

Supporting Information

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Mathematical Modeling

Here we discuss in detail the assumptions underlying the theoretical considerations presented in the main text. An effort is made toward an untechnical, yet rigorous, treatment. First we introduce our model, and then four specific points are discussed.

Presentation of the Model. We consider a membrane tube pulled by optical tweezers and connected to a membrane tension reservoir (the aspiration-controlled vesicle). The tube has a length L and is partially covered by a possibly discontinuous coat of dynamin over a total length L_d . We neglect the transition regions between bare and dynamin-covered sections of the tube. Taking into account a force f externally applied on the tube (by the optical tweezers), we write the free energy of the system as the sum of the free energies of the two types of regions, plus the work of the force:

$$F = \mu_b(L - L_d) + \mu_d L_d - fL, \quad [\text{S1}]$$

where μ_b and μ_d stand for the free energy per unit length of the bare region and the dynamin-covered region, respectively. For μ_b we use the classic expression (1)

$$\mu_b = \frac{\pi\kappa}{r} + 2\pi r\sigma, \quad [\text{S2}]$$

where κ is the bending modulus of the cylindrical membrane, r is its radius, and σ is its surface tension. We consider timescales that are large compared with the mechanical equilibration of the tube and the equilibration of the lipids with the reservoir (≈ 1 s). Therefore, we assume in all of the following that the bare sections minimize their free energy with respect to r and that σ is equal to the value imposed by the reservoir. This yields

$$r = \sqrt{\frac{\kappa}{2\sigma}}, \quad \mu_b = 2\pi\sqrt{2\sigma\kappa}. \quad [\text{S3}]$$

To construct the free energy of the dynamin-covered part, we take into account the bending and tension energies of the membrane as well as a dynamin-related term P . This term accounts for the free energy difference between dynamin in solution and in the membrane-bound polymerized state. This includes the following effects:

- The binding energy of a dynamin dimer to an already existing helix—this quantity might depend on the salinity of the solution.
- The energy gain of dynamin upon binding the membrane, and the change of the membrane energy upon dynamin binding [e.g., insertion of dynamin's hydrophobic loops into the bilayer (2)]—this quantity depends on the chemical composition of the membrane.
- The loss of entropy for leaving the solution and going into the immobilized polymerized state—this quantity depends on the concentration c of the dynamin solution.
- The elastic cost of deforming the helix away from its preferred radius—this quantity depends on the inner radius r_d of the dynamin helix.

The free energy per unit length of the dynamin-covered tube then reads

$$\mu_d = \frac{\pi\kappa}{r_d} + 2\pi r_d\sigma - P(c, r_d), \quad [\text{S4}]$$

where $P > 0$ denotes a situation where dynamin dimers are more stable in the polymerized state than in solution.

Throughout this work, we do not consider the dependence of P on r_d , and write $P(c)$. This assumption is justified theoretically in *SI Section 1*, where we argue a priori that dynamin is so stiff that the radius r_d is not influenced by membrane tension. This makes the r_d dependence of P irrelevant. This point is demonstrated experimentally in the main text. In *SI Section 2*, we prove that thermodynamic stability implies that forming a dynamin helix is only possible in a certain (c -dependent) range of membrane tensions, which according to Eq. S3 can be translated into a certain range of initial tubule radii. In *SI Section 3*, we show that under mechanical and membrane equilibrium conditions, the force exerted by the tube on the optical tweezers is $f_b = \mu_b$ as long as the tube is not fully covered by dynamin ($L_d < L$) and drops to $f_d = \mu_d$ when full coverage is achieved ($L_d = L$).

1. A Priori Argument for a Constant Dynamin Radius

We argue here that the radius of the dynamin helices described in the main text does not depend on the tension of the membrane. Electron microscopy data point in that direction, as they consistently show that dynamin on soft membrane templates has a radius in the vicinity of 10 nm (3–6). However, one could argue that all these references use floppy membrane templates and that the higher membrane tensions used in our experiments might exert stronger stresses on dynamin, therefore deforming it substantially.

