Structural insights on the dynamics of proteasome formation

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Abstract

Molecular organization in biological systems comprises elaborately programmed processes involving metastable complex formation of biomolecules. This is exemplified by the formation of the proteasome, which is one of the largest and most complicated biological supramolecular complexes. This biomolecular machinery comprises approximately 70 subunits, including structurally homologous, but functionally distinct, ones, thereby exerting versatile proteolytic functions. In eukaryotes, proteasome formation is non-autonomous and is assisted by assembly chaperones, which transiently associate with assembly intermediates, operating as molecular matchmakers and checkpoints for the correct assembly of proteasome subunits. Accumulated data also suggest that eukaryotic proteasome formation involves scrap-and-build mechanisms. However, unlike the eukaryotic proteasome subunits, the archaeal subunits show little structural divergence and spontaneously assemble into functional machinery. Nevertheless, the archaeal genomes encode homologs of eukaryotic proteasome assembly chaperones. Recent structural and functional studies of these proteins have advanced our understanding of the evolution of molecular mechanisms involved in proteasome biogenesis. This knowledge, in turn, provides a guiding principle in designing molecular machineries using protein engineering approaches and de novo synthesis of artificial molecular systems.

Keywords Assembly chaperone · Proteasome · Biomolecular machinery · Transient interaction

Introduction

Living systems are characterized as dynamic processes of assembly and disassembly of a variety of biomolecules, interacting with varying external environments. The omics-based approaches developed in recent decades have provided comprehensive information regarding biomolecules as dynamic building blocks in living organisms. Furthermore, by the technical advancement of structural biology and computa-

tional techniques, 3D protein structure data have rapidly accumulated (135,359 entries; November 21, 2017, <https://www.rcsb.org>). In particular, recently advanced cryo-electron microscopy (EM) techniques have markedly accelerated atomic visualization of biomolecular machineries (Cheng 2015; Vinothkumar 2015). Given these developments, elucidating the mechanisms underlying the organization of biomolecular systems has become one of the most important areas of research in biomolecular science.

Molecular organization in biological systems comprises elaborately programmed steps involving the organization of metastable complexes, as exemplified by the formations of viruses, phages (Arisaka et al. 2016; Schur et al. 2016), and ribosomes (Peña et al. 2017; Shajani et al. 2011). The formation of biomolecular machineries involves transiently generated complexes mediated by weak interactions. In this article, we will focus on the proteasome, one of the largest and most complicated biological supramolecular complexes, and outline our current understanding of the mechanisms involved in its formation, particularly from a structural point of view, while providing comparative views on eukaryotic and archaeal proteasome systems.

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Proteasome structure and function

The proteasome is a major proteolytic machine for the selective degradation of unnecessary proteins in cells. In eukaryotes, the proteasome comprises approximately 70 subunits (Budenholzer et al. 2017; Collins and Goldberg 2017; Finley et al. 2016; Kish-Trier and Hill 2013; Tanaka 2009). The proteolytic active sites are sequestered within a cavity of a cylindrical chamber called the 20S core particle (CP). The 20S CP is composed of four, stacked $\alpha\beta\alpha$ rings, each of which is composed of seven different subunits, $\alpha 1-7$ or $\beta 1-7$ (Fig. 1) (Löwe et al. 1995; Unno et al. 2002). At the center of each α ring is a gating pore that leads to the sequestered active sites formed by the $\beta 1$, $\beta 2$, and $\beta 5$ subunits (Heinemeyer et al. 1997). This gate can be opened by protein complexes, collectively referred to as the proteasome activator. In the eukaryotic 26S proteasome, the gate key is regulated by the 19S regulatory particle (RP), which is composed of at least 19 subunits and is divided into two subcomplexes, base and lid (Fig. 1). While the lid (Rpn3, Rpn5–9, Rpn11, Rpn12, and Rpn15) removes the ubiquitin chain from the substrates in cooperation with the ubiquitin receptors, Rpn10 and Rpn13, the base activates 20S CP and acts as AAA+ ATPase for unfolding and loading the substrates

