Supporting Information

Mechanical energy of the Z-ring

The energy stored in the Z-ring as a function of its radius R, total subunits S , S_D of which are hydrolyzed, assuming linear elasticity, is given by [1]

$$\mathcal{H} = \frac{1}{2}\int B\left(\kappa(s) - \frac{1}{R}\right)^2 ds = \frac{B\delta}{2}\left((S - S_D)\frac{1}{R^2} + S_D\left(\kappa_D - \frac{1}{R}\right)^2\right)$$

where B is the bending modulus of a filament. $\kappa(s)$ is the preferred curvature as a function of position in the ring (s) and is defined piecewise as either 0 or κ_D depending on the hydrolysis state of the ring at that point (GTP- or GDP-bound respectively).

Energy barrier of lateral bonds

Lan et al. [2] estimate the lateral bond energy between subunits in neighbouring filaments to be about $E_l = 0.2k_BT$. It is not clear if the energy barrier associated with breaking these lateral bonds can be overcome during constriction which is an implicit assumption of the model. The number of bonds that must be broken during sliding is approximately $(N-1)\lambda/2$ where N is the number of wraps the Z-ring forms around the cell and λ is the mean filament length. For a subunit size of $\delta = 4\,\text{nm}$ and a Z-ring seven subunits wide (estimated here and by Anderson et al. [3]), the force required is $(N-1)\lambda/2\ E_l/\delta \approx 18\,\text{pN}$. This barrier must be added to the 8 pN force prediction of Lan et al. [4]. Although this barrier is significant, as seen in Figure 2B, the forces generated by the Z-ring are greater than 18+8 pN at all values of R, although barely so for the largest radii plotted. For a Hill sleeve mechanism, the barrier is a problem because thermal fluctuations cannot easily overcome it.

Quasi-steady state of kinetic equations

The model for the ring in which subunits detach only at filament tips within the ring lattice is given by the equations

$$\frac{\partial p}{\partial t} = 4\pi R k_{in}\left(Z_T - \frac{S}{N_C V}\right)\frac{1}{\lambda}e^{-l/\lambda} + k_{off}\,\frac{S_D}{S}\frac{\partial p}{\partial l}.$$

$$\frac{\partial S_D}{\partial t} = k_{hyd}(S - S_D) - k_{off}\,\frac{S_D}{S}F.$$

The quasi-steady state for this subsystem is given by the following:

$$S_{Dqss} = 4\pi R\frac{k_{in}}{k_{off}}Z_T\left(\frac{1}{1+G}\right)\lambda^2$$

$$S_{qss} = N_C V Z_T\frac{G}{1+G}$$

$$p_{qss}(l) = \frac{S_{qss}}{\lambda^2}e^{-l/\lambda}$$

where $G = 4\pi R k_{in}\lambda\left(\frac{\lambda}{k_{off}} + \frac{1}{k_{hyd}}\right)/(N_C V)$

Prediction of hydrolyzed fraction in Z-ring

In the Supplemental Material we present a simpler kinetic model that ignores the spatial structure of the Z-ring entirely. It predicts that the steady state ratio of S_D to S (f_D) is independent of R and has a value of $f_D = 0.036$, clearly too low to explain force generation based on the results in Figure 2A

For the more structurally explicit model, the ratio of S_{Dqss} to S_{qss} is still independent of R and quite a bit higher than for the simple model:

$$f_D = \frac{S_{Dqss}}{S_{qss}} = \frac{k_{hyd}}{k_{hyd} + \frac{k_{off}}{\lambda}} = \frac{\tau_{off}}{\tau_{hyd} + \tau_{off}} = 0.53$$

This slight modification to the model (restricting disassembly to the tips) therefore allows for the possibility of generating a sufficient constriction force. Also, comparing this expression to the one for the spaceless model allows us to see the effect of tip-only disassembly; in particular, it decreases the effective disassembly rate by a factor of λ, the mean length of incorporated filaments.

