Membrane constriction and thinning by sequential ESCRT-III polymerization

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16   **Abstract**

17   The Endosomal Sorting Complexes Required for Transport (ESCRTs) mediate diverse

18   membrane remodeling events. These activities typically require ESCRT-III proteins to stabilize

19   negatively-curved membranes, although recent work has indicated that certain ESCRT-IIIs also

20   participate in positive-curvature membrane shaping reactions. ESCRT-IIIs polymerize into

21   membrane-binding filaments, but the structural basis for negative versus positive membrane

22   curvature shaping by these proteins remains poorly understood. To learn how ESCRT-IIIs shape

23   membranes, we determined structures of human membrane-bound CHMP1B-only, membrane-

24   bound CHMP1B+IST1, and IST1-only filaments by electron cryomicroscopy. Our structures show

25   how CHMP1B first polymerizes into a single-stranded helical filament, shaping membranes into

26   moderate-curvature tubules. Subsequently, IST1 assembles a second strand upon the CHMP1B

27   filament, further constricting the membrane tube and reducing its diameter nearly to the fission

28   point. Each step of constriction, moreover, thins the underlying bilayer and lowers the barrier to

29   membrane fission. Together, our structures reveal how a two-component, sequential

30   polymerization mechanism drives membrane tubulation, tube constriction, and bilayer thinning.

31

32 **Introduction**

33   The Endosomal Sorting Complexes Required for Transport (ESCRT) belong to an

34 evolutionarily conserved pathway that mediates membrane remodeling and fission events

35 throughout the cell. The ESCRT machinery comprises staged complexes, including the early-

36 acting ALIX, ESCRT-I, and -II factors and the late-acting ESCRT-III factors and VPS4 family of

37 AAA+ ATPases. Early-acting factors bind to site-specific adaptors and then recruit the late-acting

38 factors that constrict and sever the target membrane. First discovered for their role in the formation

39 of multivesicular bodies (MVBs), ESCRT proteins serve essential functions in an expanding range

40 of cellular processes. Beyond MVBs, these processes include: cytokinetic abscission; egress of

41 enveloped viruses; sealing holes in nuclear, endosomal, and plasma membranes (*1-9*); and in

42 peroxisome biogenesis and function (*10, 11*). ESCRT-III proteins primarily shape negatively-

43 curved membranes, such as the necks of budding viruses or intralumenal vesicles, but we and

44 others have shown that some ESCRT-III proteins can also stabilize positively-curved membranes

45 (*12-15*). Despite their importance to the cell, the mechanisms that govern how ESCRT-III proteins

46 assemble and catalyze membrane remodeling reactions—of either positive or negative

47 membrane curvature—remain unclear.

48   Humans have 12 different ESCRT-III proteins that share a conserved secondary structure

49 core, including helices α1-α5. X-ray crystal structures of IST1 and CHMP3 revealed how these

50 helices fold into a compact conformation referred to as a "closed" state (*16-18*). Other ESCRT-

51 III proteins can adopt more elongated "open" states that can also polymerize (*16, 19-21*).

52 Structures of such open, elongated and assembled states are available for human CHMP1B (*14*),

53 *S. cerevisiae* Snf7 (*22*), and *D. melanogaster* Shrub (*23*). ESCRT-III polymerization may be

54 regulated by reversible switching between closed and open conformations. Such conformational

55 transitions could be regulated by protein-protein interactions with nucleating factors like the early-

56 acting ALIX or ESCRT-II factors, by membrane curvature (*24*), or by post-translation

57 modifications, such as ubiquitination, which can sterically hinder membrane-binding or

58 polymerization (*25*).

59   We previously reported a ~4 Å resolution electron cryomicroscopy (cryoEM) structure of

60 a helical filament containing CHMP1B and IST1 (*14*). This copolymer structure was surprising as

61 it consisted of two distinct strands: an inner strand of CHMP1B in an open conformation and an

62 outer strand of IST1 in a closed conformation. The IST1 strand was tightly associated with the

63 CHMP1B strand with 1:1 stoichiometry. The lumenal cavity of the copolymer was strongly

64 positively charged and capable of shaping negatively charged membranes into positive-curvature

65 membrane tubes in vitro and in vivo. Consistently, studies in living cells have shown that CHMP1B

66 and IST1 co-localize with the VPS4 family member SPASTIN along the positive curvature

67 surfaces of endosomal tubules and contact sites between lipid droplets and peroxisomes (*10, 14,*

68 *15, 26*). To better understand these new properties and roles for CHMP1B and IST1, and the still

69 unknown structural mechanism by which any ESCRT-III protein interacts with lipid bilayers, we

70 sought to understand how CHMP1B and IST1 work together to bind and constrict membranes.

71

72 **Results**

73 *Structure of the membrane-bound CHMP1B filament*

74   To learn how CHMP1B and IST1 remodel positively curved membranes, we sought to

75 capture stable membrane-bound polymers composed of these proteins. We previously showed

76 that incubating liposomes with CHMP1B led to formation of membrane tubules coated with protein

77 filaments (*14*). To increase the yield and stability of these membrane tubules, we optimized the

78 lipid composition and found that CHMP1B could remodel a variety of different liposome

79 compositions into tubules. Two factors, in particular, enhanced the prevalence and stability of

80 membrane-bound filaments for cryoEM analysis (Figure 1A and 1D): 1) incorporation of

81 polyunsaturated lipids (16:0-22:6 phosphocholine, SDPC) to increase membrane malleability (*27,*

82 *28*); and 2) increasing the concentration of negatively charged phospholipids such as PI(3)P

83   (phosphatidylinositol 3-phosphate), PI(3,5)P$_2$, or PI(4,5)P$_2$ to complement the highly basic charge

84   of the CHMP1B lumen (*14*). For our in-depth studies, we settled on liposomes containing 58 mol%

85   SDPC / 18 mol% POPS / 18 mol% cholesterol / 6 mol% PI(3,5)P$_2$.

86   From this optimized lipid mixture, we first analyzed CHMP1B-only membrane tubes and

87   found that they exhibited a range of diameters (~26-30 nm). This heterogeneity precluded high-

88   resolution studies, but by sorting the tubes based on diameter, we were able to reconstruct a 28

89   nm diameter tube to ~6 Å resolution. This reconstruction unambiguously showed the open-state

90   conformation of CHMP1B and the interconnected network of protomers within the single-

91   stranded, right-handed filament (Figure 1B–1C, Figure 1—figure supplement 1, Tables 1-3). Upon

92   membrane tubulation, the membrane tube diameter, the distance between outer leaflet phosphate

93   headgroups, narrows from >50 nm in the starting, spherical liposomes down to ~18 nm in the

94   CHMP1B-constricted cylindrical state. The inner leaflet headgroups are separated by ~12 nm.

95   Thus, the energy of CHMP1B self-assembly upon the membrane is sufficient to remodel low-

96   curvature membrane spheres into moderate-curvature membrane cylinders.

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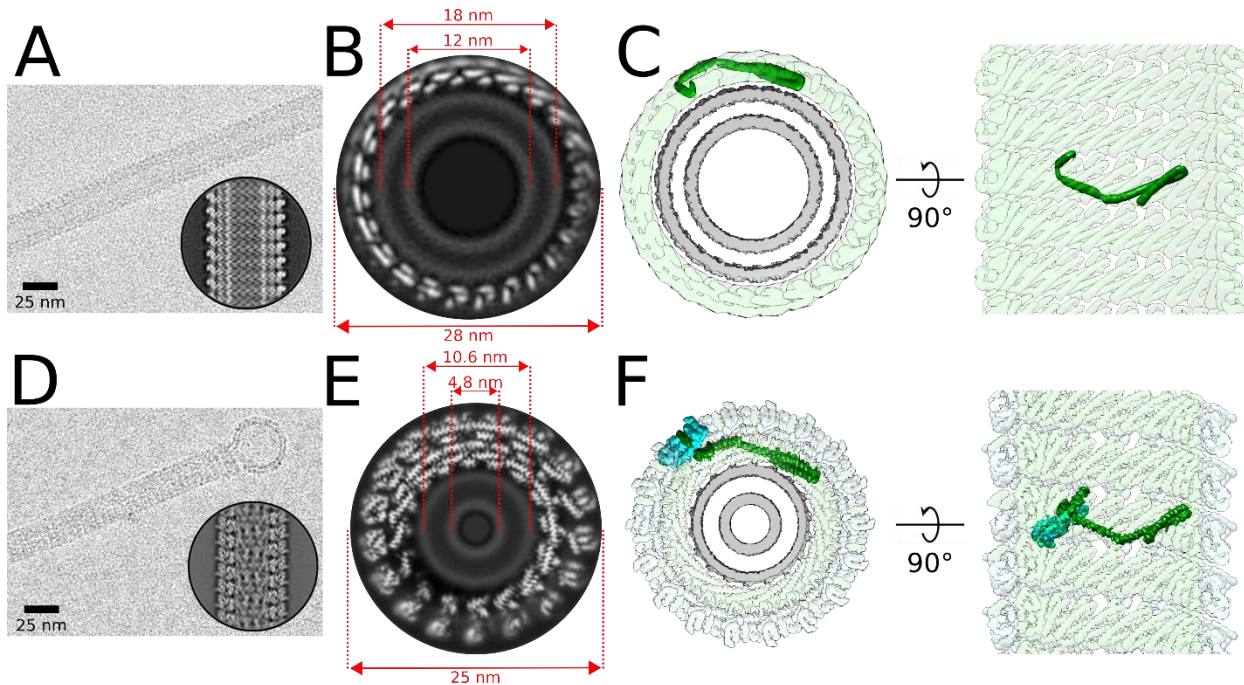
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99 **Figure 1. CHMP1B and IST1 sequentially constrict membrane tubes.**
100 (**A**) CryoEM micrograph of a membrane-bound CHMP1B tubule. Scale bar: 25 nm. Inset,
101 representative 2D class average. (**B**) A grey-scale slice looking down the helical axis of the 3D
102 cryoEM reconstruction of the membrane-bound CHMP1B filament. Diameters of the entire tube
103 and the membrane leaflet peak-to-peak distances are annotated. (**C**) *Left*, surface
104 representation of the same end-on view as in B down the helical axis. CHMP1B (green) coats
105 the exterior of the membrane bilayer (grey). A CHMP1B protomer is highlighted in dark green.
106 *Right*, internal view looking outward from the surface of the membrane. (**D**) IST1 further
107 constricts the CHMP1B-membrane filament nearly to the hemifission point. CryoEM micrograph
108 of a membrane-bound CHMP1B+IST1 filament with a vesicle protruding from the end. Scale
109 bar: 25 nm. Inset, representative 2D average. (**E**) A grey-scale slice looking down the helical
110 axis of the 3D cryoEM reconstruction of the membrane-bound, right-handed CHMP1B+IST1
111 filament. Diameters of the entire tube and membrane leaflet peak-to-peak distances are
112 annotated. (**F**) *Left*, surface representation of the same end-on view as in E down the helical
113 axis. (*Right*), internal view looking outward from the surface of the membrane. IST1 protomers
114 (cyan) binds to the exterior of CHMP1B (green), leading to constriction of the membrane (grey).
115 IST1 and CHMP1B promoters are highlighted in dark cyan and dark green, respectively.
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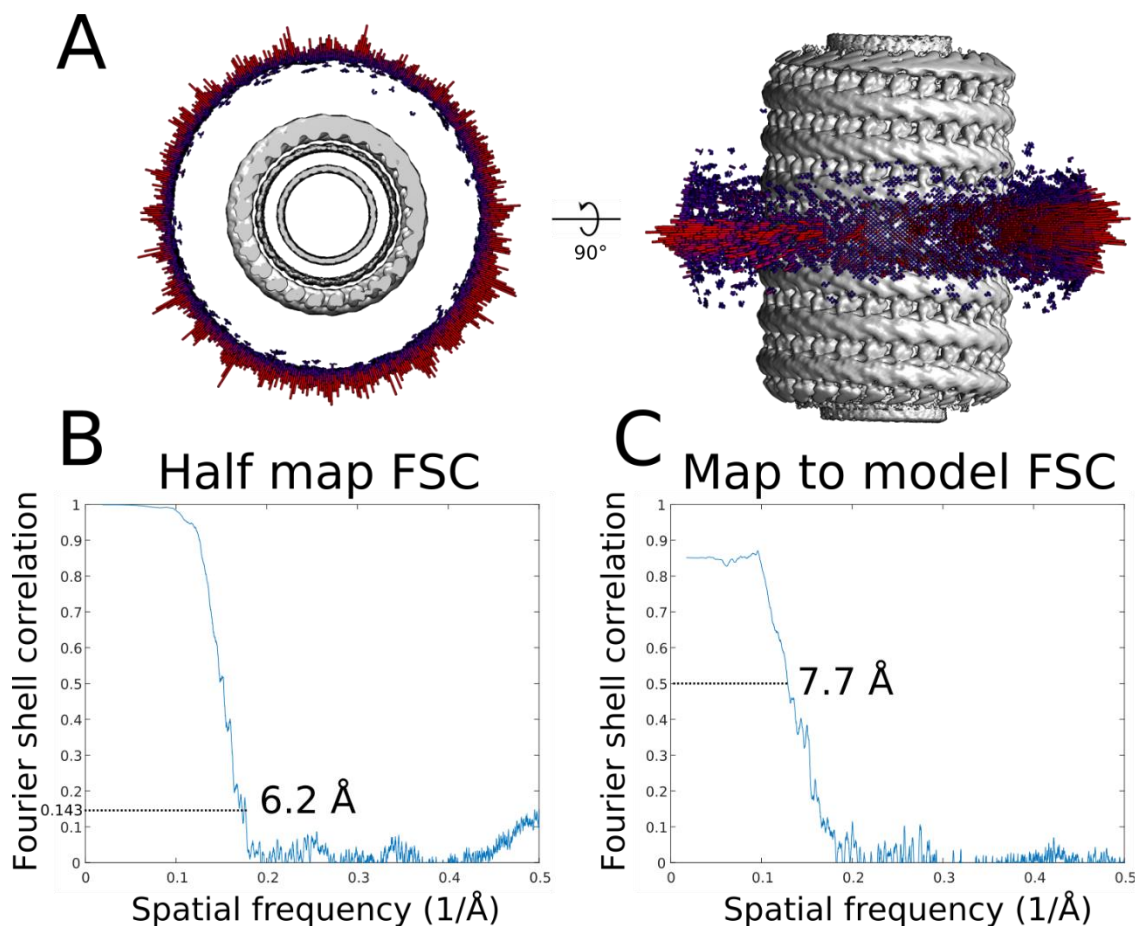
Figure 1—figure supplement 1. CryoEM validation of membrane-bound CHMP1B-only filament.