The argument given here is a comparison of the elasticities of the membrane and the dynamin helix. The elasticity of the membrane is known to be well-described by its bending modulus $\kappa = 16.1 k_B T$ (see main text). To our knowledge, the only data available in the literature concerning the elasticity of dynamin are a measurement of the persistence length of dynamin-covered tubules performed in ref. 7, which yields $\ell_p = 37.3 \pm 4.6 \mu\text{m}$. We recall that the persistence length is the length over which an elastic filament is curved by the thermal fluctuations of the surrounding medium. It therefore characterizes how difficult it is to bend the filament (a stiff filament will have a large ℓ_p). It is possible to use this value to evaluate the bending rigidity of the dynamin coat. A detailed derivation of this bending stiffness for a particular toy model of dynamin elasticity is proposed in appendix C of ref. 8 and commented on in section IV.B of the same paper. Here we propose an order-of-magnitude argument leading to the same conclusions. We need to compare two quantities:

- On the one hand the elasticity of the dynamin helix, characterized by the elastic constant $k_B T \ell_p \approx 2 \times 10^{-25}$ J.m, which quantifies to what extent the dynamin coat wants to be curved to its preferred radius r_d , assuming the elastic properties of the helix are reasonably isotropic.
- On the other hand the bending modulus κ of the membrane, which quantifies how much the membrane wants to be flat.

Because the former is an energy \times length and the latter an energy, we need to introduce a characteristic length scale of the system to compare them. The appropriate length is obviously the radius of the tube $r_d \approx 10^{-8}$ m. Therefore, we can define the dimensionless ratio characterizing the relative stiffness of the membrane and the helix:

$$\frac{\text{stiffness of the membrane}}{\text{stiffness of the helix}} \approx \frac{\kappa}{k_B T \ell_p / r_d} \approx 3 \times 10^{-3}. \quad [\text{S5}]$$

Because this ratio is much smaller than one, we conclude that the influence of the elastic stresses exerted by the membrane on the helix will be small, and therefore that the radius r_d of the dynamin coat will not be substantially influenced by the membrane, justifying that we consider it constant throughout this work. This assumption is verified experimentally in Fig. 2C, where it is found that $f_d = \mu_d$ depends linearly on σ , which according to Eq. S4 shows that r_d is constant.

2. Threshold Radii and Concentration Regimes

Here we present a thermodynamic criterion for the stability of the dynamin polymer. At equilibrium, we expect that the tube will either be dynamin-coated or bare depending on which situation has the lowest free energy. Therefore, we expect that no nucleation of dynamin oligomers will be observed if a bare tube is the preferred state for a large system.

It is fairly obvious that the polymer stability criterion we are looking for is $\mu_d < \mu_b$ (for instance, one can consider whether minimizing F with respect to L_d yields $L_d = 0$ or $L_d = L$). Using Eq. S2 and S4, it is seen to be equivalent to

$$\sigma - \frac{\sqrt{2\kappa}}{r_d} \sqrt{\sigma} + \left(\frac{\kappa}{2r_d^2} - \frac{P}{2\pi r_d} \right) < 0, \quad [\text{S6}]$$

which with the help of Eq. S3 can be expressed as a condition on the initial bare tube radius r :

$$1 - 2\frac{r}{r_d} + \left(1 - \frac{Pr_d}{\pi\kappa} \right) \left(\frac{r}{r_d} \right)^2 < 0. \quad [\text{S7}]$$

This criterion is illustrated in Fig. S7.

There are three regimes for this inequality, as pictured in Fig. 4D:

- (i) For $P < P_1 = 0$, Eq. S7 has no solution.
- (ii) For $P_1 < P < P_2 = \pi\kappa/r_d$, Eq. S7 is satisfied on the interval

$$r_c^- = \frac{r_d}{1 + \sqrt{\frac{Pr_d}{\pi\kappa}}} < r < r_c^+ = \frac{r_d}{1 - \sqrt{\frac{Pr_d}{\pi\kappa}}}. \quad [\text{S8}]$$

- (iii) For $P_2 < P$, the upper threshold radius diverges and Eq. S7 is equivalent to $r > r_c^-$.

To express r_c^- and r_c^+ as functions of c , we have to specify a particular form for the dependence of P on c . In the limit of a very dilute solution, one can use the ideal solution hypothesis

$$P(c) = P \left(12 \mu\text{M} \right) + \frac{k_B T}{a} \ln \left(\frac{c}{12 \mu\text{M}} \right), \quad [\text{S9}]$$

where $a = 0.85$ nm is the length increment of the polymer upon binding of a dynamin dimer and where the value of $P(12 \mu\text{M})$ is deduced from the measurement of $f_d(12 \mu\text{M})$ (see main text). Eq. S9 allows us to express the boundaries c_1^* and c_2^* of the three concentration regimes described in the main text as the concentrations at which $P = P_1$ and $P = P_2$, respectively. This yields

$$c_1^* = \left(12 \mu\text{M} \right) \times \exp \left[-a \frac{P(12 \mu\text{M})}{k_B T} \right] \approx 280 \text{ nM}, \quad [\text{S10}]$$

$$c_2^* = \left(12 \mu\text{M} \right) \times \exp \left[a \frac{(\pi\kappa/r_d) - P(12 \mu\text{M})}{k_B T} \right] \approx 12.6 \mu\text{M}. \quad [\text{S11}]$$

Finally, we note that the argument presented here concerns equilibrium situations, but that kinetic effects could prevent the observation of dynamin nucleation even at concentrations and tensions where it is favored.

