1 **Structural Basis of Mitochondrial Receptor Binding and GTP-Driven**

2 **Conformational Constriction by Dynamin-Related Protein 1**

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4 Raghav Kalia[1,2,3,5], Ray Yu-Ruei Wang[1,3,4], Ali Yusuf[1,3], Paul V. Thomas[1,3,5], David A.

5 Agard[1,3,4], Janet M. Shaw[2,4], and Adam Frost[1,2,3,5]

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7 [1]Department of Biochemistry and Biophysics, University of California, San Francisco, San

8 Francisco, CA 94158 USA

9 [2]Department of Biochemistry, University of Utah, Salt Lake City, UT, 84112 USA

10 [3]California Institute for Quantitative Biomedical Research, San Francisco, CA 94158 USA

11 [4]Howard Hughes Medical Institute

12 [5]Chan Zuckerberg Biohub, San Francisco, CA 94158 USA

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14 Corresponding author. Email: adam.frost@ucsf.edu

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16

17 **Abstract**

18 Mitochondrial inheritance, genome maintenance, and metabolic adaptation all depend on

19 organelle fission by Dynamin-Related Protein 1 (DRP1) and its mitochondrial receptors.

20 DRP1 receptors include the paralogs Mitochondrial Dynamics 49 and 51 (MID49/MID51)

21 and Mitochondrial Fission Factor (MFF), but the mechanisms by which these proteins

22 recruit DRP1 and regulate its activities are unknown. Here we present a cryoEM structure

23 of human, full-length DRP1 bound to MID49 and an analysis of structure- and disease-

24 based mutations. We report that GTP binding allosterically induces a remarkable

25 elongation and rotation of the G-domain, Bundle-Signaling Element (BSE) and connecting

26 hinge loops of DRP1. In this nucleotide-bound conformation, a distributed network of

27 multivalent interactions promotes DRP1 copolymerization into a linear filament with

28 MID49, MID51 or both. Subsequent GTP hydrolysis and exchange within the filament

29 leads to receptor dissociation, shortening through disassembly, and concomitant curling

30 of DRP1 oligomers into closed rings. The dimensions of the closed DRP1 rings are

31 consistent with DRP1-constricted mitochondrial tubules observed in human cells. These

32 structures are the first views of full-length, receptor- and nucleotide-bound dynamin-family

33 GTPases and—in comparison with nucleotide-free crystal structures—teach us how

34 these molecular machines perform mechanical work through nucleotide-driven allostery.

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Introduction

Pioneering work in yeast and other model systems revealed that fragmentation of the mitochondrial reticulum disperses units of the organelle during cell division[1-3], coordinates morphological adaptation with metabolic demand[4-7], and quarantines damaged units for turnover[8-10]. Recent work also led to the discovery of the role mitochondrial fission plays in regulated cell death pathways[11-19], brain development and synaptic function[20-23], and how certain pathogens disrupt these processes and hijack mitochondrial resources[24-26]. Finally, there is a growing understanding of how inter-organelle contacts between the ER and mitochondria initiate mitochondrial fission[27,28], and how this process regulates mitochondrial genome duplication and integrity[29,30]. The master regulator that unites these processes across eukaryotic evolution is the membrane-remodeling GTPase DRP1[4,7,31-37].

DRP1 is necessary but not sufficient for mitochondrial fission because receptor proteins must recruit the enzyme to the Outer Mitochondrial Membrane (OMM). In mammals, these include the paralogs MItochondrial Dynamics proteins MID49 and MID51 or the Mitochondrial Fission Factor, MFF [32,35,38-41]. Following receptor-dependent recruitment, DRP1 assembles into polymers that encircle mitochondria and, through still poorly understood mechanisms, channels energy from GTP binding, hydrolysis, and nucleotide exchange into a mechanochemical constriction[13,41-46]. In addition to DRP1 and

55   its OMM receptors, a recent study revealed that a second member of the dynamin-family

56   of GTPases, dynamin-2, enacts the final fission event downstream of DRP1-driven

57   constriction of a mitochondrial tubule[47]. Thus, mitochondrial division is a stepwise reaction

58   regulated by DRP1 receptor binding, oligomerization and nucleotide-dependent

59   conformational dynamics.

60   We and others have reported that the OMM receptors MFF or MID49/51 are

61   independently sufficient to recruit DRP1 in order to divide mitochondria[38,39,41,48]. We also

62   reported that MID49/51 can coassemble with DRP1 forming a copolymer with a

63   dramatically different morphology than reported for dynamin-family members[41]. While

64   these results suggest that an adaptor protein could alter the architecture of a dynamin

65   polymer to facilitate a mitochondria-specific activity, the architecture and functions of this

66   coassembly remain unclear.

67   Here, we report the structural basis of DRP1 coassembly with MID49/MID51. Our

68   cryoEM structure reveals how nucleotide binding to the G-domain induces conformational

69   changes that allosterically propagate through the BSE to open and elongate DRP1 and

70   thereby expose multiple receptor-binding surfaces. MID49/51 binding stabilizes a specific

71   alignment of DRP1 tetramers to nucleate polymerization of a linear co-filament. Then, in

72   a path-dependent reaction, we show how GTP hydrolysis and nucleotide exchange lead

73   to conformational constriction by the polymer. Specifically, when DRP1 subunits within

74   the cofilament exchange and hydrolyze nucleotide, they dissociate from MID49/51

75   receptors and the entire polymer dynamically shortens and curls into a closed ring.

76   Analysis of structure-based and disease-causing mutations indicate that allosterically

77   driven rearrangements of the stalk helices—and the critical L1N^S loop—support curling

78   from linear strings into constricted rings following receptor dissociation.

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80   **Results and Discussion**

81   To date, many structural studies of dynamin-family proteins have relied upon

82   mutated or truncated constructs to facilitate crystallization. We purified wild-type, full-

83   length human DRP1 including the N-terminal GTPase domain (G-domain), Bundle

84   Signaling Element (BSE), and four-helix bundle known as the stalk (Fig. 1a). This

85   construct also contained the lipid binding ~100 amino acid region referred to as the

86   variable domain (VD) that sits between the third and fourth alpha helices of the stalk,

87   analogous to the Pleckstrin Homology (PH) domain found in endocytic dynamin proteins.

88   A crystal structure of a nucleotide-free and truncated DRP1 mutant revealed the

89   organization of these domains and an overall similarity with the structure of nucleotide-

90   free endocytic dynamin[42,49,50]. We also purified soluble truncations of MID49 and MID51

91   engineered to lack their N-terminal transmembrane anchors but include the cytoplasmic

92   nucleotidyltransferase-like domain and the "Dynamin Recruitment Region" (DRR)

93   required for DRP1 binding (Fig 1a)[38,51-54].

94   Incubating equimolar ratios of DRP1 with soluble $MID49_{126-454}$, $MID51_{132-463}$, or

95   both proteins together, in the presence of GTP resulted in cofilament assembly (Extended

96   Data Figs. 1-2). We focused on the filaments formed with $MID49_{126-454}$ in the presence of

97   the non-hydrolyzable analog GMPPCP and determined their structure from cryoEM

98   images (Extended Data Figs. 3-9 and Table 1). 3D reconstruction resolved elongated

99   DRP1 dimers bound stoichiometrically to $MID49_{126-454}$, without assignable density for the

100   variable domain (Fig. 1b, Extended Data Fig. 4). Surprisingly, each chain of DRP1 bound

101   MID49 at four different sites, and each MID49 in turn bound four DRP1 molecules to yield

102   a vast and highly avid interaction network (Figs. 1b-c, Extended Data Figs. 4 and 8).

103   MID49 binding to four separate DRP1 molecules stabilized a linear arrangement of inter-

104   DRP1 interfaces reminiscent of those observed for other dynamin-family proteins by X-

105   ray crystallography (Fig. 1b, Extended Data Figs. 4 and 8)[42,49,50,55-57].

106   MID49's DRR motif occupied the space between two neighboring G-domains and

107   contacted both via MID-interaction interfaces 1 and 2 (buried surface areas of ~530$Å^2$

108   and ~200 $Å^2$, respectively, see Fig. 1b-d). The precise spacing required for this bivalent

109   G-domain interaction explains why previous mutagenesis efforts suggested that the size

110   and topology of the β4–α4 loop, rather than its exact sequence (which differs between

111   MID49 and MID51) are critical determinants of binding. Single point mutations within this

112   loop for MID49 and MID51 did not disrupt binding, while mutations that altered the length,

113   topology or positioning of the loop did[51,52,54]. We found that mutating conserved DRP1

114   residues involved in interface 1—the energetically most significant interface—did prevent

115   coassembly. Specifically, the D190A mutation should neutralize an interface 1 salt bridge

116   and the D221A mutation should alter the conformation of an interface 1 loop (Fig. 1d).

117   We observed that both mutants prevented assembly with MID49 and altered DRP1's self-

118   assembly properties (Extended Data Figs. 10-12).

119   Unexpectedly, MID49 also made contact with the stalk loops of a third and fourth

120   DRP1 molecule through MID-interaction interfaces 3 and 4 (buried surface areas of

121   ~450Å^2 and ~230Å^2, respectively, Fig. 1c, e, and f). The loops involved in both interaction

122   interfaces 3 and 4 determine the assembly properties of higher-order dynamin-family

123   oligomers [42,49,50,55-57]. MID-interaction surface 3, in particular, harbors the conserved loop

124   L1N^S and is the site of multiple disease alleles that lead to elongated mitochondrial

125   morphology, including G362D and G363D (Fig. 1e, Extended Data Figs. 10-11 and [58-60]).

126   Prior work has also established that this loop comprises part of the intra-molecular PH

127   domain binding site for the soluble state of endocytic dynamin tetramers (Extended Data

128   Fig. 13)[57], and is a determinant of conformational heterogeneity for these and other

129   dynamin-family proteins[55,57,61]. The presence of disease alleles near this interface

130 suggests that these mutations may compromise receptor interactions and that defects in

131 the recruitment of DRP1 to mitochondria may contribute to pathogenesis. As discussed

132 below, we found that the G362D mutant (Fig. 1e) failed to coassemble with MID49 and

133 displayed altered assembly and conformational properties (Extended Data Figs. 12-15).

134 Understanding the allosteric coupling between nucleotide binding, hydrolysis or

135 exchange and the conformational repertoire of dynamin-family GTPases remains an

136 unmet challenge[62,63]. We observed that the GMPPCP-bound G-domains and the BSE of

137 DRP1 adopt strikingly different conformations in the cryoEM density compared to the

138 nucleotide-free crystal structure[42]. In addition to other nucleotide-induced conformational

139 changes within the G-domain, the most salient are the closing of the G2/switch-1 loop to

140 form a closed "lid" over the nucleotide (Figs. 2a-c). The closure of the switch-1 lid

141 propagates through the adjacent beta sheet to push the α-helices of the BSE into an

142 orthogonal position (Extended Data Movie 1). When evaluated in the context of a stalk

143 interface-2 DRP1 dimer, this conformational change is an impressive 90° rotation of the

144 G-domain and a 40Å translation toward the stalk (Fig. 2d, Extended Data Movies 2-3).

145 Two of the three DRP1 surfaces that engage the DRR of MID49 (MID receptor interaction

146 interfaces 1 and 2, Figs. 1-2) are inaccessible in the nucleotide-free state but become

147 available for binding upon nucleotide-driven elongation (Fig. 2d, Extended Data Movies

148 2-3).

149   Since DRP1's ability to polymerize into linear filaments with MID49 or MID51

150   depended on non-hydrolyzable GTP analogs, we next evaluated the dynamics of these

151   filaments in the presence of hydrolyzable GTP. Following copolymerization in the

152   presence of the non-hydrolyzable analog, we exchanged GMPPCP for GTP through

153   dialysis and followed the reaction using negative stain transmission electron microscopy

154   at sequential time points until the GTP was exhausted. We observed that the linear DRP1-

155   MID49 cofilaments disassembled into small oligomers upon complete hydrolysis to GDP

156   and exhibited a fascinating dynamic instability at intermediate time points (Fig. 3).

157   Specifically, the long and linear filaments seen at early time points disassembled into

158   shorter, curling oligomers that—upon reaching a reproducibly narrow range of lengths—

159   spontaneously closed into constricted rings that were remarkably uniform in diameter

160   (Fig. 3).

161   In a separate but related experiment, we evaluated the assembly properties of the

162   DRP1 mutant G362D with and without MID49$_{126-454}$. As described above, this disease-

163   associated residue sits at the base of the L1N^S loop that forms part of the third interface

164   with MID49 (Figs. 1c, 1e, Extended Data Fig. 13). We found that DRP1$_{G362D}$ purified as a

165   nearly monodisperse and stable dimer, rather than a mix of tetramers and higher order

166   species observed for the wild-type, full-length protein (Extended Data Fig. 12). In addition,

167   DRP1$_{G362D}$ exclusively formed rings, not filaments, with or without MID49$_{126-454}$ and in the

168   presence of GMPPCP or GTP (Fig. 4a, Extended Data Figs. 14-15). These rings

169   resembled those observed with wild-type DRP1 in all respects except that the wild-type

170   protein only formed closed rings from the pre-formed linear MID49 copolymers through

171   the path-dependent reaction described above (Fig. 3 versus Fig. 4). Using the non-

172   hydrolyzable GTP analog GMPPCP with the DRP1$_{G362D}$ rings also improved structural

173   homogeneity, presumably because the wild-type rings remain dynamic and eventually

174   disassembled in the presence of GTP upon complete hydrolysis to GDP (Fig. 3).

175   We imaged the DRP1$_{G362D}$ rings using cryoEM and used 2D class averages of the

176   predominant 12-dimer closed ring to model the architectural differences between the

177   linear filaments and the closed rings (Fig. 4, Extended Data Fig. 14-15). To account for

178   the projected ring density, the G-domain and the BSE of DRP1 must move even further

179   down toward the stalks. Moreover, while stalk interface-2 remains constant—as revealed

180   by the X-shaped projected dimer (Fig. 4c)—the curvature of the ring dictates that stalk

181   interfaces 1 and 3 must be extensively remodeled to allow a ~30 degree bending per

182   dimer in comparison with the linear DRP1-MID49 copolymer (Fig. 4d, Extended Data Fig.

183   15, Movie 4). We did not observe any density for MID49 in the wild-type rings that form

184   by curling of the MID49-DRP1 cofilament in the presence of GTP, nor in our higher-

185   resolution analysis of the DRP1$_{G362D}$ rings that form with or without MID49 present (Fig.

186   4b-c, Extended Data Fig. 14-15).

187 We note that even the most constricted form of the closed ring is insufficient to

188 drive complete mitochondrial fission because the inner diameter is only ~16nm. This

189 length suggests that a constricted membrane tubule would be stable and that the

190 structures we have observed in vitro could correspond with the highly-constricted but pre-

191 fission state observed in living cells when another dynamin-family protein, the primarily

192 endocytic dynamin-2, is depleted[47]. Thus, initial constriction by DRP1 may stabilize the

193 high degree of membrane curvature that is suitable, perhaps even tuned, for the

194 recruitment and final fission event catalyzed by additional dynamin-family enzymes.

195 Together, these findings establish four conceptual advances. First, our cryoEM

196 structure revealed how receptor proteins like MID49/MID51 recruit and stabilize a specific

197 nucleotide-bound conformation of DRP1 and nucleate polymerization of a cofilament. We

198 speculate that the nearly linear properties of this polymer have adapted to encircle low-

199 curvature mitochondrial tubules. The selective stabilization of this open, elongated

200 conformation of DRP1 within the MID receptor cofilament explains why overexpression

201 of the MID receptors inhibits mitochondrial fission[39]. Second, analysis of the MID49-DRP1

202 copolymer exposed how nucleotide binding induces an impressive conformational

203 rearrangement to expose a network of multi-valent receptor binding sites. We now

204 understand these nucleotide-driven allosteric transformations in detail and in the context

205 of full-length and oligomeric DRP1. Third, a path-dependent constriction reaction revealed

206   intrinsic GTP-dependent DRP1 properties that are reminiscent of microtubule dynamic

207   instability[64]. In this reaction, nucleotide exchange and hydrolysis led to MID49/51 receptor

208   dissociation, disassembly from the ends of the linear filament, and concomitant curling of

209   the shortened filaments into closed rings. Fourth and finally, analysis of a disease mutant

210   in the L1N^S loop, DRP1$_{G362D}$, exposed this loop as a vital determinant of mitochondrial

211   receptor binding and the dynamic inter-stalk interactions that govern oligomer architecture

212   and the ability of dynamin proteins to perform mechanical work.

