Assembly mechanism of the pleomorphic immature poxvirus scaffold

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In Vaccinia virus (VACV), the prototype poxvirus, scaffold protein D13 forms a honeycomb-like lattice on the viral membrane that results in formation of the pleomorphic immature virion (IV). The structure of D13 is similar to those of major capsid proteins that readily form icosahedral capsids in nucleocytoplasmic large DNA viruses (NCLDVs). However, the detailed assembly mechanism of the nonicosahedral poxvirus scaffold has never been understood. Here we show the cryo-EM structures of the D13 trimer and scaffold intermediates produced in vitro. The structures reveal that the displacement of the short N-terminal α-helix is critical for initiation of D13 self-assembly. The continuous curvature of the IV is mediated by electrostatic interactions that induce torsion between trimers. The assembly mechanism explains the semiordered capsid-like arrangement of D13 that is distinct from icosahedral NCLDVs. Our structures explain how a single protein can self-assemble into different capsid morphologies and represent a local exception to the universal Caspar-Klug theory of quasi-equivalence.
Poxviruses are large, enveloped, double-stranded DNA viruses that infect humans, other vertebrates, and arthropods. Viral infection typically causes skin lesions but can also prove fatal, as in the case of smallpox. Despite eradication of smallpox, zoonotic outbreaks of smallpox-like diseases urge thorough understanding of the poxvirus replication cycle to facilitate the development of strategies for prevention and treatment.

Poxviruses share a common phylogeny and replication pathways similar to those of NCLDVs. A common feature of NCLDVs is the presence of major capsid proteins with a double-jelly-roll motif that assemble into icosahedral capsids. Notable exceptions are poxviruses, ascoviruses, and pandoraviruses, which exhibit variable capsid diameters and do not follow canonical symmetry. In Vaccinia virus (VACV), copies of the double-jelly-roll protein D13 bind to the viral membrane via interaction with its partner protein A17 and assemble into a scaffold surrounding the viral membrane. This process leads to the formation of a spherical immature virion (IV). Subsequent proteolytic processing of A17 releases the D13 scaffold from the viral membrane, leading to a dramatic transformation into the brick-shaped mature virion (MV). Unbound to the viral membrane, D13 exist as trimers and is found forming aggregated hexagonal patches and rodlets in the viral factory. Only with the expression of viral late proteins and in association with the viral membrane, D13 trimers assemble into a crescent-shaped honeycomb lattice followed by complete formation of the spherical immature virion upon DNA uptake. However, in vitro assembly of D13 demonstrated formation of spherical particles with the morphology comparable to authentic IV scaffolds. This suggests that the intrinsic curvature induced by D13 self-assembly is playing a major role in governing the shape and size of IVs. Hence, detailed molecular insights into the intermolecular D13 interactions are needed to understand the scaffold assembly mechanism.

The 62-kDa D13 protein exists as trimers both in vivo and in vitro, and structures of the recombinantly expressed D13 trimer have been determined by X-ray crystallography. D13 adopts a double-jelly-roll structure composed of 8 antiparallel \( \beta \)-strands. A head domain is inserted between \( \beta \)-strands of the C-terminal jelly roll. Recently, the structure of D13 in complex with the antibiotic rifampicin was determined by X-ray crystallography, illustrating the mechanism by which the antiviral drug prevents binding of A17, occupying the phenylalanine-rich pocket on the 3-fold axis of the D13 trimer. Inhibition of the D13-A17 interaction would impair recruitment of D13 onto the viral membrane, leading to aberrant virion formation, as previously demonstrated by mutagenesis. However, none of the crystallographic packing arrangements from any of the reported structures represent the honeycomb-shaped lattice observed on the surface of authentic IV, and intertrimer interactions that govern D13 assembly have been poorly resolved.