3. Force Drop and the Influence of Dynamin Adsorption

3.1. Tubes Partially Covered by Dynamin. Here we are interested in time scales that are large compared with the mechanical and membrane equilibration times, but short compared with the dynamin growth times. In this section, we therefore consider the growth process of dynamin to be essentially frozen, with L_d fixed. The behavior of the tube on longer time scales is described in *SI Section 3.3*. The partial equilibrium of the system is described by a minimization of F with respect to L and r , but with $L_d < L$ imposed. This yields the force needed to hold the partially dynamin-covered tube:

$$f = \mu_b = 2\pi\sqrt{2\sigma\kappa}, \quad [\text{S12}]$$

which is equal to the force f_b needed to hold a completely bare membrane tube. Hence, a partial dynamin coat does not change the force needed to pull a tube. An intuitive way of seeing this result is to say that although dynamin is able to exert a force while polymerizing, this force can only be visualized in our setup if it pushes the bead held by the optical tweezers away from its resting position. As long as the dynamin helix touches only the bead or only the vesicle or is discontinuous between the two, it will not be able to induce such a displacement, whereas it will if it is continuous. Because the membrane is a two-dimensional liquid on the timescales considered, this situation is exactly that of a swimmer who is unable to move a rock sitting in the middle of the pool, but who will be able to take two rocks apart by placing himself between them and pushing on both of them at the same time.

3.2. Adsorbed Dynamin Has a Negligible Influence on the Tube. In our model, we consider only the influence of polymerized dynamin and not of adsorbed dynamin. Here we show that the amount of dynamin adsorbed on the membrane tube is not only too low to be seen in fluorescence microscopy but also too low to have any measurable mechanical effect. Indeed, in general, proteins adsorbed on a membrane are expected to change its bending rigidity from κ to $\tilde{\kappa}$ and to induce a spontaneous curvature r_0 . Such modifications are expected to change the force needed to maintain a bare tube to

$$\tilde{f}_b = 2\pi\sqrt{2\sigma\tilde{\kappa}} \left(\sqrt{1 + \frac{\tilde{\kappa}}{2\sigma r_0^2}} - \sqrt{\frac{\tilde{\kappa}}{2\sigma r_0^2}} \right). \quad [\text{S13}]$$

Similar to what was discussed above, this result is expected to still be valid if the tube is partially coated by dynamin.

Experimentally, such deviations of the force are not observed. Indeed, we see in Fig. 2E that the force needed to hold the tube does not change upon dynamin injection until the tube is fully covered by the polymer. Hence, we can safely neglect dynamin adsorption.

3.3. Tubes Fully Covered by Dynamin. Here we deal with long time scales, where the dynamin polymer fully covers the membrane tube. This is equivalent to assuming a chemical equilibrium between polymerized dynamin and the dynamin solution. When dynamin fully covers the tube ($L_d = L$), the growing polymer touches both the vesicle and the optical tweezers' bead, hence exerting a force pushing them apart in the same way that a polymerizing microtubule held at one end can exert a force on a wall at its other end (9). Returning to our formalism, we note that $P(c)$ has units of a force, and it can be seen as the force one needs to exert on a polymerizing dynamin helix to stall its growth. This force is exactly equivalent to the stall force of polymerizing microtubules.

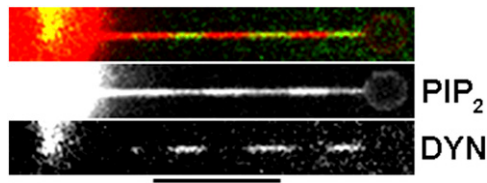


Fig. S6. Squeezing of a membrane tubule by dynamin polymerization. Under appropriate conditions (low fluorescence excitation), it is possible to see a narrowing of the tube (red fluorescence) reflected by a lower fluorescence intensity, where dynamin is polymerized (green channel). (Scale bar, 10 μm .)

