Dynamin oligomerizes into helical filaments on tubular membrane templates and, through constriction, cleaves them in a GTPase-driven way. Structural observations of GTP-dependent cross-bridges between neighboring filament turns have led to the suggestion that dynamin operates as a molecular ratchet motor. However, the proof of such mechanism remains absent. Particularly, it is not known whether a powerful enough stroke is produced and how the motor modules would cooperate in the constriction process. Here, we characterized the dynamin motor modules by single-molecule Förster resonance energy transfer (smFRET) and found strong nucleotide-dependent conformational preferences. Integrating smFRET with molecular dynamics simulations allowed us to estimate the forces generated in a power stroke. Subsequently, the quantitative force data and the measured kinetics of the GTPase cycle were incorporated into a model including both a dynamin filament, with explicit motor cross-bridges, and a realistic deformable membrane template. In our simulations, collective constriction of the membrane by dynamin motor modules, based on the ratchet mechanism, is directly reproduced and analyzed. Functional parallels between the dynamin system and actomyosin in the muscle are seen. Through concerted action of the motors, tight membrane constriction to the hemifission radius can be reached. Our experimental and computational study provides an example of how collective motor action in megaladon molecular assemblies can be approached and explicitly resolved.

Dynamin is a protein that is a central player in endocytosis, a process that mediates the entry of diverse particles into cells, from nutrients to viruses. Dynamin’s primary activity is to use guanosine triphosphate as fuel to constrict and cut membrane tubes. Key quantitative aspects of its function remain yet unclear. In this work, we determine the strength of an individual dynamin motor. Then, by building a detailed computational model resolving individual motors, we demonstrate that dynamin produces sufficient force to tightly constrict a membrane tube when most of its motors are simultaneously cooperating. Hence, we quantitatively validate the prevailing constriction-by-ratchet model for nature’s strongest torque-generating motor: the dynamin “nanomotor.”

Significance

Dynamin is a protein that is a central player in endocytosis, a process that mediates the entry of diverse particles into cells, from nutrients to viruses. Dynamin’s primary activity is to use guanosine triphosphate as fuel to constrict and cut membrane tubes. Key quantitative aspects of its function remain yet unclear. In this work, we determine the strength of an individual dynamin motor. Then, by building a detailed computational model resolving individual motors, we demonstrate that dynamin produces sufficient force to tightly constrict a membrane tube when most of its motors are simultaneously cooperating. Hence, we quantitatively validate the prevailing constriction-by-ratchet model for nature’s strongest torque-generating motor: the dynamin “nanomuscle.”
of the nonhydrolyzable GTP analog β,γ-methyleneadenosine 5′-di phosphate (GMPPCP), the MM crystallized in an open BSE conformation (6), whereas a closed conformation of the BSE was observed in the nucleotide-free state or when bound to guanosine-5′-diphosphate (GDP) or to GDP-AlF₄⁻, a mimic of the transition state of GTP hydrolysis (20, 21, 23, 24). While this structural shift is the obvious candidate for the power stroke driving motor function, it remains unknown how strong it is, since even small energy preferences might result in the different structural states captured in the available structures. For example, in crystallographic studies, a dynamin family member, MxA, shows open and closed states in the GMPPCP- and GDP-bound states, respectively (25), but only weak nucleotide-dependent preferences in solution (26).

In this study, kinetic measurements for the GTPase cycle of the dynamin MM are first performed. After that, we explore nucleotide-dependent conformational changes in the MM using single-molecule FRET experiments. Their results, in conjunction with molecular modeling, are then used to quantify the forces generated by single dynamin motors and to refine the ratchet mechanism, which reproduces collective constriction-by-ratchet mechanism in the dynamin molecular motor.

Results

To study the kinetic and energetic parameters of a single dynamin motor, we have worked with a MM construct containing only the G domain and BSE (23) (Fig. 1A). This allows clean interpretation of nucleotide on and off rates, as it removes the complicating process of tetramers (or higher oligomers) associating via their G interfaces. While clarity in this respect could also be achieved with a full-length, but nonoligomerizing mutant, a full-length monomer may enable G-domain interactions with the stalk or PH domain that are disallowed in the membrane-bound filament. Therefore, using the MM construct for the single-molecule FRET (smFRET) experiments allows us to ensure we are capturing the intrinsic G-domain/BSE conformational ensemble.

Exploring Dynamin’s GTPase Cycle. Nucleotide binding and dissociation rates to this construct were evaluated using stopped-flow experiments with fluorescently labeled 2′-O-(N-methylanthraniloyl) (mant)-nucleotides (Fig. 1B). Previous measurements (28, 29) reporting even higher GTP affinity were performed using full-length dynamin, thus also including possible contributions from nucleotide-induced assembly that enhance nucleotide occupancy. A low basal rate of GTPase hydrolysis and a linear dependence of the specific hydrolysis rate kₜₐₚ on GDP concentration were observed (Fig. 1E and F and SI Appendix, section S1.A.1), confirming that MM dimerization should precede the hydrolysis and is required for it. These GTPase measurements additionally indicated that the GTP-bound dimer has a weak affinity (Kₜₐₚ > 50 µM).

Running the GTPase assay beyond the linear regime allows GDP to compete with GTP for the active site. Therefore, the GTPase kinetics of such an experiment contain information on the relative affinity between GTP and GDP for the MM. A kinetic model was devised to fit experimental traces and determine unknown parameters (SI Appendix, section S1.A.2). Varying this setup with different initial conditions (SI Appendix, Fig. S1B and D) allowed us to estimate an apparent GTP on rate of 6.6 s⁻¹ µM⁻¹, which was not straightforward to determine with mant-GTP (SI Appendix, Fig. S1A).

