Cell

Supplemental Information

Relaxation of Loaded ESCRT-III Spiral Springs Drives Membrane Deformation

Nicolas Chiaruttini, Lorena Redondo-Morata, Adai Colom, Frédéric Humbert, Martin Lenz, Simon Scheuring, and Aurélien Roux

Supplemental experimental procedures

Giant unilamellar vesicles electroformation

20 µl of 1 mg/mL lipid mix (DOPC: DOPS, 6:4, mol:mol) were deposited on two indium tin oxide (ITO)-coated glass slides (70-100 Ω resistivity, Sigma-Aldrich) and placed in a vacuum drying oven for 60' for complete solvent evaporation. An O-ring of ~1 mm thickness was used as a non-leaky spacer between the two ITO slides, and the chamber was formed by compressing the two slides with spring metal tweezers. The formed chamber was filled with 400 µl of 500 mM sucrose solution (osmolarity adjusted to outside buffer solution, around 500 mOsm) and exposed to 1 V AC-current (10 Hz sinusoidal) at room temperature for 1 h. The resulting suspension was collected in a tube and used within the next days for experiments.

Supported membranes preparation

After the flow chamber is set-up, 80 µL of buffer with 5 µL of GUVs is injected into the chamber. Bursting of multiple vesicles on the coverslip is immediately observed on the surface of the coverslip (supplemental video 1). Then 100 µL of casein (Sigma-Aldrich ref n°C6905) solution at 1 mg/mL is incubated for 30 minutes in the flow chamber in order to passivate the bare glass around membrane patches before any experiment.

Partially adhered GUVs preparation and imaging

To image GUV over a long period of time, GUVs are partially adhered on the coverslip surface with an avidin-biotin system (see supplemental figure S5A). A coverslip is cleaned with ddH_2O, ethanol and then, plasma cleaned. Then 20 µL of avidin (Sigma-Aldrich ref n°A9275) at 0.1 mg/ml is incubated onto the coverslip for 10 minutes. The coverslip if washed thoroughly with ddH_2O then dried under nitrogen. This coverslip is assembled into a flow chamber and put into the imaging setup. 80 µL of buffer with 5 µL of GUVs supplemented with 0.03% biotinylated lipids (DSPE-PEG(2000)-Biotin, Avanti Polar Lipids, ref n°880129C) are flushed in the flow chamber. After a few minutes of incubation, several vesicles are partially adhered on the surface via Avidin-Lipid Biotin interaction. To avoid further adhesion, which could lead to GUV bursting, the remaining glass-bound avidin is blocked by flowing BSA-biotin (1 mg/mL) (Sigma-Aldrich ref n° A8549) into the chamber during 15 minutes. Partially adhered vesicles can be imaged over several hours without movement and the surrounding solution can be exchanged, provided

the shear rate is low (below 7 s^{-1}) to avoid damaging of vesicles. Time-lapse imaging is performed only at the equatorial section to limit photobleaching. For supplemental figure S4B and S4C, the anti-bleaching solution used is composed of 100 mM DTT, 100 µg/mL Catalase (Sigma-Aldrich ref n°C9322), 100 µg/mL Glucose Oxydase (Sigma-Aldrich ref n°G6125), Glucose 3 mg/mL.

AFM and HS-AFM

AFM

Peak-Force Quantitative Nanomechanical Property Mapping (PF-QNM) AFM imaging was performed on a Nanoscope-V AFM (Bruker, Santa Barbara, CA, USA) piloted by Nanoscope-8 control software. PF-QNM AFM measurements were carried out in measurement buffer (10 mM Tris-HCl, 150 mM KCl, pH 7.4) at ambient temperature and pressure. The AFM was equipped with Si_3N_4 cantilevers with nominal spring constants of 100 pN/nm featuring silicon tips with 2 nm nominal radius (MSNL, Bruker, Santa Barbara, CA, USA). The spring constant of the cantilevers was calibrated using the thermal fluctuations method before every measurement. PF-QNM AFM imaging consists of oscillating the sample support at constant rate (2 kHz) and amplitude of about 20 nm. During each oscillation cycle a force-distance curve is acquired monitoring the deflection (force) of the cantilever, which is used as feedback parameter. The vertical amplitude of the piezoelectric displacement was set to allow the tip to completely separate from the sample surface, assuring accurate determination of the zero force and then maximum (peak-force) applied indentation force. The approach trace was used to control and keep constant this maximum force applied to about 200 pN. The retraction trace was used to determine the Young's modulus. The Young's modulus (E) was calculated at each pixel of the image from the contact part of the retraction trace of each oscillation cycle by fitting the Hertz model of a spherical tip of radius 2 nm indenting an elastic half-space, assuming a Poisson ratio of 0.5. To avoid the contribution from electrostatic and van der Waals interactions, we restricted the fit to a range in between 30% and 90% of the maximum indentation force. PF-QNM images and mechanical maps were obtained at 256 by 256 pixels, at a scan rate of 2 Hz and optimized feedback gains.