(**A**) Angular distribution of the membrane-bound CHMP1B filament. (**B**) Half map Fourier shell correlation (FSC) of the membrane-bound CHMP1B filament. (**C**) Map to model FSC of the membrane-bound CHMP1B filament.

Structures of left- and right-handed membrane-bound CHMP1B+IST1 filaments reveal highly constricted bilayers and filament flexibility

We next investigated the effect of sequentially adding IST1 to these filaments by cryoEM. Interestingly, from the same sample we were able to determine two 3D reconstructions of membrane-bound CHMP1B+IST1 filaments, corresponding to approximately equal populations of right- and left-handed helical filaments, to 3.2 Å and to 3.1 Å resolution, respectively (Figure 1E–1F, Figure 1—figure supplements 2–3, Tables 1–3). The right-handed CHMP1B+IST1 copolymer is a, one-start, double-stranded filament. The outer strand comprises IST1 in the closed conformation, and the inner strand comprises CHMP1B again in the open conformation

133   (*14*). The reconstruction also reveals a continuous and highly constricted bilayer within the lumen.

134   The overall outer diameter of the double-stranded filament is 25 nm, slightly narrower than the

135   membrane-bound CHMP1B-only filament. However, due to the presence of two protein strands,

136   the distance between outer leaflet phosphate headgroups is reduced to 10.6 nm, and the distance

137   between inner leaflet headgroups is just 4.8 nm (Figure 1E). Thus, the sequential addition of IST1

138   was sufficient to drive constriction of the CHMP1B strand and the internal membrane, narrowing

139   the lumenal inner leaflet diameter from 12 nm down to 4.8 nm.

140   The left-handed helical filament of the membrane-bound CHMP1B+IST1 copolymer also

141   a one-start, double-stranded filament. The left-handed copolymer is slightly more constricted, with

142   an outer diameter of 24 nm and an inner leaflet distance of 4.4 nm (Figure 1—figure supplement

143   2A).

144   Previous work has documented that ESCRT-III filaments (*30*), like bacterial flagella (*31,*

145   *32*), can adopt both left- and right-handed helical structures in vivo. To understand the structural

146   basis of this flexibility, we compared CHMP1B protomer conformations within the left- versus right-

147   handed copolymers (Figure 1—figure supplement 2B–2C). The overall root mean square

148   deviation of the Cα backbone (RMSD) between helices α1-α5 of CHMP1B protomers in the two

149   filaments is 1.2 Å. However, the RMSD is smaller when only aligning either the N-terminal helices

150   α1-α2 (~0.7 Å) or the C-terminal helices α4-α5 (~0.6 Å) (Figure 1—figure supplement 2B–2C).

151   Therefore, switching between a left- or right-handed filament can be achieved simply by a small

152   change in the joint between helices α2 and α3 (Figure 1—figure supplement 2B–2C).

153   Sequential assembly of the IST1 strand does not discriminate between the left- and right-

154   handed filaments. The RMSD of single IST1 protomers or between "j" and "j+1" subunits between

155   the two copolymers are only 0.5 Å and 0.6 Å respectively. There are no significant differences in

156   how IST1 assembles around either the left- or right-handed filaments. We envision that as one

157   turn of CHMP1B finishes a revolution, it will either continue polymerizing 'above' or 'below' the

158   initiating subunit, and this choice will define the helical hand. Thus, it appears that a stochastic

159   flexing between helices α2-α3 will allow either handedness to propagate. We did not observe left-

160   handed helical polymers in our prior work on the lipid-free or the nucleic-acid templated copolymer

161   structure, perhaps due to the different solution and nucleation conditions that promoted lipid-free

162   polymerization (*14, 29*). While we did not reconstruct a left-handed membrane-bound CHMP1B-

163   only filament, this likely simply reflects the limited dataset size and diameter variability.

