

**Two-step process for disassembly mechanism of proteasome $\alpha 7$
homo-tetradecamer by $\alpha 6$ revealed by high-speed atomic force
microscopy**

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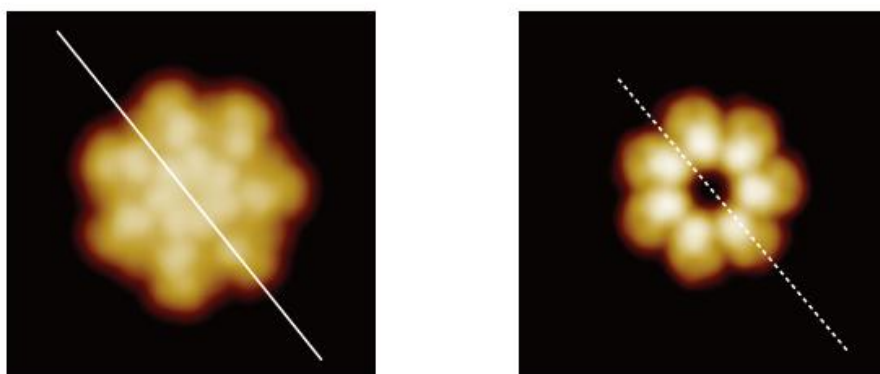
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(a)



(b)

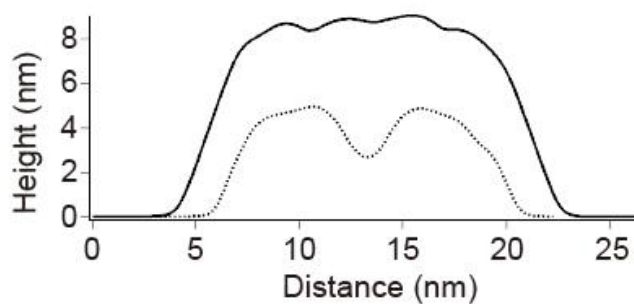


Figure S1. Comparison between simulated images of $\alpha 7$ tetradecamer and heptamer. (a) Simulated images of the $\alpha 7$ (left) tetradecamer and (right) heptamer. (b) Corresponding height profiles indicated by the lines in (a). The solid and broken lines correspond to the profiles for the double ring and the single ring, respectively.

