LETTER

A hemi–fission intermediate links two mechanistically distinct stages of membrane fission

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Fusion and fission drive all vesicular transport. Although topologically opposite, these reactions pass through the same hemi-fusion/fission intermediate1–3, characterized by a ‘stalk’ in which only the outer membrane monolayers of the two compartments have merged to form a localized non-bilayer connection1–3. Formation of the hemi-fission intermediate requires energy input from proteins catalysing membrane remodelling; however, the relationship between protein conformational rearrangements and hemi-fission/fusion remains obscure. Here we analysed how the GTPase cycle of human dynamin 1, the prototypical membrane fission catalyst4–6, is directly coupled to membrane remodelling. We used intramolecular chemical crosslinking to stabilise dynamin in its GDP–AlF4−-bound transition state. In the absence of GDP this conformer produced stable hemi-fission, but failed to progress to complete fission, even in the presence of GTP. Further analysis revealed that the pleckstrin homology domain (PHD) locked in its membrane-inserted state facilitated hemi-fission. A second mode of dynamin activity, fuelled by GTP hydrolysis, couples dynamin disassembly with cooperative diminishing of the PHD wedging, thus destabilizing the hemi-fission intermediate to complete fission. Molecular simulations corroborate the bimodal character of dynamin action and indicate radial and axial forces as dominant, although not independent, drivers of hemi-fission and fission transformations, respectively. Mirrored in the fusion reaction7,8, the force bimodality might constitute a general paradigm for leakage-free membrane remodelling.

Membrane fission and fusion both involve a pivotal stage, in which lipids rapidly rearrange into a new topology under extreme protein-driven stress3,4. It is generally accepted that lipid rearrangements proceed in distinct steps, involving the formation of transient highly curved non-bilayer intermediate(s)9,10. How conformational changes of the protein machinery orchestrate this orderly remodelling of lipids remains unknown. This knowledge gap is highlighted in dynamin, the founding member of a superfamily of large GTPases implicated in membrane fission and fusion events4–6. Self-assembly of dynamin into helical structures around the necks of deeply invaginated clathrin-coated pits and the consequent stimulated GTPase activity drive conformational changes that underpin its role in catalysing membrane fission and fusion events4–6. Crystallographic studies have provided multiple insights into the nature of these GTPase-driven conformational changes. The amino- and carboxy-terminal helices of dynamin’s GTPase (G) domain, together with the C-terminal helix from the GTPase effector domain (GED), form a three-helix bundle, termed the ‘bundle signalling element’ (BSE) (Extended Data Fig. 1a). Crystal structures of a minimal G domain–BSE dynamin construct bound to either GMPPCP or the nucleotide transition-state analogue GDP–AlF4− revealed two distinct conformations corresponding to a ~70° swing of the BSE relative to the G domain core (Fig. 1a, inset)11,12. This, akin to a lever arm in motor proteins13, it was proposed that BSE movements transmit and amplify transition-state-dependent conformational changes in the G domain to affect intra- and/or intermolecular conformational changes required for fission14. Observed only in the context of a minimal dynamin construct11,12, whether the dramatic nucleotide-dependent movement of the BSE occurs in the full-length protein and how it is transmitted to the membrane-interacting PHD and further on to lipids are unknown.

To gain insight into the functional consequences of this nucleotide-dependent conformational change, we used molecular engineering to access and control BSE motility in full-length wild-type dynamin 1 (WT-Dyn1). To this end, we introduced Cys at position 11 into a functional reactive-Cys-less (RCL) derivative of WT-Dyn1 (ref. 14) for site-specific labelling with a thiol-reactive BODIPY derivative and replaced Tyr at position 125 with Trp to yield CW-Dyn1 (Fig. 1a, inset). This mutant and its BODIPY conjugate retained near normal basal and assembly-stimulated GTPase activities (Extended Data Fig. 1b, c). To detect BSE movements we used photo-induced electron transfer (PET)15, which results in the quenching of the BODIPY label in the BSE (Fig. 1a) by the Trp residue in the G domain only if the two moieties reside within a radius of 10 Å (Fig. 1a, inset)16. When bound to lipid nanotubes (Fig. 1b), the magnitude of PET-induced quenching of BODIPY varies in a nucleotide-dependent manner, becoming progressively higher along the transition from the GDP-bound state (stabilized by GMPPCP) to the GDP–PI transition state (stabilized by GDP–AlF4−). This behaviour is consistent with the GTP-dependent BSE movement predicted by structural analyses (Fig. 1a)11,14, which further suggest that the BSE pivots around a Pro residue (P294) connecting the C-terminal helix of the G domain to the core12,17,18. Consistent with this, mutation of P294 reduces BSE motility and impairs both the GTPase and fusion activities of dynamin (Extended Data Fig. 2). Together, these data confirm that the BSE in full-length dynamin undergoes GTP-dependent conformational changes consistent with a rotation around P294 away from the G domain core.