HS-AFM

High-Speed Atomic Force Microscopy (HS-AFM, (Ando et al., 2001)) was performed on a HS-AFM 1.0 (RIBM, Tsukuba, Japan) setup equipped with 8 µm long cantilevers with nominal spring constant k = 0.15 N/m, resonance frequency in air f(r) = 1200 kHz, (NanoWorld, Neuchâtel, Switzerland),

calibrated before each experiment. HS-AFM image acquisition was performed using a dynamic feedback circuit at highest possible speed. Images were obtained at pixel sampling ranging from 200 by 200 to 500 by 500 pixels on scan areas ranging from 200 nm to 1500 nm. Under non-destructive force conditions movies at frame rates ranging from 1 to 5 images per second could be acquired. HS-AFM movies were stabilized for movie acquisition piezo drift in post-acquisition treatment.

EM Thin sectioning

The Snf7 bound LUVs were fixed with 4% glutaraldehyde, embedded in 2% agar and spun at 5000 rpm for 3-4 min. The pellet was treated with 0.4 M Millonig's buffer (sodium phosphate (monobasic) + NaOH) containing 2% osmium tetroxide for 1 h and rinsed with ddH_2O. The sample was stained with 0.25% uranyl acetate overnight and rinsed with ddH_2O followed by sequential dehydration in 30%, 50%, 70% and 90% ethanol for 5-10 min. The last dehydration step was carried out three times in 100% ethanol for 30 min each. The sample was then washed with propylene oxide twice for 10 min followed by incubation in epon-propylene oxide (1:1) for 1 h. Finally, the sample was treated with 100% epon overnight at room temperature before being embedded in 1 ml of epon resin mix and cured at 65 °C for at least 24 h.

TIRF microscope calibration

To estimate the average intensity of a single Atto647N fluorophore ($I_{Atto647N}$), the microscope is calibrated with commercially available fluorescent DNA origamis (supplemental figure S6) which are labeled with exactly 9 or 18 Atto647N molecules (GATTA-Brightness 9R and 18R, GATTAquant, Braunschweig, Germany) (Schmied et al., 2012). DNA origamis are bound at low density on a microscope coverslip, appearing as diffraction limited spots that are then quantified. For all quantifications, inhomogeneities due to the uneven TIRF illumination are reduced by applying a flat-field correction and by restricting the analyzed region to the central part of images. Then fluorescence quantification of diffraction limited spots is performed by using a custom 2D Gaussian fitting algorithm (Holtzer and Schmidt, 2009) that estimates the integrated intensity within the diffraction limited dot. Quantification of these spots shows the linear dependence of the integrated intensity with fluorophore number, laser power and exposure time (supplemental figure S6).

Snf7 oligomers quantification

The number of Snf7 molecules per dot (N_{Snf7}) is computed by dividing the intensity of the Snf7 dot (I_{Snf7_Dot}) with the estimated average intensity of a single fluorescent Snf7 ($I_{Snf7\text{-}Atto647N}$ or $I_{Snf7\text{-}Alexa488}$). As

we measured a labeling ratio of 40% with Snf7-Atto647N, an Atto647N fluorophore corresponds to 2.5 Snf7 molecules on average ($I_{Snf7\text{-}Atto647N} = I_{Atto647N}/2.5$). As no DNA origami is currently available with Alexa488, the average fluorescence of a single Snf7-Alexa488 molecule was estimated by comparing the intensity of the central part of patches made with either polymerized Snf7-Atto647N or polymerized Snf7-Alexa488. As the saturated part of patches has a fixed density of Snf7 molecules, this fluorescence ratio is the same at the single molecule level.

Snf7 filament polymerization speed quantification

The area occupied by each spiral, equals to 2π times their radius (figure 4I) to the square, has been plotted as a function of time. A linear fit ($R^2 = 0.98$) of the average area increase yields the given value. Only the first 40 seconds have been fitted.

Data processing

All fluorescent images quantifications were done using ImageJ software. AFM topography and nanomechanical maps have been analyzed in the Nanoscope-8 software (Bruker, Santa Barbara, CA). HS-AFM movies have been analyzed in ImageJ and self-written image analysis routines.

Relaxation of loaded ESCRT-III spiral springs drives membrane deformation.

—

Supplemental mathematical modeling

Chiaruttini *et al.*

Here we present the details of the mathematical modeling of the nucleation, growth and mechanics of Snf7 spirals. Our analysis relies on three interdependent levels of description. In Sec. 1 we describe the nucleation and growth dynamics of Snf7 spirals leading to full membrane coverage, allowing us to predict the distribution of spiral sizes observed by AFM. We then turn to the mechanical characterization of Snf7 filaments in Sec. 2, and estimate their persistence length from AFM data. Finally, Sec. 3 uses this estimate to model the in-plane elasticity of close-packed Snf7 spirals, thus accounting for the spontaneous curvature imparted by them to the membrane and observed in EM. The appendix presents the details of some numerical calculations presented in the main text.