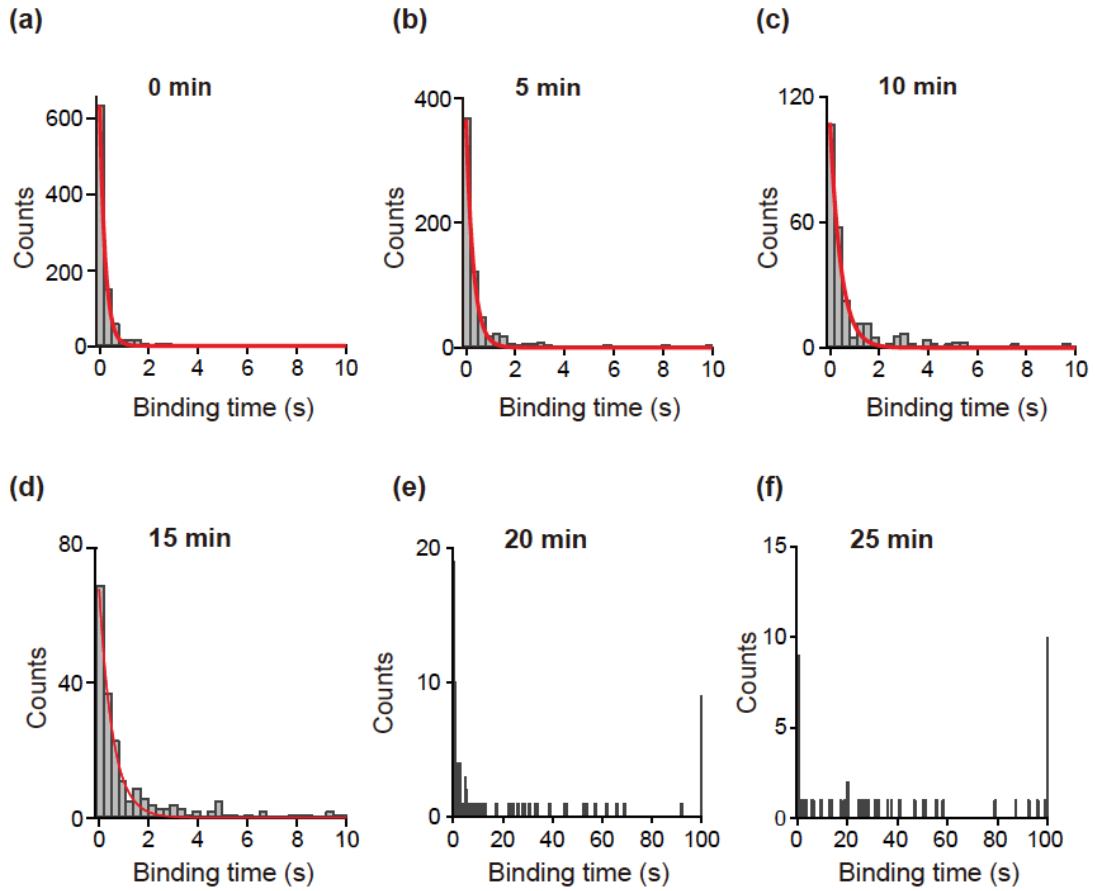


Figure S2. Dwell time analysis of $\alpha 6$ on $\alpha 7$ homo-heptamer. Histograms of dwell time of $\alpha 6$ bound to the $\alpha 7$ heptamer ring for a delimited elapsed time after the addition of $\alpha 6$. The delimited elapsed time, t_e , was (a) $0 \leq t_e < 5$ min, (b) $5 \text{ min} \leq t_e < 10$ min, (c) $10 \text{ min} \leq t_e < 15$ min, (d) $15 \text{ min} \leq t_e < 20$ min, (e) $20 \text{ min} \leq t_e < 25$ min and (f) $25 \text{ min} \leq t_e$. Red lines in (a-d) represent fitted curves with single exponential functions, giving the life time $\pm$ s.d.; (a) $\tau_{0 \text{ min}} = 0.22 \pm 0.01 \text{ s}$ ($n = 970$), (b) $\tau_{5 \text{ min}} = 0.29 \pm 0.01 \text{ s}$ ($n = 712$), (c) $\tau_{10 \text{ min}} = 0.43 \pm 0.02 \text{ s}$ ($n = 283$), (d) $\tau_{15 \text{ min}} = 0.55 \pm 0.03 \text{ s}$ ($n = 235$). (e and f) Over 20 min, the dwell time was significantly longer and histograms were not able to be fitted by single exponential curves. Mean binding times $\pm$ s.ds. were (e) $19 \pm 31 \text{ s}$ at $20 \text{ min} \leq t_e < 25 \text{ min}$ ($n = 100$) and (f) $33 \pm 38 \text{ s}$ at $25 \text{ min} \leq t_e$ ($n = 68$).