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214   **Acknowledgments**

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227 **Author Contributions**

228 R.K., J.S. and A.F. conceived of the study. R.K., R.W., A.Y. and P.T. performed all

229 experiments. R.K., R.W. and A.F. performed the computational analyses. All authors

230 evaluated the results and edited the manuscript. R.K. and A.F. wrote the manuscript with
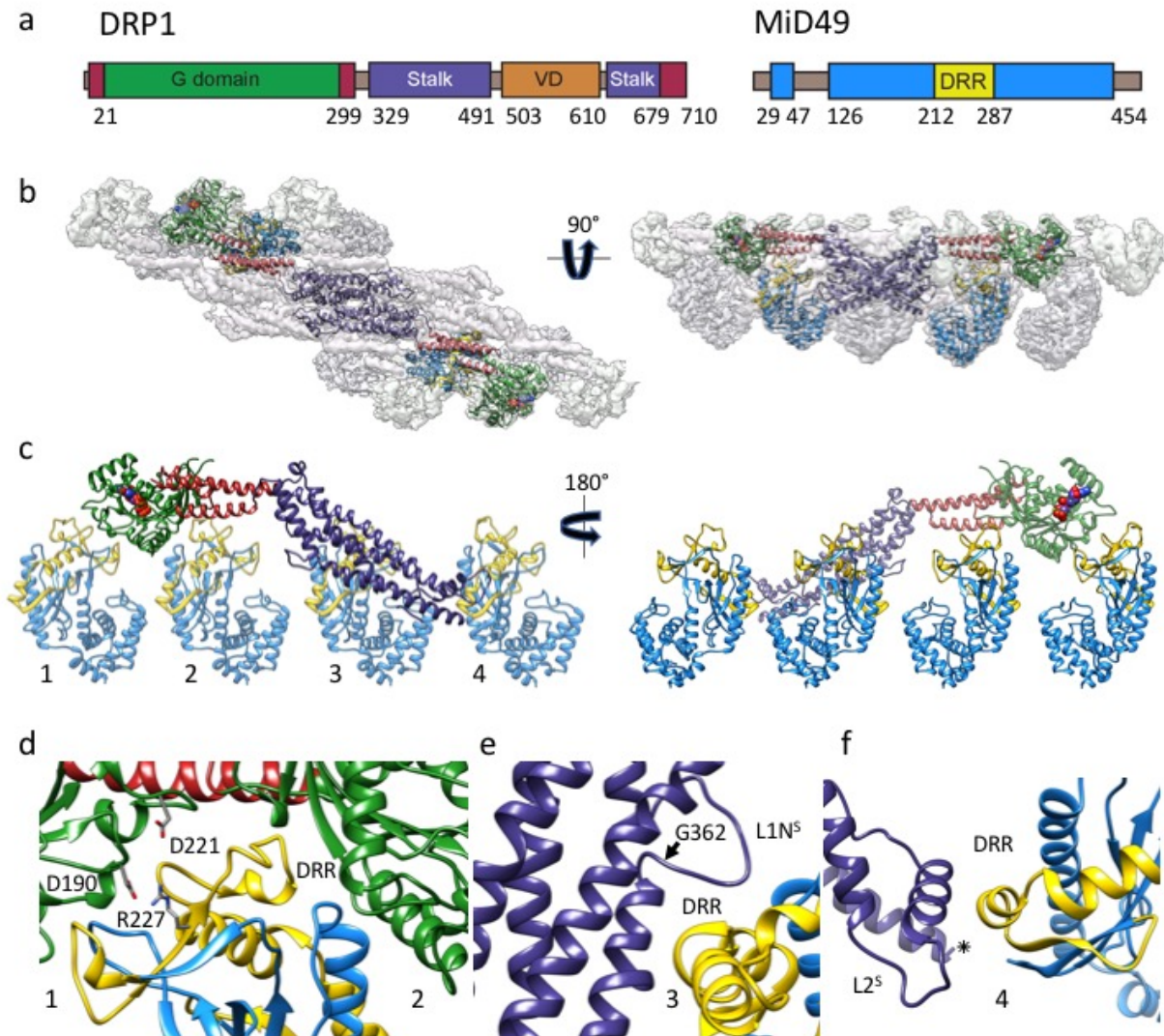
231 input from all of the authors.

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233 **Data Accessibility:**

234 All of the cryoEM density maps associated with this study have be deposited in the EMDB

235 with accession numbers EMD-8874(PROC). The atomic coordinates have been

236 deposited in the PDB as 5WP9(PROC).

237   **Figures**

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Figure 1

239 **Figure 1: Architecture of the DRP1-MID49 linear filament:** (**a**) DRP1 and MID49

240 domain arrangements. (**b**) Density map and atomic models for DRP1 and MID49$_{126-454}$.

241 Green: G-domain, Red: Bundle Signaling Element (BSE), Purple: Stalk, Blue: MID49,

242 Yellow: Dynamin Recruitment Region (DRR) of MID49. (**c**) Each DRP1 chain contacts

243 four different MID49 molecules, as numbered. (**d**) MID-interaction surfaces 1 and 2.

244 Green ribbons on either side of the DRR come from two separate G-domains, and the

245 residues involved in a key salt bridge for MID-interaction surface 1 are shown as sticks.

246 (**e**) Rotated view of MID-interaction surface 3. Disease-associated DRP1 residue G362

247 (arrow) supports the conformation of the L1N^S loop essential for linear copolymerization

248 with MID receptors. (**f**) Rotated view of MID-interaction surface 4 and the L2^S loop.

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Figure 2

a — Nucleotide-free

b — GMPPCP

c — Overlay

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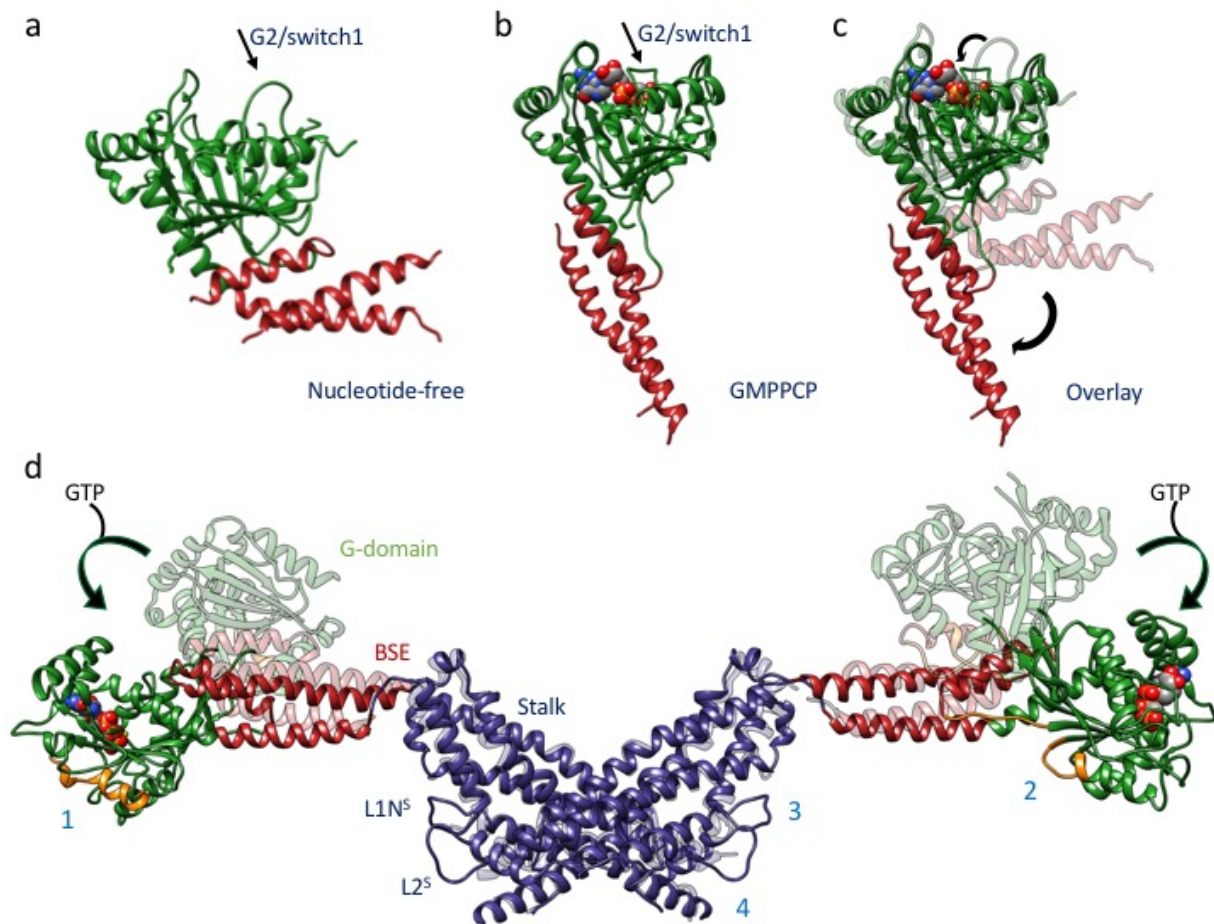
259   **Figure 2: Nucleotide-driven allosteric elongation of DRP1 exposes MID binding**

260   **sites**: **(a)** nucleotide-free state of DRP1 G-domain and BSE as seen in a crystal structure

261   (PDB ID:4BEJ). Arrow points to the G2/switch1 loop. **(b)** GMPPCP-bound G-domain-BSE

262   conformation determined by cryoEM. **(c)** Overlay of A and B. Curved arrows highlight the

263   closing of G2/switch 1 "lid" and the opening of the BSE "wrist". **(d)** Global conformational

264   change induced by nucleotide binding. Rotation and translation of the G-domain and BSE

265   elongates the dimer and exposes MID-interaction surfaces 1 and 2 (annotated on

266   separate monomers for clarity). The surfaces of the G-domains that engage MID

267   receptors are rendered orange in the nucleotide-bound and elongated conformation.

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Figure 3
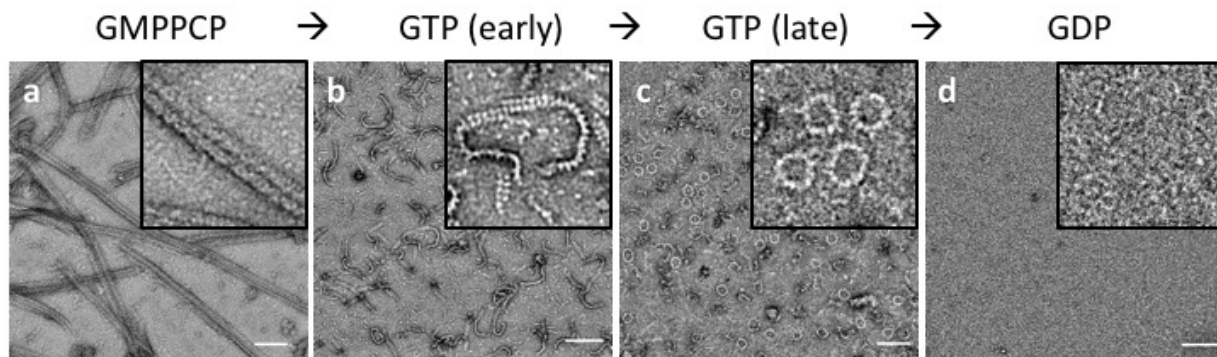
279 **Figure 3: Dynamic instability of the DRP1-MID49 linear assembly and curling into**

280 **closed DRP1 rings**: **(a)** DRP1-MID49$_{126-454}$ linear filaments copolymerized with

281 GMPPCP. **(b-c)** Subsequent exchange into GTP leads to partial disassembly and curling

282 into closed rings. **(d)** GTP exhaustion leads to complete oligomer disassembly.

283 Bars=100nm.

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Figure 4
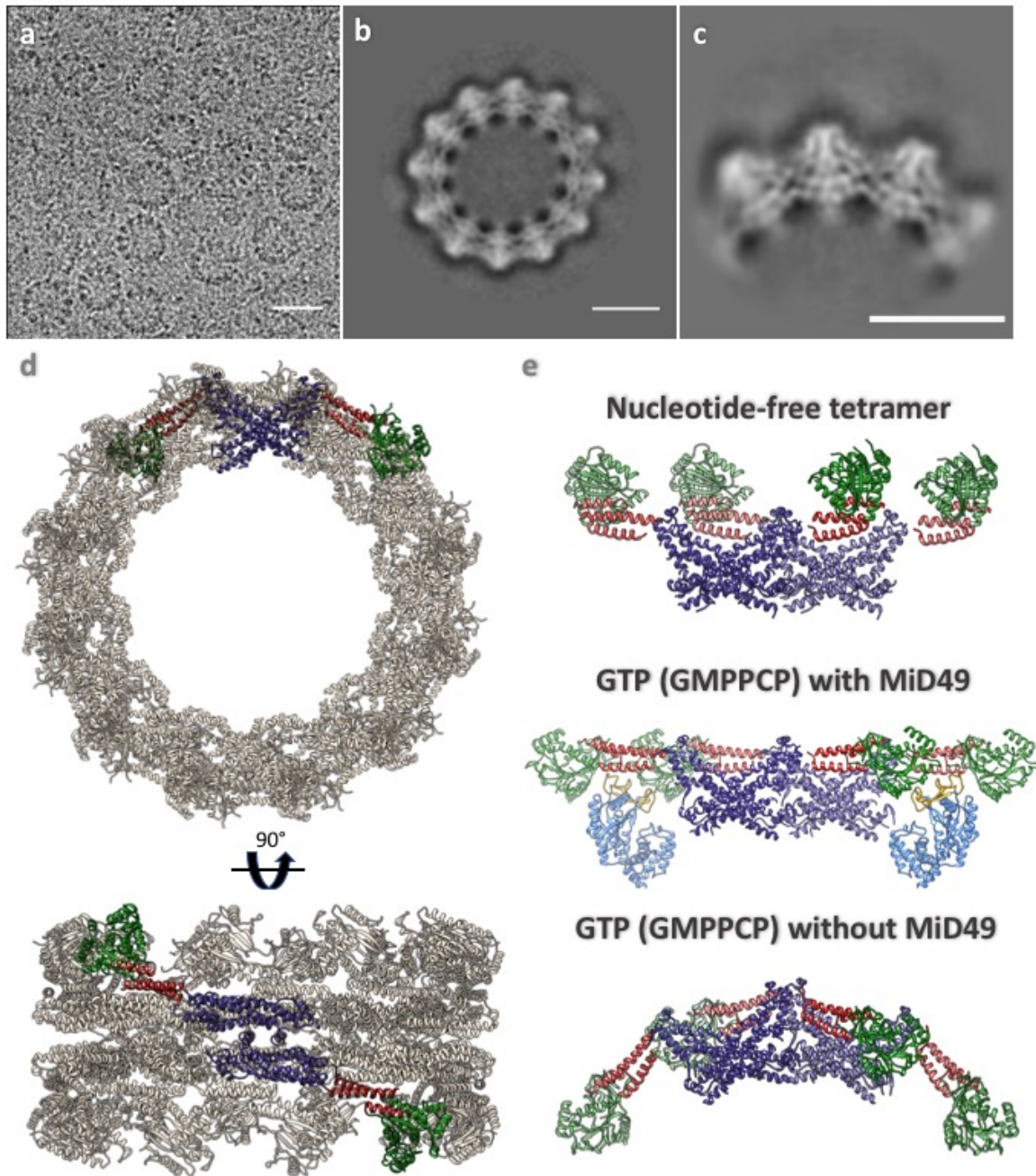
299 **Figure 4: DRP1$_{G362D}$ cannot bind MID49 and forms rings exclusively with GMPPCP**

300 **or GTP. (a)** CryoEM micrograph of DRP1$_{G362D}$ rings. **(b)** 2D class average of the

301 predominant closed ring that comprises 12 DRP1 dimers. **(c)** 2D class average of a

302 quarter of the ring revealing the secondary structure elements of the "X"-shaped DRP1

303 dimer. **(d)** 3D model of the closed ring. **(e)** Comparison between DRP1 tetramers

304 observed in the nucleotide-free state (top, PDB ID:4BEJ), the GMPPCP and MID49$_{126-}$

305 $_{454}$-bound linear state (middle), and the bent conformation modeled for the rings. Bar for

306 **(a)** = 100nm, for **(b, c)** =100Å.

307   **Extended Data**

308   Methods

309   Figures 1 to 15

310   Movies 1 to 4

311   Table 1

312   Extended Data References

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477

Extended Data

Methods

Construct design

Wild type (WT) DRP1 isoform 2 sequence was purchased from DNASU (sequence ID HsCD00043627, UNIPROT identifier: O00429-3 also known as DLP1a) and was cloned into pET16b plasmid (Novagen) between the Nde1 and BamH1 sites. The vector was kindly provided by the laboratory of Wesley I. Sundquist with a 10X-His tag followed by a PreScission protease site (Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro). Wild-type MID49$_{126-454}$ sequence was PCR amplified and cloned into pGEX6p1 vector having an N-terminal GST tag followed by a PreScission protease site. Site directed mutagenesis was performed on pET16b-DRP1 using the Gibson cloning method to introduce mutations[1]. All constructs were verified using Sanger sequencing.

Protein purification

Protein purification was performed as described[2]. Briefly, plasmids containing the WT DRP1 or MID49$_{126-454}$ sequence were transformed in the BL21-DE3 (RIPL) strain of *E. coli*. The colonies were inoculated in LB culture medium and grown overnight. Secondary inoculations were done the next morning in ZY medium for auto-induction[3,4]. The cultures were grown to an OD$_{600}$ of 0.8 at 37°C in baffled flasks and were shifted to 19°C to grow for another 12 hours. The cultures were spun down and the bacterial pellets were used for protein purification immediately or stored at -80°C.

Full length DRP1 WT and mutant variants were purified as described previously for DRP1 WT with modifications[5]. Briefly, the bacterial pellets were resuspended in buffer A (50 mM

HEPES/NaOH (pH 7.5), 400 mM NaCl, 5 mM MgCl$_2$, 40 mM imidazole, 1 mM DTT, 0.5 mg

DNase (Roche) and protease inhibitors (10 mM pepstatin, 50 mM PMSF, 0.5 mM aprotinin and

2 mM leupeptin), followed by cell disruption with a probe sonicator. Lysates were cleared by

centrifugation at 40,000xg in Beckman JA 25.5 rotors for 60 min at 4°C. The supernatant was

filtered using a 0.45 μm filter and applied to Ni-NTA Agarose beads pre- equilibrated with buffer

B (50 mM HEPES/NaOH (pH 7.5), 400 mM NaCl, 5 mM MgCl$_2$, 40 mM imidazole, 1 mM DTT).

