Discovery, Structure, and Function of Filamentous 3-Methylcrotonyl-CoA Carboxylase

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Summary

3-methylcrotonyl-CoA carboxylase (MCC) is a biotin-dependent enzyme necessary for leucine catabolism in most organisms. While the crystal structure of recombinant bacterial MCC has been characterized, the structure and potential polymerization of native MCC remain elusive. Here, we discovered that native MCC from *Leishmania tarentolae* (LtMCC) forms filaments and determined its structure at near-atomic resolution using cryoEM. $\alpha_6\beta_6$ LtMCC dodecamers assemble in a twisted-stacks architecture, manifesting as supramolecular rods extending up to approximately 400 nanometers. LtMCCs in filaments bind biotin but are not covalently biotinylated and lack coenzyme A. Filaments elongate by stacking $\alpha_6\beta_6$ LtMCCs onto the exterior α-trimer of the terminal $\alpha_6\beta_6$ dodecamer. This stacking immobilizes the biotin carboxylase domains, sequestering the enzyme in an inactive state within the mitochondrial matrix. Our results support a new model for LtMCC catalysis, termed the dual-swinging-domains model, and cast new light on the functional significance of polymerization in the carboxylase superfamily and beyond.

Keywords

3-methylcrotonyl-CoA, carboxylase, cryo-electron microscopy, filament, leucine catabolism, biotin, mitochondria, Leishmania, atomic modeling, cryoID

Introduction

3-methylcrotonyl-CoA carboxylase (MCC) is a biotin-dependent enzyme necessary for the catabolism of leucine (Anderson et al., 1998; Knappe et al., 1961; Nikolau et al., 2003), an essential branched-chain amino acid involved in regulating cellular metabolism (Gondáš et al., 2022; Yang et al., 2010), protein synthesis (Gondáš et al., 2022; Yang et al., 2010), and anabolic signaling (Gran and Cameron-Smith, 2011; Paulussen et al., 2021). MCC belongs to a superfamily of biotin-dependent carboxylases with different substrate preferences, such as acetyl-CoA carboxylase (ACC), geranyl-CoA carboxylase (GCC), propionyl-CoA carboxylase (PCC), and pyruvate carboxylase (PC). In eukaryotes, MCC resides in the mitochondrial matrix (Tong, 2013). Documented in many species (Diez et al., 1994; Gallardo et al., 2001; Höschle et al., 2005), MCC shares high cross-species sequence homology (Figure S1). In humans, upregulated MCC expression often correlates with various cancers (Chen et al., 2021; Dai et al., 2020; Gondáš et al., 2022; He et al., 2020; Liu et al., 2019). On the other hand, MCC deficiency, one of the most common metabolic disorders in newborns (Lee and Hong, 2014), may cause vomiting, seizures, and other neurological abnormalities (Grünert et al., 2012; Kim et al., 2017).

As part of the mitochondrial leucine degradation pathway, MCC catalyzes the conversion of 3-methylcrotonyl-CoA to 3-methylglutaconyl-CoA (Gallardo et al., 2001). Upon covalent biotinylation of the apoenzyme, MCC accelerates two successive reactions: biotin carboxylation and carboxyl group transfer (Lynen et al., 1961; Moss and Lane, 1971). First, biotin is carboxylated at an α-subunit active site with bicarbonate as the carbon dioxide donor upon concomitant ATP hydrolysis (Lynen et al., 1961; Moss

43 and Lane, 1971; Tong, 2013). Next, the carboxylated biotin is translocated to the
44 corresponding β-subunit active site, where the carboxyl group is transferred from biotin
45 to 3-methylcrotonyl-CoA (Lynen et al., 1961; Moss and Lane, 1971; Tong, 2013).
46 The atomic structure of bacterial MCC has been solved by X-ray crystallography
47 using recombinant *Pseudomonas aeruginosa* MCC ($PaMCC^{rec}$) expressed in
48 *Escherichia coli* with His-tags to the β-subunits (Huang et al., 2012). $PaMCC^{rec}$ subunits
49 oligomerize into a dodecameric complex with a core of six β-subunits sandwiched by
50 two α-trimers in an $\alpha_6\beta_6$ architecture (Huang et al., 2012). Whether MCCs may exist in
51 other forms is unclear. Albeit, their supramolecular assembly was conjectured based on
52 rod-shaped aggregations of *Achromobacter* IVS MCC observed by negative-stain
53 electron microscopy (Apitz-Castro et al., 1970). Recent advances in cryo-electron
54 microscopy (cryoEM) have uncovered unexpected modes of enzyme polymerization
55 and elucidated the regulatory roles of such architectural forms (Lynch et al., 2017;
56 Mattei et al., 2020; Maud et al., 2022; Park and Horton, 2019; Wang et al., 2019; Webb
57 et al., 2017). For example, near-atomic resolution cryoEM structures illuminated the
58 regulatory functions of several filamentous forms of eukaryotic ACC (Hunkeler et al.,
59 2018). In the absence of high-resolution structures of the native MCC enzyme, it
60 remains unsettled whether native MCCs can similarly form supramolecular assemblies.
61 Here, we used a bottom-up structural proteomics cryoEM approach (Ho et al.,
62 2020; Pfab et al., 2021) to identify and determine the near-atomic resolution structure of
63 native *Leishmania tarentolae* MCC (LtMCC) in mitochondria enriched through
64 streptavidin-coated magnetic beads. We discovered that LtMCC filaments consist of
65 stacks of LtMCC $\alpha_6\beta_6$ dodecamers. The biotin carboxylase (BC) domains of adjacent

66 $\alpha_6\beta_6$ dodecamers bind with each other, inhibiting their mobility. In contrast, at the
67 filament termini, the BC domain is flexible, while the linker between the biotin carboxyl
68 carrier protein (BCCP) and the BC-CT mediating (BT) domain is rigid. These
69 observations support a dual-swinging-domains model for LtMCC catalysis and suggest
70 a regulatory role of LtMCC filamentation in the mitochondrial matrix.

Results

Discovery and identification of LtMCC filaments from mitochondrial lysate

While examining complexes enriched by sedimentation of mitochondrial lysate from parasitic protist *L. tarentolae* in 10%-30% glycerol gradient (Figure 1A) and affinity pulldown with streptavidin-coated magnetic beads, we serendipitously discovered filamentous structures (Figure 1B). We identified the constituents of these filamentous structures by following the workflow detailed in Figures 1B–1C, which is based on the cryoID bottom-up structural proteomics approach (Ho et al., 2020). Using single-particle reconstruction, we obtained two differently centered cryoEM maps of the filament middle segments, one at 3.4 Å and another at 3.9 Å resolution (Figure 1B). Additionally, we processed the termini particles and found that the two termini have the same structure, which was resolved at 7.3 Å resolution (Figure 1B).

To establish the protein identities of the cryoEM maps, we first obtained a partial model prediction from DeepTracer (Pfab et al., 2021) using the 3.9 Å map. The sequence of the output model from DeepTracer (Pfab et al., 2021) was then input into cryoID (Figure 1C). Searching for proteins detected by mass spectrometry (Table S1), cryoID identified LtMCC α-subunits as a filament component. Upon visual inspection, it was clear that the filaments also contain LtMCC β-subunits. Indeed, LtMCC α- and β-subunits produced the most peptide spectral counts of all proteins detected by mass spectrometry (Table S1). Furthermore, we ruled out GCCs as the filament components because GCCs are not found in eukaryotes (Waldrop et al., 2012). We built the atomic model for the filament *de novo*, using an AlphaFold (Jumper et al., 2021; Mirdita et al., 2022) predicted model for the α-subunit and the β-subunit as references. We found that

both the 3.4 Å and 3.9 Å maps contain the same subunits but center on the β- and α-subunits, respectively. After translating one map for half of an $\alpha_6\beta_6$ stack along the filament and a slight rotation around the filament axis, the two maps match well with one another (cross-correlation coefficient of 0.74). The slightly better resolution of the map centered on the β-subunits is probably because the architecture of the β-subunits is more stable.

Structure of native eukaryotic LtMCC filaments

LtMCC filaments are predominantly straight (Figure 2A) although some are curved (Figure 2B), with a maximum observed curvature reaching 3.1 μm^{-1}. With a mean length of 993 Å, the length distribution resembles a Poisson distribution (Figure 2C), similar to that of ACC filaments isolated from animal tissues and observed by negative stain electron microscopy (Kleinschmidt et al., 1969). Most filaments consist of four to six $\alpha_6\beta_6$ dodecamers, or stacks (Figure 2C). With an architecture similar to that of $PaMCC^{rec}$, each $\alpha_6\beta_6$ stack in the LtMCC filament is characterized by an $\alpha_6\beta_6$ cylindrical dodecamer exhibiting D3 symmetry (Figure 2D). Measuring to a height of 216 Å and width of 148 Å, the dodecamer is composed of a core of six β-subunits (β-core) sandwiched by two layers of trimeric α-subunits (Figure 2D, Video S1). Modeling the atomic structures of LtMCC $\alpha_6\beta_6$ stacks into our cryoEM reconstruction reveals that the filament has a twisted-stacks architecture; that is, neighboring $\alpha_6\beta_6$ stacks are related by a rotation of 23° and a translation of 216 Å (Figure 2D). In the filament, the active sites are exposed in both α- and β-subunits (indicated by stars in the middle panel of Figure 2D).