into the catalytic chamber (Budenholzer et al. 2017; Collins and Goldberg 2017; Finley et al. 2016; Kish-Trier and Hill 2013; Tanaka 2009). Recently, a series of cryo-EM structures of the 26S proteasome has been reported, enabling its visualization at the atomic level, thereby giving insights into its working mechanisms [see the reviews by Budenholzer et al. (2017) and Finley et al. (2016) for details]. The base subcomplex is composed of six different, but homologous, ATPase subunits (Rpt1–Rpt6) and three additional non-ATPase subunits (Rpn1, Rpn2, and Rpn13). Among these Rpt subunits, Rpt2, Rpt3, and Rpt5 harbor proteasome-activating motifs and are key for opening the 20S CP gates (Gillette et al. 2008; Smith et al. 2007). Thus, the 26S proteasome contains a heterohexameric Rpt ring and heteroheptameric α and β rings, each of which is composed of structurally homologous, but functionally distinct, subunits, together with thirteen Rpn subunits, creating a versatile protein degradation machine.

Eukaryotic proteasome assembly

To accomplish the aforementioned sophisticated biomolecular functions, the correct assembly of a panel of subunits with a

Fig. 1 Structures of the protein degradation machine, 26S proteasome, and the catalytic 20S core particle (CP). **a** Overall structure of the eukaryotic 26S proteasome (PDB code: 4CR2), including the α and β rings of 20S CP (green and cyan, respectively) and 19S regulatory particles (RPs), which are constituted by the lid (Rpn3, Rpn5–9, Rpn11, Rpn12, and Rpn15, gray) and the base comprising the AAA+ ATPase (Rpt1–Rpt6, brown), the ubiquitin receptors (Rpn10 and Rpn13, orange),

and the non-ATPase Rpn subunits (Rpn1 and Rpn2, yellow). **b** Overall structure of the eukaryotic 20S CP (PDB code: 4R3O), with the α and β rings (green and cyan, respectively). **c** Close-up view of the 20S CP gating pore. The electron microscopy (EM) structures in the absence and presence of the proteasome activator are shown on the left and right (EMD codes: 1740 and 1733), respectively

precise spatial arrangement is essential. Notably, assembly of the eukaryotic 26S proteasome does not occur spontaneously; rather, it requires assistance from several proteins, called proteasome assembly chaperones (Budenholzer et al. 2017; Kish-Trier and Hill 2013; Murata et al. 2009). Five dedicated assembly chaperones, Pba1–Pba4 and Ump1 in yeast (orthologous to the human PAC1–PAC4 and POMP, respectively), have been identified to be responsible for the efficient and precise construction of the 20S CP complex (Hirano et al. 2005, 2006, 2008; Kock et al. 2015; Kusmierczyk et al. 2008; Le Tallec et al. 2007; Wani et al. 2015; Yashiroda et al. 2008). Formation of 19S RP is assisted by at least four specific assembly chaperones: Nas2 (p27), Nas6 (gankyrin), Rpn14 (PAAF1), and Hsm3 (S5b) (Funakoshi et al. 2009; Kaneko et al. 2009; Le Tallec et al. 2009; Roelofs et al. 2009; Saeki et al. 2009; Tomko et al. 2010). Extensive structural studies, including ours, have helped elucidate the 3D structure of the CP- and RP-assembly chaperones (Barrault et al. 2012; Ehlinger et al. 2013; Kim et al. 2010; Kock et al. 2015; Kurimoto et al. 2017; Nakamura et al. 2007; Satoh et al. 2014; Stadtmueller et al. 2012; Takagi et al. 2012; Yashiroda et al. 2008). Among these proteasome assembly chaperones, the structure of Ump1 is uniquely characterized by its intrinsically disordered nature (Sá-Moura et al. 2013; Uekusa et al. 2014).

The accumulated structural information, along with biochemical data, has provided mechanistic insights into the functions of the proteasome assembly chaperones. For instance, our structural and biochemical data demonstrated that

