Cryo-EM of the dynamin polymer assembled on lipid membrane

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Membrane fission is a fundamental process in the regulation and remodelling of cell membranes. Dynamin, a large GTPase, mediates membrane fission by assembling around, constricting and cleaving the necks of budding vesicles1. Here we report a 3.75 Å resolution cryo-electron microscopy structure of the membrane-associated helical polymer of human dynamin-1 in the GMPPCP-bound state. The structure defines the helical symmetry of the dynamin polymer and the positions of its oligomeric interfaces, which were validated by cell-based endocytosis assays. Compared to the lipid-free tetramer form2, membrane-associated dynamin binds to the lipid bilayer with its pleckstrin homology domain (PHD) and self-assembles across the helical rungs via its guanine nucleotide-binding (GT) domain3. Notably, interaction with the membrane and helical assembly are accommodated by a severely bent bundle signalling element (BSE), which connects the GT domain to the rest of the protein. The BSE conformation is asymmetric across the inter-rung GT interface, and is unique compared to all known nucleotide-bound states of dynamin. The structure suggests that the BSE bends as a result of forces generated from the GT complex dimer interaction that are transferred across the stalk to the PHD and lipid membrane. Mutations that disrupted the BSE kink impaired endocytosis. We also report a 10.1 Å resolution cryo-electron microscopy map of a super-constricted dynamin polymer showing localized conformational changes at the BSE and GT domains, induced by GT hydrolysis, that drive membrane constriction. Together, our results provide a structural basis for the mechanism of action of dynamin on the lipid membrane.

Dynamin family members are mechanochemical GTPases that catalyse membrane remodelling during essential cellular processes1. Mutations in dynamins are associated with neuropathies4 and atypical expression of dynamin is associated with diverse cancers5, while several viruses (for example, HIV) hijack dynamin-dependent pathways6,7. All dynamins are elongated, modular proteins, sharing a structurally conserved N-terminal GT domain connected to a four-helix stalk by a three-helix BSE8. The prototypical member, dynamin, contains the lipid-binding PHD and a proline/arginine-rich domain (PRD) that interacts with dynamin partners that contain an SRC homology 3 (SH3) domain9. Crystal structures have suggested that, in the absence of lipid, dynamin exists as a homo-tetramer formed from two dimers2. The dimer is held together by an extensive interface at the stalk domain (interface 2)10,11, whereas the tetramer is stabilized at the junction between the stalk and the BSE (interface 1) and at the membrane-facing end of the stalks (interface 3)12 (Fig. 1). In all crystal structures, the PHD is either disordered or tucked up into its own stalk. In the assembled state, at the necks of budding vesicles or bound to lipid in vitro, low-resolution cryo-electron microscopy (cryo-EM) structures have suggested that dynamin further oligomerizes into a helical polymer encasing a lipid tube with an additional GT domain dimer interface (interface G2) between the rungs of the helix13. When it binds and hydrolyses GT, the helical polymer constrains the underlying membrane from a thick (more than 20 nm) inner lumen diameter down to a hemi-fission state with a diameter below 3.4 nm12,13 and catalyses membrane fission. Although these points are well established, the function of GTP energy in relation to membrane constriction and fission and the molecular details of the membrane-bound conformations remain unknown for biologically relevant forms of dynamin14.

To provide a structural basis for the mechanism by which dynamin acts, we determined a 3.75 Å cryo-EM map of the constricted dynamin-1 (Dyn) polymer lacking the intrinsically disordered PRD, assembled on lipid and treated with the non-hydrolysable GTP analogue GMPPCP (DynGMPDPCP) (Fig. 1a). We have complemented this structure with a cryo-EM reconstruction of a 10.1 Å resolution super-constricted dynamin polymer treated with GTP (DynGTP) (Extended Data Figs. 1, 2). Whereas DynGMPDPCP represents the GTP-bound form of the dynamin polymer, DynGTP may constitute an intermediate conformation between GTP binding and GTP hydrolysis.

Fig. 1 | Cryo-EM map of assembled dynamin in the GTP-bound state (GMPPCP) on membrane at 3.75 Å. a, Cryo-EM images (left) of helical dynamin tubes were processed to generate a 3D map (right) and subsequently a model of the tetramer was built (green, GT domain; pink, BSE; blue, stalk; gold, PHD) (Electron Microscopy Data Bank (EMDB) code: EMDB-7957; RCSB Protein Data Bank (PDB) ID: 6DLU). n = 3 independent experiments with similar results. b, Regions in map showing high-resolution features: β-sheet in the GT domain (top left), GMPPCP molecule (top right), and side chains of the L477–R453 helix in the stalk (bottom; dashed box in c). c, Tetramer model of assembled dynamin with surrounding density and domains coloured as described above. The assembly interfaces are labelled 1–3. d, Comparison of the crystal structure of dynamin in the apo state (coloured as above) with our 3D map (grey).