117 Each α-subunit contains the BC, BT, and BCCP domains, and two linkers: the
118 BC-BT linker and the BT-BCCP linker, the latter of which was unmodeled in PaMCC^{rec}
119 (Figures 2E). A prominent feature of the BC domain is the presence of two large central
120 β-sheets surrounded by α-helices. The active site of each α-subunit is located inside the
121 central pocket of the BC domain (Figure 2F) and is responsible for carboxylating BCCP-
122 bound biotin. In contrast to the BT domain of PaMCC^{rec}, which contains a central α-helix
123 accompanied by a seven-stranded antiparallel β-barrel (Huang et al., 2012), the LtMCC
124 BT domain features eight β-strands (Figure S2). When the dodecameric PaMCC^{rec} and
125 LtMCC are superimposed globally, the BC, BT, and BCCP domains in LtMCC are
126 rotated with respect to those in PaMCC^{rec} (Figure S3A–B). However, when individual
127 domains are superimposed independently, they fit well with each other (Figure S3C–E),
128 suggesting that the above observed structural differences between the LtMCC filament
129 and PaMCC^{rec} are likely due to filamentation.
130 Like in PaMCC^{rec}, each LtMCC β-subunit possesses an N-carboxyltransferase
131 (N-CT) domain and a C-carboxyltransferase (C-CT) domain. We have designated two
132 new domains in LtMCC: the N-dock domain and CT-linker (Figure 2E), located before
133 and after the N-CT domain, respectively. The N-dock domain was introduced to
134 facilitate description of interactions between α- and β-subunits in the filament (see
135 below). Designating the helix-loop-helix-loop fragment between the N-CT and C-CT
136 domains as a separate domain (CT-linker) renders the N-CT and C-CT domains to have
137 near-identical backbone folds (Figure S4), despite sharing only 19% sequence identity.
138 Each of the six β-core active sites is wedged at the N-CT and C-CT interface between a
139 top and a bottom β-subunit (Figures 2G, 3A) and is responsible for carboxylating 3-

140 methylcrotonyl-CoA. Similar to that in PaMCC^{rec}, each LtMCC β-core active site is
141 roughly 80 Å away from its corresponding α-subunit active site.
142
143 LtMCC filaments bind biotin but lack coenzyme A
144 PaMCC^{rec} was crystalized as both a holoenzyme (with bound CoA and covalently-linked
145 biotin) and an apoenzyme (without CoA and biotin). By contrast, $\alpha_6\beta_6$ stacks in the
146 LtMCC filament contain non-covalently-bound biotin but lack CoA (Figure 3A). For
147 LtMCC to perform its two-step catalytic process, covalent biotinylation must occur first.
148 Although biotin is observed at the expected β-core active sites, it is untethered to
149 Lys651 in the highly conserved Ala-Met-Lys-Met sequence in the BCCP domain.
150 Indeed, the carboxyl group of biotin is about 10 Å apart from the ε-amino group of
151 Lys651 (Figure 3A), which is too far to form a prosthetic group (Moss and Lane, 1971).
152 The existence of non-biotinylated BCCP domains in LtMCC filaments suggests that the
153 filamentous LtMCC is inactive. Similarly, inactive forms of PCC also bind biotin without
154 forming a covalent adduct (Arabolaza et al., 2010; Diacovich et al., 2004).
155 The bicyclic ring system in biotin consists of an ureido ring and a thiophane ring
156 (Hu and Cronan, 2020). Carboxylation requires the transfer of the carboxyl group
157 attached to the N1′ atom of the biotin uredo ring to acyl-CoA (Knowles, 1989).
158 Therefore, the orientation of the N1′ atom of biotin is important for carboxylation. In
159 LtMCCs, the N1′ atom in the ureido ring of biotin is proximal to the CoA binding site, but
160 not in PaMCC^{rec} (Figure 3A). This is because the biotin bicyclic ring in LtMCC is
161 approximately 180° rotated relative to that of PaMCC^{rec} (Figure 3B). The spatial
162 arrangement of LtMCC biotin is consistent with PCC biotin (Huang et al., 2010;

163 Scheffen et al., 2021) and PC biotin (Fry et al., 1985) (Figure 3B). In contrast, the
164 arrangement of PaMCC^{rec} biotin is consistent with ACC biotin (Huang et al., 2012; Wei
165 and Tong, 2015) (Figure 3B). Therefore, biotin orientation is variable at the β-core active
166 site for different carboxylases and carboxylases in different catalytic states.
167

168 α-subunits associate more with β-subunits than with intra-trimer α-subunits

169 Many interactions are observed between an α-subunit and the β-core, with 1,515 Å^2 of
170 buried surface area as calculated by ChimeraX (Goddard et al., 2018) and a binding
171 affinity of -11.7 kcal mol^{-1} as calculated by PRODIGY (Vangone and Bonvin, 2015; Xue
172 et al., 2016). Interactions between each α-subunit and the β-core are mainly mediated
173 by a hook between the central α-helix and β1 strand of one BT domain that curls into a
174 groove of a β-subunit (yellow segment from Asp494–Thr506 in Figure 4A). This groove
175 is formed by the N-CT, C-CT, and N-dock domains (Figure S5). Due to its shorter
176 sequence, the LtMCC hook contains only one hairpin in contrast to two hairpins in the
177 PaMCC^{rec} hook (Figure S2). In both structures, the hook is crucial for interactions with
178 the β-subunit to reinforce inter-subunit associations (Huang et al., 2012). The additional
179 structures resolved in our cryoEM map also unveil previously unrecognized associations
180 among domains of three neighboring subunits: the BT-BCCP linker of an α-subunit, N-
181 dock domain of a β-subunit, and N-CT domain of another β-subunit (Figure 4B). Here,
182 the BT-BCCP linker "docks" onto the crescent N-dock domain (Figure 4B).

183 Fewer interactions are observed between intra-trimer α-subunits, contributing just
184 579 Å^2 of buried surface area and a binding affinity of -6.1 kcal mol^{-1}. Within an α-trimer,
185 each α-subunit interacts with its neighboring α-subunits through the BC-BC and BC-BT

186 interfaces. At the BC-BC interface, a loop from one BC domain (Glu34–His36) interacts
187 with a loop (Phe379–Pro385) and a helix (Arg423–Gln427) from a neighboring BC
188 domain (Figure 4C). At the BC-BT interface, strand β6 (Gly572–Ala581) from the β-
189 barrel in the BT domain interacts with a short β-strand (Ala59–Cys61) in the BC domain
190 through β-sheet augmentation (Harrison, 1996) (Figure 4D).
191

192 ## Structure of filament termini reveals mechanism of LtMCC polymerization

193 To investigate the mechanism of LtMCC polymerization, we reconstructed the filament
194 termini at 7.3 Å resolution (Figure 5A). We found that both ends of the filament
195 terminate at α-trimers, with partially resolved densities attributed to the BC domains
196 (Figure 5A). When displayed at a high threshold, cryoEM densities for the BC, BCCP
197 domain, and the BC-BT linker disappear, while density for the BT domain and BT-BCCP
198 linker of the same α-subunit remain visible (Figure 5A). Therefore, the weaker densities
199 of the BC and BCCP domains are due to their flexibility, rather than low occupancy of
200 the α-subunit at the termini, which would make all domains of the α-subunit disappear.
201 The flexibility of the BC and BCCP domains is further supported by limited intra-trimer α-
202 subunit associations (Figures 4C–D). In contrast, the BT-BCCP linker is mostly rigid as
203 it interacts extensively with the β-subunits (Figure 4B).
204 Our observation that LtMCC filaments terminate at α-subunits suggests that the
205 building block for the filament is an $\alpha_6\beta_6$ stack. Filaments plausibly elongate one stack at
206 a time, attaching through BC domains to form an α-α interface (Video S2), which is
207 defined as the associations between two α-trimers from adjacent stacks (Figure 5B).
208 The α-α interface has a total buried surface area of 1,689 $Å^2$ and a binding affinity of -

209 11.6 kcal mol^{-1}. At the α-α interface, the BC domain of an α-subunit from the lower α-
210 trimer interacts with the BC domains of two α-subunits from the upper α-trimer (Figure
211 5B). Interactions contributing to the α-α interface occur primarily between loops, along a
212 local twofold axis (right panel of Figure 5B): the first site of interaction is between two
213 loops (Leu338–Asp344), with its neighboring residues Lys319 and Arg231 also
214 participating in α-α interactions; the second site of interaction occurs between two loops
215 connected to two α-helices (His257–Glu261). These interactions at the α-α interface
216 stabilize the BC domains between neighboring stacks in the LtMCC filament (Figure
217 5B).

Discussion

This study reveals an important, yet previously unrecognized role of the BC domain in MCC catalysis. During catalysis, the BCCP domain must shuttle biotin between the CT active site (β-subunit) and the BC active site (α-subunit), located approximately 80 Å apart (Figure 5A). However, we observed that the BT-BCCP linker is fixed (Figure 5A). Therefore, the maximum distance that the BCCP domain can translocate by itself is about 60 Å, which is insufficient for the BCCP domain to reach the BC active site (right panel of Figure 5A). As such, for catalysis to occur, both the BCCP and active-site-containing BC domain must move so that BCCP-bound biotin can reach the BC active site. Indeed, at the filament termini, the BC domain is flexible, thus permitting BC domain movement. We term this the dual-swinging-domains model to highlight the difference from the original swinging-domain model (Tong, 2013), in which only the BCCP domain swings. Beyond MCC, both the BCCP and BC domains have been documented to move in pyruvate carboxylase (Maurice et al., 2007; Xiang and Tong, 2008). These observations suggest that carboxylase catalysis in general may involve a dual-swinging domains mechanism.