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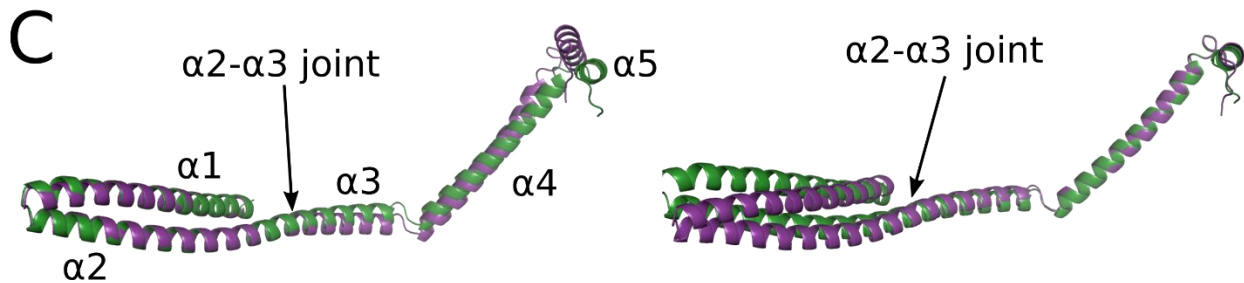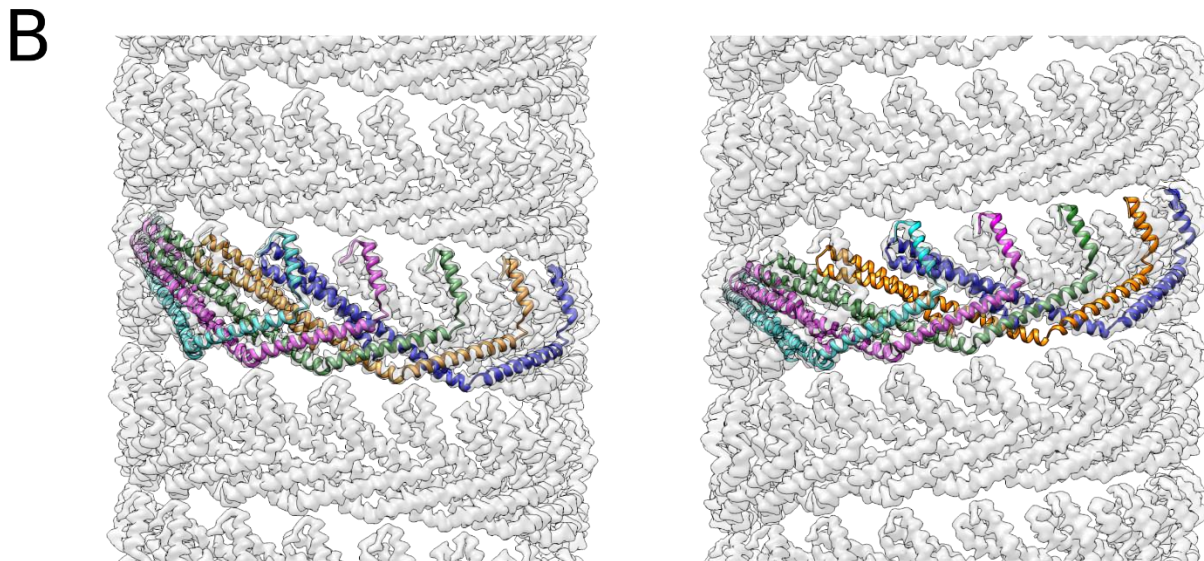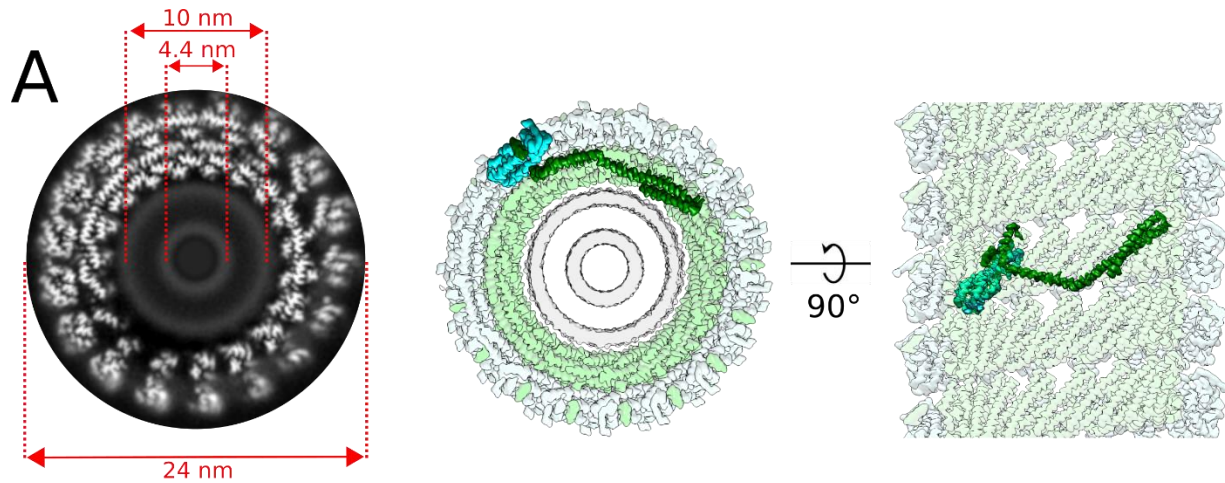
165 **Figure 1—figure supplement 2. CryoEM reconstruction of the membrane-bound**
166 **CHMP1B+IST1 filament at higher curvature and comparison of left- and right-handed**
167 **CHMP1B+IST1 filaments.**
168 (**A**) CryoEM 3D reconstruction of the membrane-bound left-handed CHMP1B+IST1 filament.
169 End-on view down the helical axis in grey-scale (*left*) or colored (*middle*). *Right*, internal view
170 looking outward from the membrane surface along the helical axis. IST1 protomers (cyan) bind
171 to the exterior of CHMP1B (green), leading to constriction of the membrane (grey). IST1 and
172 CHMP1B promoters are highlighted in dark cyan and green, respectively. Diameters of the
173 entire tube and membrane leaflet peak-to-peak distances are annotated. (**B**) Electron density
174 maps of CHMP1B from the left-handed (*left*) or right-handed (*right*) membrane-bound
175 CHMP1B+IST1 filaments. Five copies of CHMP1B are shown as ribbons. (**C**) Superposition of a
176 CHMP1B protomer from the left-handed (purple) and right-handed (green) CHMP1B+IST1
177 filaments aligned to the CHMP1B N-terminal α1-α2 helices (*left*) or C-terminal α4-α5 helices
178 (*right*).
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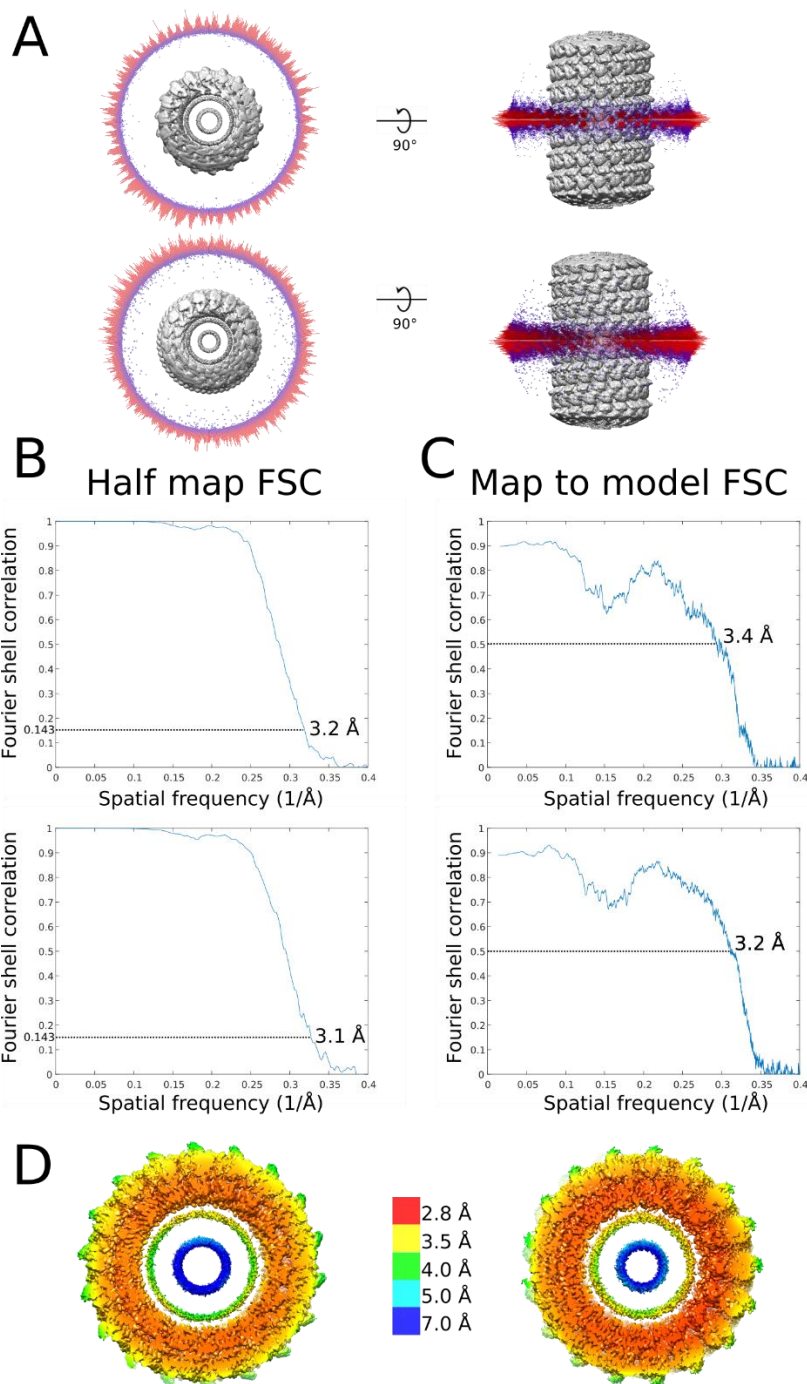
180
181 **Figure 1—figure supplement 3. Local resolution estimates and cryoEM validation of**
182 **membrane-bound CHMP1B+IST1 filaments.**
183 (**A**) Angular distribution of right-handed (*top*) and left-handed (*bottom*) membrane-bound
184 CHMP1B+IST1 filaments. (**B**) Half map FSCs of right-handed (*top*) and left-handed (*bottom*)
185 CHMP1B+IST1 filaments. (**C**) Map to model FSCs right-handed (*top*) and left-handed (*bottom*)
186 CHMP1B+IST1 filaments. (**D**) Local resolution estimates of right-handed (*left*) and left-handed
187 (*right*) CHMP1B+IST1 filaments.
188

189   *The joint between CHMP1B helices α3 and α4 allows for different curvatures*

190   To understand how CHMP1B polymers adopt different curvatures, we examined how the

191   CHMP1B conformation changes between the "moderate" constriction CHMP1B-only and the

192   "high" constriction CHMP1B+IST1 filaments. In all reconstructions, a single CHMP1B protomer

193   ("i") interacts with eight other protomers in an interconnected network. Among these, helix α5 of

194   the "i" subunit passes behind three neighboring subunits and binds the closed end of the α1-α2

195   hairpin of the "i+4" subunit (Figure 2A). Thus, CHMP1B subunits always interweave with the same

196   protomers, regardless of the filament's overall degree of curvature. There is little contact between

197   neighboring turns of the CHMP1B filament in either state, suggesting that turns can slide past one

198   another during constriction (Figure 2D, Figure 2—movie supplement 1, Figure 2—movie
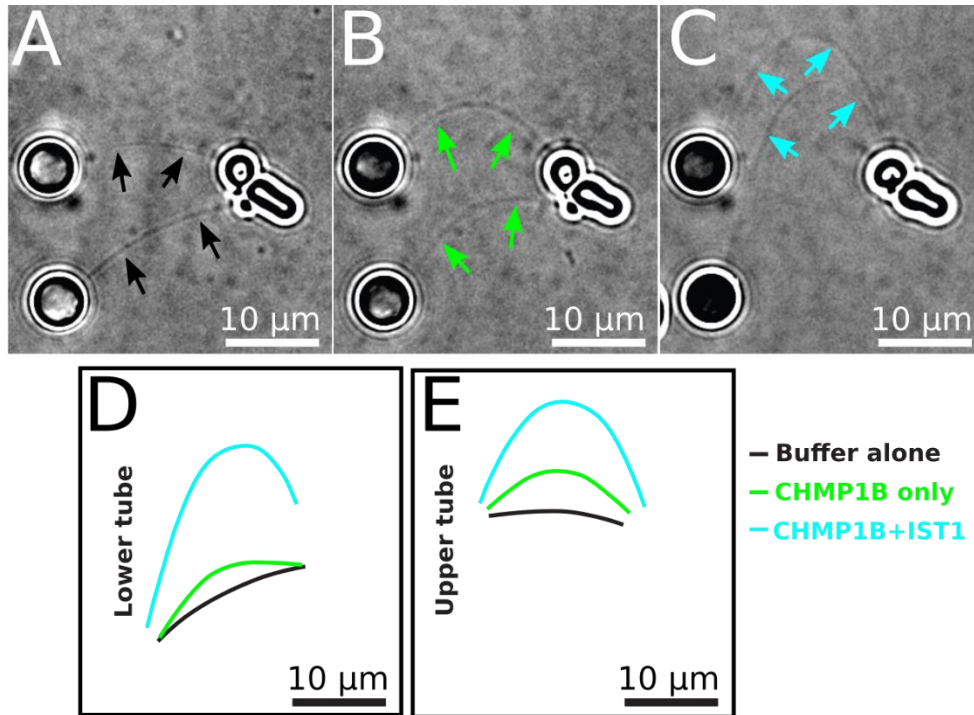
199   supplement 2).

200   We next compared the conformation of a CHMP1B protomer between the CHMP1B-only

201   and the right-handed CHMP1B+IST1 membrane-bound structures (Figure 2C). The RMSD of

202   helices α1-α5 is 2.4 Å between the two states. However, the RMSD drops to 1.1 Å when

203   comparing only the N-terminal helices α1-α2. Similarly, comparing just the C-terminal helices α4-

204   α5 lowers the RMSD to 1.5 Å. While there is again a small change in the angle between helices

205   α2-α3, the largest conformation difference is at the "elbow" between helices α3-α4 (Figure 2B-C).

206   This flex in the elbow joint, when propagated across an entire turn of the helical assembly, leads

207   to membrane constriction and also tubule elongation (Figure 2D, Figure 2—movie supplement 1,

208   Figure 2—movie supplement 2). To confirm this tube elongation, we used holographic optical

209   tweezers to hold traptavidin-coated beads and pull membrane tubes from giant unilamellar

210   vesicles (GUVs), and then visualized changes in membrane tube lengths as a function of ESCRT-

211   IIIs. CHMP1B addition elongated the tubes slightly, and subsequent incorporation of IST1

212   produced even longer tubes (Figure 2—figure supplement 1, Figure 2—movie supplement 3). In

213   agreement with our structural studies, tube elongation was dependent on CHMP1B as addition of

214   IST1 alone did not induce membrane elongation (Figure 2—movie supplement 4).

215   Previous work has shown that other ESCRT-IIIs like CHMP2, CHMP3, and CHMP4 also

216   form filaments with a wide range of curvatures (*33-35*). Owing to the high homology of the ESCRT-

217   III core, we suggest that these proteins also have dynamic elbow joints that will accommodate

218   changes in filament curvature. As noted above, there are minimal contacts between turns of

219   CHMP1B to stabilize inter-turn interactions (Figure 2—movie supplement 2). Indeed, in vivo

220   images of ESCRT-IIIs at different sites of action reveal conical spirals with significant gaps

221   between turns (*35-37*). Thus, the inherent flexibility of ESCRT-III subunits could allow such loosely

222   packed filaments to form with a range of diameters and helical pitches, and to slide past one