Fig. 1. Kinetic characterization of the dynamin motor module. (A) The dynamin monomer has four structurally characterized domains (Protein Data Bank 3SNH) (20). The motor module (MM) contains the G domain and the BSE. A continuous MM construct was created by fusing the third helix of the BSE onto the N-terminal portion of the dynamin sequence. (B) Association rate constants are obtained from the slope of kₐp versus MM concentration. See SI Appendix, Fig. S1 for mant-GTP association curves. (C) Mant-nucleotide dissociation rates were measured in stopped-flow experiments by mixing with excess unlabeled nucleotide. (D) Table of determined mant-nucleotide binding/dissociation rates of the MM construct. Kₑff is defined by the ratio kₐp/Kₜₐₚ. GTP on rate value is determined from kinetic modeling of GTPase activity (SI Appendix, section S1.A.1) because mant binding did not show a single exponential fluorescence increase for mant-GTP (SI Appendix, Fig. S1). (E) GDP production as a function of time in the linear regime for increasing concentrations of MM and an initial GTP concentration of 1 mM. Dashed lines are linear fits to three independent experiments. (F) Slope from E divided by the protein concentration gives the specific hydrolysis rate (kₜₐₚ)ₐpp. Linear dependence of the specific hydrolysis rate on protein concentration indicates that GTPase activity is controlled by dimerization (SI Appendix, section S1.A.1).
Measuring nucleotide exchange kinetics within the dimer is complicated by the low MM dimerization affinity. To resolve this difficulty, we engineered a Zn$^{2+}$-dependent metal bridge by introducing histidines into the G interface (MM-HH). X-ray crystallography confirmed that Zn$^{2+}$ stabilized a similar dimer arrangement as previously observed in the GDP-bound MM dimer (24) (SI Appendix, Fig. S2 and Table S1). In line with our predictions, addition of Zn$^{2+}$ to this double histidine (MM-HH) construct induced dimerization in gel-filtration (SI Appendix, Fig. S3A) and FRET experiments (SI Appendix, Fig. S3B). Of note, addition of GTP$\gamma$S reduced mutar variant dimer formation (SI Appendix, Fig. S3B).

Dimer dissociation rates were measured by FRET experiments and stopped-flow. Addition of GDP slowed the dimer dissociation rate of the Zn$^{2+}$-stabilized MM-HH dimer sixfold relative to the apo state (SI Appendix, Fig. S3 D and E), consistent with a long-lived posthydrolysis dimeric state. Addition of GTP greatly accelerated dimer dissociation, suggesting that the GTP-bound state is not compatible with the engineered Zn$^{2+}$ bridge. Relevant to the motor cycle, mant-GDP did not bind to empty Zn$^{2+}$-stabilized MM-HH dimers. Furthermore, mant-GDP dissociation from MM-HH dimers was 100-fold slower in the presence of Zn$^{2+}$ (SI Appendix, Fig. S3 G–J). These observations imply that nucleotide exchange is greatly reduced in the MM dimer, therefore simplifying dynamin’s motor cycle (see below).

**Nucleotide-Dependent Motor Module Conformation in Solution.** To characterize nucleotide-dependent conformational changes in the MM, single-molecule FRET (smFRET) was employed. We monitored the relative orientation of the G domain and BSE by introducing a pair of FRET dyes, one in BSE and the other in the G domain (Fig. 2A and B and SI Appendix, Fig. S4). The experiments were performed on freely diffusing MMs.

In the presence of GTP, three populations could be discerned (Fig. 2A and SI Appendix, Fig. S4C) by fitting to Gaussian distributions: a dominant open state with a FRET efficiency peak ($E$) at $E = 0.18$, an intermediate state at $E = 0.39$, and a closed state at $E = 0.77$. The peak values of the open and closed states were in excellent agreement with the predicted mean FRET efficiencies from simulations based on the crystal structures of 0.19 and 0.79, respectively (Fig. 2C). The GTP analogs (GMPPCP and GTP$\gamma$S) showed bimodal distributions. Interestingly, the low FRET peak for GTP$\gamma$S coincided with the intermediate peak found in the presence of GTP. Note that the nonhydrolyzable GTP analogs produce different structural ensembles from those found with GTP. Care has therefore to be taken when interpreting experiments using GTP analogs. Remarkably, in absence of nucleotides or in presence of GDP, the conformational distributions were strongly shifted toward the closed state. For the apo protein, the position of the FRET peak coincided with that of the GTP-bound closed state. For GDP, the closed state was shifted slightly higher to $E = 0.8$, consistent with crystal data (SI Appendix, Fig. S4D).

Ensemble stopped-flow measurements were used to relax rates to the equilibrium distributions. The rate of opening, i.e., of a transition from apo (closed) to the GTP-bound state, was fitted to a single exponential with a time constant of approximately 300 s$^{-1}$ (Fig. 2 D and E). This rate contains both nucleotide binding and the conformational change. However, since nucleotide binding at 1 mM GTP is much faster than opening, the measured rate must correspond to the conformational change. Because 300 s$^{-1}$ is large compared to the overall turnover rate of the GTPase cycle, GTP binding and opening are combined in our summary of the GTPase cycle (Fig. 3). The rate of closing in solution, induced by GTP dissociation, is larger than 105 s$^{-1}$ (SI Appendix, Fig. S4E). However, this parameter is of little relevance for dynamin’s function since, in the filament, closing takes place within a dimer and under load.

**Operation Cycle of the Dynamin Motor.** The kinetic and conformational results are summarized in the diagram of the GTPase cycle (Fig. 3). The GTP-bound MM monomers are predominantly

![Fig. 2](https://doi.org/10.1073/pnas.2101144118)
Dissociation (k_{diss}) rates of MM monomers in the GTP-bound state, remain yet unknown, meaning the entire GTPase cycle could not be precisely specified. Therefore, in our model simulations, various values of the effective hydrolysis rate k^{spec}_{hyd} and of k_{diss} were chosen and explored (see SI Appendix, section S1.B for details). Measurements of GTPase activity in the presence of membranes set a lower limit of 4 s^{-1} (20) for its turnover rate. The slowest measured rate in the forward direction is that of GDP dissociation at 120 s^{-1}, yielding an upper limit for the overall turnover rate.

**Determination of Generated Forces.** In the helical filament, a pair of GTP-bound MMs belonging to adjacent rungs can dimerize to form a cross-bridge (Fig. 4A). Upon hydrolysis in both MMs, the equilibrium conformation of the dimer shifts from the open to the closed ensemble, thus shortening its equilibrium bridging length and creating a contracting force along it.