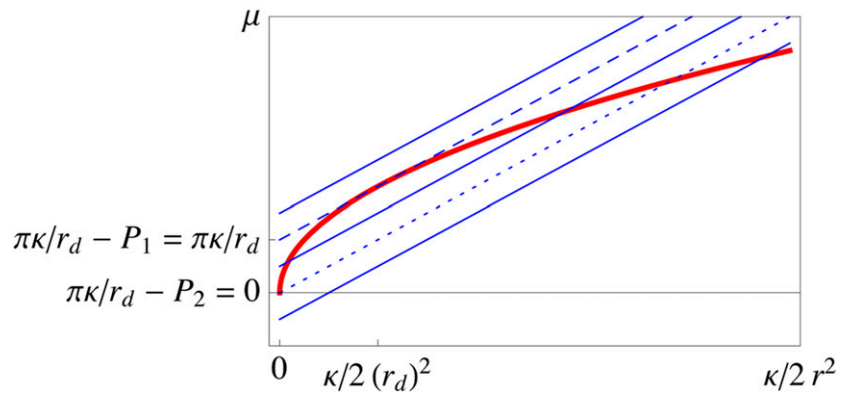
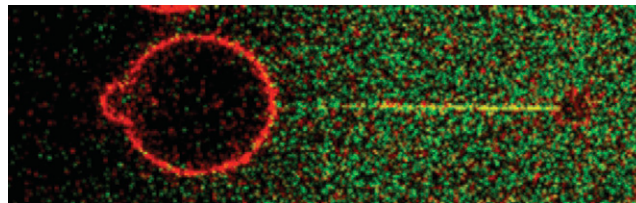
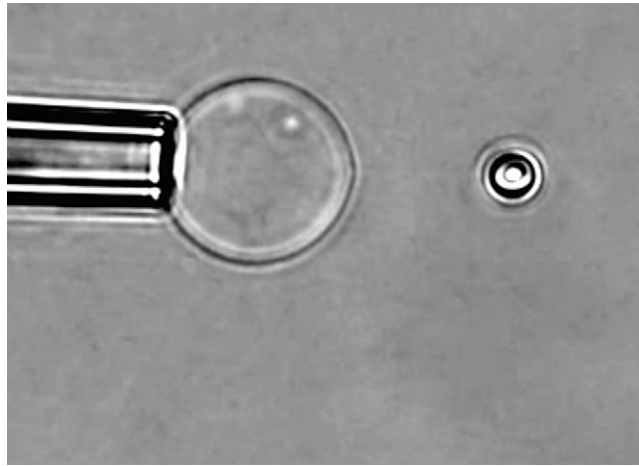


Fig. S7. Plot of the free energies per unit length of the bare tube μ_b (thick red line) and of the coated tube μ_d (blue lines) as a function of $\kappa/2r^2$. The various blue curves are plotted for different values of P . The lower the curve, the larger the P . The parameter regimes where the blue curve lies below the red curve represent situations where dynamin polymerization is energetically favored. We note two changes of behavior as P is varied: For low values of P , the bare tube is always more stable (upper solid blue line); for intermediate values of P , the coated tube is more stable only for intermediate tensions (middle solid blue line); and for large values of P , the coated tubes are always more stable at low tensions, but not at high tensions (lower solid blue line). The value of P_1 limiting the two first regimes is indicated by the dashed blue line, and the value of P_2 limiting the two last regimes is indicated by the dotted blue line.



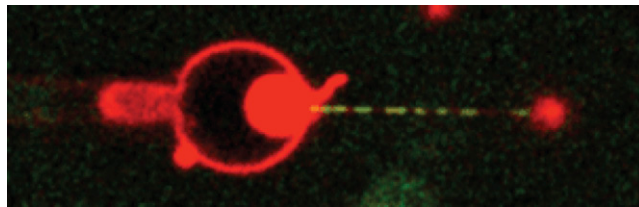
Movie S1. Using optical tweezers, a tube is extracted from a GUV. Then, a second micropipette filled with a 12 μM green-labeled dynamin solution is brought close to the tube. The tube is then rapidly covered with dynamin. See also Fig. 2A.

[Movie S1](#)



Movie S2. DivX codec is needed to read this file. A bead held in optical tweezers is used to pull a tube from a giant vesicle. First, a connection is established between bead and vesicle before the tube is then pulled. In the absence of dynamin, the tube is readily reincorporated after the tweezers are switched off. Next, a tube is again pulled with the optical tweezers and dynamin is injected through a second micropipette. The tweezers are then switched off again, but the tube does not retract fully.

[Movie S2](#)



Movie S3. Movie of data presented in Fig. 2D.

[Movie S3](#)