223   another upon constriction.
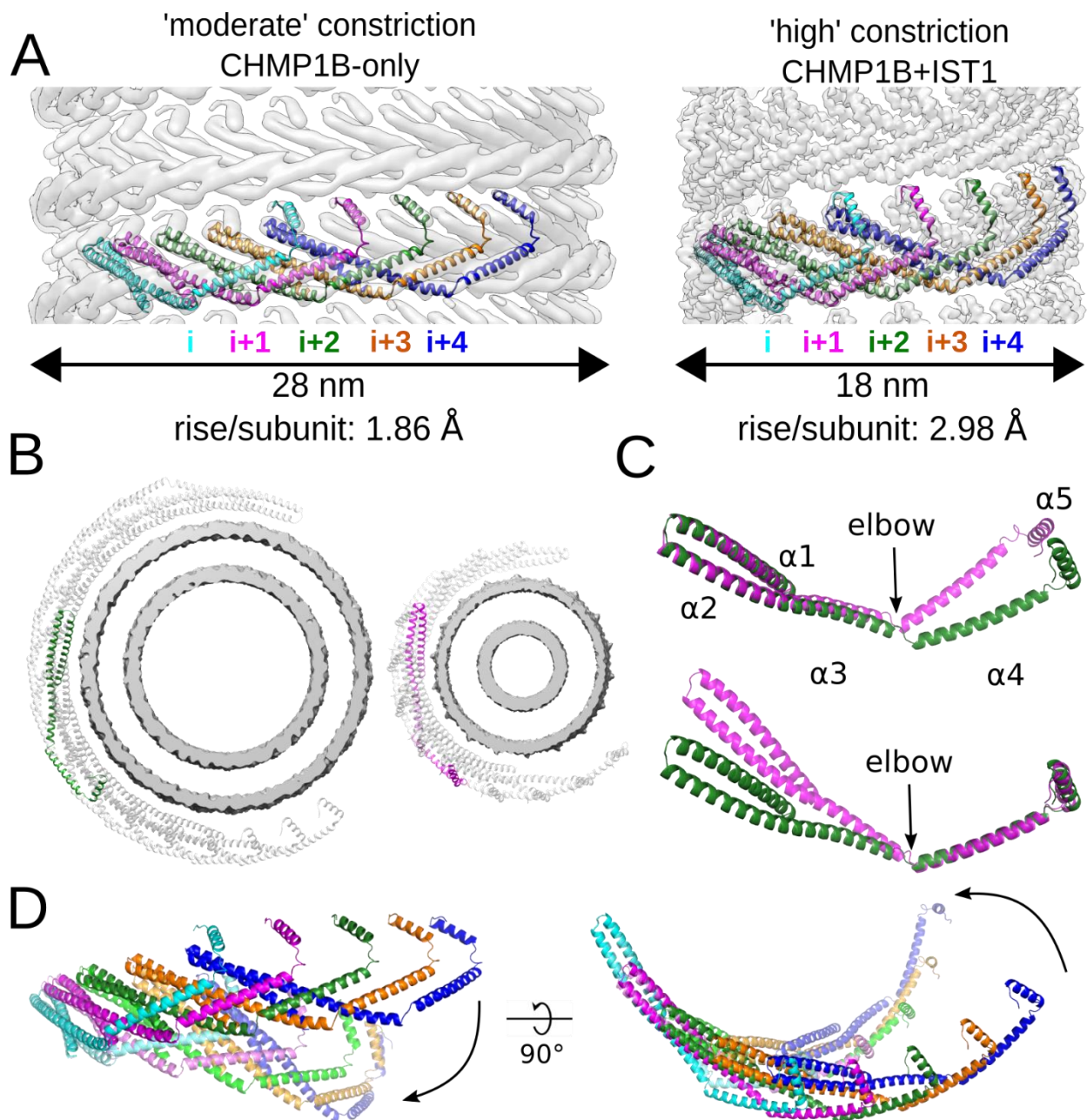
Figure 2. CHMP1B interlocks in the same arrangement in all structures and flexes at the α3-α4 elbow to accommodate different curvatures.

(**A**) CryoEM density maps of CHMP1B from the membrane-bound CHMP1B (*left*) or right-handed CHMP1B+IST1 (*right*) filaments. Five interlocked copies of CHMP1B are shown as ribbons. The C-terminal helix α5 of the i protomer always engages helices α1-α2 of the i+4 protomer. The rise per subunit for each helical filament is denoted. (**B**) Comparison of arc curvatures of CHMP1B across the two filaments. Top-down views of half a turn of CHMP1B subunits are shown for either the CHMP1B (*left*) or CHMP1B+IST1 (*right*) membrane filaments. The membrane bilayers are shown in grey and the central promoters are shown in green and magenta for the respective filaments. (**C**) Superposition of a CHMP1B protomer from the CHMP1B (green) and CHMP1B+IST1 (magenta) filaments aligned to the CHMP1B N-terminal helices α1-α2 (top) or C-terminal helices α4-α5 (bottom). The biggest conformational change

237   occurs at the elbow joint. (**D**) Superposition of 5 consecutive subunits (colored from left to right
238   in cyan, magenta, green, orange, and blue) of CHMP1B from the CHMP1B (opaque) and the
239   CHMP1B+IST1 (semi-transparent) filament. The respective first protomers from each are
240   aligned as in (C).

Figure 2—figure supplement 1. Real-time monitoring of CHMP1B and IST1 membrane constriction and elongation.

(**A-C**) Still images representing deformation of two membrane tubes due to transverse flow of (A) buffer alone, (B) then 0.5 µM CHMP1B, (C) and a final addition of 0.5 µM IST1 is shown. Solid arrows in (A)-(C) highlight tubule locations. (**D-E**) Contours of lower (D) and upper (E) membrane tubes extracted from panels (A)-(C) showing the extension of the tubes upon addition of CHMP1B and IST1.

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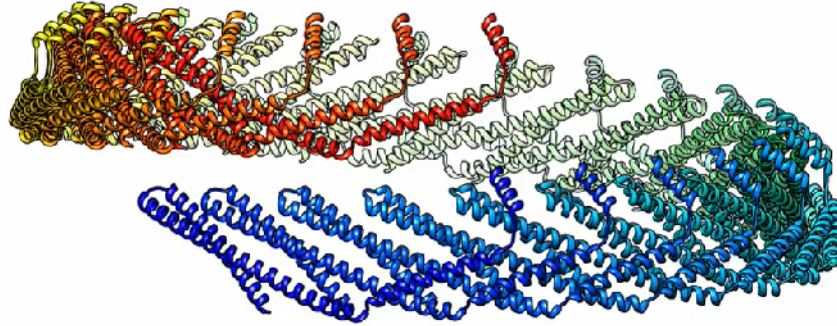251   **Figure 2—movie supplement 1. Elbow flexing of one CHMP1B subunit.**
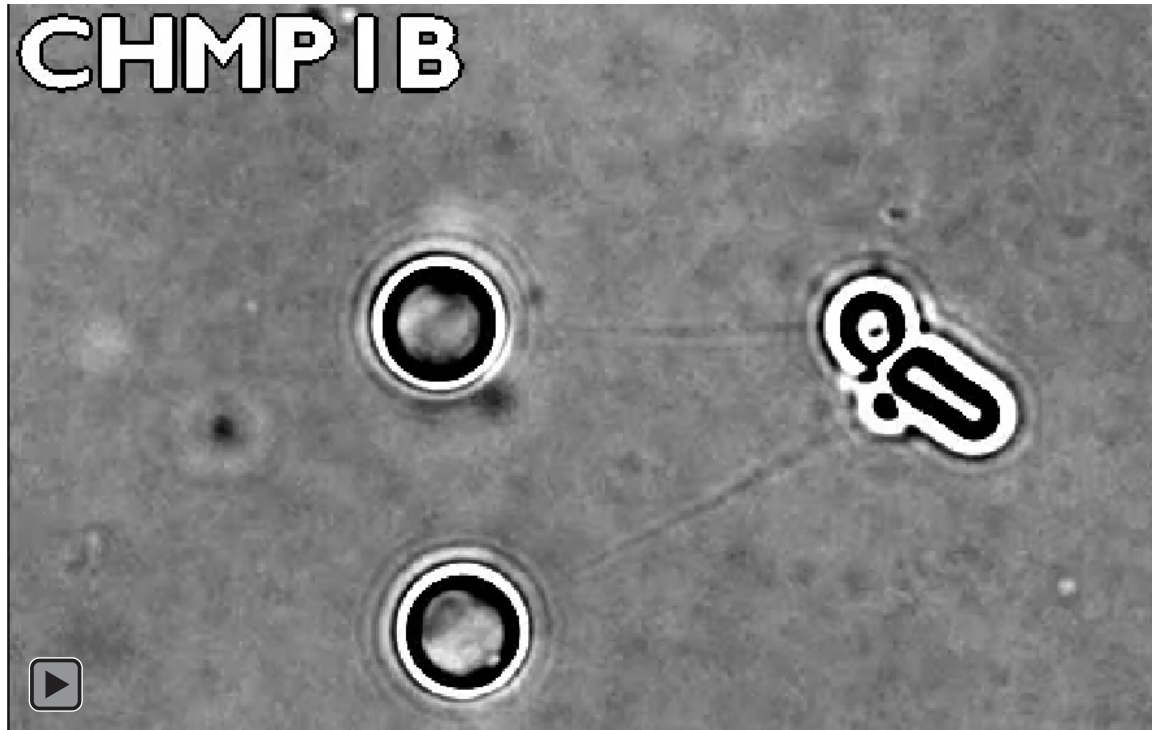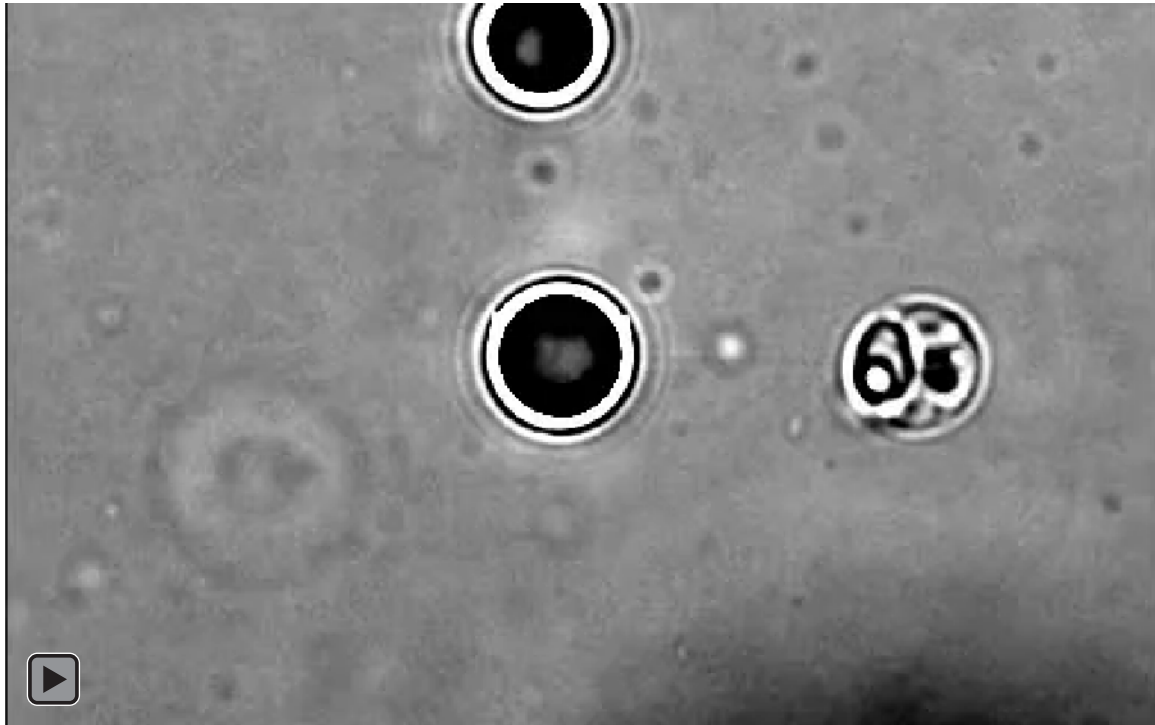
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Figure 2—movie supplement 2. Flexing of a full turn of CHMP1B subunits from low to high constriction.

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257

258 **Figure 2—movie supplement 3. Real time recording of membrane tube elongation by**
259 **CHMP1B and IST1.**
260

261

Figure 2—movie supplement 4. IST1 alone does not promote membrane tube elongation.