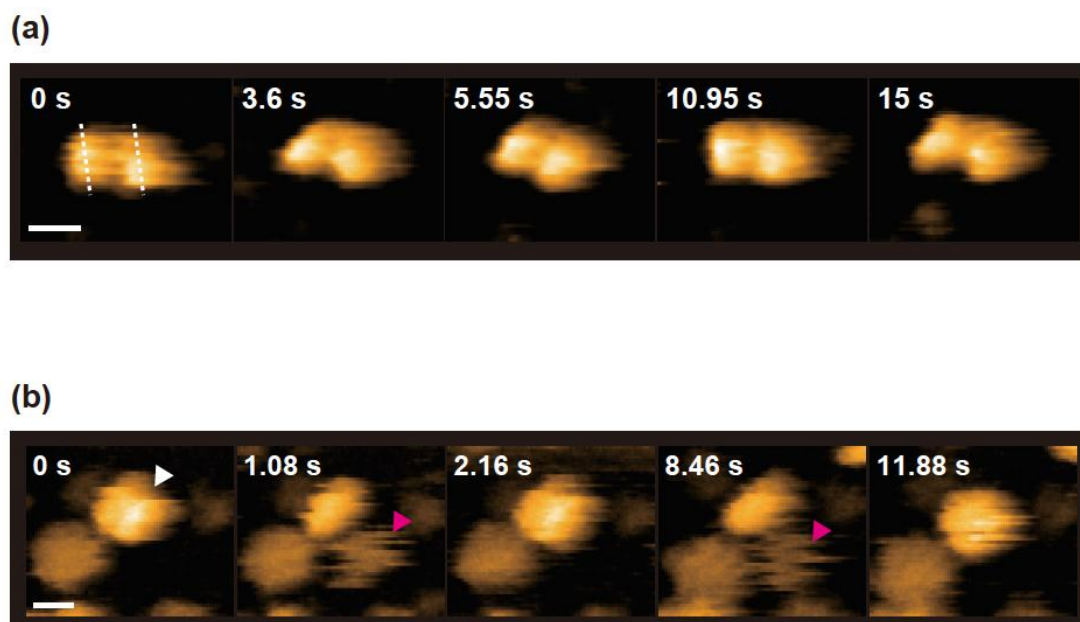


Figure S3. Weak stacking interaction between the stacked $\alpha 7$ single rings.

(a) The double ring is adsorbed on the substrate with an orientation where the ring face is perpendicular to the scanning direction. Wobbling motion of the $\alpha 7$ rings is still observed. Scale bar, 10 nm. (b). Complete dissociation of the $\alpha 7$ rings and reassembly to the double ring. A white arrow head indicates the double ring, while magenta arrow heads indicate the dissociated single ring. Scale bar, 10 nm.