The length of a bridging MM dimer is quantified by the end-to-end length distributions P_{GTP}(ℓ) and P_{GDP}(ℓ). Although distance distributions can be yielded by smFRET, our FRET experiments are limited to MM monomers. Therefore, probability distributions over ℓ for the two nucleotide states were obtained from MD simulations of a dual-basin structure-based model (31) (see SI Appendix, Fig. S5 and section S1.C for a detailed discussion). The MM dimer representation was built by connecting two MM monomers, each described by the dual-basin model, at their G interface (Fig. 4B). Importantly, the simulations incorporated the experimentally observed energetics by enforcing that the simulated FRET distribution in each MM monomer match the observed smFRET distribution of open and closed states (SI Appendix, Fig. S5).

The free energy profile of a GTP-bound MM dimer along ℓ shows a single broad basin (Fig. 4C). This implies that initial cross-bridges can form over a wide range of distances.

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**Fig. 3.** GTPase cycle for the MM. Ligand binding kinetics are taken from Fig. 1 assuming physiological concentrations of 300 μM GTP and 30 μM GDP. [MM] refers to the concentration of GTP-bound MM. The green dot indicates the presence of inorganic phosphate. Experimental measurements of GTPase activity in the presence of membranes set a lower limit of 4 s^{-1} (20) for the whole cycle.

Open and must dimerize (GTP-D) to enable hydrolysis. Hydrolysis leads to a strong preference in the MM dimer (GDP-D) for the closed state. Using the MM-HH mutant, we confirmed that the GDP-bound dimer is strongly closed in a similar fashion to the GDP-bound monomer (SI Appendix, Fig. S3C). The MM dimer in its GDP-inorganic phosphate (Pi) or GDP-bound states dissociates only slowly, consistent with the high stability of GTP-bound MM dimer in its GDP-bound state. [MM] refers to the concentration of GDP-bound MM. The green dot indicates the presence of inorganic phosphate.

**Fig. 4.** Determination of forces. (A) (Center) An MM dimer forms a cross-bridge (red) between two neighboring filament rungs. (Left) Definition of cross-bridge geometry. The length ℓ is defined as the distance between hinge 1 connections (pink circles); green circles indicate hinges 2. (Right) A cartoon representation based on the cryo-electron microscopy structure of the “nonconstricted” filament (30) shows the overall architecture of the dynamin filament. (B) Overlay of open and closed G-domain dimer crystal structures. Estimates for ℓ, i.e., the distance between hinge 1 attachment points, are shown. Cyan spheres show the nucleotide-binding location. (C) Free energies G_{GTP}(ℓ) (orange) and G_{GDP}(ℓ) (blue) obtained by MD simulations for MM dimers. The respective distributions P_{GTP}(ℓ) and P_{GDP}(ℓ) of distances are shown by orange and blue dashed lines. The dashed green line has a slope of F_s = 1.3 k_B/T nm (5.2 pN). (D) Mean stall forces along the filament (black) and in the transverse direction (red) as functions of the separation h between the rungs (see SI Appendix for details).
This property is enabled by the heterogeneity of the GTP-bound conformations (Fig. 2A). In contrast, the free energy $G_{\text{GDP}}$ for GDP-bound MM dimers has a single narrow minimum at a shorter $\ell$ (Fig. 4C). The effective contracting force along $\ell$ is given by $F(\ell) = -dG_{\text{GDP}}(\ell)/d\ell$. In the simulations of membrane constriction, this instantaneous force, produced by a MM dimer when it is in the GDP-bound state and has length $\ell$, is employed. Remarkably, the force remains approximately constant (Fig. 4C) at $F_\ell = 1.3$ kN/T nm over the range of $\ell$ from 8 to 14 nm.

To transform the cyclic changes of the GTPase cycle (Fig. 3) into a directed force acting on the helical filament, a ratchet mechanism must be employed. The principal feature of the ratchet is a differential response to the power stroke and the recovery stroke. The transition from an open to a closed conformation, representing a power stroke, takes place when the two MMs from the neighboring rungs are dimerized and forming a cross-bridge. The recovery stroke, corresponding to the reverse transition to the open state, however, occurs only in the monomeric MM state, i.e., when the bridge is absent. Therefore, force is applied to the filament only in one part of the GTPase cycle.

There is, however, an additional component to the ratchet effect. If it were equally probable that the cross-bridges slanted to the right and to the left, shortening of such links could not generate a net force. Hence, a directional bias in slanting is needed. For dynamin’s right-handed helix, preferentially slanting the upper MM to the right and the lower MM to the left with respect to the stalk filament would generate a constricting torque (i.e., as in Fig. 4A). Indeed, the comparison of all available dynamin structures has shown that hinge 1 is biased in this way, and our molecular dynamics (MD) simulations have indicated that the biased orientation is robust to thermal fluctuations (SI Appendix, Fig. S6).

One can calculate the mean force generated by a pair of MMs over many GTPase cycles by averaging the force applied between two immobilized stalk filaments (under stalk conditions). Note that time averaging is equivalent to statistical averaging over an ensemble. Using the distributions for $\ell$ from MD simulations and the geometry of Fig. 4C, the component of the mean force acting along the filament is $F_\ell = \omega \int_0^{\ell_{\text{max}}} P_{\text{CUTP}}(\ell) F(\ell) \sin \theta(\ell)d\ell$, where $\theta$ is the slant angle and $\omega$ is the probability (often referred to as the duty ratio) $<F_\ell>$ to find a MM in the dimerized state. Because of the ratchet property of hinge 1, only configurations with positive displacements $\Delta s$ are included into the average. The transverse component $<F_s>$ is given by the same equation with $\sin \theta$ replaced by $-\cos \theta$.

The mean forces $<F_\ell>$ and $<F_s>$ are shown as functions of the rung separation $h$ in Fig. 4D. Note that the derived estimates are sensitive to the relative weights of the open and closed conformations in a GDP-bound MM (SI Appendix, Fig. S5C and D). Since the observed frequency of the open conformation in smFRET is likely enhanced by experimental noise, they should be viewed as providing the lower bounds for the force.