263

264   *IST1 polymerization drives constriction*

265   To understand how IST1 induces constriction of CHMP1B-membrane filaments, we first

266   docked two IST1 subunits (j and j+1) onto sequential CHMP1B protomers (i and i+1) of the

267   moderate-curvature CHMP1B-only membrane, based on the CHMP1B-IST1 intersubunit

268   interactions observed in the copolymer filaments (Figure 3A). We then compared how the

269   interactions between IST1 subunits would change between this 'initial IST1 binding' state and

270   those observed in the CHMP1B+IST1 filaments (Figure 3B–3C). The IST1 j+1 subunit from the

271   copolymer subunit swings closer (~8 Å) to the j subunit when compared to the j+1 subunit from

272   the initial IST1 binding state, adding 480 $Å^2$ of buried surface area (BSA) (Figure 3—movie

273   supplement 1, Figure 3—movie supplement 2). This swing enables the formation of hydrogen

274   bonds between IST1 residues D77 and R82 on helix α3 of the j subunit with R55 and E57 on helix

275   α2 of the j+1 subunit (Figure 3C). In contrast, IST1 subunits make only minimal contacts between

276   adjacent turns (15 $Å^2$ of BSA between the j and j+18 subunits and no contacts between the j+1

277   and j+18 subunits). Thus, interactions between the j and j+1 subunits along the IST1 strand

278   provide the force that flexes the CHMP1B elbow and consequently constricts and elongates the

279   filament. These sliding, lateral interactions to promote changes in filament architecture have also

280   been observed for the yeast ESCRT-III proteins Snf7 and Vps24 (*38*).

281   IST1 has recently been reported to induce constriction of ESCRT-III assemblies in vitro

282   (*39*). To understand how IST1 polymerization could drive constriction, we determined a 3D

283   reconstruction of a protein-only IST1 filament (Figure 3D). We previously showed that an N-

284   terminal construct of IST1 (residues 1-189) harboring R16E and K27E mutations (IST1$_{NTD}^{R16E/K27E}$)

285   cannot co-assemble with CHMP1B as the mutations destabilize the CHMP1B-IST1 interface, but

286   can still form tightly-packed homopolymeric helical tubes (*14*). The IST1-only filaments were

287   heterogeneous in diameter (~18-28 nm), with the majority of filaments narrower than the

288   CHMP1B+IST1 copolymers (24-25 nm). We identified a major subset of IST1$_{NTD}^{R16E/K27E}$ tubes

289   that were ~20 nm wide and reconstructed this class to moderate resolution, revealing the

290   secondary structure elements of closed-conformation IST1$_{NTD}$ subunits (Figure 3—figure

291   supplement 1). IST1$_{NTD}$$^{R16E/K27E}$ forms a single-stranded, right-handed filament (Figure 3D). In the

292   absence of a membrane or CHMP1B, therefore, IST1 alone polymerizes into even narrower

293   helical assembly than either of the copolymers. Interestingly, the reconstruction of these IST1-

294   only filaments revealed that IST1 always adopted the closed conformation, suggesting that IST1

295   may exclusively function in the closed state.

296   To understand how the arrangement of IST1 subunits between IST1-only and

297   CHMP1B+IST1 filaments differ, we compared the interactions of IST1 subunits at the inter-turn

298   interface between the copolymer (j, j+1, and j+18 subunits) and the IST1$_{NTD}$$^{R16E/K27E}$ (j, j+1, and

299   j+14 subunits) filaments. The RMSD between a protomer from the IST1$_{NTD}$$^{R16E/K27E}$ filament and

300   an IST1 protomer from the copolymer filament is ~1 Å, with minimal changes in the Cα backbone.

301   The j and j+1 contact that defines nearest-neighbor IST1-IST1 interactions is conserved in both

302   structures, but the IST1 j and j+1 subunits swing slightly closer together (~2 Å) in the

303   IST1$_{NTD}$$^{R16E/K27E}$ filaments (Figure 3E). Propagation of this subtle change actually decreases the

304   BSA between the j and j+1 subunits (from 480 Å^2 to 340 Å^2), but increases inter-turn contacts,

305   which are predominantly made by helix α5 of the j+1 subunit contacting helix αA of the j+14

306   subunit (220 Å^2) and additional packing between the j+1 and j+14 subunits (100 Å^2). This results

307   in an overall increase of 165 Å^2 of BSA at the inter-turn interface for the IST1$_{NTD}$$^{R16E/K27E}$ filament

308   (Figure 3E). Thus, unlike the constriction seen upon addition of IST1 to the CHMP1B filament,

309   this second constriction step appears to be driven by inter-turn contacts.

310   We note that the inter-turn interactions involving the j and j+14 subunit in the IST1-only

311   filament are unattainable in the CHMP1B+IST1 filament, as the presence of the CHMP1B helix

312   α6, the MIT interacting motif (MIM), sterically blocks the IST1 j and j+14 packing (Figure 3—figure

313   supplement 2). Intriguingly, the interaction between IST1 helix α5 and the CHMP1B MIM supports

314   efficient assembly of the copolymer (*29*). We speculate that modulation of this interface by the

315   VPS4 family of ATPases could, in principle, regulate the degree of constriction achieved by the

316   CHMP1B+IST1 filament.

317
318   **Figure 3. IST1 polymerization drives constriction of the CHMP1B+IST1 filament.**
319   (**A**) Model of two IST1 subunits (j, j+1), colored pink, initially binding onto the CHMP1B filament
320   (green). (**B**) CryoEM reconstruction of the right-handed CHMP1B+IST1 filament, with CHMP1B
321   and IST1 in green and cyan, respectively. Three IST1 subunits (j, j+1, j+18) are highlighted.(**C**)
322   Superposition of the j and j+1 IST1 subunits from (A) and (B), with the j subunits used for
323   alignment. (*Inset*), new electrostatic interactions between helix α3 (pink) from the j subunit and
324   helix α2 (cyan) from the j+1 subunit help stabilize intra-IST1 contacts to drive constriction. (**D**)
325   CryoEM 3D reconstruction of the IST1$_{NTD}$$^{R16E/K27E}$ filament (bronze). Three IST1 subunits (j, j+1,
326   j+14) are highlighted. (**E**) Superposition of the j, j+1, and j+14 subunits from the IST1$_{NTD}$$^{R16E/K27E}$
327   filament in (D) with IST1 subunits from the CHMP1B+IST1 filament in (B). Protomers were
328   aligned by the j subunit. The boxed area highlights helix α5 of the j subunit and helix αA from
329   the j+14 subunit driving inter-turn interactions.

330

331
332 **Figure 3—figure supplement 1. CryoEM validation of the IST1$_{NTD}$$^{R16E/K27E}$ filament.**
333 (**A**) Angular distribution of the IST1NTDR16E/K27E filament. (**B**) Half map FSC of the
334 IST1NTDR16E/K27E filament. (**C**) Map to model FSC of the IST1NTDR16E/K27E filament.
335
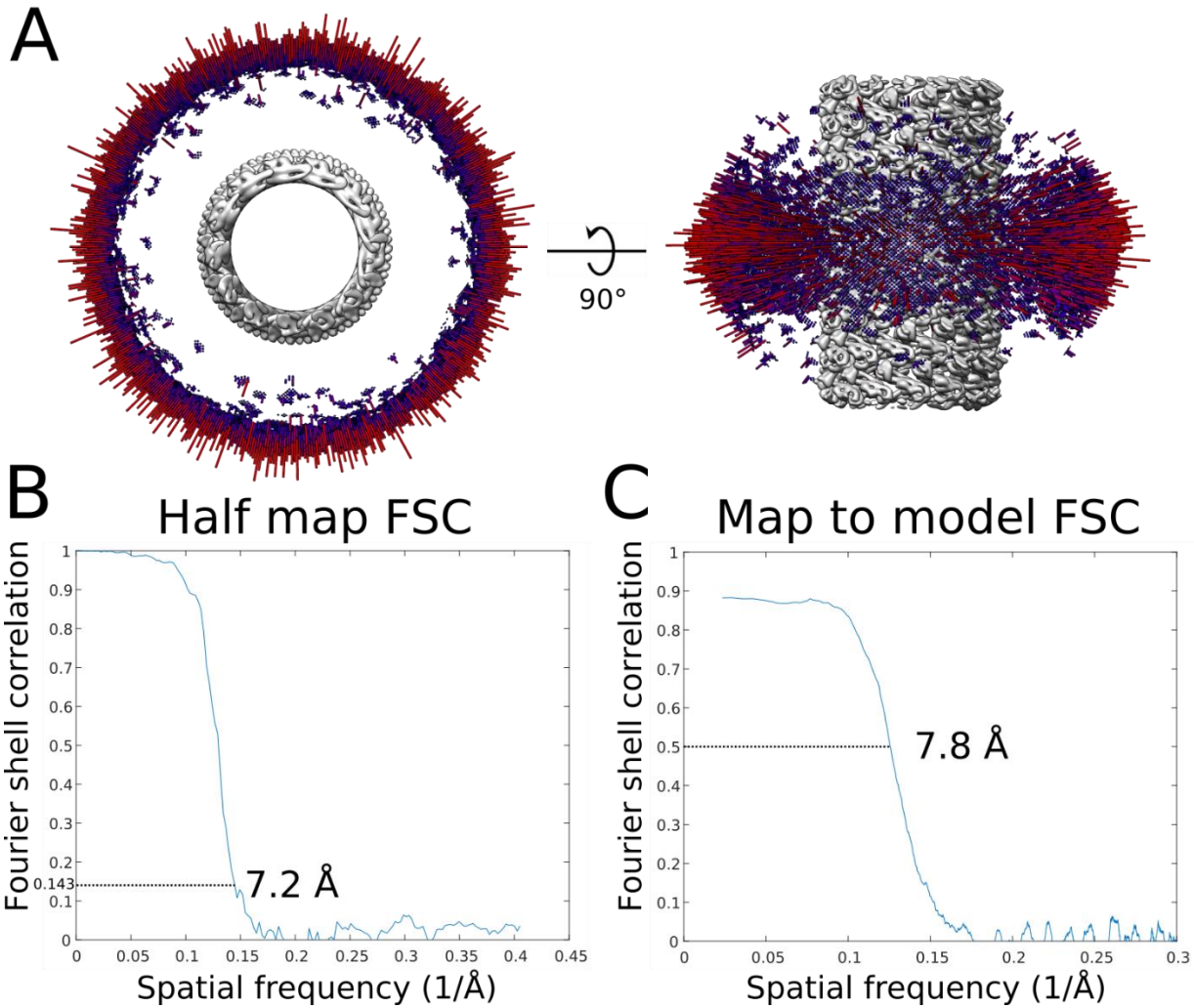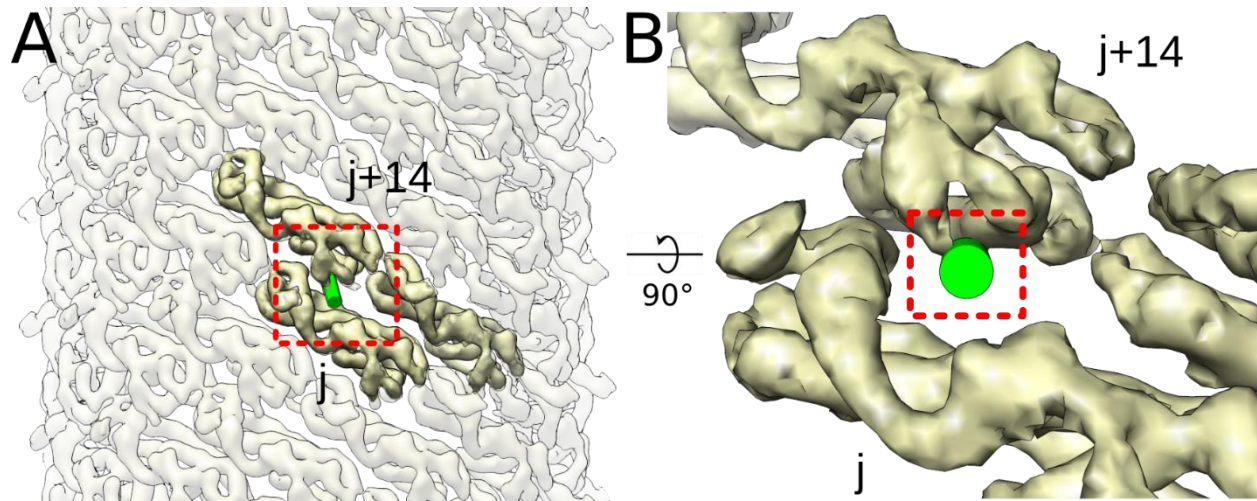
Figure 3—figure supplement 2. Steric clashing between the CHMP1B MIM and inter-turn IST1 subunits would prevent IST1 from achieving its preferred curvature in the copolymer.
(**A**) External view of the $IST1_{NTD}^{R16E/K27E}$ filament with one CHMP1B MIM (shown as a green cylinder) docked onto the $IST1_{NTD}^{R16E/K27E}$ j subunit. (**B**) Zoomed in view of boxed area in (A) highlighting how the CHMP1B MIM clashes with the IST1 j+14 subunit.