Estimation of k_{in} *in vivo*

It has been reported that approximately 30% of the total FtsZ in a cell is found in the Z-ring. As the total number of subunits is $N_C V Z_T$, to force S_{qss} to be 30% of the total subunits available, it must be that $G/(1+G) = 0.3$ or equivalently that $G = 3/7$. As estimates for all quantities other than k_{in} in the expression for G can be found in the literature, this constraint provides an estimate of $k_{in} = 2.1 \cdot 10^{-4}\,\mu\mathrm{M}^{-1}\,\mathrm{nm}^{-1}\,\mathrm{s}^{-1}$. Varying k_{in} by an order of magnitude or more up or down does not effect the force-generating capacity of the ring as much as it does the percentage of FtsZ in the ring. To be precise, over the range of $k_{in} = 3 \cdot 10^{-5}$ to $10^{-2}\,\mu\mathrm{M}^{-1}\,\mathrm{nm}^{-1}\,\mathrm{s}^{-1}$, the ring is still capable of generating forces larger than 8 pN, but across this same range, the percentage of FtsZ in the ring varies from 6% to 95%. Thus any claims we make about force scales are at least as accurate as the extent to which our model matches measurements that have been reported and repeated in the literature [5, 3]. In other words, even though our treatment of filament incorporation is phenomenological, it is relatively robust.

Spaceless model – quasi-steady state, hydrolyzed fraction, time constants

The model for the ring in which subunits detach independent of location in the lattice is given by the equations

$$\frac{dS}{dt} = 4\pi R k_{in}\left(Z_T - \frac{S}{N_C V}\right) - k_{off}\, S_D.$$

$$\frac{\partial S_D}{\partial t} = k_{hyd}(S - S_D) - k_{off}\, S_D.$$

For the *in vivo* case, we are interested in treating R as a slow variable so we solve for the quasi-steady state of the kinetic equations:

$$S_{Dqss} = Q k_{hyd}$$

$$S_{qss} = Q(k_{hyd} + k_{off}\,)$$

where

$$Q = \frac{4\pi R k_{in} Z_T N_C V}{4\pi R k_{in} k_{hyd} + 4\pi R k_{in} k_{off}\, + k_{off}\, N_C V k_{hyd}}.$$

Note that the fraction of hydrolyzed subunits is independent of R and is, in fact, quite a simple expression:

$$\frac{S_{Dqss}}{S_{qss}} = \frac{k_{hyd}}{k_{hyd} + k_{off}} = \frac{\tau_{off}}{\tau_{hyd} + \tau_{off}} = 0.036.$$

There is a very simple interpretation of this: the fraction of hydrolyzed subunits is the ratio of the average time a hydrolyzed subunit spends in the ring to the average total time the subunit spends in the ring. Note that $\tau_{off} = 1/k_{off}$ for this simple model, instead of λ/k_{off} for the full model.

Both k_{hyd} and k_{off} are well characterized in the literature but even if the measured values are only accurate within an order of magnitude, it is still clear that $S_{Dqss}/S_{qss} \approx 10^{-2}$. As explained in the Results section, this is insufficient to generate the required force for driving the cell-wall constriction. For this reason, we consider the more spatially explicit model described in the Model section.

Because this system is linear, it is a simple matter to calculate analytically the time constants. We find that the eigenvalues of the system are

$$\alpha_{1,2} = -\frac{1}{2}\left(k_{hyd} + k_{off} + \frac{4\pi R k_{in}}{N_C V} \pm \sqrt{k_{hyd}^2 - 2k_{hyd}k_{off} + {k_{off}}^2 - 2\frac{4\pi R k_{in}}{N_C V}k_{hyd} - 2\frac{4\pi R k_{in}}{N_C V}k_{off}}\right)$$

Recognizing that $4\pi R k_{in}/(N_C V) \ll k_{hyd} + k_{off}$, the eigenvalues can be approximated as $\alpha_1 = k_{hyd}$ and $\alpha_2 = k_{off}$. This provides us with estimates of the time constants for the system: $\tau_{hyd} = 1/k_{hyd} = 8$ seconds and $\tau_{off} = 1/k_{off} = 0.3$ seconds. Hydrolysis is rate-limiting in this case.