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Each nucleotide treatment of dynamin yielded distinct distributions of polymer diameters (Extended Data Figs. 1a, 2). Consistent with previous reports, the dyn\textsuperscript{GMPPCP} reconstruction has a 40-nm outer diameter and a 7.4-nm inner lumen diameter (Extended Data Fig. 1b). The more constricted dyn\textsuperscript{GTP} reconstruction has a 36-nm outer diameter and a 3.4-nm inner lumen diameter, which is narrow enough to induce spontaneous fission without a protein scaffold\textsuperscript{15}. In addition, dyn\textsuperscript{GTP} has two-start helical symmetry (Extended Data Fig. 3), similar to a previously published structure of GTP-bound dynamin containing the GTP hydrolysis-deficient mutation K44A\textsuperscript{12}.

Molecular details of the 3.75 Å map of dyn\textsuperscript{GMPPCP} could not be resolved when relying on previously published helical parameters\textsuperscript{3}. New helical parameters (rise 6.3 Å, twist 23.7°) were determined that led to the elucidation of secondary structure, side chains and the nucleotide density as appropriate for the nominal resolution (Fig. 1b), enabling us to derive a precise molecular model of the dynamin tetramer across most of the molecule. The PHDs were of lower local resolution (over 7.0 Å), suggesting they exhibit conformational flexibility and may not conform to a fixed helical symmetry (Extended Data Fig. 1b). This is likely to be due to their unstable positioning on the dynamic lipid membrane while linked to the stalk by long flexible loops, which are disordered in published X-ray crystal structures\textsuperscript{2,10,11}.

Compared to the crystal structure of the lipid-free tetramer, dyn\textsuperscript{GMPPCP} adopts an extended form, with the GTPase domain positioned more distal from the stalk and the PHD placed atop the lipid bilayer instead of tucked beneath interface 3 (Fig. 1c, d). The oligomeric interfaces in the stalk domain are similar between the lipid-free tetramer and dyn\textsuperscript{GMPPCP} except at interface 1. Interface 1 was originally postulated from the crystal structures of the dynamin-like GTPase Mx\textsuperscript{10} but is not clearly defined in crystal structures of dynamin, consisting of only 190 Å\textsuperscript{2} buried solvent-accessible surface area\textsuperscript{17}. By contrast, interface 1 in the dyn\textsuperscript{GMPPCP} structure has 726 Å\textsuperscript{2} of buried solvent accessible surface area (Fig. 2a). To probe the functional importance of interface 1 in endocytosis, we conducted transferrin uptake assays on cells transfected with interface mutants (Fig. 2b, c). Compared to cells with wild-type dynamin, cells with mutations in interface 1 (L330R/Q334R/L702R) exhibited marked endocytosis defects that were similar to those of cells with the GTPase mutation K44A, which is known to disrupt GTP hydrolysis\textsuperscript{17}, or with interface-3-disrupting mutations (D406R/M407R/T488W). The defects were associated with poor transferrin uptake that did not affect clathrin colocalization\textsuperscript{18} (Fig. 2b). This is consistent with recruitment of dynamin to clathrin before dynamin polymer assembly\textsuperscript{19–21} and suggests that either polymerization or the mechano-enzyme function of dynamin were inhibited. Double or single mutations at interface 1 only partially disrupted endocytosis (Extended Data Figs. 4, 5), suggesting that interface 1 is highly robust. The most notable differences between dyn\textsuperscript{GMPPCP} and the lipid-free crystal structures are in the conformations and dispositions of the GTPase and BSE domains (Fig. 1c, d), which are known to depend on nucleotide state. Previously published studies have sought to capture the different dynamin conformational states associated with the GTPase cycle through crystal structures of a dynamin GTPase–BSE dimer (GG)\textsuperscript{3,14}. While interface G2 in dyn\textsuperscript{GMPPCP} is equivalent to the GG interface G2 in crystal structures, with an average root mean squared deviation (r.m.s.d.) of 0.8 Å, the GG crystal structures do not fit well into the cryo-EM density of dyn\textsuperscript{GMPPCP} (Fig. 3a), suggesting that the cryo-EM structure represents a different hydrolysis intermediate. Notably, the BSE exhibits marked asymmetry across interface G2 in dyn\textsuperscript{GMPPCP} (Fig. 3b). Of the two dynamin molecules that form the interface G2 in dyn\textsuperscript{GMPPCP}, one is well ordered (Fig. 3a, b) and the other is disordered (Fig. 4b). In all dynamin crystal structures, the αS\textsuperscript{5G} and αS\textsuperscript{2B} helices are continuous and form an extended helix (T274–E310) with only a slight bend at T294 (Extended Data Fig. 4b), suggesting that a severe kink at T294 is not energetically favourable. The cryo-EM density of the bent BSE is more disordered than that of the unbent BSE, particularly at residues 20–31 (the N-terminal helix and