The beads were washed with 20 column volumes each of buffer B and buffer C (50 mM

HEPES/NaOH (pH 7.5), 800 mM NaCl, 5 mM MgCl$_2$, 40 mM imidazole, 1 mM DTT, 1 mM ATP,

10 mM KCl) followed by buffer D (50 mM HEPES/NaOH (pH 7.5), 400 mM NaCl, 5 mM MgCl$_2$,

80 mM imidazole, 1 mM DTT, 0.5% (w/v) CHAPS). A final pre-elution wash was done with 20

column volumes of buffer B. Bound DRP1 was eluted with buffer E (50 mM HEPES/NaOH (pH

7.5), 400 mM NaCl, 5 mM MgCl$_2$, 300 mM imidazole, 1 mM DTT) and dialyzed overnight at 4°C

against buffer B without imidazole in the presence of PreScission protease to cleave the N-

terminal 10X-His tag. The protein was re-applied to a Ni-NTA column pre-equilibrated with

dialysis buffer and was observed to bind the column without the 10X-His tag as well.

Subsequently, the protein was eluted with buffer B containing 80 mM imidazole.

Pure protein was concentrated with a 30kDa molecular weight cutoff (MWCO) centrifugal

concentration device (Millipore). In a final step, DRP1 was purified by size-exclusion

chromatography (SEC) on a Superdex-200 column (GE) in buffer F containing 20 mM HEPES/

NaOH (pH 7.5), 300 mM NaCl, 2.5 mM MgCl$_2$ and 1 mM DTT. Fractions containing DRP1 were

pooled, concentrated, flash frozen as single use aliquots in liquid nitrogen and stored at -80°C.

Exact masses for purified DRP1 proteins were validated by MALDI-TOF mass spectrometry.

MID49$_{126-454}$ was purified as described with modifications[6]. pGEX6p1-MID49$_{126-454}$ plasmid DNA (human, UNIPROT identifier: Isoform 1 Q96C03-1, also known as MIEF2) was transformed in BL21 (DE3) RIPL cells. The colonies were grown overnight in LB medium and secondary cultures were grown in ZY medium. Cells were grown to an OD$_{600}$ of 0.8-1, collected by centrifugation and processed immediately or stored at -80°C as described above. 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 and 0.1% (v/v) Triton X-100). The lysates were pre-cleared at 40,000xg and filtered using a 0.45 μm filter before applying to 3 ml glutathione sepharose beads (GE). After overnight binding to beads, the unbound protein was removed and the beads were washed using 20 column volumes each of MID-buffer A and MID-buffer B (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 20 mM reduced glutathione). The eluate was cleaved overnight with PreScission protease while dialyzing against MID-buffer D (20 mM Tris pH 8.0, 100 mM NaCl, 5% glycerol, 1 mM DTT). Cleaved protein was further purified using ion-exchange chromatography using a Q sepharose (GE) column. 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 an SEC 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, EM sample preparation, data acquisition and processing

To assemble DRP1-MID49$_{126-454}$ filaments, the proteins were mixed to a final concentration of 2 μM each and kept for an hour at room temperature. The mixture was dialyzed against assembly

buffer (20 mM HEPES pH 7.5, 50 mM KCl, 3 mM $MgCl_2$, 1 mM DTT, 200 μM GMPPCP and the detergent octyl-glucopyranoside (Anatrace) at 0.2% final concentration). The filaments were observed by screening in negative stain or vitrified for cryo-EM. For vitrification, the sample was applied to Quantifoil holey carbon grids (R2/2) using a Vitrobot Mark III with 3.5 μl sample, 5 seconds blotting time and a 0 mm offset at 19°C and 100% humidity. Images were collected on an FEI T30 Polara operating at 300kV at a magnification of 31000X. Images were recorded on a Gatan K2 summit camera in super resolution mode that had a binned pixel size of 1.22 Å/pixel. The images were dose fractionated, containing 30-40 frames with a total exposure time of 6-8 seconds, 0.2 seconds per frame and a per frame dose of 1.42 electrons/$Å^2$. SerialEM was used to automate data collection[7]. The defocus range was 0.8-3 μm under focus. The data was motion corrected and dose-weighted using UCSF Motioncorr2[8]. CTF parameter estimation on the non-dose-weighted but motion-corrected stacks was done using CTFFIND4 and GCTF[9,10].

Filaments were boxed using the program e2helixboxer.py from the EMAN2 suite[11]. Particle coordinates were used to extract discrete particles using RELION 1.3-1.4[12] and all further processing was done within the RELION suite. Multiple rounds of 2D classification identified the most well-ordered segments, and 3D autorefine was run using a Relion1.2 version with the IHRSR algorithm implemented[13,14]. The consensus helical structure was used to classify the particles without refining helical symmetry (using RELION 1.4), resulting in 2 major classes that differed slightly in rise and twist (Extended data figure 4). Particles from each class were selected and independently refined again with helical RELION 1.2 and IHRSR. Analysis of these reconstructions revealed that each structure was comprised of three linear filaments that bundle together to form a structure that resembled a triangle in cross-section (Extended data figures 3-4). The vertices of the triangle are formed through asymmetric interactions between the G-domains in adjacent filaments. The significance of these asymmetric G domain interactions has

not been evaluated. The triangular arrangement of the bundled helices is unlikely to correspond to a biologically meaningful architecture, and this structure cannot form if the MID49 receptor is embedded in the outer mitochondrial membrane.

To further improve signal-to-noise, each of the three filaments in each independent half-map was segmented, extracted, resampled on a common grid and summed using UCSF Chimera[15-19]. The respective symmetrized but unfiltered half maps from each class were again aligned to a common grid, summed, averaged along 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 figure 4). These half maps and the final summed map, with differential B-factor sharpening per region (Extended data figures 4-9), were used for atomic modeling using Rosetta as described below.

For the projection structure of the DRP1$_{G362D}$ rings, 2 μM protein was mixed 1:1 molar ratio with MID49$_{126-454}$ and was allowed to sit at room temperature for an hour. The mixture was dialyzed against the assembly buffer (without detergent) overnight. The sample was collected after 12 hours and vitrified using ultra-thin 3 nm carbon support films (Ted Pella). For vitrification, a Mark III vitrobot was used with 3.5 μl sample, 0 mm offset, 100% humidity and 3.5 seconds 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 using a binned pixel size of 1.234 Å/pixel. 40 frames were collected per stack (0.2 seconds per frame and 1.42 electrons/Å^2). The movie stacks were motion corrected and the parameters of the transfer function were estimated as described above. Approximately 2000 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 averaging of the entire rings versus quarter segments of the rings were computed using Relion1.4.

Model building

The general procedure for atomic model interpretation and validation using Rosetta were performed as described[20]. To obtain an initial model for DRP1, the crystal structure of nucleotide-free DRP1 (PDB ID: 4BEJ)[5] was used for the stalk region and DRP1 G domain-BSE structures bound to GMPPCP (PDB ID: 3W6O) were used for the G-domain and BSE regions. Density-guided model completion for DRP1 was carried out with RosettaCM[21] 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 503-612 were found to be extremely flexible without cryoEM density constraints and therefore were omitted for further coordinate refinement. For MID49, the highly homologous mouse MID49 crystal structure (81.3% identity, PDB ID: 4WOY, Extended Data Fig. 7)[6] was used to generate a homology model using RosettaCM and used as the starting model.

To enable fragment-based, density-guided model refinement with missing residues (503-612, DRP1), Rosetta iterative local rebuilding tool was customized to disallow backbone rebuilding at breaks within a single chain. Multiple rounds of refinement were done for each component against one half map (training map), and the other half map (validation map) was used to monitor overfitting according to the detailed procedure described in Wang et al.[20].

With the refined model of DRP1 and MID49, we further refined the model in the context of a full assembly that included 8 identical copies of each protein, Mg^{2+} and nucleotide which included all possible inter-domain molecular interactions in the filament (Extended data figure 8).

Pseudo-symmetry was used[22] to enable and facilitate the energy evaluations of all neighboring interactions around the asymmetric unit (Green model, shown in Extended data figure 8) 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[23]. Finally, quantification of buried surface area and the number and nature of the bonds involved for each DRP1-MID49 interaction interface modeled by Rosetta were performed with the PISA server (http://www.ebi.ac.uk/pdbe/pisa/).

To design a molecular model for the closed 12-dimer DRP1 rings, we used the diameter, thickness, and angles revealed by the 2D cryoEM 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 4b-c) and the side view (Extended data Figure 15) were used as constraints. The complete atomic model of ring was finally refined in Phenix[24] to minimize clashes.

Extended data Table 1

Data Collection	Filaments	Ring
Particles	412684	61574
Pixel Size (Å)	1.22	1.234
Defocus Range (microns)	0.3-4.0	0.3-5.0
Defocus Mean (microns)	1.3	2.1
Defocus Median (microns)	1.3	2.08
Voltage (kV)	300	200
Per frame electron dose ($e^/Å^2$)	1.42	1.42

Refinement		
Resolution	4.2 Å ($FSC_{average}$)	6.1 Å (FRC)
Map sharpening B-factor	-225 (average)	
DRP1 stalk only	-300 to -400	
MID49 only	-150 to -200	
G-domain & BSE	-200	

Validation		
Molprobity score	1.41	1.84
Clashscore, all atoms	2.13	4.94
Favored rotamers (%)	99.25	99.44
RMS (bonds)	0.0107	0.011
RMS (angles)	0.97	1.62
Ramachandran Statistics		
Favored (%)	93.6	88.9
Outliers (%)	1.32	0.37

Extended data figures

Kalia, et al.
Extended Data Fig. 1
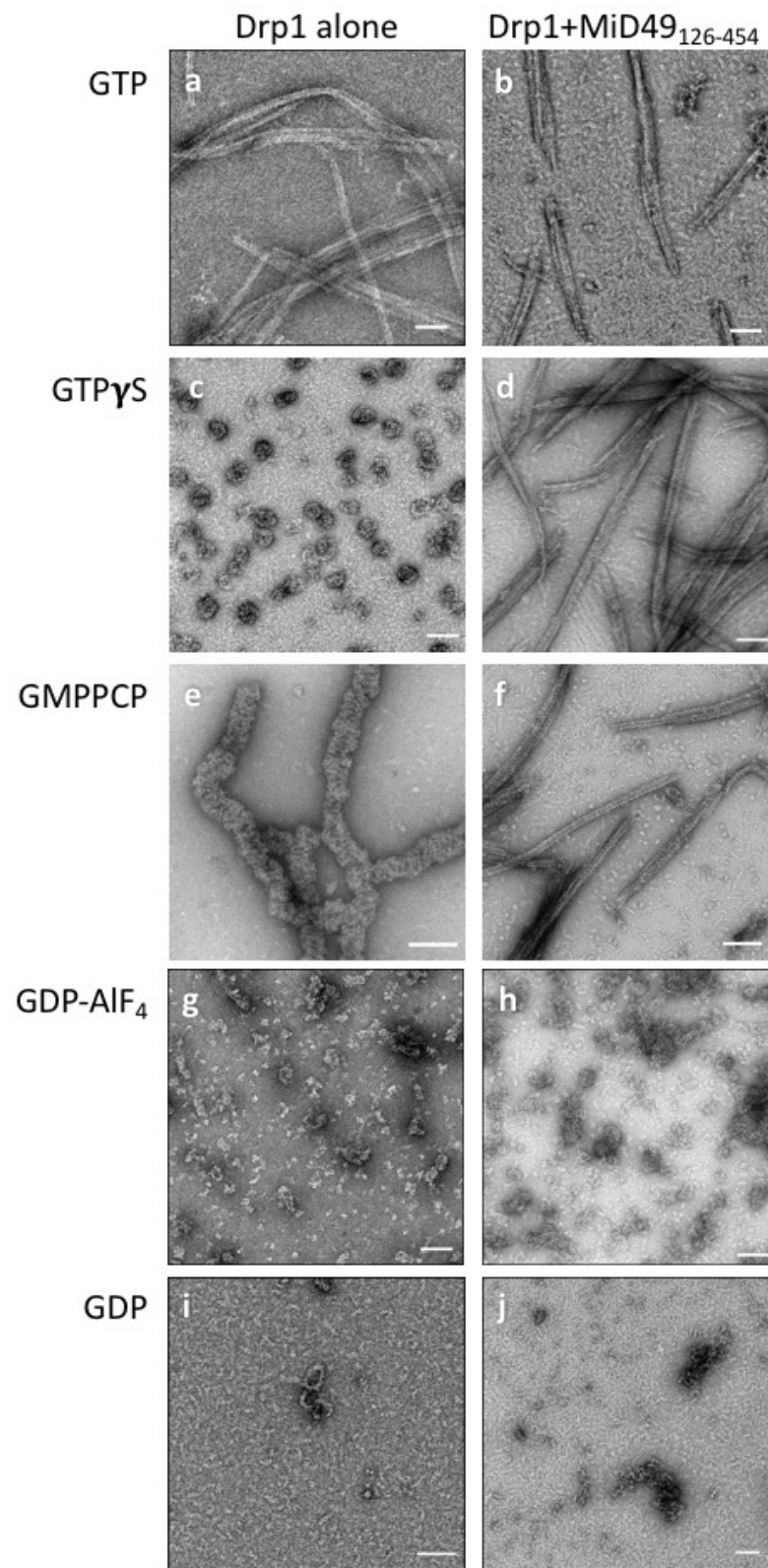
Extended data figure 1: DRP1 assembly states visualized with negative stain electron microscopy in the presence of different guanine nucleotides and stoichiometric MID49$_{126-454}$ ([2μM] for both proteins). Bars = 100nm.

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Extended Data Fig. 2

Drp1+MiD49$_{126-454}$ Drp1+MiD51$_{132-463}$ Drp1+MiD49$_{126-454}$ +MiD51$_{132-463}$
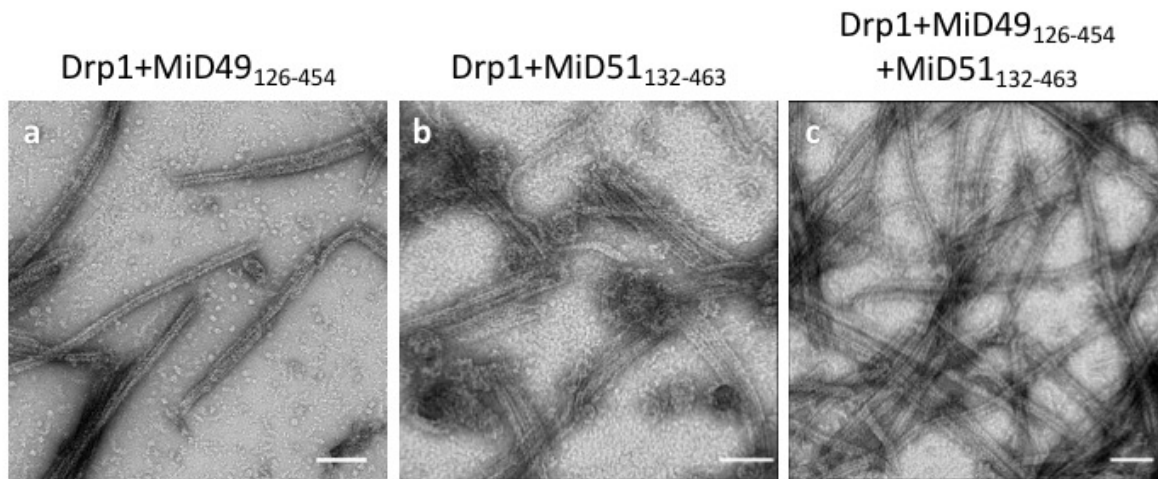
Extended data figure 2: MID49$_{126\text{-}454}$ and MID51$_{132\text{-}463}$ form indistinguishable assemblies with

DRP1 by negative stain electron microscopy. **(a)** DRP1 plus MID49$_{126\text{-}454}$ and GMPPCP; **(b)**

DRP1 plus MID51$_{132\text{-}463}$ and GMPPCP, **(c)** DRP1 plus both MID49 and MID51. Bars = 100nm.

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Extended Data Fig. 3
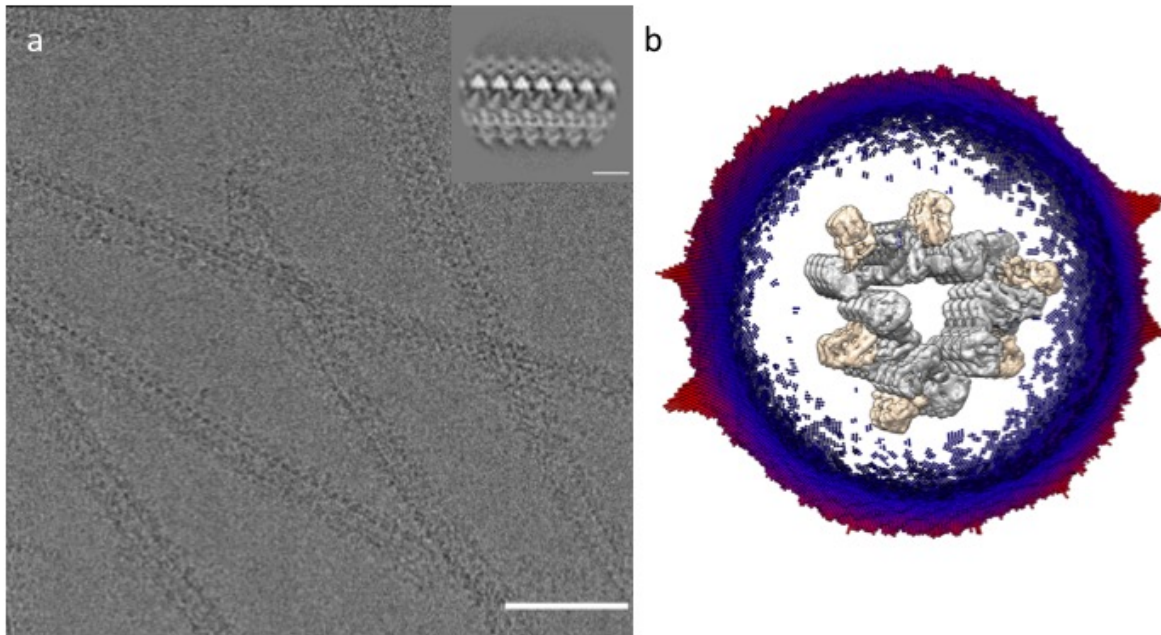
Extended data figure 3: CryoEM imaging and reconstruction. **(a)** An electron cryo-micrograph of DRP1-MID49$_{126-454}$ filaments formed with GMPPCP. Inset shows a representative 2D class average. Bar =100nm, Inset bar =10nm. **(b)** Oblique cross-section of the 3D reconstruction and the distribution of views determined during helical reconstruction. DRP1 density is rendered in grey, MID49 in golden yellow.

