

Supplementary Information

A Role for the Proteasome Alpha2 Subunit N-Tail in Substrate Processing

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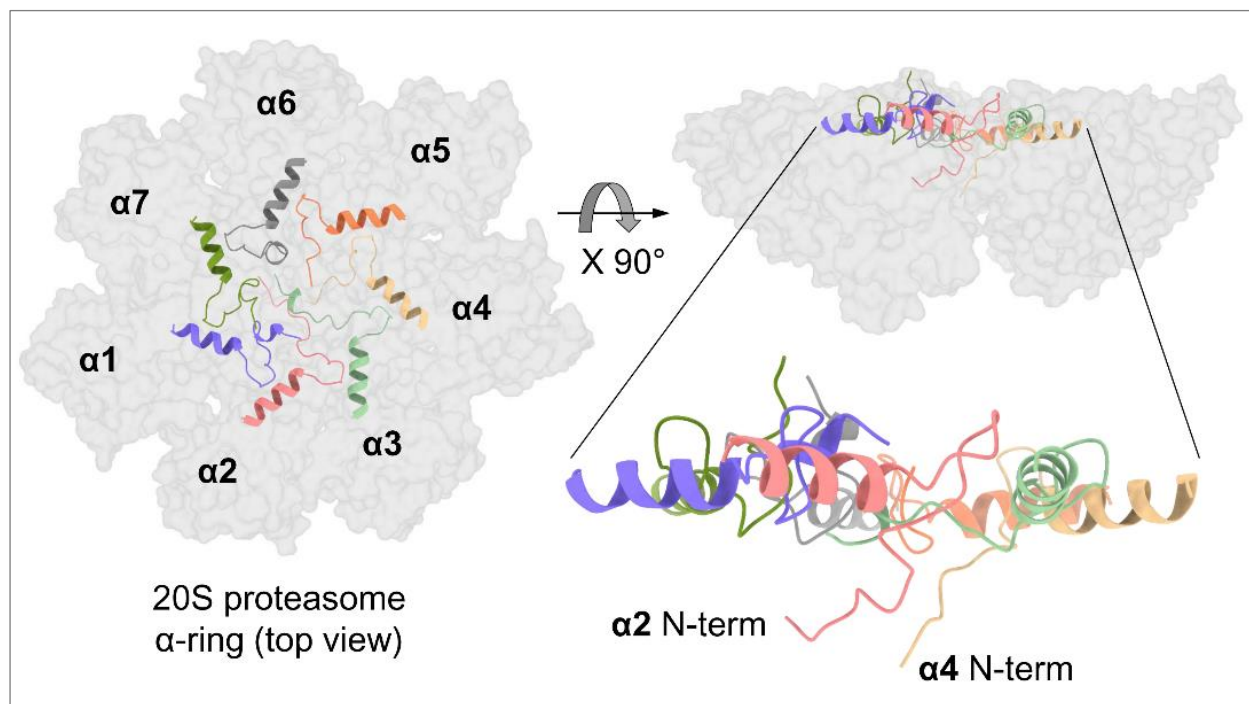
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This file contains **Supplementary Figures S1-S5** on the following pages 2-6:

Figures:

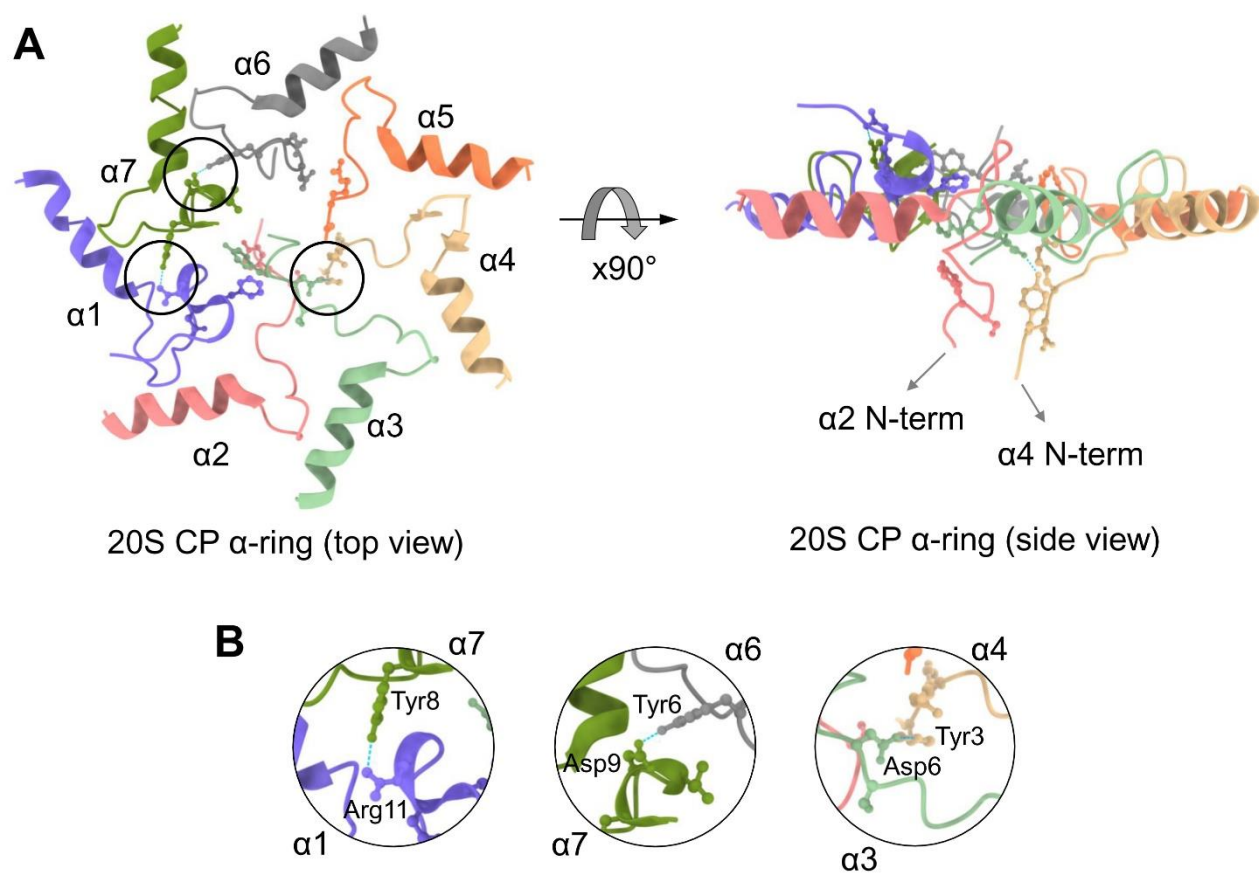


Supplementary Figure S1: The α -ring of closed 20S gate. Position of α -subunit N-termini in closed yeast 20S proteasomes (*Model PDB 1ryp*). Left: Top view of 20S-CP α -ring structure showing in grey color surface contour. The N-terminal tails and the H0-helix of all α -subunits (α N-ring) are shown in colored cartoon. Right: The side view of the figure on left showing the downward position of $\alpha 2$ and $\alpha 4$ N-terminal tails.

A	N-Term	YDX	Reverse	H0 helix
		-motif	turn	
Hs α 1	MSRGSSAG	FDR	HITIFSP	EGRLYQVEYAFKAINQGG
Hs α 2	MAERG	YSF	SLTTFSP	SGKLVQIEYALAAVAG
Hs α 3	MSRR	YDS	RTTIFSP	EGRLYQVEYAMEAI
Hs α 4	MS	YDR	AITVFSP	DGHLFQVEYAQEAVKKG
Hs α 5	MFLTRSE	YDR	GVNTFSP	EGRLFQVEYAIEAIKL
Hs α 6	MFRNQ	YDN	DVTWSP	QGRVHQIEYAMEAVK
Hs α 7	MSSIGTG	YDL	SASTFSP	DGRVFQVEYAMKAVE

