Structural Basis For Antigenic Peptide Precursor Processing by the Endoplasmic Reticulum Aminopeptidase ERAP1

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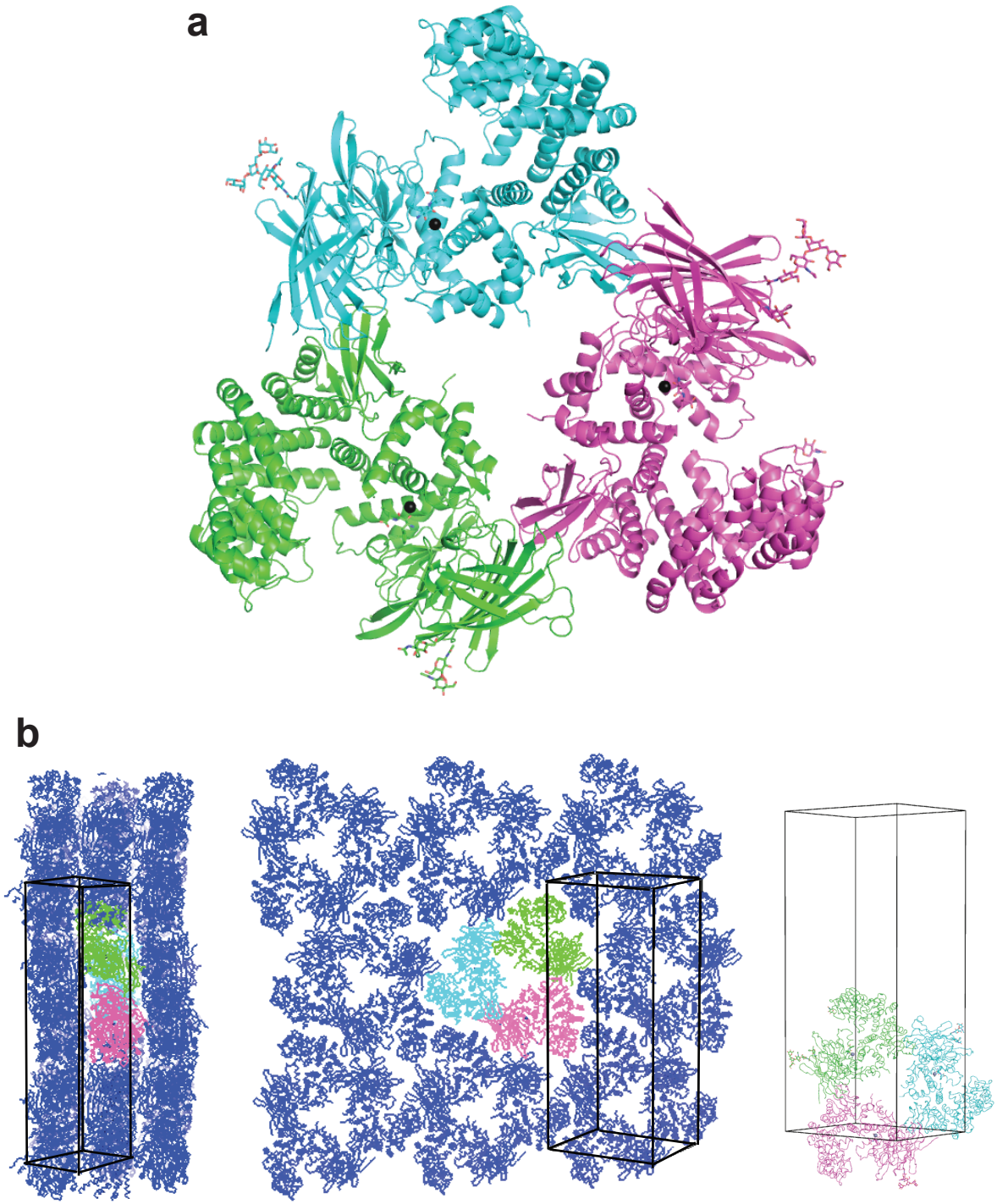
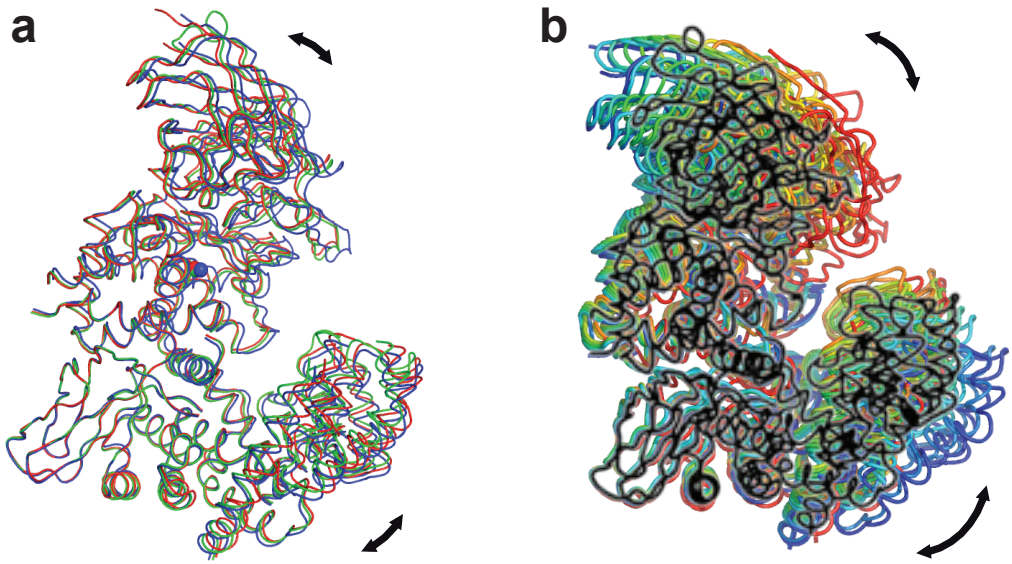
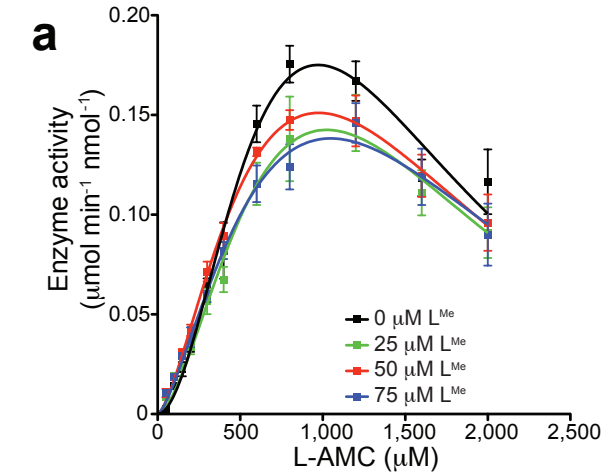
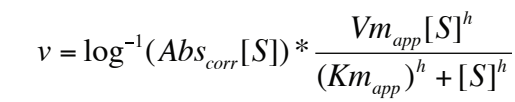


