Interactions of PAN's C-termini with archaeal 20S proteasome and implications for the eukaryotic proteasome-ATPase interactions

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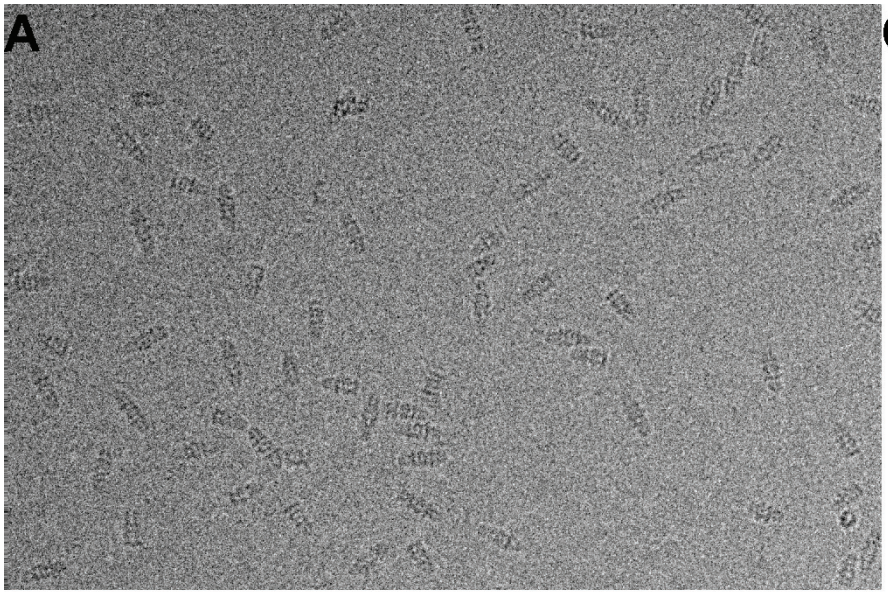
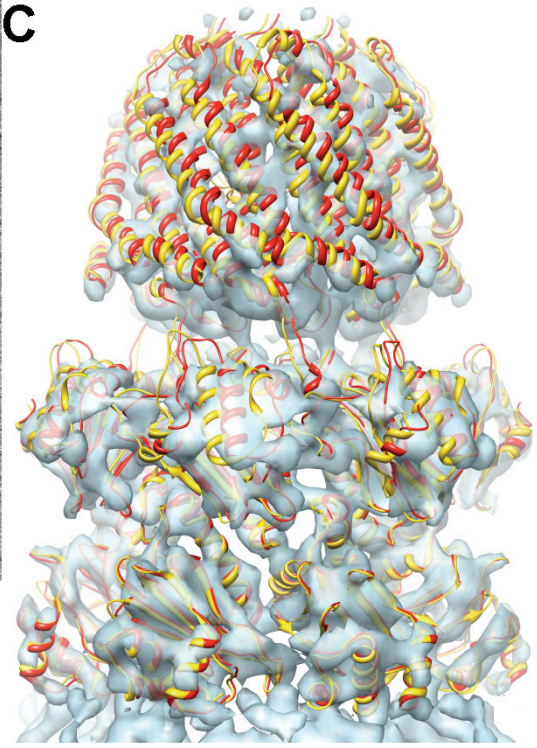
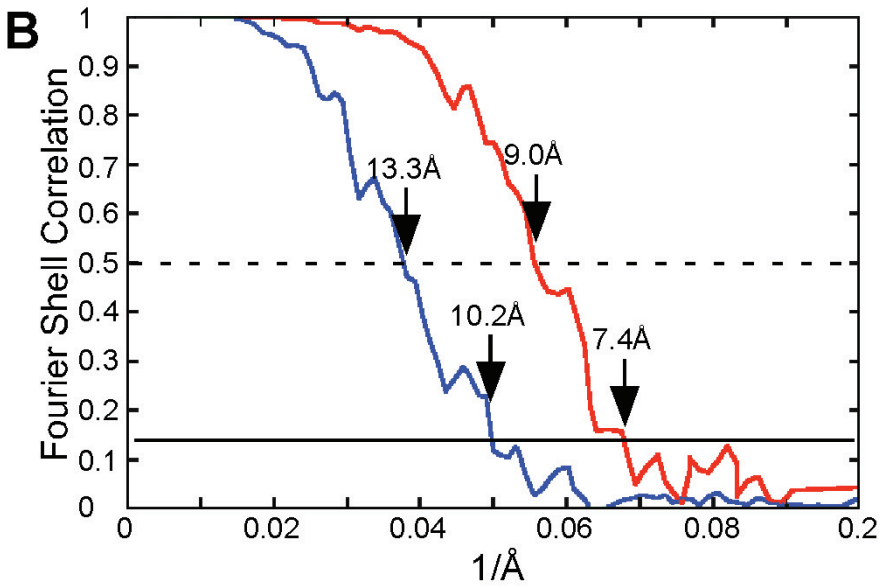
Running Title