**Results**

**Structure of D13 trimer.** We purified recombinant D13 trimer protein for structure determination using single-particle cryo-electron microscopy (cryo-EM). Initial attempts were hampered by problems associated with preferred particle orientation and spontaneous protein assembly into honeycomb-like crystalline patches that consist of rings of five or six trimers (Supplementary Fig. 1). This problem was mitigated using holey EM grids coated with a graphene oxide support film (Supplementary Fig. 2a), and the cryo-EM structure of the D13 trimer was determined at 2.3 Å resolution (Fig. 1a and Supplementary Fig. 2). In our atomic model, we followed the convention for the jelly-roll fold with BIDG-CHEF \( \beta \)-strand-order (Fig. 1b). The refined atomic model based on this structure was largely identical to previously reported crystal structures (average RMSD 0.495 Å). However, the presence of the short N-terminal tail \( \alpha \)-helix (aa 1-10) was inconsistent with previously reported models. In the first two crystal structures, electron density for this helix was only observed in the D13D513G mutant but not in wild type protein, whereas subsequently reported crystal structures contain the homologous capsid proteins of icosahedral NCLDVs. Our structures demonstrate how a single protein can self-assemble into different capsid morphologies induced by a small conformational change of a peripheral helix.
corresponding density in only two of the three subunits\textsuperscript{12}. Our cryo-EM map showed intact N-terminal tail \(\alpha\)-helices in all three subunits, with or without imposition of 3-fold symmetry, although reduced local map resolution and the increased B-factor suggested that the helix was prone to dislocation (Supplementary Fig. 2f–h). In our structure, the hydrophobic face of the N-terminal helix (M1, I5, I6 and L9) was held in a pocket of surrounding hydrophobic residues from the adjacent subunits. Additional hydrogen bonds were identified between the side chains of T4, N2, Q35 and the carboxylic groups of V451, L452, and N2 (Fig. 1c). The contact between the N-terminal helix and the interaction pocket is nearly identical when compared to previously determined crystal structures (PDB 3SAM, 2YGC, 6BE1)\textsuperscript{9,11,12}, with marginal discrepancy of the contact surface area (Supplementary Fig. 3a).

**Intertrainer interactions in D13 trimer doublet.** Image processing of D13 trimers revealed a small subpopulation of adjacent trimers bound together, forming doublets of trimers. Further 2D and 3D classifications enabled segregation of doublet particle images from the trimer singlets. The scarcity of intermediate angular views within the dataset was supplemented by additional micrographs acquired from tilted specimen grids without the graphene oxide support film (Supplementary Fig. 4a). Although the resolution of the resulting 3D reconstruction (3.9 Å on average) was not fully isotropic (Supplementary Fig. 4e), it allowed unambiguous positioning of an atomic model for identification of the intertrainer interfaces. There are two major interfaces that mediate doublet formation (Fig. 2a). The first is located between one of the head domains on each of the two trimers. Although map resolution at this interface was insufficient to determine the precise positioning of all the interacting residues, a potential salt bridge interaction between R353 and D325 is observed in a magnified view in a red box. The cation-\(\pi\) interaction between Y62 and R498 is shown in a red box.

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**Fig. 2 Cryo-EM structures of the VACV D13 trimer doublet.** a Isoelectron potential surface (contoured at 3.0σ above average) of D13 trimer doublet with the docked molecular model. Intertrainer contacts are revealed in slabs (boxes with bold and dashed lines) extracted at different clipping planes. b Head-to-head contact between the trimers in the doublet model superposed on the cryo-EM map. A potential salt bridge interaction between R353 and D325 is shown in a magnified view in a red box. c Base-to-base contact between trimers in the doublet model superposed on the cryo-EM map. Positions of the missing N-terminal tail \(\alpha\)-helix in the doublet structure are depicted as purple circles, with its isoleucine (I6) rotamer indicated by a protruding dashed line. The cation-\(\pi\) interaction between Y62 and R498 is shown in the red box. d Relative position of neighboring trimers in the doublet as shown by cylinders, indicating the 3-fold symmetry axis of each trimer. The torsion angle between the axes is 8.0°. e, f Linear and planar expansions of the D13 doublet interface. The side view of the linear expansion exhibits convex curvature with the trimer base facing the center of the arch. Planar expansion results in a ring of six trimers, but the ring does not close (arrow) due to geometrical clash. Scale bars, 5 nm.
to clearly define side chain interactions, it was possible to trace the loops extending toward the neighboring trimer (Fig. 2b). These include an extended loop that connects β-strands E and F (EFH loop) and a hairpin loop between β-strands C and D (CDH loop) in the head domain. The interface is clustered with polar (N355, Q324, S356) and charged amino acid residues (D325, R353, K354) around the two-fold symmetry axis. Side chains of R353 in the EFH loop and D325 in the CDH loop extend toward each other, suggesting salt bridge interactions. Another interface is located at the base of the trimers, which would be proximal to the viral membrane in the IV. At this interface, the major difference of doublet structure in comparison to the singlet trimer is the unambiguous absence of the N-terminal tail α-helix (Fig. 2c and Supplementary Fig. 3b). The interface involves the loop that connects β-strands C and D of the N-terminal jelly-roll (CDN loop) and the loop connecting β-strands G and H of the C-terminal jelly-roll (GHc loop) in the neighboring trimer. Unlike the head-to-head interface, this interaction occurs pairwise between two monomers in each of the two trimers. The clearly resolved features in the reconstructed electron potential map between Y62 and R498 suggests cation–π interactions as the main stabilizer at this interface. We found Y62 in close proximity to J6 of the N-terminal tail α-helices in the singlet trimer structure, suggesting that the hydrophobic interaction between these residues may have been altered in favor of intertrimeric interaction in the doublet structure, upon dislocation of the N-terminal tail α-helix. Furthermore, the doublet is characterized by a slight twist of the trimer axes defined by a torsion angle of 8.0°, leading to an arched arrangement when the doublets are linearly expanded (Fig. 2d, e). The bases of D13 trimers face the center of the arch, which supports the idea that remodeling of the viral membrane into a convex curvature is induced by the self-assembled D13 scaffold. However, planar expansion of the doublet prohibits closure of the dome-shaped curvature, suggesting that additional intertrimer interfaces or oligomers are involved in the formation of spherical poxvirus scaffolds (Fig. 2f).

