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Supplemental Information

Molecular Simulation of Mechanical Properties and Membrane Activities of the ESCRT-III Complexes

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Table S1: Summary of MD simulations conducted for Vps32 filament models and their interaction with lipid membrane

System ($X \times Y \times Z\ nm^3$)	Number of proteins and lipids	Total number of atoms	Simulation time (ns)
1-D filament structure ($17.7 \times 5.3 \times 10.8$)	6 protein monomers	99,267	100
2-D filament structure (two configurations) ($17.7 \times 5.3 \times 10.8$)	12 protein monomers	100,077	100
1-D filament mechanical properties ($68.\ 7\times 8.4 \times 10.6$)	21 protein monomers	617,044	140
Protein-membrane interface with HMMM model (four configurations) ($14.\ 6\times 14.6 \times 14.8$)	3 proteins + 360 lipids	277,914	75
Protein-membrane interface with full lipid model[a] (four configurations) ($15.\ 4\times 15.4 \times 11.1$)	3 proteins + 646 lipids	270,932	140
Membrane curvature simulation with full protein ($32.2 \times 14.4 \times 10.9$)	3 proteins + 1172 lipids	491,638	85
Membrane curvature simulation without protein ($32.2 \times 14.4 \times 10.9$)	1172 lipids	484,399	85
Membrane curvature simulation without α_0 helix ($32.2 \times 14.4 \times 10.9$)	3 proteins + 1172 lipids	491,131	85
Membrane curvature simulation with two trimers ($61.5 \times 14.4 \times 11.0$)	6 proteins + 2584 lipids	950,196	20
Membrane curvature simulation with two trimers ($108.0 \times 14.4 \times 12.5$)	6 proteins + 4484 lipids	1,872,098	20

a. To set up the system, simulations with the HMMM model (the row above) was first conducted at low lipid density or high area/lipid condition to better sample protein insertion and equilibrate lipid distributions around the protein. The full lipid model was then generated by re-growing the tails of the equilibrated HMMM lipids using CHARMM-GUI. The system is then replicating twice along the X and Y directions. Then using VMD, the final full lipid model was generated keeping a single protein trimer and desired number of lipids such that the ratio of POPC:POPS lipids is ∼70:30 with the same number of POPC/POPS lipids in both leaflets.

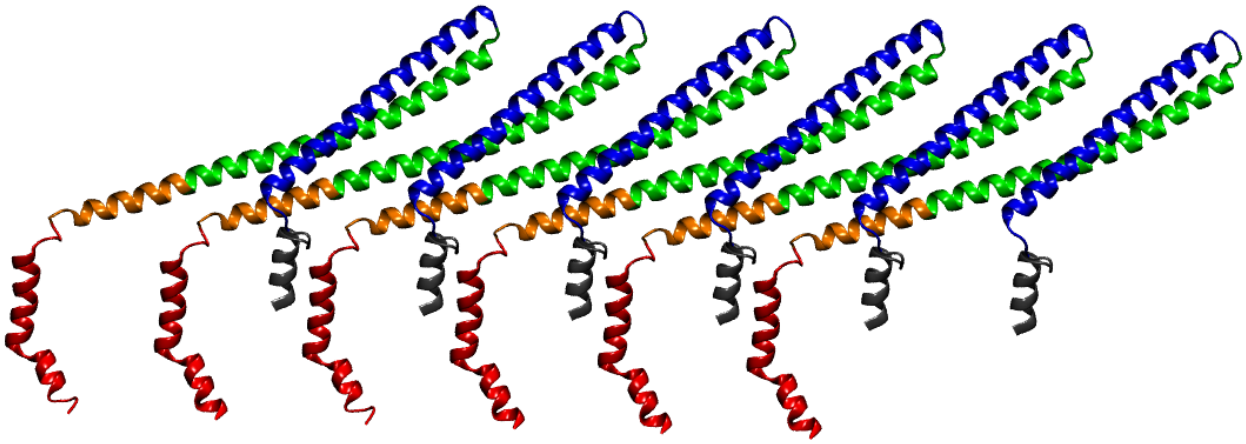

Figure S1: The initial structure of a one-dimensional Vps32 filament, which contains 6 protein monomers.