1 Snf7 spiral nucleation and growth

We describe the nucleation and growth of a population of Snf7 spirals in agreement with observations described in the main text. Our model takes the form of a dynamical equation describing the time evolution for $c(a,t)$, the in-plane concentration of Snf7 spirals of area a at time t. In this analysis, we model Snf7 spirals as hard disks of variable radius. The quantity $c(a,t)$ has units of $(\text{length})^{-4}$, and the (dimensionless) number of disks of area comprised between a and $a+\mathrm{d}a$ on a membrane of area $\mathcal{A}$ is thus given by $c(a,t)\mathcal{A}\mathrm{d}a$. We assume throughout that $\mathcal{A}$ is much larger than the area occupied by any disk, implying that fluctuations of that number are negligible.

The observations of the main text indicate that Snf7 spirals form from rings with a 25 nm radius, following which their radius increases due to Snf7 polymerization. Accordingly, in the model new disks appear with a radius of $r_0 = 25\,\text{nm}$, implying $c(a < \pi r_0^2, t) = 0$. A disk grows continuously as long as there is space left on the membrane, *i.e.*, as long as the total area $A(\{c\},t)$ occupied by existing disks is smaller than $\mathcal{A}$. Here the notation $\{c\}$ denotes a functional dependence on $c(a,t)$ to be made explicit later. We assume that a disk grows by the ends of the Snf7 filament, implying a rate of monomer addition constant in time. Therefore, that the area of the disk grows at a constant rate, which we denote by w. We neglect the spontaneous nucleation of new isolated disks, and thus assume that the formation of new disks is catalyzed at the rim existing ones. This is analogous to a thermodynamic situation where homogeneous nucleation (in the middle of the bare membrane) is negligible compared to heterogeneous nucleation (in contact with existing disks). In the absence of steric constraints, each unit of existing rim length thus catalyses the formation of a number λ of new disks of area πr_0^2 per unit time. Denoting by $L(\{c\},t)$ total rim length of all existing disks, the nucleation rate in the absence of steric constraints is thus $\lambda L(\{c\},t)$. When the membrane becomes crowded by many existing disks, steric hindrance becomes important, and the probability of success of a nucleation event is proportional to the probability $P(\{c\},t)$ for the attempted nucleation site (a disk of radius r_0) to be free of other disks. As a result of these assumptions, the evolution equation for $c(a,t)$ reads

$$\frac{\partial c}{\partial t} = \begin{cases} -w\frac{\partial c}{\partial a} + \lambda\delta(a-\pi r_0^2)\frac{L(\{c\},t)}{\mathcal{A}}P(\{c\},t) & \text{if } A(\{c\},t) < \mathcal{A} \\ 0 & \text{if } A(\{c\},t) \geqslant \mathcal{A} \end{cases} \tag{1}$$

and in the following we use an initial condition reflecting the fact that the nucleation and growth process start from one or a few sparsely distributed disks of radius r_0:

$$c(a, t=0) = c_0\delta(a-\pi r_0^2), \tag{2}$$

where we will see below that the exact value of c_0 is of little consequence as long as the initial disks cover only a small fraction of the membrane (*i.e.*, as long as $c_0 \mathcal{A} \pi r_0^2 \ll 1$).

Here we first discuss the full functional form of $A(\{c\},t)$, $L(\{c\},t)$ and $P(\{c\},t)$ in Sec. 1.1. We then solve a linearized form of the equation in Sec. 1.2, yielding insights into the intermediate-time behavior of the solution. Finally, we present the numerical procedure used to predict the final disk size distribution, in Sec. 1.3.

1.1 Functional forms of $A(\{c\},t)$, $L(\{c\},t)$ and $P(\{c\},t)$

The total area occupied by disks is obtained by summing over all disks, simply yielding:

$$\frac{A(\{c\},t)}{\mathcal{A}} = \int_{r_0}^{+\infty} a c(a,t)\,\mathrm{d}a. \tag{3}$$

The total rim length is similarly obtained, noting that the rim length of a disk of area a is equal to $2\sqrt{\pi a}$:

$$\frac{L(\{c\},t)}{\mathcal{A}} = \int_{r_0}^{+\infty} 2\sqrt{\pi a} c(a,t)\,\mathrm{d}a. \tag{4}$$

Contrary to these two quantities, the probability $P(\{c\},t)$ is a complex object dependent on the positions of all existing disks. To obtain a tractable form of the equations, we assume that disk diffusion is sufficient for the distribution of the disk positions to be reasonably approximated by a random distribution of non-overlapping disks in the plane. This assumption breaks down only at large length scales, which gives rise to the front dynamics described in the main text. We thus need to estimate the probability that the intended nucleation site, a circle of radius r_0, does not overlap with any existing disk. Denoting the total number of disks as N, this probability can be written as

$$P(\{c\},t) = p(N|1\ldots N-1) \times p(N-1|1\ldots N-2) \times \ldots \times p(3|1,2) \times p(2|1) \times p(1), \tag{5}$$

