Structural basis of mitochondrial receptor binding and constriction by DRP1

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Mitochondrial inheritance, genome maintenance and metabolic adaptation depend on organelle fission by dynamin-related protein 1 (DRP1) and its mitochondrial receptors. DRP1 receptors include the paralogues mitochondrial dynamics proteins of 49 and 51 kDa (MID49 and MID51) and mitochondrial fission factor (MFF); however, the mechanisms by which these proteins recruit and regulate DRP1 are unknown. Here we present a cryo–electron microscopy structure of full-length human DRP1 co-assembled with MID49 and an analysis of structure- and disease-based mutations. We report that GTP induces a marked elongation and rotation of the GTPase domain, bundle–signalling element and connecting hinge loops of DRP1. In this conformation, a network of multivalent interactions promotes the polymerization of a linear DRP1 filament with MID49 or MID51. After co-assembly, GTP hydrolysis and exchange lead to MID receptor dissociation, filament shortening and curling of DRP1 oligomers to constricted and closed rings. Together, these views of full–length, receptor– and nucleotide–bound conformations reveal how DRP1 performs mechanical work through nucleotide-driven allostery.
domain and the dynamin recruitment region (DRR) required for DRP1 binding
(Extended Data Figs. 2, 3). A combination of helical reconstruction, segmentation, and single-particle alignment and averaging resolved the elongated DRP1 subunits bound stoichiometrically to MID49 (126–454), but no density was assignable for the majority of the variable domain (Fig. 1b, Extended Data Figs. 2–6). Surprisingly, each chain of DRP1 bound MID49 through four different surfaces, and each MID49 in turn bound four DRP1 molecules to yield a vast interaction network (Fig. 1b, Extended Data Figs. 2, 3a–c). MID49 binding to four DRP1 molecules stabilized a linear arrangement of inter-DRP1 interfaces, similar to those observed for other dynamin-family oligomers (Extended Data Figs. 6f–h), and is a determinant of conformational heterogeneity for these and other dynamin-family proteins (Extended Data Figs. 6e, 7a). Previous work has established that this loop comprises part of the binding site for the pleckstrin homology domain within the endocytic dynamin tetramers (Extended Data Fig. 6f–h), and is a determinant of conformational heterogeneity for these and other dynamin-family proteins (Extended Data Figs. 6e, 7a). Previous work has established that this loop comprises part of the binding site for the pleckstrin homology domain within the endocytic dynamin tetramers (Extended Data Fig. 6f–h), and is a determinant of conformational heterogeneity for these and other dynamin-family proteins (Extended Data Figs. 6e, 7a).

Structure-based mutants disrupt DRP1–MID49 assembly

The DRR motif of MID49 occupied the space between two neighbouring G domains and contacted both via receptor interfaces 1 and 2 (buried surface areas of around 450 Å² and around 230 Å², respectively, Figs. 1b, c, 2a). The precise spacing required for this bivalent G-domain interaction explains why previous mutagenesis efforts suggested that the size and topology of the 34–44 loop, rather than its exact sequence (which differs between MID49 and MID51), are critical determinants of binding. Nevertheless, the structure indicated that R235 of MID49 (analogous to R243 in MID51, Extended Data Fig. 7) within the DRR makes key contacts between two neighbouring G domains (Fig. 2a). Accordingly, the MID49(R235E) point mutant could not co-assemble with DRP1 (Fig. 2d–f). In addition, we mutated conserved DRP1 residues involved in receptor interface 1, which is the largest interaction interface. Both the D190A mutation, which should neutralize a salt bridge with the receptor, and the D221A mutation, which
GTP-binding enables receptor binding
Understanding the allosteric coupling between the binding, hydrolysis and exchange of nucleotides and the conformational repertoire of dynamin-family GTPases remains a challenge. From the cryo-EM density we observed that the GMPPCP-bound G domains and the BSE of DRP1 adopt markedly different conformations compared to the nucleotide-free crystal structure. In addition to other nucleotide-induced conformational changes within the G domain, the most evident are the closing of the G2 switch 1 loop to form a closed ‘lid’ over the nucleotide. Analogous to the conformational change reported for dynamin-1, the closure of the switch-1 lid propagates through the adjacent β-sheet to push the α-helices of the BSE into an orthogonal position (Supplementary Video 1). When evaluated in the context of the dynamin interface-2 containing X-shaped DRP1 dimer, this conformational change is a 90° rotation of the G domain and a 40 Å translation towards the stalk (Fig. 3d, Supplementary Video 2). Two of the four DRP1 surfaces that engage the DRR of MID49 or MID51 (receptor interfaces 1 and 2, Fig. 2a) are inaccessible in the nucleotide-free state but become available for binding upon nucleotide-driven elongation (Fig. 3d, Supplementary Video 2).