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Extended Data Fig. 4

412684 particles consensus
Colors: DRP1, MiD49

2 Major classes:

Class A: 22% images
Rise= 54.9 Å, Turn= -0.6°

Class B: 77% images
Rise= 54.8 Å, Turn= -1.0°

Segment 3 faces x 2 3D classes for each independent half map

Impose C2 symmetry for each independent half map

Sum independent half maps
Average resolution ~ 4.2 Å

Turn y 90°
Turn x 90°
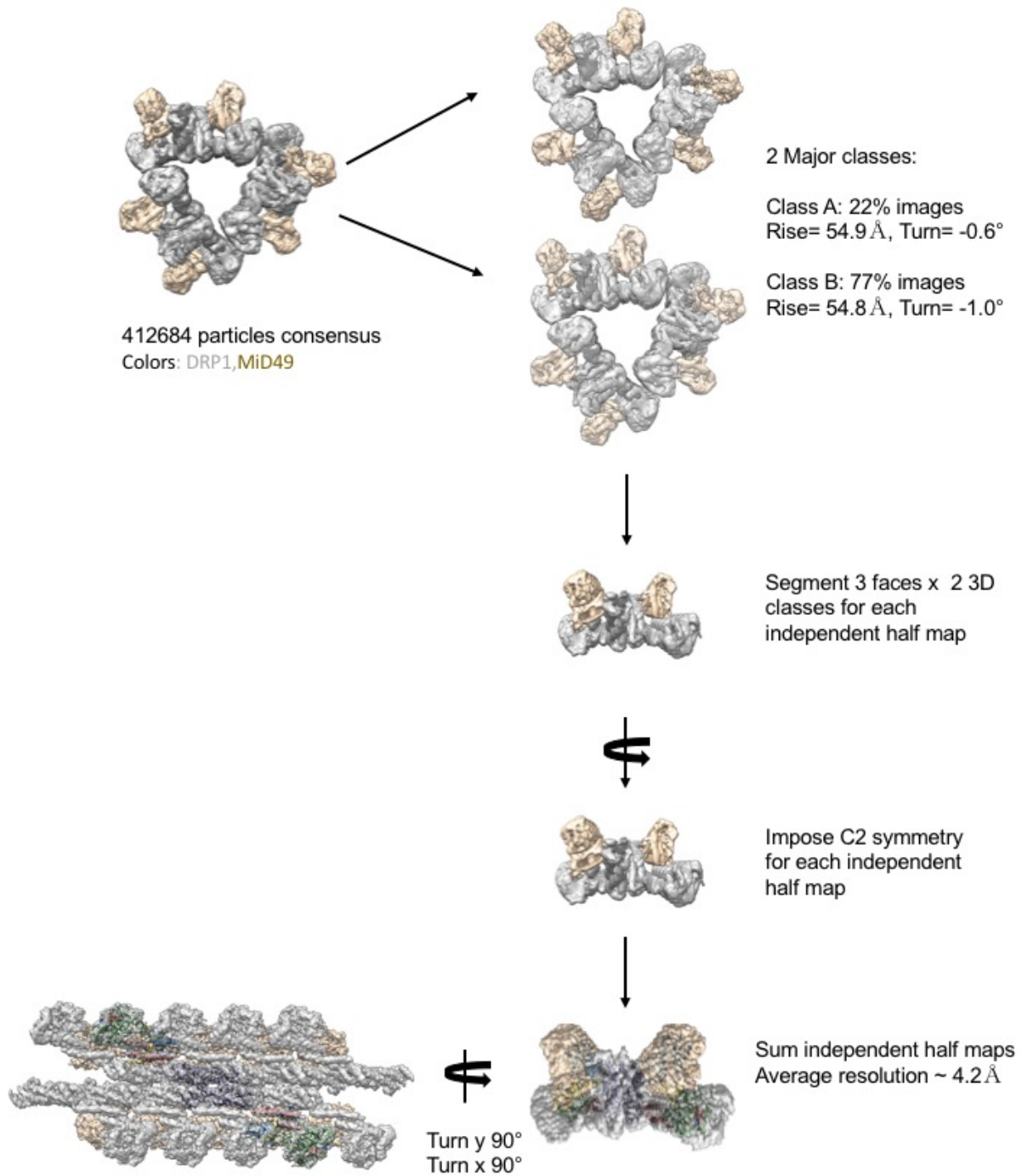
Extended data figure 4: Raw particle numbers and workflow for the reconstruction protocol

and imposition of symmetries. DRP1 density is rendered in grey, MID49 in golden yellow.

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Extended Data Fig. 5

a

b

DRP1,MiD49
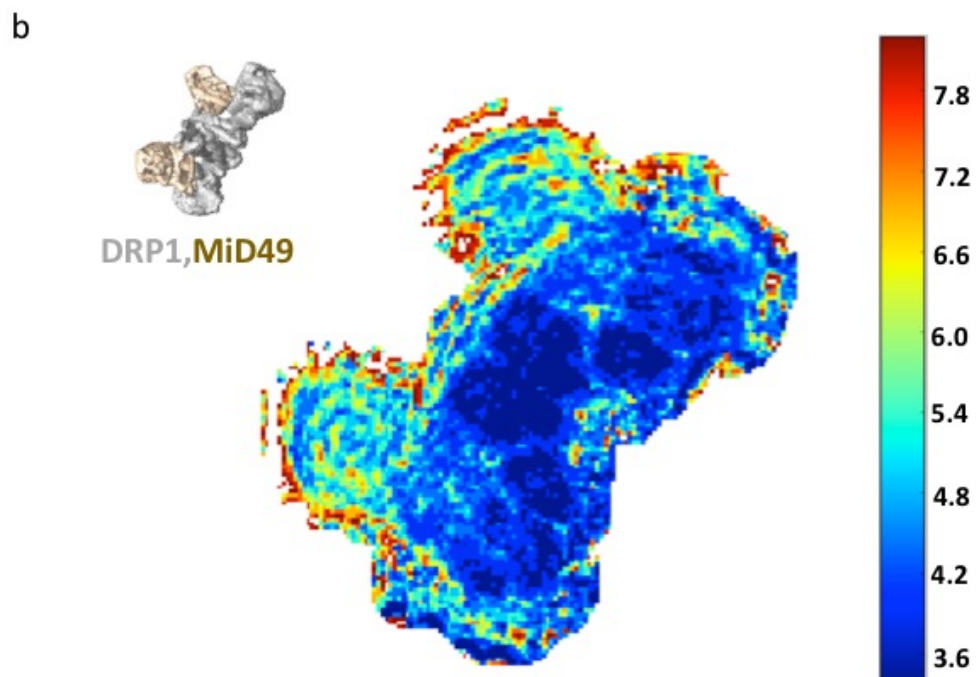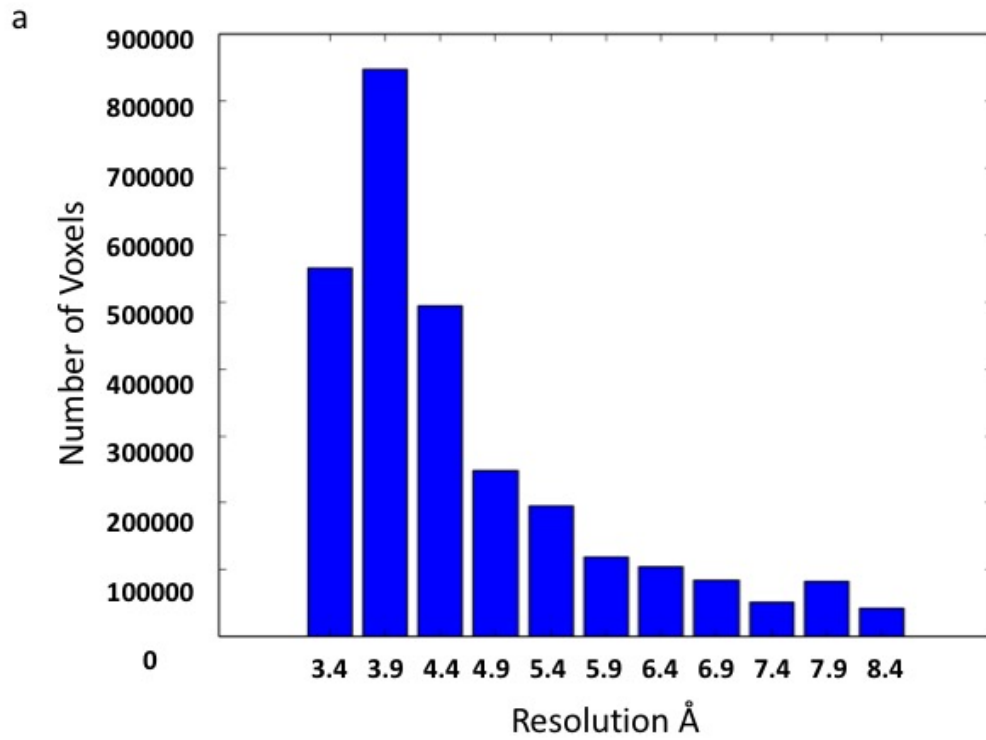
Extended data figure 5: Local resolution estimates computed by Resmap[25]. **(a)** Histogram of voxels values, and **(b)** Heat map of local resolution estimates displayed for a cross-section through the reconstruction.

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Extended Data Fig. 6
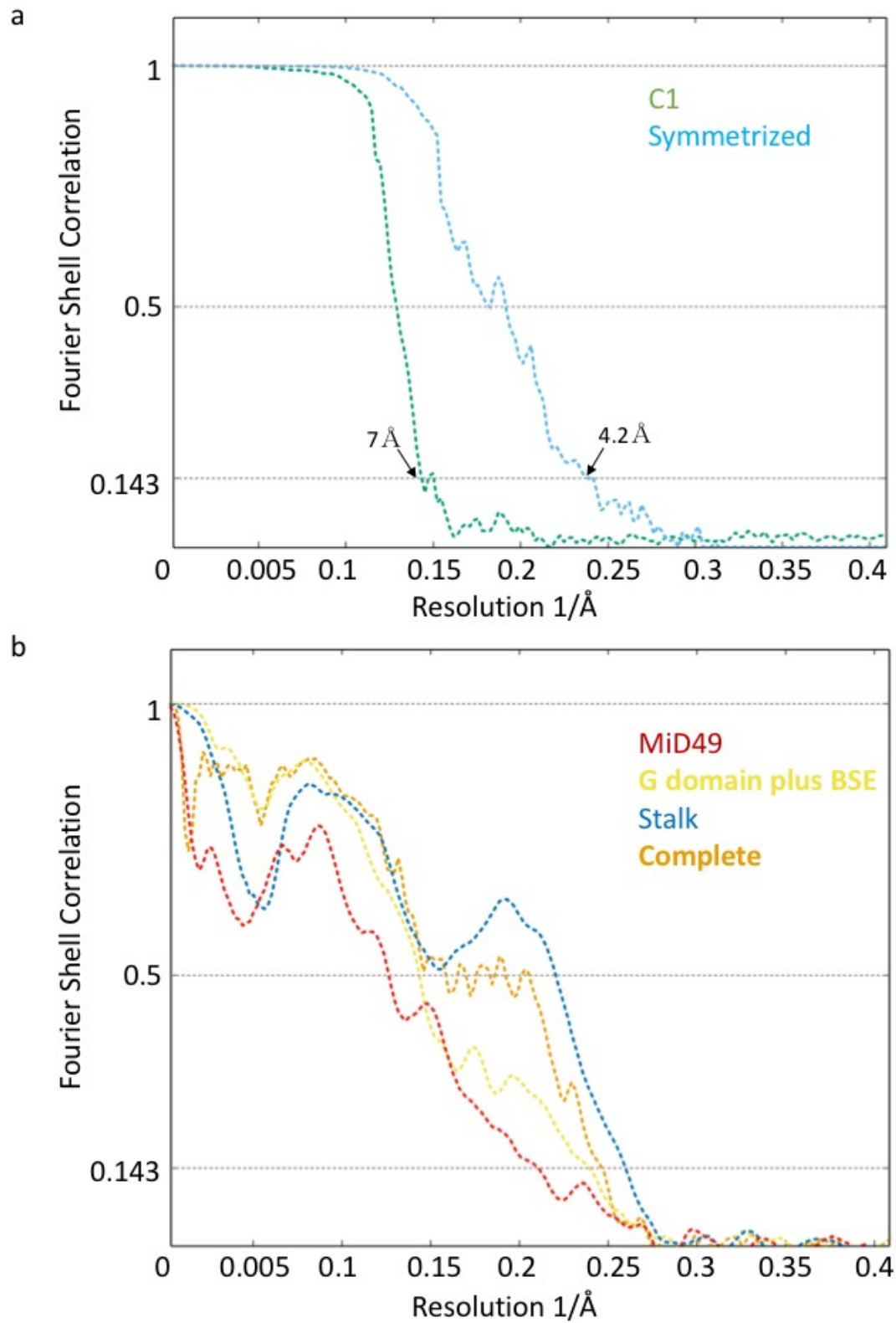
Extended data figure 6: Fourier Shell Correlation plots for **(a)** the half-maps with and without imposed symmetry; and **(b)** model-to-map correlations for the averaged as well as each sub-region of the structure using the atomic coordinates and B-factors determined using Rosetta.

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Extended Data Fig. 7
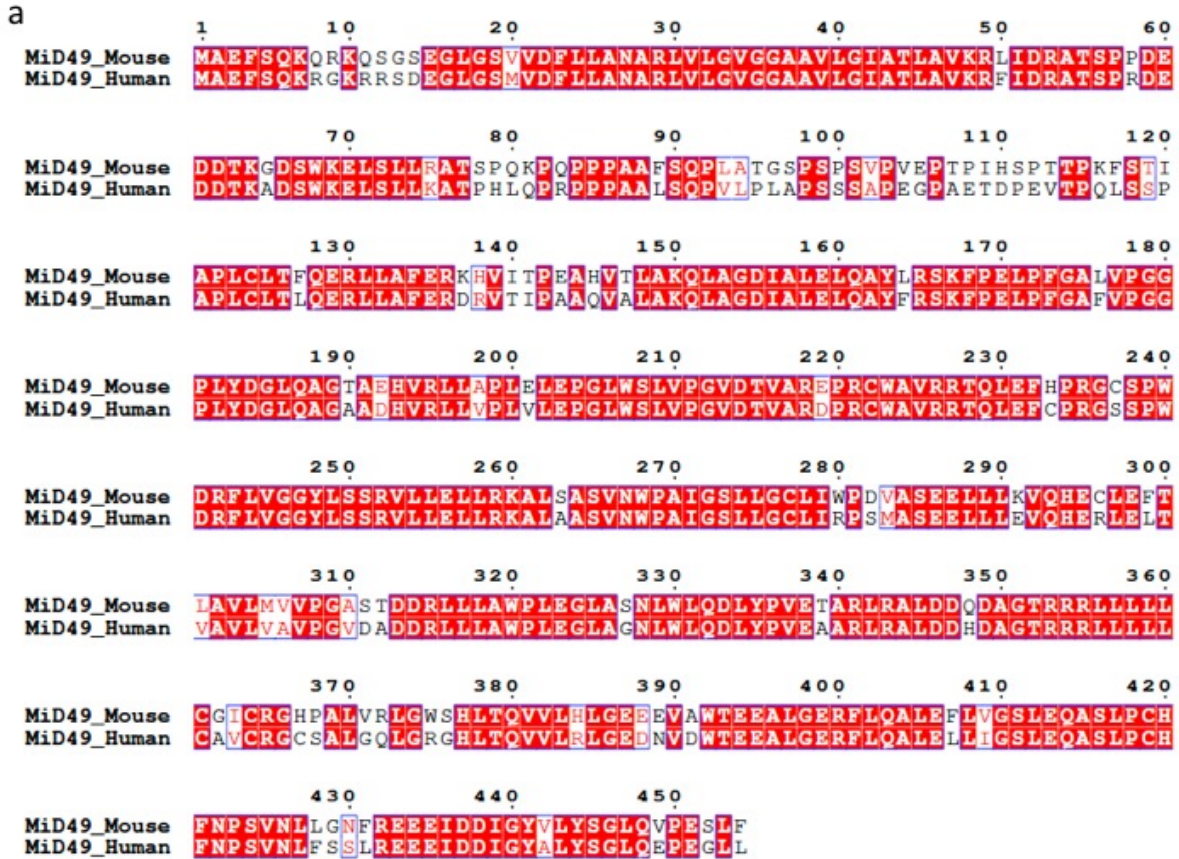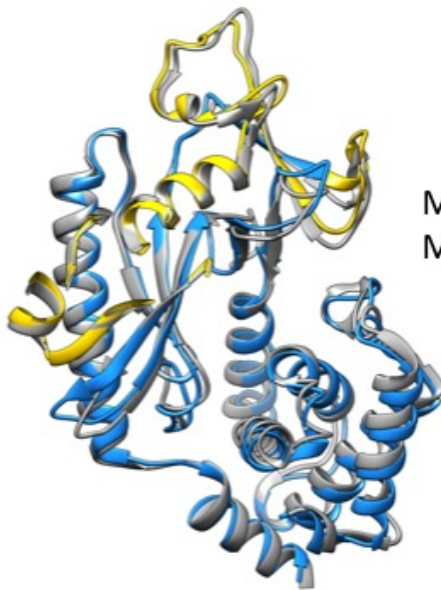
Extended data figure 7: Modeling of human MID49. **(a)** Sequence alignment between human and mouse MID49 sequences. **(b)** Overlay of the homology model of human MID49$_{126-454}$ (blue, with yellow DRR, ribbon) modeled within the cryoEM density versus the mouse MID49 crystal structure (PDB ID: 4WOY, grey ribbon)[6].