We next applied site-specific crosslinking between the G domain and the BSE to stabilize the ‘transition-state’ conformer. Trp 125 in CW-Dyn1 was replaced with Cys to produce CC-Dyn1. Using a series of variable-length thiol-specific homo-bifunctional methanethiosulfonate (MTS) reagents, we identified MTS-4-MTS, which has a theoretical crosslinking span of 7.8 Å, as the shortest reagent able to yield ~100% crosslinking efficiency of CC-Dyn1, as evidenced by a gel shift to a faster migrating species (Fig. 1c). This is in good agreement with the distance separating the two Cys residues in the transition state (Fig. 1a). Hereafter, we refer to the crosslinked species as CxC-Dyn1.

In solution, CxC-Dyn1 exhibited enhanced GTPase activity and self-assembled into rings, similar to GDP–AlF4−-bound WT-Dyn1 (Extended Data Fig. 3a, b), verifying that crosslinking stabilizes the

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BSE at or near its transition-state conformation. CxC-Dyn1 retains the ability of WT-Dyn1 to produce high membrane curvature from flat lipid templates (Fig. 1d and Extended Data Fig. 4). Furthermore, like CC-Dyn1 (and WT-Dyn1)\(^{19}\), CxC-Dyn1 rapidly assembles on and constricts tubular membrane templates (Fig. 1e). However, unlike CC-Dyn1, CxC-Dyn1 failed to produce membrane fission either in the presence (Fig. 1e, f and Supplementary Videos 1 and 2) or absence (Fig. 1d and data not shown) of GTP. Reversal of the crosslink with dithiothreitol (DTT) led to full recovery of fission activity (Fig. 1f), indicating that inhibition was due to disruption of dynamin’s conformational changes and not to chemical modification of the cysteine residues.

To determine at which stage fission is disrupted, we analysed the membrane activity of CxC-Dyn1 by measuring protein-induced changes of the ionic conductance of the lumen of thin lipid nanotubes pulled from a planar reservoir membrane\(^{20,21}\). In the presence of GTP, CC-Dyn1 behaved like WT-Dyn1 (ref. 21): it caused a decrease in conductance due to nanotube constriction, followed (in 3 out of 3 cases) by an acute drop in conductivity to zero, indicating complete closure of the tube lumen (Fig. 2a), which for WT-Dyn1 correlated with membrane fission\(^{21}\). In contrast, CxC-Dyn1 failed to trigger lumen closure in the presence of GTP in 11 out of 11 cases, although it retained the ability to constrict and lower nanotube conductance (Fig. 2a). In the absence of nucleotide (apo) or with GMPPCP molecular weight. d, Representative images showing membrane tubulation of SUPER templates (≥5 independent experiments) or GUVs (3 independent experiments) by CC- and CxC-Dyn1 in the absence of nucleotides. Images are inverted for clarity. e, Constriction (seen as dark patches) and fission activity of CC- and CxC-Dyn1 on fluorescently labelled membrane tethers incubated in the presence of 1 mM GTP (see Supplementary Videos 1 and 2; representative data from 3 independent experiments). f, Fission activity assessed by vesicle release into the supernatant (Sup) from SUPER templates of WT-Dyn1 (filled squares) CC-Dyn1 (filled circles) and CxC-Dyn1 (open upward-pointing triangles), and CxC-Dyn1 treated with DTT (open downward-pointing triangles) (average ± s.d., \(n = 3\)).
Figure 2 | CxC-Dyn1 produces stable hemi-fission. a–d, Representative traces of nanotube conductance changes in the presence of CC-Dyn1 (red traces) or CxC-Dyn1 (black traces) obtained in the presence (a) or absence (b) of GTP, or in the presence of GMPPCP (c, d). d, Expanded timescale of the flickering hemi-fission phenotype, boxed in c. $G_n$ indicates conductance normalized to the nanotube conductance before protein addition. e, Cryo-EM images (representative examples from 4 independent experiments) of membrane tubulation by CxC-Dyn1 in the presence of GMPPCP. Arrows indicate putative hemi-fission events detected by the loss of a defined inner leaflet of the bilayer occurring at sites of super-constriction (see inset). Scale bars, 100 nm.