the yeast Pba3/Pba4 heterodimeric chaperone acts as a molecular matchmaker during α -ring formation (Takagi et al. 2014; Yashiroda et al. 2008). This heterodimeric complex interacts primarily with the proteasome $\alpha 5$ subunit and also with the neighboring $\alpha 4$ subunits, thereby reinforcing $\alpha 4$ – $\alpha 5$ interactions (Fig. 2). In yeast, knock-out of this matchmaking chaperone complex results in the accumulation of non-productive complexes lacking the $\alpha 4$, $\beta 1$, $\beta 5$, $\beta 6$, and $\beta 7$ subunits and aggregates of $\alpha 4$ subunits. Because of steric hindrance, interaction of Pba3/Pba4 with the α ring competes with recruitment of the $\beta 4$ subunit onto the same interaction surface of this ring, suggesting that the chaperone complex is released from the protease assembly intermediates during β -ring formation after the completion of the α ring (Yashiroda et al. 2008). Our recent crystallographic data of human PAC4, an ortholog of yeast Pba4, suggest that this matchmaking function is evolutionally conserved across eukaryotes, despite their low sequence similarity (Kurimoto et al. 2017). Like Pba3/Pba4, Pba1 also forms a heterodimer with Pba2 (Kock et al. 2015; Stadtmueller et al. 2012) and preferentially binds to α -ring intermediates, rather than the mature form of 20S CP, through its C-terminal proteasome-binding motifs, thus functioning as a molecular matchmaker in α -ring formation (Wani et al. 2015). In contrast to Pba3/Pba4, the Pba1/Pba2 heterodimer binds to the apical surface of the α ring, and not the β -subunit interface, causing potential steric hindrance for the proteasome activators, including 19S RP (Kock et al. 2015; Stadtmueller et al. 2012). It has been shown that

Fig. 2 The 20S CP assembly mechanism mediated by the assembly chaperone complex Pba3/Pba4. **a** Crystal structure of the Pba3/Pba4/ $\alpha 5$ ternary complex (PDB code: 2Z5C). **b** Docking model of the α ring (PDB code: 1RYP) complexed with the Pba3/Pba4 heterodimer. Pba3 (orange)

and Pba4 (red) are indicated as ribbon models, whereas the α subunits are shown as surface models highlighting $\alpha 4$ (blue) and $\alpha 5$ (green). **c** The Pba3/Pba4 heterodimer operates as a molecular matchmaker during α -ring formation through the association of multiple α subunits ($\alpha 4$ and $\alpha 5$)

Hsm3 also exerts a similar matchmaker function during base formation (Barrault et al. 2012; Takagi et al. 2012). Transient association is also observed between 20S CP assembly intermediates and assembly chaperone Ump1 (Kock et al. 2015), which is responsible for β -ring formation and considered the first substrate of the proteasome in its biogenesis (Hirano et al. 2008).

The elaborate mechanisms of proteasome assembly chaperones are also exemplified by Nas2, which operates as a checkpoint in the 19S base formation (Sato et al. 2014) (Fig. 3). Nas2 is composed of an N-terminal helical domain and a C-terminal PDZ domain connected via a flexible linker. Our crystallographic and NMR spectroscopic data demonstrated that Nas2 binds bivalently to the Rpt5 subunit; the N-terminal domain of Nas2 masks the flanking Rpt1-binding interface of Rpt5, whereas the C-terminal domain caps its C-terminal proteasome-activating motif. These data suggest that Nas2 blocks proteasome activation during the base formation and is released from Rpt5 upon completion of Rpt-ring formation, thereby enabling its fully prepared docking onto 20S CP. Thus, assembly chaperones intervene in proteasome formation

by forming transient complexes with assembly intermediates, which eventually dissociate once proteasome formation is complete.

Unique self-assembling properties of eukaryotic proteasome subunits

Experiments using negative-stain EM and small-angle neutron scattering (SANS) have shown that *Trypanosoma brucei* $\alpha 5$ and human proteasome $\alpha 7$ subunits have unique properties of self-assembling into four and two stacked heptameric rings, respectively (Gerards et al. 1997; Sugiyama et al. 2009, 2011; Yao et al. 1999). Our recent X-ray crystallographic data revealed that the $\alpha 7$ subunits make a double-ring tetradecameric structure in which the single-ring architecture of $\alpha 7$ is similar to those of the archaeal homoheptameric and eukaryotic heteroheptameric α rings (Fig. 4) (Ishii et al. 2015). Intriguingly, deuteration-assisted SANS experiments revealed that only two of the 14 subunits were exchangeable in each $\alpha 7$ homotetradecamer, suggesting an asymmetric nature of the $\alpha 7$ tetradecamer (Sugiyama et al. 2011). However,

Fig. 3 Nas2-regulated 19S RP assembly. **a** Crystal structure of the Nas2/Rpt5 binary complex (PDB code: 3WHL). **b** Docking model of the Rpt ring (PDB code: 4B4T) complexed with Nas2. Nas2 (red) is indicated as a ribbon model, whereas the Rpt subunits are shown as surface models; Rpt1 (gray), Rpt2 (green), Rpt3 (pink), Rpt4 (slate), Rpt5 (brown), and Rpt6 (yellow). **c** Nas2-mediated checkpoint in Rpt-ring formation prior to its docking onto the proteolytic 20S CP