The torque, locally applied to the filament by a MM dimer motor, is $<F_\ell>R$, where $R$ is the filament radius. Assuming that a MM is found predominantly in the dimerized state, i.e., $\omega$ is near unity, $<F_\ell>$ is 2.9 pN (0.7 kN/T nm) and the mean torque generated by a single motor in the nonconstricted state (with $h = 8$ nm and $R = 25$ nm as in Fig. 4A) is 72 pN nm. When many MMs are simultaneously cooperating, large torques on the order of 1 nN nm can be produced, similar in size to what has been experimentally observed (10). These collective effects are discussed in the next section.

Collective Operation of Motor Modules and Constriction of Membranes. The MM dimers, connecting neighboring rungs, produce forces that tend to rotate them with respect to one another. Collectively, they generate the torque applied to the filament. We directly demonstrate this via computer simulations of a mesoscopic model. It was obtained by augmenting the recently developed coarse-grained description of a dynamin polymer filament on a deformable membrane tube (16) to additionally account for the GTPase motor activity of dynamin. In the model, MM dimers are introduced as elastic cross-bridges that connect the polymer beads and apply forces between them. Since each bead corresponds to a dynamin stalk dimer, it has two MMs associated with it. The nucleotide state of each MM is tracked and the GTPase cycle is implemented within it. Dimerization, i.e., formation of a cross-bridge, can occur if both MMs are GTP bound. After the hydrolysis-induced conformational change, the cross-bridge becomes strained and produces, as outlined above, a contracting force $F(\ell)$ for a cross-bridge of instantaneous length $\ell$. The MM dimers dissociate (and thus cross-bridges disappear) at rate $k_{\text{diss}}$. See Materials and Methods and SI Appendix, Fig. S7 for the details.

In endocytosis, constriction and cleavage of membrane necks are performed by short dynamin filaments with only a few tens of MMs (33, 34). Filaments with 28 or 40 beads were therefore probed in our simulations. They were carried out for various MM dimer dissociation rates $k_{\text{diss}}$ (Movie S1), thus yielding the regimes with different duty ratios $\omega$ (SI Appendix, Eq. S8). Fig. S4 gives example snapshots of the initial and final states. The dependence of the final steady-state inner lumen radius (ILR) of the membrane tube on the duty ratio is displayed in Fig. 5B. Tight constriction from 10.5 to 2 to 3 nm ILR is found above $\omega > 0.65$. Increasing the filament length from 28 to 40 significantly decreases the ILR; however, increasing the length beyond 40 beads does not lead to further decreases in ILR.

Imaging of single endocytic pits during CME suggests that dynamin is active from a few seconds to tens of seconds before scission occurs (33, 34). Consistent with this, simulations showed that ILR below 3 nm could be reached within about 1 s for the duty ratios of 0.6 to 0.7 and within 15 s for $\omega = 0.94$ (Fig. 5C). Examination revealed that constriction speed was limited by $k_{\text{diss}}$. When MM dimer dissociation is slow (corresponding to large duty ratios), cross-bridges continue to stay after the power stroke and are driven along with the sliding filament to reverse their slants (Fig. 5D). The misoriented cross-bridges get oppositely stretched and block the constriction.

In the actomyosin system, key kinetic steps are sensitive to strain (35–38) and similar sensitivity may be present in dynamin. In our model, strain control could be implemented by introducing immediate dissociation of MM dimers once their slant is reversed and they become stretched. For a 40-bead filament and $\omega = 0.94$, this reduced the time of constriction to 3 nm from 15 s down to 300 ms (Fig. 5C and Movie S2). This effect could underlie the reported fast endocytosis within hundreds of milliseconds (39). Note that strain control affects only the kinetics, since the standing filament in the steady state cannot drive cross-bridges to alter their slants (and therefore the same ILR dependence as in Fig. 5B holds).

In vitro experiments, scission of membrane tubes by long dynamin filaments can be observed. Surprisingly, such experiments find that breaking of the tube takes place in the middle of the dynamin coat, but rather at its flanks (10, 40). In line with this finding, our simulations show that constriction by a long filament begins near its ends (Fig. 6A and B and Movie S3) and only later propagates toward the middle. Fission may thus occur at a flank before the steady state has been reached. A simple explanation is that, in the middle of a long filament, a rung is pulled in opposite directions by its two neighboring rungs (Fig. 6C and D). Such cancellation is absent for the terminal turns and, thus, only the motors on the flanks of a long helical filament apply a net torque and contribute to constriction of the membrane. Hence, the work produced by a long dynamin...
filament becomes independent of its length. Note that, in the employed model, dynamin-coated membrane tubes are assumed to be straight. Therefore, the effects of supercoiling, reported for very long tubes (8, 9), could not be considered here.

Discussion

Since its discovery in the early 1990s (41, 42), dynamin has become the paradigm for studying GTPase-driven membrane remodeling. Biophysical experiments and structural studies have revealed dynamin to be a helical-shaped, torque-producing motor. In the current study, we provide the quantitative basis for the proposed ratchet operation and describe in detail the molecular mechanism whereby dynamin’s motor modules collectively induce filament sliding. Our smFRET experiments showed strong nucleotide-dependent shifts, which, in conjunction with molecular modeling, allowed us to estimate the force generated by a single cross-bridge. Then, by incorporating these interactions into an energetically realistic filament-on-a-membrane model, we directly demonstrate that through cooperative action, dynamin’s cross-bridges generate sufficient torque to drive strong constriction of biomembrane tubes. Importantly, the principal experimental features of membrane constriction by dynamin are either reproduced in our study or consistent with its results. Dynamin’s ability to create torque over a dynamin helix (9) is shown to arise from repeated cycles of interaction between neighboring MMs. It is confirmed that short dynamin helices with 1.5 to 2 turns are sufficient to produce tight constriction (33, 34) and the torque production in the model is consistent with the levels measured experimentally (10). The fission at the edge of a dynamin coat, observed in the experiments with fluorescent membrane tubes pulled from giant unilamellar vesicles (10) or for nanotubes imaged by high-speed atomic force microscopy (40, 43), can be explained by us. Typical durations of dynamin scission events (33, 39) are in agreement with the theoretic predictions.