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**Fig. 2 | Mutations in interfaces 1 and 3 inhibit endocytosis.** **a,** Mutations in interface 1 (L330R/Q334R/L702R) and interface 3 (D406R/M407R/T488W) generated for endocytic assays. Middle panels, the dyn\textsuperscript{GMPPCP} polymer has a tighter interface 1 (left, blue) than the soluble crystal tetramer (right, green). Distances between stalks in interface 1 are shown above. **b,** Total internal reflectance fluorescence (TIRF) images of dynamin and clathrin colocalization at the plasma membrane (n = 2 independent experiments). Scale bar, 20 μm. Insets, 10-μm squares. **c,** Transferrin uptake is defective with interface 1 (L330R/Q334R/L702R) and interface 3 (D406R/M407R/T488W) mutations. Uptake in wild type and K44A mutant are shown for comparison. Mean ± propagated s.d. from n = 3 biological replicates are shown with single replicates (grey dots) background subtracted and referenced to mean values. Trends were verified with n = 2 biologically independent experiments (Extended Data Fig. 5).
To gain additional insight into this, we aligned the coordinates of a dynamin with an unbent BSE to the structure of a bent dynamin in the dyn GMPPCP map at the GTPase domain (Fig. 3c). Unexpectedly, the stalk and PHD of the aligned unbent dynamin were positioned deep inside the lipid bilayer. This result suggests that the BSE bends to accommodate the forces generated at interface G2, which are then transferred across the stalk domain and the PHD to the underlying lipid membrane. Indeed, the cryo-EM density around the PHD of the bent dynamin is better defined than that of the unbent dynamin, as if the PHD from the bent dynamin is stabilized from the transferred force against the lipid membrane (Fig. 3c). To evaluate the functional relevance of bending dynamin, we investigated mutations that disrupt the BSE kink using cell-based endocytosis assays. A triple mutant that increases the helical propensity of the kink (T292A/L293A/P294A)—which presumably resists bending—resulted in substantially reduced transferrin uptake, nearly to the level of the K44A mutation (Fig. 3d, e). The single P294A mutant and the triple R290A/D291A/T292A mutant did not significantly alter transferrin uptake (Fig. 3d, e, Extended Data Figs. 4, 5). An additional mutation on the back side of the GTPase domain (T92R/L84R/T118R) also had little effect on endocytosis, even though there is close contact here between neighbouring GTPase domains in the assembled polymer (Fig. 3d, e).
at its interfaces. Previous low-resolution cryo-EM studies have shown that interface G2 is formed in this apo state, but probably in a different configuration. Notably, the crystal structure of the apo conformation of dynamin is inconsistent with assembly around a lipid tube (Extended Data Fig. 6). Upon GTP binding, dynamin polymers sample a much more restricted range of conformations, favouring a distinct set of interfaces and a marked asymmetry in the BSE and GTPase domains that applies a force onto the underlying lipid membrane. Localized conformational changes at the GTPase and BSE domains as GTP energy is harnessed drive global changes to the helical symmetry, making room for a second strand to assemble on the membrane tube. This process would require disassembly of dynamin from the lipid bilayer upon GTP hydrolysis, as has been previously reported. Furthermore, in crystal structures, interface G2 has been observed only in the presence of GMPPCP or GDP and ALF (aluminium fluoride), but not in the presence of GDP or in the apo state. The flexibility of the PHDs should accommodate the transition from the one-start helix to the two-start helix. In summary, these molecular snapshots of the biologically relevant form of dynamin provide a framework for understanding the complex orchestration of GTP-driven conformational changes that mediate membrane constriction.

Online content
Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0378-6.

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**Author contributions**

L.K. and J.E.H. designed the research; L.K. and J.E.H. prepared protein samples; L.K., H.W., W.J.R. and J.E.H. collected cryo-EM data; L.K., S.F., A.D.K., H.W., B.C. and J.E.H. processed and analysed the data; K.A.S. and J.W.T. designed and performed cell-based assays; M.-P.S. generated all constructs for cell-based assays; L.K. and J.E.H. wrote the paper; all authors were asked to comment on the manuscript.