Cytosolic filament diffusion

Anderson et al. [3] reported that FRAP experiments on cytosolic FtsZ-GFP gave a recovery half-time of roughly twice that reported for cytosolic GFP [6]. One might expect larger differences in half-times between monomers and filaments consisting of, on average, 30 subunits. Here we estimate the half-time for an exponential length distribution of filaments with mean length 30 subunits given that the monomeric form has a half-time of 0.5s. The diffusion coefficient for a filament of length l is given by

$$D(l) = \frac{k_B T}{6\pi\rho R}$$

where $\rho = 7 \cdot 10^{-8}$ is the viscosity of cytosol (chosen to match the monomer data), $R = l/(2s)$ is the hydrodynamic radius, $s = \ln\left(\alpha + \sqrt{\alpha^2+1}\right) - \alpha^{-1}\sqrt{\alpha^2+1} + \alpha^{-1}$ and $\alpha = l/\delta$ [7]. Each filament length has a time constant of $\tau(l) = L^2/(2D(l))$ where $L = 1000$ nm is the recovery length scale. The normalized intensity in the bleached zone after photobleaching is roughly described by the equation

$$I(t) = \int_0^\infty \left(1 - e^{-t/\tau(l)}\right) e^{-l/\lambda}\, dl.$$

Numerically integrating this expression gives a half-time of 0.8s. Although this is a crude means of estimating the effective half-time for a filament population, it gives a rough indication that oligomers in this length distribution do not affect the recovery half-time significantly compared to a pure monomer population.

Incorporation of partially hydrolyzed filaments

The model described in the Model section includes the simplifying assumption that all incorporated subunits are GTP-bound. As subunits can hydrolyze within filaments before getting incorporated,

this is certainly a simplification. The appropriate modification to the model is to add an incorporation term to the S_D equation. If the fraction of hydrolyzed subunits in an incorporated filament is f_0 then the term can be derived by multiplying the incorporation term in equation (1)by l and integrating from zero to ∞, to get the total number of incorporated subunits, and multipling that by f_0:

$$f_0 4\pi R k_{in} \left(Z_T - \frac{S}{N_C V} \right) \lambda$$

With this modification, the steady state can still be calculated. Interestingly, the effect is minor. The only change in the steady state is that the factor G becomes

$$G = 4\pi R k_{in} \lambda \left(\frac{\lambda}{k_{off}} + \frac{1 - f_0}{k_{hyd}} \right) / (N_C V).$$

Essentially, this says that introducing a small fraction of hydrolyzed subunits to the incorporated filaments is equivalent to increasing the hydrolysis rate by a factor $1/(1 - f_0)$.

Estimation of k_{in} *in vitro*

In the *in vitro* experiments of Osawa et al. [8], the FtsZ protein was modified to include a membrane binding domain so that it did not require the presence of FtsA for membrane attachment as is the case *in vivo* [9]. Because of this, we do not assume a priori that the *in vivo* estimate of k_{in} is appropriate for modeling the *in vitro* system. Note that the total pool of subunits Z_T and the total volume V are different for the *in vitro* case. In the multiple-ring liposome, we consider the volume as a "per ring" quantity. Osawa et al. [8] reported a total of 44 rings in 575 μm of tubular liposomes so we set $V = 575\pi R_0^2$. Unfortunately, no estimate of the average ring mass in the liposome was available but given a radius of 1 μm, a minimum of 1500 subunits are required to encircle the liposome. Examining images from Osawa et al. [8], it appears as though Z-ring can be much larger than estimates for *in vivo* Z-rings. This gives a lower bound of $k_{in} = 6 \cdot 10^{-5}$. Remarkably, if we assume the average ring size *in vitro* is similar to the ring size *in vivo* ($S_R = 4000$), the predicted value of $k_{in} = 1.7 \cdot 10^{-4}$ falls surprisingly close to the *in vivo* value of $2 \cdot 10^{-4}$. These estimates are only made for the purpose of choosing parameters for numerics because the steady state results only depend on the ratio F_M/k_{in}.