The force generated by a single cross-bridge is much less than the overall force the dynamin filament has been experimentally measured to produce (10) and less than the force that is theoretically required for strongly constricting the membrane tube (SI Appendix, Fig. S7E). Therefore, strong constriction requires that, at any time, most of the possible cross-bridges are formed (i.e., a high duty ratio) to cooperatively generate a large torque. Note that this cooperation does not come from synchronizing GTPase activity between MMs. Importantly, our simulations have shown that relative sliding of neighboring turns is possible even when MMs are working asynchronously and with large duty ratios. Sliding can be expedited with a strain-dependent dissociation rate.

We noted that nucleotide-dependent conformational changes are much more pronounced in dynamin than in the related MxA protein (26). Whereas for MxA, the ratio of populations of the open to closed states in the presence of GDP was 0.43 (i.e., 30 to 70%), the GDP-bound open state was practically undetectable for dynamin (i.e., a ratio < 0.05). Thus, the resulting forces generated are at least three times stronger for dynamin. This highlights the functional difference of dynamin with MxA: MxA works as an antiviral machine that most likely acts on viral nucleocapsids rather than in the remodeling of membranes (44). We therefore
expect that the functions of dynamin superfamily members are modulated by tuning the nucleotide-dependent conformational preferences of the MM.

In our simulations, the stalk filament is treated as an elastic ribbon whose local orientation with respect to the membrane remains fixed. The elastic description, developed in ref. 16 and used in the present study, accounts for subtle changes to the membrane due to curvature variations. The interface 2, defining the inner lumen radius of about 2 nm, is assumed to be rigid in our simulations and is taken as a constraint on the filament.

The membrane is treated in the elastic Helfrich approximation, and lipid flows within it are conserved. Moreover, axial symmetry of the membrane is assumed. The filament sits on the membrane, but is free to slide over it, subject to viscous friction. To account for membrane breakup, a more detailed, molecular-level description for lipid bilayers would have been needed (such as in refs. 18 and 48). Generally, the instability of a membrane tube and hemifission is expected when an ILR below 2 nm is reached (17). Fission can be, however, facilitated by buckling of the tube (49). It can also be promoted by membrane-bound PH domains whose conformations change within the GTPase cycle (7, 18, 50, 51). The combination of such effects would probably enable membrane breakup even at ILRs of 2 to 3 nm.

As has been previously suggested (2, 12), we find that collective motor activity within the dynamin “nanomuscle” resembles that of actomyosin in the muscle (Fig. 7). In the muscle, a thick myosin filament slides over a thin filament of actin (32). Myosin heads bind to actin so that cross-bridges connecting both filaments are formed. As proposed by H. E. Huxley (52), muscle contraction is effectively due to a combination of cyclic conformational changes and a ratchet effect. Depending on its nucleotide state, myosin alternates between open and closed conformations. The transition from the open to the closed state takes place when the bridge exists; it leads to shortening of the bridge and generation of a pulling force. The reverse transition, however, occurs when the cross-bridge is absent and, therefore, the ratchet is lifted and no force is produced. The muscle is a large assembly where thousands of motors over many filaments operate in parallel.

For dynamin, in the presence of GTP, cross-bridges between adjacent rungs of a helical stalk filament of dynamin are formed through dimerization of neighboring MMs and preferentially in an open state. A posthydrolysis shift to the closed state generates a force pulling the filaments with respect to one another, and hence a torque is produced. Binding of GTP and return to the open state can occur only after the disappearance of the cross-bridge. Remarkably, only tens of motor modules are typically involved in this nanomuscle.

The posthydrolysis dimer dissociation rate $k_{\text{diss}}$ plays an important role in the motor mechanism because it introduces a lag time during which force can be generated. It is possible that Pi release from the GDP-Pi-bound dimer is the dominant contributor to the lag time because it is known that the G interface can be strongly stabilized by the transition-state analog GDP-AlF$_4^-$ (23). Pi is produced while buried within the G interface and, therefore, slow release of Pi is to be expected. Modulation of the Pi release by the conformational state of the MM can also provide a mechanism for the strain control. Further work is needed to elucidate the role played by Pi in constriction. In addition, our computational model can be further validated and, possibly, refined by sophisticated force measurements of the MM using optical tweezers, further high-speed atomic force microscopy imaging of dynamin-mediated membrane remodeling (40, 43), or detailed

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**Fig. 6.** Long filaments first constrict near their ends. (A and B) Evolutions of ILR profile under strain control for short ($N = 40$) and long ($N = 900$) filaments. Circles mark the positions of terminal beads. The membrane has stiffness of $24 \kappa T (6 \times 10^{-20})$, tension of $0.03 \kappa T \text{nm}^{-2}$, and $\omega = 0.94$. (C) Motor forces in a dynamin coat tend to cancel each other, except for the flanks. Shown is an $N = 200$ filament and cross-bridges are not shown. (D) Net motor torque along the helical filament axis, summed over 15 consecutive beads (approximately corresponding to one rung). The bead index specifies the position starting from which the summation is performed. The plotted data are additionally averaged over 200 ms (from 600 to 800 ms in B). The net torque almost vanishes everywhere, except the flanks.

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**Fig. 7.** A sliding-filaments cross-bridge mechanism for dynamin is suggested by our results. (A) smFRET indicates that the motor module exhibits strong nucleotide-dependent conformational shifts in solution. The transitions to closed and open represent, respectively, the power and the recovery strokes in the MM. (B and C) Comparison of operation schemes in dynamin and muscle myosin.
kinetics experiments with dynamin mutants in combination with deformable membrane templates (53).

In summary, through integrating smFRET measurements, structural modeling, and filament simulations, we have shown that the conformational shifts in the dynamin motor module are strong enough to generate the necessary force to concert biomicelle tubes when summed over several cooperating cross-bridges. In so doing, we have quantitatively validated the constricted-by-ratchet hypothesis and developed a model that can be generally applied to other membrane-remodeling motors, e.g., those involved in the maintenance and scission of mitochondria.