In LtMCC filaments, the α-α interface fixes the BC domain, thus preventing its swinging. According to our dual-swinging-domains model, immobilization of the BC domain inhibits catalysis. Therefore, filamentation likely sequesters LtMCCs in a quiescent state in the mitochondrial matrix (Figure 5C), which may provide a capacity to reactivate the enzyme in response to environmental signals. Polymerization appears to be a readily deployable and economic mechanism for regulating the activity of abundant mitochondrial matrix enzymes in response to rapid changes in cellular metabolism

241 requirements. Such capacity is particularly relevant for parasitic protists cycling between
242 the amino acid-rich gut environment of insect vectors and the high glucose
243 concentration bloodstream of their hosts.

244 Our inquiries into MCC polymerization further reflect the diversity of
245 polymerization in the carboxylase superfamily. It is known that both isoforms of ACC,
246 ACC1 and ACC2, form filaments (Beaty and Lane, 1983; Hunkeler et al., 2018; Kim et
247 al., 2010; Kleinschmidt et al., 1969; Meredith and Lane, 1978; Park et al., 2013; Wei
248 and Tong, 2018). Recently, high-resolution structures of filamentous ACC1 have been
249 reported (Hunkeler et al., 2018). In contrast to LtMCC, ACC1 assembles into two
250 filamentous forms: an active single-stranded and inactive double-stranded helical
251 structure. Eukaryotic MCC and ACC filamentation exhibit different architectures and
252 regulatory functions, supporting the distinct evolutionary lineages of these enzymes
253 (Huang et al., 2012). Nonetheless, filament formation may be a general way to regulate
254 enzymatic activity in the carboxylase superfamily.

255 Evolutionarily, employing the same gene costs less than “inventing” a new gene
256 for regulation. Polymerization is a remarkable example of such a cost-effective strategy.
257 In the case of LtMCC, the stacking of active $\alpha_6\beta_6$ dodecamers can inhibit biotin shuttling
258 (Figure 5C), thus sequestering enzymes by polymerization. In other enzymes,
259 polymerization can alter specificity (Kim et al., 2005), increase activity (Polley et al.,
260 2019), and even introduce non-enzymatic functions such as conducting electrons
261 between bacterial cells (Wang et al., 2019) and regulating cell curvature (Ingerson-
262 Mahar et al., 2010). Just like how complex life forms arose by the aggregation and

263 polymerization of unicellular forms, the diverse functions arising from enzyme

264 polymerization can be considered as a rudimentary form of evolution.

265 **STAR★Methods**

266 Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
L. tarentolae TATII/UC strain	(Parrot, 1949)	TATII/UC was isolated from Algerian gecko in 1939 and maintained in axenic culture in various laboratories. Parasite culture used in this study was received from Prof. Larry Simpson laboratory at UCLA in 2004 and transfected with pX based plasmid exoressing RNA editing ligase 1 (KREL1).
Chemicals, peptides, and recombinant proteins		
Complete protease cocktail tablet	Roche	N/A
Software and algorithms		
MotionCorr2	(Zheng et al., 2017)	https://emcore.ucsf.edu/ucsf-software
Gctf	(Zhang, 2016)	https://www2.mrc-lmb.cam.ac.uk/research/locally-developed-software/zhang-software/#gctf
RELION 3.1	(Zivanov et al., 2018)	https://www3.mrc-lmb.cam.ac.uk/relion/index.php/Main_Page
ChimeraX	(Goddard et al., 2018)	https://www.cgl.ucsf.edu/chimerax/
Coot	(Emsley et al., 2010)	https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/
Phenix	(Liebschner et al., 2019)	https://phenix-online.org/
DeepTracer	(Pfab et al., 2021)	https://deeptracer.uw.edu/

cryoID	(Ho et al., 2020)	https://github.com/EICN-UCLA/cryoID
ESPript 3	(Robert and Gouet, 2014)	https://espript.ibcp.fr/ESPript/ESPript/
AlphaFold	(Jumper et al., 2021; Mirdita et al., 2022)	https://github.com/sokrypton/ColabFold
IMOD	(Kremer et al., 1996)	https://bio3d.colorado.edu/imod/
SerialEM 3.8	(Mastronarde, 2005)	https://bio3d.colorado.edu/SerialEM/
ExPASy Translate	(Gasteiger et al., 2003)	https://web.expasy.org/translate/
Clustal Omega	(Sievers and Higgins, 2018)	https://www.ebi.ac.uk/Tools/msa/clustalo/
PRODIGY	(Vangone and Bonvin, 2015; Xue et al., 2016)	https://wenmr.science.uu.nl/prodigy/
ResMap	(Kucukelbir et al., 2014)	http://resmap.sourceforge.net/
Deposited data		
Crystal Structure of PaMCC^{rec} apoenzyme	(Huang et al., 2012)	PDB: 3U9T
Crystal Structure of PaMCC^{rec} holoenzyme	(Huang et al., 2012)	PDB: 3U9S
Crystal Structure of PCC D422-mutant β-subunit	(Arabolaza et al., 2010)	PDB: 3IB9
Crystal Structure of PCC wild-type β-subunit	(Diacovich et al., 2004)	PDB: 1XNY
CryoEM Structure of ACC–Citrate filament	(Hunkeler et al., 2018)	PDB: 6G2D
CryoEM Structure of ACC–BRCT filament	(Hunkeler et al., 2018)	PDB: 6G2I
Crystal structure of ACC holoenzyme	(Wei and Tong, 2015)	PDB: 5CSL
Crystal structure of PCC holoenzyme	(Huang et al., 2010)	PDB: 3N6R

CryoEM map of α-subunit centered LtMCC	This paper	EMDB: XXXX
CryoEM map of β-subunit centered LtMCC	This paper	EMDB: XXXX
CryoEM map of LtMCC termini	This paper	EMDB: XXXX
CryoEM structure of the LtMCC filament	This paper	PDB: XXXX
Other		
MagStrep XT beads	IBA Lifesciences	2-4090-002
Strep-Tactin XT 4Flow High-Capacity agarose resin	IBA Lifesciences	2-5030-002
Zeba™ Spin Desalting Columns	Thermo Fisher Scientific	N/A

Resource availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hong Zhou (hong.zhou@ucla.edu).

Experimental model and subject details

L. tarentolae cells were grown at 27 °C in brain heart infusion and harvested during late-exponential growth phase at ~$2x10^8$ cell/ml.

Method details

Preparation of mitochondrial lysate

279 Mitochondrial fraction was enriched by hypotonic cell lysis and sequential separation of
280 membrane-containing fraction on RenoCal76 density gradients (Pelletier et al., 2007).
281 Mitochondrial pellets (0.5-0.8 g wet weight) were lysed in 1 ml of pH 7.3, 50 mM
282 HEPES, 150 mM KCl, 2 mM EDTA, 1%NP40, and 50 µl of 20x Complete protease
283 cocktail by sonication at 24W for 15 seconds and centrifuged at 30,000 RPM in an
284 SW55 rotor for 15 minutes. The supernatant was recovered and separated on a
285 continuous 10-30% gradient glycerol in pH 7.3, with 20 mM HEPES, 100 mM KCl, and 1
286 mM EDTA and prepared in SW28/32 Setton clear tubes for 15 hours at 24,000 RPM.
287 Glycerol gradient fractions of 1.5 ml were collected and those corresponding to 20S-40S
288 region were combined.
289
290 **Purification of LtMCC by streptavidin affinity pulldown**
291 Glycerol gradient fractions were supplemented octylglucoside to 2 mM and incubated
292 on Strep-Tactin®XT magentic beads in a Binding Buffer (50 mM Tris-HCl, pH 8.0, 100
293 mM KCl, 1 mM EDTA, 2 mM OG) for 1 hour at 4 °C on a nutating mixer. Beads were
294 washed with 5 ml of Binding Buffer twice. For elution, the beads were incubated in 0.2
295 ml of Elution Buffer (20 mM Tris-HCl, pH 8.0, 100 mM KCl, 1 mM EDTA, 2 mM OG, 100
296 mM biotin) at 4 °C for 10 minutes. The concentrated sample was buffer exchanged to
297 0.2 ml uL of Sample Buffer (20 mM Tris-HCl, pH 7.5, 60 mM KCl, 5 mM $MgCl_2$, 1 mM
298 DTT, 5 mM OG) using Zeba™ Spin Desalting Columns, 7K MWCO (0.5 ml). The
299 sample was then centrifuged at 15,000 RPM for 10 minutes and the supernatant was
300 stored on ice before grid preparation.
301

302 **Protein identification by LC-MS/MS**

303 Affinity-purified complexes were sequentially digested with LysC peptidase and trypsin.
304 LC-MS/MS was carried out by nanoflow reversed phase liquid chromatography (RPLC)
305 using an UltiMate 3000 RSLC (Thermo Fisher Scientific) coupled on-line to an Orbitrap
306 Fusion Lumos mass spectrometer (Thermo Fisher Scientific). A cycle of full FT scan
307 mass spectrum (m/z 375-1500, resolution of 60,000 at m/z 400) was followed by
308 MS/MS spectra acquired in the linear ion trap for 3 seconds at top speed with
309 normalized collision energy (HCD, 30%). Following data extraction to MGF format using
310 MSConvert from ProteoWizard (Chambers et al., 2012), resultant peak lists for each
311 LC-MS/MS experiment were submitted to Protein Prospector (UCSF) for database
312 searching (Fang et al., 2012). We mapped the protein interaction network of the human
313 COP9 signalosome complex using a label-free QTAX strategy (Fang et al., 2012). Each
314 project was searched against a normal form concatenated with the random form of the
315 *L. tarentolae* Parrot Tar II from the TriTrypDB database (Amos et al., 2022). The mass
316 accuracy for parent ions and fragment ions were set as ± 10 ppm and 0.6 Da,
317 respectively. Trypsin was set as the enzyme, with a maximum of two missed cleavages
318 allowed. Cysteine carbamidomethylation was set as a fixed modification, and protein N-
319 terminal acetylation, methionine oxidation, and N-terminal conversion of glutamine to
320 pyroglutamic acid were selected as variable modifications.

