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

SEMINAR

Monday, 3 April 2017 | 2pm | DBS Conference Room 1

Hosted by Dr Lu Gan

Visualizing dynamic cellular machinery with electron microscopy

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In spite of recent technological advancements in transmission electron microscopy (EM), determining structures of flexible macromolecules continue to be a major challenge. We show how EM can be used to elucidate structural details of cellular macromolecules of different sizes and complexities, and involved in diverse biological functions. The largest of these is the microtubule (MT) associated dynein-dynactin (DD) complex (~2.6 MDa in size), whose conformational flexibility has stymied structure determination for decades. By combining 2D and 3D EM analyses, we obtained structural snapshots of isolated dynein complex and DD complexes attached to MTs. From our analyses we not only discerned the first molecular details of native dynein, but also observed unique orientations of dynactin relative to the dynein tail, with the dynein heads positioned uni-directionally on MTs. This suggests a mechanism of overcoming auto-inhibited conformation of dynein. While these studies provide domain-level resolution, we also used EM to determine atomic-level details. To answer how phages evade the CRISPR-based bacterial defense system, we obtained near-atomic resolution reconstruction of phage-encoded anti-CRISPRs (ACRs) bound to the *P. aeruginosa* type I-F CRISPR-crRNA surveillance complex (~450kDa in size). New image processing techniques were used to overcome the intrinsic structural heterogeneity of the complex, revealing for the first time structural details of the type I-F CRISPR surveillance complex, and also explaining the molecular mechanism by which ACRs prevent binding of phage DNA to the CRISPR complex.

Artwork by Ann Nee, DBS, NUS