The hemi-fission activity of CxC-Dyn1 in the apo state contrasts with its inability to produce either hemi-fission or complete fission in the presence of GTP (Fig. 2a–d). To understand this paradoxical effect of GTP on CxC-Dyn1, we further examined the nature of the membrane constriction of membrane nanotubes produced by CxC-Dyn1.
Figure 3 | CxC-Dyn1 displays enhanced membrane wedging activity and altered scaffolding properties. a, Cartoon illustrating transmission of transition-state BSE conformational information through the stalk to the PHD. b, FRET between PHD Trp residues and dansyl lipids measuring the relative membrane insertion of CC- and CxC-Dyn1 (average ± s.d., n = 3) (see Extended Data Fig. 5 for complete spectra). F0 and F correspond to fluorescence intensities of dansyl-labelled liposomes in the absence and presence of FRET donors, respectively. c, Hydrophobic character of membrane insertion of WT-Dyn1 (filled squares), CC-Dyn1 (filled circles) and CxC-Dyn1 (open upward-pointing triangles) measured by resistance to salt extraction (average ± s.d., n = 3). d, Differential behaviour of nanotubes to vertical displacement of the patch-pipette depending on the nature/persistence of the protein scaffold. Long scaffolds formed by WT- or CC-Dyn1 in the presence of GMPPCP prevent retraction of the nanotube into the reservoir when shortened, and hence there is no change in tube conductance (left). Short/ flexible scaffolds formed by CxC-Dyn1 in the presence of GTP allow free movement of membranes back into the reservoir, with concomitant increase in conductance (right). e, Addition of GTP to GUVs previously tubulated by preassembled CxC-Dyn1 (3 independent experiments) promotes tubule retraction towards the vesicle membrane. The tubules remained constricted during retraction (see Supplementary Video 3). Images are inverted for clarity. f, Concentration dependence and cooperativity of the assembly-stimulated GT-Pase activity of WT-Dyn1 (filled squares), CC-Dyn1 (filled circles) and CxC-Dyn1 (open upward-pointing triangles) measured on 100 nm 1-γ-phosphatidylinositol-4,5-bisphosphate (P1P2)-containing liposomes by quantifying the release of inorganic phosphate (P0) (average ± s.d., n = 3).

The nanotube conductance characterizing stationary constriction produced by CxC-Dyn1 in the presence of GTP (G0 = 0.22 ± 0.1) was comparable to that produced by CC-Dyn1 (G0 = 0.27 ± 0.05) in the absence of nucleotide (Fig. 2a, b). Such tight membrane constriction is traditionally associated with polymerization of a rigid helical scaffold that, as with CC-Dyn1, prevents retraction of the underlying constrained nanotube to the reservoir, and the accompanying increase in the tube conductance (Fig. 3d, left). In contrast, the length of the nanotube constrained by CxC-Dyn1 could be freely decreased in the presence of GTP, seen as an increase in conductance (Fig. 3d, right).
intermediate using similar simulation modelling. Closely imitating the localized constriction of the membrane nanotube by CxC-Dyn1 (Fig. 4a and Extended Data Fig. 6a, b), we induced self-mergers of the inner monolayer of the tube that further developed into an extended wormlike micelle structure (Fig. 4a and Extended Data Fig. 6b). The micelle geometry was reproducible in different simulation runs (length (L) = 9.0 ± 0.9 nm, standard deviation (s.d.); n = 8; four independent simulation runs). These micelles remained stable throughout the observation period even under application of moderate membrane tension (see Methods). Relaxation of the geometric constraints imposed by the ring system (ring ‘disassembly’46) caused shortening of the micelle (to L = 5.2 ± 0.6 nm, s.d.; n = 10) without rupture (Extended Data Figs 6 and 7), demonstrating that the hemi-fission intermediate does not spontaneously rupture even in the absence of the protein support. Hence, as for membrane fusion23, completion of the fission reaction requires additional energy input to overcome the intrinsic lipid resistance and the stabilizing effect of the protein scaffold.

This energy input apparently comes from GTP hydrolysis. Importantly, the connection between the G domains and PHD, mediated by BSE and disrupted in CxC-Dyn1, is required to deliver energy to the hemi-fission intermediate. It is unlikely that this GTP hydrolysis-driven conformational change causes additional membrane constriction because progression of the GTP cycle past the transition state diminishes the curvature activity of dynamin20 and structural studies clearly associate membrane super-constriction with the pre-transition-state dynamin conformer21. Interestingly, in computer simulations, application of moderate (~0.6 dyn cm⁻¹) membrane tension27 in combination with ring disassembly produced immediate rupture of the hemi-fission intermediate. The combination of tenfold weaker tension and a gradual increase of the separation between rings (for example, due to abrupt loosening of the scaffold46) also mediated the transition from hemi-fission to complete fission (Extended Data Fig. 7). Although the mechanisms of this transition require further investigation, our data suggest that they differ from radial constriction and probably involve production of an axial force in coordination with disassembly of the dynamin scaffold.

These findings demonstrate that dynamin implements different strategies while mediating sequential topological transitions of inner and outer membrane monolayers for fission (Fig. 4b and Extended Data Fig. 8). This bimodality, which is probably embedded in the molecular design of the proteins that catalyse fission and fusion, may constitute a fundamental feature required to coordinate the sequential, two-step, remodelling of membrane monolayers required for non-leaky formation of hemi-fusion/fission intermediates and subsequent fusion/fission. It is tempting to speculate that the current controversies regarding mechanistic models of dynamin1,4,8 are related to the previously unappreciated bimodal nature of the fission process. That is, the different models may reflect sequential modes of dynamin action required for formation and rupture of hemi-fission.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 11 January; accepted 5 May 2015.

Published online 29 June 2015.

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**Supplementary Information** is available in the online version of the paper.