321

322 **CryoEM sample preparation**

323 Lacey carbon cryoEM grids with a 2 nm continuous carbon film (Ted Pella) were first
324 glow discharged for 45 seconds with a target current of 15 mA, using PELCO

325 easiGlowTM. Then a 2.5 μL sample was applied to the grids. After waiting for 5
326 seconds, the grids were blotted for 4 seconds with blot force 0, at 100% humidity and
327 4°C temperature; after blotting, the grids were plunge-frozen into liquid ethane using an
328 FEI Mark IV Vitrobot (Thermo Fisher Scientific). The grids were stored in a liquid
329 nitrogen dewar before imaging.
330
331 **CryoEM image acquisition**
332 The cryoEM grids were loaded and imaged using a Titan Krios (Thermo Fisher
333 Scientific) operated at 300 kV, with a Gatan K3 camera and a Gatan Imaging Filter
334 Quantum LS. Movies were recorded with SerialEM (Mastronarde, 2005) by electron
335 counting in super-resolution mode at a pixel size of 0.55 Å/pixel. The exposure time was
336 2 seconds and fractionated to 40 frames. The defocus range was between -1.5 to -2.5
337 μm. The total dosage was approximately 40 electrons/Å2. A total of 3,328 movies were
338 collected.
339
340 **CryoEM image processing**
341 The movies were processed with MotionCor2 (Zheng et al., 2017), leading to dose-
342 weighted and drift-corrected electron micrographs with a calibrated pixel size of 1.1 Å.
343 We discarded the first frame due to severe drift of this frame. The defocus of the
344 micrographs was determined by Gctf (Zhang, 2016). Using IMOD (Kremer et al., 1996),
345 the micrographs were binned by a factor of 10 to make a stack for particle picking. After
346 directly observing filaments on the micrographs, we manually picked the start and end
347 of each filament using the 3dmod command in IMOD (Kremer et al., 1996). A total of

348 4,496 filaments were picked. To derive the constituents of the filaments, we coded a
349 Python script to subdivide the filaments into equally spaced segments of 87 Å. The
350 coordinates were then converted through a Bash script to RELION format. A total of
351 51,322 particles were extracted in total through RELION 3.1, with a box size of 384.
352 After extraction, we performed reference-free 2D class averaging on the particles
353 with RELION 3.1 (Zivanov et al., 2018). We chose the best classes and proceeded to
354 3D classification. We made an initial 3D model from the best 3D class averages through
355 RELION 3.1. The 3D classification was done with D3 symmetry and yielded α- and β-
356 centered 3D class averages. After removing duplicates, the α- and β-centered classes
357 contained 7,582 and 13,250 particles, respectively. We did 3D refinement for the α- and
358 β-centered map independently with D3 symmetry using RELION 3D refinement. We
359 then performed CTF refinement for both maps independently using RELION 3.1. We
360 used CTF refinement (Zivanov et al., 2018) to improve the accuracy of the defocus for
361 each particle and to correct beam tilt for the dataset. After performing CTF refinement,
362 we performed 3D refinement for both maps again, independently. At the end of all
363 processing, based on RELION's gold-standard Fourier shell correlation (FSC) at the
364 0.143 criterion, we obtained α- and β-centered maps at 3.9 and 3.4 Å resolution,
365 respectively (Figure S6). The resolution towards the center of the map is better than the
366 edge of the map (Figure S6).
367 For the filament termini map, we used RELION 3.1 to extract the previously
368 picked filament start-and-end points at a box size of 384 pixels. We calculated the in-
369 plane rotation angle using the direction of vectors from the start to end of the filament.
370 We used a Python code to include the in-plane rotation angle information in the RELION

371 star file for the particles. We performed 2D classification for these particles without local
372 search around the precalculated in-plane rotation angle. To generate an initial 3D
373 model, we used the IMOD clip function (Kremer et al., 1996) to change the center of the
374 β-centered map. The best particles were selected for 3D reconstruction with C3
375 symmetry. We performed 3D classification and removed bad particles. During 3D
376 classification, we set the “limit_tilt” parameter to 85 to keep side-view particles. We
377 proceeded to 3D refinement with 3,380 particles. We obtained a final reconstruction with
378 C3 symmetry at 7.3 Å resolution based on the gold-standard Fourier shell correlation at
379 the 0.143 criterion.
380
381 **Sequence determination**
382 We re-sequenced the α-subunit gene with the same strain of *L. tarentolae* as used in
383 cryoEM sample preparation. The amino acid sequence for the LtMCC β-subunit was
384 obtained by analyzing the genome sequencing data of *L. tarentolae* Parrot Tar II (Goto
385 et al., 2020), since the putative, shotgun LtMCC β-subunit sequence on the NCBI
386 protein database was notably shorter than MCCs from other organisms, deviating from
387 the high sequence homogeneity observed across MCC sequences. A frameshift occurs
388 near the middle of the sequence, likely caused by a sequencing error. The frameshift is
389 not present in the previous genome sequencing data of *L. tarentolae* Parrot Tar II
390 (Raymond et al., 2012). Since MCC plays an essential role in the catabolism of leucine
391 across many species, it is unlikely that MCC exhibits a highly different sequence in *L.*
392 *tarentolae*.
393

394 **Atomic modeling**

395 An initial AlphaFold (Jumper et al., 2021) model prediction was generated from
396 ColabFold (Mirdita et al., 2022) using the LtMCC α-subunit and β-subunit sequences.
397 Atomic model building was conducted de novo in regions of low sequence conservation.
398 First, an individual α-subunit and β-subunit were modeled into the cryoEM map in Coot
399 (Emsley et al., 2010) and then real-space refined in Phenix (Liebschner et al., 2019).
400 The α-subunit was modeled using the α-centered map, and respectively for the β-
401 subunit. Biotin was modeled de novo into its observed density. Then, the subunits were
402 duplicated according to D3 symmetry, fitted into the cryoEM maps, and refined
403 iteratively using Phenix (Liebschner et al., 2019) and Coot (Emsley et al., 2010).
404

405 **Model analysis**

406 Buried surface area and map cross-correlation coefficient were calculated in ChimeraX
407 (Goddard et al., 2018). Binding affinity calculations were done using the PRODIGY web
408 server (Vangone and Bonvin, 2015; Xue et al., 2016). Sequence alignments and
409 similarity calculations were performed using Clustal Omega (Sievers and Higgins, 2018)
410 from the EMBL-EBI web server and visualized with ESPript 3 (Robert and Gouet, 2014).
411

412 **Data and code availability**

413 CryoEM maps of the α-subunit centered, β-subunit centered, and filament termini map
414 have been deposited in the Electron Microscopy Data Bank under accession numbers
415 EMD-XXXX, EMD-XXXX, and EMD-XXXX, respectively. The coordinates of LtMCC
416 have been deposited in the Protein Data Bank under accession number XXXX. All

aforementioned deposited data are publicly available as of the date of publication and accession numbers are also listed in the key resources table. This paper does not report original code. DOIs are listed in the key resources table. This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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Author contributions

Z.H.Z. and R.A. initiated and supervised the project. I.A. prepared the sample. C.Y. and L.H. conducted mass spectrometry. Y-T.L. carried out cryoEM imaging and data processing with assistance from J.K.J.L. and J.J.H. J.K.J.L. and J.J.H. built the atomic models. J.K.J.L., J.J.H., and Y-T.L. analyzed the data. J.J.H., J.K.J.L., and Y-T.L. made

illustrations and wrote the original draft. Z.H.Z., Y-T.L., J.J.H., and J.K.J.L. finalized the manuscript. All authors reviewed and approved the paper.

Declaration of interests

The authors declare no competing interests.