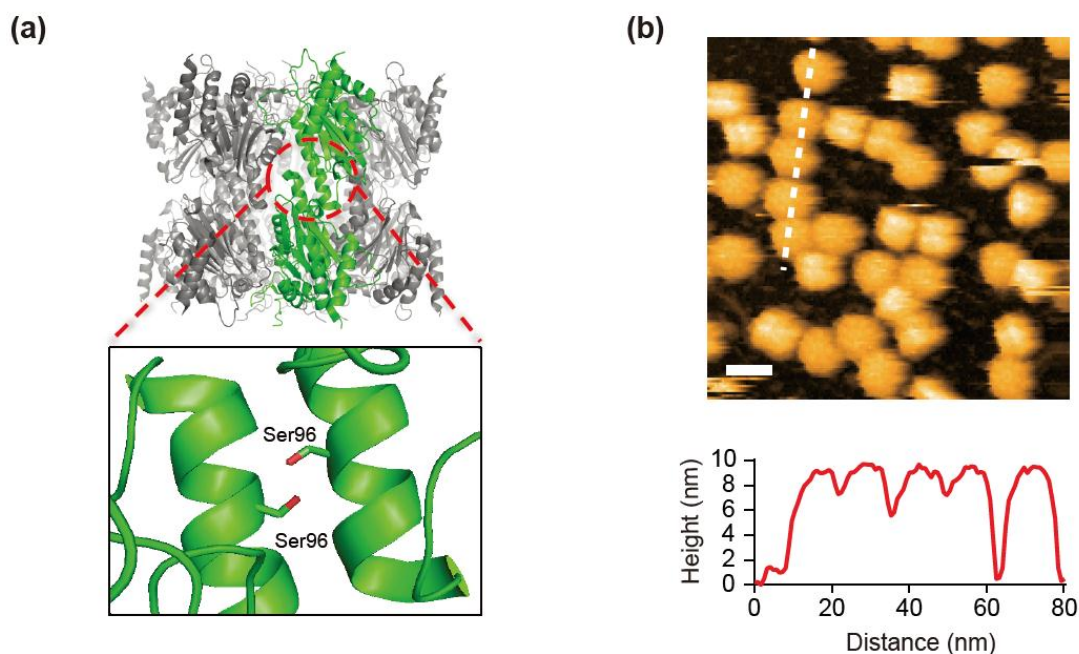


Figure S4. Schematic of position of Ser96 in $\alpha 7$ and AFM image of cross-linked S96C double rings. (a) Structure of the wild-type $\alpha 7$ double ring showing positions of S96 residues, which were replaced by Cys residues. Chemically cross-linked $\alpha 7$ double ring was prepared using the S96C variant. (b) HS-AFM image of cross-linked S96C double rings, which are stable even on the APTES-mica.

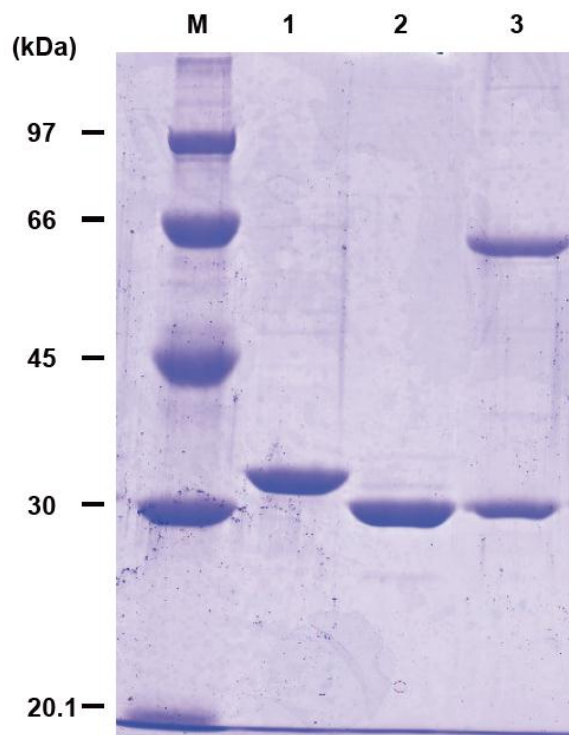


Figure S5. Purities of proteasome $\alpha 6$ and $\alpha 7$ subunits. Purified samples were analyzed by SDS-PAGE; M: molecular weight of marker proteins, lane 1: $\alpha 6$ subunit, lane 2: $\alpha 7$ subunit, lane 3: chemically cross-linked $\alpha 7$ S96C variant.

Supplementary Movies

Movie 1. Bisection of the $\alpha 7$ double ring by adsorption on APTES-mica.

Acquired frame rate: 1 fps. The movie is x 1 playback.

Movie 2. Interaction between $\alpha 6$ and $\alpha 7$ heptameric ring. Acquired frame

rate: 2 fps. The movie is x 3 playback.

Movie 3. Binding and dissociation of $\alpha 6$ to $\alpha 7$ heptamer just after the

addition of $\alpha 6$. Acquired frame rate: 10 fps. The movie is x 1 playback.

Movie 4. Binding and dissociation of $\alpha 6$ to $\alpha 7$ heptamer after 20 min of the

addition of $\alpha 6$. Acquired frame rate: 10 fps. The movie is x 2 playback.

Movie 5. Wobbling motion of the stacked $\alpha 7$ single rings. Acquired frame

rate: 3.3 fps. The movie is x 2 playback.

Movie 6. Transient dissociation of the $\alpha 7$ single rings. Acquired frame rate:

6.6 fps. The movie is x 1 playback.

Movie 7. Binding and dissociation of $\alpha 6$ to the cleft between $\alpha 7$ single rings.

Acquired frame rate: 10 fps. The movie is x 1 playback.

Movie 8. Non-binding of $\alpha 6$ to cross-linked $\alpha 7$ -S96C double ring. Acquired

frame rate: 10 fps. The movie is x 1 playback.

Movie 9. Binding of $\alpha 6$ to $\alpha 7$ double ring and disassembly of the double

ring. Acquired frame rate: 6.6 fps. The movie is x 2 playback.