GTP hydrolysis induces filaments to curl into rings
We next evaluated the dynamics of the DRP1 + MID49 filaments in the presence of hydrolysable GTP, rather than the non-hydrolysable analogue used for 3D reconstruction. After copolymerization in the presence of the non-hydrolysable analogue, we exchanged GMPPCP for GTP by dialysis and followed the reaction using negative-stain transmission electron microscopy (TEM) at sequential time points until the GTP was consumed. We observed that the linear, three-sided DRP1–MID49 cofilaments disassembled into shorter, single-sided filaments before disassembling entirely upon complete hydrolysis to GDP (Fig. 4). The thinner single-sided filaments seen at intermediate time points resembled the single-sided filaments that we observed after mutating a salt bridge that appears to hold the triangular structure together (Extended Data Fig. 3e–i). Moreover, the dynamics of the single-sided filaments at intermediate time points were of interest (Fig. 4b, c). Specifically, upon reaching a reproducibly narrow range of lengths, the nearly linear single-sided filaments spontaneously curled into closed rings of markedly uniform diameter (Fig. 4c).

A model for closed DRP1 rings
In a separate but related experiment, we also evaluated the assembly properties of the DRP1 mutant G362D, which disrupts receptor interface 3, with and without MID49 (126–454). As described above, this disease-associated residue sits at the base of the L1NS loop and this loop is a key site of inter-stalk interaction between adjacent DRP1 molecules in the linear filament (Figs. 1c, 2b, Extended Data Fig. 6b, e). We found that DRP1(G362D) purified as a nearly monodisperse and stable dimer, rather than as a mix of tetramers and higher-order species as observed for the wild-type, full-length protein (Extended Data Fig. 8a). In addition, DRP1(G362D) exclusively formed rings, not filaments, with or without MID49 (126–454) and in the presence of GTP or GMPPCP (Fig. 5a, Extended Data Fig. 8i–l). These rings resembled those observed with wild-type DRP1 in all respects except that the wild-type protein formed closed rings via the linear MID49 copolymer only through the path-dependent reaction described above (Fig. 4c) compared with Fig. 5a). We also observed that these apparently closed DRP1(G362D) rings could constrict liposomes into membrane tubules and circumscribe lipid nanotubes (Extended Data Fig. 9).

DRP1(G362D) rings showed improved structural homogeneity when formed using GMPPCP, presumably because when assembled with GTP, the rings remain dynamic and eventually disassemble upon hydrolysis to GDP (Fig. 4). We imaged the GMPPCP-bound DRP1(G362D) rings using cryo-EM and used 2D class averages of the predominant 12-dimer closed ring to model the differences between the linear filaments and the closed rings (Fig. 5, Extended Data Fig. 10). To account for the projected ring density, the G domain and the BSE of DRP1 must move even further down towards the stalks. Stalk interface 2 appears to remain constant in conformation, as revealed by the

Fig. 3 | Nucleotide-driven allosteric elongation of DRP1 exposes MID49 and MID51 receptor binding sites. a, Nucleotide-free state of the DRP1 G domain and BSE as seen in a crystal structure (PDB ID: 4BEJ). The arrow points to the G2 switch 1 loop. b, Conformation of GMPPCP-bound G domain and BSE determined by cryo-EM. c, Overlay of A and B. The curved arrows highlights the closing of the G2 switch 1 ‘lid’ and the opening of the BSE ‘wrist’. For comparison, the G2 switch 1 loop from 4BEJ was chosen from the only chain (B) in which it was completely resolved. d, Global conformational change induced by nucleotide binding. Rotation and translation of the G domain and BSE elongates the dimer and exposes receptor interfaces 1 and 2 (annotated on separate monomers for clarity). The surfaces of the G domains that engage the receptors are rendered orange in the nucleotide-bound and elongated conformation.