**Acknowledgements** We thank J. Chappie for helpful discussions, A. Mohanakrishnan and D. Reed for technical assistance. S.L.S. was supported by National Institutes of Health grant RO1-GM42455 and the Welch Foundation Grant I-1823. V.A.F. was supported by grants from the Spanish Ministry of Economy and Competitiveness BFU2012-34885, the Basque Government Program Etoriek IE12-332 and European FEDER funds. J.-P.M. was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Intramural Research Program, M.F. and M.M. were supported by the Volkswagen foundation and the DFG-CRC803 “Functionality controlled by organization in and between membranes” (B03). Simulations were performed at the Jülich Supercomputing Center and the North-German Supercomputer Alliance Hannover/Berlin. J.-P.M. was supported by a postdoctoral research grant from the Academy of Finland.

**Author Contributions** J.-P.M. and S.L.S. initiated the project. J.-P.M. designed and generated the CC- and CxC-Dyn1 mutants and performed all of the FRET and PET analysis, as well as their biochemical and functional characterization on liposomes and SUPER templates. A.V., E.R.H. and V.A.F. performed the nanotube conductance and GTP digestion assays and S.N. characterized the P234 mutants. A.C.S. and J.E.H. performed the cryo-EM and negative-stain EM analyses. M.F. and M.M. performed the molecular simulations. All authors discussed and interpreted the experimental data and the results of molecular simulation. S.L.S. and V.A.F. coordinated the project and wrote the manuscript with considerable contributions from all co-authors who also approved the final version.

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METHODOLOGIES

Protein expression and purification. Sf9 insect cells were transiently transfected with complementary DNAs encoding wild-type human dynamin 1 or 1 mutant subcloned in pLex-6 vector (EMD Millipore) for protein production. Proteins were purified by affinity chromatography using glutathione S-transferase (GST)-tagged Aphthipsin-II SH3 domain as an affinity ligand as described previously27. Purified proteins were dialysed overnight in 20 mM HEPES (pH 7.5), 150 mM KCl, 1 mM EDTA, 1 mM DTT and 10% (v/v) glycerol, aliquoted, flash-frozen in liquid N2, and stored at −80 °C. Protein concentrations were determined by absorbance at 280 nm using a molar absorbity coefficient of 59,820 M−1 cm−1 for Dyn1RC(P11C/Y125W), and Dyn1RC(C11V/P125C) (P294A), 54,455 M−1 cm−1 for Dyn1RC(I15V/P125C), and 56,185 M−1 cm−1 for other dynamin-1 proteins.

Protein labelling. The Cys residues at positions 11 in Dyn1RC(P11C/Y125W), Dyn1RC(P11C/Y125W/P294A), and 752 in Dyn1RC(V752C) were selectively labelled in the absence of reducing agent using tenfold molar excess of the thiol-reactive iodoacetamide derivative of BODIPY-F1 (Life Technologies). After 30 min incubation at room temperature, DTT was added to 5 mM to quench the reaction. The solution was extensively dialysed against buffer containing 20 mM HEPES (pH 7.5), 150 mM KCl, 1 mM EDTA and 1 mM DTT to separate unreacted dye molecules. After high-speed ultracentrifugation (100,000g) to discard any precipitated protein, the efficiency of labelling was determined using a molar absorbity coefficient of 76,000 M−1 cm−1 at 502 nm for BODIPY-F1.

Protein crosslinking. MTS-based homobifunctional crosslinking reagents were obtained from Toronto Research Chemicals. Unless otherwise indicated, crosslinking of Dyn1RC(11C/Y125W) was carried out at room temperature for several time-points and transferred to wells of a 96-well microplate for visualization of the crosslinked proteins. For reversal of crosslinking, the templates were subsequently washed four times with 1 ml of Milli-Q H2O by a low-speed spin (2600 rpm for 2 min in a swinging-bucket rotor at room temperature, leaving behind after each wash a 100 μl volume for resuspension of the pelletted templates.

GTPase assay. Basal and assembly-stimulated GTP hydrolysis rates were determined by means of a sedimentation assay, as described elsewhere19,31. In brief, an aliquot of template suspension was added without mixing to a final volume of 100 μl containing 100 mM KCl, 1 mM MgCl2, 1 mM GTP, and indicated protein concentrations. The samples were left undisturbed for 30 min at room temperature, the templates subsequently pelleted at 2600 rpm for 2 min and the supernatants mixed with Triton X-100 to dissolve released vesicles. Total membrane fluorescence of templates was determined in a separate reaction by excitation and emission monochromators using a plate reader. Free phosphate was determined from the absorbance values using a standard curve. The initial rates of GTP hydrolysis were calculated from the linear phase of the time course.