Fig. 3 Assembly of D13 requires displacement of the N-terminal tail α-helix. Transmission electron micrographs of a wtD13, b His6-tagged D13 and c D1318-548, before (left column) and after (right column) low-salt buffer-mediated assembly. Images are of negatively stained samples, except in the right column of b and c where images of vitrified samples captured by cryo-EM are shown for clear morphological observation. Insets show details of the respective contoured cryo-EM reconstructions. 130,384, 173,354 and 156,813 particle images were used to reconstruct the maps shown in a, b, and c, respectively. Their structures reveal that the wild-type trimer has an intact N-terminal tail helix (colored purple), whereas the corresponding density is missing in His6-tagged protein and in the truncation mutant. Proteins exist as homogenous trimers in 600-mM NaCl storage buffer. Reduction of salt concentration to 150 mM leads to aggregation in case of wtD13, to spherical assemblies for His6-tagged D13, or to a mixture of tubular and spherical assemblies for D1318-548. The spherical particles in b were sometimes imaged as concentric shells containing several layers, or incompletely closed structures. Isoelectron potential surfaces were contoured at 3.0σ above average. Map resolutions are 2.3 Å, 2.6 Å and 4.1 Å (a, b, c, respectively). Scale bars, 100 nm.

Critical role of the N-terminal α-helix in poxvirus scaffold assembly. The absence of the N-terminal tail α-helix in the doublet structure suggests that its displacement likely helps to initiate intertrimer interactions, promoting ordered assembly of oligomers and leading to formation of spherical IV-like particles. To test this hypothesis, we used in vitro D13 self-assembly. The protein solution turned turbid after dialysis into low-salt buffer, but examination by transmission electron microscopy (TEM) showed aggregated honeycomb-like patches and rodlets without any IV-like particles (Fig. 3a). Under these conditions, the D13 protein with an N-terminal His6-tag and an extended linker (28 amino acid residues) assembled into spherical particles with an average diameter of approximately 340 nm, revealing a dis-
confirming the essential function of the N-terminal tail helix for D13 self-assembly. Based on the observation that both spherical and tubular assembly products co-exist under the same assembly condition, these objects are believed to be variants of curved assembly.