**Competing interests** The authors declare no competing interests.

**Additional information**

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

ΔPRD dynamin expression and purification. HA-tagged ΔPRD dynamin (86 kDa) was expressed in baculovirus-infected TN5 cells and purified as previously described. In brief, cells were collected after ~48 h and flash frozen in liquid nitrogen. Cell pellets were thawed quickly in ~ 50 ml of HCB100 (20 mM HEPES, pH 7.2, 100 mM NaCl, 2 mM EGTA, 1 mM MgCl2, 1 mM DTT) at 37 °C and homogenized by N2-cavititation at 500 psi for 25 min. The homogenate was diluted with HCB0 (no NaCl) to a final concentration of HCB50 (50 mM NaCl) and then centrifuged for 1 h at 50,000 rpm. To concentrate and enrich for dynamin, 30% ammonium sulfate was added to the supernatant and centrifuged for 12 min at 10,000 g. The pellets were resuspended in HCB50, containing protease inhibitors (Roche), and centrifuged at 10,000 g for 8 min to pellet aggregated protein. The protein was further purified by a Mono-Q column followed by a Macro-Prep Ceramic Hydroxyapatite (HAP) Type I column. Dynamin was eluted with 400 mM KPO4 off the HAP column and frozen in liquid nitrogen. The purity was ~95%, judged by Coomassie blue staining, and the final dynamin concentration was 2 mg/ml.

Liposome preparation. Synthetic phosphatidylserine in chloroform (50 µl of 10 mg/ml, DOPS, Avanti) was dried down under argon gas in a glass tube and stored overnight under vacuum to remove excess solvent. The lipid was resuspended in 250 μl HCB150 (150 mM NaCl) and extruded 21 times through a 0.8 μm pore-size polycarbonate membrane (Avanti).

ΔPRD dynamin polymorphism. ΔPRD dynamin polymers were generated as described previously. Three dynamin treatments were performed to explore a wide range of polymer conformation states (Extended Data Figs. 1, 2). In brief, dynamin was centrifuged at 13,000 rpm (table top centrifuge at 4 °C) for 5 min to remove aggregated protein and then diluted 1:3 with HCB0 for a final concentration ~0.5 mg/ml. The protein was then incubated with DOPS liposomes for 2 h at room temperature with or without 1 mM GMPPCP. For dyn488/GFP polymers, ΔPRD dynamin was pre-incubated for 5 min before the addition of the DOPS vesicles followed by further incubation for 1 h. For dyn561/GFP polymers, 1 mM GTP was added to preformed ΔPRD dynamin tubes 5–10 s before freezing.

Cryo-EM sample preparation and imaging. Aliquots of 3.5 ml of each sample were applied to plasma-cleaned (Fisichone) C-flat grids (Protochips, CF-1.2/1.3-4C), blotted on the sample side with filter paper for 2 s (22 °C, 90% humidity) and then plunged into liquid ethane with a Leica EM Grid Plunger (Leica Microsystems). For the dyn488/GFP samples, after 3.5 ml sample was applied to the grids in the grid plunger, GTP was added and plunged into ethane after 5–10 s. The vitrified samples were stored in liquid nitrogen before examination by cryo-EM. For the dyn561/GFP samples, images were recorded during three sessions on a Titan Krios (1.25/1.45-cm CTF corrected) at 300 kV (Cryo-EM, FEI) or 300 kV (Cryo-EM, FEI) and 300 kV (Cryo-EM, JEOL) at cryo-EM. The grid was first docked into the cryo-EM density manually in UCSF Chimera followed by rigid-body refinement with Modeler. Upon convergence, all-atom real space refinement was done using the Phenix 1.13-2998 software suite along with manual model building in Coot 0.8.7. The final refinement statistics are shown in Extended Data Table 1. Surface burial analysis was performed using the EMBL PISA server.

Cell culture. All flow cytometry and microscopy was performed on HeLa cells (ATCC CCL-2) that were maintained in phenol red-free DMEM growth media (DMEM, Life Technologies 31053-036; 10% fetal bovine serum, Life Technologies 26140-079; 2 mM glutamax, Life Technologies 35050-061; 1 mM sodium pyruvate, Sigma S8636-100ML) at 37 °C with 5% CO2. HeLa cells were early passaged stocks directly obtained from ATCC and were tested and shown to be mycoplasma free. The human dynamin1–GFP mutants created for this work were fully sequenced and have been deposited in the Addgene repository database.

Fluorescence microscopy. Cells were trypsinized (0.05% trypsin, Thermo-Fisher 252000506) and plated on poly-d-lysine-coated coverslips (Neuvitro, GG -25-1.5-pdl) 24–36 h before imaging. Transfection was performed overnight (1–1.5 µg of cDNA per 100 µl of Lipofermin). Cells were incubated on T25 flasks for 0.5 h at room temperature with or without 1 mM GMPPCP. The resolutions of the dynamin–GFP mutant of interest and mCherry–clathrin (light chain a, addgene #27680) were transfected in imaging buffer (10 mM Hepes, 10 mM glucose, 130 mM NaCl, 2.8 mM KCl, 5 mM CaCl2, 1 mM MgCl2, pH 7.4). Cells were imaged on a Nikon Eclipse TI inverted fluorescence microscope with a 100× apotIRF 1.49 NA objective, 488-nm, and 561-nm excitation lasers. TIRF images displayed in Figs. 2, 3 are characteristic examples from n > 20 cells over two independent experiments for each mutant.