Preparation of giant unilamellar vesicles. Giant unilamellar vesicles (GUVs) were formed by spontaneous swelling of lipid films deposited on 40 μm silica beads. Briefly, DOPC:DOPG:DOPS:Chol:PIP2:RhPE = 28:24:15:30:2:1 mixture in chloroform (0.05 mg total lipid) was dried in a vacuum for 1 h. Then the mixture was rehydrated by adding 10 μl of 1 mM HEPES buffer, pH 7.0. After vigorous mixing, the multilamellar lipid solution was doped with 40 μm plain silica beads and deposited on a Teflon film as 4–5 drops of ~2 μl and then vacuum-dried for 30 min. The beads covered by lipid film were picked from the Teflon film by a thin glass pipette, pre-hydrated for 5 min under H2O saturated N2 atmosphere, and then added from the top to a vertically placed plastic pipette tip filled with 5 μl of a pH-buffered sucrose solution. GUVs formed spontaneously on the bead surface upon 10 min of gentle hydration at 60 °C. Then the lower end of the tip was briefly immersed into a homemade observation chamber filled with 1 ml of buffer (150 mM KCl, 1 mM EDTA, 2 mM MgCl2), then transferring the beads with the attached and detached GUVs into the chamber. The 0.13–0.16-mm-thick cover glass of the chamber was pretreated with bovine serum albumin (BSA) solution (0.1 g/l, 3 min at room temperature) to inhibit lipid attachment to the glass surface. GUVs were further monitored by fluorescence microscopy, as described later.

Preparation of lipid nanotubes for the ionic conductance measurements. Bilayer lipid membranes (BLMs) were formed from the same lipid composition as GUVs on a gilded copper grid (mesh 200, Agar Scientific) pretreated with the same lipid mixture (10 g/l total lipid) dissolved in decaneoform (1:1 v/v): a small drop of the mixture was dropped across the grid and the solvents were then evaporated under argon stream. The grid was mounted on the bottom of an observation chamber that was subsequently filled with the buffer containing 150 mM KCl, 10 mM HEPES, 1 mM EDTA, 2 mM MgCl2. Finally, a small amount of lipid mixture in squalane (20–30 g/l total lipid) was painted over the grid using a thin brush. Lipid bilayers formed spontaneously on each mesh covered by a thick film deposited by the brush. The excess lipid material, expelled to the periphery of the mesh, formed a toroidal meniscus maintaining the lateral tension of the lipid bilayer.

Lipid membrane nanotubes were pulled from the parent BLM using a nano-pulling system based upon high-resolution NanoPZ actuators (Newport Corporation) and calibrated piezo-micromanipulator (Newport; 30 mm travel). Fire-polished borosilicate patch-pipettes (tip diameter of ~1 mm) were used for pulling. The tube formation and manipulation were performed as described earlier20,21. Proteins were delivered with a second micropipette, back-filled with a 7 mM solution of the CC- or CxC-Dyn1 solution in 150 mM KCl, 20 mM HEPES, 1 mM EDTA and 2 mM MgCl2. For experiments conducted in the presence of nuclease, the nucleotides were added in equal concentration both to the observation chamber and the protein delivery pipette.

Fission assay. The efficiency of wild-type and mutant dynamins to catalyse the release of membrane vesicles from RhPE-labelled S2 tubes was analysed by means of a sedimentation assay, as described elsewhere19,31. In brief, an aliquot of template suspension was added without mixing to a final volume of 100 μl of 20 mM HEPES (pH 7.5), 150 mM KCl, with 1 mM MgCl2, 1 mM GTP, and indicated protein concentrations. The samples were left undisturbed for 30 min at room temperature, the templates subsequently pelleted at 2600 rpm for 2 min and the supernatants mixed with Triton X-100 to dissolve released vesicles. Total membrane fluorescence of templates was determined in a separate reaction by excitation and emission monochromators set at 530/25 and 590/25 nm, respectively.

Sedimentation assay. Self-assembly of wild-type and mutant dynamins and their GTP hydrolysis-triggered disassembly were assessed by sedimentation after high-speed centrifugation. Two identical sets of samples were prepared by incubating dynamin (1 μM) for 30 min with or without 400 μM DOPS liposomes (total lipid concentration = 300 μM) in 20 mM HEPES (pH 7.5), 150 mM KCl, 1 mM MgCl2, in a final volume of 30 μl at room temperature. One millimolar GTP or GMPPCP was added to one set of samples and both sets were transferred to a 37 °C water bath for 5 min. Mixture were then spun at 20,800g for 20 min in a microfuge (Heraeus Megafuge 16) at 37 °C to obtain supernatant and pellet fractions containing liposomes and assembled protein was resuspended in 30 μl of the same buffer to obtain equal volumes of S and P fractions. Samples were subsequently resolved on a 7.5% polyacrylamide gel and visualized by Coomassie staining to evaluate protein levels. Dynamin self-assembly on
The nanotube conductance was measured at 50–100 mV holding nitrogen. The vitrified samples were imaged at liquid nitrogen temperature on a Leica EM GP (Leica Microsystems). The grids were subsequently stored in liquid ethane using a Tecnai 12 (FEI) transmission electron microscope at 120 kV using a 2 mesh Cu/Rh grids (Ted Pella), stained with 2% uranyl acetate, and imaged in a Tecnai-12 (T2752C) was used to establish the level of BODIPY emission intensity corresponding to complete loss of PE-induced quenching.