**Intertrimer interactions in tubular D13 assembly.** To investigate intertrimeric interactions in the context of higher-order assembly, tubular assembly products of the N-terminal truncation mutant D13_{18-548} were subjected to cryo-EM and helical reconstruction (Supplementary Fig. 6a–d). Refined helical parameters indicated a 3-start helix with a 33.9 Å rise and a 77.0° twist. A 3D reconstruction at 7.3 Å resolution exhibited a continuous honeycomb-shaped lattice rolled into a tube (Fig. 4a). Single-particle analysis of symmetry-expanded, signal-subtracted particle images of trimer sextets resulted in improved cryo-EM map resolution at 3.9 Å, enabling further analysis of intertrimer interactions in tubular D13 assembly.
interfaces (Fig. 4b and Supplementary Fig. 6e–h). The structure exhibits two alternating modes of intertrimer arrangements, which are mostly stabilized by electrostatic interactions (Supplementary Fig. 7) and agree with the self-assembly behavior of D13 with varying salt concentrations of the buffer solution. The first mode (mode I) is similar to the doublet structure in which the interaction between the head domains appears most extensive between the EF₃ and CD₃ loops. The base-to-base interface involves cation–π interactions between Y62 of the CD₃ loop and R498 of the GH₂₁ loop (Fig. 4c). The second mode (mode II) also implicates two intertrimer interfaces. However, in this arrangement, two monomers in each trimer are involved, forming pairwise interactions (Fig. 4d). In one moiety, contacts between the EF₃ and FG₃ loops (P144 and N145) appeared most prominent. In the other moiety, the EF₃ loop is in close proximity to charged residues in a short β-strand in the DE₃ loop (E77 and K79) and a short α-helix in the HI₀ loop (E205 and K204). Symmetric Y62–R498 interactions were no longer found in the base-to-base interface of the mode II arrangement. One moiety appears to involve a cation–π interaction between Y62 and R446 δ-strand E of the C-terminal jelly roll (E₃), replacing R498 in the mode I arrangement. The other moiety exhibits close contact between R498 of one monomer and Y62 and D60 of the other monomer. Residues R353 and Y62 were critical in all modes of arrangements for head-to-head and base-to-base interfaces, respectively. This was confirmed by mutation of these residues to alanine, resulting in assembly incompetence in low-salt buffer (Supplementary Fig. 8). Each trimer was positioned with a slight torsion in relation to neighboring trimers in the sextet. Torsion angles ranged from 7.6° to 9.4° and from 12.5° to 13.5° in mode I and mode II arrangements, respectively (Fig. 4e). The torsion angle varied among mode I arrangements, indicating slight plasticity. While the twist direction was consistent for mode I arrangements, it alternated depending on the asymmetric contact points between the trimers. Variability of the intertrimer torsion angle and direction found in the helical assembly suggests a possible continuous curvature in all directions, inducing the spherical morphology observed in authentic VACV IV. To examine this, two types of intertrimer arrangements were alternatively modeled instead of two consecutive mode I arrangements, followed by a mode II arrangement, as originally found in the tubular assembly. This allowed for closure of a crown-shaped ring of six trimers (Fig. 4f). However, expansion of the sextet failed to generate a continuous spherical morphology.

Discussion

Poxvirus scaffolds consist of loosely connected rings of six trimers in contrast to tightly packed capsomers of icosahedral NCLDV capsids that require additional minor capsid proteins and accessory proteins. Poxviruses require concerted interactions between viral late proteins for the formation of the capsid-like scaffold in vivo, with the exception of the D13D₁₃G mutation that results in stacks of planar honeycomb lattices in the absence of viral late proteins. However, we show that scaffold morphology is mainly governed by self-assembly of D13 without accessory proteins. The charge distribution on the surface of the D13 trimer roughly resembles that of the capsomers of NCLDVs and hence is likely to follow their capsid assembly scheme based on electrostatic interactions. The intertrimer torsion and its variation are unique features of the poxvirus scaffolding protein. We revealed that the curvature that results from alternating torsion between the D13 trimers forms the basis of semiregular, spherical immature poxvirus particles. Mild curvature between the capsomers has been described for African swine fever virus (ASFV) major capsid protein, although such curvature depends on the microenvironment within the icosahedron and is dissimilar to continuous curvature in the case of poxvirus scaffolds.

We found that the N-terminal short tail α-helix of D13 is critical for in vitro assembly into ordered structures. We propose that repositioning of the N-terminal tail α-helix away from D13 toward the surface of the lipid bilayer may act as a molecular trigger that initiates scaffold assembly in vivo. This scenario implies that D13 assembly into a scaffold occurs in situ only when bound to the viral membrane via the D13-A₁₇ interaction, but not before, thereby preventing the virion assembly sequence without a lipid membrane. This is supported by cellular cryo-electron tomography (cryo-ET) of immature poxvirus, which showed that intact scaffold formation requires association with a lipid membrane. N-terminal helices have been found to act as anchors on proximal lipid membranes in double-jelly-rolloid capsids of dsDNA bacteriophages and African swine fever virus (ASFV). However, such interactions were only proposed to stabilize the capsid and not to initiate assembly.

The N-terminal helix and residues involved in the intertrimer interactions of D13 are widely conserved among chordopoxviruses, suggesting a common scaffold mechanism. In contrast, this conservation is poor in entomopoxviruses; most notably the absence of the N-terminal helix (Supplementary Fig. 9). The intertrimer interfaces are distal from the rifampicin binding site and from residues that confer rifampicin resistance in VACV, indicating that these intertrimeric interactions are independent of drug binding. (Supplementary Fig. 10a). Aspartic acid residues 513 (D513) from neighboring trimers were within 7 Å along the 2-fold symmetry axis in the mode I arrangement, but mutation D13D₁₃G, which leads to abrogation of the curved lattice, could not be explained by our structure (Supplementary Fig. 10b).

