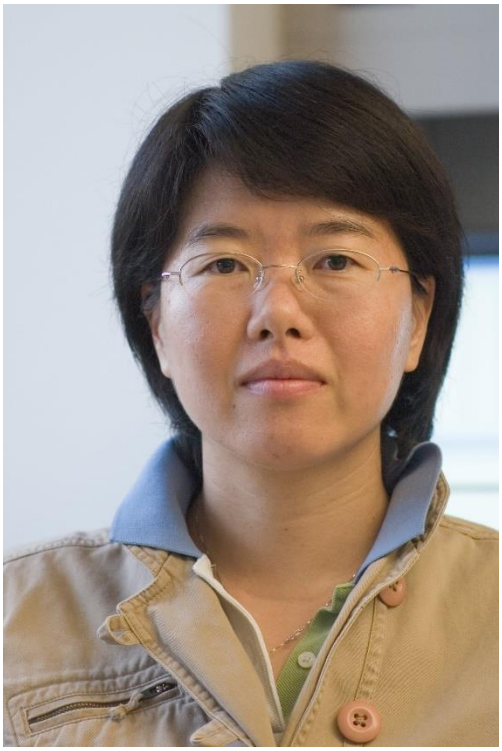
NUS National University of Singapore | Department of Biological Sciences, Faculty of Science

BIOLOGY COLLOQUIUM

Friday , 6 April 2018 | 4pm | DBS Conference Room 1

Hosted by Dr Lu Gan



Protein Quality Control Nanomachines in Action Revealed by Near-atomic Resolution Cryo-EM

By **Yao Cong**

Professor, National Center for Protein Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, China

Cellular protein homeostasis is maintained by protein synthesis, correct protein folding and efficient proteolysis. Two ATP-consuming molecular machines help balance these processes: TRiC and proteasomes. The eukaryotic chaperonin TRiC/CCT assists the folding of 10% of cytosolic proteins. The 26S proteasome is a dynamic 2.5 MDa protease that regulates numerous essential cellular functions through degradation of ubiquitinated substrates. Here we present two cryo-EM structures of S. cerevisiae TRiC in a newly identified nucleotide partially preloaded (NPP) state and in the ATP-bound state at near-atomic resolution. Through our newly developed yeast internal-subunit eGFP tag labeling (YISEL) strategy, we unambiguously identified the subunit locations in open-state TRiC for the first time and found that the CCT2 subunit pair forms an unexpected Z shape, which plays an essential role in TRiC allosteric cooperativity. Our structural and biochemical data reveal a staggered ATP binding mechanism of TRiC. This work offers insight into how the TRiC nucleotide cycle coordinates with its mechanical cycle in preparing folding intermediates for further productive folding. We also present a near-atomic-resolution cryo-EM map of the S. cerevisiae 26S proteasome in complex with ADP-AlFx, which is in an activated state, displaying a distinct conformational configuration especially in the AAA-ATPase motor region. Noteworthy, this map demonstrates an asymmetric nucleotide binding pattern with four consecutive AAA-ATPase subunits bound with nucleotide. The remaining two subunits, Rpt2 and Rpt6, with empty or only partially occupied nucleotide pocket exhibit pronounced conformational changes, which could play an important role in substrate processing of proteasome. Our results provide new insights into the mechanisms of nucleotide-driven allosteric cooperativity of the complex and of the substrate processing by the proteasome. Interestingly, our comparative study revealed ATP triggered opposite modes of action of the two ATPases in protein quality control.