same lattice surface area). Hence, the total energy cost per protein increases with lattice asymmetry, and these pathways are thus less favorable thermodynamically. A primary reason

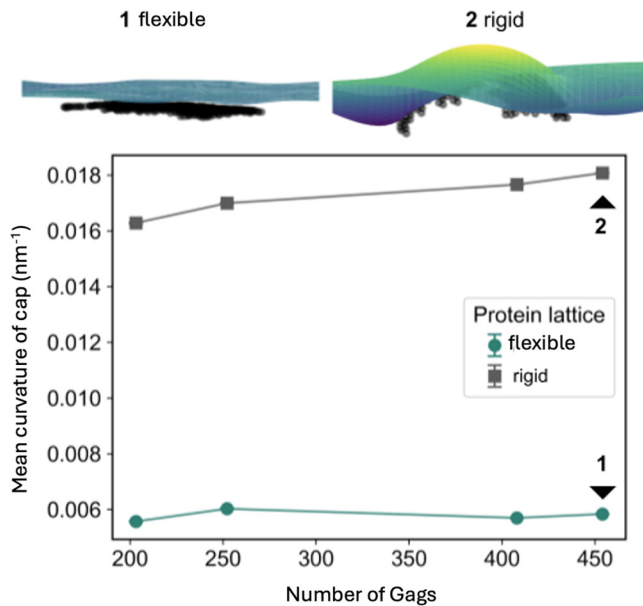


FIG. 10. Brownian dynamics simulations using HIV Gag lattices retain the radially symmetric membrane invagination for the rigid lattice, but flatten for the flexible lattice. We introduced stochastic fluctuations at a finite temperature $T = 310$ K using the FSBD method. For the rigid HIV-1 Gag lattices (gray squares), the membrane retains a curvature that is very close to the curvature of the energy-minimized (zero-temperature) structures as the lattice grows, despite surface fluctuations and irregularities in these stochastically assembled Gag structures. For the flexible lattices with protein lattice rigidity of 1040 pN nm or pN/nm (green circles), the membrane largely outcompetes and flattens the lattice, resulting in minimal curvature. This arises due to a very large number of bonds, some of which are missing, within this stochastically assembled lattice, in contrast to the ideal eccentric lattices of Fig. 8. Snapshots on the top correspond to the largest lattice with 450 Gag monomers, where only the lattice flexibility was changed between the simulations.

for this is because, despite asymmetry or irregularity in the rigid lattices, the membrane still prefers to bend into a nearly radially symmetric invagination to minimize total bending energy, as it produces a significantly lower energy compared to more tightly wrapped, irregularly shaped deformations (Fig. S5 of the Supplemental Material [81]). As a result, we can predict relatively accurately what the minimal membrane energy cost will be for a rigid lattice regardless of its eccentricity using analytical theory, by calculating the radius of the cylinder that fully circumscribes the lattice. For flexible protein lattices, we show that the cost of eccentric growth pathways goes beyond energy per protein, as these structures are also significantly worse at inducing curvature. Our findings emphasize that membrane deformation due to protein scaffolding is sensitive to lattice perimeter geometry much more so than the density of contacts between the lattice and membrane for these structures with a constant spherical curvature. This suggests that the shape and not just the total area of the deforming lattice strongly impacts the bending energy penalty and the amount of curvature induced. Given that the probability of observing intermediate structures during coupled assembly and remodeling is dependent on

their energy and kinetics (not assessed here), the dramatic increase in bending energy costs associated with eccentric or irregular self-assembly pathways on the membrane would strongly select against their occurrence. This distinguishes these membrane-coupled assembly pathways from those stochastically accessible in solution where smaller energetic variations can support distinct lattice structures.