Fig. 4 Structures of the (a) $\alpha 7$ homotetradecamer and (b) eukaryotic half-proteasome model derived from the 20S proteasome (PDB code: 4R30). **a** Ribbon models of the single and double $\alpha 7$ rings derived from the crystal structure of the $\alpha 7$ tetradecamer (PDB code: 5DSV), as shown

such an asymmetric structure could not be found in the crystal structure, probably due to limited resolution (3.75 Å) and/or crystal packing. Therefore, the unique subunit-exchange mechanism of the $\alpha 7$ homotetradecamer remains to be clarified. Interestingly, our analytical ultracentrifugation and mass spectrometric analyses under non-denaturing conditions demonstrated that the $\alpha 7$ double-ring structure is disassembled upon the addition of the $\alpha 6$ subunit, giving rise to a 1:7 heterooctameric $\alpha 6$ – $\alpha 7$ complex (Ishii et al. 2015). Furthermore, recently emerged high-speed atomic microscopy data demonstrated a two-step process for the disassembly mechanism, whereby the $\alpha 6$ initially cracks at the interface between the stacked two $\alpha 7$ single rings and the subsequent interaction of $\alpha 6$ onto the central pore of the $\alpha 7$ single ring prevents its re-association into the double ring (Kozai et al. 2017). Although the significance of these non-native assembly states of the proteasome subunits remains undefined, their existence and unique behaviors suggest that scrap-and-build processes are involved in proteasome formation.

in the upper and lower panels, respectively, related by a 90° rotation around a horizontal axis. **b** The α subunits are colored as follows: $\alpha 1$ (orange), $\alpha 2$ (red), $\alpha 3$ (cyan), $\alpha 4$ (blue), $\alpha 5$ (forest green), $\alpha 6$ (magenta), and $\alpha 7$ (green). The β subunits are colored in gray

Archaeal proteasome assembly

Unlike eukaryotic proteasomes, archaeal proteasomes are structurally simple because the 20S CP typically comprises only a single form of each α and β subunit (Löwe et al. 1995). These subunits spontaneously assemble into four homoheptameric rings without help from the proteasome assembly chaperones (Sprangers and Kay 2007). Intriguingly, bioinformatics analysis showed that archaeal genomes encode homologs of eukaryotic proteasome assembly chaperones, PAC1 and PAC2 (termed PbaA and PbaB, respectively) (Kusmierczyk et al. 2011). Like their eukaryotic counterparts, the archaeal proteins harbor C-terminal proteasome-binding motifs (Gillette et al. 2008; Smith et al. 2007). It is enigmatic how the archaeal proteins contribute to proteasome assembly, which can proceed in an autonomous manner. Our biochemical and structural studies have revealed that the archaeon *Pyrococcus furiosus* PbaB forms a homotetramer (Fig. 5) and preferentially binds to the mature 20S CP, rather than its assembly intermediate, through the proteasome-binding

Fig. 5 Structures of yeast Pba1/Pba2 and the archaeal proteasome assembly homologs PbaA and PbaB. Ribbon models of (a) PbaA (PDB code: 3WZ2), (b) PbaB (PDB code: 3VR0), and (c) Pba1/2 (PDB code: 4G4S) are shown in the upper and lower panels, respectively, which are related by a 90° rotation around a horizontal axis

motif, thus functioning as an ATP-independent proteasome activator (Kumoi et al. 2013). Furthermore, the PbaB homotetramer is capable of interacting with unstructured proteins through its tentacle-like C-terminal segments (Kumoi et al. 2013; Sugiyama et al. 2014), thereby suppressing their aggregation. This implies that the PbaB homotetramer can simultaneously bind to the 20S proteasome and its substrate via dual interactions mediated by the C-terminal segments. In contrast, the biological function of PbaA remains largely unknown; it forms a homopentamer (Fig. 5) (Sikdar et al. 2014) and does not interact with the proteasome, despite the presence of its C-terminal proteasome-binding motif, which is potentially capable of activating the 20S CP (Kumoi et al. 2013; Yagi-Utsumi et al. 2017).