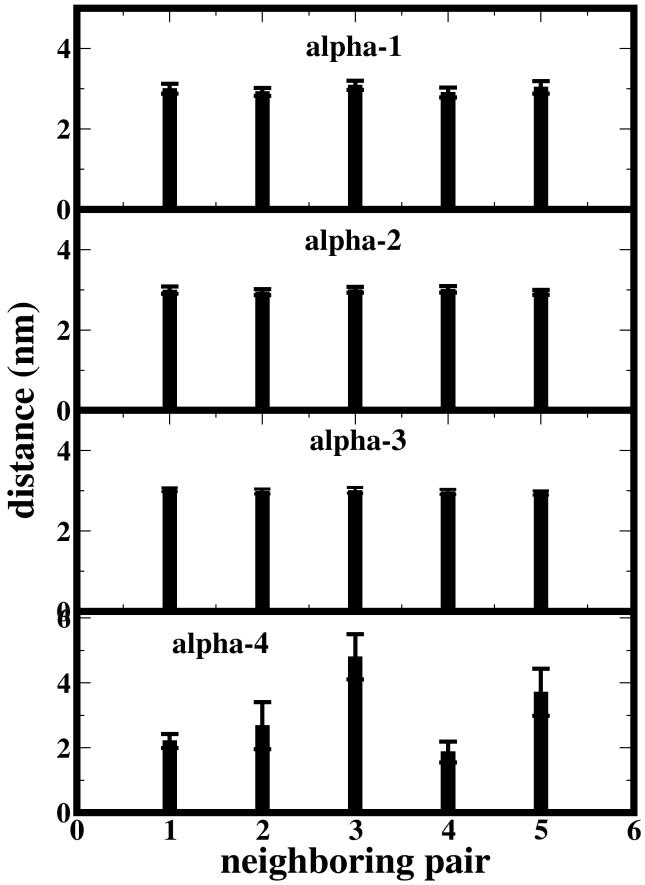

Figure S2: Average distances between the neighboring α_1, α_2, α_3 and α_4 helices in a one-dimensional Snf7 filament from molecular dynamics simulations. The distance between neighboring α_4 helices may significantly vary.