Nearly 60 years ago, Donald Caspar and Aaron Klug introduced their theory of quasi-equivalence of macromolecular assemblies, which predicts arrangements of protein subunits at the surface of icosahedral virus particles in a regular lattice, parametrized by a triangulation number (T). The Caspar-Klug theory has been instrumental in understanding the molecular symmetry of icosahedral viruses and many other macromolecular assemblies. With the advent of cryo-EM at near-atomic resolution, a number of deviations from ideal symmetry have been discovered. The VACV scaffold is an example of such a deviation, in which D13 is uniquely responsible for generating various building blocks and continuous curvature. The observation of pentameric tiling (Fig. 5b and Supplementary Fig. 1) supports the hypothesis that the underlying symmetry of the VACV scaffold as a result of D13 assembly is an icosahedral lattice, but local deviations result in an imperfect, yet semi-regular scaffold (Fig. 3b, Supplementary Fig. 5 and Supplementary Movie 1). The N-terminal helix represents a mobile element that regulates the initiation of scaffold assembly and electrostatic intertrimer interactions, resulting in different local symmetries that follow the principle of quasi-equivalence within a patch of common assembly mode, but not throughout the entire scaffold (Fig. 5c). Using only a single gene product, such locally irregular transient scaffold assembly may serve as an economic and efficient way of packaging the large poxvirus genome in its immature virion. The interactions identified in our study may inspire the design of a new generation of assembly inhibitor drugs (e.g., peptidomimetics based on the N-terminal helix) and further structural investigation of other pleomorphic viral capsids, such as HIV.

Methods

Protein expression and purification. The gene encoding D13 of Western Reserve strain Vaccinia virus in the pPROEX-Hta vector was expressed in BL21(DE3) cells...
Fig. 5 Capsid-like assembly hypothesis of the immature VACV scaffold in vivo. a Schematic illustration of D13 assembly initiation. D13 exists as individual trimers in the cytoplasm. Once D13 is recruited onto the surface of a viral membrane (depicted by a curved gray line) through an interaction with A17 (depicted as dashed ellipses), the N-terminal helix (purple) is dislocated away from D13 and exposes the base-to-base intertrimer interface. D13 trimers can subsequently dimerize with two distinct intertrimer torsions (clockwise and counterclockwise arrows), inducing curvature. b Gallery of D13 oligomers that are basic building blocks of the honeycomb-shaped lattice. Class averages were generated from electron micrographs of honeycomb-like patches (Supplementary Fig. 1). c Expansion of the honeycomb-like lattice into a spherical scaffold. The cryo-electron tomography reconstruction shows a spherical IV scaffold-like particle (diameter 340 nm) with a typical honeycomb-like lattice. Although the limited resolution precluded detailed modeling of the scaffold, it is likely that additional patterns of oligomers such as pentameric building blocks or different combinations of intertrimer arrangements are required to form the authentic immature VACV scaffold. Scale bars, 10 nm in a, b, and 100 nm in c, respectively.
and a 1.8-µm beam diameter area were used. A dose-symmetric tomography acquisition scheme was applied on a Falcon 3 direct electron detector in EC mode using the transfer function (FTF) over magnitude. For D13 trimer doublet maps, the dose total applied to each tomogram was approximately 120 e/Å², and 5 movie fractions were collected for each tilt image at a dose rate of 0.14 e/Å²/s.

**Cryo-EM Image processing.** All single-particle datasets were processed with Relion 3.1 software unless stated otherwise. Motion correction and contrast transfer function (CTF) estimation were performed using MotionCor2 and CTFFIND49, respectively. Statistics of individual datasets can be found in Supplementary Table 2. Cryo-ET data were processed using IMOD. Visual examination of maps and figure preparations were performed with UCSF Chimera38 and PyMol (The PyMol Molecular Graphic System, Version 2.4.0, Schrödinger, LLC).

For wtD13 trimers, 2,168 dose-weighted micrographs were manually inspected. Images without graphene oxide support, poor estimated maximum resolution or strong astigmatism from CTFFIND calculations were discarded. From 1,862 assorted micrographs, particles were automatically picked using 2D class averages without downsampling. Because of the clear 2-fold symmetry of the 3D class, two copies of the 3D reconstruction was estimated at 4.10 Å resolution.