Structure of archaeal 20S with PAN's C-terminus

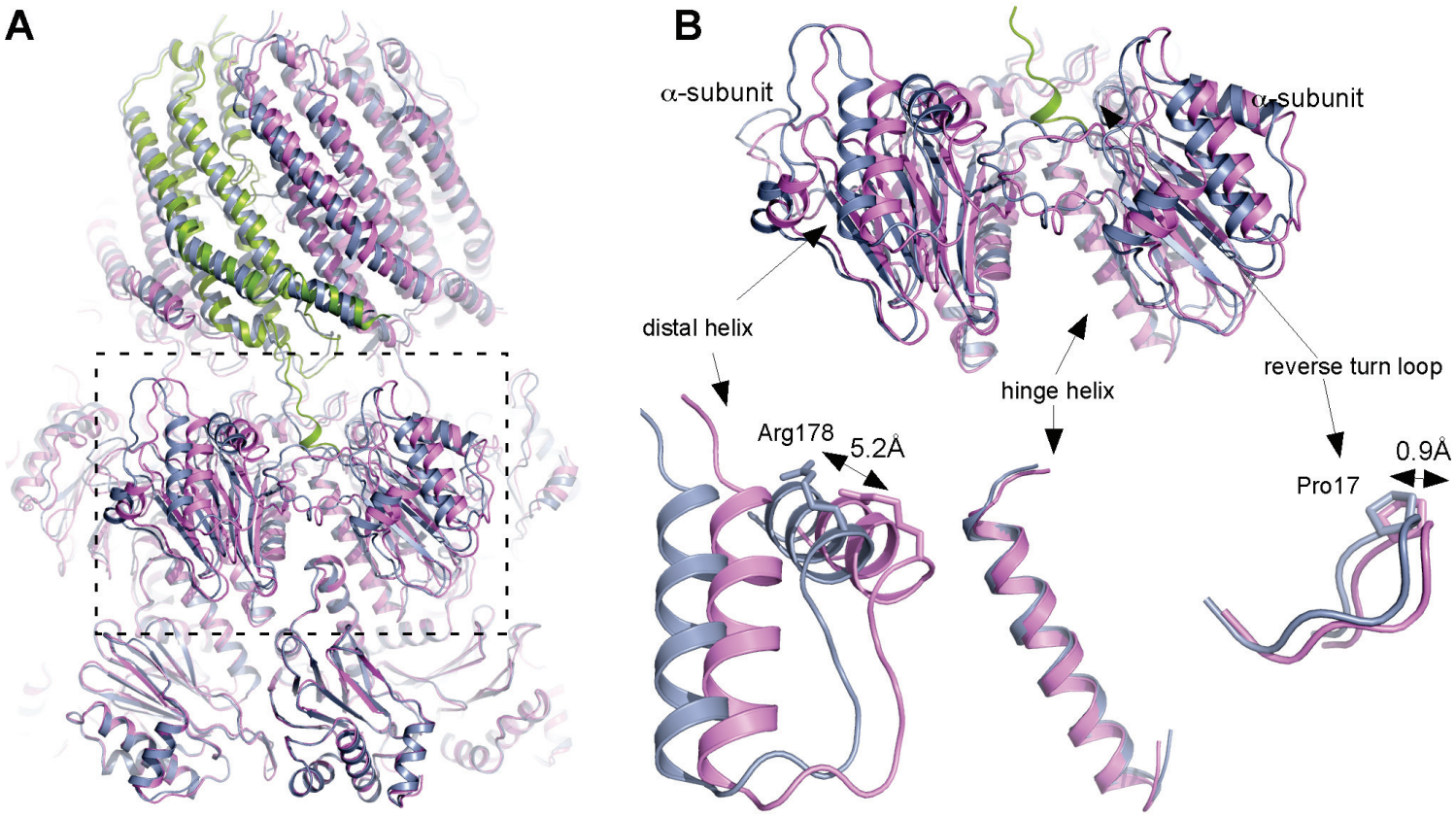






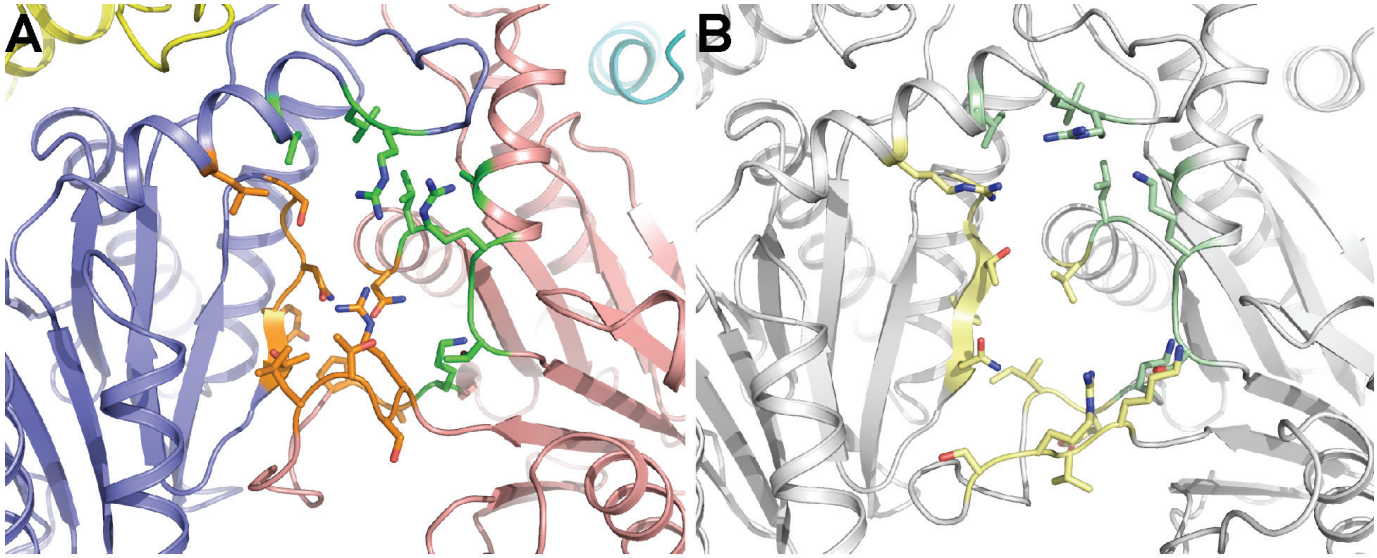
Supplementary Figure 1

Supplementary Figure 1 Single particle cryoEM of the 20S-PA26/PAN. (**A**) A typical image of 20S-PA26/PAN complexes embedded in vitreous ice. The defocus of this image is about -3µm. (**B**) FSC curves of 3D reconstructions of 20S-PA26$^{E102A\text{-}PAN9}$ (blue) and of 20S core (red). The two 3D reconstructions were calculated from the same particle images with the same parameters (Euler angles, in-plan shift and defocuses). **(C)** A side view of 3D reconstruction of 20S- PA26$^{E102A\text{-}PAN9}$. When β-ring of the wild type PA26 (yellow ribbon) was fitted into this 3D reconstruction, its α-ring does not fit the density map. A better docking can be achieved by fitting the β-ring, individual α-subunits and PA26 separately as rigid bodies (red ribbon).