Figures

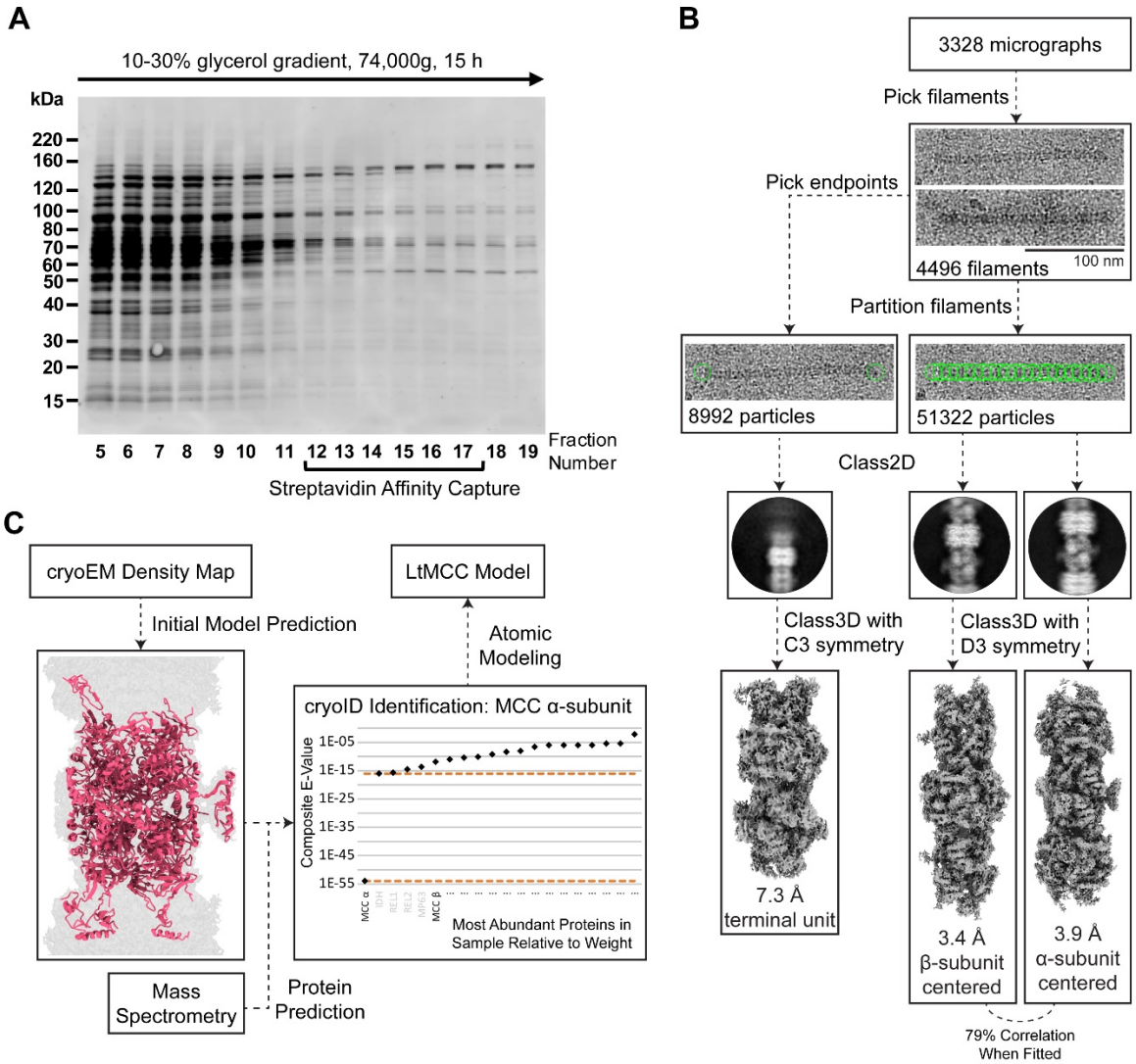


Figure 1. Discovery of filamentous 3-methylcrotonyl-CoA carboxylase from mitochondrial lysate of *L. tarentolae*

(A) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of the glycerol gradient fractions obtained from mitochondrial lysate. The gel was stained by Sypro Ruby.

(B) Workflow for cryoEM imaging and reconstruction. Filaments observed in cryoEM micrographs were visually identified, partitioned into segments of equal length, classified in 2D and 3D. Refinement of 3D reconstructions yielded an α-centered and β-centered cryoEM map. The filament ends were picked and reconstructed similarly, converging into a single map for both ends of the filament (i.e., the termini map).

(C) Workflow for protein identification and atomic modeling. From an input cryoEM map, DeepTracer (Pfab et al., 2021) generated an initial model with predicted amino acid sequence, which was input into cryoID (Ho et al., 2020) together with mass spectrometry data. CryoID predicted that the filaments contained LtMCC. Finally, de novo atomic modeling of LtMCC was performed in Coot (Emsley et al., 2010) using an AlphaFold predicted model as a reference (Jumper et al., 2021; Mirdita et al., 2022).

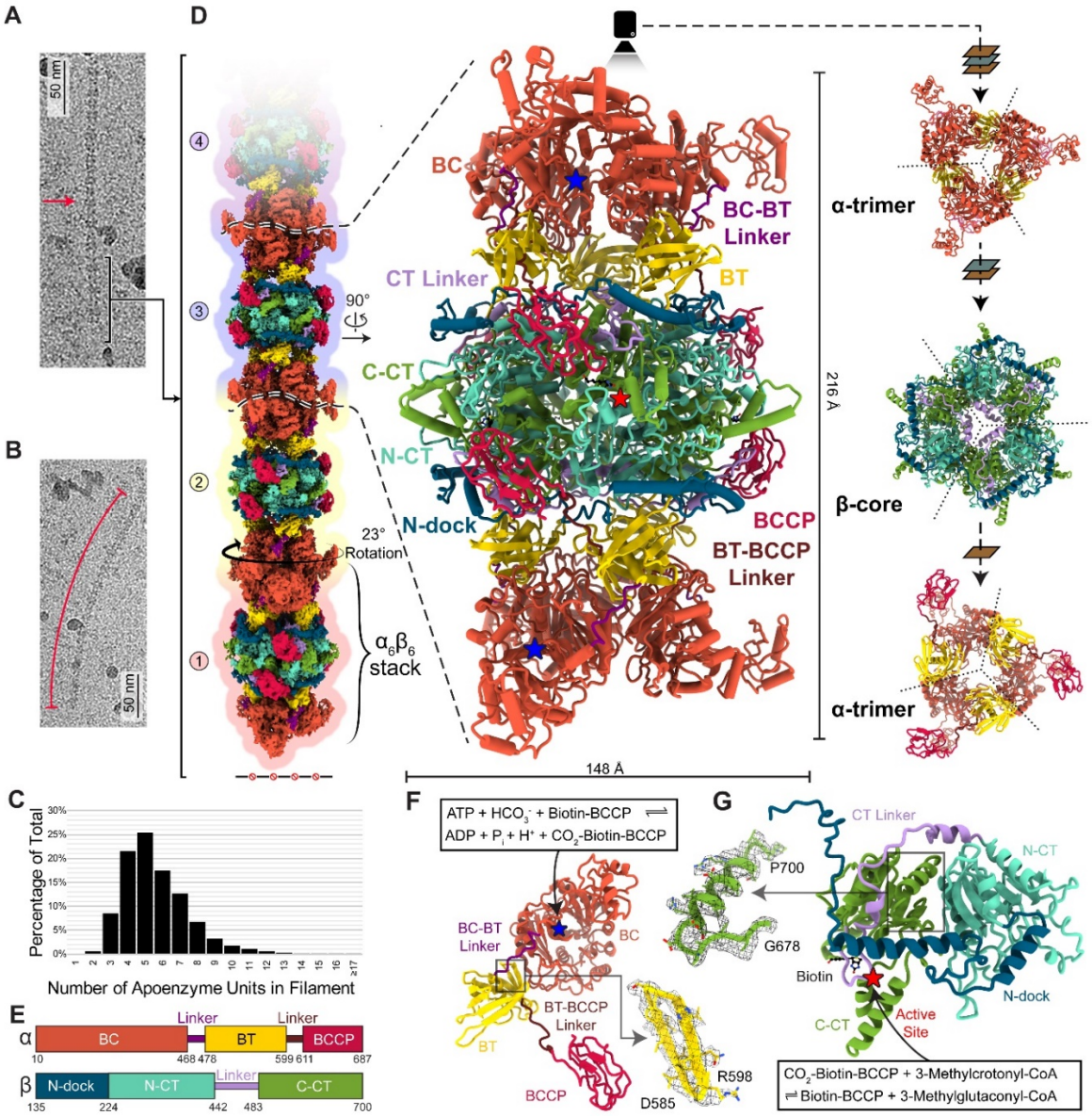


Figure 2. Structure of the filamentous LtMCC

(A-C) CryoEM images of a straight (A) and curved (B) LtMCC filament, and distribution of filament lengths (C).

(D-E) CryoEM map and atomic models of the LtMCC filament. The composite cryoEM map of the filament (left column in D) is composed of three copies of the α-centered map, three copies of the β-centered map, and one copy of the termini map, with overlapping subunits. The atomic models (middle and right columns in D) of an $\alpha_6\beta_6$ dodecamer and its trimeric stacks are shown in various views as ribbons, colored by domains according to the scheme in (E). The view directions are indicated by arrows, planes, and the camera. The dashed lines in the right column demarcate the boundaries between neighboring subunits (β-core is two-layered).