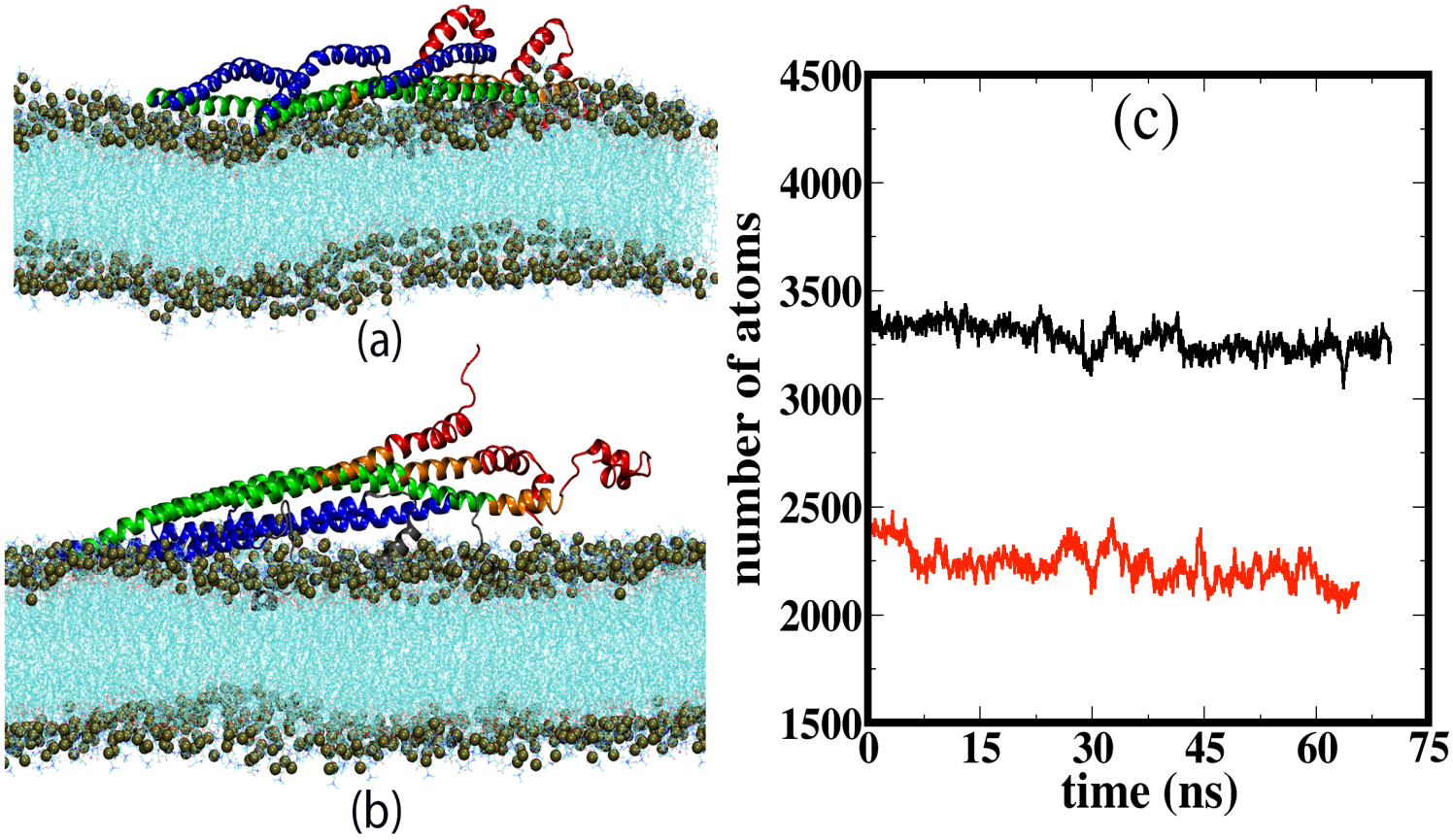

Figure S3: (a) Equilibrated protein-membrane interface structure with (a) the elongated α_2 and α_3 helices facing the membrane surface and (b) with the α_1 helices facing the membrane surface. Gray, blue, green, brown and red color represent the α_0, α_1, α_2, α_3, α_4 helices, respectively. Water and ions are removed for clarity. (c) Black and red plots represent the number of protein atoms within 6 Angstrom from the membrane surface when the elongated α_2 and α_3 helices and the α_1 helices are in contact with the membrane, respectively.