Our model framework is the first to systematically characterize how the eccentricity of spherically curved lattices, both flexible and rigid, pays a steep price in energy costs for all but the smallest structures. We further showed that this penalty persists even if the total membrane bending energy cost is reduced by matching the spontaneous curvature of the membrane to that of the rigid lattice (Fig. 7). A limitation of this study is that our thermodynamic comparison of snapshots along distinct growth pathways ignores possible kinetic barriers along these growth pathways that could disfavor recruitment or structural transitions. Our assumptions here are similar in spirit to the protein folding energy landscape paradigm, where the lowest energy configurations should dominate growth/folding pathways. Our Brownian dynamics (BD) simulations here indicate that the membrane deformations around a single (rigid) snapshot will still adopt a curvature that matches our energy-minimized structure and retain the radial symmetry, which provides support for a driving role of membrane energetics under thermal fluctuations. However, membrane budding driven by self-assembly is not an equilibrium process. While the membrane shape relaxes rapidly under these deformations (at $<$ millisecond timescales) [97], reaching a pseudoequilibrium, the transitions between discrete intermediates may face variable barrier heights. Because spontaneous curvature can also promote or suppress protein aggregation [98] and drive membrane constriction [21], its variation during growth may further impact growth pathways. Based on our results, we would predict that a dynamically coupled recruitment, self-assembly, and remodeling simulation would eliminate proteins that bound to the edge of the lattice and substantially extended its eccentricity. The membrane bending cost would effectively increase the dissociation rate of these types of events much higher than they experience in solution. Extending our dynamic simulations to incorporate these dynamics of protein recruitment and reversible self-assembly will be important to allow the exploration of kinetic growth trajectories as they couple to shape changes and protein interactions [99].

Another limitation of our study is that we stopped short of inducing the fully spherical, near-fission structure. This is a numerical challenge rather than a physical limit, as the finite-element method we use here can capture these topological changes [30,31]. Under such large curvature changes, the discretization of the surface via a triangular mesh becomes increasingly irregular, which leads to instabilities with numerical integration. Remeshing or edge flipping of the mesh discretization resolves these issues [85,86] and will be an important future step. With increasing deformations, it would also be important to monitor the membrane tension to ensure it remains in the physiologic regime, and to otherwise increase the simulated membrane area. We also used a finite-system boundary condition in both the simulations and our theoretical models, asserting that the membrane relaxes to a flat

curvature at or near the boundary. Our benchmarks (Sec. 3.3.2 and Fig. M9 of the Supplemental Material [81]) demonstrate that the deformation energy of a spherical invagination does depend on the finite-system boundary condition. However, we find that roughly 90% of the mechanical energy derives from the spherical-cap region, and not the relaxation region (Fig. 6). This is qualitatively consistent with previous studies that attached a spherical coat to a membrane but allowed the boundary to flatten at infinite distance from the attachment. They reported $\sim 80\%$ of mechanical energy arising in the cap region [20,100,101]. Physiologically, it is unlikely that the curvature can relax infinitely far from the attached coat. Cytoskeletal scaffolding that attaches to the membrane can enforce local boundary conditions to the membrane structure, acting like a picket fence [102]. This effective compartmentalization of membrane regions can limit the free propagation of tension and restrict curvature at the fence boundaries [103].

Our comparison of rigid and flexible lattices found that both produced the same trend in higher energy costs per protein for asymmetric growth. However, they did not have the same trends in curvature induction. For rigid lattices, the curvature and symmetry of the membrane deformations were relatively similar across all eccentricities with respect to the circumscribing radial extent, as supported by the reasonable collapse of data in Fig. 6. For flexible lattices, however, the curvature induction was significantly lower for eccentric lattices despite having the same major radial extent as their symmetric counterparts. These flexible eccentric lattices thus sacrifice curvature due to their asymmetric perimeter. Notably, this response is even stronger when we consider the stochastically assembled HIV Gag lattice compared to the ideal lattices quantified in Fig. 9. The trihexagonal architecture of the HIV Gag lattice cannot perfectly tile a spherical surface and must inevitably incorporate defects [14], and the stochastic assembly also leaves some bonds free along the more irregular perimeter. While these differences do not matter for rigid lattices as shown in our BD simulations (Fig. 10), it renders the flexible lattice much worse at inducing curvature than the corresponding idealized lattice architecture. This creates something of a paradox, as the incompleteness of this HIV Gag lattice is important for facilitating remodeling that is necessary for maturation [26]. We therefore speculate that the Gag lattices must be significantly more rigid than the flexible lattices we simulated here to retain their ability to induce successful budding. While one explanation is that the force constants should simply be far stiffer, the values we used here were sufficiently strong to drive successful curvature induction by clathrin lattices on dynamic membranes, where the primary difference is that the much larger clathrin subunits have far fewer bonds within the lattice [53]. This indicates that the number of bond flexion points per surface area of the lattice impacts emergent rigidity. Another possibility is that the packaging of nucleic acids helps to stiffen or buttress

the lattice against the forces from the membrane. A promising future route to clarify these observations is to measure force constants from atomic structures by mapping through coarse-grained molecular dynamics simulations [104,105]. The mechanical properties of the lattices are critical not only for deforming membranes but for withstanding forces externally during processes like nuclear translocation of the HIV capsid [106,107], representing an important target for such multiscale methods.