**FRET between PH domain tryptophans and dansyl-PE-containing 400 nm liposomes (DOPs:dansyl-PE = 90:10) was used to investigate membrane interaction of dynamin proteins, as described elsewhere. Briefly, 2.4 ml samples composed of either 0.1 M protein (donor only) or 5 M lipid (acceptor only) were excited at 280 nm (2 nm bandpass) and their emission spectra recorded between 10 s intervals (5 s signal integration). Nucleotides or AlCl3 (1 mM final concentration) were added to the cuvette at indicated time-points. For experiments involving GDP-AlF4−, 10 mM NaF was added to the buffer before data collection was initiated. Concentration-matched sample of BODIPY conjugated to Dyn1L18 (T752C) was used to establish the level of BODIPY emission intensity corresponding to complete loss of PE-induced quenching.

**Fluorescence microscopy.** Fluorescence imaging of RhPE-labelled SUPER templates was performed in BSA-coated Nunc Lab-Tek chambered microscope slides (Thermo Scientific) using a Nikon Eclipse Ti (Nikon instruments) inverted microscope equipped with ×100, 1.45-NA oil-immersion objective and ORCA-Flash 4.0 CMOS camera (Hamamatsu). An aliquot of template suspension was added to 200 µl 20 mM HEPES (pH 7.5), 150 mM KCl, 1 mM MgCl2 in the presence or absence of indicated nucleotides (1 mM final concentration) and allowed to settle to the bottom of the chamber. For curvature generation (tubulation) experiments, 0.1 M dynamin was added to the observation chamber before templates, and imaging was performed after 10–15 min incubation at room temperature. Membrane mets were generated by rolling 20 µm silica beads over the surface of the SUPER templates through tilting of the observation chamber.

The GUVs were monitored using an Olympus IX-70 inverted microscope (×150, 1.45-NA objective) equipped with an AndorXon+ camera (Andor Technology). A halogen lamp was used as the excitation source, ensuring minimal photobleaching. 550/590 nm excitation/emission wavelengths were used. All images were collected and processed using the ImageJ open source software.

**Electron microscopy.** For negative-stain EM, samples (1-3 µM dynamin and 200 M DOPs liposomes incubated in the presence or absence of 1 mM GTP or GMPPCP for 30 min at room temperature) were absorbed onto carbon-coated 400 mesh Cu/Rh grids (Ted Pella), stained with 2% uranyl acetate, and imaged in a Tecnai 12 (FEI) transmission electron microscope at 120 kV using a 2 × 2 Gatan CCD camera. For cryo-EM, a 3.5 µl sample (prepared as described earlier) was placed on a plasma-cleaned (Fishione) Quantifoil holey carbon EM grid (SPI Supplies), blotted with filter paper, and flash-frozen in liquid ethane using a Leica EM GP (Leica Microsystems). The grids were subsequently stored in liquid nitrogen. The vitrified samples were imaged at liquid nitrogen temperature on a Tecnai G2 microscope (FEI) operating at 200 kV and images were collected with a 4 × 4 CCD camera.

**Measurement of ionic conductance through lipid nanotubes.** The equivalent electrical circuit for nanotubes pulled from planar BLMs has been described previously. The nanotube conductance was measured at 50–100 mV holding potential using an Axopatch 200B (Molecular Devices) amplifier. The signal was digitized using a PC-44 acquisition board (Signalogic) as described previously. The current was acquired at voltage-clamp mode of the amplifier, collected using the acquisition board and processed offline using Origin software (OriginLab). The measured conductance of the nanotube in the presence of the protein was normalized to the conductance level measured for the nanotube just before protein addition.

**Molecular simulations.** The simulation method20 and the model parameters25 were used as were previously described. The simulations were conducted using a molecular dynamics scheme with a dissipative particle dynamics thermostat as well as lipid phase behaviour and topological transitions32,34.

The cylindrical tubes of lipid bilayers were assembled by using estimates for the number of lipids in the inner and outer monolayer based on their radii. These configurations were then relaxed by simulating the system in an ensemble that allowed the cylinder length to dynamically vary, keeping the tension along the cylinder axis at zero, resulting in a radius of 6.2 nm and an inside-to-outside lipid ratio of 126:205. The cylinders used in our simulations had a length of 38.8 nm at zero axial tension and consisted of 7,200 lipids.

To explicitly test the effects of the insertion of the PH domains of the dynamin complex, we modelled the PH domains as amphiphilic hexagonal disks consisting of one layer of polar particles connected to one layer of hydrophobic particles. Each layer had three particles per edge and an edge length of 1.3 nm, and the two layers had a separation of 0.6 nm. The particles in each peptide disk were held together by a network of stiff, elastic bonds.