Materials and Methods
Cloning, Expression, and Purification. The pET-M11 vector was used to express a codon-optimized His6-tagged GTPase-BSE (MM construct) (Eurofins; residues 6 to 322 fused to residues 712 to 746 with a GS5GS5 linker) in BL21 (DE3) Rosetta 2. Cells were grown at 37 °C to an OD600 of 0.4 and the protein was expressed in the presence of 0.15 mM Isopropyl β-d-1-thiogalactopyranoside (IPTG) overnight at 25 °C in terrific broth (TB) medium, supplemented with 34 μM of chloramphenicol and 50 μg/mL of kanamycin. Cells were harvested by centrifugation, resuspended in buffer A (20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)-NaOH, pH 7.5, 500 mM NaCl, 15 mM imidazole, 5% glycerol) and lysed by a microfluidizer (Avestin). After centrifugation, the supernatant was filtered through a 5 mL Ni-NTA bead at 4 °C on a rocking platform. Afterward, Ni-NTA beads were washed in batch three times with buffer A and transferred into a gravity-flow 20-mL column. Column material was further washed with 10 column volumes (CV) of buffer A supplemented with 2 mM ATP and 5 mM MgCl2 and then with 2 CV of buffer A containing 50 mM imidazole. Eventually, the protein was eluted with 5 CV of buffer A containing 250 mM imidazole. The His6 tag was cleaved by incubation with Tobacco Etch Virus nuclear-inclusion-a endopeptidase during overnight dialysis against buffer A without imidazole. Subsequently, the protein was reapplied to the Ni-NTA column, collected in the flow-through fraction, and further purified via size-exclusion chromatography (SEC) on an S200 26/600 Superdex column with running buffer C (20 mM HEPES-NaOH, pH 7.5, 150 mM NaCl). Fractions containing the pure protein were pooled, concentrated to 30 to 50 mg/mL, aliquoted, and shock frozen in liquid nitrogen. The MM wild-type construct as well as various mutant variants were purified following the same protocol. For protein labeling with Alexa fluorophores or for experiments exploring metal-mediated dimerization of the MM constructs, the following mutations were introduced individually or in combination: T165C, R313C, N112C, K142H, N183H. Point mutations in the MM construct were introduced using QuikChange site-directed mutagenesis (Agilent). Cysteine mutations were used for covalent attachment of Alexa Fluor dyes and their locations were chosen to avoid any obvious functional disruption. See SI Appendix for methods specific to the crystallization and characterization of the Zn2+-stabilized dimer.

Protein Labeling with Fluorescent Probes. Thiol-reactive fluorescent dyes (ThermoFisher Scientific) were used for site-specific labeling of cysteines in MM variants. Using Alexa Fluor 488 as donor and Alexa Fluor 594 as acceptor provides a Foster distance of 5.4 nm (54, 55). The T165C variant was used to determine the dissociation constants of the artificial dimer and the R313C/N112C double mutant for smFRET measurement. To reduce cysteines, 250 μL of a protein solution at 30 to 40 mg/mL was incubated with 20 mM dithiothreitol (DTT) for 1 h on ice, following DTT removal by gel filtration on an S200 26/600 Superdex column with running buffer C (20 mM HEPES-NaOH, pH 7.5, 150 mM NaCl). Fractions containing the pure protein were pooled, concentrated to 30 to 50 mg/mL, aliquoted, and shock frozen in liquid nitrogen. The MM wild-type construct as well as various mutant variants were purified following the same protocol. For protein labeling with Alexa fluorophores or for experiments exploring metal-mediated dimerization of the MM constructs, the following mutations were introduced individually or in combination: T165C, R313C, N112C, K142H, N183H. Point mutations in the MM construct were introduced using QuikChange site-directed mutagenesis (Agilent). Cysteine mutations were used for covalent attachment of Alexa Fluor dyes and their locations were chosen to avoid any obvious functional disruption. See SI Appendix for methods specific to the crystallization and characterization of the Zn2+-stabilized dimer.

Stopped-Flow Kinetic Measurements. We carried out a Chirascan stopped-flow accessory unit equipped with an independent light-emitting diode power source (Applied Photophysics). For nucleotide-binding studies, fluorescent mannose-substituted nucleotides were used. The dye fluorescence was excited at 350 nm and the fluorescence change monitored through a Schott 395-nm cutoff filter. To measure koff, values, increasing concentrations of MM construct (2, 3, 4, 5, 7, 10, 15, and 20 μM) were titrated to 1 mM mann-nucleotide. For each concentration, at least five traces originated from the same sample were averaged. A linear regression was performed and the slope provided koff with error given by the standard deviation in the slope. For quenching experiments to measure koff, 20 μM protein and 1 μM mann-nucleotide were mixed with 500 μM GTP•S or GDP. Three independent experiments were performed. Rates were determined by single exponential fits and the presented rate represents the average over the three experiments and the error the corresponding variation. Error in koff is determined by propagating the errors in koff and koff.

To detect conformational changes in the MM construct, FRET between Alexa Fluor 488 and 596 fluorescent pairs was recorded. Signals from both dyes (quartz 25-mm diameter round coverslips [Esco Optics], borosilicate glass 6-mm diameter cloning cylinder [Hilgenberg], Norland 61 optical adhesive [Thorlabs]) were detected with a 100-μm pinhole. The sample fluorescence was detected with two channels. Donor fluorescence and acceptor fluorescence were separated via a dichroic mirror (TS58 LPXR; Chroma) and each color was focused onto a single-photon avalanche diode (SPAD) (Excelitas) with additional bandpass filters: FF03-525/50 (Semrock) for the donor SPAD and FF02-650/100 (Semrock) for the acceptor SPAD. The arrival time of every detected photon was recorded with a Hydrel-Amp 40 MHz time-correlated single-photon counting module (PicoQuant) and stored with a resolution of 16 ps.

Photons from individual molecules, separated by less than 100 ps, were combined into bursts if the total number of photons exceeded 30. Photon counts were corrected for background, acceptor direct excitation, and diffusion collection efficiency of the individual detector. A background ratio S < 0.7 was used to identify molecules with an active acceptor. Identified bursts were corrected for background, differences in quantum yields of donor and acceptor, different collection efficiencies in the detection channels, cross-talk, and direct acceptor excitation. FRET histograms were fitted with a combination of Gaussian functions.