Supplementary Figure 2

Supplementary Figure 2 Comparison of structures of 20S-PA26$^{E102A\text{-}PAN9}$ and wild type 20S-PA26. (**A**) The atomic structure of 20S-PA26$^{E102A\text{-}PAN9}$ complex (purple and green) is superimposed with that of 20S-PA26 complex (PDB code: 1YA7; grey). All Cα atoms of two central β-rings were used to align the two structures. (**B**) An enlarged view of adjacent α-subunits. 20S-PA26/PAN is colored in purple, C-terminus of PAN is colored in green and the wild type 20S-PA26 is colored in light gray. The rotation of α-subunit is also illustrated in the enlarged fragments. The two distal helices linked by a flexible loop has the largest movement with Arg178 moved ~5.2 Å. The hinge helix has the smallest RMSD of only 0.7 Å. The reverse turn loop (Pro17) moved less than 1 Å.



Supplementary Figure 3

Supplementary Figure 3 Residues within the intersubunit pocket of archaeal 20S proteasome were mutated to mimic specific intersubunit pocket of yeast 20S proteasome.
(a) The pocket of yeast 20S proteasome between α3- and α4-subunit. **(b)** The intersubunit pocket of T. acidophilum 20S proteasome. Residues shown in yellow in (b) were mutated to the corresponding residues shown in orange in (a). Residues shown in light green in (b) were similar as the corresponding residues in (a) and were not changed.

Supplementary Table I. Data collection and refinement statistics

Data collection	
Space group	P4(2)22
Cell dimensions	
a,b,c (Å)	166.90,166.90,412.08
α,β,γ (°)	90,90,90
Resolution range (Å)	89.51-4.00 (4.22-4.00)
Rmerge*	0.226 (0.449)
I/σ(I)	2.9 (1.6)
Number of reflections	156551 (23145)
Number of unique reflections	48850 (7045)
Redundancy	3.2(3.3)
Completeness	98.4% (98.6%)
Refinement	
Resolution range (Å)	89.51-4.00
Reflections work/test	48849/2435
Rfactor† / Rfree‡	0.249/0.284
No. atoms	
Protein	35119
Ligand/Ion	0
Water	0
B-factors	
Protein	94.6
Ligand/Ion	0
Water	0
R.m.s deviations ¶	
bond lengths (Å)	0.008
bond angles (°)	1.2
Ramanchadran plot	
most favoured	80.2%
additionally allowed	18.8%
generally allowed	1.0%

These data were from collected from one crystal.
Values in parentheses refer to the highest resolution shell.
* Rmerge=Σ|I-<I>|/ΣI, where I is the intensity of an individual measurement and <I> is the corresponding mean value.
† Rfactor=Σ||Fo|-|Fc||/Σ|Fo|, where |Fo| is the observed and |Fc| the calculated structure factor amplitude
‡ Rfree is the same as Rfactor calculated with a randomly selected test set of 2,435 reflections that were never used in refinement calculations.
¶ The r.m.s deviations in bond lengths and bond angles from ideal values.

Supplement Table II. Two sets of mutations on *T. acidophilum* 20S α-subunit residues to mimic the 2 *S. cerevisiae* 20S pockets separately. Residues are numbered according to *T. acidophilum* 20S proteasome.

	To mimic the pocket between yeast α1- α2		To mimic the pocket between yeast α3- α4	
	α1	α2	α3	α4
H0	R28F	K33Q, S35V	R28L	-
S2-S3 loop	-	V54S, R55S	-	K53R, V54S, R55T, S56L, I64P, E65S
S4-H1 loop	-	L81M, V82G, A83P	-	V82N
S6-S7 loop	T156Y, I157Y, N158V	-	A154S, T156N, I157Y, N158T	-