Kalia, et al.
Extended Data Fig. 8

a

b
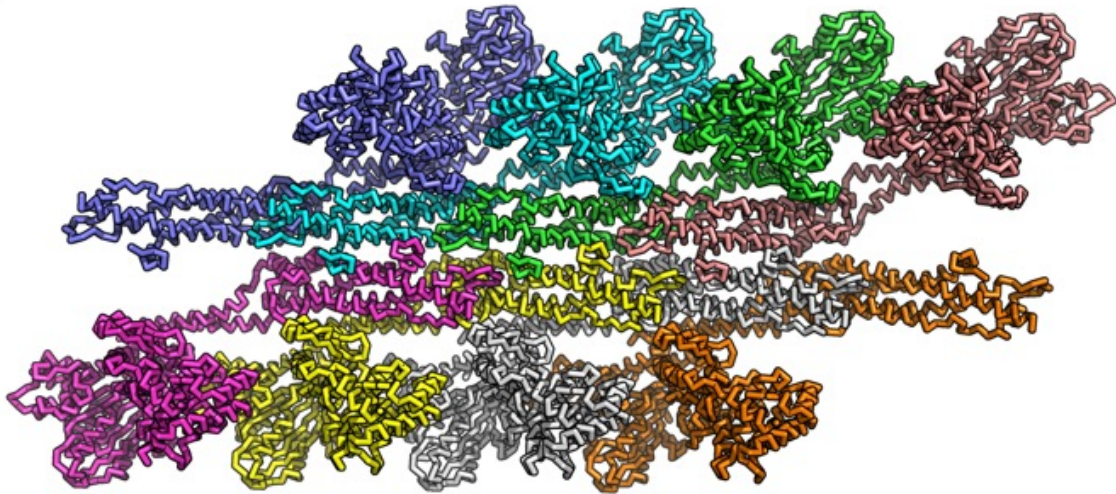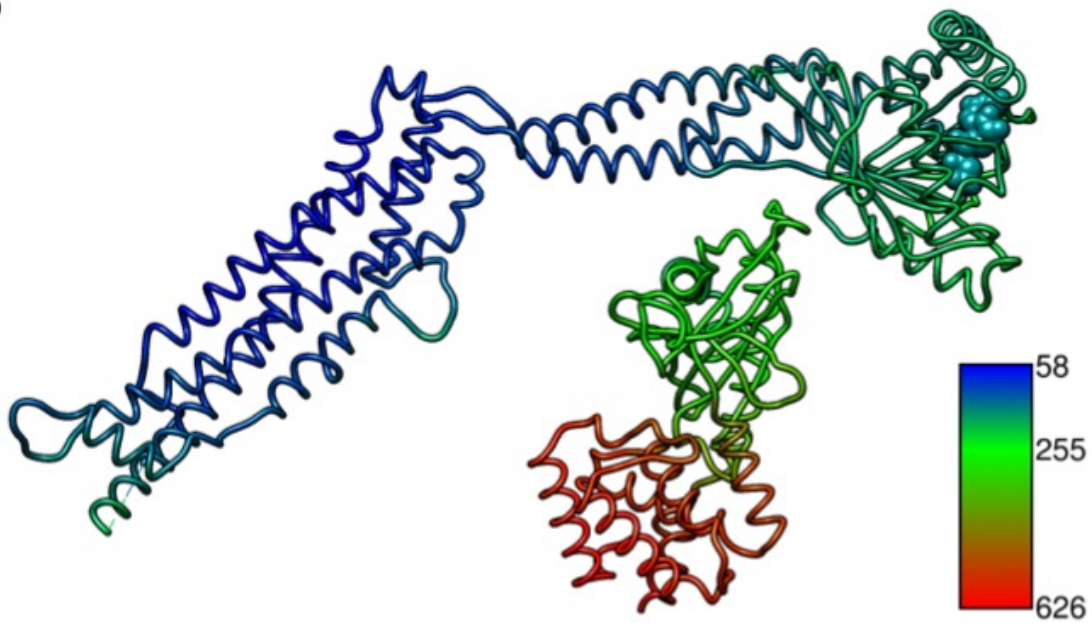
Extended data figure 8: Rosetta refinement. **(a)** Symmetric unit of the filament with 8 chains of DRP1 and 8 chains of MID49 refined using Rosetta to enforce symmetry and account for all possible inter-molecular interfaces. **(b)** Atomic B-factors for the DRP1 and MID49 models (ribbon) and bound GMPPCP (space filling).

Kalia, et al.
Extended Data Fig. 9
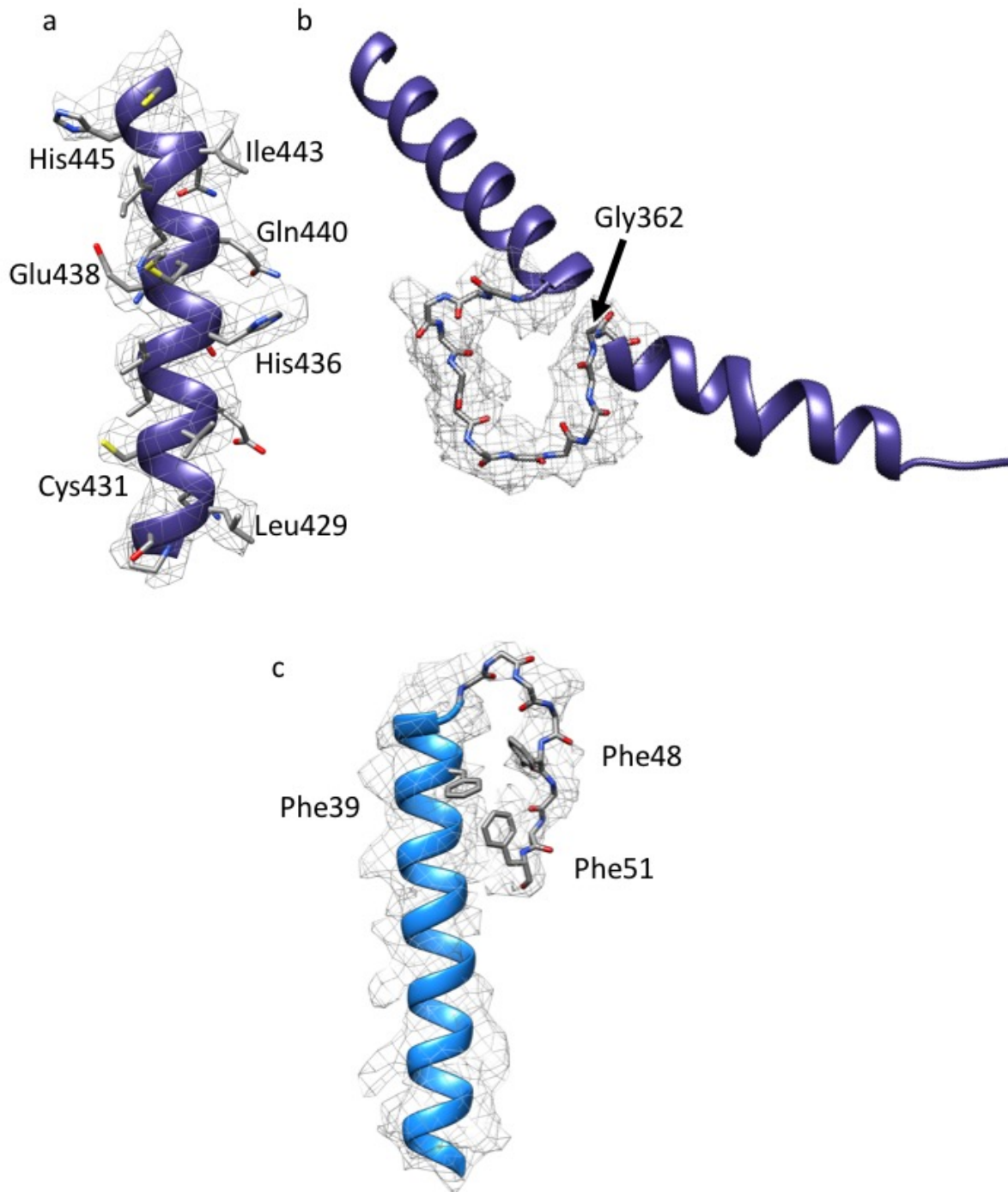
Extended data figure 9: Examples of model fitting to B-factor sharpened density for **(a)** a helix from the DRP1 stalk, **(b)** the backbone of the L1N^S loop, and **(c)** a helix and loop from MID49.

Kalia, et al.
Extended Data Fig. 10

DRP1$_{D190A}$ alone
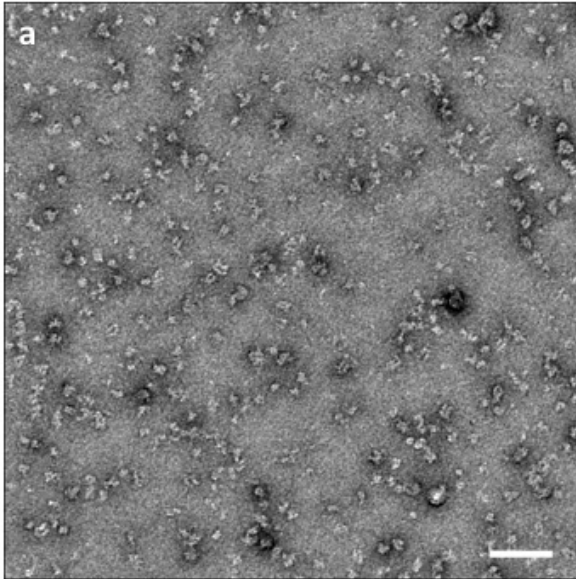
DRP1$_{D190A}$+MiD49
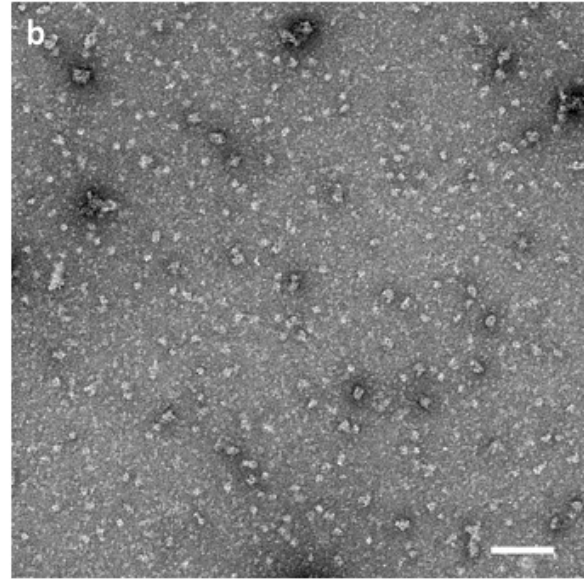
DRP1$_{D221A}$ alone
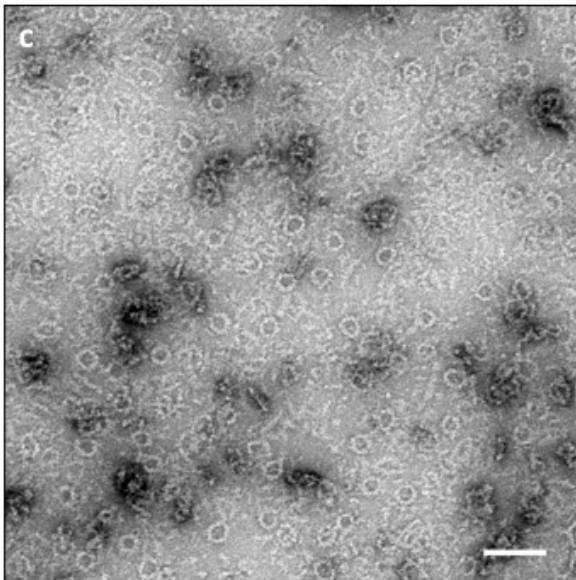
DRP1$_{D221A}$+MiD49
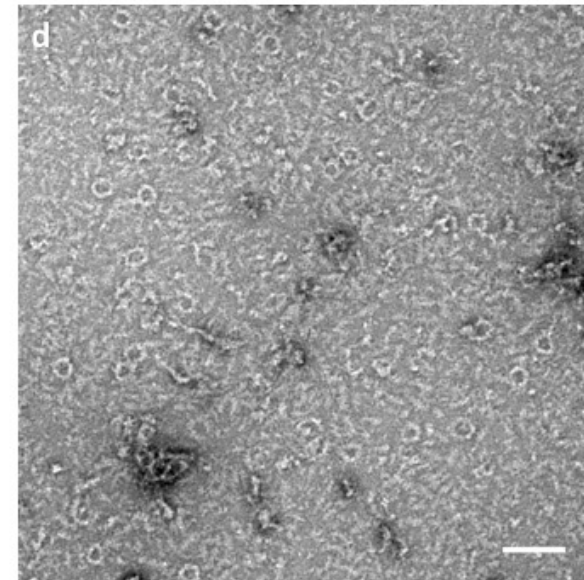
Extended data figure 10: DRP1 assembly and coassembly reactions with GMPPCP for **(a)** DRP1$_{D190A}$ alone, **(b)** DRP1$_{D190A}$+MID49, **(c)** DRP1$_{D221A}$ alone, and **(d)** DRP1$_{D221A}$+MID49. Bars = 100nm.

Kalia, et al.
Extended Data Fig. 11

	G domain	G domain	L1N^S
Human	TAANTDMATSE	TKLDLMDAGT	IETSELCGGAR
Chimpanzee	TAANTDMATSE	TKLDLMDAGT	IETSELCGGAR
Mouse	TAANTDMATSE	TKLDLMDAGT	IETSELCGGAR
Chicken	TAANTDMATSE	TKLDLMDAGT	IETSELCGGAR
Zebrafish	TAANTDMATSE	TKLDLMDAGT	IETAELCGGAR
Drosophila	TAANTDMATSE	TKLDLMDAGT	IETTELCGGAR
Worm	TPANQDFATSE	TKLDLMDQGT	IETTELCGGAR
Yeast	SPANVDLVNSE	TKLDLMDSGT	INTKELCGGAR

D190　　　　　D221A　　　　　GG362-363

Extended data figure 11: Multiple sequence alignment of the regions near and including the

DRP1 residues mutated in this study: D190, D221 and G362. The residue numbers apply to

human DRP1, isoform 2 (UNIPROT identifier: O00429-3 also known as DLP1a).

Kalia, et al.
Extended Data Fig. 12
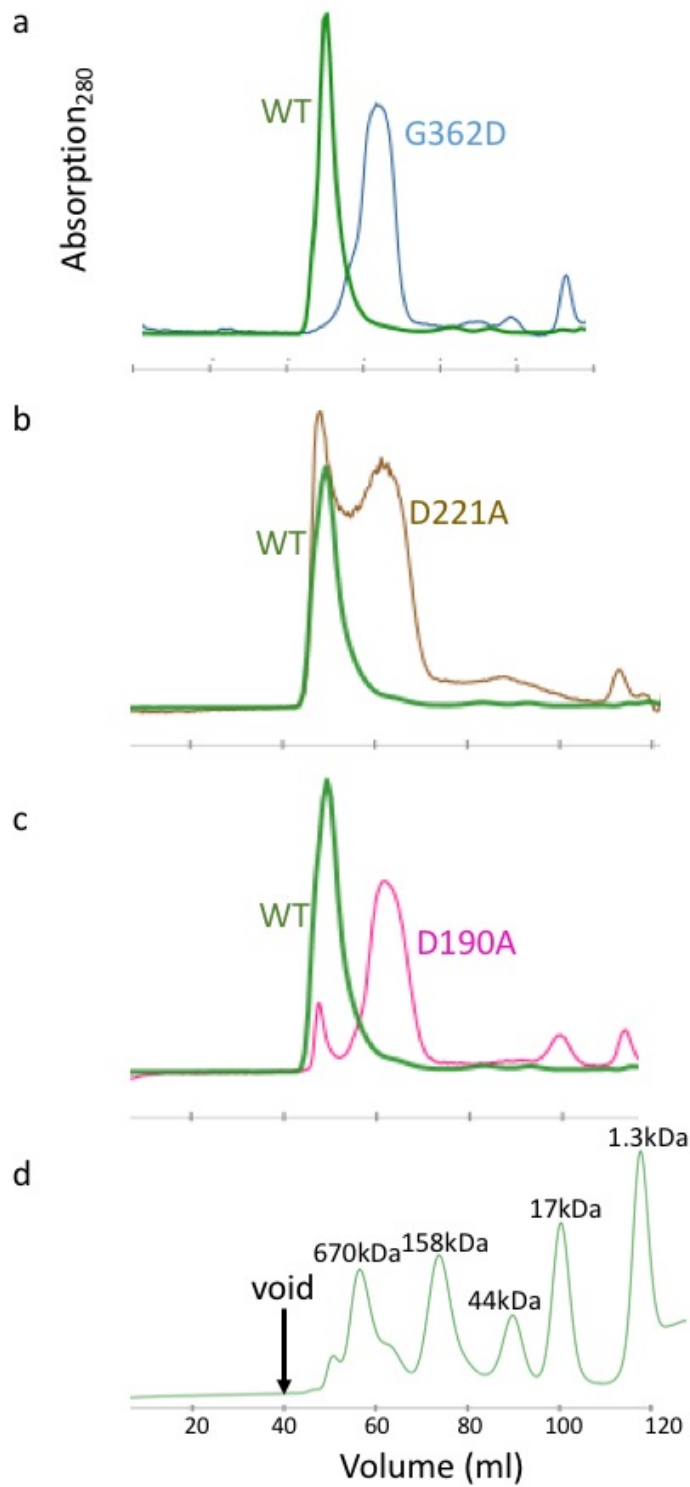
Extended data figure 12: Size exclusion chromatography traces for DRP1 wild-type and mutants used in the study. **(a)** Comparison between wild type (WT) versus DRP1$_{G362D}$, **(b)** WT versus DRP1$_{D221A}$, and **(c)** WT versus DRP1$_{D190A}$.