Fig. 4 | Dynamic instability of the DRP1–MID49 linear assembly and curling into closed DRP1 rings. a, Three-sided DRP1–MID49 (126–454) linear filaments copolymerized with GMPPCP, as in Extended Data Figs. 2, 3. b, c, Subsequent exchange for GTP leads to disassembly of the three-sided filaments and partial disassembly of the single-sided filaments (b), and curling of single-sided filaments into closed rings (c). d, Complete consumption of GTP leads to complete oligomer disassembly. Scale bars, 100 nm.
X-shaped dimer seen in projection (Fig. 5d, e). The curvature of the ring, however, dictates that stalk interfaces 1 and 3, and the conformations of the L1N5 and L25 loops, must be extensively remodelled to allow an inter-dimer bending of approximately 30° in comparison with the linear DRP1–MID49 copolymer (Fig. 5d, e, Extended Data Fig. 10e, f, Supplementary Videos 2, 3). We did not observe any density for MID49 in the wild-type rings that form by curling of the DRP1–MID49 coilfament in the presence of GTP, nor in our higher-resolution analysis of the DRP1(G362D) rings that form with or without MID49 present (Fig. 5b, c, Extended Data Figs. 8l–10). This suggests that MID49 binding is incompatible with the curled state of the ring-shaped oligomer, and that constriction therefore requires receptor dissociation (Supplementary Video 3).

Discussion
We note that with an inner diameter of around 16 nm, the closed ring may be sufficient to sever a double-membrane mitochondrion if both the outer and inner membranes are compressed together. Alternatively, if inner membrane fission is distinct and precedes outer membrane fission, a 16-nm diameter suggests that a single membrane tubule would be stabilized by these rings. The structures we observe in vitro may therefore correspond to a highly constricted but pre-fission state observed in vitro50 and in living cells when another dynamin-family protein, dynamin-2, is depleted29. Constriction by DRP1, therefore, may stabilize the high degree of membrane curvature that is suitable for the recruitment and final fission event catalysed by additional dynamin-family enzymes.

Together, these findings establish four advances. First, our cryo-EM structure and mutagenesis studies reveal how receptor proteins such as MID49 and MID51 recruit and stabilize a specific nucleotide-bound conformation of DRP1 and initiate polymerization of a cofilament. We speculate that the nearly linear properties of this polymer have adapted to encircle low-curvature mitochondrial tubules. Second, analysis of the DRP1–MID49 copolymer revealed how the binding of a guanine nucleotide induces a conformational rearrangement to expose an avid network of receptor-binding sites. We now understand these nucleotide-driven allosteric transformations in the context of both full-length and oligomeric DRP1. Third, a path-dependent constriction reaction revealed GTP-dependent conformational dynamics. In this reaction, nucleotide exchange and hydrolysis led to MID49/51 receptor dissociation, disassembly from the ends of the linear filament, and concomitant curling of the shortening filaments into closed rings. The requirement for MID49 or MID51 receptor dissociation before constriction may explain how overexpression of the MID receptors inhibits mitochondrial fission51. Fourth, analysis of a disease mutant in the L1N5 loop, DRP1(G362D), highlights this loop as a fundamental determinant of receptor binding as well as the inter-stalk interactions that govern oligomer geometry. Together, these observations reveal how DRP1 performs mechanical work by curling from linear filaments into closed rings around mitochondria.

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/s41586-018-0211-2.

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Competing interests
The authors declare no competing interests.

Data and code availability
Nucleotide-free tetramer DRP1-GTP-MID49 and MID49 cofilament in the presence of GTP, nor in our higher-resolution analysis of the DRP1(G362D) rings that form with or without MID49 present (Fig. 5b, c, Extended Data Figs. 8l–10). This suggests that MID49 binding is incompatible with the curled state of the ring-shaped oligomer, and that constriction therefore requires receptor dissociation (Supplementary Video 3).