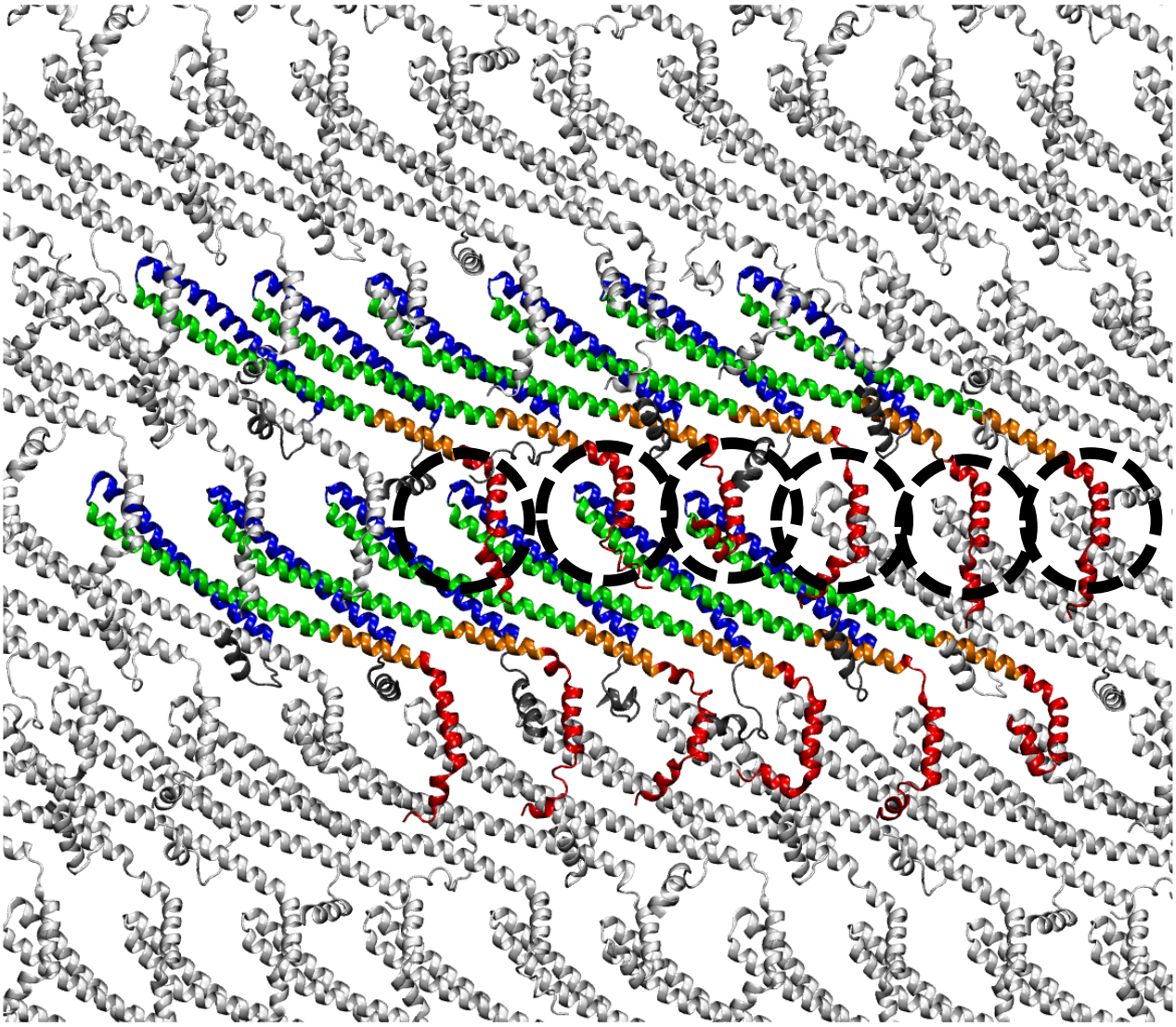

Figure S4: Equilibrated two-dimensional filament structure sampled after 100 ns MD simulations with the initial configuration taken as the same in the crystal structure. Black dotted circles highlight the binding of α_4 helices (red) of a protomer with the α_2 helices (green) of the neighboring protomer.