During evolution, eukaryotes became able to form precisely arranged hetero-oligomeric proteasomes with non-redundantly functioning catalytic sites from divergent proteasomal subunits, by acquiring a series of assembly chaperones. Further structural and functional studies of the archaeal homologs of proteasome assembly chaperones will provide deeper insights into the evolution of molecular mechanisms involved in proteasome biogenesis.

Summary and perspectives

Proteasome assembly is characterized as a dynamic process, assisted by transient associations of assembly chaperones functioning as molecular matchmakers and checkpoints, and involving scrap-and-build mechanisms. Such sophisticated

mechanisms could be realized based on exquisite energy balances in protein–protein interactions, in marked contrast to artificial supramolecular assemblies with highly symmetric, stable structures. Understanding the programmed pathway of proteasome assembly that has evolved divergent functions will offer a guiding principle not only in the design of molecular machineries using protein-engineering approaches, but also in the de novo creation of artificial molecular systems.

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Compliance with ethical standards

Conflict of interest Koichi Kato declares that he has no conflict of interest. Tadashi Satoh declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Arisaka F, Yap ML, Kanamaru S, Rossmann MG (2016) Molecular assembly and structure of the bacteriophage T4 tail. *Biophys Rev* 8: 385–396. <https://doi.org/10.1007/s12551-016-0230-x>
- Barraut MB, Richet N, Godard C, Murciano B, Le Tallec B, Rousseau E, Legrand P, Charbonnier JB, Le Du MH, Guérois R, Ochsenbein F, Peyroche A (2012) Dual functions of the Hsm3 protein in chaperoning and scaffolding regulatory particle subunits during the proteasome assembly. *Proc Natl Acad Sci U S A* 109:E1001–E1010. <https://doi.org/10.1073/pnas.1116538109>