where $p(i|1\ldots i-1)$ is the probability that disk i does not overlap with the intended nucleation site, assuming none of the $i-1$ first particle do. To compute this conditional probability, we thus consider a non-overlapping distribution of the $i-1$ first disks that does not interfere with the intended nucleation site, pictured by a dashed circle in supplemental figure S3A. We then position the i^{th} disk at a random location not overlapping the existing disks, and ask what the probability is that this random location does not interfere with the intended nucleation site. The locations accessible to the center of mass of the i^{th} particle are colored in white in supplemental figure S3A. Neglecting overlaps between the forbidden (grey) areas[1], this area reads $\mathcal{A}_i^{\text{eff}} = \mathcal{A} - \sum_{j=1}^{i-1} \pi (r_j + r_i)^2$, where r_j is the radius of disk j. On the other hand, if the center of the i^{th} disk falls within the dotted circle, this circle will overlap with the intended nucleation site. As a result (and again neglecting overlaps), the probability that disk i does not overlap with the intended nucleation site reads

$$p(i|1\ldots i-1) = 1 - \frac{\pi (r_0+r_i)^2}{\mathcal{A}_i^{\text{eff}}} \simeq \exp\left[-\frac{\pi (r_0+r_i)^2}{\mathcal{A}_i^{\text{eff}}}\right], \tag{6}$$

with $\pi(r_0+r_i)^2$ the area of the dotted circle, and where the approximate equality is obtained by noting that $\pi(r_0+r_i)^2 \ll \mathcal{A}_i^{\text{eff}}$ typically. As a result:

$$P(\{c\},t) \simeq \exp\left[-\sum_{i=1}^{N} \frac{\pi (r_0+r_i)^2}{\mathcal{A}_i^{\text{eff}}}\right]. \tag{7}$$

1.2 Linearized solution to the nucleation and growth equation

For low membrane coverages [$A(\{c\},t) \ll \mathcal{A}$], the argument of the exponential in Eq. (7) is much smaller than one, and we may approximate $P(\{c\},t) \simeq 1$, yielding

$$\frac{\partial c}{\partial t} = -w\frac{\partial c}{\partial a} + \lambda \delta(a - \pi r_0^2) \int_{r_0}^{+\infty} 2\sqrt{\pi a} c(a,t)\,\mathrm{d}a. \tag{8}$$

[1]While this assumption breaks down at high disk densities, this turns out to be of little consequence to our final results as nucleation rates at such densities are negligibly small anyway.

Our goal here is thus to solve this equation with the initial condition Eq. (2). We first make the following changes of variables:

$$\tilde{a} = \frac{a - \pi r_0^2}{(w/\lambda)^{2/3}} \tag{9a}$$

$$\tilde{z} = (w\lambda^2)^{1/3} t - \tilde{a} \tag{9b}$$

$$c(a,t) = c_0 \left[\delta(\tilde{z}) + \Theta(\tilde{a})\tilde{c}(z)\right], \tag{9c}$$

where Θ is the Heaviside step function and $\tilde{c}$ a smooth function of z. The change of variable of Eq. (9c) makes use of the fact that for $a > \pi r_0^2$ Eq. (8) is simply an advection equation for $c(a,t)$, implying that the concentration of disks depends on a and t only through $\tilde{z}$. As a result, any function of the form given in Eq. (9c) is a solution of Eq. (8) for $\tilde{a} > 0$. Now focusing on the vicinity on $\tilde{a} = 0$, we use our new variables and integrate Eq. (8) in a small neighborhood of $\tilde{a} = 0$, thus retaining only the contributions of the terms with a $\delta(\tilde{a})$. This results in a first-order evolution equation in one variable, a considerable simplification compared to the integro-differential PDE Eq. (8):

$$\tilde{c}(\tilde{z}) = 2\sqrt{\pi(\tilde{z} + \tilde{a}_0)} + 2\sqrt{\pi} \int_0^{\tilde{z}} \sqrt{(\tilde{z} - \tilde{z}' + \tilde{a}_0)}\tilde{c}(\tilde{z}')\, \mathrm{d}\tilde{z}', \tag{10}$$

where

$$\tilde{a}_0 = \pi r_0^2 \left(\frac{\lambda}{w}\right)^{2/3}. \tag{11}$$

We now solve Eq. 10 using a Laplace transform. Defining $\tilde{f}(\tilde{z}) = 2\sqrt{\pi(\tilde{z} + \tilde{a}_0)}$, we find that this equation can be rewritten as

$$\tilde{c} = \tilde{f} + \tilde{f} * \tilde{c}, \tag{12}$$

where $*$ denotes the convolution. Using hats to denote Laplace transforms, this yields in Laplace space

$$\hat{c} = \hat{f} + \hat{f} \times \hat{c} \Rightarrow \hat{c} = \frac{\hat{f}}{1 - \hat{f}}. \tag{13}$$

As a result, the $\tilde{t} \to \infty$ behavior of the disk concentration is dominated by the rightmost pole s^* of $\hat{c}$, *i.e.*.