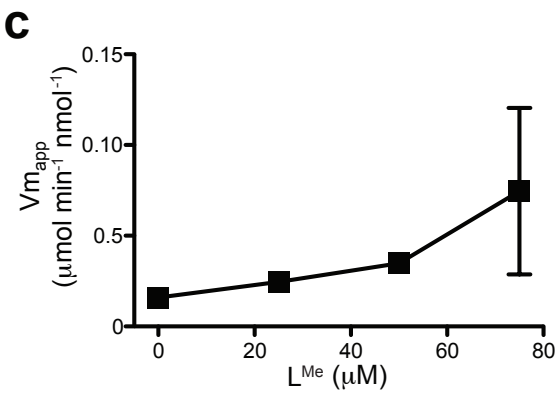
Supplementary Figure 1 ERAP1 trimer and packing in unit cell
(a) Ribbon representation of an asymmetric unit showing trimeric arrangement of the crystallographically distinct molecules. ERAP1 main chain is represented in ribbon, the zinc atoms are shown as black spheres, and N-linked sugars and bestatin are shown in stick representation. **(b)** Orientation of ERAP1 trimer and packing in unit cell.

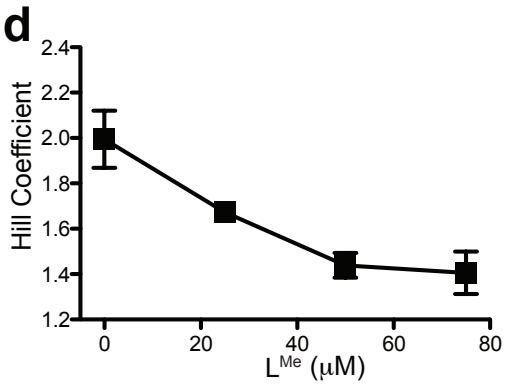


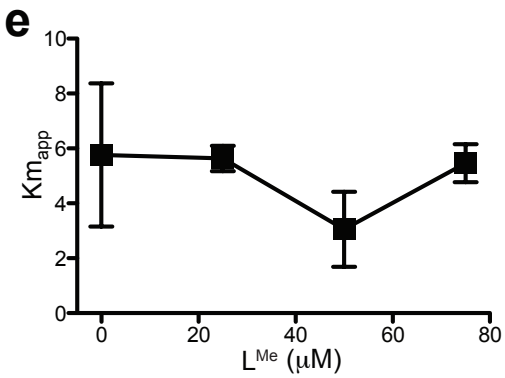
Supplementary Figure 2 Domain Motions **(a)** Hinge movement suggested by different inter-domain orientations among the three molecules observed in the ERAP1 crystallographic asymmetric unit, shown here as C-alpha traces aligned at domain III.
(b) Samples of ERAP1 motions as indicated by normal mode analysis, shown as C-alpha traces colored blue to red representing extremes of conformational alteration. The ERAP1 structure is overlayed in black.