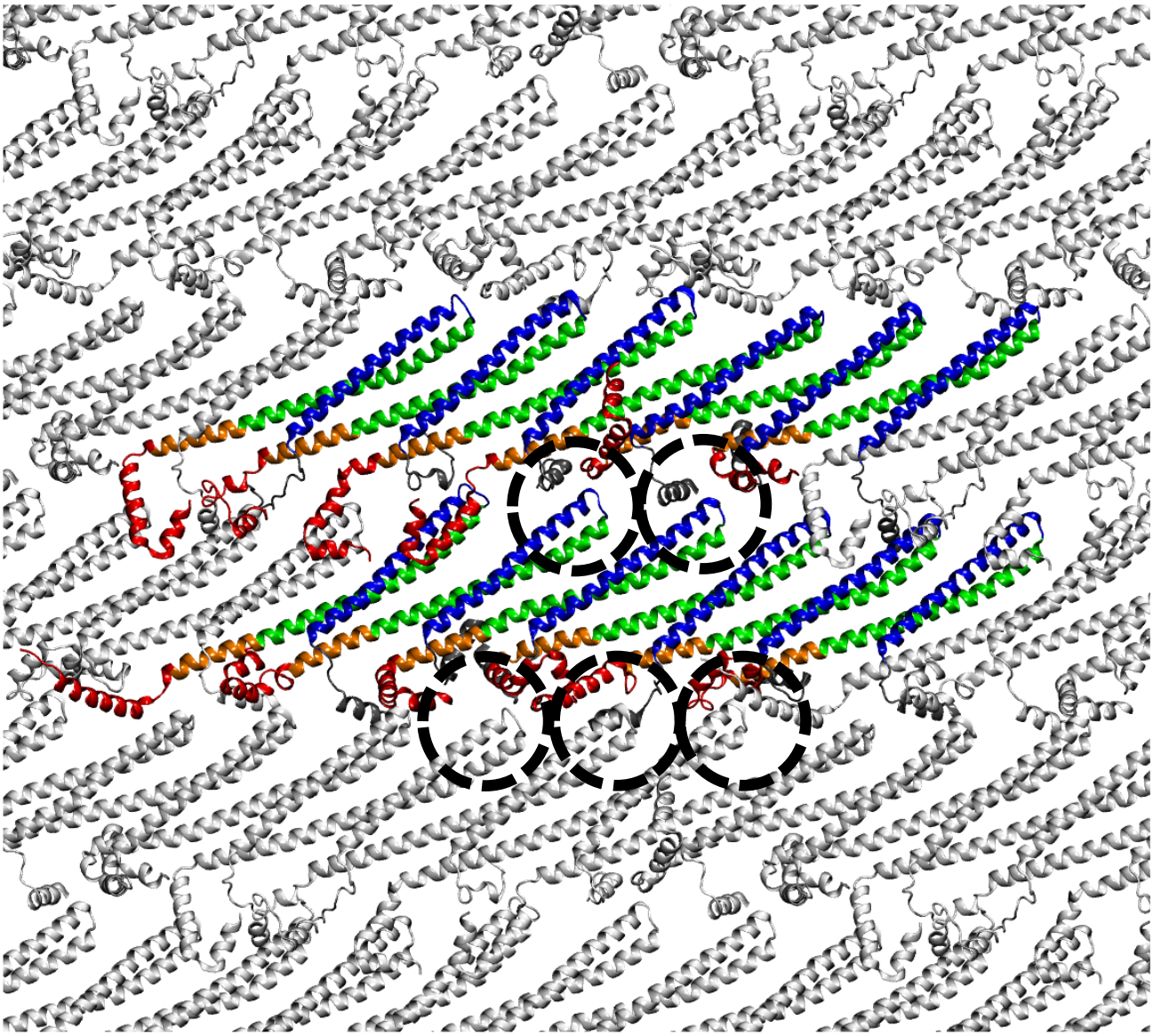

Figure S5: Equilibrated two-dimensional filament structure sampled after 100 ns MD simulations when the initial structure is built such that the α_4 helices of one protofilament are in contact with the α_1 helices of the neighboring protofilament. Black dotted circles highlight that many of the α_4 helices (red) of a protomer do not bind with the α_1 helices (blue) of the neighboring protomer.

To build the initial configuration illustrated in Fig. S5, we first take a unit cell containing a single protein with the crystal structure information (Tang et al. *eLife* 2015; 4:e12548). Then restraining α_0 α_1 α_2 α_3 helices, we rotate the α_4 helix in the unit cell such that it remains close to the α_1 helix of the neighboring protomer when the unit cell is replicated. The two-dimensional structure is then generated by replicating the unit cell.