344
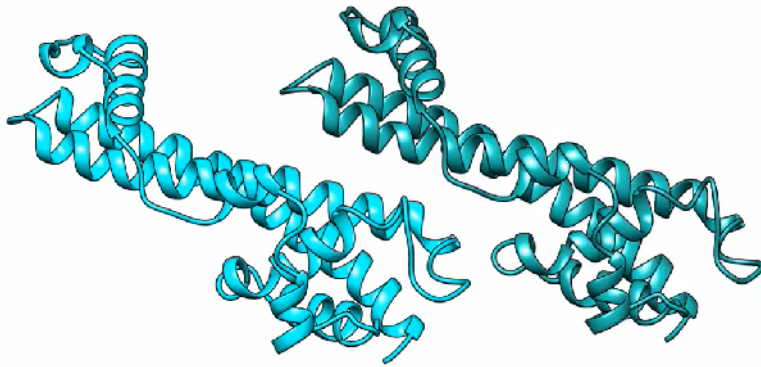
Figure 3—movie supplement 1. Swinging of two IST1 subunits from initial binding to the CHMP1B filament to the constricted state (side view).

347
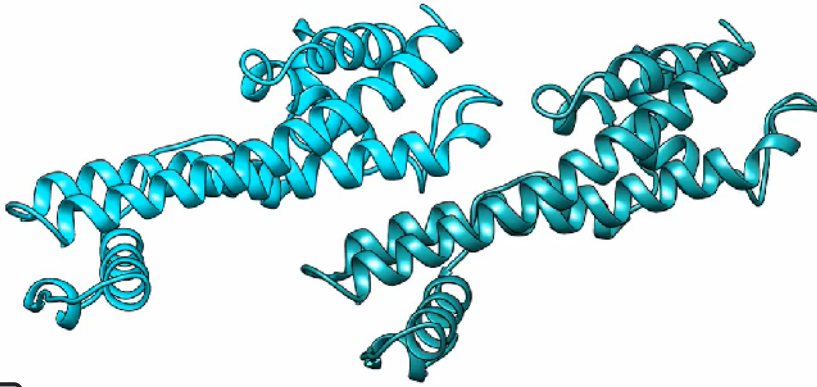
348

349 **Figure 3—movie supplement 2. Swinging of two IST1 subunits from initial binding to the**
350 **CHMP1B filament to the constricted state (top-down view).** The membrane would lie at the
351 top of the animation.
352

353   *The membrane bilayer thins and the inner leaflet compresses at high curvature*

354   We also examined the consequences to the membrane as a function of increasing

355   curvature stress (Figure 4). In both copolymer reconstructions, helix α1 of CHMP1B faced the

356   membrane, and the surface of the bilayer appeared to be "dimpled" by certain amino acids (Figure

357   4A). Specifically, the conserved CHMP1B residues F9, F13, K16, and R20, which lie on the same

358   face of helix α1, comprise the protein-membrane interface (Figure 4B). No residues appeared to

359   insert deeply into the membrane. Rather, they appear to sit at the hydrated surface of the bilayer.

360   The mixed aromatic and cationic character of the CHMP1B region that most closely approaches

361   the membrane does not suggest any lipid recognition specificity beyond net anionic charge, with

362   the basic residues complementing the negatively charged membrane. It is somewhat surprising

363   that the hydrophobic residues remain fully hydrated at this degree of constriction, but we

364   speculate that they may be poised to insert into the membrane (see below).

365   By measuring the peak-to-peak distances between the outer leaflet and inner leaflet

366   headgroups, we observed a correlation between bilayer thinning and the degree of membrane

367   constriction by CHMP1B and by CHMP1B+IST1. To determine the bilayer thickness of our initial,

368   unconstricted bilayers, we performed small-angle X-ray scattering (SAXS) of our relaxed

369   liposomes. This experiment yielded a thickness estimate of 38.6 ± 0.6 Å (Figure 4—figure

370   supplement 1), consistent with previously published SAXS measurements of membranes

371   composed primarily of SDPC (*40*). We compared this measurement with bilayer profiles from

372   cryoEM 2D averages of segments of our liposomes, which yielded a thickness of 36.9 ± 1.8 Å,

373   which is within the experimental uncertainty of the SAXS measurement (Figure 4C–4D). Upon

374   constriction by CHMP1B alone, the bilayer compressed to 29.9 ± 0.0 Å. The sequential addition

375   of IST1 led to further compression of the membrane to 28.6 ± 0.1 Å and 28.4 ± 0.1 Å for the right-

376   handed and left-handed copolymers, respectively (Figure 4C–4D). In addition, the intensity of the

377   inner leaflet increased as a function of constriction, with inner/outer leaflet peak intensities of 1.08

378   ± 0.03, 1.12 ± 0.01, 1.40 ± 0.01, and 1.46 ± 0.03 for the liposomes, moderate-constriction

379   CHMP1B filaments, and the high-constriction right-handed and left-handed CHMP1B+IST1

380   filaments, respectively (Figure 4E). Thus, the lipid density in the inner leaflet increases

381   significantly as the membrane tubule constricts towards the fission point.

382   In agreement with physical models of membrane behavior (*41, 42*), our reconstructions

383   indicate that the bilayer thins as the membrane is constricted (Figure 4C–4D) and that the outer

384   leaflet headgroups separate while the inner leaflet headgroups become more crowded (Figure

385   4E). To accommodate this thinning, the acyl chains from both leaflets likely become more

386   disordered and less extended (Figure 4F). It has also been suggested that curvature stress

387   causes local lipid composition changes that increases the membrane line tension and promote

388   fission (*43, 44*). We speculate that these changes may lower the activation barrier for fission. With

389   this specific lipid composition, however, the tubes remain stable with only a 4.2 nm gap between

390   the inner leaflet headgroups across the lumen.
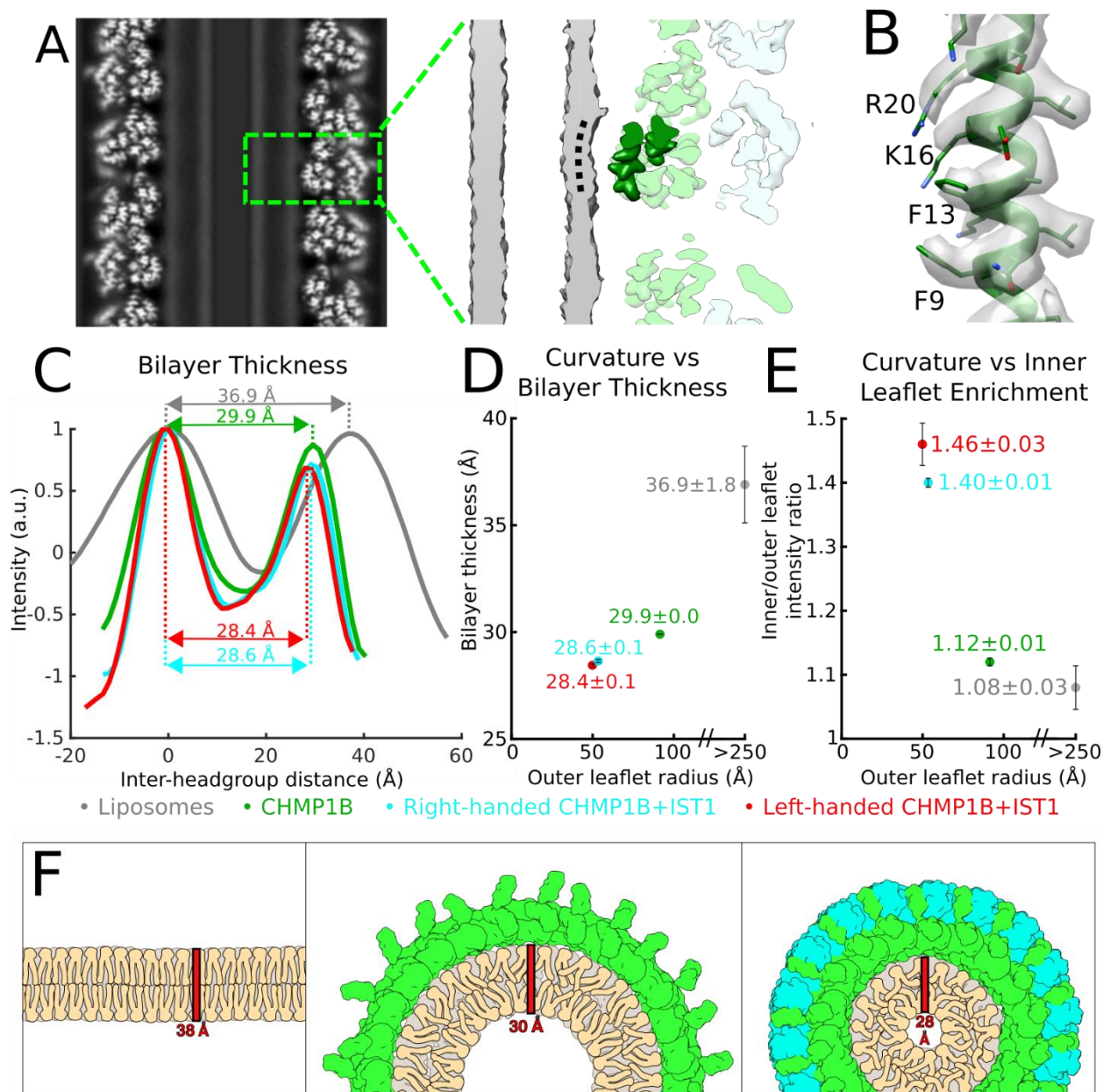
Figure 4. The bilayer thins and the inner leaflet crowds under high curvature.
(**A**) The outer leaflet buckles under extreme curvature. *Left*, central slice along the helical axis.
Right, zoomed view of boxed area in left showing CHMP1B dimpling (black dashed curved line)
in the outer leaflet of the bilayer. A CHMP1B helix α1 sitting at the membrane is highlighted in
dark green. (**B**) CryoEM density map and model of CHMP1B helix α1 from (A). The residues
involved in membrane binding are labeled. (**C**) Intensity plots of membrane bilayer thickness for
liposomes only (from 2D class averages, colored in grey), membrane-bound CHMP1B (green),
and right- or left-handed membrane-bound CHMP1B+IST1 filaments (cyan and red
respectively) as determined by cryoEM. The bilayer thickness is labeled for each. Intensities
were normalized to the peak intensity of the inner leaflet. (**D**) Plot of membrane thickness as a
function of radius of the outer leaflet. The dots are colored as in (left). For (C) and (D), the
liposome values were determined from 2D averages (n=6) while the others were determined
from half maps from each 3D reconstruction (n=2). (**E**) The inner membrane density increases
as a function of curvature. Plot of ratio of inner leaflet to outer leaflet peak intensity as a function

406   of radius of the outer leaflet. Dots are labeled as in (C). (**F**) Schematic illustration of lipid
407   behavior as the bilayer is remodeled from planar (*left*), to moderate curvature by CHMP1B
408   (*middle*), to high curvature by CHMP1B+IST1 (*right*). The outer leaflet headgroups spread out,
409   while the inner leaflet headgroups crowd and the aliphatic tails become more disordered and
410   therefore less extended.