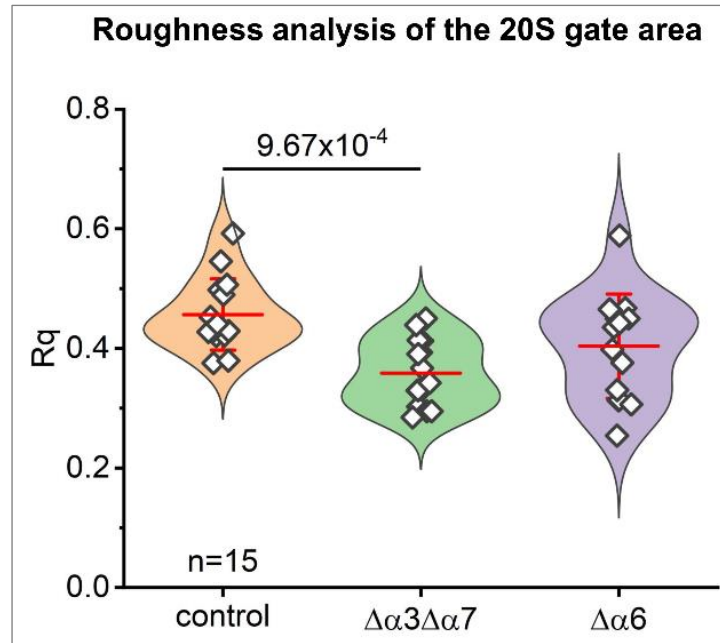
B	YDX -motif	
	Yeast	Human
α 1	YDR	FDR
α 2	YSF	YSF
α 3	YDS	YDS
α 4	YDR	YDR
α 5	YDR	YDR
α 6	YDG	YDN
α 7	YDL	YDL

Supplementary Figure S2: YD(X) motifs of α -subunit N-termini of human proteasome. (A) N-terminal tail sequences of human proteasome α -subunits highlighting the embedded YD(X) motif. (B) Conservation of YD(X) motifs between yeast and human proteasome α subunits.