Kalia, et al.
Extended Data Fig. 13

a

b

c

DRP1+MiD49   Dynamin-3 (PDB:**5A3F**)   Overlay
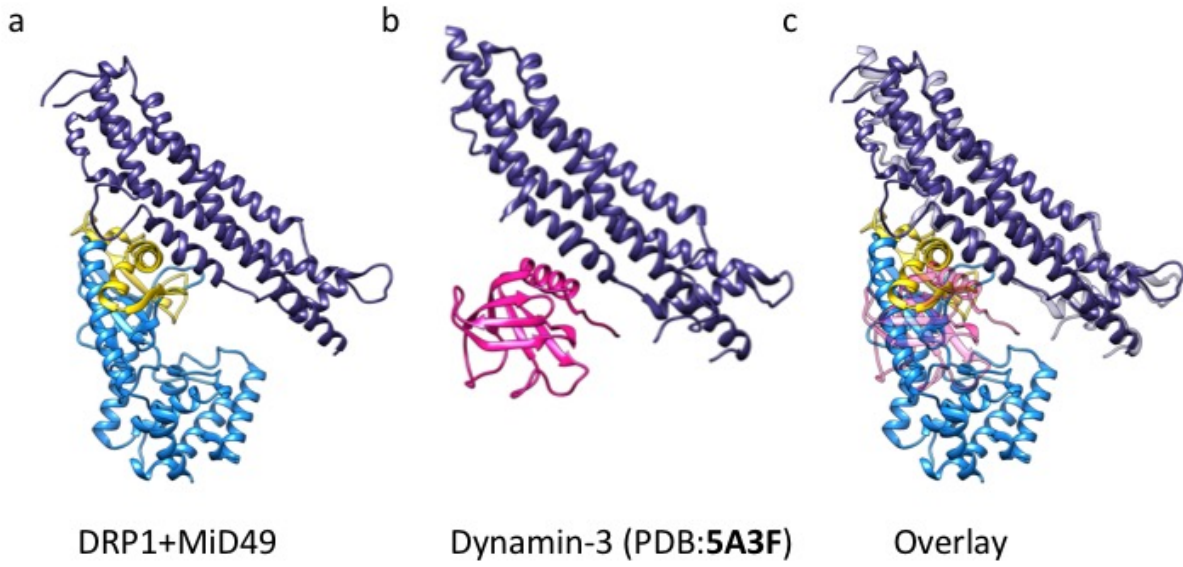
Extended data figure 13: Structural similarities between the third DRP1-MID49 interaction interface that includes the L1N^S loop and the interaction between the Pleckstrin Homology (PH) domain and the stalk of Dynamin-3 (PDB ID:5A3F)[26]. **(a)** DRP1-MID49 interaction at MID-interaction interface 3, **(b)** the PH domain bound to the stalk of Dynamin-3, and **(c)** Overlay of a and b.

Kalia, et al.
Extended Data Fig. 14

DRP1$_{G362D}$ alone   GMPPCP   DRP1$_{G362D}$+MiD49

DRP1$_{G362D}$ alone   GTP   DRP1$_{G362D}$+MiD49
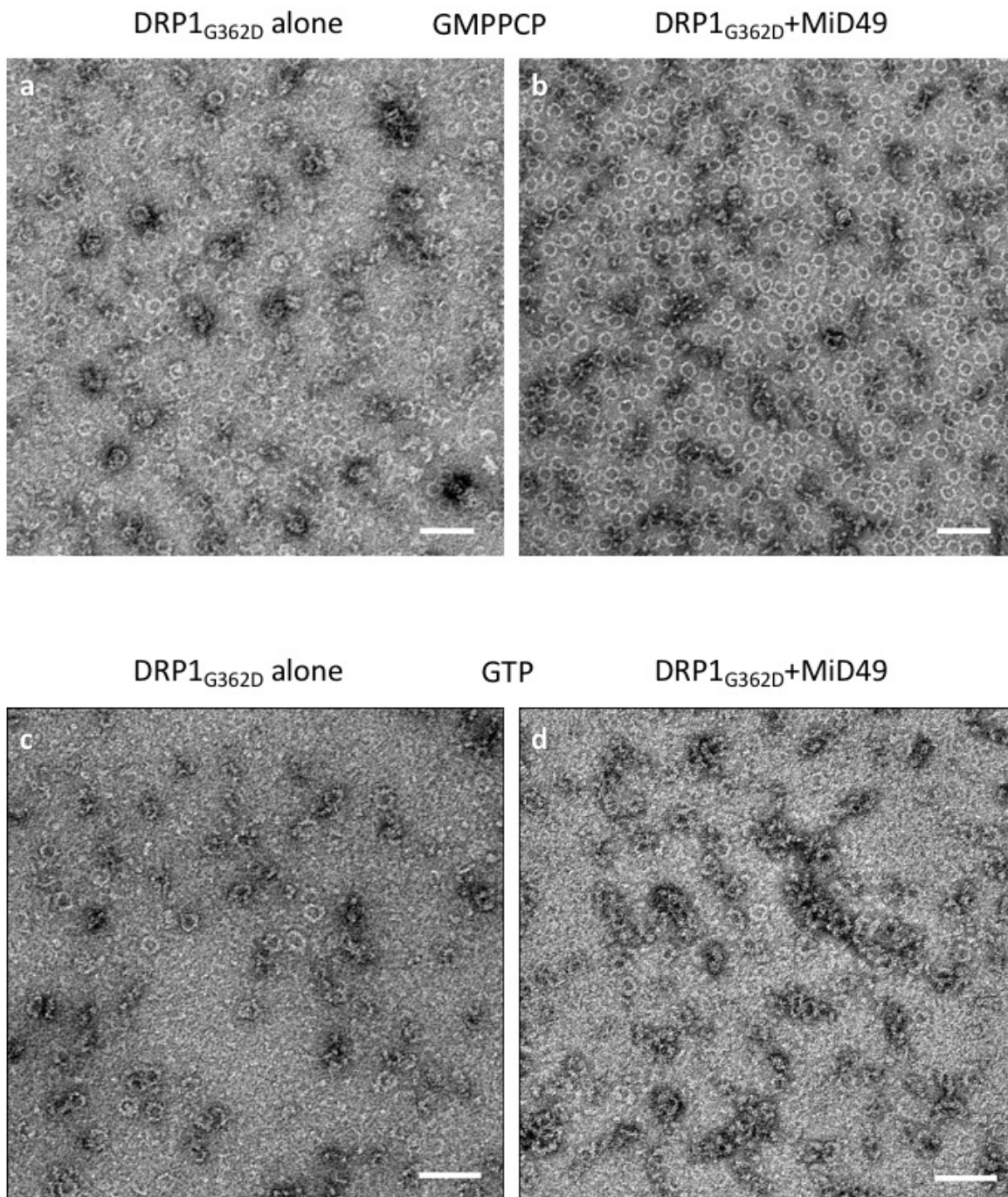
Extended data figure 14: DRP1$_{G362D}$ assembly and coassembly reactions with GMPPCP or

GTP. DRP1$_{G362D}$ forms rings but not linear filaments without MID49 **(a,c)**; and with MID49

present **(b,d)**; with GMPPCP **(a-b)**; or with GTP **(c-d)**. Bars = 100 nm.

Kalia, et al.
Extended Data Fig. 15
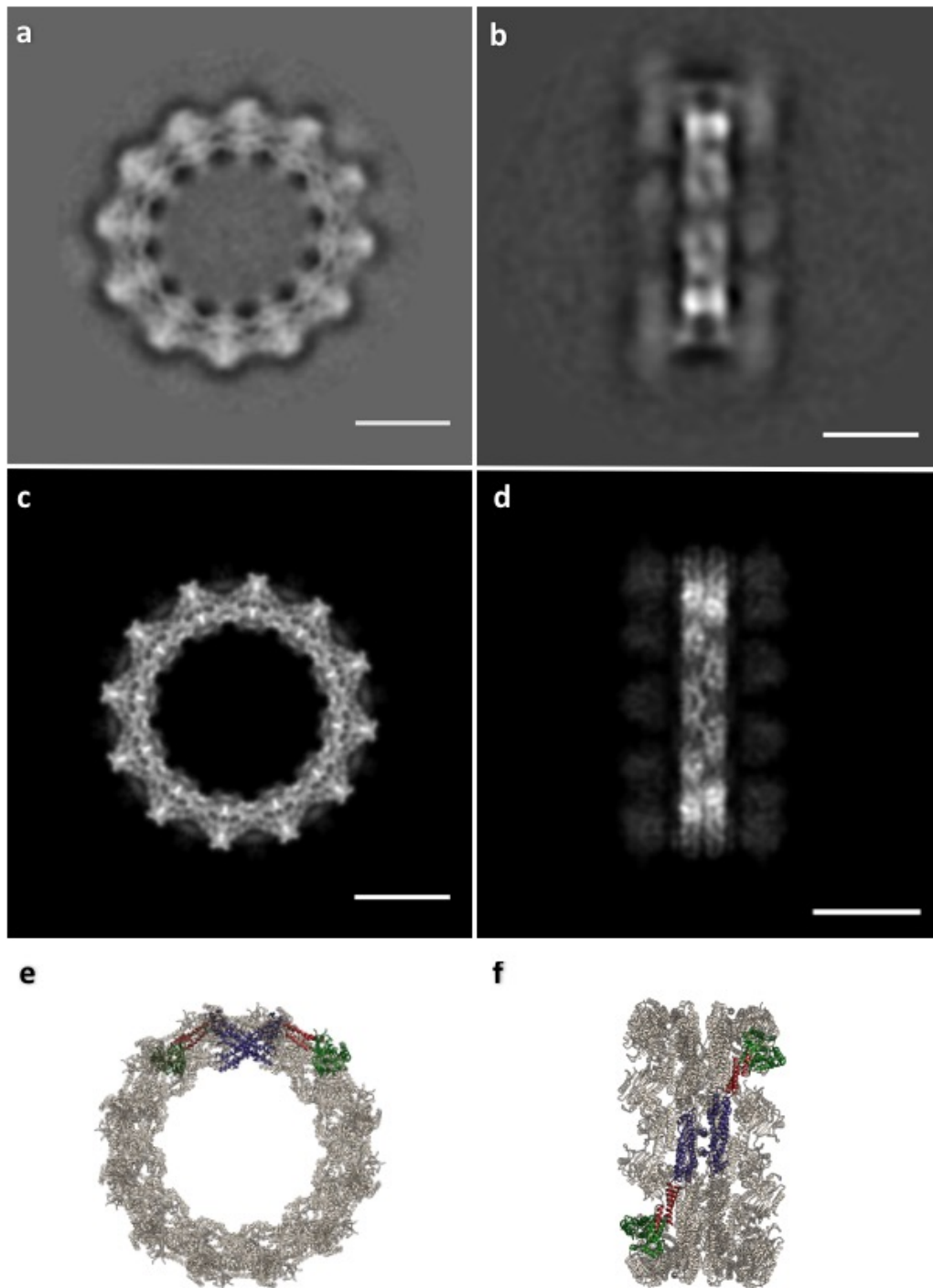
Extended data figure 15: DRP1$_{G362D}$ forms 12-dimer closed rings. **(a)** 2D class average of the rings; **(b)** 2D class average of infrequent, orthogonal or "side" views used as a constraint during model building; **(c)** "top" and **(d)** "side" projections of the model; **(e)** "top" and **(f)** "side" views of the final model rendered as ribbons. Bars =100Å. Green: G-domain, Red: Bundle Signaling Element (BSE), Purple: Stalk region.

Extended data movie Legends

Extended data movie 1: Nucleotide-induced conformational changes in the G-domain and Bundle Signaling Element (BSE).

Extended data movie 2: Nucleotide-induced conformational changes in the G-domain and Bundle Signaling Element (BSE) in the context of a full-length DRP1 dimer ("side" view).

Extended data movie 3: Nucleotide-induced conformational changes in the G-domain and Bundle Signaling Element (BSE) in the context of a full-length DRP1 dimer ("top" view).

Extended data movie 4: MID receptors engage a specific nucleotide-bound conformation of DRP1 tetramers and promote formation of a linear copolymer. Subsequent nucleotide hydrolysis and exchange promotes receptor dissociation and tetramer bending in the closed rings.

Extended data References

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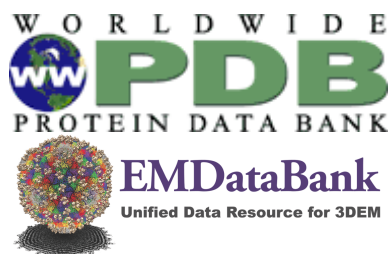
Full wwPDB/EMDataBank EM Map/Model Validation Report ⓘ

Aug 2, 2017 – 01:55 PM EDT

This is a Full wwPDB/EMDataBank EM Map/Model Validation Report.

This report is produced by the standalone wwPDB validation server.
The structure in question has not been deposited to the wwPDB.
This report should not be submitted to journals.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
http://wwpdb.org/validation/2016/EMValidationReportHelp
with specific help available everywhere you see the ⓘ symbol.

MolProbity	:	4.02b-467
Percentile statistics	:	20161228.v01 (using entries in the PDB archive December 28th 2016)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et. al. (1996)
Validation Pipeline (wwPDB-VP)	:	rb-20029824

1   Overall quality at a glance ⓘ

The following experimental techniques were used to determine the structure:
ELECTRON MICROSCOPY

The reported resolution of this entry is unknown.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.

Metric	Percentile Ranks	Value
Clashscore		2
Ramachandran outliers		1.3%
Sidechain outliers		0.4%

Worse   *Better*

▌ Percentile relative to all structures
▯ Percentile relative to all EM structures
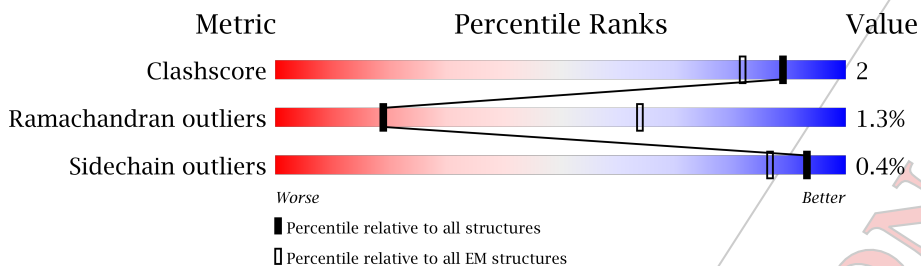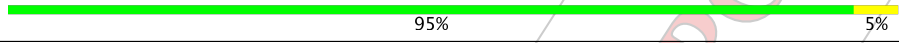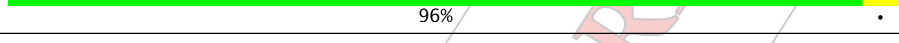
Metric	Whole archive (#Entries)	EM structures (#Entries)
Clashscore	125131	1336
Ramachandran outliers	121729	1120
Sidechain outliers	121581	1026

The table below summarises the geometric issues observed across the polymeric chains. The red, orange, yellow and green segments on the bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5%

Mol	Chain	Length	Quality of chain
1	A	591	94% · ·
1	C	591	94% · ·
1	E	591	94% 5% ·
1	G	591	94% 5% ·
1	I	591	94% · ·
1	K	591	94% 5% ·
1	M	591	95% · ·
1	O	591	95% · ·
2	B	329	97% ·

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Mol	Chain	Length	Quality of chain
2	D	329	95% · 5%
2	F	329	95% ·
2	H	329	95% · 5%
2	J	329	97% ·
2	L	329	96% ·
2	N	329	95% · 5%
2	P	329	96% ·

2   Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 116960 atoms, of which 59040 are hydrogens and 0 are deuteriums.

In the tables below, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 1 is a protein.