Fig. 5 | Drp1(G362D) cannot bind MID49 and forms rings exclusively
Together, these findings establish four advances. First, our cryo-EM structure and mutagenesis studies reveal how receptor proteins such as MID49 and MID51 recruit and stabilize a specific nucleotide-bound conformation of DRP1 and initiate polymerization of a cofilament. We speculate that the nearly linear properties of this polymer have adapted to encircle low-curvature mitochondrial tubules. Second, analysis of the DRP1–MID49 copolymer revealed how the binding of a guanine nucleotide induces a conformational rearrangement to expose an avid network of receptor-binding sites. We now understand these nucleotide-driven allosteric transformations in the context of both full-length and oligomeric DRP1. Third, a path-dependent constriction reaction revealed GTP-dependent conformational dynamics. In this reaction, nucleotide exchange and hydrolysis led to MID49/51 receptor dissociation, disassembly from the ends of the linear filament, and concomitant curling of the shortening filaments into closed rings. The requirement for MID49 or MID51 receptor dissociation before constriction may explain how overexpression of the MID receptors inhibits mitochondrial fission51. Fourth, analysis of a disease mutant in the L1N5 loop, DRP1(G362D), highlights this loop as a fundamental determinant of receptor binding as well as the inter-stalk interactions that govern oligomer geometry. Together, these observations reveal how DRP1 performs mechanical work by curling from linear filaments into closed rings around mitochondria.
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METHODS

Data reporting. 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.

Construct design. Wild-type DRP1 isofrom 2 sequence was purchased from DNASU (sequence ID HS/DD00043627, UNIPROT identifier: O00429-3, also known as Dlamp1) and was cloned into pET16b plasmid (Novagen) between the NdeI and BamH I sites. The vector was kindly provided by the laboratory of W. Sundquist with a 10×-His tag followed by a PreScission protease site (Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro). The wild-type MID49 (126–454) sequence was amplified by PCR and cloned into a pGEX6p1 vector with a N-terminal glutathione-S-transferase tag followed by a PreScission protease site. Site-directed mutagenesis was performed on pET16b-DRP1 and pGEX6p1-MID49(126–454) using the Gibson cloning method to introduce mutations. All constructs were verified using Sanger sequencing.

Protein purification. Protein purification was performed as described. In brief, plasmids containing the wild-type DRP1 or MID49 (126–454) sequence were transformed in the BL21-DE3 (RIPL) strain of E. coli. The colonies were inoculated in LB medium and grown overnight. Secondary inoculations were performed the next morning in ZY medium for autoinduction. The cultures were grown to an optical density at 600 nm (OD600) of 0.8 at 37°C in baffled flasks and were transferred to 19°C to grow for another 12 h. The cultures were spun down and the bacterial pellets were used for protein purification immediately or stored at −80°C.

Full-length DRP1 wild-type and mutant variants were purified as described previously and dialyzed against and desiccated with modifications. In brief, the bacterial pellets were resuspended in buffer A (50 mM HEPES/NaOH (pH 7.5), 400 mM NaCl, 5 mM MgCl₂, 40 mM imidazole, 1 mM dithiothreitol (DTT), 0.5 mg DNase (Roche) and protease inhibitors (10 mM peptatin, 50 mM phenylmethylsulfonyl fluoride, 0.5 mM aprotinin and 2 mM leupeptin), followed by cell disruption with a probe sonicator. Lysates were cleared by centrifugation at 40,000 × g for 30 min in Beckman JA 25.50 rotors for 60 min at 4°C. The supernatant was filtered before use. The clarified lysate was loaded onto a Ni-NTA column containing imidazole and equilibrated with buffer A. The column was washed with buffer A containing 20 mM imidazole. Filaments were pooled, concentrated, flash-frozen in liquid nitrogen and stored as single-use aliquots at −80°C.

MID49 (126–454) was purified as described previously for wild-type DRP1 with modifications. In brief, the bacterial pellets were lysed as described above in MID-buffer A (50 mM Tris pH 8.0, 500 mM NaCl, 5% glycerol, 1 mM DTT). The protein was eluted with MID-buffer C (50 mM Tris pH 8.0, 500 mM NaCl, 5% glycerol, 1 mM DTT) and dialysed overnight against buffer A containing 20 mM reduced glutathione. The eluate was cleaved overnight with PreScission protease site (Glu-Leu-Gln-Leu-Pro). Filaments were pelleted by centrifugal concentration device (Millipore). In the final step, DRP1 was purified by ion-exchange chromatography using a Q Sepharose column (GE Healthcare). The low-salt buffer for ion exchange was the same as MID-buffer D and the high-salt buffer was MID-buffer E (20 mM Tris pH 8.0, 1 M NaCl, 5% glycerol, 1 mM DTT). The relevant MID49 (126–454) fractions were pooled, concentrated and further purified using a size-exclusion chromatography column pre-equilibrated with MID-buffer F (20 mM Tris pH 8.0, 200 mM NaCl, 5% glycerol, 1 mM DTT). The fractions containing MID49 (126–454) were pooled, concentrated, flash-frozen in liquid nitrogen and stored as single-use aliquots at −80°C.