$$c(a,t) \underset{\tilde{t} \gg 1}{\propto} \Theta(a - \pi r_0^2)\Theta(a_{\max} - a) \exp\left[s^* \left(w^{1/3}\lambda^{2/3} t - \frac{\lambda^{2/3}(a - \pi r_0^2)}{w^{2/3}}\right)\right], \tag{14}$$

where $a_{\max} = \pi r_0^2 + wt$. Since

$$\hat{f}(s) = \frac{\pi}{s^{3/2}} \left[2\sqrt{\frac{\tilde{a}_0 s}{\pi}} + e^{\tilde{a}_0 s} \mathrm{erfc}\left(\sqrt{\tilde{a}_0 s}\right)\right], \tag{15}$$

s^* is given as the solution of

$$(s^*)^{3/2} - 2\sqrt{\pi \tilde{a}_0 s^*} = \pi e^{\tilde{a}_0 s^*} \mathrm{erfc}\left(\sqrt{\tilde{a}_0 s^*}\right). \tag{16}$$

The solutions of this equation are plotted in supplemental figure S3B.

Therefore, Eq. (14) shows that following a short transient regime during which the exponential a-dependence of the concentration becomes established, the concentration starts growing exponentially as discussed in the main text. Since the linearized Eq. (8) is valid as long as $A/\mathcal{A} \approx C(w/\lambda)^{2/3} \ll 1$, this regime properly describes the disk concentration profile for $c_0 \ll C \ll (w/\lambda)^{-2/3}$.

1.3 Numerical integration of the fully nonlinear equation

To obtain the full distribution of disk sizes at full membrane coverage, we numerically simulate the growth process described above. The simulation uses the dimensionless units described in Sec. 1.2, and thus the only dimensionless fitting parameter is $\tilde{a}_0$. The values of the initial concentration of the disks and the total area of the membrane have no influence on the final size distribution as long as the area of the total membrane is much larger than the one of a typical disk and the initial concentration is very small.

In practice we choose a ratio of membrane area to typical disk area $\mathcal{A}(\lambda/w)^{2/3} = 10^5$ with an initial number of particles $c_0\mathcal{A} = 10^2$, implying that the initial fraction of the area occupied by particles is $\tilde{a}_0 \times 10^{-3} \approx 10^{-3}$.

We explicitely keep track of the size of all disks during the simulation, starting the initial ones at area $\tilde{a}_0$. We implement time stepping with a time step equal to $\mathrm{d}\tilde{t} = 10^{-2}$, small enough that its value has no bearing on our results. At each time step, each existing disk is grown by a dimensionless area $\mathrm{d}\tilde{t}$. The total dimensionless perimeter of all disks $\tilde{L}(\{c\}, t)$ and the probability $P(\{c\}, t)$ of a successful nucleation event are computed. According to the process described above, the average number of nucleation events during a time step is thus $P(\{c\}, t)\tilde{L}(\{c\}, t)\mathrm{d}\tilde{t}$. This number is generally not an integer, and therefore at each time step we draw a number n from a Poisson distribution with average $\langle n\rangle = P(\{c\}, t)\tilde{L}(\{c\}, t)\mathrm{d}\tilde{t}$ and create a number n of disks with area $\tilde{a}_0$. We check that the initial number of disks $c_0\mathcal{A} = 10^2$ is sufficiently large that the statistical fluctuations due to this stochastic process are negligible.

We then use $\tilde{a}_0$ as an adjustable parameter to minimize the difference between the predicted and measured disk size cumulative probability distribution (figure 3D of the main text). As shown in supplemental figure S3C,the best fit is obtained for $\tilde{a}_0 = 0.043$, and a variation of this parameter by $\pm 10\%$ induces a significantly worse fit. Inverting Eq. (11) we find

$$\frac{w}{\lambda} = \left(\frac{\pi r_0^2}{\tilde{a}_0}\right)^{3/2} \simeq 9.8 \pm 1.5 \times 10^{-21}\,\mathrm{m}^3, \tag{17}$$

where we used the experimentally determined value $r_0 \simeq 25\,\mathrm{nm}$.

2 Snf7 filament persistence length inferred from AFM data

To extract mechanical information from the Snf7 polymer profiles observed in AFM, we develop a data analysis framework that makes the best use of the available dataset, as well as filters out the unphysical high-frequency noise induced by local displacements of the filament by the AFM tip during scanning.

We assume that the Snf7 polymer is a persistent chain of discrete elements (reflecting the discrete pixels of the chain in the AFM data) labeled by n with possibly unequal lengths Δs_n. As a result, the angle ϕ_n characterizing the orientation of the nth element of the chain satisfies the recursion:

$$\phi_n = \phi_{n-1} + \sqrt{\frac{\Delta s_n}{2\ell_p}}\xi_n^\phi, \tag{18}$$

with ℓ_p the persistence length of the filament and ξ_n^ϕ a random number drawn from the distribution

$$P_\xi(\xi) = \frac{1}{\sqrt{2\pi}} e^{-\xi^2/2}. \tag{19}$$

Collisions between the tip of the atomic force microscope generate localized shifts of the filament during the imaging procedure, thus introducing an error in the determination of the true polymer angle ϕ_n. We model this error by introducing the measured angle θ_n as a noisy version of ϕ_n:

$$\theta_n = \phi_n + \alpha\xi_n^\theta, \tag{20}$$

where α characterizes the amplitude of the noise and ξ_n^θ is also drawn from the probability distribution of Eq. (19). These rules allow us to compute the effect of the noise on the usual angle correlation function:

$$\begin{aligned}\langle\cos(\theta_n - \theta_0)\rangle &= \left\langle\cos\left[\phi_n - \phi_0 + \alpha(\xi_n^\theta - \xi_0^\theta)\right]\right\rangle \\ &= \langle\cos(\phi_n - \phi_0)\rangle\left\langle\cos\left[\alpha(\xi_n^\theta - \xi_0^\theta)\right]\right\rangle \\ &= e^{-s_n/\ell_p}e^{-\alpha^2},\end{aligned} \tag{21}$$

where $s_n = \sum_{i=1}^n \Delta s_n$. Therefore, the effect of noise is simply to renormalize the angle correlation function by a constant factor $e^{-\alpha^2}$.

To compare this prediction to the experimental data, we now define

$$\zeta_n = e^{i\theta_n}, \tag{22}$$

the Fourier transform of which is easily extracted from the data as a function of wavevector q:

$$\hat{\zeta}(q) = \sum_n e^{i(qs_n+\theta_n)}\Delta s_n \tag{23}$$

In the limit where the total length S of the filament is much longer than both its segments and its persistence length, we can re-write

$$\begin{aligned} C(q) &= \left\langle \hat{\zeta}(q)\hat{\zeta}^*(q) \right\rangle \\ &= S\int_{-\infty}^{\infty} \langle \zeta(s)\zeta^*(s+\delta s)\rangle \, e^{iq\delta s}\,\mathrm{d}\delta s \\ &= \frac{2\ell_p e^{-\alpha^2}}{1+(q\ell_p)^2}, \end{aligned} \tag{24}$$

where the theoretical result of Eq. (21) is used to derive the last equality. Computing $C(q)$ by averaging over the available dataset, we can compare the predicted distribution with the experimental one as shown in supplemental figure S3E. The agreement is satisfactory, especially in the small-q region characterizing the filaments' long-range behavior. Due to the AFM-induced small-scale fluctuations of the filament, we expect that the most reliable information about its persistence length will be given by this small-q region, which is reflected in the two first even terms of the Taylor expansion of Eq. (24):

$$\begin{aligned} \left(\sum_n e^{i\theta_n}\Delta s_n\right)^2 &= 2\ell_p e^{-\alpha^2} \\ -q^2\left(\sum_n e^{i\theta_n} s_n \Delta s_n\right)^2 &= -2\ell_p e^{-\alpha^2}(q\ell_p)^2, \end{aligned} \tag{25}$$

yielding a simple, reliable determination of ℓ_p that averages over all available data[2]:

$$\ell_p = \frac{\sum_n e^{i\theta_n} s_n \Delta s_n}{\sum_n e^{i\theta_n}\Delta s_n}. \tag{26}$$

As discussed in the main text, we find $\ell_p \simeq 260\,\mathrm{nm}$.

3 Elasticity of Snf7 spirals and membrane curling

Although the calculation presented in Sec. 1 discusses the nucleation and growth of perfectly circular Snf7 disks until full membrane coverage, in practice this process leaves small gaps between such disks that are too small to be filled by newly nucleated disks. As the existing disks continue to grow, they fill the voids by deforming into a more polygonal profile, as shown in figure 5A of the main text. In this deformed state, elastic energy is stored in the (deformed) disks. This elastic energy can be liberated if the membrane is ruptured, yielding membrane curling as shown in figure 6 of the main text. Here we use our independent measurements of the disks' polymerization energy μ (Eq. 1 of the main text) and our quantification of their deformability throught the measurement of ℓ_p (see Sec. 2) to predict how much elastic energy they are able to store. This allows us to predict the amount of curling expected upon membrane rupture.

Following the polymerization-deformation process described here, we represent the Snf7-covered membrane as a triangular lattice of deformed disks shown in supplemental figure S3F (a). Upon membrane rupture, these disks relax into their resting, circular form, increasing the area effectively occupied by the membrane coat from a deformed value A' to the resting value A supplemental figure S3F (b). The coat is bound to an initially flat inextensible membrane whose area remains constant during this relaxation; the resulting area mismatch between the relaxed coat and the membrane implies that the composite lipid-protein layer has a spontaneous (curling) curvature

$$r_c = \frac{d}{2}\frac{A+A'}{A-A'}, \tag{27}$$

[2]as opposed to, *e.g.*, the often-used measurement of the filaments' end-to-end lengths.

where $2d \simeq 9\,\mathrm{nm}$ is the total thickness of the Snf7-coated membrane.

