Figures and figure supplements

Structure of Vps4 with circular peptides and implications for translocation of two polypeptide chains by AAA+ ATPases

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Figure 1. Linear and circular peptides bind Vps4 with similar affinities. Sequences of the linear and circular peptides used in this study are shown, together with competition fluorescence polarization binding isotherms and calculated $K_D$ and $K_i$ values.

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Figure 1—figure supplement 1. Competitive binding assays. Competition binding data for the linear and circular peptides used in this study against Vsp4 and Vps4-Hcp1.

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Figure 1—figure supplement 2. Characterization of HPLC purified peptide F by LC-MS. (A) Analytical HPLC trace of peptide F using FortisBIO C4 column (5 μM, 300 Å, 4.6 × 150 mm) at 214 nm. Sample was injected with an isocratic flow of 10% Buffer B at 1 mL/min. After 2 min, the solvent gradient was increased from 10% to 90% Buffer B over 15 min. (B) Electrospray mass spectrum of peptide F.

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Figure 1—figure supplement 3. Characterization of HPLC purified F12 by LC-MS. (A) Analytical HPLC trace of peptide F12 at 228 nm. Sample was injected with an isocratic flow of 5% Buffer B (0.1% FA in ACN) at 0.75 mL/min. After 1 min, the solvent gradient was increased from 5% to 90% Buffer B over 7 min. (B) Electrospray mass spectrum of peptide F12.
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Figure 1—figure supplement 4. Characterization of HPLC purified F30 by LC-MS. (A) Analytical HPLC trace of peptide F30. (B) Electrospray mass spectrum of F30.

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Figure 1—figure supplement 5. Characterization of HPLC purified cF30 by LC-MS. (A) Analytical HPLC trace of peptide cF30 (pre-cyclization). (B) Electrospray mass spectrum of cF30 (pre-cyclization). (C) Analytical HPLC trace of peptide cF30. (D) Electrospray mass spectrum of cF30.

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Figure 1—figure supplement 6. Characterization of HPLC purified FF30 by LC-MS. (A) Analytical HPLC trace of peptide FF30. (B) Electrospray mass spectrum of FF30.

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Figure 1—figure supplement 7. Characterization of HPLC purified cFF30 by LC-MS. (A) Analytical HPLC trace of peptide cFF30 (pre-cyclization). (B) Electrospray mass spectrum of cFF30 (pre-cyclization). (C) Analytical HPLC trace of peptide cFF30. (D) Electrospray mass spectrum of cFF30.

Calculated: 2943.2 Da
Observed: 2943.2 Da

Calculated: 2911.2 Da
Observed: 2911.2 Da

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Figure 1—figure supplement 8. High-resolution LC-MS analysis of trypsin-digested peptides. Extracted ion chromatograms after trypsin digestion of linear and cyclic peptides. Colors of peptides are matched to colors of ion signals. The brown tryptic peptide of cF30 and cFF30 differ from the corresponding blue tryptic peptides of the linear F30 and F30 by the presence of a glycine at the N-terminus.

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Figure 1—figure supplement 9. Comparison of MS2 spectra from trypsin-digested linear and cyclic peptides. (A) Representative MS2 spectrum for trypsin digested F30. MS2 spectra of FF30 are essentially identical. (B) Representative MS2 spectrum for trypsin digested cF30. MS2 spectra of cFF30 are essentially identical. Successful cyclization is indicated by the 57 m/z shifts of b₆ and b₇ ions compared to the b₅ and b₆ ions of the linear peptide MS2 spectrum (panel A).

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Figure 2. Structure determination. (A) Overall structure of the Vps4-cyclic peptide complex. The close up view of the pore region shows the primary strand (dark green) and returning strand (light green) of the cyclic peptide. (B) Representative section of density in the large domain of the Vps4 B subunit. (C) Density around the nucleotides and coordinating residues for the active sites of Vps4 subunits A–E. These binding sites occur at the interface with the following subunit. (D) Density around the circular peptides. Shown as side views separately for the cF30, cFF30, and combined maps. DOI: https://doi.org/10.7554/eLife.44071.014
**Figure 2—figure supplement 1.** Cryo-EM structure determination and validation of Vps4 bound to cyclic peptides. (A) Cryo-EM micrograph, representative 2D classes (before and after Hcp1 subtraction), orientation distribution, and local resolution of Vps4 bound to cF30 and cFF30. (B) Gold-standard FSC plots of Vps4 (Hcp1-subtracted) bound to cF30 (purple), cFF30 (orange), and the combined dataset (blue). Dotted lines represent unmasked FSC plots, solid lines represent masked, corrected FSC plots from RELION post-processing. (C) Orientation distribution and local resolution of the combined dataset. (D) FSC plots of the combined (cF30 and cFF30) dataset (as in B), and the refined model versus the 3.6 Å map (blue). (E) Cross-validation of the refined model. Overlay of FSC\textsubscript{work} and FSC\textsubscript{test} plots.

