Figures and figure supplements

The AAA ATPase Vps4 binds ESCRT-III substrates through a repeating array of dipeptide-binding pockets

Han Han et al
Figure 1. Overall structure of the Vps4 complex. (A) Ribbon representation of the complex viewed from the ‘top’ N-terminal side of Vps4 and N-terminal end of the peptide. (B) Similar orientation as panel A showing a segmented map contoured around Vps4 and peptide. (C) Same as panel B viewed from the side with density for subunit F removed for clarity.

DOI: https://doi.org/10.7554/eLife.31324.002
Figure 1—figure supplement 1. Cryo-EM of the Vps4 complex. (A) Representative cryo-EM micrograph of Vps4101-437-Hcp1 particles. (BC) Representative 2D class averages, (B) before and (C) after Hcp1 signal subtraction. (D) Gold-standard FSC of the Hcp1-subtracted particle reconstructions on independent (odd:even particles) halves of the data (blue) and FSC between the refined model and the density map (orange). (E) Cross-validation of refined model (see Materials and methods). (F) Angular distribution plot based on orientation assignments in RELION and visualized in UCSF Chimera. Cylinders scaled (low to high) and colored (blue to red) proportional to number of particles in the assigned orientation. (G) Local resolution estimates determined by ResMap (Kucukelbir et al., 2014).

DOI: https://doi.org/10.7554/eLife.31324.003
Figure 1—figure supplement 2. Classification and signal-subtraction scheme for the Vps4 complex. 140,958 particles were input for 3D classification. 109,241 particles were sorted into classes with good Vps4 features and used to generate a consensus reconstruction of the entire Vps4\(^{101-437}\)-Hcp1.

Han et al. eLife 2017;6:e31324. DOI: https://doi.org/10.7554/eLife.31324
complex at 4.1 Å resolution. Signal subtraction of Hcp1 was performed using a previously described strategy (see Materials and methods). An additional round of 3D classification was performed using the Hcp1-subtracted particles. 82,225 particles were sorted into a single class with high-resolution Vps4 features and used to generate the final 3.2 Å Vps4 reconstruction.

DOI: https://doi.org/10.7554/eLife.31324.004
Figure 1—figure supplement 3. Focused classification of Vps4 subunit F and Vta1. (A) Representative masking scheme for subunit F. Custom masks were generated for structurally heterogeneous features and focused 3D classification was performed using the masks. Classes with good features were used to isolate particles for additional rounds of RELION auto-refinement. (B) Representative masking scheme for Vta1.

DOI: https://doi.org/10.7554/eLife.31324.005
Figure 1—figure supplement 4. Surface Representation. Similar orientation to Figure 1A. Shows the gaps between the subunit F large ATPase domain and its neighboring subunits, and the highly solvated channel between subunit F and the peptide.

DOI: https://doi.org/10.7554/eLife.31324.006
Figure 2. Nucleotide coordination and subunit interfaces. (A) Stereoview of a representative ADP·BeF₆ coordination shown at subunit B (BC interface). Subunits color-coded as in Figure 1. (B) Stereoview of nucleotide-binding sites at subunits A, B, C, D, and E following superposition on the large domains of the first subunit at each interface.

DOI: https://doi.org/10.7554/eLife.31324.009
Figure 3. ESCRT-III peptide conformation and coordination. (A) Left – tilted view of a surface representation showing how the pore loop residues form an array of class I and II binding pockets through the hexamer pore. W206 and M207 from subunits A-E are highlighted. Right – close up of the pore region. (B) Distances between Cα atoms of the peptide and pore loop 1 W206 and M207 indicate equivalent binding in the different class I and class II pockets. (C) Superposition of the four Class I pockets following superposition on Cα atoms of the class I pocket residues of subunits A and B. (D) Superposition of the four class II pockets following superposition on Cα atoms of the class II pocket residues of subunits A and B. (E) The H-bond seen between the NH of even-numbered ESCRT-III residues and the K205 CO of Vps4 subunits A-D – here centered on the bond between ESCRT-III V4 and subunit B. The bond between E2 and subunit A is also visible.

DOI: https://doi.org/10.7554/eLife.31324.020
Figure 3—figure supplement 1. Fit of peptide to density when refined in the assigned and reversed orientations. Visual inspection shows that the assigned peptide orientation is a better fit to the map than the inverted orientation. Arrowheads indicate notably poor agreement between model and map in the inverted orientation. Chimera RSCC and EMRinger scores also support the assigned orientation.

DOI: https://doi.org/10.7554/eLife.31324.021
Figure 4. Mechanism of translocation. (A) Proposed mechanism of ESCRT-III translocation by Vps4. W206 and M207 residues of the six Vps4 subunits are shown, with the peptide passing through the Vps4 hexamer. The peptide model was constructed by changing the side chains to leucine without adjusting the main chain, and building out in the N and C directions by overlapping copies of the peptide model. The proposed mechanism envisions that Vps4 progresses through states A to E while bound to successive dipeptides of its substrate. ATP hydrolysis at subunit D destabilizes the DE interface and promotes displacement of subunit E toward the transitioning subunit F configuration, which allows displacement of ADP. Subsequent ATP binding allows subunit F to pack against subunit A, bind to the next dipeptide of ESCRT-III, and assume the subunit A configuration. (BC) Conservation of helical pore loop structure in AAA ATPases. Overlap on the large ATPases of multiple AAA ATPase structures gives a similar helical arrangement of pore loop 1 residues from five subunits. (B) Top and (C) side views are shown of the ESCRT-III peptide (green) and Vps4 pore loop 1 (red) with the equivalent residues of: VAT (Ripstein et al., 2017) (pdbid 5vca), HSP104 (Gates et al., 2017) (5vjh), NSF (Zhao et al., 2015) (3j94), human 26S proteasome (Huang et al., 2016) (5gjr), yeast 26S proteasome (Wehmer et al., 2017) (5mp9), katanin (Zehr et al., 2017) (5wc0, 5wcb).

DOI: https://doi.org/10.7554/eLife.31324.026
Figure 4—figure supplement 1. Structure-based alignment of pore loop 1 sequences. Pore loop 1 residues that contact the ESCRT-III peptide (red font) are shown with four flanking residues on either side.

DOI: https://doi.org/10.7554/eLife.31324.027