- Budenholzer L, Cheng CL, Li Y, Hochstrasser M (2017) Proteasome structure and assembly. *J Mol Biol* 429:3500–3524. <https://doi.org/10.1016/j.jmb.2017.05.027>
- Cheng Y (2015) Single-particle cryo-EM at crystallographic resolution. *Cell* 161:450–457. <https://doi.org/10.1016/j.cell.2015.03.049>
- Collins GA, Goldberg AL (2017) The logic of the 26S proteasome. *Cell* 169:792–806. <https://doi.org/10.1016/j.cell.2017.04.023>
- Ehlinger A, Park S, Fahmy A, Lary JW, Cole JL, Finley D, Walters KJ (2013) Conformational dynamics of the Rpt6 ATPase in proteasome assembly and Rpn14 binding. *Structure* 21:753–765. <https://doi.org/10.1016/j.str.2013.02.021>
- Finley D, Chen X, Walters KJ (2016) Gates, channels, and switches: elements of the proteasome machine. *Trends Biochem Sci* 41:77–93. <https://doi.org/10.1016/j.tibs.2015.10.009>
- Funakoshi M, Tomko RJ Jr, Kobayashi H, Hochstrasser M (2009) Multiple assembly chaperones govern biogenesis of the proteasome regulatory particle base. *Cell* 137:887–899. <https://doi.org/10.1016/j.cell.2009.04.061>
- Gerards WL, Enzlin J, Häner M, Hendriks IL, Aepli U, Bloemendal H, Boelens W (1997) The human α -type proteasomal subunit HsC8 forms a double ringlike structure, but does not assemble into proteasome-like particles with the β -type subunits HsDelta or HsBPROS26. *J Biol Chem* 272:10080–10086
- Gillette TG, Kumar B, Thompson D, Slaughter CA, DeMartino GN (2008) Differential roles of the COOH termini of AAA subunits of PA700 (19 S regulator) in asymmetric assembly and activation of the 26 S proteasome. *J Biol Chem* 283:31813–31822. <https://doi.org/10.1074/jbc.M805935200>
- Heinemeyer W, Fischer M, Krimmer T, Stachon U, Wolf DH (1997) The active sites of the eukaryotic 20 S proteasome and their involvement in subunit precursor processing. *J Biol Chem* 272:25200–25209
- Hirano Y, Hendil KB, Yashiroda H, Iemura SI, Nagane R, Hioki Y, Natsume T, Tanaka K, Murata S (2005) A heterodimeric complex that promotes the assembly of mammalian 20S proteasomes. *Nature* 437:1381–1385. <https://doi.org/10.1038/nature04106>
- Hirano Y, Hayashi H, Iemura SI, Hendil KB, Niwa SI, Kishimoto T, Kasahara M, Natsume T, Tanaka K, Murata S (2006) Cooperation of multiple chaperones required for the assembly of mammalian 20S proteasomes. *Mol Cell* 24:977–984. <https://doi.org/10.1016/j.molcel.2006.11.015>
- Hirano Y, Kaneko T, Okamoto K, Bai M, Yashiroda H, Furuyama K, Kato K, Tanaka K, Murata S (2008) Dissecting β -ring assembly pathway of the mammalian 20S proteasome. *EMBO J* 27:2204–2213. <https://doi.org/10.1038/emboj.2008.148>
- Ishii K, Noda M, Yagi H, Thammaporn R, Seetaha S, Satoh T, Kato K, Uchiyama S (2015) Disassembly of the self-assembled, double-ring structure of proteasome $\alpha 7$ homo-tetradecamer by $\alpha 6$. *Sci Rep* 5: 18167. <https://doi.org/10.1038/srep18167>
- Kaneko T, Hamazaki J, Iemura SI, Sasaki K, Furuyama K, Natsume T, Tanaka K, Murata S (2009) Assembly pathway of the mammalian proteasome base subcomplex is mediated by multiple specific chaperones. *Cell* 137:914–925. <https://doi.org/10.1016/j.cell.2009.05.008>
- Kim S, Saeki Y, Fukunaga K, Suzuki A, Takagi K, Yamane T, Tanaka K, Mizushima T, Kato K (2010) Crystal structure of yeast Rpn14, a chaperone of the 19 S regulatory particle of the proteasome. *J Biol Chem* 285:15159–15166. <https://doi.org/10.1074/jbc.M110.104042>
- Kish-Trier E, Hill CP (2013) Structural biology of the proteasome. *Annu Rev Biophys* 42:29–49. <https://doi.org/10.1146/annurev-biophys-083012-130417>
- Kock M, Nunes MM, Hemann M, Kube S, Dohmen RJ, Herzog F, Ramos PC, Wendler P (2015) Proteasome assembly from 15S precursors involves major conformational changes and recycling of the Pba1–Pba2 chaperone. *Nat Commun* 6:6123. <https://doi.org/10.1038/ncomms7123>