To constrict the lipid cylinder, we arranged the disks into a ring system described previously. Twelve disks were restrained at positions equally distributed on a ring forming a belt around the cylindrical lipid bilayer (Extended Data Fig. 6a). Only the disks’ centres of mass were restrained, while the orientation of the disks could freely change in response to interactions with the lipids. To represent one ‘ring’ of the dynamin spiral formed by the protein dimers, we used pairs of such rings separated by a = 0.45 nm. This distance was smaller than the disk size so the disks from the juxtaposed rings overlapped while preserving their independent mobility (Extended Data Fig. 6a). This way the disk pair created a flexible membrane-interacting surface imitating the adaptive membrane wedging by a pair of PH domains of dynamin dimers. The radius of one of the two juxtaposed rings was slightly smaller, so that this disk pair exerted a direct influence on the orientation of the membrane at the location of the peptides, thus stimulating formation of an hourglass-shaped lipid morphology. Two of the juxtaposed ring pairs situated 9 nm apart (separation between the inner rings corresponding to ~10 nm separation between the midpoints of the ring pairs) constituted the ring system used in simulations (Extended Data Fig. 6a).

In simulations with restrained disks, the ring radii and the separation between the rings were fixed and the position of the disks on the rings were tied to their respective anchor points with a harmonic potential. In the experiments with gradually changing separation between the rings, we fixed the position of one ring pair and slowly moved the other pair away along the axis of the membrane cylinder. In other simulations, we effectively ‘disassemble’ the rings by omitting the positional restraints and allowing the disks to move freely along the membrane surface after the stable hemic-fission intermediate has formed. A more detailed description of the peptide model and the simulation setup can be found elsewhere.

**Stability and rupture of the hemic-fission intermediate.** The time unit in our simulations, obtained from the self-diffusion coefficient for lipid at room temperature, was = 2 ns. The characteristic time for a local relaxation process in the cylindrical bilayer system described can be estimated as ~100 (ref. 26). The total lifetime of the wormlike micelle obtained in the restrained system (9 nm ring separation) under zero tension was 30,200 ns (four independent simulations). In the simulations of rings that could produce rupture of the micelle (9,900 ns). To probe the stability of this unrestrained system we applied small axial tension. The system remained stable for 18,200 under 0.6 dyn cm−1 tension (Extended Data Fig. 7; three independent simulations) and for 15,800 under 0.12 dyn cm−1 tension (two independent simulations), indicating that moderate membrane tensions are not sufficient to make the hemic-fission intermediate unstable. From the lifetime of the hemic-fission state, the lower boundary for the barrier separating hemic-fission from fusion can be estimated to be on the order of ~10 kBT (where kBT is the Boltzmann constant and T is ambient temperature); however, the exact pathway(s) of the membrane transformations leading to complete fission and the corresponding free energy profiles require further investigation.

To induce rupture of the pre-formed hemic-fission intermediate we applied an axial tension of 0.6 dyn cm−1, typical for the planar bilayer systems used in the experiments. This tension produces immediate (lifetime <100s, five independent simulations) rupture in the unrestrained systems and also destabilized the
restrained systems, although much less efficiently (lifetime of 3,400 ± 1,400 s.d.; three independent simulations). To augment the effect of tension we add an additional axial force-factor by slowly (<0.03 nm per t) increasing the separation distance between the two double rings. This ring movement augmented the tension effect so that immediate rupture (<100 t; three independent simulations) was produced under 0.06 dyn cm⁻² tension. The pathways of the hemi-fission rupture explored here are summarized in Extended Data Fig. 7.

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**Extended Data Figure 1** | Domain structure and biochemical characterization of dynamin constructs. a, Domain structure of dynamin and cartoon illustrating that the GTPase domain (G domain, blue) connects through the bundle signalling element (BSE), composed of the N- and C-terminal helices of the G domain and the C-terminal helix from GED (yellow) to the stalk formed by the middle domain and GED (magenta). The pleckstrin homology domain (PHD, green) interacts with membrane lipids. b, c. Basal (b) and assembly-stimulated (c) rates of GTP hydrolysis for 0.5 μM WT-Dyn1 and CW-Dyn1 before and after BODIPY conjugation. Data are shown as average ± s.d., n = 3.
Extended Data Figure 2 | Role of P294 in BSE conformational dynamics.

**a**, Changes in emission intensity of BODIPY-labelled CW-Dyn1 and CW-Dyn1(P294A) due to loss of PET after addition of 1 mM GMPPCP. Although the BSE partially opens upon addition of GMPPCP, its movements are constrained relative to wild type by the mutation of P294.

**b**, Assembly-stimulated GTPase activity of 0.5 mM P294A, P294G and P294V Dyn1 measured on 100 nm liposomes relative to WT-Dyn1. The mutants show near wild-type activity, indicating their ability to self-assemble onto and tubulate liposomes (data shown as average ± s.d., n = 4).