Filament assembly, cryo-EM sample preparation, data acquisition and processing. To assemble DRP1-MID49 (126–454) filaments, the proteins were mixed at a final concentration of 2 μM each and maintained for an hour at room temperature. The mixture was dialysed against assembly buffer: 20 mM HEPES pH 7.5, 50 mM KCl, 3 mM MgCl₂, 1 mM DTT and 200 mM 1,3,5-triphenylsulfonate sodium salt (GMPPCP) with or without 0.2% octyl-glucopyranoside (Anatrace). The filaments were observed using negative-stain TEM or cryo-EM after vitrification. Under these conditions, the mutant DRP1(S611D) failed to co-assemble with MID49, but upon further lowering the ionic strength to 25 mM KCl, DRP1(S611D) displayed detectable but greatly reduced co-assembly compared to wild-type protein. For vitrification, the sample was applied to Quantifoil holey carbon grids (R2/2) using a Vitrrobot Mark III with 3.5 μl of sample, a 5-s blotting time and a 0-mm offset at 19°C and 100% humidity. Images were collected on a FEI T30 Polara operating at 300 kV at a magnification of 31,000×. Images were recorded on a Gatan K2 summit camera in super resolution mode for a final binned pixel size of 1.22 Å per pixel. The movies were dose-fractionated, containing 30–40 frames, had a total exposure time of 6–8 s with 0.2 s per frame and a per-frame dose of 1.1 to 1.4 electrons per Å². SerialEM was used to automate data collection. The defocus range was 0.8–3 μm under focus. The data was processed using the Relion version 1.4 program and UCSF Motioncor2. Contrast transfer function (CTF) parameter estimation on the non-dose-weighted but motion-corrected stacks was carried out using CTFIND4D and GCTE. Filaments were boxed using the program e2helixboxer.py from the EMAN2 suite. Particle coordinates were used to extract discrete particles using RELION 1.3–1.4 and all further processing was carried out within the RELION suite. Multiple rounds of 2D classification identified the well-ordered segments. 3D autoselection was run using a customized Relion1.2 version with the HRSR algorithm implemented. The consensus helical structure was used to classify the particles without redefining helical symmetry (using RELION 1.4), resulting in two major classes that differed slightly in rise and twist (Extended Data Fig. 2c). Particles from each class were selected and independently refined again with helical RELION 1.2 and HRSR. Analysis of these reconstructions revealed that each structure comprised three linear filaments that bundle together to form a structure that resembled a triangle in cross-section (Extended Data Figs. 2, 3). The vertices of the triangle are formed through asymmetric interactions between the G domains in adjacent filaments. The triangular arrangement of the bundled helices is unlikely to correspond to a biologically meaningful architecture, and this structure cannot form a stable condensed state on the inner mitochondrial membrane.

To further improve the signal-to-noise ratio, each of the three filaments in each independent half-map was segmented, extracted, resampled on a common grid and summed using UCSF Chimera. The symmetrized but unfilmed half-maps from each class were again aligned to a common grid and summed according to the C2 symmetry axis of the DRP1 dimer. In a last step, relion_postprocess was used to add the resulting and fully symmetrized half-maps (Extended Data Fig. 2c). These half-maps and the final summed map, with differential B-factor sharpening per region (Extended Data Figs. 2c, 4, 5), were used for atomic modeling using Rosetta as described below.

For the projection structure of the DRP1(G362D) rings, 2 μM protein was mixed in a 1:1 molar ratio with MID49 (126–454) and was allowed to stand at room temperature for an hour. The mixture was dialysed against the assembly buffer (without detergent) overnight. The sample was collected after 12 h and vitrified using ultra-thin 3-nm carbon support films (Ted Pella). For vitrification, a Mark III vitrobot was used with 3.5 μl of sample, a 0-mm offset, 100% humidity and a 3.5-s blot time. The images were collected using an FEI TF20 microscope and SerialEM for automated data collection. The data were recorded with a Gatan K2 camera operating in super resolution mode to collect dose-fractionated movie stacks with a final binned pixel size of 1.23 Å per pixel. 40 frames were collected per stack (0.2 and 1.42 electrons per Å² per frame). The movie stacks were motion-corrected and the parameters of the transfer function were estimated as described above.