- Kozai T, Sekiguchi T, Satoh T, Yagi H, Kato K, Uchiyama T (2017) Two-step process for disassembly mechanism of proteasome $\alpha 7$ homo-tetradecamer by $\alpha 6$ revealed by high-speed atomic force microscopy. *Sci Rep* 7:15373. <https://doi.org/10.1038/s41598-017-15708-8>
- Kumoi K, Satoh T, Murata K, Hiromoto T, Mizushima T, Kamiya Y, Noda M, Uchiyama S, Yagi H, Kato K (2013) An archaeal homolog of proteasome assembly factor functions as a proteasome activator. *PLoS One* 8:e60294. <https://doi.org/10.1371/journal.pone.0060294>
- Kurimoto E, Satoh T, Ito Y, Ishihara E, Okamoto K, Yagi-Utsumi M, Tanaka K, Kato K (2017) Crystal structure of human proteasome assembly chaperone PAC4 involved in proteasome formation. *Protein Sci* 26:1080–1085. <https://doi.org/10.1002/pro.3153>
- Kusmierczyk AR, Kunjappu MJ, Funakoshi M, Hochstrasser M (2008) A multimeric assembly factor controls the formation of alternative 20S proteasomes. *Nat Struct Mol Biol* 15:237–244. <https://doi.org/10.1038/nsmb.1389>
- Kusmierczyk AR, Kunjappu MJ, Kim RY, Hochstrasser M (2011) A conserved 20S proteasome assembly factor requires a C-terminal HbYX motif for proteasomal precursor binding. *Nat Struct Mol Biol* 18:622–629
- Le Tallec B, Barrault MB, Courbeyrette R, Guérois R, Marsolier-Kergoat MC, Peyroche A (2007) 20S proteasome assembly is orchestrated by two distinct pairs of chaperones in yeast and in mammals. *Mol Cell* 27:660–674. <https://doi.org/10.1016/j.molcel.2007.06.025>
- Le Tallec B, Barrault MB, Guérois R, Carré T, Peyroche A (2009) Hsm3/S5b participates in the assembly pathway of the 19S regulatory particle of the proteasome. *Mol Cell* 33:389–399. <https://doi.org/10.1016/j.molcel.2009.01.010>
- Löwe J, Stock D, Jap B, Zwickl P, Baumeister W, Huber R (1995) Crystal structure of the 20S proteasome from the archaeon *T. acidophilum* at 3.4 Å resolution. *Science* 268:533–539
- Murata S, Yashiroda H, Tanaka K (2009) Molecular mechanisms of proteasome assembly. *Nat Rev Mol Cell Biol* 10:104–115
- Nakamura Y, Nakano K, Umehara T, Kimura M, Hayashizaki Y, Tanaka A, Horikoshi M, Padmanabhan B, Yokoyama S (2007) Structure of the oncoprotein gankyrin in complex with S6 ATPase of the 26S proteasome. *Structure* 15:179–189
- Peña C, Hurt E, Panse VG (2017) Eukaryotic ribosome assembly, transport and quality control. *Nat Struct Mol Biol* 24:689–699. <https://doi.org/10.1038/nsmb.3454>
- Roelofs J, Park S, Haas W, Tian G, McAllister FE, Huo Y, Lee BH, Zhang F, Shi Y, Gygi SP, Finley D (2009) Chaperone-mediated pathway of proteasome regulatory particle assembly. *Nature* 459:861–865. <https://doi.org/10.1038/nature08063>
- Saeki Y, Toh-e A, Kudo T, Kawamura H, Tanaka K (2009) Multiple proteasome-interacting proteins assist the assembly of the yeast 19S regulatory particle. *Cell* 137:900–913. <https://doi.org/10.1016/j.cell.2009.05.005>
- Sá-Moura B, Simões AM, Fraga J, Fernandes H, Abreu IA, Botelho HM, Gomes CM, Marques AJ, Dohmen RJ, Ramos PC, Macedo-Ribeiro S (2013) Biochemical and biophysical characterization of recombinant yeast proteasome maturation factor Ump1. *Comput Struct Biotechnol J* 7:e201304006. <https://doi.org/10.5936/csbj.201304006>
- Satoh T, Saeki Y, Hiromoto T, Wang YH, Uekusa Y, Yagi H, Yoshihara H, Yagi-Utsumi M, Mizushima T, Tanaka K, Kato K (2014) Structural basis for proteasome formation controlled by an assembly chaperone Nas2. *Structure* 22:731–743. <https://doi.org/10.1016/j.str.2014.02.014>
- Schur FK, Obr M, Hagen WJ, Wan W, Jakobi AJ, Kirkpatrick JM, Sachse C, Kräusslich HG, Briggs JA (2016) An atomic model of HIV-1 capsid-SP1 reveals structures regulating assembly and maturation. *Science* 353:506–508. <https://doi.org/10.1126/science.aaf9620>
- Shajani Z, Sykes MT, Williamson JR (2011) Assembly of bacterial ribosomes. *Annu Rev Biochem* 80:501–526. <https://doi.org/10.1146/annurev-biochem-062608-160432>