Transferrin uptake assay. For the transferrin uptake assay, the cells were prepared as for fluorescence microscopy but were plated onto six-well plates (Fisher Scientific 08-772-1B) and transfected only with the dynamin1–GFP mutant of interest. After overnight transfection, cells were serum starved for 45 min (DMEM; 2 mM glutamax; 1 mM sodium pyruvate). The medium was replaced with ice cold PBS+ (PBS with 1 mM CaCl2, 1mM MgCl2, 0.2% BSA, 5 mM glucose) and placed on ice then replaced by PBS containing 5 µg/ml Alexa-Fluor 647 conjugated transferrin (Thermo Fisher T23366). The cells were incubated on ice with transferrin for 5 min before the cells were placed into a 37 °C chamber (Safeguard, Thermo Scientific) and the cells were washed twice with ice cold PBS and incubated on ice with 1 ml 2 mg/ml pronase (Sigma 10165921001) in PBS for 10 min. The cells were no longer adherent and were pipetted gently to separate clumps before adding 0.25 ml of 16% paraformaldehyde (Electron Microscopy Sciences 15710) to fix for 20 min. The cells were then spun down and resuspended in 300 µl PBS for flow cytometry. Experiments were performed on a BD LSR II flow cytometer and acquired using BD FACSdiva Software version 8.0.1. Single cells were gated away from debris using forward and side scattered light. In one experiment, 1.6 ng/ml DAPI was added to help gate cells away from debris. The results did not differ from a replicate using scattered light (Extended Data Fig. 5). Each experiment when presented together in a plot had identical gating throughout and included each condition in triplicate. The isolated single cells were plotted with Alexa Fluor 647 transferrin fluorescence versus GFP fluorescence. Gates for GFP positive cells and GFP negative cells was chosen based on untransfected controls. Average Alexa Fluor 647 fluorescence was obtained for each population and were background subtracted with a no uptake control. Their ratio determined the fraction of transferrin uptake. In duplicate experiments, the exact uptake ratio could change due to slightly different GFP gating parameters but the relative trends remained constant (Extended Data Fig. 5). In Figs. 2c, 3e, and Extended Data Figs. 4a, 5d, h, the standard deviations are propagated to include the standard deviations from the subtracted background and the GFP-negative reference. The single grey points shown are the average fluorescence from Alexa Fluor 647 transferrin in GFP positive cells in single replicates that have been background subtracted and referenced to the mean values from n = 3 replicates.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability. Data that support the findings of this study have been deposited in EMDB with the accession codes EMDB-7957, EMDB-7958, PDB ID: 6DLU and PDB ID: 6DLV. The dynamin1–GFP mutant plasmids produced for this study have been deposited at the Addgene plasmid repository.
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Extended Data Fig. 1 | Cryo-EM parameters and data analysis.

**a**, Diameter distribution of dynamin tubes in the absence or presence of GMPPCP or GTP. Scale bar, 200 nm. Each experiment was repeated three independent times with similar results. **b**, Local resolution and helical parameters. R, rise; T, twist; SPT, subunits per helical turn. **c**, Gold standard FSC curves of the dynamin 3D maps. **d**, Model-to-map FSC curves. Dotted blue line indicates gold standard resolution at 0.5 FSC.
Extended Data Fig. 2 | Cryo-EM data collection and processing flowchart. Starting from the top, the flowchart details the pathways of three separate samples (red, green and blue) of dynamin protein through imaging and processing. The samples were imaged by two different microscopes, and then three different conformational states were selected manually. Each of these states was processed separately by Spider and then Relion. The red stars in the polymer diameter histograms indicate the diameters of the particles used for the final reconstructions. See Methods for details.
Extended Data Fig. 3 | Dynamin helical tubes and their Fourier transforms. a, b. Representative cryo-EM images (left) of two dynamin polymers in different conformations. Each experiment was repeated three independent times with similar results. Right, Fourier transforms of the polymer images. The strong layer lines associated with the pitch (red arrows) are highlighted. The distance of the layer lines from the meridian (m), highlighted by dotted black lines, indicate that the dynGTP polymer is a two-start helix, whereas the dynGMPPCP polymer is a one-start helix.

c, d. Power spectra of 2D class averages from dynGTP (c) and dynGMPPCP (d), highlighting the differences between a two-start and one-start helix.