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Figure 2—figure supplement 2. Cryo-EM processing workflow of the Vps4-cF30 complex.
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Figure 2—figure supplement 3. Cryo-EM processing workflow of Vps4-cFF30 complex.
DOI: https://doi.org/10.7554/eLife.44071.017
Figure 2—figure supplement 4. Data processing workflow for combining the cyclic peptide datasets and subunit F classification. Focused classification was performed using a spherical mask over subunit F (yellow), leading to three populated conformations (F1, F2, and F3) and junk classes with poor densities. Particles sorting into F1, F2, F3 were processed separately within RELION.

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Figure 2—figure supplement 5. Validation of subunit F classes. (A) Rigid body fits of the Vps4 protomer (PDBID: 3EIE [Gonciarz et al., 2008]) into subunit F (arrowhead) after focused 3D classification. (B) Local resolution heat map generated from ResMap showing a surface view. (C) Same as (B), cut away view. (D) Gold standard, corrected FSC plots of each of the subunit F classes after RELION auto-refinement.

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Figure 2—figure supplement 6. The three subunit F conformations. These models were derived from the cF30/cFF30 focused classification maps, and are shown after overlap on subunits A-E.

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Figure 2—figure supplement 7. Fit of peptide to the cF30/cFF30 combined density when refined in the assigned and reversed orientations. The peptide primary strand sequence was built optimally in the assigned and reversed orientations, and refined equivalently. Visual inspection does not give a strong indication of peptide orientation. Chimera (RRID:SCR_004097) RSCC (Pettersen et al., 2004) and EMRinger (Barad et al., 2015) scores support the assigned orientation by small margins. The assigned orientation agrees with that of a previously reported structure with an extended, linear peptide that was determined at slightly higher resolution (Han et al., 2017).

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Figure 2—figure supplement 8. Focused 3D classification of Vta1\textsuperscript{VSL}. (A) Focused classification schematic of Vta1\textsuperscript{VSL}. A mask covering all Vta1\textsuperscript{VSL} binding sites was applied to the cFF30 reconstruction. Particles were sorted into ten classes, one of which showed robust Vta1\textsuperscript{VSL} densities at all six binding sites. Particles in this class were then used for RELION 3D auto-refinement. (B) Gold-standard FSC plot of Vps4-Vta1\textsuperscript{VSL}. (C) Local resolution of Vps4-Vta1\textsuperscript{VSL}.

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Figure 3. Cyclic peptide structure and coordination. (A) Model of the entire cyclic peptide, including the residues that lack density (gray), with the Vps4 pore loop 1 and 2 residues. (B) Superposition of the cyclic peptide structure (colors) on the previously determined structure of the linear peptide F (gray). The returning strand of the cyclic peptide is omitted for clarity. (C) Ordered residues of the cyclic peptide are shown as green ribbons. Pore loop one residues K205, W206, and M207 of the five Vps4 subunits that form the helical assembly that binds the substrate peptide are shown as sticks and molecular surfaces. Alternating side chains bind to class I pockets between pairs of W206 residues of adjacent subunits (two examples labeled), and to class II pockets between pairs of M207 side chains from adjacent subunits (one example labeled).

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Figure 4. Cyclic peptide aligns with the helical axis. Side and top views of the cyclic peptide and Vps4 pore loop one residues. The pseudo two-fold axis that relates the path of the two peptide strands to each other (albeit with opposite direction) aligns with the helical axis of Vps4 subunits A-E (gray). This ensures that the second strand is maximally distant from subunits A-E, thereby explaining the lack of contacts between Vps4 and the second strand.

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Figure 5. Subunit F conformation and contacts. (A) Overlap of subunits showing variation in the hinge angle between large and small domains, as defined in Gonciarz et al. (2008). The overlaps were performed on the large domains. Left, Subunits A-E. Right, three classification structures of subunit F. (B) The large domain interfaces for AB, BC, CD, and DE subunit pairs are closely associated. These interfaces are much more open for the EF and FA subunit pairs, as seen by the openings in this slightly tilted view. (C) Side view of the Vps4 circular peptide complex with all of the classified subunit F models from all structures: cF30, cFF30, and combined cF30 and cFF30, plus the two previously reported data sets of Vps4 with the linear peptide F (Han et al., 2017; Monroe et al., 2017). Subunit F is colored based on position along its proposed trajectory.
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Figure 6. Transition of subunit F is coupled to movement of subunit E. The maps reconstructed by focused classification over subunit F show that the uppermost subunit F position from the cyclic peptide data correlates with displacement of the subunit E small domain (cyan). This domain is connected by an extended 10-residue stretch of residues to the finger arginines that complete the subunit D active site.

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Figure 7. Overlap of cyclic peptide structure suggests compatibility with YME1, Hsp104, and NSF. (A) Vps4 color, YME1 (Puchades et al., 2017) gray. Overlap is on the Ca atoms of the peptide and pore loop 1 residues of the five helical subunits. (B) Same as (A) but without Vps4 and showing the zoomed in region of panels (C), (D), and (E). (C) Vps4 cyclic peptide after overlap on YME1. (D) Same as (C) for Hsp104 ring 1 (Gates et al., 2017). (E) Same as (C) for Hsp104 ring 2. (F) Same as (C) for NSF (White et al., 2018).

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