The total number of particles was subjected to 3D classiﬁcation. A total of 746,660 particles were subjected to 3D classiﬁcation without symmetry imposition. A total of 835,797 particles from a combined 1,695 micrographs were binned by a factor of 2 and extracted into 256-pixel square boxes. A consensus reconstruction was generated from helical parameters (77° twist, 33.5 Å rise with the command ‘cylinder –cyld_interal_diameter 900 –angix 5.6 –rise 33.5 –twist 77 –boxdim 256’). The particles that belong to each class were re-extracted into 512-pixel square boxes after binning by a factor of 2, followed by 3D ﬁltering. Based on the detailed structural features of D13 trimers in the tubes and the nominal resolutions of the reconstructions, a total of 75,079 particle images from four classes were selected and combined for the final reconstruction (Supplementary Fig. 6b). After 3D ﬁltering, CTF reﬁnement and Bayesian particle polishing were performed, followed by reconstruction with Ewald sphere correction. The final map resolution was 7.3 Å at 0.143 FSC. Particles were re-extracted into 1024-pixel square boxes and a 3D reconstruction was generated prior to symmetry expansion and signal subtraction. From the helical reconstruction, 5 asymmetric units were symmetry-expanded based on the final estimated helical operator (76.98° twist, 33.86 Å rise). For signal subtraction, a volume segment that corresponds to a ring of six trimers in the tube was generated from the reconstruction using UCSF Chimera. The map was then used to create a binary mask embracing six trimers, which was applied to the symmetry-expanded stack of particle images. Signalsubtracted sextet images were subjected to 2D class averaging using cisTEM48, and 305,052 particles from good class averages were selected for further analysis in 3D (Supplementary Fig. 6e, f). A total of 247,311 particles were subjected to 3D classiﬁcation of 42,854 particles in 3D classes with detailed structural features were selected for ﬁnal reconstruction in cisTEM. The resulting half-maps were examined and postprocessed using Relion 3.1. Final resolution of the map was 3.87 Å at 0.143 FSC (Supplementary Fig. 6h, g).

For Cryo-ET data, movie frames were aligned and summed using MotionCor2. Images were phase-adjusted using cifphaseadjust49 and the tilt series was aligned based on the fiducial markers. Tomograms were reconstructed from images that were binned by 2 using the simultaneous iterative reconstruction technique (SIRT) implemented in IMOD43.

**Protein structure modeling.** 3D reconstructions were subjected to density modiﬁcation with the ResolveCryoEM tool45 in the Phenix software suite prior to model reﬁnement. All coordinate reﬁnements were performed using the real-space reﬁnement routine in Phenix45. The X-ray crystal structure of D13 (PDB ID 6BEI) was manually ﬁtted into the cryo-EM map of our D13 trimer in UCSF Chimera and used as a reference model for atomic coordinate reﬁnement in Phenix, while enforcing a noncrystallographic symmetry constraint. The resulting reﬁned trimer model was then used as a reference to reﬁne models of the trimer doublet and trimer sextet, respectively. These reﬁned models were evaluated in Coot46, followed by further postrefinement with Phenix. The quality of the ﬁnal models and map-to-model correlations were calculated using Phenix' Cryo-EM validation and Mtiage tools47.

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

**Data availability.** Atomic coordinates of cryo-EM based models have been deposited under PDB IDs 7VFD (wtD13 trimer), 7VFE (His₆-tagged D13 trimer), 7VF7 (D13₆-548 trimer), 7VFG (D13 trimer doublet) and 7VH7 (D13 trimer sextet). Cryo-EM maps have been deposited in the EM database under accession codes EMD-31949 (wtD13 trimer), EMD-31950 (His₆- tagged D13 trimer), EMD-31951 (D13₆-548 trimer), EMD-31952 (D13 trimer doublet),
EMD-31953 (D13 trimer tubular assembly) and EMD-31954 (D13 trimer sextet). Raw image data are available from the authors upon reasonable request.

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Author contributions

J.H. conceived and designed experiments. J.H. and T.K. performed molecular cloning and protein purification. J.H. created mutants. J.H. and M.W. collected cryo-EM data. J.H. and M.W. processed cryo-EM data. H.M. and J.H. carried out atomic modeling and refinement into cryo-EM maps. M.W. supervised the project and provided advice on cryo-EM and image processing. J.H. and M.W. wrote the manuscript. All authors analyzed results and contributed to writing the paper.

Competing interests

The authors declare no competing interests.
Additional information

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