e, f. Sections of the dynGMPPCP (e) and dynGTP (f) maps starting from the outside and going to the middle section. A top middle section looking down the centre of the tubes is shown in the top left panels.
Extended Data Fig. 4 | Functional cell-based assays probing dynamin mutants and structural comparison of the BSE hinge. a, Transferrin uptake of additional interface 3 mutants. Wild-type and K44A are shown for comparison. Mean ± propagated s.d. from n = 3 biological replicates are shown. Also shown are single GFP+ biological replicates that are each background subtracted and referenced to mean values (grey dots). Experiment was repeated and trends verified with n = 2 biologically independent experiments. b, Comparison of α5i and α2b helices from dyn^{GMPPCP} (cryo-bent and cryo-unbent, respectively) and available crystal structures, including dynamin bound to GMPPCP (PCP 1 and 2, PDB ID: 3ZYC), dynamin bound to GDP-AlF (GDP-AlF 1 and 2, PDB ID: 2X2E) and dynamin bound to GDP (GDP 1 and 2, PDB ID: 5D3Q). The number after each structure (1 or 2) represents the first or the second member of the dynamin dimer presented in each structure.
Extended Data Fig. 5 | FACS gating. a–h, To test the robustness of our gating, we used either side-scattering (a–d) or DAPI (e–h) to isolate single cells in two different experimental replicates. In both cases, single cells were isolated from debris (a, e). Single cells were then separated into GFP– and GFP+ gates (b, c, f, g). Controls lacking transfection or transferrin uptake (b, f) informed gating choices. Inhibitory mutants exhibited a characteristic dip in transferrin fluorescence in the GFP+ cells (c, g). The exact fraction of transferrin uptake was dependent on GFP+ gating choices (d, h) while qualitative trends were consistent. Mean ± propagated s.d. from n = 3 biological replicates are shown. Also shown are single GFP+ biological replicates that are each background subtracted and referenced to mean values (grey dots).
Extended Data Fig. 6 | Comparison of dyn$^{\text{GMPPCP}}$ and dyn$^{\text{APO}}$ at interface G2.  

**a**. A large swing in the BSE of the tetramer in the apo conformation (PDB ID: 5A3F) disrupts interface G2. The apo tetramer and cryo-EM dyn$^{\text{GMPPCP}}$ structure (GMPPCP-bound conformation) were aligned by the stalk. Curved arrow indicates the movement of the G domain. Domains are coloured green for GTPase, pink for BSE, blue for stalk in monomer B, and purple for GTPase, orange for BSE, tan for stalk in monomer A.  

**b**. Interface G2, coloured as above, in cryo-EM map of dyn$^{\text{GMPPCP}}$ (grey mesh).
# Extended Data Table 1 | Cryo-EM data collection, refinement and validation statistics

|                      | Dyn<sup>OMPPCP</sup> (EMDB-7957) | Dyn<sup>GTP</sup> (EMDB-7958) |
|----------------------|-----------------------------------|-------------------------------|
| **Data collection and processing** |                                   |                               |
| Microscope           | FEI Titan Krios                   | FEI TF20                      |
| Magnification        | 22,500X                           | 29,000X                       |
| Voltage (kV)         | 300                               | 200                           |
| Electron exposure (e-/Å<sup>2</sup>) | 67                               | 36                            |
| Defocus range (µm)   | 1.0-3.0                           | 1.5-3.0                       |
| Pixel size (Å)       | 1.07                              | 1.27                          |
| Image processing software | RELION v2.0.6                  | RELION v2.0.6                 |
| Symmetry imposed     | Helical                           | Helical                       |
| Initial particle images (no.)<sup>a</sup> | 989,911                          | 58,260                        |
| Final particle images (no.)<sup>b</sup> | 452,959                          | 14,322                        |
| Map resolution (Å)   | 3.75                              | 10.1                          |
| FSC threshold        | 0.143                             | 0.143                         |
| Map resolution range (Å) | 3.57-5.67                      | 7.8-21                        |
| **Helical Parameters** |                                   |                               |
| Inner diameter (nm)  | 7.4                               | 3.4                           |
| Outer diameter (nm)  | 40.0                              | 36.0                          |
| Pitch (Å)            | 96.4                              | 201.5                         |
| Rise (Å)             | 6.35                              | 14.63                         |
| Twist (°)            | 23.68                             | 26.14                         |
| Dynamin dimers per turn (no.) | 15.2                             | 13.8                          |
| Start (no.)          | 1                                 | 2                             |
| **Refinement**       |                                   |                               |
| Refinement Software  | Phenix 1.13-2998                  | Phenix 1.13-2998              |
| Initial model used (PDB code) | 3SNH, 3ZYC                      | 3SNH, 3ZYC                    |
| Model resolution (Å) | 3.86                              |                               |
| FSC threshold        | 0.5                               |                               |
| Model resolution range (Å) | -146.8                          | Not used                      |
| Map sharpening B factor (Å<sup>2</sup>) | Phenix Mask CC<sup>b</sup> | 0.793                          | 0.789                          |
| Non-hydrogen atoms   | 11,993                            | 22,031                        |
| Protein residues     | 1,453                             | 2,678                         |
| Ligand atoms         | 66                                | 0                             |
| B factors (Å<sup>2</sup>) |                                   |                               |
| Protein              | 88.3                              | 482                           |
| Ligands              | 46.0                              | N/A                           |
| R.m.s. deviations    | Bond lengths (Å)                  | 0.007                         | 0.007                         |
| Bond angles (°)      | 0.829                             | 1.468                         |
| Validation           | Molprobity score<sup>c</sup>     | 1.97                          | 1.76                          |
| Clashscore           | 11.78                             | 9.69                          |
| Poor rotamers (%)    | 0.23                              | 0.57                          |
| EM Ringer score<sup>d</sup> | 1.92                             | -0.24                         |
| Ramachandran plot    | Favored (%)<sup>e</sup>           | 94.2                          | 96.3                          |
| Allowed (%)<sup>e</sup> | 5.8                               | 3.7                           |
| Disallowed (%)<sup>e</sup> | 0                                 | 0                             |