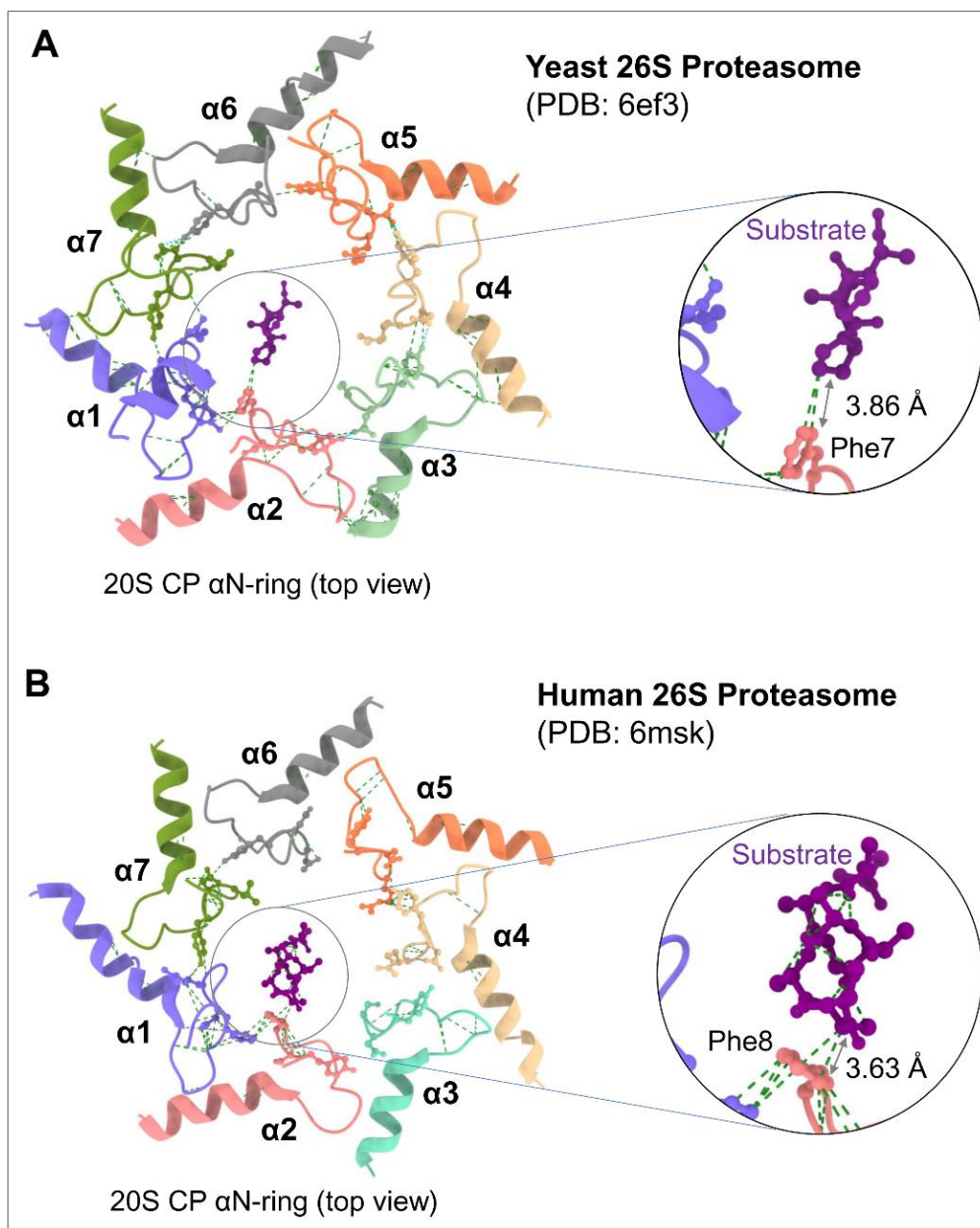


Supplementary Figure S3: YD(X) motif Interactions in closed state human 20S proteasome. (A)

Left: A model figure of the α N-ring region in resting human 20S (pdb:7pg9) with cartoon representation. The black circle shows the hydrogen bonds between $\alpha1$ - $\alpha7$, $\alpha3$ - $\alpha4$ and $\alpha6$ - $\alpha7$ at the YDX motifs. Right: Side view of the α N-ring shows the deeper extension of $\alpha2$ and $\alpha4$ N-termini. The YDX motif of each α -subunit tails are shown in ball-stick representation. **(B)** The zoomed in view of the hydrogen bonds between $\alpha1D - Y\alpha7$, $\alpha3D - Y\alpha4$ and $\alpha6Y - D\alpha7$.



Supplementary Figure S4: Roughness analysis of the 20S gate area. Roughness analysis was performed on the eight-pixel α face scan-lines of randomly selected single images of core particles, fifteen each from WT (control; closed), $\alpha3\alpha7\Delta N$ (pseudo-open), and $\alpha6\Delta N$ (closed) (NanoScope Analysis and SPIP/Scanning Probe Image Processor v. 6.013; Image Metrology, Denmark). The RMS (root mean square) values were considered a measure of the flatness of the gate area.



Supplementary Figure S5: Substrate interaction with Phe residue in N-terminal tail of the $\alpha 2$ subunit. Cartoon view models of the α N-ring of Yeast proteasome (**A**) and Human proteasome (**B**) show a substrate polypeptide (in purple ball & stick model) interacting with the Phe residue (in pink ball & stick model) of the corresponding YDF sequence of $\alpha 2$ subunits. Each YD(X) motif of the different α subunits is shown in ball & stick models. The green dash lines represent the Van-der-Wall interactions between atoms within specific residues measured by Chimera X with interaction parameters as “Find pairs of atoms with VDW overlap” ≥ 0.30 Å. The blue dash lines represent the H-bonds between the Y and D residues of adjacent YD(X) motifs.