Compared to other viruses like SARS-CoV-2, HIV budding relies on a highly structured hexagonal lattice that, despite incorporating defects and assembling lattices that are not 100% complete [27], produces virions with a relatively narrow range of radii that are consistent with the curvature of the hexagonal lattice assembled in solution [42]. For SARS-CoV-2, however, the assembly of the M protein is known to be structurally heterogeneous, not regular. Yet this assembly still gives rise to a regular, spherical viral particle during membrane-enveloped budding, as observed in previous studies [37]. Coupling protein assembly to membrane bending thus may produce enough selective pressure to narrow assembly pathways even with more disordered structures to favor a single, spherical protein lattice.

Finally, we note that from a design perspective, one might actually prefer the asymmetric lattice growth. Although these eccentric structures produce a higher bending energy cost per protein, for sufficiently rigid lattices they can produce a larger spherical membrane invagination with a smaller number of proteins. In this case, if lattice growth is limiting, arranging the subunits into a rigid eccentric lattice can preserve large membrane deformations. Our simulations clearly show that these lattices must be even stiffer than required for symmetric growth to retain high curvature induction. To facilitate development and future studies discussed here, our code is all provided open source in Ref. [64]. This provides a community resource for carefully validated models that support mechanistic understanding of membrane remodeling in cell biology.

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DATA AVAILABILITY

The software used to produce these results and analyze the simulations is available open source at GitHub [64]. The data presented here were generated by numerical simulations or analytical theory. All data are available within the manuscript or in the accompanying Supplemental Material in a Zenodo repository [81]. The Supplemental Material includes Methods, Figs. S1–S12, and Tables S1–S4.

[1] F. A. Barr and U. Gruneberg, Cytokinesis: Placing and making the final cut, *Cell* **131**, 847 (2007).

[2] M. Kaksonen and A. Roux, Mechanisms of clathrin-mediated endocytosis, *Nat. Rev. Mol. Cell Biol.* **19**, 313 (2018).