<sup>a</sup>Number of particles is equivalent to number of asymmetric units as calculated by (number of boxes) × (number of unique asymmetric units per box).

<sup>b</sup>Model-to-map fit (CC<sub>Mask</sub>) as reported by phenix.real_space_refine.

<sup>c</sup>As reported by Molprobity (http://molprobity.biochem.duke.edu).

<sup>d</sup>As reported by Phenix.
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**Statistical parameters**

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| Item                                                                 | Confirmed |
|----------------------------------------------------------------------|-----------|
| n/a                                                                  | Confirmed |
| The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement | Confirmed |
| An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly | Confirmed |
| The statistical test(s) used AND whether they are one- or two-sided | Confirmed |
| *Only common tests should be described solely by name; describe more complex techniques in the Methods section.* | Confirmed |
| A description of all covariates tested | Confirmed |
| A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons | Confirmed |
| A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) | Confirmed |
| For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted | Confirmed |
| Give P values as exact values whenever suitable. | Confirmed |
| For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings | Confirmed |
| For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes | Confirmed |
| Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated | Confirmed |
| Clearly defined error bars | Confirmed |
| *State explicitly what error bars represent (e.g. SD, SE, CI)* | Confirmed |

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**Software and code**

**Policy information about availability of computer code**

Data collection

Cryo-EM data was collected on either a FEI Krios or TF20 microscope, with Gatan K2 direct detectors, using the program Leginon and Serial EM respectively. For transferrin uptake, experiments were collected using a BD LSR II flow cytometer and acquired using BD FACSDiva Software Version 8.0.1.

Data analysis

For drift correction of the cryo-EM images we used motioncorr2 and Unblur. For data classification based on polymer diameter, we used Spider. Scripts implementing this process in Spider are available upon request. Further data processing was done in RELION 2.0.3 and 2.0.6, which generated the final reconstructions. CTF was corrected in Relion using Ctfind4 estimations. Model refinement was implemented by the Phenix-dev2747 and Coot 0.8.7 software suites, using geometry-based minimization and real-space refinement. The model was first docked into the cryo-EM density manually in UCSF Chimera 1.12 followed by rigid-body refinement with Modeller. Helical propensity was calculated by PROFphd. Transferrin uptake data was analyzed in FlowJo.

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For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

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*Our web collection on statistics for biologists may be useful.*
### Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

There are no restrictions on availability of unique materials.

### Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
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For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

### Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

#### Sample size

Our cryo-EM reconstructions of GMPPCP-constricted and GTP-constricted dynamin polymers contained 452,959 and 5,316 asymmetric units respectively. These sample sizes were determined through image processing software, and were sufficient to converge the structure calculation to detailed maps that are consistent with crystal structures and functional studies. Furthermore, all Euler angles were well sampled in the reconstructions. For the transferrin uptake experiments, the number of cells in each FACS analyzed data point after preliminary gating was between 7000 and 30000 (one replicate in one case was only 2000). Cell number was determined to be sufficient because population shifts were visibly apparent in the raw data comparing positive and negative controls and deviation among biological replicates was low.

#### Data exclusions

Particles were excluded from the final reconstructions in order to enhance the homogeneity of the final image set, which enabled a higher resolution reconstruction. This was achieved by only selecting particles of polymers with a defined range of diameters, and then further sieving the particles based on 2D classification.

