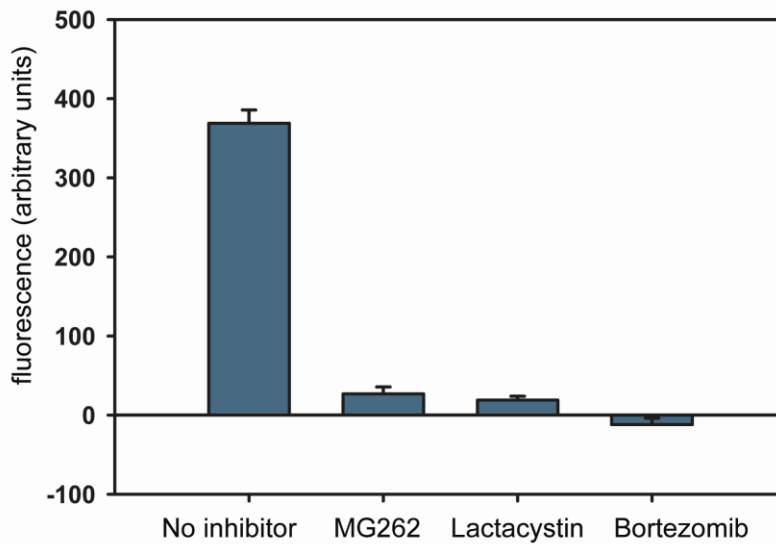


SUPPLEMENTARY INFORMATION

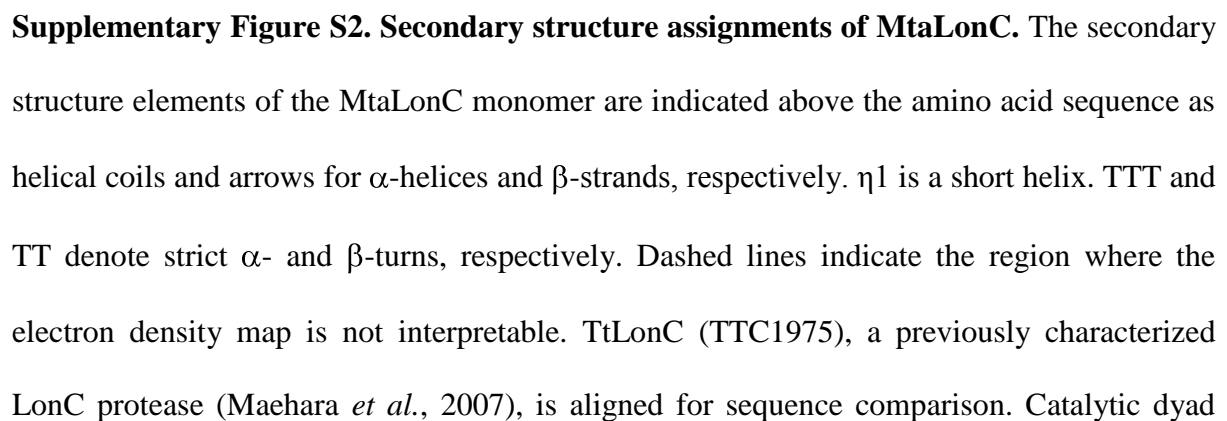
(a)



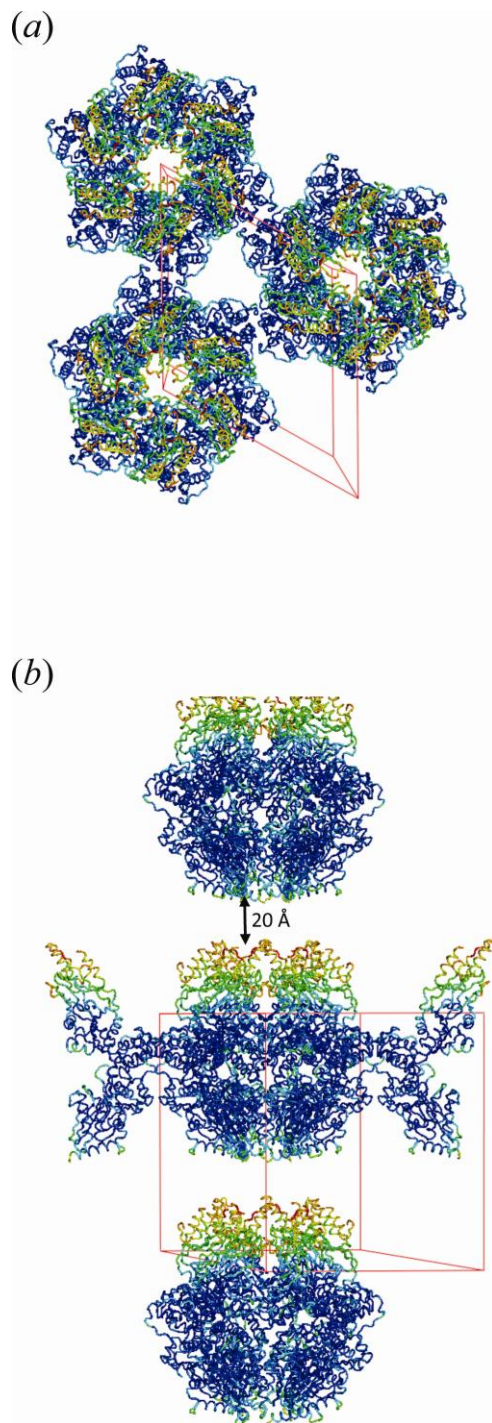
(b)