**c**, Fission activity of 0.5 mM P294A, P294G and P294V Dyn1 relative to WT-Dyn1 measured as the percentage of total membrane released from SUPER templates during 30 min incubation in the presence of GTP (data shown as average ± s.d., n = 3). Substitution of P294 with the more rigid valine residue has a greater effect on fission activity.
Extended Data Figure 3 | Characterization of CxC-Dyn1.  

a, Concentration dependence of the specific GTPase hydrolysis rates of WT-Dyn1 (filled squares), CC-Dyn1 (filled circles) and CxC-Dyn1 (open triangles) measured in solution at 1 mM GTP (data shown as average ± s.d., n = 3). 

b, EM micrographs (representative images from four independently prepared samples) showing CxC-Dyn1 assembled into rings and short spirals (arrows) in the presence of GMPPCP visualized by negative stain. Insets: top view, rings; side view, short spirals (arrows). These rings are reminiscent of those previously observed with WT-Dyn1 only in the presence of transition state nucleotide analogues (for example, GDP-αF₄). Unlike CxC-Dyn1, CC-Dyn1 remained unassembled in the presence of GMPPCP (data not shown). Scale bars, 100 nm.
Extended Data Figure 4 | Negative-stain and cryo-EM images of CC- and CxC-Dyn1 assembled onto PS liposomes in the absence of nucleotides. 

a, b, Negative-stain (a) and cryo-EM (b) images are shown. Note the disordered nature of CxC-Dyn1 spirals relative to CC-Dyn1 structures seen via negative stain in a. Scale bars, 100 nm. b, Arrow points to relatively ordered CxC-Dyn1 assemblies, while arrowheads point to sparse dynamin assemblies appearing as single or double rings.
Extended Data Figure 5 | Altered membrane interactions of the CxC-Dyn1 transition-state conformer. a, Fluorescence emission spectra of 0.1 μM CC-Dyn1 or CxC-Dyn1 (donor) as well as dansyl-PE (acceptor)-containing liposomes (5 μM total lipid; 90 mol% PS, 10 mol% dansyl-PE) upon excitation at 280 nm. FRET between the PH domain Trp residues and dansyl is evident in the donor plus acceptor samples as a decrease in donor and an increase in acceptor emission. b, Self-assembly of the indicated proteins (1 μM) on liposomes identical to those used in the GTPase assay (300 μM total lipid; Fig. 3f) examined by sedimentation followed by SDS–PAGE analysis of the supernatant (S) and pellet (P) fractions. c, Percentages of proteins pelleted after incubation with or without 400 nm PS liposomes (1 μM protein, 300 μM total lipid) and 1 mM GTP, as indicated, was quantified by sedimentation followed by SDS–PAGE and densitometric analyses of the protein levels in supernatant and pellet fractions (data shown are average ± s.d., n = 3).
Extended Data Figure 6 | Coarse-grained approach to modelling localized membrane constriction by CxC-Dyn1. a, Schematic representation of the geometry of the ring system used to produce local constriction of a prototype membrane tube. Two pairs of rings are shown, each formed by two closely juxtaposed rings (separated by a small distance $\Delta x$). The inner ring in each pair has the radius $r$ and the outer ring has a slightly larger radius $r + \Delta r$ so that the ring pair promotes creation of an hourglass membrane shape. The PHDs of dynamin are represented as amphiphilic disks evenly distributed over the rings with the centre of mass of each disk being restrained to a position on the ring (marked by blue and orange points). Two overlapping disks (purple and brown) attached to the right juxtaposed ring pair are shown. The orientations of the disks are not fixed, so the normal to the disk surface (purple and brown arrows) can have an arbitrary direction. b, Axial cross-section of a stable hemifission intermediate, the cylindrical micelle, created by the ring system shown in a. The rectangular box indicates the dimensions of the cylindrical micelles (the diameter $D$ and the length $L$).
Extended Data Figure 7 | Molecular simulations of the hemi-fission and fission transformations. The red box shows a representative sequence of simulation snapshots (axial cross-sections) demonstrating the formation of the stable hemi-fission intermediate. Radial constriction of a membrane tube resulted in reversible closure of the tube lumen, that is, flicker, followed by formation of a stable cylindrical micelle structure. The blue box summarizes the simulation runs exploring the stability of the hemi-fission intermediate and its rupture. The top part shows stable structures corresponding to the constrained intermediate (left, taken at zero tension) and the unconstrained intermediate (right, taken at 0.06 dyn cm\(^{-1}\) tension). The bottom part shows the rupture of the intermediates by 0.6 dyn cm\(^{-1}\) tension (left and right) or by elongation of the ring system at 0.06 dyn cm\(^{-1}\) tension (middle). The characteristic times for the rupture are indicated near the corresponding blue arrows.
Extended Data Figure 8 | Dynamin-catalysed membrane fission occurs in two mechanistically distinct stages through a hemi-fission intermediate. Model overlaying the distinct dynamin activities and conformational changes onto the two energy barriers (green curve) that must be overcome, first to catalyse formation of the metastable hemi-fission intermediate and subsequently to drive full fission. When trapped in the transition state and in the absence of GTP, CxC-Dyn1 can drive the formation of a metastable and flickering hemi-fission state (solid blue curve) through the assembly of small scaffolds and enhanced wedging activity of the PHD. However, without subsequent GTPase-driven conformational changes required to loosen the scaffold, generate axial force and retract the PHD, as occurs for WT-Dyn1 (dotted blue line), the membrane-bound CxC-Dyn1 creates an insurmountable barrier to fission (dashed blue line).