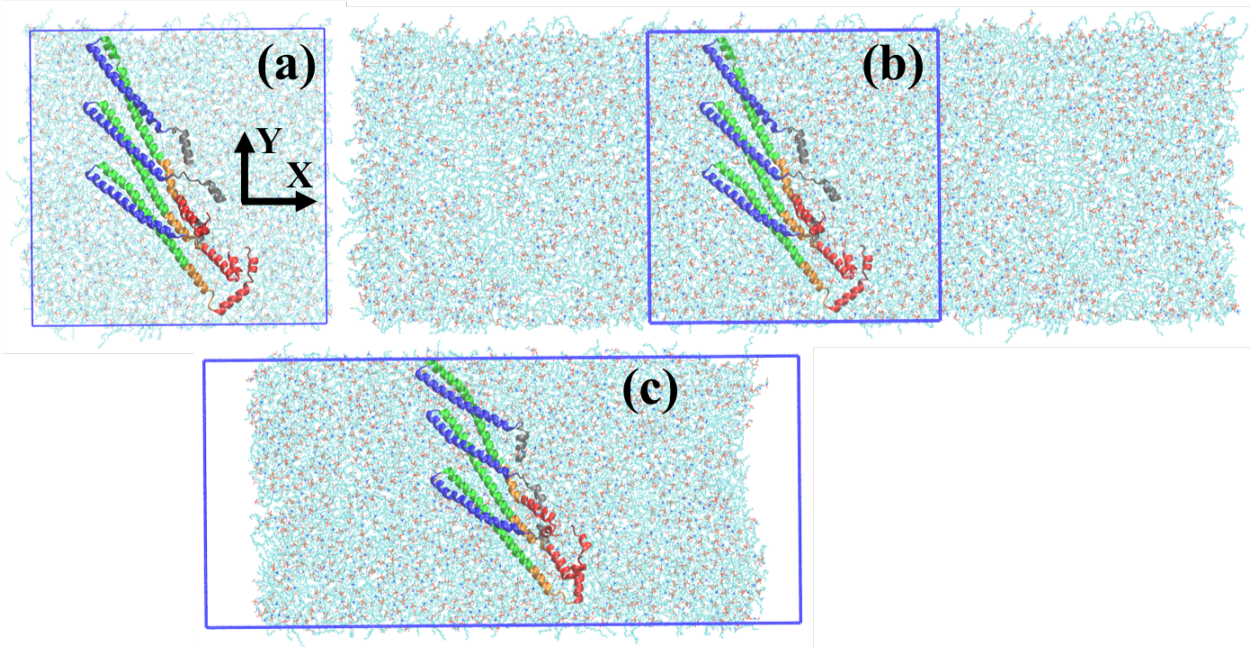

Figure S6: Preparation of the membrane ribbon system. (a) Snapshot of the simulation box containing the protein trimer. (b) The system is replicated twice to generate a longer simulation box along X; the two protein trimers at the ends are removed, leaving only one trimer in the middle. (c) Some lipids from both ends are removed to break the membrane periodicity along the X direction. Water and ions are not shown for clarity.

To generate the membrane ribbon, first we take an equilibrated structure of protein adsorbed on the membrane surface (Fig. S6a), which is then replicated twice keeping the original structure at the center. Two protein trimers at the sides are then removed (Fig. S6b). Finally, some lipid molecules are removed from the two ends such that (i) the ratio of the POPC:POPS lipids is ~70:30 and (ii) the numbers of POPC and POPS lipids in both leaflets are the same. The box length along the X direction is taken to be larger than the membrane length to break the membrane periodicity. However, continuity of the membrane along Y is maintained (Fig. S6c). To investigate the role of protein or the α_0 helices in membrane bending (see main text), either the protein trimer or the α_0 helices are removed from the structure shown in Fig. S6c.