- [3] E. O. Freed and A. J. Mouland, The cell biology of HIV-1 and other retroviruses, *Retrovirology* **3**, 77 (2006).
- [4] T. Baumgart, B. R. Capraro, C. Zhu, and S. L. Das, Thermodynamics and mechanics of membrane curvature generation and sensing by proteins and lipids, *Annu. Rev. Phys. Chem.* **62**, 483 (2011).
- [5] F. Campelo, H. T. McMahon, and M. M. Kozlov, The hydrophobic insertion mechanism of membrane curvature generation by proteins, *Biophys. J.* **95**, 2325 (2008).
- [6] N. Yandrapalli, Q. Lubart, H. S. Tanwar, C. Picart, J. Mak, D. Muriaux, and C. Favard, Self assembly of HIV-1 Gag protein on lipid membranes generates PI(4,5)P₂/Cholesterol nanoclusters, *Sci. Rep.* **6**, 39332 (2016).
- [7] A. J. Bryer, T. Reddy, E. Lyman, and J. R. Perilla, Full scale structural, mechanical and dynamical properties of HIV-1 liposomes, *PLoS Comput. Biol.* **18**, e1009781 (2022).
- [8] Z. G. Denieva, P. I. Kuzmin, T. R. Galimzyanov, S. A. K. Datta, A. Rein, and O. V. Batishchev, Human immunodeficiency virus type 1 Gag polyprotein modulates membrane physical properties like a surfactant: Potential implications for virus assembly, *ACS Infect. Dis.* **10**, 2870 (2024).
- [9] E. R. May and C. L. Brooks, Determination of viral capsid elastic properties from equilibrium thermal fluctuations, *Phys. Rev. Lett.* **106**, 188101 (2011).
- [10] I. L. Ivanovska, P. J. de Pablo, B. Ibarra, G. Sgalari, F. C. MacKintosh, J. L. Carrascosa, C. F. Schmidt, and G. J. Wuite, Bacteriophage capsids: Tough nanoshells with complex elastic properties, *Proc. Natl. Acad. Sci. USA* **101**, 7600 (2004).
- [11] J. Harrison, D. O. Passos, J. F. Bruhn, J. D. Bauman, L. Tuberty, J. J. DeStefano, F. X. Ruiz, D. Lyumkis, and E. Arnold, Cryo-EM structure of the HIV-1 Pol polyprotein provides insights into virion maturation, *Sci. Adv.* **8**, eabn9874 (2022).
- [12] F. K. M. Schur, M. Obr, W. J. H. Hagen, W. Wan, A. J. Jakobi, J. M. Kirkpatrick, C. Sachse, H.-G. Kräusslich, and J. A. G. Briggs, An atomic model of HIV-1 capsid-SP1 reveals structures regulating assembly and maturation, *Science* **353**, 506 (2016).
- [13] S. Mattei, A. Tan, B. Glass, B. Müller, H.-G. Kräusslich, and J. A. G. Briggs, High-resolution structures of HIV-1 Gag cleavage mutants determine structural switch for virus maturation, *Proc. Natl. Acad. Sci. USA* **115**, E9401 (2018).
- [14] A. Tan, A. J. Pak, D. R. Morado, G. A. Voth, and J. A. G. Briggs, Immature HIV-1 assembles from Gag dimers leaving partial hexamers at lattice edges as potential substrates for proteolytic maturation, *Proc. Natl. Acad. Sci. USA* **118**, e2020054118 (2021).
- [15] L. Mendonça, D. Sun, J. Ning, J. Liu, A. Kotecha, M. Olek, T. Frosio, X. Fu, B. A. Himes, A. B. Kleinpeter, E. O. Freed, J. Zhou, C. Aiken, and P. Zhang, CryoET structures of immature HIV Gag reveal six-helix bundle, *Commun. Biol.* **4**, 481 (2021).
- [16] J. A. G. Briggs, M. N. Simon, I. Gross, H.-G. Kräusslich, S. D. Fuller, V. M. Vogt, and M. C. Johnson, The stoichiometry of Gag protein in HIV-1, *Nat. Struct. Mol. Biol.* **11**, 672 (2004).
- [17] J. Hendrix, V. Baumgärtel, W. Schrimpf, S. Ivanchenko, M. A. Digman, E. Gratton, H.-G. Kräusslich, B. Müller, and D. C. Lamb, Live-cell observation of cytosolic HIV-1 assembly onset reveals RNA-interacting Gag oligomers, *J. Cell Biol.* **210**, 629 (2015).