Approximately 2,000 particles were picked manually for initial 2D classification in RELION 1.4 and these averages were used as templates for further particle picking by Gautomatch (http://www.mrc-lmb.cam.ac.uk/kzhang/). Final 2D averages of the entire rings versus quarter segments of the rings were computed using RELION 1.4.

Liposome and nanotube reactions. Liposomes were made as described previously using 1,2-DOPC and 1,2-DOPS (Avanti Polar Lipids). The total molar ratio of DOPC to DOPS was purchased from Avanti Polar Lipids. DOPS dissolved in chloroform was dried under a steady stream of nitrogen and dried under vacuum for an hour. The dried lipid film was resuspended in n-hexane and dried again under nitrogen. The
resulting lipid film was dried under vacuum for 4 h and was finally resuspended in 20 mM HEPES pH 7.5 and 150 mM KCl. The same protocol was followed for making nanotubes, in which the mixture contained 60% d-galactosyl-(3-1,4)-N-nervonoyl-d-erythro-sphingosine (Galactosyl Ceramide), 30% DOPS and 10% Ni^{2+}-NTA DOGS (1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1-carboxypentyl) imidodiacetic acid)succinyl] nickel salt).

For assembly reactions of DRP1(G362D) over lipid, 0.5–2 μM protein was incubated with liposomes or nanotubes for an hour and dialysed against the assembly buffer without detergent.

**Model building.** The general procedure for atomic model interpretation and validation using Rosetta were performed as described. To obtain an initial model for DRP1, the crystal structure of nucleotide-free DRP1 (PDB ID: 4BEJ) was used for the stalk region and DRP1 G-domain-BSE structures bound to GMPPCP (PDB ID: 3W60) were used for the G-domain and BSE regions. Density-guided model completion for DRP1 was carried out with RosettaCM using this hybridization of DRP1 crystal structures. A converged solution appeared from the low-energy ensemble of the complete models generated by RosettaCM. However, among the low-energy ensemble, residues 503–610 were found to be extremely flexible without cryo-EM density constraints and therefore were omitted for further coordinate refinement. For MID49, the highly homologous mouse MID49 crystal structure was used.

We further refined the model in the context of a full assembly that included eight identical copies of each protein, Mg^{2+} and nucleotide that included all possible inter-domain molecular interactions in the filament (Extended Data Fig. 5a, b). Pseudo-symmetry was used to enable and facilitate the energy evaluations of all neighbouring interactions around the asymmetric unit (green model, shown in Extended Data Fig. 5a) for final model refinement of the full assembly. To this end, refinement was done against the training map. Finally, the half-maps were used to determine a weight for the density map that did not introduce overfitting. Using the weight and with the symmetry imposed, the whole assembly of DRP1 and MID49 was refined in the full map, followed by B-factors refinement. Finally, quantification of the buried surface area and the number and nature of the bonds involved for each DRP1–MID49 interaction interface modelled by Rosetta were performed with the PISA server (http://www.ebi.ac.uk/pdbe/pisa/).

Visual evaluation of the model-to-map correspondence was carried out in UCSF Chimera using unfiltered and unsharpened maps, maps uniformly sharpened with a range of ad hoc B-factors, and maps processed with a model-based local sharpening and local low-pass filtering procedure to optimize contrast and the visibility of high-resolution features of the model.

To build a molecular model for the closed 12-dimer DRP1 rings, we used the diameter, thickness and angles revealed by the 2D cryo-EM class averages of the DRP1(G362D) rings stabilized with GMPPCP. The atomic coordinates determined above using RosettaCM were used to build the ring in sections, first with repeating dimers of the interface-2 X-shaped stalk, then the BSE and finally the G domains and the angles between these sections were iteratively adjusted until calculated projections of the molecular model corresponded with the features of the experimental projection densities. Both the top (Fig. 5b, c) and the side view (Extended Data Fig. 10b) were used as constraints. The complete atomic model of the ring was finally refined in Phenix to minimize clashes.

**Statistics and reproducibility.** All electron microscopy experiments in this study were repeated at least three times.