(F-G) Atomic model of an α-subunit (F) and a β-subunit (G) of LtMCC shown as ribbons colored by domains with active sites labeled. Insets: example cryoEM densities superimposed with the atomic model.

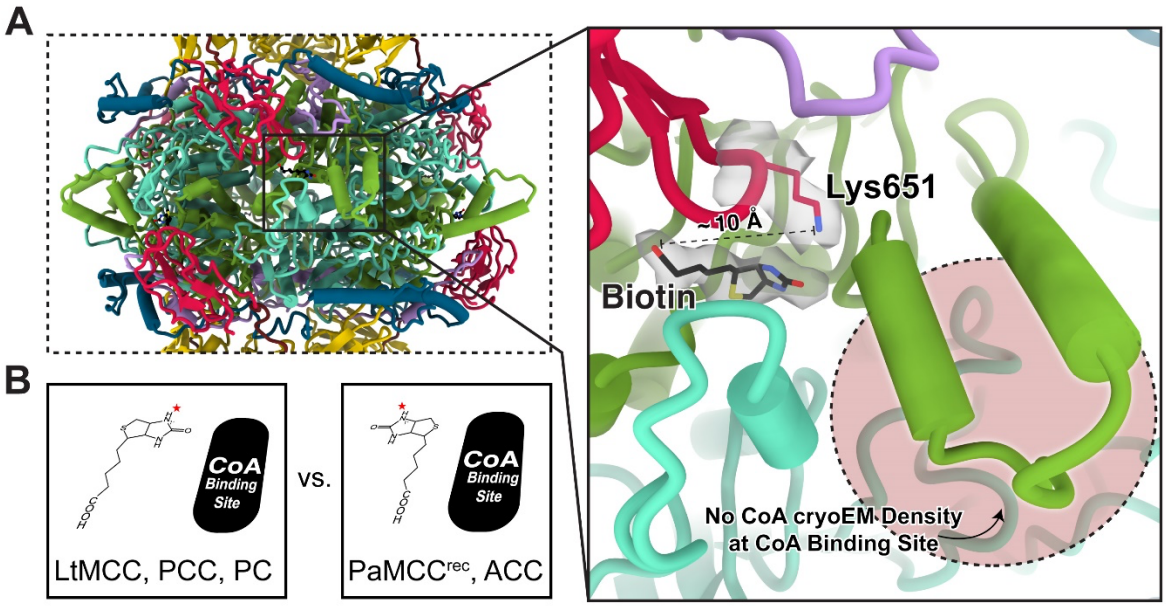


Figure 3. Presence of non-covalently bound biotin and lack of CoA in the LtMCC filament

(A) Close-up view of the middle column of Figure 2D, showing the biotin-containing site surrounded by the CT linker (purple), the BCCP (red), N-CT (turquoise), and C-CT (green) domains. The conserved Lys651 (red) and the non-covalently bound biotin (black) are shown as sticks, superimposed with their cryoEM densities (semi-transparent gray). The distance between the carboxyl group of biotin and the ε-amino group of Lys651 is about 10 Å. Notably, the CoA binding site (pink circle region) lacks cryoEM density, indicating absence of CoA.

(B) Comparison of biotin and CoA orientations documented in various carboxylases (Fry et al., 1985; Huang et al., 2010, 2012; Scheffen et al., 2021; Wei and Tong, 2015). The red star indicates the location of the N1′ atom of biotin.

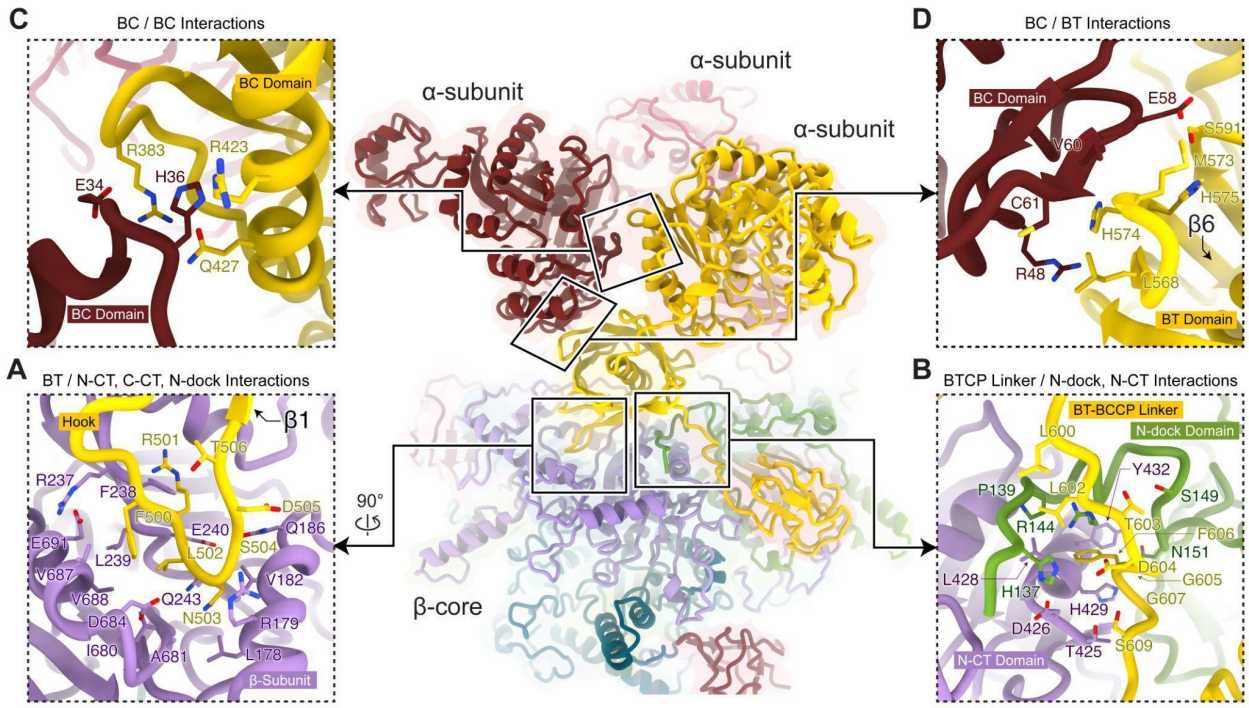


Figure 4. Subunit interactions in the filamentous LtMCC

Interacting regions are boxed and examined in close-up views (A-D) with their interfacial residues labeled. Interactions between α- and β-subunits (A-B) are more extensive than interactions among α-subunits (C-D).