- [18] M. Tritel and M. D. Resh, Kinetic analysis of human immunodeficiency virus type 1 assembly reveals the presence of sequential intermediates, *J. Virol.* **74**, 5845 (2000).
- [19] S.-K. Guo, A. J. Sodt, and M. E. Johnson, Large self-assembled clathrin lattices spontaneously disassemble without sufficient adaptor proteins, *PLoS Comput. Biol.* **18**, e1009969 (2022).
- [20] M. Deserno, Elastic deformation of a fluid membrane upon colloid binding, *Phys. Rev. E* **69**, 031903 (2004).
- [21] C. Vanhille-Campos, K. D. Whitley, P. Radler, M. Loose, S. Holden, and A. Šarić, Self-organization of mortal filaments and its role in bacterial division ring formation, *Nat. Phys.* **20**, 1670 (2024).
- [22] I. Kucharska, P. Ding, K. K. Zadrozny, R. A. Dick, M. F. Summers, B. K. Ganser-Pornillos, and O. Pornillos, Biochemical reconstitution of HIV-1 assembly and maturation, *J. Virol.* **94**, e01844 (2020).
- [23] D. L. Bush and V. M. Vogt, *In vitro* assembly of retroviruses, *Annu. Rev. Virol.* **1**, 561 (2014).
- [24] M. J. Varga, Y. Fu, S. Loggia, O. N. Yogurtcu, and M. E. Johnson, NERDSS: A nonequilibrium simulator for multi-body self-assembly at the cellular scale, *Biophys. J.* **118**, 3026 (2020).
- [25] Y. Qian, D. Evans, B. Mishra, Y. Fu, Z. H. Liu, S. Guo, and M. E. Johnson, Temporal control by cofactors prevents kinetic trapping in retroviral Gag lattice assembly, *Biophys. J.* **122**, 3173 (2023).
- [26] S. Guo, I. Saha, S. Saffarian, and M. E. Johnson, Structure of the HIV immature lattice allows for essential lattice remodeling within budded virions, *eLife* **12**, e84881 (2023).
- [27] F. K. M. Schur, W. J. H. Hagen, M. Rumlová, T. š Ruml, B. Müller, H.-G. Kräusslich, and J. A. G. Briggs, Structure of the immature HIV-1 capsid in intact virus particles at 8.8 Å resolution, *Nature (London)* **517**, 505 (2015).
- [28] A. J. Pak, M. Gupta, M. Yeager, and G. A. Voth, Inositol hexakisphosphate (IP6) accelerates immature HIV-1 Gag protein assembly toward kinetically trapped morphologies, *J. Am. Chem. Soc.* **144**, 10417 (2022).
- [29] A. Jhaveri, S. Loggia, Y. Qian, and M. E. Johnson, Discovering optimal kinetic pathways for self-assembly using automatic differentiation, *Proc. Natl. Acad. Sci. USA* **121**, e2403384121 (2024).
- [30] F. Feng and W. S. Klug, Finite element modeling of lipid bilayer membranes, *J. Comput. Phys.* **220**, 394 (2006).
- [31] Y. Fu, W. F. Zeno, J. C. Stachowiak, and M. E. Johnson, A continuum membrane model can predict curvature sensing by helix insertion, *Soft Matter* **17**, 10649 (2021).
- [32] F. Mohajerani, B. Tyukodi, C. J. Schlicksup, J. A. Hadden-Perilla, A. Zlotnick, and M. F. Hagan, Multiscale modeling of hepatitis B virus capsid assembly and its dimorphism, *ACS Nano* **16**, 13845 (2022).
- [33] M. F. Hagan and R. Zandi, Recent advances in coarse-grained modeling of virus assembly, *Curr. Opin. Virol.* **18**, 36 (2016).
- [34] A. J. Pak, J. M. A. Grime, P. Sengupta, A. K. Chen, A. E. P. Durumeric, A. Srivastava, M. Yeager, J. A. G. Briggs, J. Lippincott-Schwartz, and G. A. Voth, Immature HIV-1 lattice assembly dynamics are regulated by scaffolding from nucleic acid and the plasma membrane, *Proc. Natl. Acad. Sci. USA* **114**, E10056 (2017).