- Sikdar A, Satoh T, Kawasaki M, Kato K (2014) Crystal structure of archaeal homolog of proteasome-assembly chaperone PbaA. *Biochem Biophys Res Commun* 453:493–497. <https://doi.org/10.1016/j.bbrc.2014.09.114>
- Smith DM, Chang SC, Park S, Finley D, Cheng Y, Goldberg AL (2007) Docking of the proteasomal ATPases' carboxyl termini in the 20S proteasome's α ring opens the gate for substrate entry. *Mol Cell* 27:731–744
- Sprangers R, Kay LE (2007) Quantitative dynamics and binding studies of the 20S proteasome by NMR. *Nature* 445:618–622. <https://doi.org/10.1038/nature05512>
- Stadtmueller BM, Kish-Trier E, Ferrell K, Petersen CN, Robinson H, Myszka DG, Eckert DM, Formosa T, Hill CP (2012) Structure of a proteasome Pba1–Pba2 complex: implications for proteasome assembly, activation, and biological function. *J Biol Chem* 287:37371–37382. <https://doi.org/10.1074/jbc.M112.367003>
- Sugiyama M, Hamada K, Kato K, Kurimoto E, Okamoto K, Morimoto Y, Ikeda S, Naito S, Furusaka M, Itoh K, Mori K, Fukunaga T (2009) SANS simulation of aggregated protein in aqueous solution. *Nucl Instr Meth Phys Res A* 600:272–274
- Sugiyama M, Kurimoto E, Yagi H, Mori K, Fukunaga T, Hirai M, Zaccari G, Kato K (2011) Kinetic asymmetry of subunit exchange of homooligomeric protein as revealed by deuteration-assisted small-angle neutron scattering. *Biophys J* 101:2037–2042. <https://doi.org/10.1016/j.bpj.2011.09.004>
- Sugiyama M, Yagi H, Yamaguchi T, Kumoi K, Hirai M, Oba Y, Sato N, Porcar L, Martel A, Kato K (2014) Conformational characterization of a protein complex involving intrinsically disordered protein by small-angle neutron scattering using the inverse contrast matching method: a case study of interaction between α -synuclein and PbaB tetramer as a model chaperone. *J Appl Crystallogr* 47:430–435
- Takagi K, Kim S, Yukii H, Ueno M, Morishita R, Endo Y, Kato K, Tanaka K, Saeki Y, Mizushima T (2012) Structural basis for specific recognition of Rpt1p, an ATPase subunit of 26 S proteasome, by proteasome-dedicated chaperone Hsm3p. *J Biol Chem* 287:12172–12182. <https://doi.org/10.1074/jbc.M112.345876>
- Takagi K, Saeki Y, Yashiroda H, Yagi H, Kaiho A, Murata S, Yamane T, Tanaka K, Mizushima T, Kato K (2014) Pba3–Pba4 heterodimer acts as a molecular matchmaker in proteasome α -ring formation. *Biochem Biophys Res Commun* 450:1110–1114. <https://doi.org/10.1016/j.bbrc.2014.06.119>
- Tanaka K (2009) The proteasome: overview of structure and functions. *Proc Jpn Acad Ser B Phys Biol Sci* 85:12–36
- Tomko RJ Jr, Funakoshi M, Schneider K, Wang J, Hochstrasser M (2010) Heterohexameric ring arrangement of the eukaryotic proteasomal ATPases: implications for proteasome structure and assembly. *Mol Cell* 38:393–403. <https://doi.org/10.1016/j.molcel.2010.02.035>
- Uekusa Y, Okawa K, Yagi-Utsumi M, Serve O, Nakagawa Y, Mizushima T, Yagi H, Saeki Y, Tanaka K, Kato K (2014) Backbone ^1H , ^{13}C , and ^{15}N assignments of yeast Ump1, an intrinsically disordered protein that functions as a proteasome assembly chaperone. *Biomol NMR Assign* 8:383–386. <https://doi.org/10.1007/s12104-013-9523-1>
- Unno M, Mizushima T, Morimoto Y, Tomisugi Y, Tanaka K, Yasuoka N, Tsukihara T (2002) The structure of the mammalian 20S proteasome at 2.75 Å resolution. *Structure* 10:609–618
- Vinothkumar KR (2015) Membrane protein structures without crystals, by single particle electron cryomicroscopy. *Curr Opin Struct Biol* 33:103–114. <https://doi.org/10.1016/j.sbi.2015.07.009>
- Wani PS, Rowland MA, Ondracek A, Deeds EJ, Roelofs J (2015) Maturation of the proteasome core particle induces an affinity switch that controls regulatory particle association. *Nat Commun* 6:6384. <https://doi.org/10.1038/ncomms7384>
- Yagi-Utsumi M, Sikdar A, Kozai T, Inoue R, Sugiyama M, Uchihashi T, Yagi H, Satoh T, Kato K (2017) Conversion of functionally undefined homopentameric protein PbaA into a proteasome activator by mutational modification of its C-terminal segment conformation. *Protein Eng Des Sel*. <https://doi.org/10.1093/protein/gzx066>
- Yao Y, Toth CR, Huang L, Wong ML, Dias P, Burlingame AL, Coffino P, Wang CC (1999) $\alpha 5$ subunit in *Trypanosoma brucei* proteasome can self-assemble to form a cylinder of four stacked heptamer rings. *Biochem J* 344(2):349–358
- Yashiroda H, Mizushima T, Okamoto K, Kameyama T, Hayashi H, Kishimoto T, Niwa SI, Kasahara M, Kurimoto E, Sakata E, Takagi K, Suzuki A, Hirano Y, Murata S, Kato K, Yamane T, Tanaka K (2008) Crystal structure of a chaperone complex that contributes to the assembly of yeast 20S proteasomes. *Nat Struct Mol Biol* 15:228–236. <https://doi.org/10.1038/nsmb.1386>