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

**Data accessibility.** All of the 3D cryo-EM density maps associated with this study have been deposited in the Electron Microscopy Data Bank with accession number EMD-8874. The atomic coordinates have been deposited in the Protein Data Bank as 5WP9. Raw data, models and image processing scripts are also available from the corresponding author upon reasonable request.
Extended Data Fig. 1 | DRP1 and MID49 assembly states. a–j, DRP1 assembly states visualized with negative-stain electron microscopy in the presence of different guanine nucleotides and MID49(126–454). Both proteins were incubated at concentrations of 2 μM. Scale bars, 100 nm. k–m, MID49(126–454) and MID51(132–463) form indistinguishable assemblies with DRP1: DRP1 + MID49(126–454) and GMPPCP (k), DRP1 + MID51(132–463) and GMPPCP (l), DRP1 + both equimolar MID49 and MID51 (m). Scale bars, 100 nm.
Extended Data Fig. 2 | Cryo-EM and 3D reconstruction. a, A cryo-EM micrograph of DRP1–MID49(126–454) filaments formed with GMPPCP. Scale bar, 100 nm. Inset, a representative 2D class average. Scale bar, 10 nm. b, Cross-section of the 3D reconstruction of the filament and the distribution of views determined during helical reconstruction. The length of the cylinders and the colour code correspond to the number of particles for that viewing direction (from few to many, blue to red). The 3D structure has been segmented and coloured with DRP1 in grey and MID49 in golden yellow. c, Particle numbers and workflow for the reconstruction protocol. DRP1 density is shown in grey and MID49 is in golden yellow.
**Extended Data Fig. 3 | Intra- and inter-filament interactions.**

**a**, The triangular structure seen in cross section. Side 1 of the triangular structure has the atomic model placed in the density. The G-domain-to-G-domain contact between adjacent sides is circled. **b**, The sum of the three sides with the model fit in density. **c**, Ribbon diagram of the same atomic model as in **b**. The rotated view shows eight chains each of DRP1 and MID49. The chains further from the reader are rendered transparent. **d**, An isolated tetramer of DRP1 from the filament, rendered to highlight the stalk interfaces 1, 2 and 3 observed for DRP1 and other GTPases of the dynamin family. **e**, Expanded view of the circular region in **a** illustrating a salt bridge between adjacent G domains. **f–i**, Negative-stain micrographs of: DRP1-only wild-type polymers incubated with GMPPCP (**f**), DRP1 co-assembly with wild-type MID49(126–454) and GMPPCP (**g**; inset, high-magnification view), DRP1(E116R) mutant polymers (**h**), DRP1(E116R) mutant co-assembly with MID49(126–454) (**i**). Shorter, single-sided filaments predominate. Disordered ‘triangular assemblies’ were also observed, but were much shorter and infrequent compared with the wild-type proteins. Scale bars, 50 nm.
Extended Data Fig. 4 | Resolution estimates. a, b, Local resolution estimates computed by Resmap\textsuperscript{74}. Histogram of voxel values (a), and results in a depicted as a heat map of a cross-section through the reconstruction (b). c, d, Fourier shell correlation plots for the half-maps with and without symmetry (c) and model-to-map correlations for each sub-region of the structure (d).
Extended Data Fig. 5 | Rosetta-based model refinement. a, Complete assembly used for Rosetta-based model building with the asymmetric unit shown in green. b, Atomic B-factors for one asymmetric unit, DRP1 versus MID49 models (ribbon) and bound GMPPCP (space filling). c, Sequence alignment between human and mouse MID49 sequences.

d, Overlay of the homology model of human MID49(126–454) (blue, with DRR in yellow, ribbon) modelled within the cryo-EM density overlaid with the mouse MID49 crystal structure (PDB: 4WOY, grey ribbon) which was used as a constraint. No density attributable to ADP within the nucleotidytransferase domain was observed.

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Extended Data Fig. 6 | Map-to-model fits and role of the L1N\(^\text{S}\) and L2\(^\text{S}\) loops. a–d. Examples of models fit within B-factor-sharpened cryo-EM density for a helix from the DRP1 stalk (a), the backbone of the L1N\(^\text{S}\) loop from the stalk (b), an elongated helix and turn found in MID49 (c) and GMPPCP and Mg within the G domain (d). e. Roles of L1N\(^\text{S}\) and L2\(^\text{S}\) in linear filament formation. Top, an isolated Drp1 tetramer from the cryo-EM model. The circled region is expanded in the lower panel. Bottom, interactions that the conserved loop L1N\(^\text{S}\) makes within the assembly. G362 is highlighted with an arrow. f. DRP1 stalk and MID49 at receptor interface-3. g. Dyn3 stalk and pleckstrin homology domain from PDB 5A3F\(^\text{40}\). h. Overlay of f and g.
Extended Data Fig. 7 | Sequence conservation and key interaction sites. 

