# A Lon Story

*This report features the work of Chung-I Chang, Shih-Hsiung Wu and their co-workers published in Acta Cryst. D* ***69****, 1395 (2013) and of Chung-I Chang and his co-workers published in Acta Cryst. D* ***69****, 1789 (2013).*

Proteins are molecular machines in cells that are responsible for most cellular activities. They are synthesized as long polypeptides and folded into defined three-dimensional structures spontaneously or with the aid of chaperones, a class of proteins. The control of quality of synthesized proteins is important to the health of cells. Newly synthesized proteins might require modification, including cleavage, to function properly. Misfolded ones might be rescued by chaperones or recycled by proteases. Proteases digest misfolded proteins into small pieces that are then recycled in cells.

Some proteases are big; some are small. The 26S proteasome is huge, with molecular mass 2000 kDa. It contains a catalytic core of 20S hexamer and one of many regulatory parts, which itself has multiple components. In contrast, Lon is small, with only one protein forming a hexamer. Bacterial LonA is the target of the development of antibiotics. Human mitochondrial LonA is also targeted with an anticancer drug because of its involvement in lymphoma cell death.

The Lon family contains an AAA+ (ATPases associated with diverse cellular activities) domain and a C-terminal protease domain with a conserved serine-lysine catalytic dyad. The AAA+ domain hydrolyzes ATP as an energy source and is believed to unfold proteins and to translocate the substrates through a narrow channel into the proteolytic site. Unlike LonA and LonB, LonC has no activity of ATP hydrolysis but can still digest substrates. This cleavage activity can be inhibited with drugs such as bortezomib, MG262 and lactacystin. The conserved residues in the AAA+ domain are missing in LonC, explaining the loss of ATPase activity. LonC has an extra LID segment extending from the AAA+ domain. Chung-I Chang in Academia Sinica, Taiwan, and Soichi Wakatsuki in KEK, Japan (now in SLAC in USA) led a group of scientists to solve structures of LonC protease from *Meiothermustaiwanensis* (MtaLonC) in complexes with several inhibitors to understand its mechanism.[1] This is the first LonC structure and the first in the Lon family with an intact catalytic site. Previously solved structures have this site modified or inactivated to prevent protein aggregation.

The solved MtaLonC forms a symmetrical hexamer; each protomer is crescent-shaped. Unlike LonB from *Thermococcus onnurineus*, in which the entrance of a substrate is gated with aromatic and hydrophobic residues, MtaLonC has a more open pore, allowing a diffusion-based mechanism for the entrance of substrates into the chamber. Many important residues involving ATP hydrolysis are missing and replaced with other amino acids. Extensive interdomain interactions might prohibit a rotation between the AAA+ domain and the proteolytic domain. Such movement is commonly seen in AAA+-containing proteins.