To determine the values of A and A' and the resulting spontaneous curvature, we parametrize the disk deformation by ℓ', which we define as the distance of flat contact between two neighboring disks (supplemental figure S3F) (a).We also introduce the sides L' and L of the deformed and undeformed hexagonal unit cell, and the corresponding filament curvature radii R' and R (we approximate the curved part of the deformed filaments to a circle). AFM measurements yield $R \simeq 130\,\mathrm{nm}$. Assuming that the number of filaments and their lengths is conserved upon compression, simple geometry yields:

$$R = \frac{\sqrt{3}}{2} L \tag{28a}$$

$$L = L' + \left(\frac{2\sqrt{3}}{\pi} - 1\right)\ell' \tag{28b}$$

$$R' = \frac{\sqrt{3}}{2}(L' - \ell') \tag{28c}$$

where the two last equation implies that the disks can be compressed by up to $1-L'/L = 1-\pi/(2\sqrt{3}) \simeq 9\%$ until full membrane coverage is reached (corresponding to $R' = 0$ and $L' = L$).

As shown in supplemental figure S3F (a), the spacing between the filaments within a deformed disk is the denoted by $b \sim 17\,\mathrm{nm}$, a length of the order of the filament thickness. We assume for simplicity that the shape of these filaments are identical up to a scaling factor ν, with $0 < \nu \leqslant 1$, with $\nu = 1$ corresponding to the outermost ring. We now focus on the elastic energy of these filaments, which we describe as elastic beams with persistence length ℓ_p given by Eq. (26) and spontaneous curvature c, yielding a single-filament elastic energy

$$e = \frac{k_B T \ell_p}{2} \int [\phi'(s) - c]^2 \,\mathrm{d}s, \tag{29}$$

where the arclength s is defined as in Sec. 2 and the angle $\phi(s)$ is a continuum version of the local filament orientation used there. The continuum description of Eq. (29) is essentially identical to that of Sec. 2, with the addition of the spontaneous curvature possibly induced by self-assembly into a ring as in (Lenz et al., 2009). Interestingly, we show below that the value of c does not in any way affect the results of this section, and thus we need not make any assumption about its origin or numerical value. In the situation of supplemental figure S3F, the energy of the filament described by scaling factor ν reads

$$\begin{aligned} e(\nu) &= 6\nu L' \times \frac{k_B T \ell_p}{2} c^2 + 2\pi\nu R' \times \frac{k_B T \ell_p}{2}\left(\frac{1}{\nu R'} - c\right)^2 \\ &= \frac{\pi k_B T \ell_p}{\nu R'} + \text{terms independent of the amplitude of the deformation,} \end{aligned} \tag{30}$$

where the second equation uses Eqs. (28).

To obtain the full elastic energy of a deformed disk, we sum over all filaments composing it, assuming the initial radius of the innermost filament is b:

$$\begin{aligned} E &= \sum_{\nu R/b=1}^{R/b} e(\nu) \\ &\simeq \int_{b/R}^{1} e(\nu)\frac{R}{b}\,\mathrm{d}\nu \\ &= \frac{\pi k_B T \ell_p \ln(R/b)}{b}\frac{R}{R'}. \end{aligned} \tag{31}$$

The derivative of this energy with respect to the area $3\sqrt{3}L'^2/2$ of the hexagonal unit cell enclosing the disk is equal to $-\mu$, where μ is the lateral two-dimensional pressure within the Snf7 coat. In the main text we measure $\mu \simeq 3.1 \times 10^{-4}\,\mathrm{N/m}$. Using Eqs (28) to express L' as a function of R', we find that this equality is equivalent to:

$$1 = \frac{\mu}{\mu^*}\left(\frac{R'}{R}\right)^2 \left[1 + \left(\frac{2\sqrt{3}}{\pi} - 1\right)\frac{R'}{R}\right], \tag{32}$$

with

$$\mu^* = \frac{1}{2 - \pi/\sqrt{3}} \frac{k_B T \ell_p}{R^2 b} \ln(R/b) \simeq 4.1 \times 10^{-5}\,\mathrm{N/m} \tag{33}$$

the typical lateral pressure required to deform the disks. Note that the calculation presented here breaks down if $\mu \lesssim \mu^*$, as μ^* is of the order of the threshold pressure required to flatten part of the disks as represented in supplemental figure S3F (a). Here we are however in the opposite limit $\mu \gg \mu^*$, implying that the following simple asymptotic solution to Eq. (32) gives R' with a precision of order 2%:

$$R' = R\sqrt{\mu^*/\mu} \simeq 47\,\mathrm{nm}. \tag{34}$$

Using Eqs. (28) to express the areas of Eq. (27) as functions of R', we find

$$r_c = \frac{d}{2}\frac{1+\left[\frac{\pi}{2\sqrt{3}}+\left(1-\frac{\pi}{2\sqrt{3}}\right)\frac{R'}{R}\right]^2}{1-\left[\frac{\pi}{2\sqrt{3}}+\left(1-\frac{\pi}{2\sqrt{3}}\right)\frac{R'}{R}\right]^2} \simeq 37\,\mathrm{nm}, \tag{35}$$

a value compatible with our EM observations, implying that membrane curling is indeed induced by the stresses accumulated in the Snf7 coat.