- [35] M. Gupta, A. J. Pak, and G. A. Voth, Critical mechanistic features of HIV-1 viral capsid assembly, *Sci. Adv.* **9**, eadd7434 (2023).
- [36] S. Timmermans, A. Ramezani, T. Montalvo, M. Nguyen, P. van der Schoot, J. C. M. van Hest, and R. Zandi, The dynamics of viruslike capsid assembly and disassembly, *J. Am. Chem. Soc.* **144**, 12608 (2022).
- [37] S. Li and R. Zandi, Biophysical modeling of SARS-CoV-2 assembly: Genome condensation and budding, *Viruses* **14**, 2089 (2022).
- [38] I. Saha and S. Saffarian, Dynamics of the HIV Gag lattice detected by localization correlation analysis and time-lapse iPALM, *Biophys. J.* **119**, 581 (2020).
- [39] T. Ruiz-Herrero and M. F. Hagan, Simulations show that virus assembly and budding are facilitated by membrane microdomains, *Biophys. J.* **108**, 585 (2015).
- [40] G. S. Aytton and G. A. Voth, Multiscale simulation of protein mediated membrane remodeling, *Semin. Cell Dev. Biol.* **21**, 357 (2010).
- [41] B. Li and S. M. Abel, Shaping membrane vesicles by adsorption of hinge-like nanoparticles, *J. Chem. Phys.* **160**, 194901 (2024).
- [42] W. I. Sundquist and H. G. Krausslich, HIV-1 assembly, budding, and maturation, *Cold Spring Harb. Perspect. Med.* **2**, a006924 (2012).
- [43] M. D. Tomasini, D. S. Johnson, J. S. Mincer, and S. M. Simon, Modeling the dynamics and kinetics of HIV-1 Gag during viral assembly, *PLoS One* **13**, e0196133 (2018).
- [44] C. Floderer, J.-B. Masson, E. Boilley, S. Georgeault, P. Merida, M. El Beheiry, M. Dahan, P. Roingeard, J.-B. Sibarita, C. Favard, and D. Muriaux, Single molecule localisation microscopy reveals how HIV-1 Gag proteins sense membrane virus assembly sites in living host CD4 T cells, *Sci. Rep.* **8**, 16283 (2018).
- [45] Y. Fu and M. E. Johnson, Modeling membrane reshaping driven by dynamic protein assemblies, *Curr. Opin. Struct. Biol.* **78**, 102505 (2023).
- [46] Y. Fu, D. H. Johnson, A. H. Beaven, A. J. Sodt, W. F. Zeno, and M. E. Johnson, Predicting protein curvature sorting across membrane compositions, *Biophys. J.* **125**, 1007 (2026).
- [47] W. F. Zeno, U. Baul, W. T. Snead, A. C. M. DeGroot, L. Wang, E. M. Lafer, D. Thirumalai, and J. C. Stachowiak, Synergy between intrinsically disordered domains and structured proteins amplifies membrane curvature sensing, *Nat. Commun.* **9**, 4152 (2018).
- [48] B. R. Capraro, Y. Yoon, W. Cho, and T. Baumgart, Curvature sensing by the epsin N-terminal homology domain measured on cylindrical lipid membrane tethers, *J. Am. Chem. Soc.* **132**, 1200 (2010).
- [49] B. J. Reynwar, G. Illya, V. A. Harmandaris, M. M. Müller, K. Kremer, and M. Deserno, Aggregation and vesiculation of membrane proteins by curvature-mediated interactions, *Nature (London)* **447**, 461 (2007).
- [50] L. Harker-Kirschneck, B. Baum, and A. E. Šarić, Changes in ESCRT-III filament geometry drive membrane remodelling and fission in silico, *BMC Biol.* **17**, 82 (2019).
- [51] L. Harker-Kirschneck, A. E. Hafner, T. Yao, C. Vanhille-Campos, X. Jiang, A. Pulschen, F. Hurtig, D. Hryniuk, S. Culley, R. Henriques, B. Baum, and A. Šarić, Physical mechanisms of ESCRT-III-driven cell division, *Proc. Natl. Acad. Sci. USA* **119**, e2107763119 (2022).
- [52] L. C. Lin and F. L. Brown, Brownian dynamics in Fourier space: Membrane simulations over long length and time scales, *Phys. Rev. Lett.* **93**, 256001 (2004).
- [53] S. L. Foley and M. E. Johnson, Hybrid dynamical simulation reveals apparent stiffening of flexible protein lattices driving membrane bending, *arXiv:2607.06378*.
- [54] B. Li and S. M. Abel, Shaping membrane vesicles by adsorption of a semiflexible polymer, *Soft Matter* **14**, 185 (2018).
- [55] S. Kutti Kandy and R. Radhakrishnan, Crowding-induced membrane remodeling: Interplay of membrane tension, polymer density, architecture, *Biophys. J.* **121**, 3674 (2022).
- [56] H. Ni and G. A. Papoian, Membrane-MEDYAN: Simulating deformable vesicles containing complex cytoskeletal networks, *J. Phys. Chem. B* **125**, 10710 (2021).
- [57] A. Sciortino, H. A. Faizi, D. A. Fedosov, L. Frechette, P. M. Vlahovska, G. Gompper, and A. R. Bausch, Active membrane deformations of a minimal synthetic cell, *Nat. Phys.* **21**, 799 (2025).
- [58] N. Cordella, T. J. Lampo, S. Mehraeen, and A. J. Spakowitz, Membrane fluctuations destabilize clathrin protein lattice order, *Biophys. J.* **106**, 1476 (2014).
- [59] N. Cordella, T. J. Lampo, N. Melosh, and A. J. Spakowitz, Membrane indentation triggers clathrin lattice reorganization and fluidization, *Soft Matter* **11**, 439 (2015).
- [60] M. Giani, W. K. den Otter, and W. J. Briels, Early stages of clathrin aggregation at a membrane in coarse-grained simulations, *J. Chem. Phys.* **146**, 155102 (2017).
- [61] W. Helfrich, Elastic properties of lipid bilayers: Theory and possible experiments, *Z. Naturforsch. C* **28**, 693 (1973).
- [62] E. A. Evans, Bending resistance and chemically induced moments in membrane bilayers, *Biophys. J.* **14**, 923 (1974).
- [63] P. B. Canham, The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell, *J. Theor. Biol.* **26**, 61 (1970).
- [64] Y. M. Ying and M. E. Johnson, SLIMED code repository, GitHub, 2026, <https://github.com/JohnsonBiophysicsLab/SLIMED>.
- [65] G. Niggemann, M. Kummrow, and W. Helfrich, The bending rigidity of phosphatidylcholine bilayers: Dependences on experimental method, sample cell sealing and temperature, *J. Phys. II* **5**, 413 (1995).
- [66] J. R. Henriksen and J. H. Ipsen, Measurement of membrane elasticity by micro-pipette aspiration, *Eur. Phys. J. E Soft Matter* **14**, 149 (2004).
- [67] F. T. Doole, T. Kumarage, R. Ashkar, and M. F. Brown, Cholesterol stiffening of lipid membranes, *J. Membr. Biol.* **255**, 385 (2022).
- [68] D. Dean and M. Manghi, Fluctuation-induced interactions between domains in membranes, *Phys. Rev. E* **74**, 021916 (2006).
- [69] J. Steinkühler, E. Sezgin, I. Urbančič, C. Eggeling, and R. Dimova, Mechanical properties of plasma membrane vesicles correlate with lipid order, viscosity and cell density, *Commun. Biol.* **2**, 337 (2019).
- [70] B. W. Koenig, H. H. Strey, and K. Gawrisch, Membrane lateral compressibility determined by NMR and X-ray diffraction:

- Effect of acyl chain polyunsaturation, *Biophys. J.* **73**, 1954 (1997).
- [71] W. Rawicz, K. C. Olbrich, T. McIntosh, D. Needham, and E. Evans, Effect of chain length and unsaturation on elasticity of lipid bilayers, *Biophys. J.* **79**, 328 (2000).
- [72] M. Deserno, Fluid lipid membranes: From differential geometry to curvature stresses, *Chem. Phys. Lipids*. **185**, 11 (2015).
- [73] C. Zhu, C. T. Lee, and P. Rangamani, Mem3DG: Modeling membrane mechanochemical dynamics in 3D using discrete differential geometry, *Biophys. Rep.* **2**, 100062 (2022).
- [74] A. L. Le Roux, C. Tozzi, N. Walani, X. Quiroga, D. Zalvidea, X. Trepas, M. Staykova, M. Arroyo, and P. Roca-Cusachs, Dynamic mechanochemical feedback between curved membranes and BAR protein self-organization, *Nat. Commun.* **12**, 6550 (2021).
- [75] C. Tozzi, N. Walani, and M. Arroyo, Out-of-equilibrium mechanochemistry and self-organization of fluid membranes interacting with curved proteins, *New J. Phys.* **21**, 093004 (2019).
- [76] G. Kumar and A. Srivastava, Membrane remodeling due to a mixture of multiple types of curvature proteins, *J. Chem. Theory Comput.* **18**, 5659 (2022).
- [77] A. Ono, S. D. Ablan, S. J. Lockett, K. Nagashima, and E. O. Freed, Phosphatidylinositol (4, 5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane, *Proc. Nat. Acad. Sci. USA* **101**, 14889 (2004).
- [78] J. S. Saad, J. Miller, J. Tai, A. Kim, R. H. Ghanam, and M. F. Summers, Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly, *Proc. Natl. Acad. Sci. USA* **103**, 11364 (2006).
- [79] J. Lidmar, L. Mirny, and D. R. Nelson, Virus shapes and buckling transitions in spherical shells, *Phys. Rev. E* **68**, 051910 (2003).
- [80] M. Buenemann and P. Lenz, Mechanical limits of viral capsids, *Proc. Natl. Acad. Sci. USA* **104**, 9925 (2007).
- [81] See Supplemental Material at <http://link.aps.org/supplemental/10.1103/tnv7-fjh5> for Methods, Figures, and Tables (doi: <https://doi.org/10.5281/zenodo.22307447>).
- [82] J. H. Michell, On the direct determination of stress in an elastic solid, with application to the theory of plates, *Proc. Lond. Math. Soc.* **s1-31**, 100 (1899).
- [83] T. L. Charles, Smooth subdivision surfaces based on triangles, MS thesis, University of Utah, 1987.
- [84] F. Cirak, M. Ortiz, and P. Schröder, Subdivision surfaces: A new paradigm for thin-shell finite-element analysis, *Int. J. Numer. Methods Eng.* **47**, 2039 (2000).
- [85] X. Wang and G. Danuser, Remeshing flexible membranes under the control of free energy, *PLoS Comput. Biol.* **18**, e1010766 (2022).
- [86] A. Mandal, P. Chaudhuri, and S. Chaudhuri, Real-time physics-based mesh deformation with haptic feedback and material anisotropy, in *Proceedings of the 18th International Joint Conference on Computer Vision, Imaging and Computer Graphics Theory and Applications* (SCITEPRESS—Science and Technology Publications, Setúbal, Portugal, 2023), pp. 153–161.
- [87] N. C. Gauthier, T. A. Masters, and M. P. Sheetz, Mechanical feedback between membrane tension and dynamics, *Trends Cell Biol.* **22**, 527 (2012).
- [88] Y. Schweitzer, A. D. Lieber, K. Keren, and M. M. Kozlov, Theoretical analysis of membrane tension in moving cells, *Biophys. J.* **106**, 84 (2014).