a, Multiple sequence alignment of the regions near and including the DRP1 residues mutated in this study: D190, D221 and G362, G363. The residue numbers apply to human DRP1, isoform 2 (UNIPROT identifier: O00429-3 which is also known as DLP1a). b, Sequence alignment of MID49 and MID51 at the region around residue R235 of MID49. R235 of MID49 corresponds to R243 of MID51. c, Sequence alignment of DRP1 isoform 1 and isoform 2 showing the correspondence of S637 (isoform 1) and S611 (isoform 2).
Extended Data Fig. 8 | Biochemical and structural characterization of mutants. a–c, Size-exclusion chromatography traces for DRP1 wild-type and mutants used in the study. Comparison between wild-type (WT) and G362D (a), wild-type and D221A (b), and wild-type and D190A (c). d, Gel filtration standards with annotated molecular weights from the same column and chromatography system. e–h, DRP1 assembly and co-assembly reactions with GMPPCP for DRP1(D190A) alone (e), DRP1(D190A) + MID49 (f), DRP1(D221A) alone (g) and DRP1(D221A) + MID49(126–454) (h). Scale bars, 100 nm. i–l, DRP1(G362D) assembly and co-assembly reactions with GMPPCP or GTP. DRP1(G362D) forms rings but not linear filaments without MID49 (i, k), with MID49 (j, l), with GMPPCP (i, j) or with GTP (k, l). Scale bars, 100 nm.
Extended Data Fig. 9 | DRP1(G362D) rings on model membranes.

a, DOPS liposomes used in the study. b, DOPS liposomes after incubation with DRP1(G362D) showing ring-like assemblies on the membrane and in the background. c Lipid nanotubes incubated with DRP1(G362D).

d, Power spectrum of the area shown within the dashed square in b. e, Power spectrum of the area shown within the dashed square in c. In both d and e, layer lines indicative of helical geometry are not detectable. Scale bars, 50 nm.
Extended Data Fig. 10  |  DRP1(G362D) forms 12-dimer closed rings.

a, 2D class average of the rings. The red dashed circle indicates density that may be attributable to the variable domain. b, 2D class average of infrequent, orthogonal or side views used as a constraint during model building. c–f, Top (c) and side (d) projections of the model; top (e) and side (f) views of the final model rendered as ribbons. Scale bars, 100 Å. Green, G domain; red, BSE; purple, stalk.
Reporting Summary

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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).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
- Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

- Data collection
  - Serial EM was used to collect cryoEM data.
- Data analysis
  - RELION (version 1.3, 1.4, 1.2ah), EMAN2, Rosetta, Gautomach, GCTF, MotionCor2, CTFFIND4, UCSF Chimera were used for analysis. All are open source softwares. All softwares were cited in the methods section.

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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Policy information about availability of data

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

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All of the 3D cryoEM density maps associated with this study have been deposited in the EMDB with accession numbers EMD-8874. The atomic coordinates have been deposited in the PDB as 5WP9. Raw data, models and image processing scripts are also available from Adam Frost (adam.frost@ucsf.edu) upon request.
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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     | Data collection was performed until the sample size comprised all necessary views for 3D reconstruction. Given the uncertainty and lack of experimental control of preferred orientation, no predetermination of sample size could be estimated. |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | Single particle images of damaged, incomplete, or contaminated structures were filtered out by 2D and 3D classification procedures.                                                                                                           |
| Replication     | All attempts at replication were successful.                                                                                                                                                      |
| Randomization   | Random assignments of orientation parameters and classification parameters is integral to the reconstruction process.                                                                             |
| Blinding        | All data points were treated equally. The investigators were blinded to the group allocation and outcomes.                                                                                  |

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods            |
|---------------------------------|--------------------|
| n/a                             | n/a                |
| Involved in the study           | Involved in the study |
| Unique biological materials     | ChIP-seq           |
| Antibodies                      | Flow cytometry     |
| Eukaryotic cell lines           | MRI-based neuroimaging |
| Palaeontology                   |                     |
| Animals and other organisms     |                     |
| Human research participants     |                     |