Although the modifications to the protein might have also effected k_{off} and k_{hyd} *in vitro*, this is not a certainty and so for simplicity we assume they remain unchanged. FRAP experiments on the liposome rings would be one way of testing this hypothesis.

Estimation of F_M

From dimensional arguments, the force-scale for membrane resistant to constriction by a Z-ring should scale as κ_B/L where κ_B is the bending modulus of the membrane and L is some relevant length scale. Here, we are neglecting the influence of membrane stretch. In this case, there are two length scales to consider, the radius of the liposome ($R \approx 1000$ nm) and the width of the Z-ring ($w \approx 30$ nm). As there is one to two orders of magnitude difference between these, we suggest the relevant force scale is proportional to the larger of these two, κ_B/w, with a possible unknown pre-factor. For a liposome with a single lipid bilayer, $\kappa_B = 60$ pN nm so the force scale is in the range of pN. However, the liposomes used by Osawa et al. [8] were multilamellar and, in fact, Osawa et al. [8] report being unable to generate tubular liposomes with thin walls. As a function of the number of bilayers in a liposome wall n, the bending modulus scales like n^3 [1], assuming layers cannot sheer relative to one another. Thus, a four-layer wall can already put the force scale into

the hundreds of pN range. With sheer between layers, a lesser but still significant increase would be possible. The unknown pre-factor, which we have ignored, could increase this parameter further.

In vitro force-balance analysis

As described in the Model section, the force-balance equation for membrane and Z-ring, after substituting in the steady state value of S and writing it in dimensionless form, is

$$\alpha\,(1-r) = \frac{r-\beta}{r^2(r+1/\gamma)}$$

where $\alpha = F_M R_0^2/(B\delta Z_T f_D \kappa_D N_C V)$ is the ratio of membrane to Z-ring force scales, $\beta = (f_D\kappa_D R_0)^{-1} = 0.024$ is the ratio of GDP-bound-filament preferred curvature to membrane-preferred curvature scaled by GDP fraction and

$$\gamma = \frac{4\pi R_0 k_{in}\lambda\left(\frac{\lambda}{k_{off}}+\frac{1}{k_{hyd}}\right)}{N_C V}$$

is approximately the fraction of the total FtsZ in a liposome that is bound up in Z-rings. Each side of this equation is plotted in Figure 3. Notice that for each value of α and γ, or dimensionally F_M and k_{in}, there are either one, two or three crossing and hence solutions.

It is worthwhile to consider when the upper root disappears. For the range of values of k_{in} given in Table 1, γ varies from 0.015 to 0.1 and so its inverse dominates the factor in the denominator. As we are interested in the upper solution, we can also take advantage of the fact that $\beta << 1$. The solutions of the following equation are therefore good approximates of the two upper solutions to equation (4):

$$r\,(1-r) = \frac{\gamma}{\alpha}$$

This equation loses its roots for $\gamma > \alpha/4$. Translating this back into dimensional terms, we find the upper two roots disappear when

$$k_{in} > \frac{R_0}{16\pi B\delta Z_T f_D\kappa_D\lambda\left(\frac{\lambda}{k_{off}}+\frac{1}{k_{hyd}}\right)}F_M = qF_M$$