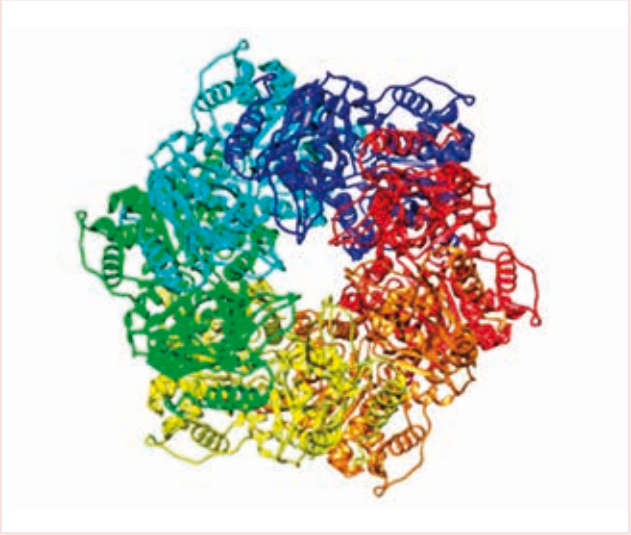

**Fig. 1:** Hexameric structure of LonC protease viewed through the entrance of substrates.

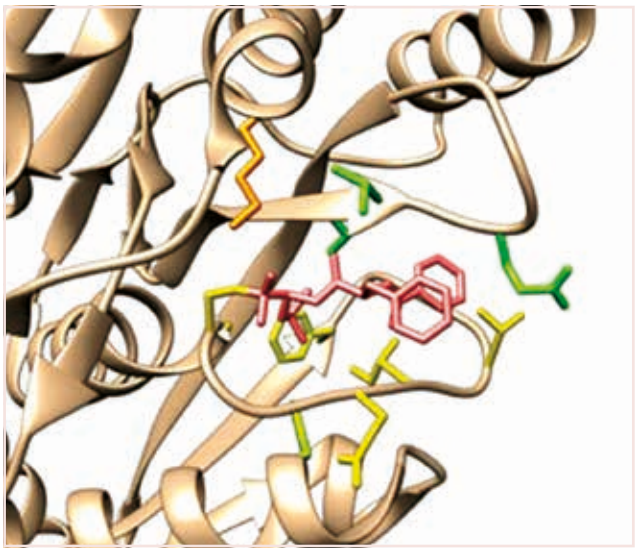

Fig. 2: Residues of LonC interacting with inhibitor bortezomib (colored in salmon).

Fig. 3: Extended HHE domain.

The binding of inhibitors allows scientists to define a contiguous substrate-binding groove at a proteolytic site. They conclude that Ser582 and Lys625 are involved directly in a cleavage of the substrate. They also reveal that the S1 subpocket interacts critically with all inhibitors. This S1 subpocket has an extra space for the development of inhibitors.

The LID segment is not seen in the crystal structure, but appears to mediate the protein-protein contact. This property is seen as a missing layer of thickness about 20 Å between two layers of electronic density. This segment might be important for the recognition and interaction of a substrate. Chang's group also solved the structure of the N-terminal region of LonC containing a LID segment and part of an AAA+ domain (α/β) of this protease.[2] Based on this part of structure and the full-length ones, they built a model of the entire LonC structure. The structure shows that the LID segment forms a large α-helical hairpin extension, termed HHE, with a flexible reverse loop from which about 50 residues extend as a series of two arched α-helical segments. The HHE forms a fence-shaped basket over the top of a LonC pore. These HHE extensions can indeed occupy the 20-Å missing gap in crystals of full-length LonC. The conserved hydrophobic residues in HHE might trap unfolded protein substrates. HHE might adopt many conformations in solution. After searching against a database of protein structures, these scientists found that HHE resembles a chaperone Skp, which is located on the surface of cells and protects unfolded substrates from aggregation. Their overall structures are similar. By engineering MtaLonC, they proved that HHE can prevent unfolded proteins from aggregation, which is important for the cleavage activity of MtaLonC. The LID segment of LonC has thus dual functions – to collect a substrate like tentacles around the opening of the chamber and to protect an unfolded substrate from aggregation.

Their work describes extensively the structure of this newly found LonC protease, elucidates its mechanism in substrate recognition and proteolytic activity and provides opportunities to design drugs against it. This example is classic to demonstrate how the use of a synchrotron can improve our understanding of science and human life. This work used beamline AR-NE3A and BL-1A at the Photo Factory, **BL13B1** and **BL13C1** at the TLS, and **BL44XU** at SPring-8.

## References


1. J.-K. Li, J.-H. Liao, H. Li, C.-I. Kuo, K.-F. Huang, L.-W. Yang, S.-H. Wu, and C.-I. Chang, Acta cryst. D **69**, 1789 (2013).
2. J.-H. Liao, K. Ihara, C.-I. Kuo, K.-F. Huang, S. Wakatsuki, S.-H. Wu, and C.-I. Chang, Acta cryst D **69**, 1395 (2013).