#### Replication

The different conformations of the dynamin polymer were reproducible under different nucleotide conditions. This consistency was necessary in order to accrue sufficient sample to be imaged for cryo-EM. The transferrin uptake experiments were reproducible as indicated by the standard deviation error bars. For the transferrin uptake experiment, all attempts at replication were successful.

#### Randomization

Image particles were allocated to different classes based on a maximum likelihood algorithm implemented by the software RELION. Furthermore, in order to minimize model bias, the initial model used for 3D reconstruction was a featureless tube.

#### Blinding

The assignment of particles to classes during reconstruction is implemented automatically by the RELION software, without direct supervision by the investigator.

### Reporting for specific materials, systems and methods

#### Materials & experimental systems

- [x] Involved in the study
- [ ] Unique biological materials
- [x] Antibodies
- [x] Eukaryotic cell lines
- [x] Palaeontology
- [x] Animals and other organisms
- [x] Human research participants

#### Methods

- [x] Involved in the study
- [x] ChIP-seq
- [x] Flow cytometry
- [x] MRI-based neuroimaging
Unique biological materials

Policy information about availability of materials

Obtaining unique materials

There are no restrictions on obtaining unique biological material. The human dynamin1-GFP mutants created for this work are being deposited in Addgene repository database.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

TNS and SF9 insect cells (invitrogen) were used for protein expression, and HeLa cells (ATCC CCL-2) were used for fluorescence microscopy and Flow cytometry.

Authentication

None of the cell lines were authenticated but the HeLa cells were early passaged stocks directly obtained from ATCC.

Mycoplasma contamination

HeLa cells were tested and mycoplasma free.

Commonly misidentified lines

No commonly misidentified cell lines were used.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

All flow cytometry was performed on HeLa cells (ATCC CCL-2) that were maintained in phenol red free DMEM growth media (DMEM, Life Technologies 31053-036; 10% Fetal Bovine Serum, Life Technologies 26140-079; 2 mM Glutamax, Life Technologies 35050-061; 1 mM Sodium pyruvate, Sigma SB636-100ML) at 37°C with 5% CO2. HeLa cells were early passaged stocks directly obtained from ATCC. For the transferrin uptake assay, cells were plated onto six-well plates (Fisher Scientific 08-772-1B) and transfection was performed overnight (1-1.5 ug of DNA, 3 uL of lipofectamine 2000 in 0.5 mL of optiMEM and 2 mL of DMEM growth media) with the dynamin-GFP mutant of interest. After overnight transfection, cells were serum starved for 45 minutes (DMEM; 2 mM Glutamax; 1 mM Sodium pyruvate). The media was first replaced with ice cold PBS4+ (PBS with 1 mM CaCl2, 1mM MgCl2, 0.2%BSA, 5 mM glucose) and placed on ice then replaced by PBS4+ containing 5 μg/mL Alexa-fluor 647 conjugated transferrin (Thermo Fisher T23366). The cells were incubated on ice with transferrin for 5 minutes before the cells were placed into a 37°C incubator for 20 minutes. The transferrin was then removed and the cells were rinsed twice with ice cold PBS, placed on ice, and incubated on ice with 3mL, 2 mg/ml pronase (Sigma 10165921001) in PBS for 10 minutes. The cells were no longer adherent and were pipetted gently to separate clumps before adding 0.25 mL of 16% paraformaldehyde (Electron Microscopy Sciences 15710) to fix for 20 minutes. The cells were then spun down and resuspended in 300 μL PBS for flow cytometry.

Instrument

Experiments were performed on a BD LSR II flow cytometer.

Software

BD FACSDiva Software Version 8.0.1

Cell population abundance

The number of cells in each sample after initial gating to single cells was between 7000-30000. One outlier was only 2000 cells. The amount of debris that was filtered out with gating was highly variable and dependent on the turbidity of the pronase solution used to remove the cells from the substrate.

Gating strategy

Single cells were gated away from debris using forward and side scattered light. In one experiment, 1.6 ng/mL DAPI was added to help gate cells away from debris. The results did not differ from a replicate using scattered light. Each experiment when presented together in a plot had identical gating throughout and included each condition in triplicate. The isolated single cells were plotted with Alexa Fluor (AF) 647 transferrin fluorescence vs GFP fluorescence. Gating for GFP positive cells and GFP negative cells was chosen based on untransfected controls. Average AF647 fluorescence was obtained for each population and were background subtracted with a no uptake control. Their ratio determined the fraction of transferrin uptake. In duplicate experiments, the exact uptake ratio could change due to slightly different GFP gating parameters but the relative trends remained constant.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.