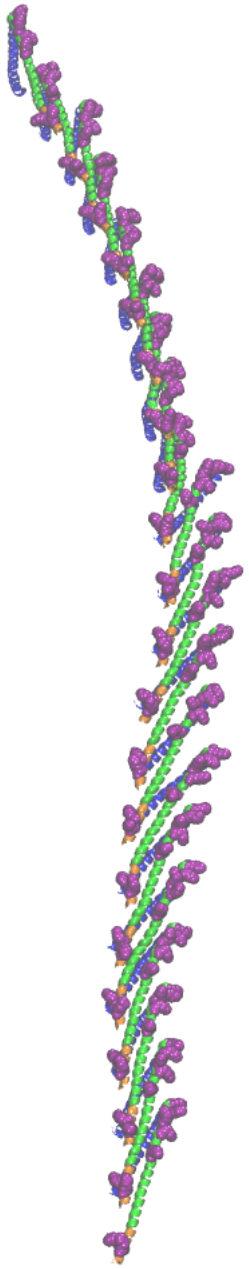

Figure S7: The equilibrated structure showing the intrinsic twist present in the filament. The positively charged Lys60, Lys64, Lys68, Lys71, Lys79, Lys112, and Lys115 residues that form the cationic surface are marked by pink color.

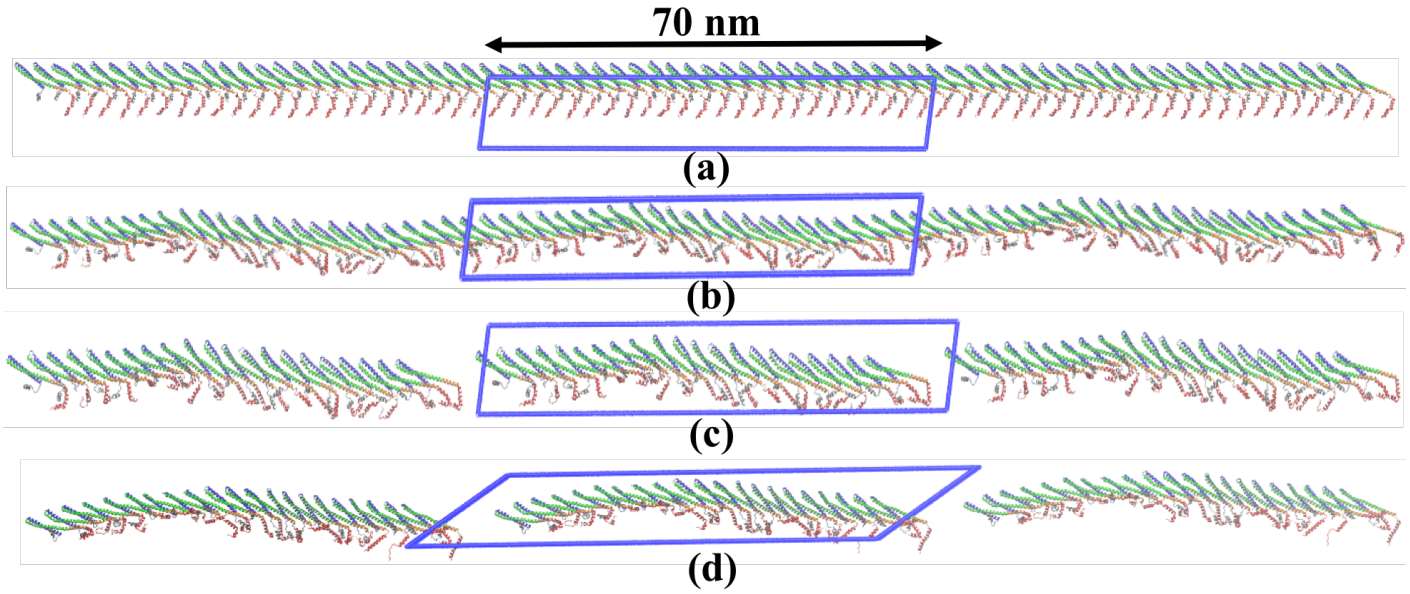

Figure S8: Various steps for simulating a finite filament. (a) First, a one-dimensional periodic filament with 24 monomers is built. (b) The periodic filament is simulated for 60 ns. (c) Three monomers from the equilibrated periodic structure are removed to break the periodicity of the filament. (d) Finally, the non-periodic 21-monomer-long filament is simulated for 80 ns. Water and ions are removed for clarity.

