MS12-P3 **Interaction Surface of Get3/Get4/Get5, a Complex Involved in Tail-Anchored Proteins Targeting.** Chwan-Deng Hsiao,[a] Yi-Wei Chang,[ab] Tai-Wen Lin,[a] Yi-Chuan Li,[a] Yu-Shan Huang,[c] Yuh-Ju Sun,[b] *[a]Institute of Molecular Biology, Academia Sinica, Taipei, 115, Taiwan, [b]Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu, 300, Taiwan, [c] National Synchrotron Radiation Research Center, Hsinchu, 300, Taiwan*
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During the biosynthesis of tail-anchored (TA) membrane proteins, their single C-terminal trans-membrane segment is inserted into the endoplasmic reticulum (ER) membrane for orientating the functional domain(s) towards the cytosolic side of the cell. Recent work has uncovered the "Get pathway (guided entry of Tail-anchored protein)", which is responsible for ER targeting of tail-anchored proteins. The Get system consists of Get1, Get2, Get3, Get4 and Get5 proteins. Although structural information and the individual roles of most components of this system have been defined, the interactions and interplay between them remain to be elucidated. Here, we investigated the interactions between Get3 and the Get4/Get5 complex (Get4/5) from *Saccharomyces cerevisiae* [1]. We show that Get3 interacts with Get4/5 via an interface dominated by electrostatic forces. Using isothermal titration calorimetry (ITC) and small-angle X-ray scattering (SAXS), we further demonstrate that the Get3 homodimer interacts with two copies of the Get4/5 complex to form an extended conformation in solution.

[1] Chang, Y.-W., Lin, T.-W., Li, Y.-C., Huang, Y.-S., Sun, Y.-J. and Hsiao, C.-D. (2012). *J. Biol. Chem*. 287, 4783-4789.

Keywords: tail-anchored protein; SAXS; ITC

MS12-P4 **Crystal structure of Lon protease** Chang-Sook Jeong[a], Young Jun An[a], Min-Kyu Kim[a], Sangmin Lee[a], Sun-Shin Cha[a,b] *[a]Marine Biotechnology Research Center, Korea Ocean Research and Development Institute, Ansan, Republic of Korea [b]Department of Marine Biotechnology, University of Science and Technology, Daejeon, Republic of Korea*
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Lon proteases are distributed in all kingdoms of life and are required for survival of cells under stress. Lon is a tandem fusion of an AAA+ molecular chaperone and a protease with a serine-lysine catalytic dyad. We report the 2.0-Å resolution crystal structure of Thermococcus onnurineus NA1 Lon (TonLon). The structure is a three-tiered hexagonal cylinder with a large sequestered chamber accessible through an axial channel. Conserved loops extending from the AAA+ domain combine with an insertion domain containing the membrane anchor to form an apical domain that serves as a gate governing substrate access to an internal unfolding and degradation chamber. Alternating AAA+ domains are in tight- and weak-binding nucleotide states with different domain orientations and intersubunit contacts, reflecting intramolecular dynamics during ATP-driven protein unfolding and translocation. The bowl-shaped proteolytic chamber is contiguous with the chaperone chamber allowing internalized proteins direct access to the proteolytic sites without further gating restrictions.

Keywords: AAA+ protein, ATP-dependent protease, *Ton*Lon