where $q = 5\cdot 10^{-6}\,\mu\mathrm{M}^{-1}\,\mathrm{nm}^{-1}\,\mathrm{s}^{-1}\,\mathrm{pN}^{-1}$. Using the estimate $F_M = n^3\kappa_B/w$ where κ_B is the membrane bending modulus, w is the width of the Z-ring and n is the number of bilayers, F_M is roughly 2.4, 19, 65, and 154 pN for $n = 1, 2, 3$ and 4 respectively. Multiplying by q gives the threshold values of k_{in} above which the upper roots disappear: $1.2\cdot 10^{-5}, 9.7\cdot 10^{-5}, 3.3\cdot 10^{-4}$ and $7.7\cdot 10^{-4}$. Note that the first two of these is smaller than our *in vivo* estimate of k_{in}, although the second is close. The other two are above k_{in}. This indicates that, for this particular choice of parameters, multilamellar liposome with three or more layer have a stable constriction radius whereas unilamellar liposomes clearly do not. This seems also be the case for two-layered liposomes. The dividing line between having and not having a stable constriction radius is monotone in k_{in}. If the minimal number of bilayers in the tubular liposomes could be measured experimentally, it might suggest an upper bound for k_{in}.

Depletion of GTP

In order to account for experiments in which GTP is in limiting supply [8], we track total cytosolic FtsZ-GTP (Z_T), FtsZ-GDP ($Z_D = Z - Z_T$) and GTP (T):

$$\frac{dZ_T}{dt} = -k_{hyd}Z_T + k_{ex}(Z_{tot}-Z_T)T$$

$$\frac{dT}{dt} = -k_{ex}(Z_{tot} - Z_T)T$$

Note that we have omitted the contribution of the Z-ring to the overall hydrolysis rate because most of the FtsZ is in solution (see estimate above) so GTP dynamics are primarily dictated by the solution concentrations of FtsZ-GTP and -GDP. Due to the large difference in concentration scales of Z and T (4μM and $100 - 400\mu$M respectively [8]), the value of the parameter k_{ex} makes little impact on the time course unless it is unrealistically small ($< 0.01\mu\text{M}^{-1}\text{s}^{-1}$). This Michaelis-Menten scenario leads to a gradual almost linear depletion of GTP. However, the depletion of FtsZ-GTP is barely noticeable until the GTP concentration reaches the range of k_{hyd}/k_{ex} which is in the μM range. For convenience, in our simulations, we do not implement this model explicitly but instead simply artificially set Z_T to zero at a predetermined time.

Liposome rupture

Lipid bilayers can tolerate a maximal strain of $2-4\%$ before failure, depending on lipid composition [10]. Therefore the liposomes studied in Osawa et al. [8] can sustain a maximal amount of distortion, above which the membranes will rupture. Here we estimate this maximal distortion.

The liposomes were cylinders with radius $R_0 \approx 1\,\mu$m and Z-rings appeared on average every $2L \approx 13\,\mu$m. We assume each Z-ring has a radius R and is centered in a tube of width $2L$ whose ends are perfect circles. The solution to the linearization of the membrane elasticity equations [4] with boundary conditions $R(0) = (1-r)R_0$ and $R(\pm L) = R_0$ is

$$R(z) = R_0(1 - re^{-|z/R_0|}).$$

The surface area is

$$A = 2\pi \int_{-L}^{L} R(z)dz = 4\pi R_0 \left(L + rR_0\left(1 - e^{-L/R_0}\right)\right)$$

compared to $4\pi R_0 L$ for an unconstricted membrane. This increase in area leads to a strain ϵ, which occurs when the invagination's fractional amplitude is

$$r = \frac{\epsilon L}{R_0\left(1 - e^{-L/R_0}\right)}.$$

For a critical strain of 4%, this occurs when the liposome is constricted to 740 nm. For 2%, the maximal constriction has radius 870 nm. Note that since several Z-rings share the total lipid content of one liposome, these maximal constrictions represent liposome-wide averages – one Z-ring can constrict beyond the maximum, provided others constrict less. An accurate prediction of when rupture occurs would require information about all constrictions within a liposome but, assuming a roughly uniform distribution of sizes, it is reasonable to expect some Z-rings to get as small as 500 nm but perhaps not much smaller than that.

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