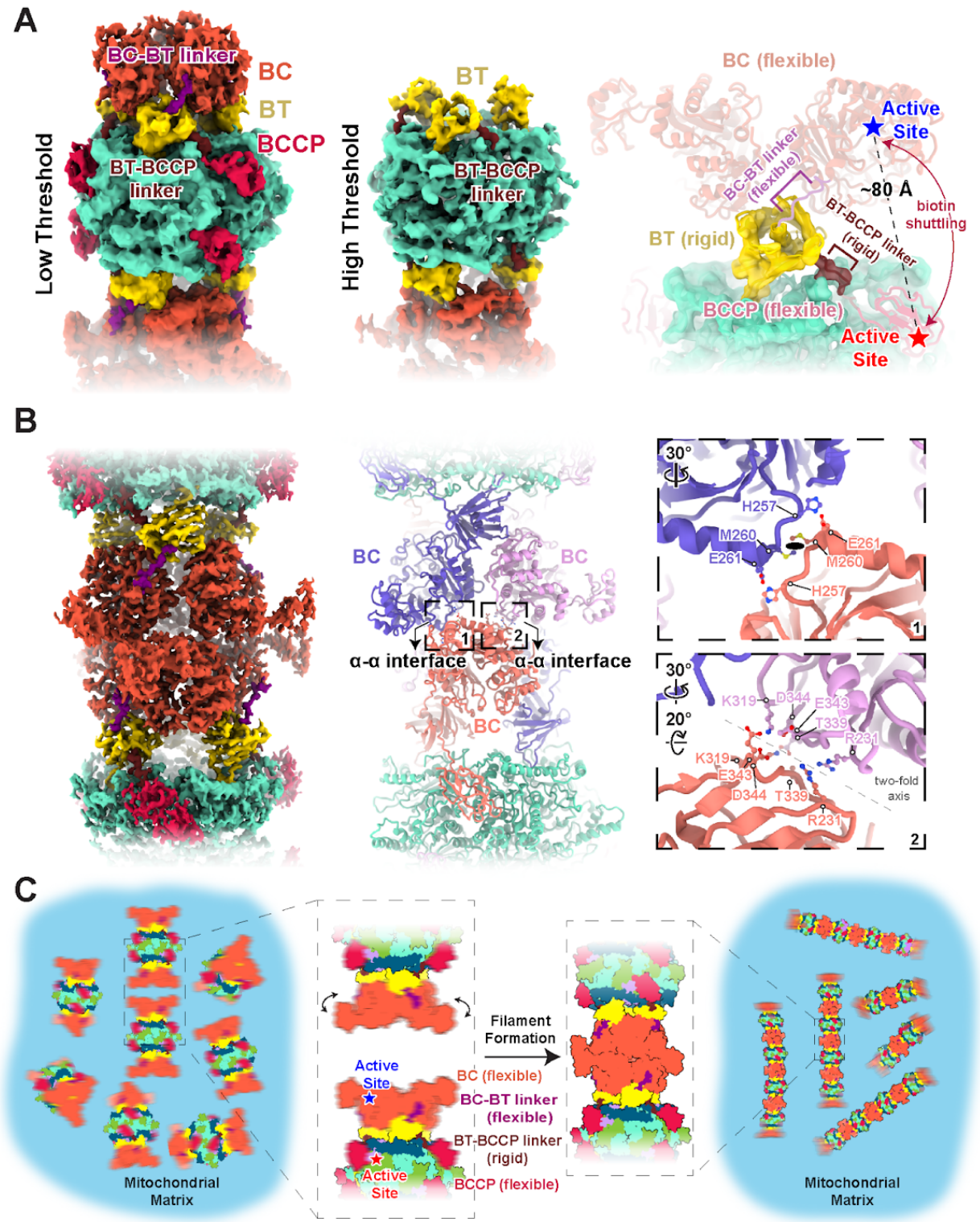


Figure 5. Filament formation stabilizes flexible BCCP and BT domains in LtMCC
(A) Structure of the filament termini. The left and middle panels show the 7.3 Å cryoEM map of the filament termini at a low and high density threshold, respectively. The α-subunit is colored by domain according to the scheme in Figure 2E and the entire β-core is turquoise. Note, while visible at low threshold, the BCCP and BC domain

502 densities disappear at high threshold. The right panel shows the atomic model of the
503 filament terminus with the rigid and flexible domains in harder and softer shades,
504 respectively.
505 (B) Structure of the α-α interface in the LtMCC filament. The cryoEM map (left panel) is
506 colored as in (A) but the atomic models (middle panel) of the interfacing α-subunits are
507 colored differently from each other to delineate individual α-subunits. Close-up views
508 (right panel) of the two boxed areas in the middle panel show interfacing residues at the
509 α-α interface. The ellipse in the top right panel denotes the local two-fold symmetry axis.
510 (C) Proposed model of filament formation and stabilization. Blurry domains indicate their
511 flexibility.

512 **Tables**

	α-subunit centered map	β-subunit centered map	Termini map
Data collection and processing			
Magnification	81,000	81,000	81,000
Voltage (kV)	300	300	300
Electron exposure (e^-/Å^2)	40	40	40
Defocus range (μm)	-1.5 to -2.5	-1.5 to -2.5	-1.5 to -2.5
Pixel size (Å)	1.1	1.1	1.1
Symmetry imposed	D3	D3	C3
Initial particle images (no.)	51,322	51,322	8,992
Final particle images (no.)	7,582	13,250	3,380
Map resolution (Å)	3.9	3.4	7.3
FSC threshold	0.143	0.143	0.143
Estimated resolution range (Å)	3-5	3-5	6-8
Map sharpening B factor (Å^2)	-112	-85	-153
Model refinement			
Model-to-map fit, mask CC	0.47	0.62	0.02
Model composition			
Non-hydrogen atoms	57846	57846	57846
Protein residues	7464	7464	7464
Ligands	BTI: 6	BTI: 6	BTI: 6
Mean isotropic B factor (Å^2)			
Protein	70.80	70.80	70.80
Ligand	150.43	150.43	150.43
R.M.S. deviations			
Bond lengths (Å)	0.006	0.006	0.006
Bond angles (°)	1.089	1.088	1.089
Validation			
MolProbity score	2.35	2.35	2.35

Clashscore	21.26	21.31	21.29
Rotamer outliers (%)	0.39	0.39	0.39
Ramachandran statistics			
Outliers (%)	0.00	0.00	0.00
Allowed (%)	9.40	9.38	9.38
Favored (%)	90.60	90.62	90.62

513 **Table 1. CryoEM map and model statistics.**

Supplemental Figures

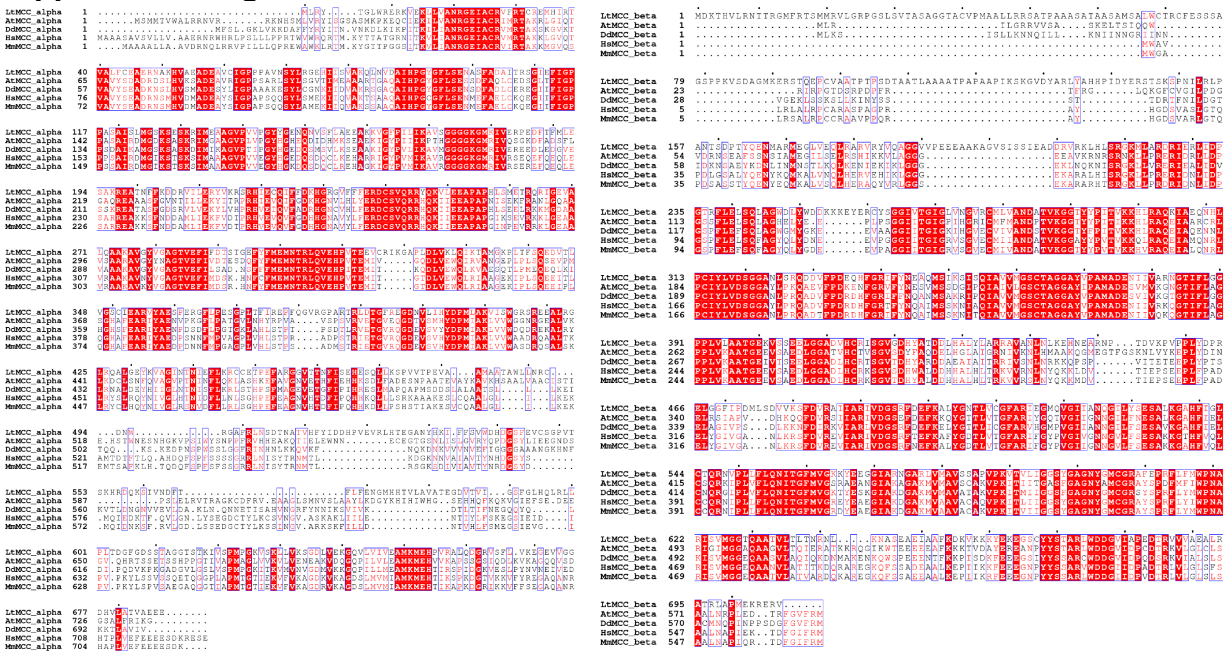


Figure S1. Multiple sequence alignment from *ESPript 3.0* (Robert and Gouet, 2014).

Left panel compares LtMCC α-subunit with α-subunits from *Arabidopsis thaliana* MCC (AtMCC), *Dictyostelium discoideum* MCC (DdMCC), *Homo sapien* MCC (HsMCC), and *Mus Musculus* MCC (MmMCC). Right panel compares LtMCC β-subunit with β-subunits from AtMCC, DdMCC, HsMCC, and MmMCC. Boxes with and without solid red background indicate identical residues and similar residues, respectively. Dots on top of the residues demarcate intervals of ten residues.

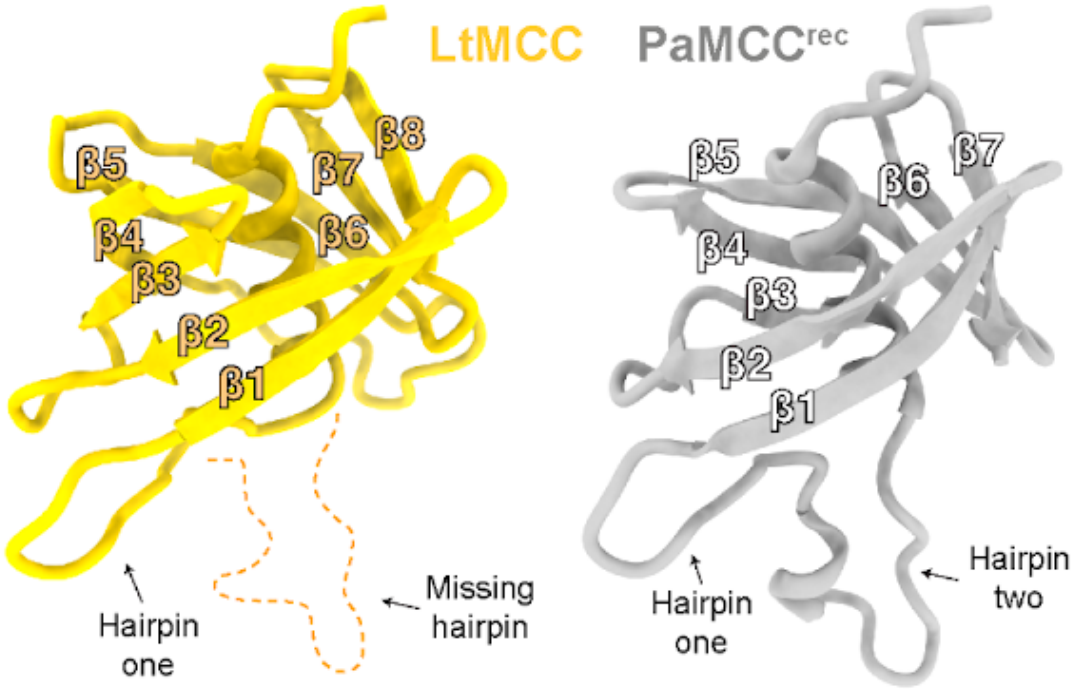


Figure S2. Structural comparison of the BT domain from LtMCC and PaMCC^{rec}
The LtMCC BT domain exhibits an 8-stranded β-sheet fold while the PaMCC^{rec} BT domain contains a 7-stranded β-sheet fold. The PaMCC^{rec} BT domain possesses a hook with two hairpins. The LtMCC BT domain possesses a hook with only one hairpin.