The labeled MM construct was diluted to a concentration of approximately 50 pM in 20 mM HEPES-NaOH, pH 7.5, 150 mM KCl, 4 mM MgCl2 in the absence or presence of increasing amounts of GTP, GTP•S, GMP-PCP, and GDP. To prevent surface adsorption of the protein and to maximize photon emission, 0.001% Tween 20 (Pierce) was included in the buffer. All measurements were performed at 23 °C.

In the presence of saturating concentrations of GTP and GTP analogs (e.g., GTP•S, Fig. S4, A), a significant closed population remained, which could not be fully explained by unbound MM (predicted to >2% based on the determined affinities) or GDP contamination. The purity of GTP was 99.4% and that of GTP•S was 95%, as determined by high-performance liquid chromatography (HPLC).

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Chroma ET525/50nm bandpass and Schott 645-nm cutoff filter. G-domain/BSE opening was performed by mixing 5 μM of protein and 300 μM of GTP-S. Experiments for measuring G-domain/BSE closure were performed as follows: A total of 5 μM protein and 100 μM GTP-S were mixed with 2 μM GDP, where GDP will outcompete GTP-S for binding to the protein. Concentrations of reactants in both syringes of all stopped-flow experiments were two times higher compared to the measurement cell since they were diluted upon mixing in 1:1 ratio. Throughout this paper, indicated concentrations are those after mixing.

GTase Assay. GTase assays were performed in 20 mM HEPS-NaOH, pH 7.5, 150 mM KCl, 4 mM MgCl2 containing 5 μM of the MM construct, and 1 mM GTP. The reaction was carried out at 37 °C. Upon starting the reaction, 2-μL reaction aliquots were withdrawn at different time points and mixed with 2 μL of 1 M HCl to stop the reaction and then diluted with 26 μL of HPLC buffer (100 mM potassium phosphate buffer, pH 6.5, 10 mM tetrabutylammonium bromide, 7.5% acetonitrile). Reaction products were quantified on an HPLC system from Agilent equipped with a Hypersil ODS-2 C18 column (250 × 4 mm). Each time-point measurement (five time points) was done in triplicate with the same sample. The specific hydrolysis rate for each protein concentration was computed by linear regression with error given by the standard deviation of the linear fit. To investigate the effect of Zn2+ on the dimer activity, 2, 5, 10, 40, 60, and 140 μM ZnSO4 were added to the standard reaction. For testing concentration-dependent GTase activity, reactions were performed in standard buffer at RT with 5, 15, 30, and 50 μM enzyme. The competition GTase assay was performed by adding 0.21 or 0.85 mM of GDP to the reaction. See SI Appendix, section S1.A.2 for details of the kinetic modeling of the competition assay.

MD Simulations. The conformational dynamics of the MM were studied using simplified structure-based models (SBMs) (57, 58), a method commonly employed to investigate protein dynamics. The dual-domain SBM was constructed in such a way that both the known open and closed MM structures were explicit energetic minima of it. The open structure was defined by chain A of the GMPPCP-bound MM (S2) (6) and the closed structure by chain A of the GDP-bound MM (S2) (24). The G domains between the two structures are nearly identical. The structural differences mainly reside in the positioning of the stalk relative to the G-domain (SI Appendix, Fig. S5). The weighting λ between open and closed in the simulation was determined by matching the simulated open/closed distributions to the smFRET data. See SI Appendix for details of the energy potential. Simulations were performed using GROMACS 4.5.3 (59) containing code edits implementing Gaussian contact interactions (available at ). Single-basin simulation topologies were generated using the SMOG2 software (https://smog-server.org) (31, 60) with the forcefield *SBM.calpha + gaussian* (61) and combined using an in-house script. The temperature (T = 0.92 in reduced units) was chosen such that the average Cα rmsd in the closed MM agreed between a 100-ns explicit solvent simulation using AMBER99SB-ILDN at 310 K and the SBM. The temperature was maintained with stochastic dynamics with coupling constant 0.1 and the time step was 0.0005 fs.

The immobilized MB model in the free energy landscape was observed in smFRET, explicit dyes were attached at T165C and R318C. The dye consisted of a linear chain of six Cys beads connected by bonds 3.8 Å in length (as in Fig. 2C). The FRET efficiency for each snapshot was calculated based on E = (1 + (d/3d)cosθ)−1 using the distance d between the two terminal dye beads and d0 = 5.4 nm. To compute the estimated FRET expected in either the open or the closed ensemble including thermal fluctuations (Fig. 2C) a λ of 0.5 (always open) and 1.2 (strongly closed) was used. λ of 0.98 and 1.1 matched the smFRET histograms for GTP bound or GDP bound, respectively (SI Appendix, Fig. SSD). Note that the photon counting statistics in the experimental FRET system are sensitive to the timescale of moving between the open and closed states. If the conformational time motion is comparable to the time a single molecule dwells in the laser point (∼1 ms), the FRET distribution will become unimodal. Since separated peaks were observed in the experimental data, the dynamics observed must have been slower than 1 ms. Nonetheless, since averaging always tends to compress the peaks, the simulation data, which were not averaged, were expected to have wider separation between the peaks.

We note that the experimental smFRET signature for GTP-bound MM appears to possess an intermediate state (Fig. 2A), although a truly intermediate state is not trivially resolvable due to this intermediate state into the model because there is no crystal structure of it. For our purposes here, such an intermediate within GTP-bound dimers would have little effect, serving only to further flatten the already flat δ distribution.

The flexibility of hinge 1 (SI Appendix, Fig. S8) was estimated by a Cα SMB simulation equivalent to that just described except single basin, meaning that any term in the energy function containing the open structure was zero and λ = 1. A full monomer (chain A) taken from the apo (closed) crystal structure (20) was used to build the SMB. The PH domain along with stalk residues farther than 2 nm from hinge 1 were frozen during the simulation, mimicking its filament-bound state. The temperature was the same as above (T = 0.92) and the simulation was run for 106 time steps. For analysis of the structural fluctuations in terms of the helical coordinates, the frozen part of the stalk was fitted to a stalk domain in the helical reconstruction of Fig. 4A. Hinge 1 was identified by Proline 322 and hinge 2 was identified by Proline 27.