Mol	Chain	Residues	Atoms								AltConf	Trace
1	A	591	Total 9493	C 2954	H 4801	Mg 1	N 821	O 893	P 3	S 20	0	1
1	C	591	Total 9493	C 2954	H 4801	Mg 1	N 821	O 893	P 3	S 20	0	1
1	E	591	Total 9493	C 2954	H 4801	Mg 1	N 821	O 893	P 3	S 20	0	1
1	G	591	Total 9493	C 2954	H 4801	Mg 1	N 821	O 893	P 3	S 20	0	1
1	I	591	Total 9493	C 2954	H 4801	Mg 1	N 821	O 893	P 3	S 20	0	1
1	K	591	Total 9493	C 2954	H 4801	Mg 1	N 821	O 893	P 3	S 20	0	1
1	M	591	Total 9493	C 2954	H 4801	Mg 1	N 821	O 893	P 3	S 20	0	1
1	O	591	Total 9493	C 2954	H 4801	Mg 1	N 821	O 893	P 3	S 20	0	1

- Molecule 2 is a protein.

Mol	Chain	Residues	Atoms						AltConf	Trace
2	B	329	Total 5127	C 1630	H 2579	N 445	O 465	S 8	0	0
2	D	329	Total 5127	C 1630	H 2579	N 445	O 465	S 8	0	0
2	F	329	Total 5127	C 1630	H 2579	N 445	O 465	S 8	0	0
2	H	329	Total 5127	C 1630	H 2579	N 445	O 465	S 8	0	0
2	J	329	Total 5127	C 1630	H 2579	N 445	O 465	S 8	0	0
2	L	329	Total 5127	C 1630	H 2579	N 445	O 465	S 8	0	0
2	N	329	Total 5127	C 1630	H 2579	N 445	O 465	S 8	0	0

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Mol	Chain	Residues	Atoms						AltConf	Trace
			Total	C	H	N	O	S		
2	P	329	5127	1630	2579	445	465	8	0	0

3 Residue-property plots ⓘ

These plots are drawn for all protein, RNA and DNA chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry. Residues are color-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

• Molecule 1:

Chain A:

• Molecule 1:

Chain C:

• Molecule 1:

Chain E:
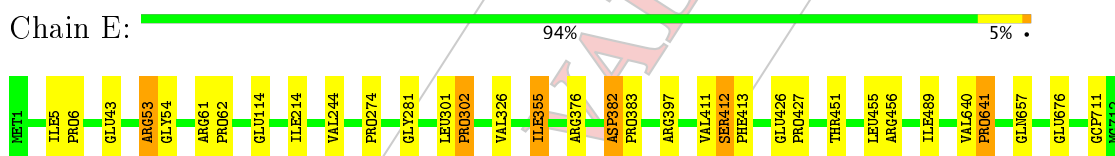
• Molecule 1:

Chain G:

• Molecule 1:

Chain I:

• Molecule 1:

Chain K:　94%　5%

Chain M:　95%

- Molecule 1:

Chain O:　95%

- Molecule 1:

- Molecule 2:

Chain B:　97%

- Molecule 2:

Chain D:　95%　5%

- Molecule 2:

Chain F:　95%

- Molecule 2:

Chain H:　95%　5%

THR1 PRO17 PHE51 GLU78 PRO79 PRO144 MET158 GLU166 VAL167 GLU170 PRO183 PRO196 TYR210 PRO211 THR227 ARG275 ASN297 LEU329

- Molecule 2:

Chain J: 97%   .

THR1 PRO17 PHE51 PRO144 GLU166 VAL167 PRO183 PRO196 THR227 ARG275 LEU329

- Molecule 2:

Chain L: 96%   .

THR1 PRO17 PHE51 GLU78 PRO79 PRO144 SER148 MET158 GLU166 VAL167 GLU170 PRO183 PRO196 THR227 ARG275 LEU329

- Molecule 2:

Chain N: 95%   5%

THR1 PRO17 PHE51 GLU78 PRO79 PRO144 SER148 MET158 GLU166 VAL167 GLU170 PRO183 PRO196 TYR210 PRO211 THR227 ARG275 ASN297 LEU329

- Molecule 2:

Chain P: 96%   .

THR1 PRO17 PHE51 GLU78 PRO79 PRO144 SER148 MET158 GLU166 VAL167 GLU170 PRO183 PRO196 THR227 ARG275 LEU329

4　Experimental information　ⓘ

Property	Value	Source
Reconstruction method	Not provided	Depositor
Imposed symmetry	POINT, Not provided	Depositor
Number of images used	Not provided	Depositor
Resolution determination method	Not provided	Depositor
CTF correction method	Not provided	Depositor
Microscope	Not provided	Depositor
Voltage (kV)	Not provided	Depositor
Electron dose $(e^-/\text{Å}^2)$	Not provided	Depositor
Minimum defocus (nm)	Not provided	Depositor
Maximum defocus (nm)	Not provided	Depositor
Magnification	Not provided	Depositor
Image detector	Not provided	Depositor

5 Model quality ⓘ

5.1 Standard geometry ⓘ

Bond lengths and bond angles in the following residue types are not validated in this section: GCP, MG

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 5$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	#$|Z| > 2$	RMSZ	#$|Z| > 2$
1	A	0.72	0/4727	0.51	0/6400
1	C	0.72	0/4727	0.51	0/6400
1	E	0.72	0/4727	0.51	0/6400
1	G	0.72	0/4727	0.51	0/6400
1	I	0.72	0/4727	0.51	0/6400
1	K	0.72	0/4727	0.51	0/6400
1	M	0.72	0/4727	0.51	0/6400
1	O	0.72	0/4727	0.51	0/6400
2	B	0.72	0/2601	0.49	0/3543
2	D	0.72	0/2601	0.49	0/3543
2	F	0.73	0/2601	0.49	0/3543
2	H	0.72	0/2601	0.49	0/3543
2	J	0.72	0/2601	0.49	0/3543
2	L	0.72	0/2601	0.49	0/3543
2	N	0.72	0/2601	0.49	0/3543
2	P	0.72	0/2601	0.49	0/3543
All	All	0.72	0/58624	0.50	0/79544

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

5.2 Too-close contacts ⓘ

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in the chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes within the asymmetric unit, whereas Symm-Clashes lists symmetry related clashes.

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	4692	4801	4813	27	0
1	C	4692	4801	4813	25	0
1	E	4692	4801	4813	24	0
1	G	4692	4801	4813	26	0
1	I	4692	4801	4813	25	0
1	K	4692	4801	4813	26	0
1	M	4692	4801	4813	23	0
1	O	4692	4801	4813	23	0
2	B	2548	2579	2581	5	0
2	D	2548	2579	2581	10	0
2	F	2548	2579	2581	8	0
2	H	2548	2579	2581	9	0
2	J	2548	2579	2581	5	0
2	L	2548	2579	2581	9	0
2	N	2548	2579	2581	10	0
2	P	2548	2579	2581	9	0
All	All	57920	59040	59152	249	0

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including hydrogen atoms). The all-atom clashscore for this structure is 2.

All (249) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:657:GLN:OE1	1:A:657:GLN:N	2.15	0.80
1:C:657:GLN:OE1	1:C:657:GLN:N	2.15	0.80
1:M:657:GLN:OE1	1:M:657:GLN:N	2.14	0.80
1:O:657:GLN:OE1	1:O:657:GLN:N	2.15	0.79
1:K:657:GLN:OE1	1:K:657:GLN:N	2.14	0.79
1:G:657:GLN:OE1	1:G:657:GLN:N	2.15	0.79
1:E:657:GLN:N	1:E:657:GLN:OE1	2.15	0.79
1:O:355:ILE:HG22	1:O:355:ILE:O	1.83	0.77
1:I:657:GLN:N	1:I:657:GLN:OE1	2.15	0.77
1:K:355:ILE:O	1:K:355:ILE:HG22	1.83	0.77
1:E:355:ILE:HG22	1:E:355:ILE:O	1.83	0.77
1:M:355:ILE:HG22	1:M:355:ILE:O	1.83	0.77
1:G:355:ILE:O	1:G:355:ILE:HG22	1.83	0.76
1:C:355:ILE:HG22	1:C:355:ILE:O	1.83	0.76
1:A:355:ILE:HG22	1:A:355:ILE:O	1.83	0.76
1:I:355:ILE:O	1:I:355:ILE:HG22	1.83	0.75
1:E:397:ARG:NH1	2:L:148:SER:OG	2.30	0.65
2:D:227:THR:OG1	2:D:275:ARG:NH2	2.31	0.64

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
2:J:167:VAL:O	2:J:167:VAL:HG13	1.96	0.64
2:N:227:THR:OG1	2:N:275:ARG:NH2	2.31	0.64
2:B:227:THR:OG1	2:B:275:ARG:NH2	2.31	0.64
2:J:227:THR:OG1	2:J:275:ARG:NH2	2.31	0.64
2:B:167:VAL:O	2:B:167:VAL:HG13	1.97	0.64
2:D:167:VAL:O	2:D:167:VAL:HG13	1.96	0.64
2:D:148:SER:OG	1:I:397:ARG:NH1	2.31	0.64
2:L:227:THR:OG1	2:L:275:ARG:NH2	2.31	0.64
2:H:227:THR:OG1	2:H:275:ARG:NH2	2.31	0.64
2:F:227:THR:OG1	2:F:275:ARG:NH2	2.31	0.63
2:P:167:VAL:HG13	2:P:167:VAL:O	1.96	0.63
2:P:227:THR:OG1	2:P:275:ARG:NH2	2.31	0.63
2:N:167:VAL:HG13	2:N:167:VAL:O	1.96	0.63
2:L:167:VAL:O	2:L:167:VAL:HG13	1.96	0.63
2:H:167:VAL:HG13	2:H:167:VAL:O	1.96	0.63
2:F:167:VAL:O	2:F:167:VAL:HG13	1.96	0.62
1:I:43:GLU:OE1	1:I:43:GLU:HA	2.02	0.58
1:O:43:GLU:HA	1:O:43:GLU:OE1	2.03	0.58
1:A:43:GLU:OE1	1:A:43:GLU:HA	2.03	0.58
1:K:43:GLU:HA	1:K:43:GLU:OE1	2.02	0.58
1:C:43:GLU:HA	1:C:43:GLU:OE1	2.03	0.57
1:E:43:GLU:OE1	1:E:43:GLU:HA	2.03	0.57
1:G:43:GLU:OE1	1:G:43:GLU:HA	2.03	0.57
1:M:43:GLU:OE1	1:M:43:GLU:HA	2.02	0.57
1:A:397:ARG:NH1	2:N:148:SER:OG	2.40	0.55
1:G:397:ARG:NH1	2:P:148:SER:OG	2.40	0.55
1:A:382:ASP:CB	1:A:383:PRO:HA	2.39	0.52
1:I:382:ASP:CB	1:I:383:PRO:HA	2.39	0.52
1:C:382:ASP:CB	1:C:383:PRO:HA	2.39	0.52
1:M:382:ASP:CB	1:M:383:PRO:HA	2.39	0.52
1:E:382:ASP:CB	1:E:383:PRO:HA	2.39	0.51
1:G:382:ASP:CB	1:G:383:PRO:HA	2.39	0.51
1:K:382:ASP:CB	1:K:383:PRO:HA	2.39	0.51
1:O:382:ASP:CB	1:O:383:PRO:HA	2.39	0.51
1:I:214:ILE:O	1:I:244:VAL:N	2.44	0.50
1:A:214:ILE:O	1:A:244:VAL:N	2.44	0.49
1:M:214:ILE:O	1:M:244:VAL:N	2.44	0.49
2:B:51:PHE:CD1	2:B:51:PHE:N	2.80	0.49
1:C:214:ILE:O	1:C:244:VAL:N	2.44	0.49
2:D:51:PHE:CD1	2:D:51:PHE:N	2.80	0.49
1:K:214:ILE:O	1:K:244:VAL:N	2.44	0.49

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:G:214:ILE:O	1:G:244:VAL:N	2.44	0.49
1:O:214:ILE:O	1:O:244:VAL:N	2.44	0.49
2:N:51:PHE:N	2:N:51:PHE:CD1	2.80	0.49
1:E:214:ILE:O	1:E:244:VAL:N	2.44	0.48
1:C:412:SER:OG	1:C:413:PHE:N	2.47	0.48
1:E:376:ARG:O	2:H:158:MET:SD	2.72	0.48
2:B:158:MET:SD	1:I:376:ARG:O	2.71	0.48
2:J:51:PHE:N	2:J:51:PHE:CD1	2.80	0.48
1:A:412:SER:OG	1:A:413:PHE:N	2.47	0.48
1:E:412:SER:OG	1:E:413:PHE:N	2.47	0.48
1:G:412:SER:OG	1:G:413:PHE:N	2.47	0.48
1:I:412:SER:OG	1:I:413:PHE:N	2.47	0.48
1:K:412:SER:OG	1:K:413:PHE:N	2.47	0.47
1:O:412:SER:OG	1:O:413:PHE:N	2.47	0.47
2:F:51:PHE:N	2:F:51:PHE:CD1	2.80	0.47
2:L:51:PHE:CD1	2:L:51:PHE:N	2.80	0.47
2:P:51:PHE:CD1	2:P:51:PHE:N	2.80	0.47
1:E:54:GLY:O	1:E:711:GCP:H5'1	2.14	0.47
2:H:51:PHE:N	2:H:51:PHE:CD1	2.80	0.47
1:G:54:GLY:O	1:G:711:GCP:H5'1	2.14	0.47
1:K:640:VAL:N	1:K:641:PRO:CD	2.78	0.47
1:K:54:GLY:O	1:K:711:GCP:H5'1	2.14	0.47
1:O:640:VAL:N	1:O:641:PRO:CD	2.78	0.47
1:G:640:VAL:N	1:G:641:PRO:CD	2.78	0.46
1:E:640:VAL:N	1:E:641:PRO:CD	2.78	0.46
1:O:54:GLY:O	1:O:711:GCP:H5'1	2.14	0.46
1:I:54:GLY:O	1:I:711:GCP:H5'1	2.14	0.46
1:M:54:GLY:O	1:M:711:GCP:H5'1	2.14	0.46
1:A:54:GLY:O	1:A:711:GCP:H5'1	2.14	0.46
1:A:640:VAL:N	1:A:641:PRO:CD	2.78	0.46
1:C:54:GLY:O	1:C:711:GCP:H5'1	2.14	0.46
1:C:640:VAL:N	1:C:641:PRO:CD	2.78	0.46
1:I:640:VAL:N	1:I:641:PRO:CD	2.78	0.46
1:M:640:VAL:N	1:M:641:PRO:CD	2.78	0.46
1:G:376:ARG:O	2:L:158:MET:SD	2.74	0.46
1:E:640:VAL:HB	1:E:641:PRO:HD3	1.98	0.46
1:G:640:VAL:HB	1:G:641:PRO:HD3	1.98	0.46
1:K:640:VAL:HB	1:K:641:PRO:HD3	1.98	0.46
1:M:640:VAL:HB	1:M:641:PRO:HD3	1.98	0.45
1:O:640:VAL:HB	1:O:641:PRO:HD3	1.98	0.45
1:A:382:ASP:CB	1:A:383:PRO:CA	2.94	0.45

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:I:382:ASP:CB	1:I:383:PRO:CA	2.95	0.45
1:C:382:ASP:CB	1:C:383:PRO:CA	2.94	0.45
1:A:489:ILE:HD12	1:G:643:ALA:HA	1.98	0.45
1:E:5:ILE:HB	1:E:6:PRO:CD	2.47	0.45
1:G:5:ILE:HB	1:G:6:PRO:CD	2.47	0.45
1:K:5:ILE:HB	1:K:6:PRO:CD	2.47	0.45
1:M:382:ASP:CB	1:M:383:PRO:CA	2.94	0.45
1:C:640:VAL:HB	1:C:641:PRO:HD3	1.98	0.45
1:E:382:ASP:CB	1:E:383:PRO:CA	2.94	0.45
1:O:382:ASP:CB	1:O:383:PRO:CA	2.94	0.45
1:O:5:ILE:HB	1:O:6:PRO:CD	2.47	0.45
1:G:382:ASP:CB	1:G:383:PRO:CA	2.95	0.45
1:A:640:VAL:HB	1:A:641:PRO:HD3	1.98	0.45
1:M:5:ILE:HB	1:M:6:PRO:CD	2.47	0.45
1:K:382:ASP:CB	1:K:383:PRO:CA	2.95	0.45
1:C:5:ILE:HB	1:C:6:PRO:CD	2.47	0.45
1:E:355:ILE:CG2	1:E:355:ILE:O	2.56	0.45
1:A:643:ALA:HA	1:G:489:ILE:HD12	1.98	0.45
1:I:640:VAL:HB	1:I:641:PRO:HD3	1.98	0.45
1:G:355:ILE:CG2	1:G:355:ILE:O	2.56	0.44
1:A:5:ILE:HB	1:A:6:PRO:CD	2.47	0.44
2:D:166:GLU:O	2:D:167:VAL:HB	2.18	0.44
1:I:643:ALA:HA	1:K:489:ILE:HD12	1.99	0.44
1:I:5:ILE:HB	1:I:6:PRO:CD	2.47	0.44
2:B:166:GLU:O	2:B:167:VAL:HB	2.18	0.44
1:K:355:ILE:CG2	1:K:355:ILE:O	2.56	0.44
2:N:166:GLU:O	2:N:167:VAL:HB	2.18	0.44
1:G:411:VAL:O	1:G:412:SER:C	2.57	0.44
2:J:166:GLU:O	2:J:167:VAL:HB	2.18	0.44
1:K:411:VAL:O	1:K:412:SER:C	2.57	0.44
1:O:355:ILE:O	1:O:355:ILE:CG2	2.56	0.44
1:A:355:ILE:O	1:A:355:ILE:CG2	2.56	0.43
1:E:411:VAL:O	1:E:412:SER:C	2.57	0.43
2:F:166:GLU:O	2:F:167:VAL:HB	2.18	0.43
1:O:411:VAL:O	1:O:412:SER:C	2.57	0.43
2:H:166:GLU:O	2:H:167:VAL:HB	2.18	0.43
2:L:166:GLU:O	2:L:167:VAL:HB	2.18	0.43
2:P:166:GLU:O	2:P:167:VAL:HB	2.18	0.43
1:A:382:ASP:HB3	1:A:383:PRO:CA	2.49	0.43
1:C:355:ILE:O	1:C:355:ILE:CG2	2.56	0.43
1:C:382:ASP:HB3	1:C:383:PRO:CA	2.49	0.43