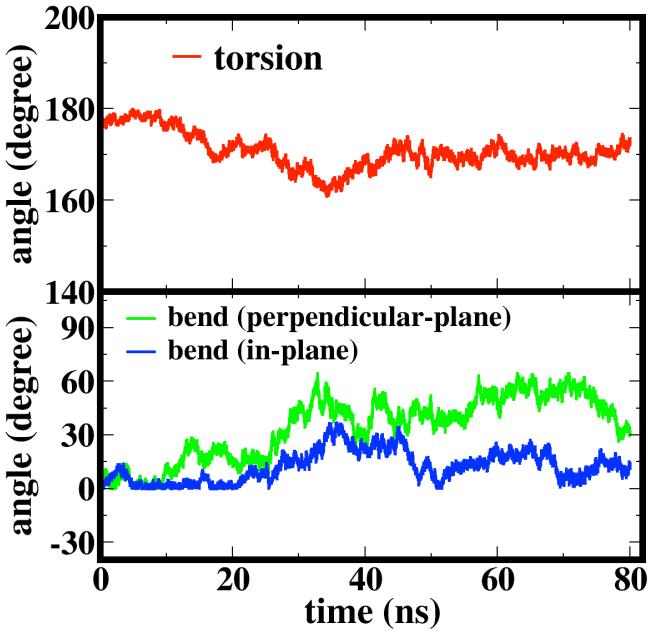

Figure S9: Variation of twist/torsion and bending angles of the 21-monomer-long filament during the MD simulation.

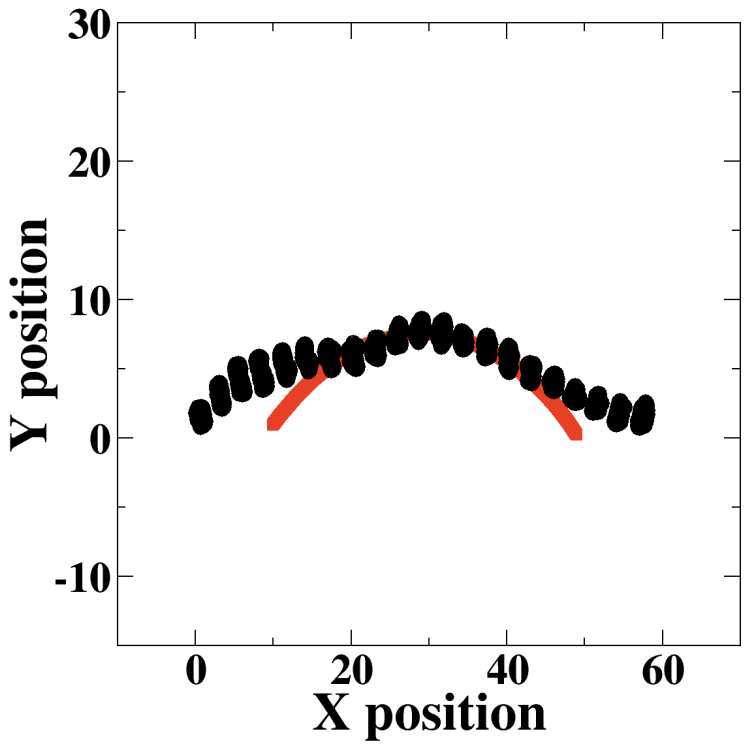

Figure S10: Estimate of radius of curvature of the ESCRT-III filament from MD simulations. The filament structure (e.g., shown in Fig. 4 of the main text) is projected on to the XY plane (the initial filament in oriented along X and lies in the XZ plane), and the traces for centers of mass of the monomers in the central part of the filament are used to estimate the radius of curvature; only the seven monomers in the central part were used to avoid end effects. The average radius of curvature is 30.5+-9.6 nm.