**Mean Stall Forces.** The probability distribution for the end-to-end lengths ℓ of cross-bridges is determined by the equilibrium distance distribution in prehydrolysis GTP-bound dimers, P(ℓ) = P0(ℓ)/ℓ. On the other hand, the contraction force is F(ℓ) = −dG0(ℓ)/dℓ = kT d ln P0(ℓ)/dℓ, where G0(ℓ) is the Helmholtz free energy of a posthydrolysis GDP-bound MM dimer. The distance distributions for GTP- and GDP-bound MM dimers are presented in Fig. 4C. Thus, the mean force acting along the filament under the stall condition can be determined as

\[
\langle F_s \rangle = \int_{0}^{\infty} \frac{P_0(\ell)}{\ell} \sin(\theta) d\ell
\]

where θ is the slant angle and ω is the probability (often referred to as the duty ratio) (32) to find a MM in the dimerized state. Refer to Fig. 4A for the definition of the geometry.

The transverse component (Ft) is given by the same equation with sin θ replaced by −cos θ.

The integral in Eq. 1 was computed by using the distribution P(ℓ) = A exp[−G0(ℓ)/kT] and the force F(ℓ) = −dG0(ℓ)/dℓ with the free energies G0 and GGP in MD simulations, where A is a normalization constant for the probability distribution P(ℓ). In Eq. 1, we have sin θ = Δs/ℓ with Δs = √(s2 − s12). Because of the ratchet property of hinge 1, only configurations with positive displacements Δs should be included when estimating the force. The minimal possible length of a link is lmin = h. Functions P(ℓ) and F(ℓ) are discretized in O(50) steps and it was assumed that the filament radius is the same in the two rungs.

**Elastic Treatment of the Filament and Membrane Tube.** The code used to implement the constrictor model (written in Java) is publicly available at https://bitbucket.org/jkncl/constrictionSimulation. A detailed formulation and analysis of the model for a dynamin filament on a membrane tube, in absence of the motor activity, was given in a previous publication (16). See SI Appendix for a full description. The dynamin filament was represented as an elastic polymer with each bead corresponding to a dynamin dimer. The membrane tube description was based on an axially symmetric continuous Helfrich elastic membrane with stiffness earch and under tension γ. The membrane stiffness urch = 24 kT and the tension γ = 0.03 kBT/m were chosen. At such a stiffness, the elastic energy cost of constricting the filament/membrane tube is dominated by the membrane energy (SI Appendix, Fig. S7E). It should be noted that membrane composition and protein insertion can affect the resulting stiffness (62). As a point of reference a pure 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane has a stiffness around 30 kBT/m. While the true stiffness at the scissor neck remains unknown, the known active remodeling of the membrane composition and protein insertions should presumably act to facilitate the scission by shifting the membrane stiffness as low as possible.

**Dynamin Motors in the Constriction Model.** In the current study, the effects of motor GTase activity were additionally incorporated into the above-described model. This entailed including explicit cross-bridges representing MM dimers and resolving the kinetics of GTase cycles. Again, each simulation bead corresponds to a stalk dimer and, thus, has two MMs associated with it. A MM undergoes stochastic transitions between the five ligand states of the GTase cycle, i.e., Apo and with the nucleotides GDP, GTP, GDP-D, or GDP-D (Fig. 3). The transitions between three monomeric states are characterized by the nucleotide on/off rates displayed in Fig. 1D. The bulk concentrations of GDP and GTP are chosen as 300 μM and 30 μM, respectively (these are typical physiological concentrations) (27). The Apo and GDP-D states cannot form cross-bridges. Binding of GTP by a MM alters the conformational state from the predominantly closed to the predominantly open at the rate of approximately 300 s−1 (Fig. 2D and E). Since opening transitions are thus relatively fast, they are not explicitly resolved in the model. Instead,
all MMs in the GTP state are assumed to immediately go to the equilibrium state, i.e., the probability of a transition with rate $k_{\text{off}}$ = 3 k$_{\text{eff}}$ is constant regardless of the displacement $\Delta s$. The transition from a prehydrolysis GDP-D dimer to the posthydrolysis GDP-D dimer takes place at rate $k_{\text{dis}}$. Note that hydrolysis is chosen to occur simultaneously in both monomers; i.e., high cooperativity is assumed. Such cooperativity is suggested by our kinetic measurements showing that the hydrolysis is equally fast for the dimers containing either two GTP molecules or one GTP and one GDP (SI Appendix, section S1.A.2). The GDP-D dimer dissociates into two GDP MMs at rate $k_{\text{dis}}$.

In kinetic simulations involving transitions between the states, the Gillespie algorithm is often used (63). In the present study, explicit stochastic simulations of the GTbPase cycle were, however, carried out, because most of the calculations reported here are only needed for integrating the equations of motion for the membrane and the beads. The weighting times of transitions obeyed an exponential distribution with a single rate parameter; i.e., the probability of a transition with rate $k$ within time $\Delta t$ is $1 - \exp(-k \Delta t) \approx k \Delta t$ for $k \Delta t \ll 1$. The transition times (inverse of transition rates) between states were all much longer than the integration time step $\Delta t$ of 10 ns. Therefore, the probability of a transition occurring during a single time step was taken to be $k \Delta t$. The small time step was dictated by the membrane description that had to capture the lipid flows. As a characteristic example, a 1-s simulation (i.e., $10^6$ time steps) for a 200-bead filament on a 600-nm-long membrane tube required 5 h on a single core of the Intel Xeon E5-2695 v3 2.30-GHz central processing unit.

The nucleotide association/dissociation rates are given in Fig. 1D. Some GTbPase cycle parameters could not be directly measured (Fig. 3). Unless otherwise noted, $k_{\text{on}}$[MM] = $3 \times 10^5$ s$^{-1}$, $k_{\text{off}}$ = 175 s$^{-1}$, and $k_{\text{hyd}}$ = 10$^6$. The duty ratio $\omega$ was varied between 0.37 and 0.94 by changing $k_{\text{off}}$ between 100 s$^{-1}$ and 3.3 s$^{-1}$. See SI Appendix, section S1.B for further discussion of the choice of the GTbPase-cycle kinetic parameters.

**Data Availability.** All study data are included in this article and/or SI Appendix.

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