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:M:382:ASP:HB3	1:M:383:PRO:CA	2.49	0.43
1:M:355:ILE:O	1:M:355:ILE:CG2	2.56	0.43
1:I:382:ASP:HB3	1:I:383:PRO:CA	2.49	0.43
1:I:411:VAL:O	1:I:412:SER:C	2.57	0.43
1:A:411:VAL:O	1:A:412:SER:C	2.57	0.43
2:P:78:GLU:HA	2:P:79:PRO:HD3	1.87	0.43
1:E:382:ASP:HB3	1:E:383:PRO:CA	2.49	0.43
1:C:411:VAL:O	1:C:412:SER:C	2.57	0.42
1:G:382:ASP:HB3	1:G:383:PRO:CA	2.49	0.42
1:I:489:ILE:HD12	1:K:643:ALA:HA	2.01	0.42
1:O:382:ASP:HB3	1:O:383:PRO:CA	2.49	0.42
2:L:78:GLU:HA	2:L:79:PRO:HD3	1.87	0.42
1:M:411:VAL:O	1:M:412:SER:C	2.57	0.42
1:A:376:ARG:O	2:D:158:MET:SD	2.77	0.42
1:K:382:ASP:HB3	1:K:383:PRO:CA	2.49	0.42
1:C:376:ARG:O	2:N:158:MET:SD	2.77	0.42
1:A:451:THR:O	1:A:455:LEU:N	2.53	0.42
1:I:451:THR:O	1:I:455:LEU:N	2.53	0.42
1:C:451:THR:O	1:C:455:LEU:N	2.53	0.42
1:A:456:ARG:O	1:A:676:GLU:HA	2.20	0.42
2:H:78:GLU:HA	2:H:79:PRO:HD3	1.87	0.42
1:I:456:ARG:O	1:I:676:GLU:HA	2.20	0.42
1:M:412:SER:OG	1:M:413:PHE:N	2.47	0.42
1:M:451:THR:O	1:M:455:LEU:N	2.53	0.42
1:O:451:THR:O	1:O:455:LEU:N	2.53	0.42
1:K:451:THR:O	1:K:455:LEU:N	2.53	0.42
1:G:451:THR:O	1:G:455:LEU:N	2.53	0.41
1:C:456:ARG:O	1:C:676:GLU:HA	2.20	0.41
1:E:382:ASP:HB2	1:E:383:PRO:HA	2.02	0.41
1:E:451:THR:O	1:E:455:LEU:N	2.53	0.41
1:M:456:ARG:O	1:M:676:GLU:HA	2.20	0.41
1:I:53:ARG:N	1:I:53:ARG:HD2	2.36	0.41
1:C:382:ASP:HB2	1:C:383:PRO:HA	2.02	0.41
1:O:426:GLU:HB3	1:O:427:PRO:HD3	2.03	0.41
1:A:53:ARG:N	1:A:53:ARG:HD2	2.36	0.41
1:G:382:ASP:HB2	1:G:383:PRO:HA	2.02	0.41
1:I:23:ILE:HG23	1:I:23:ILE:O	2.20	0.41
1:O:53:ARG:HD2	1:O:53:ARG:N	2.36	0.41
1:A:382:ASP:HB2	1:A:383:PRO:HA	2.02	0.41
1:C:301:LEU:HB2	1:C:302:PRO:HD3	2.03	0.41
1:E:456:ARG:O	1:E:676:GLU:HA	2.20	0.41

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:G:426:GLU:HB3	1:G:427:PRO:HD3	2.03	0.41
1:K:426:GLU:HB3	1:K:427:PRO:HD3	2.03	0.41
1:K:53:ARG:HD2	1:K:53:ARG:N	2.36	0.41
1:M:382:ASP:HB2	1:M:383:PRO:HA	2.02	0.41
1:A:23:ILE:O	1:A:23:ILE:HG23	2.20	0.41
1:A:301:LEU:HB2	1:A:302:PRO:HD3	2.03	0.41
1:C:53:ARG:HD2	1:C:53:ARG:N	2.36	0.41
1:C:643:ALA:HA	1:E:489:ILE:HD12	2.02	0.41
2:F:78:GLU:HA	2:F:79:PRO:HD3	1.87	0.41
1:K:456:ARG:O	1:K:676:GLU:HA	2.20	0.41
1:M:301:LEU:HB2	1:M:302:PRO:HD3	2.03	0.41
1:O:456:ARG:O	1:O:676:GLU:HA	2.20	0.41
1:C:382:ASP:HB3	1:C:383:PRO:HA	2.02	0.41
1:E:426:GLU:HB3	1:E:427:PRO:HD3	2.03	0.41
1:G:456:ARG:O	1:G:676:GLU:HA	2.20	0.41
1:I:280:ASN:O	1:I:281:GLY:C	2.59	0.41
1:I:382:ASP:HB2	1:I:383:PRO:HA	2.02	0.41
2:P:170:GLU:N	2:P:170:GLU:OE1	2.54	0.41
1:A:280:ASN:O	1:A:281:GLY:C	2.59	0.41
1:A:61:ARG:HA	1:A:62:PRO:HD3	1.94	0.41
1:C:23:ILE:O	1:C:23:ILE:HG23	2.20	0.41
1:G:53:ARG:N	1:G:53:ARG:HD2	2.36	0.41
2:L:170:GLU:OE1	2:L:170:GLU:N	2.54	0.41
1:M:382:ASP:HB3	1:M:383:PRO:HA	2.02	0.41
1:M:53:ARG:N	1:M:53:ARG:HD2	2.36	0.41
1:A:382:ASP:HB3	1:A:383:PRO:HA	2.02	0.41
2:H:170:GLU:N	2:H:170:GLU:OE1	2.54	0.41
1:I:301:LEU:HB2	1:I:302:PRO:HD3	2.03	0.41
2:P:167:VAL:CG1	2:P:167:VAL:O	2.65	0.41
1:C:280:ASN:O	1:C:281:GLY:C	2.60	0.41
2:F:170:GLU:OE1	2:F:170:GLU:N	2.54	0.41
1:G:301:LEU:HB2	1:G:302:PRO:HD3	2.03	0.41
1:K:382:ASP:HB2	1:K:383:PRO:HA	2.02	0.41
2:N:78:GLU:HA	2:N:79:PRO:HD3	1.87	0.41
1:O:23:ILE:O	1:O:23:ILE:HG23	2.20	0.41
1:O:382:ASP:HB3	1:O:383:PRO:HA	2.02	0.41
2:D:78:GLU:HA	2:D:79:PRO:HD3	1.87	0.40
2:J:167:VAL:O	2:J:167:VAL:CG1	2.65	0.40
1:O:61:ARG:NH1	1:O:114:GLU:O	2.54	0.40
1:E:53:ARG:HD2	1:E:53:ARG:N	2.36	0.40
1:I:382:ASP:HB3	1:I:383:PRO:HA	2.02	0.40

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:K:382:ASP:HB3	1:K:383:PRO:HA	2.02	0.40
2:L:167:VAL:O	2:L:167:VAL:CG1	2.65	0.40
1:M:280:ASN:O	1:M:281:GLY:C	2.59	0.40
1:O:301:LEU:HB2	1:O:302:PRO:HD3	2.03	0.40
1:O:382:ASP:HB2	1:O:383:PRO:HA	2.02	0.40
2:F:297:ASN:C	2:F:297:ASN:OD1	2.60	0.40
1:K:301:LEU:HB2	1:K:302:PRO:HD3	2.03	0.40
1:M:61:ARG:NH1	1:M:114:GLU:O	2.54	0.40
1:M:23:ILE:HG23	1:M:23:ILE:O	2.20	0.40
1:C:61:ARG:NH1	1:C:114:GLU:O	2.54	0.40
2:D:297:ASN:C	2:D:297:ASN:OD1	2.60	0.40
1:E:301:LEU:HB2	1:E:302:PRO:HD3	2.03	0.40
1:G:61:ARG:NH1	1:G:114:GLU:O	2.54	0.40
1:K:61:ARG:NH1	1:K:114:GLU:O	2.54	0.40
1:K:23:ILE:HG23	1:K:23:ILE:O	2.20	0.40
2:N:170:GLU:OE1	2:N:170:GLU:N	2.54	0.40
2:D:170:GLU:N	2:D:170:GLU:OE1	2.54	0.40
2:D:210:TYR:N	2:D:211:PRO:HD2	2.37	0.40
1:E:61:ARG:NH1	1:E:114:GLU:O	2.54	0.40
2:F:210:TYR:N	2:F:211:PRO:HD2	2.37	0.40
1:G:382:ASP:HB3	1:G:383:PRO:HA	2.02	0.40
2:H:210:TYR:N	2:H:211:PRO:HD2	2.36	0.40
2:H:297:ASN:OD1	2:H:297:ASN:C	2.60	0.40
2:N:210:TYR:N	2:N:211:PRO:HD2	2.36	0.40
2:N:297:ASN:C	2:N:297:ASN:OD1	2.60	0.40
1:K:376:ARG:O	2:P:158:MET:SD	2.80	0.40

There are no symmetry-related clashes.

5.3 Torsion angles ⓘ

5.3.1 Protein backbone ⓘ

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all EM entries.

The Analysed column shows the number of residues for which the backbone conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
1	A	583/591 (99%)	550 (94%)	25 (4%)	8 (1%)	13	13
1	C	583/591 (99%)	550 (94%)	25 (4%)	8 (1%)	13	13
1	E	583/591 (99%)	550 (94%)	25 (4%)	8 (1%)	13	13
1	G	583/591 (99%)	550 (94%)	25 (4%)	8 (1%)	13	13
1	I	583/591 (99%)	550 (94%)	25 (4%)	8 (1%)	13	13
1	K	583/591 (99%)	550 (94%)	25 (4%)	8 (1%)	13	13
1	M	583/591 (99%)	550 (94%)	25 (4%)	8 (1%)	13	13
1	O	583/591 (99%)	550 (94%)	25 (4%)	8 (1%)	13	13
2	B	327/329 (99%)	302 (92%)	21 (6%)	4 (1%)	15	15
2	D	327/329 (99%)	302 (92%)	21 (6%)	4 (1%)	15	15
2	F	327/329 (99%)	302 (92%)	21 (6%)	4 (1%)	15	15
2	H	327/329 (99%)	302 (92%)	21 (6%)	4 (1%)	15	15
2	J	327/329 (99%)	302 (92%)	21 (6%)	4 (1%)	15	15
2	L	327/329 (99%)	302 (92%)	21 (6%)	4 (1%)	15	15
2	N	327/329 (99%)	302 (92%)	21 (6%)	4 (1%)	15	15
2	P	327/329 (99%)	302 (92%)	21 (6%)	4 (1%)	15	15
All	All	7280/7360 (99%)	6816 (94%)	368 (5%)	96 (1%)	19	14

All (96) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	382	ASP
1	A	412	SER
2	B	167	VAL
1	C	382	ASP
1	C	412	SER
2	D	167	VAL
1	E	382	ASP
1	E	412	SER
2	F	167	VAL
1	G	382	ASP
1	G	412	SER
2	H	167	VAL
1	I	382	ASP
1	I	412	SER
2	J	167	VAL
1	K	382	ASP

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Mol	Chain	Res	Type
1	K	412	SER
2	L	167	VAL
1	M	382	ASP
1	M	412	SER
2	N	167	VAL
1	O	382	ASP
1	O	412	SER
2	P	167	VAL
1	A	281	GLY
1	C	281	GLY
1	E	281	GLY
1	G	281	GLY
1	I	281	GLY
1	K	281	GLY
1	M	281	GLY
1	O	281	GLY
1	A	274	PRO
1	A	326	VAL
2	B	17	PRO
1	C	274	PRO
1	C	326	VAL
2	D	17	PRO
1	E	274	PRO
1	E	326	VAL
2	F	17	PRO
1	G	274	PRO
1	G	326	VAL
2	H	17	PRO
1	I	274	PRO
1	I	326	VAL
2	J	17	PRO
1	K	274	PRO
1	K	326	VAL
2	L	17	PRO
1	M	274	PRO
1	M	326	VAL
2	N	17	PRO
1	O	274	PRO
1	O	326	VAL
2	P	17	PRO
1	A	641	PRO
2	B	196	PRO

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Mol	Chain	Res	Type
1	C	641	PRO
2	D	196	PRO
1	E	641	PRO
1	G	641	PRO
1	I	641	PRO
1	K	641	PRO
1	M	641	PRO
1	O	641	PRO
1	A	62	PRO
1	A	355	ILE
2	B	183	PRO
1	C	62	PRO
1	C	355	ILE
2	D	183	PRO
1	E	62	PRO
1	E	355	ILE
2	F	183	PRO
2	F	196	PRO
1	G	62	PRO
1	G	355	ILE
2	H	183	PRO
2	H	196	PRO
1	I	62	PRO
1	I	355	ILE
2	J	183	PRO
2	J	196	PRO
1	K	62	PRO
1	K	355	ILE
2	L	183	PRO
2	L	196	PRO
1	M	62	PRO
1	M	355	ILE
2	N	183	PRO
2	N	196	PRO
1	O	62	PRO
1	O	355	ILE
2	P	183	PRO
2	P	196	PRO

5.3.2 Protein sidechains ⓘ

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all EM entries.

The Analysed column shows the number of residues for which the sidechain conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
1	A	527/527 (100%)	525 (100%)	2 (0%)	93	93
1	C	527/527 (100%)	525 (100%)	2 (0%)	93	93
1	E	527/527 (100%)	525 (100%)	2 (0%)	93	93
1	G	527/527 (100%)	525 (100%)	2 (0%)	93	93
1	I	527/527 (100%)	525 (100%)	2 (0%)	93	93
1	K	527/527 (100%)	525 (100%)	2 (0%)	93	93
1	M	527/527 (100%)	525 (100%)	2 (0%)	93	93
1	O	527/527 (100%)	525 (100%)	2 (0%)	93	93
2	B	268/268 (100%)	267 (100%)	1 (0%)	93	93
2	D	268/268 (100%)	267 (100%)	1 (0%)	93	93
2	F	268/268 (100%)	267 (100%)	1 (0%)	93	93
2	H	268/268 (100%)	267 (100%)	1 (0%)	93	93
2	J	268/268 (100%)	267 (100%)	1 (0%)	93	93
2	L	268/268 (100%)	267 (100%)	1 (0%)	93	93
2	N	268/268 (100%)	267 (100%)	1 (0%)	93	93
2	P	268/268 (100%)	267 (100%)	1 (0%)	93	93
All	All	6360/6360 (100%)	6336 (100%)	24 (0%)	93	93

All (24) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	53	ARG
1	A	302	PRO
2	B	144	PRO
1	C	53	ARG
1	C	302	PRO
2	D	144	PRO
1	E	53	ARG
1	E	302	PRO

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Mol	Chain	Res	Type
2	F	144	PRO
1	G	53	ARG
1	G	302	PRO
2	H	144	PRO
1	I	53	ARG
1	I	302	PRO
2	J	144	PRO
1	K	53	ARG
1	K	302	PRO
2	L	144	PRO
1	M	53	ARG
1	M	302	PRO
2	N	144	PRO
1	O	53	ARG
1	O	302	PRO
2	P	144	PRO

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains ⓘ

There are no non-standard protein/DNA/RNA residues in this entry.

5.5 Carbohydrates ⓘ

There are no carbohydrates in this entry.

5.6 Ligand geometry ⓘ

There are no ligands in this entry.

5.7 Other polymers ⓘ

There are no such residues in this entry.

5.8   Polymer linkage issues ⓘ

The following chains have linkage breaks:

Mol	Chain	Number of breaks
1	G	2
1	K	2
1	E	2
1	I	2
1	C	2
1	A	2
1	O	2
1	M	2

All chain breaks are listed below:

Model	Chain	Residue-1	Atom-1	Residue-2	Atom-2	Distance (Å)
1	A	503:ASP	C	612:ALA	N	20.73
1	C	503:ASP	C	612:ALA	N	20.73
1	E	503:ASP	C	612:ALA	N	20.73
1	G	503:ASP	C	612:ALA	N	20.73
1	I	503:ASP	C	612:ALA	N	20.73
1	K	503:ASP	C	612:ALA	N	20.73
1	M	503:ASP	C	612:ALA	N	20.73
1	O	503:ASP	C	612:ALA	N	20.73
1	A	73:GLU	C	87:ALA	N	9.30
1	C	73:GLU	C	87:ALA	N	9.30
1	E	73:GLU	C	87:ALA	N	9.30
1	G	73:GLU	C	87:ALA	N	9.30
1	I	73:GLU	C	87:ALA	N	9.30
1	K	73:GLU	C	87:ALA	N	9.30
1	M	73:GLU	C	87:ALA	N	9.30
1	O	73:GLU	C	87:ALA	N	9.30