Appendix: Supplementary calculations

Estimate of the Snf7 filament growth rate. The data from the main text indicate an area growth rate $w = 8.0 \times 10^{-17}\,\mathrm{m}^2 \cdot \mathrm{s}^{-1}$ at a bulk concentration $[\mathrm{Snf7}] = 1\,\mu\mathrm{M}$. To translate this value into a number of subunits per seconds, we assumed that (i) The Snf7 spiral grows by its external tip, located at the outermost ring of the spiral, (ii) the tip growth rate is proportional to [Snf7], as shown in figure 1H of the main text, (iii) spirals consist of concentric double stranded turns separated by the distance $b = 17\,\mathrm{nm}$ (figure 2E of the main text), and (iv) The distance between consecutive monomers of a single Snf7 strand is $l = 3.2\,\mathrm{nm}$ (Shen et al., 2014). With these assumptions, the subunit addition rate reads

$$\frac{2w}{[\mathrm{Snf7}] \times b \times l} \simeq 3 \pm 1\,\mathrm{subunits} \cdot \mathrm{s}^{-1} \cdot \mu\mathrm{M}^{-1}. \tag{36}$$

Basis for neglecting the patch nucleation rate in front of the spiral nucleation rate. The number of patch nucleation event per unit time on a membrane domain of area $\mathcal{A}$ reads $\Lambda\mathcal{A}$, with $\Lambda = 1.4 \times 10^8\,\mathrm{m}^{-2} \cdot \mathrm{s}^{-1}$ the patch nucleation rate (value estimated for $[\mathrm{Snf7}] = 1\,\mu\mathrm{M}$; see figure 1C of the main text). If a fraction f of the membrane is covered by spirals, this area hosts $\approx f\mathcal{A}/(\pi R^2)$ spirals, with $R \approx 130\,\mathrm{nm}$ the radius of a spiral; thus the total disk perimeter in the domain is $\mathcal{P} = 2\pi f R \times [\mathcal{A}/(\pi R^2)] = 2f\mathcal{A}/R$ and the resulting spiral nucleation rate reads $2f\lambda\mathcal{A}/R$ with $\lambda = 8.2 \times 10^3\,\mathrm{m}^{-1} \cdot \mathrm{s}^{-1}$. Therefore patch nucleation becomes negligible in front of spiral nucleation as soon as the fraction of the membrane covered by spirals exceeds

$$f^* = \frac{\Lambda R}{2\lambda} \simeq 0.11\%, \tag{37}$$

which is verified in virtually all of our experimental conditions.

Computation of the Snf7 polymerization force μ. We use a standard micromanipulation technique (figure 5G and references in the main text) where a giant unilamellar vesicle is held by an aspiration pipette, which allows control over the membrane tension σ. We next pull a membrane tether from the vesicle using optical tweezers. Assuming a membrane bending modulus κ, the initial force required to hold the tether is $F_i = 2\pi\sqrt{2\sigma\kappa}$. We next polymerize an Snf7 coat on the membrane of the giant unilamellar vesicle. No significant polymerization occurs on the tether. The coat is under lateral compression due to the protein's propensity to polymerize. At equilibrium, this results in a lateral two dimensional pressure equal to the coat's polymerization energy per unit surface μ. As a result, the effective membrane consisting of the lipid bilayer and coat has an effective surface tension $\sigma' - \mu$, where σ' is the final tension of the membrane alone. This effective tension is equal to the tension σ imposed by the aspiration pipette, and thus the final force required to hold the tether is $F_f = 2\pi\sqrt{2\sigma'\kappa} = 2\pi\sqrt{2(\sigma+\mu)\kappa}$. Combining the expressions of F_i and F_f yields Eq. (1) of the main text.

Computation of elastic energy stored in a single Snf7 spiral. We use Eq. (31) to estimate the elastic energy accumulated in one Snf7 spiral due to the polymerization pressure:

$$E(R') - E(R) = \frac{\pi k_B T \ell_p \ln(R/b)}{b} \left(\frac{R}{R'} - 1 \right) \simeq 7.0 \times 10^{-19}\,\mathrm{J} \simeq 170 k_B T, \tag{38}$$

where Eq. (34) was used to estimate R'.

Supplemental references

Ando, T., Kodera, N., Takai, E., Maruyama, D., Saito, K., and Toda, A. (2001). A high-speed atomic force microscope for studying biological macromolecules. Proc. Natl. Acad. Sci. U. S. A. *98*, 12468–12472.

Holtzer, L., and Schmidt, T. (2009). The tracking of individual molecules in cells and tissues. In Single Particle Tracking and Single Molecule Energy Transfer, (John Wiley & Sons (Hoboken, New Jersey)), pp. 25–42.

Schmied, J.J., Gietl, A., Holzmeister, P., Forthmann, C., Steinhauer, C., Dammeyer, T., and Tinnefeld, P. (2012). Fluorescence and super-resolution standards based on DNA origami. Nat. Methods *9*, 1133–1134.