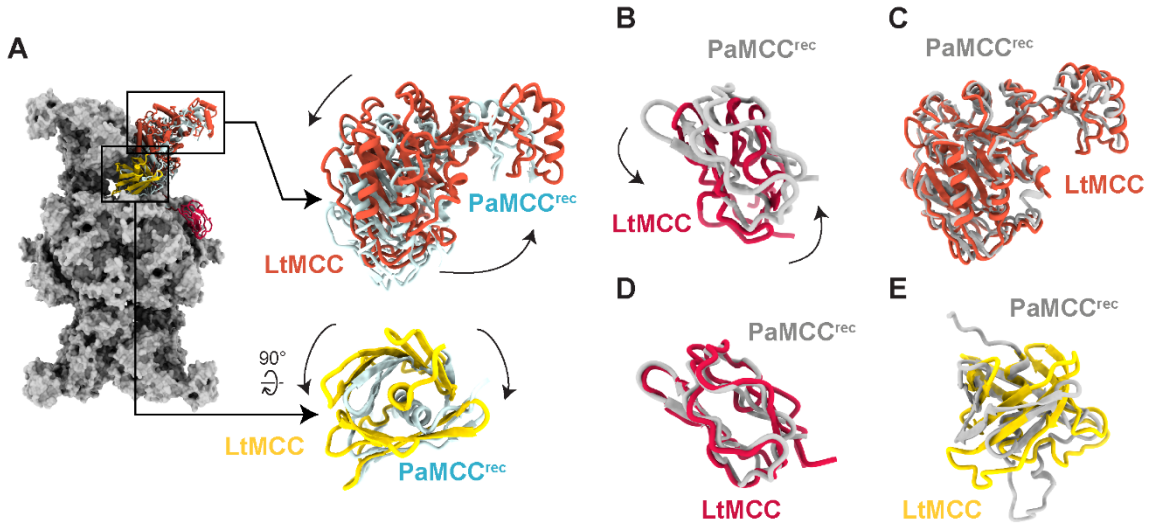
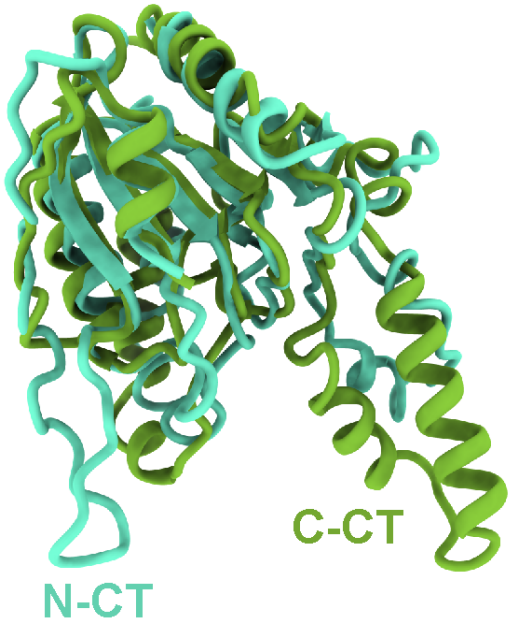


Figure S3. Structural comparison of PaMCC^{rec} and LtMCC

(A-B) Mismatched domain structures when the PaMCC^{rec} and LtMCC $\alpha_6\beta_6$ dodecamers are superimposed globally. PaMCC^{rec} apoenzyme (A) and holoenzyme (B) are globally superimposed with the LtMCC dodecamer. In (A), the BC and BT domains are rotated in LtMCC compared to the PaMCC^{rec} apoenzyme, as indicated by arrows. The BCCP domain is unmodeled in the PaMCC^{rec} apoenzyme. The entire PaMCC^{rec} apoenzyme is shown as a gray surface. In (B), the BCCP domain in LtMCC is rotated relative to the BCCP domain in the PaMCC^{rec} holoenzyme, as indicated by arrows.

(C-E) Matched domain structures when individual domains from PaMCC^{rec} and LtMCC are superimposed locally. BC (C), BCCP (D), and BT (E) domains from the two dodecamers are treated as individual models and superimposed independent from the global dodecameric structure.



542
543 **Figure S4. The N-CT and C-CT domain local alignment for LtMCC**
544 The N-CT and C-CT domains are homologous, despite low sequence similarity.

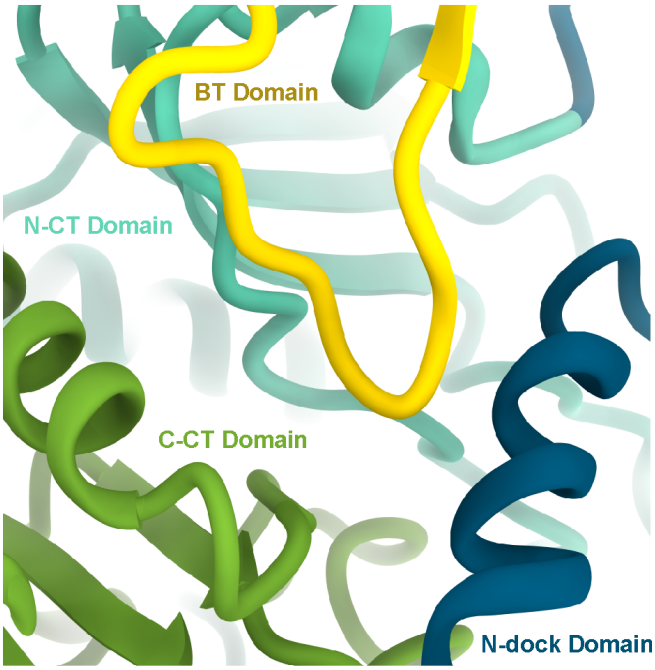


Figure S5. Close-up view of the α-subunit BT hook interacting with the domains that make up the β-subunit groove

The BT hook is colored yellow. The N-CT, C-CT, and N-dock domains from the same β-subunit make up the β-subunit groove. The N-CT domain is colored in light blue, the C-CT domain is colored in green, and the N-dock domain is colored in dark blue.

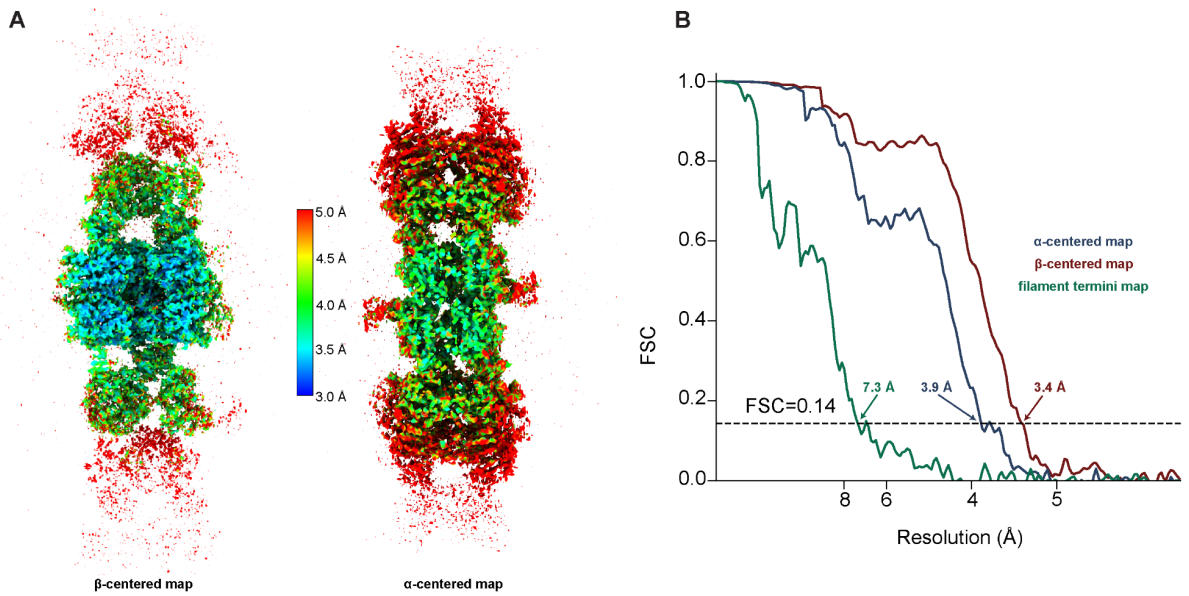


Figure S6. Resolution evaluation of cryoEM reconstruction for LtMCC filaments
(A) Local resolution evaluation by ResMap (Kucukelbir et al., 2014) for the α-centered and β-centered cryoEM maps of LtMCC filaments. The color key between the two maps shows their coloring by resolution.
(B) FSC curves of the final α-centered, β-centered, and filament termini cryoEM maps.

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