- [89] A. Pietuch, B. R. Brückner, and A. Janshoff, Membrane tension homeostasis of epithelial cells through surface area regulation in response to osmotic stress, *Biochim. Biophys. Acta* **1833**, 712 (2013).
- [90] P. Wolfe, Convergence conditions for ascent methods, *SIAM Rev.* **11**, 226 (1969).
- [91] D. Frenkel and B. Smit, *Understanding Molecular Simulation: From Algorithms to Applications* (Academic Press, Cambridge, Massachusetts, 2001).
- [92] P. Chlanda, E. Mekhedov, H. Waters, A. Sodt, C. Schwartz, V. Nair, P. S. Blank, and J. Zimmerberg, Palmitoylation contributes to membrane curvature in influenza A virus assembly and hemagglutinin-mediated membrane fusion, *J. Virol.* **91**, e00947 (2017).
- [93] N. Kaiser, G. Squires, and T. Broadhurst, A method for weak lensing observations, *Astrophys. J.* **449**, 460 (1995).
- [94] F. W. High, H. Hoekstra, N. Leethochawalit, T. de Haan, L. Abramson, K. A. Aird, R. Armstrong, M. L. N. Ashby, M. Bautz, M. Bayliss, *et al.*, Weak-lensing mass measurements of five galaxy clusters in the south pole telescope survey using magellan/megacam, *Astrophys. J.* **758**, 68 (2012).
- [95] J. A. Anderson, J. Glaser, and S. C. Glotzer, hoomd-blue: A Python package for high-performance molecular dynamics and hard particle Monte Carlo simulations, *Comput. Mater. Sci.* **173**, 109363 (2020).
- [96] github.com/PhysFoley/BrownianProteinFSBDbmem.
- [97] N. Bouzos, S. L. Foley, A. Potamianos, C. O. Jacobs, M. E. Johnson, and W. F. Zeno, Clathrin is an intrinsic driver of membrane fission, *bioRxiv* (2026).
- [98] A. Šarić and A. Cacciuto, Mechanism of membrane tube formation induced by adhesive nanocomponents, *Phys. Rev. Lett.* **109**, 188101 (2012).
- [99] I. Badvaram and B. A. Camley, Physical limits to membrane curvature sensing by a single protein, *Phys. Rev. E* **108**, 064407 (2023).
- [100] L. Foret, Shape and energy of a membrane bud induced by protein coats or viral protein assembly, *Eur. Phys. J. E Soft Matter* **37**, 42 (2014).
- [101] F. Frey, F. Ziebert, and U. S. Schwarz, Dynamics of particle uptake at cell membranes, *Phys. Rev. E* **100**, 052403 (2019).
- [102] A. Kusumi, C. Nakada, K. Ritchie, K. Murase, K. Suzuki, H. Murakoshi, R. S. Kasai, J. Kondo, and T. Fujiwara, Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: High-speed single-molecule tracking of membrane molecules, *Annu. Rev. Biophys. Biomol. Struct.* **34**, 351 (2005).
- [103] R. Dharan, A. Barnoy, A. K. Tsaturyan, A. Grossman, S. Goren, I. Yosibash, D. Nachmias, N. Elia, R. Sorkin, and M. M. Kozlov, Intracellular pressure controls the propagation of tension in crumpled cell membranes, *Nat. Commun.* **16**, 91 (2025).
- [104] S. Aland, A. Rätz, M. Röger, and A. Voigt, Buckling instability of viral capsids—A continuum approach, *Multiscale Model. Simul.* **10**, 82 (2012).

- [105] S. D. Hicks and C. L. Henley, Coarse-grained protein-protein stiffnesses and dynamics from all-atom simulations, [Phys. Rev. E](#) **81**, 030903 (2010).
- [106] A. J. Bryer, J. S. Rey, and J. R. Perilla, Performance efficient macromolecular mechanics via sub-nanometer shape based coarse graining, [Nat. Commun.](#) **14**, 2014 (2023).
- [107] A. Deshpande, A. J. Bryer, J. R. Andino-Moncada, J. Shi, J. Hong, C. Torres, S. Harel, A. C. Francis, J. R. Perilla, C. Aiken, and I. Rouso, Elasticity of the HIV-1 core facilitates nuclear entry and infection, [PLoS Pathog.](#) **20**, e1012537 (2024).