Inhibitors	IC ₅₀ (nM)
Bortezomib	1,338 ± 3
MG262	327 ± 4
Lactacystin	756 ± 2

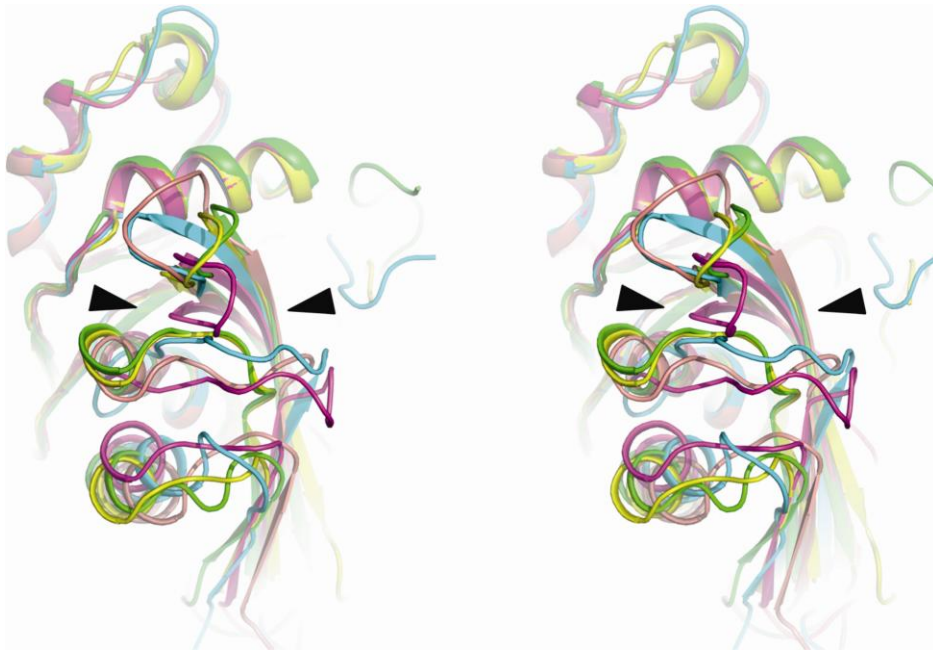
Supplementary Figure S1. Inhibition of the proteolytic activity of MtaLonC by proteasome inhibitors MG262, lactacystin, and bortezomib. (a) Increased fluorescence upon cleavage of the fluorogenic polypeptide substrate F- β 20-Q by MtaLonC in the absence or presence of the three different inhibitors (200 μ M) was measured after 30-min incubation. The error bars show mean with SD (n=3). (b) Potency of MG262, lactacystin, and bortezomib for MtaLonC.



Supplementary Figure S2. Secondary structure assignments of MtaLonC. The secondary structure elements of the MtaLonC monomer are indicated above the amino acid sequence as helical coils and arrows for α -helices and β -strands, respectively. $\eta 1$ is a short helix. TTT and TT denote strict α - and β -turns, respectively. Dashed lines indicate the region where the electron density map is not interpretable. TtLonC (TTC1975), a previously characterized LonC protease (Maehara *et al.*, 2007), is aligned for sequence comparison. Catalytic dyad



Supplementary Figure S4. Crystal packing in hexagonal MtaLonC crystals. (a) Tube diagram of three hexameric MtaLonC complexes related by P6 crystallographic symmetry, colored by temperature factor from blue (lowest) to red (highest). Crystallographic cell axes are shown in red. Shown is a view along the c-axis. (b) Lateral view of the crystal packing in MtaLonC crystals. The c-axis is vertical; the a-b plane is horizontal and perpendicular to the plane of the page.



Supplementary Figure S5. Closed conformation of the substrate-binding groove in the structures of isolated Lon protease domains. The structures of the protease domains of AfLon (cyan; 1Z0E), BsLon (green; 3M6A), EcLon (magenta; 1RR9), and MjLon (orange; 1XHK) are superimposed with that of hLonP (yellow; 2X36) in ribbon representations. Black arrowheads indicate the location of the substrate-binding groove.

Supplementary Reference

Gouet, P., Courcelle, E., Stuart, D. I. & Metoz, F. (1999). *Bioinformatics* **15**, 305-308.