A

SAXS Data Fitting

B

ρ_1	ε_1	σ_1	ρ_2	σ_2	ρ_3	ε_3
1.23 ± 0.02	-18.3 ± 0.4	7.4 ± 0.1	-1.00 ± 0.02	7.6 ± 0.1	1.84 ± 0.04	20.3 ± 0.4

σ_3	$I_0 [x10^{-7}]$	R_0	a_0	a_1	χ^2	$D = \varepsilon_3 - \varepsilon_1$
1.77 ± 0.03	6.1 ± 0.1	52 ± 1	0.362 ± 0.007	0.00 ± 0.00	0.075	38.6 ±0.6

Figure 4—figure supplement 1. SAXS analysis of liposomes and calculation of bilayer thickness.
(**A**) The small angle scattering intensities for protein-free unilamellar vesicles used in this study. The black line represents the fit to the model. The blue data points were used for fitting. (**B**) Fit results for the liposomes and the resulting thickness, D (Å). The bilayer center, ε_2, was fixed at 0, and the magnitude of the central peak, ρ_2, was fixed at -1.

420   **Conclusion and Perspective**

421   A remaining central question is how ESCRT-III proteins work with their associated AAA

422   ATPases to catalyze membrane constriction and, ultimately, membrane fission (*12, 33, 34, 45-*

423   *52*). Recent in vivo and in vitro studies have explored the roles of staged ESCRT-III assembly

424   (*39*) and ATP-dependent forces (*39, 53*). Here, we show how CHMP1B and IST1 function

425   sequentially to squeeze and thin the membrane, bringing it nearly to the fission point (Video 1).

426   Specifically, CHMP1B first binds and assembles into a flexible filament that wraps the target into

427   a moderate curvature tubule. CHMP1B flexibility allows for many degrees of filament curvature

428   and handedness, which may explain how it and other ESCRT-III proteins can adopt a wide range

429   of architectures. IST1 binding then drives CHMP1B to constrict the membrane even further,

430   exploiting IST1-intersubunit interactions to form tighter and tighter turns. Lipid composition is also

431   expected to play a role in this process, although our work does not address that issue directly.

432   Finally, our reconstructions suggest that IST1 may drive CHMP1B into an even narrower

433   constriction state during the fission step, and that this process could be regulated by a VPS4

434   family member. Comparison of the CHMP1B+IST1 and IST1-only filaments suggests that

435   CHMP1B helix α6 (MIM) may sterically limit the full potential of IST1 constriction. We therefore

436   speculate that unfolding or displacement of the CHMP1B MIM could trigger further tightening of

437   the double-stranded filament. Importantly, the CHMP1B MIM forms the binding site for the MIT

438   domains of VPS4 ATPase family members (*54-56*), and MIT domain binding could therefore

439   provide a mechanism for displacing this helix (*34, 35, 39, 48*). Further constriction of the

440   CHMP1B+IST1 filament might also push the two aromatic residues of CHMP1B helix α1 (F9 and

441   F13) from the hydrated surface layer, as seen in our structure, into the hydrophobic interior of the

442   outer membrane leaflet, thereby destabilizing the membrane, helping to drive lipid mixing, and

443   promoting fission (*57, 58*).

444
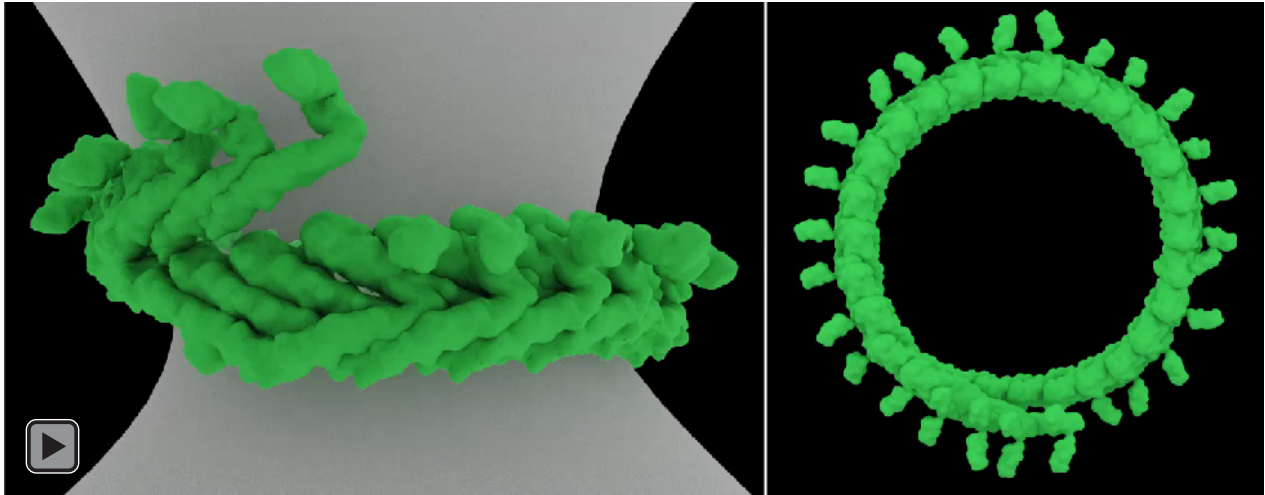
445

446   **Video 1 – Constriction of a membrane tubule by CHMP1B and IST1.**

447   Table 1. CryoEM data collection parameters

Dataset	CHMP1B-only	CHMP1B+IST1	IST1$_{NTD}$$^{R16E/K27E}$
Microscope	Titan Krios	Polara	TF20
Energy filter (slit width, eV)	20	N/A	N/A
Voltage (kV)	300	300	200
C2 aperture (µm)	70	30	70
Objective aperture (µM)	100	100	100
Camera	K2 Summit	K2 Summit	K2 Summit
Detection mode	Super resolution	Super resolution	Super resolution
Pixel size (Å/Pixel)	1.345	1.2156	1.234
Total exposure (sec)	10	8.0	8.0
Frame rate (Frames/sec)	0.2	0.2	0.2
Dose rate (e$^-$/Å^2/frame)	0.90	1.1	1.325
Total dose (e$^-$/Å^2)	45	44	53
Defocus range (µm)	0.23-1.91	0.46-2.97	1.04-3.22
Mean defocus (µm)	1.14	1.54	2.28

448

449

450   Table 2. CryoEM refinement parameters

Dataset	CHMP1B-only	CHMP1B+IST1 (right-handed)	CHMP1B+IST1 (left-handed)	IST1$_{NTD}$$^{R16E/K27E}$
Micrographs	3993	9305	9305	279
Segments in final map	9,661	66,149	57,915	4,556
Box size (Å^3)	324	352	352	320
Resolution (Å)	6.2	3.2	3.1	7.2
Helical twist (°)*	+13.86	+20.02	-20.77	+26.85
Helical rise (Å)	1.86	2.96	3.06	3.00
Map sharpening B-factor (Å^2)	-50	-25	-25	-50
Resolution (Å)	6.2	3.2	3.1	7.2

451   *denotes + for right-handed and - for left-handed twists

452

453   Table 3. Model validation statistics

Dataset	CHMP1B-only	CHMP1B+IST1 (right-handed)	CHMP1B+IST1 (left-handed)	IST1$_{NTD}$$^{R16E/K27E}$
Chains	26	72	68	14
Atoms	32,890	99,216	93,500	19,432
Protein residues	4,238	12,852	12,104	2,534
RMS bonds (Å)	0.006	0.011	0.005	0.007
RMS angles (°)	0.816	0.828	0.863	1.15
MolProbity score	1.55	1.17	1.14	1.61
Clashscore (percentile relative to PDB)	10.73 (68th)	3.82 (96th)	7.29 (85th)	5.48 (93rd)
Ramachandran favored (%)	98.76	98.58	98.86	95.53
Ramachandran allowed (%)	1.24	1.42	1.14	4.47
Ramachandran outliers (%)	0	0	0	0
Rotamer outliers (%)	0	0	0.34	0.74
C-beta outliers	0	0	0	0
Mean B-factor	N/A*	68	60	N/A*
Minimum/maximum B-factor	N/A*	51/112	45/92	N/A*
EMRinger score	N/A*	1.05	1.41	N/A*

454   *The resolutions of the maps are insufficient to determine these values
455

Materials and Methods

Protein Expression and Purification

The purification of the N-terminal domain of IST1 containing residues 1-189 ($IST1_{NTD}$) and the N-terminal IST1 R16E/K27E mutant ($IST1_{NTD}^{R16E/K27E}$) have been described previously (*14*). CHMP1B residues 1-199 were cloned into an N-terminal 6xHis-SUMO fusion to yield a native N-terminus after removal of the purification tag. Two alleles of CHMP1B have been reported, 37K or 37E, and we saw that both alleles remodeled membranes and copolymerized with IST1 with indistinguishable activity. The CHMP1B 37E allele was used for subsequent studies and was expressed in LOBSTR-BL21 (DE3) cells (*59*) in ZYP-5052 auto-induction media (*60*). Cells were harvested and frozen at -80 °C. All subsequent steps were performed at 4 °C unless otherwise noted. Thawed cells were suspended in lysis buffer (50 mM Tris, pH 8, 500 mM NaCl, 10 mM Imidazole, 1 mM DTT, 5% (v/v) glycerol) and supplemented with lysozyme. Cells were lysed by sonication. Lysate was centrifuged at 30,000 x g for 1 h, and the supernatant was filtered using a 0.45 µm membrane. Clarified lysate was loaded onto a gravity flow column with Ni-NTA resin (Qiagen), incubated for 1 hour, and washed extensively with lysis buffer. The fusion protein was eluted in lysis buffer supplemented with 400 mM imidazole. His-tagged ULP1 protease was added and then dialyzed into cleavage buffer (20 mM Tris pH 8.0, 150 mM NaCl, 1 mM DTT, 10 mM imidazole) at room temperature overnight. The cleaved product was then applied again to Ni-NTA resin to remove the purification tag, uncleaved fusion protein, and the protease. CHMP1B was further purified by Superdex-75 16/60 size exclusion chromatography (GE Healthcare Life Sciences, USA) in size exclusion buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 1 mM DTT).

Liposome Preparation

Stock lipid solutions (Avanti Polar Lipids) were resuspended in chloroform. To produce the liposomes, (18 mole% 16:0-18:1 phosphatidylserine (POPS), 58% 16:0-22:6 phosphatidylcholine (SDPC), 18% cholesterol, 6% $PI(3,5)P_2$ or equivalent phosphoinositide), 2 mg total lipid were

482   dried in a glass vial at room temperature under streaming nitrogen with vortexing. The lipids were

483   again re-dissolved in chloroform, dried under streaming nitrogen, and desiccated under house

484   vacuum (at least 4 hours in darkness). The lipid films were dispersed in 1 ml buffer (25 mM Tris,

485   pH 7.4, 150 mM NaCl, 2 mg/ml final concentration, 4 ºC overnight with gentle rocking). Liposomes

486   were freeze-thawed 10 times and then stored at -80 ºC.

487

488   *Membrane Remodeling Reactions*

489   Membrane remodeling reactions were performed at room temperature with protein

490   concentrations ranging from 5 – 15 µM and liposome concentrations ranging from 0.5 – 1 mg/ml

491   in reaction buffer (20 mM Tris, pH 7.4, 150 mM NaCl). CHMP1B was incubated with liposomes

492   overnight at room temperature. For membrane-bound CHMP1B reactions, these were then

493   directly used for EM sample preparation. For samples including IST1, the sample was pelleted

494   (13,000 x g, 5 mins), the supernatant was decanted to remove unbound CHMP1B, and the pellet

495   was resuspended in reaction buffer with equimolar $IST1_{NTD}$. This was incubated for 10 mins and

496   then subjected to EM sample preparation.