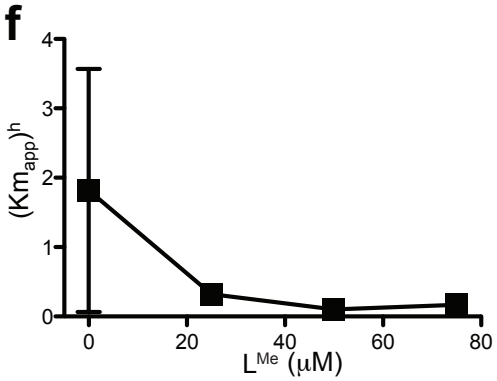




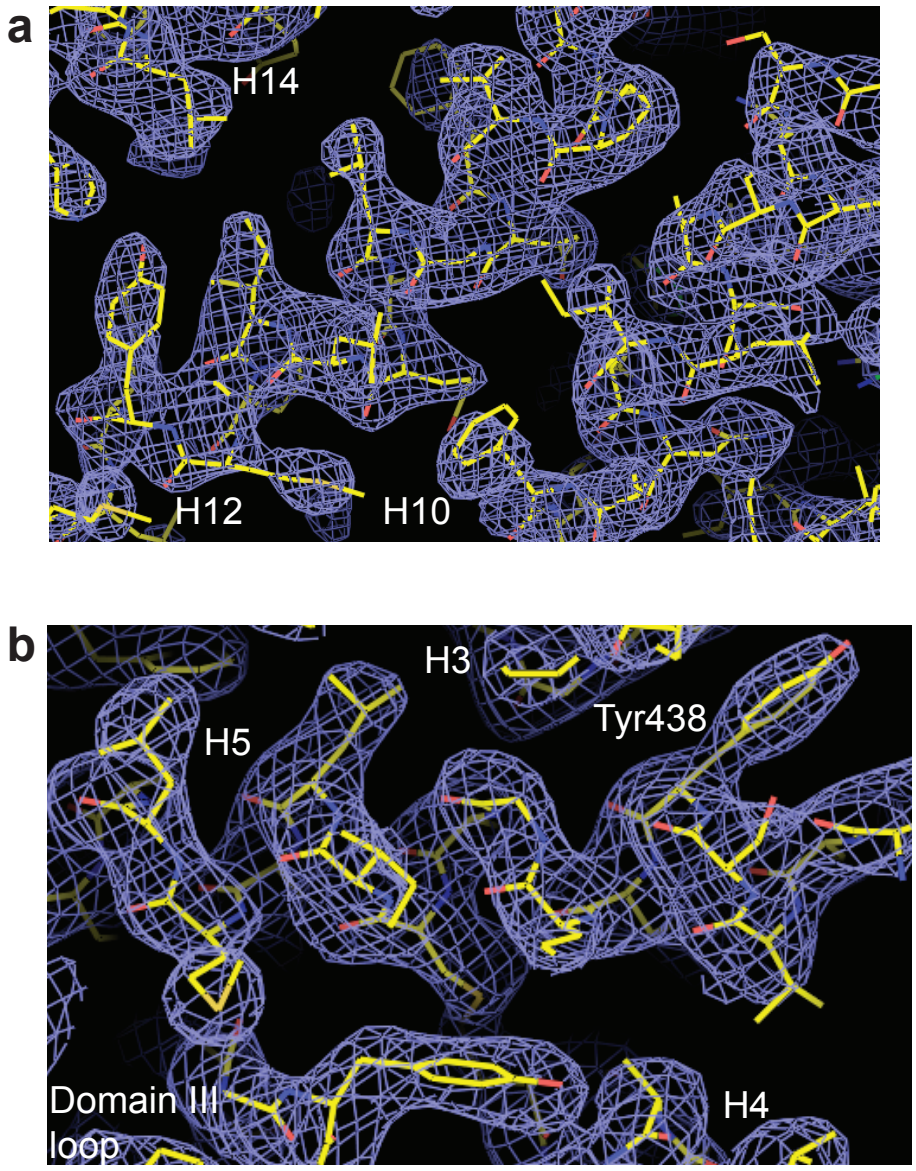








Supplementary Figure 3 Kinetic parameters for L^{Me} activation of L-AMC hydrolysis by ERAP1. **(a)** Concentration dependence of initial rate of L-AMC hydrolysis by ERAP1 in the presence of various L^{Me} peptide concentrations. Lines show fits to allosteric activation equation shown in b. **(b)** Allosteric sigmoidal equation was used to fit initial rate vs. substrate concentrations profiles containing an inner filter correction term, Abscorr. The kinetics parameters, **(c)** Apparent Vmax, **(d)** Hill Coefficient, **(e)** Apparent Km, and **(f)** Apparent Km_{app}., were extracted by fitting the curves in A using the allosteric sigmodial equation in (b).



Supplementary Figure 4 Electron Density. **(a)** Omit map showing portions of helices 10, 12, and 14 in domain IV. All atoms shown (residues 590–780) were omitted from map calculations. **(b)** Omit map showing Tyr438 on helix 5, helix 3, helix 4 in domain II and a loop from domain III. All atoms shown were omitted from map calculation. Blue mesh represents |2Fo-Fc| electron density, lines represent ERAP1 model.

Supplementary Table 1 Non-crystallographic symmetry and domain statistics

B values (Å^2)	
Mol A / Mol B / Mol C	62.8 / 63.5 / 63.4
Domain 1 (1-254)	58.2
Domain 2 (255-527)	52.3
Domain 3 (528-613)	53.2
Domain 4-n (614-750)	68.3
Domain 4-c (751-941)	105.7
NCS RMSD (Å)	
Mol A / Mol B / Mol C	0.006 / 0 / 0.006
Domain 1 (1-254)	0.19
Domain 2 (255-527)	0.14
Domain 3 (528-613)	0.15
Domain 4-n (614-750)	0.32
Domain 4-c (751-941)	0.19