497

498   *CryoEM Sample Preparation and Data Collection*

499   3.5 µL of the membrane remodeling reactions were applied to glow-discharged R1.2/1.3

500   Quantifoil 200 Cu mesh grids (Quantifoil) in a Mark III Vitrobot (FEI). Grids were blotted with

501   Whatman #1 filter paper (Whatman) for 4-8 seconds with a 0 mm offset at 19 °C and 100 %

502   humidity before plunging into liquid ethane. Grids were stored under liquid nitrogen until samples

503   were imaged for structural determination. Datasets were collected either on a 300 kV Technai

504   Polara, a 300 kV Titan Krios, or a 200 kV Technai F20, all using a K2 Summit detector operated

505   in super-resolution mode and binned by a factor of 2 for subsequent processing. Data collection

506   parameters are summarized in Table 1.

507

508   *EM Image Analysis and 3D Reconstructions*

509   All dose-fractionated image stacks were corrected for motion artifacts, 2x binned in the

510   Fourier domain, and dose-weighted using MotionCor2 (*61*). GCTF-v1.06 (*62*) was used for

511   contrast transfer function (CTF) estimation. Particles were selected manually using RELION3 with

512   helical processing (*63*), and subsequent steps were performed in RELION3 unless otherwise

513   stated. Segments were extracted with ~90% overlap between boxes. Multiple rounds of 2D

514   classification were performed to remove poor particles and to yield particles with a largely uniform

515   diameter. For determination of the helical parameters for the CHMP1B-only or $IST1_{NTD}^{R16E/K27E}$

516   filaments, the iterative helical real space refinement (IHRSR) algorithm (*64*) as implemented in

517   SPIDER (*65*) was used. For the CHMP1B+IST1 filaments, the previously determined helical

518   parameters were used as the initial values (*14*). For all reconstructions, hollow, smooth cylinders

519   were used as initial models for 3D auto-refine reconstructions with refinement of helical

520   parameters and a central Z length of 40% of the particle box and the 'ignore CTFs until first peak'

521   flag for CTF estimation was used. These particles then went through multiple rounds of 3D

522   classification without alignment with 3-4 classes and T values varying from 2-10 and a protein-

523   membrane mask. Selected particles then were subject to another 3D auto-refine reconstruction

524   with per-particle CTF estimation correction within RELION3, followed by a final 3D auto-refine

525   reconstruction. For the membrane-bound left-handed CHMP1B+IST1 reconstruction, initial 3D

526   classification without alignment yielded a class with no discernable features. 2D classification of

527   these particles still yielded class averages with secondary structure features. Refinement of the

528   helical parameters by switching the sign of the twist was then able to generate a good initial 3D

529   reconstruction that was further refined as above. Post-processing was performed using the masks

530   from refinement with ad hoc B-factors applied. Refinement parameters are listed in Table 2.

531

532   *Atomic Modeling and Validation*

533   For the high-resolution CHMP1B+IST1 filaments, a single protomer of CHMP1B and IST1

534   from the previously determined structure were initially docked into the density with UCSF Chimera

535   (*66*). The protomers were manually adjusted and rebuilt in *Coot* (*67*) and then refined in

536   phenix.real_space_refine (*68*) using global minimization, morphing, secondary structure

537   restraints, and local grid search. The refined protomers were then used to generate roughly two

538   full turns manually in real space using UCSF Chimera. Noncrystallographic symmetry (NCS)

539   constraints were then used thorough refinement in phenix.real_space_refine. Iterative cycles of

540   manually rebuilding in *Coot* and phenix.real_space_refine, with previous strategies and

541   additionally B-factor refinement, were performed. For the low resolution CHMP1B-only or IST1$_{NTD}$

542   filaments, a similar procedure was performed but only roughly one turn was built and no B-factor

543   refinement was performed in phenix.real_space_refine.

544   All final model statistics were tabulated using Molprobity (*69*) (Table 3). Map versus atomic

545   model FSC plots were computed in PHENIX (*70*). All structural figures were generated with UCSF

546   Chimera and PyMOL (*71*).

547

548   *Real-time Imaging of Membrane Constriction*

549   Lipid tube pulling and imaging were performed using a previously described holographic

550   optical trapping setup possessing an independent fluorescent imaging capability (*72*). Briefly,

551   the setup included a custom-modified Eclipse Ti microscope (Nikon Instruments; Melville, NY),

552   with a Nikon 100X, 1.49NA oil immersion objective, Sapphire 488 excitation laser (Coherent,

553   Santa Clara, CA), and a DU897 camera (Andor Technology, Oxford Instruments, USA). All

554   videos were recorded at 20 fps. Biotinylated giant unilamellar vesicles (GUVs) in Flow Buffer (50

555   mM Tris pH 8.0, 150 mM NaCl, 1 mM DTT,) were synthesized as previously described (*73*).

556   Biotinylated silica beads (5 µm diameter, Si5u-BN-1, Nanocs, USA) were incubated with 10 µM

557   traptavidin (Kerafast, USA) in PMEE buffer (35 mM PIPES, 5 mM MgSO4, 1 mM EGTA, 0.5 mM

558   EDTA, pH 7.2) for 30 min followed by two rounds of washing (centrifugation at 12,000 × g for 2

559   minutes followed by supernatant removal and re-suspension of the pellet in 50 mM Tris buffer)

560   to remove excess traptavidin. The beads were then mixed with GUVs and diluted as necessary

561   to limit bead density to 1-3 beads in each 50 μm x 50 μm field of view. The mixture was then

562   applied to a flow cell and immediately subjected to experiments.

563   Membrane tubules were formed by capturing a bead in an optical trap, moving the

564   trapped bead into contact with a GUV, and finally, upon attachment, moving the bead away from

565   the GUV. The presence of a tubule was then assessed visually in real time. The GUVs were

566   stabilized in place via other beads attached to their surface and held in independent

567   holographically defined traps or via non-specific surface attachment. Proteins were introduced

568   into the flow cell in a sequential manner: first 0.5 μM CHMP1B and then 0.5 μM IST1 (both in

569   Flow Buffer). Lipids tubules were held perpendicular to flow direction during these buffer

570   exchanges and the resulting tubule constrictions were recorded. Experiments with CHMP1B

571   and IST1 in slightly different buffers (0.5 μM CHMP1B in 10 mM Tris pH 8.0, 100 mM NaCl 1

572   mM DTT or 0.5 μM IST1 in 50 mM Tris pH7.0, 350 mM NaCl, 5% (v/v) glycerol, 5 mM 2-

573   mercaptoethanol) yielded similar results.

574

575   *Small Angle X-ray Scattering*

576   For small-angle X-ray scattering (SAXS) analysis, liposomes were formed by vortexing a

577   dry lipid film (similar to liposome preparation as stated above) in water to yield a final lipid

578   concentration of 15 mg/ml. Liposomes were extruded through a 100 nm-pore polycarbonate

579   membrane, followed by extrusion through a 50 nm-pore polycarbonate membrane. Synchrotron

580   SAXS data were collected at beamline 4-2 of the Stanford Synchrotron Radiation Lightsource

581   (SSRL), Menlo Park, CA (*74*). The sample to detector distance was set to 1.1 m, and the X-ray

582   wavelength used was $\lambda = 1.127$ Å (11 keV). Using a Pilatus 3 X 1M detector (Dectris Ltd,

583   Switzerland) the setup covered a range of momentum transfer of $q \approx 0.017 - 1.17$ Å^{-1} where q is

584   the magnitude of the scattering vector, defined as $q = 4\pi \sin\theta / \lambda$, where θ is the scattering angle,

585   and λ is the wavelength of the X-rays. Aliquots of 32 μl of freshly extruded vesicles were loaded

586   onto the automated sample loader at the beamline (*75*). Consecutive series of thirty 2 s exposures

587   were collected first from the buffer blank followed by the vesicle samples. Solutions were

588   oscillated in a stationary quartz capillary cell during data collection to reduce the radiation dose

589   per exposed sample volume. The collected data were radially integrated, analyzed for radiation

590   damage and buffer subtracted using the automated data reduction pipeline at the beam line. To

591   improve statistics and check for reproducibility, the measurements were repeated with different

592   aliquots four times. As no significant differences were found between the repeat measurements,

593   the different data sets were averaged together.

594   The buffer-subtracted and averaged data were fit using a model for unilamellar vesicles

595   (*76*) as previously described (*77*). The electron density profile of the bilayer is approximated by

596   three Gaussian peaks corresponding to the inner and outer phosphate peaks and a negative peak

597   at the center for the hydrocarbon region. The following Equation 1 was used to fit the data:

598

599     $I(q) = I_0 q^{-2} \sum_{i,j}^{n=3} (R_0 + \varepsilon_i)(R_0 + \varepsilon_j)\rho_i\rho_j\sigma_i\sigma_j \exp[-q^2(\sigma_i^2 + \sigma_j^2)/2] \cos[q(\epsilon_i - \epsilon_j)] + a_0 + a_1 q$ (1)

600

601   where R_0 is the mean radius of the vesicle measured from the center of the bilayer, ε is the peak

602   displacement from the bilayer center, σ the Gaussian width of the peak and ρ is its amplitude. I_0

603   is the overall intensity of the measured profile. A background term was added, consisting of a

604   constant a_0 and a linear term a_1, to take into account the contribution from possible lateral

605   correlations between the lipids. The measured data were fit in the q-region between q = 0.02 –

606   0.6 Å^{-1} (depicted as blue colored points in Figure 4—figure supplement 1A). First, the data were

607   fit using a simulated annealing routine, and the results were then further optimized using a non-

608   linear least square algorithm, both by using code from the open source GNU scientific

609   library project (https://www.gnu.org/software/gsl/). The final fit parameters including the final χ^2

610   value of the fits and the resulting bilayer thickness (measured as distance between the inner and

611   outer leaflet peak positions) are summarized in Figure 4—figure supplement 1B.

612

613   *Image Analysis of Membrane Bilayer*

614   To determine the bilayer thickness and relative intensity of inner and outer leaflets, Fiji

615   (*78, 79*) was used. The cryoEM half maps from the C1 reconstructions were each low-pass filtered

616   to 8 Å, summed along the central 40% along the Z-axis, and the maps were radially averaged

617   using the Radial Profile Extended plugin (*80*) to determine the intensities as a function of radius,

618   resulting in two measurements per reconstruction. 2D averages of liposomes (n=6) were low pass

619   filtered to 20 Å, parameters for a circle that defined the 2D average were determined, and a wedge

620   that best covered the image was then used to calculate the intensities. For all resulting plots, the

621   radial intensities were normalized to the peak intensity of the inner leaflet peak and then fit to a

622   three Gaussian model (*81*) similar to the SAXS measurements. The model was minimized by a

623   non-linear least square algorithm and the fit errors between the data and models ranged from

624   1.45%-3.30% with R^2 values from 0.996-0.999. The local maxima for the headgroups were used

625   to determine the bilayer thickness and inner/outer leaflet intensity ratios.

626

627   **Accession Numbers**

628   CryoEM maps and models were deposited to the PDB and EMDB with the following codes:

629   membrane-bound CHMP1B-only filament (PDB ID: 6TZ9, EMD-20590), membrane-bound right

630   handed CHMP1B+IST1 filament (PDB ID: 6TZ4, EMD-20588), membrane-bound left handed

631   CHMP1B+IST1 filament (PDB ID: 6TZ5, EMD-20589), $IST1_{NTD}^{R16E/K27E}$ filament (PDB ID: 6TZA,

632   EMD-20591).

633

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647

648   Competing Interests

649   The authors declare no competing interests.

650

651   References

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