“Single Particle EM from Low to High Resolution for Macromolecular Complex Structure”

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The knowledge gained from structural studies of biomolecules including protein domain, protein/protein complex, protein/DNA complex, and protein/RNA complex, has significantly advanced our understanding of biological phenomena at the molecular level and has catapulted the development of therapeutics. Since the structure of myoglobin was first determined in the 1950s by X-ray crystallography, this technique has been the most powerful tool in modern structural biology. Recently, a resolution revolution in single particle Cryo-EM has been led by technical breakthroughs, particularly direct electron detector (DED) with unprecedented speed and sensitivity, a state of the art electron microscope (Titan Krios), image processing software with better algorithms and GPG-based parallel computation. These achievements herald the beginning of a new era in the field of structural biology and has been appreciated as the Method of the Year in 2015 and the Nobel prizes in chemistry in 2017.

Here, I will first introduce our complementary structural research (using X-ray Crystallography and Negative stain EM), which can reveal the recognition and transfer mechanism of bacterial endotoxin for innate immune response[1] as well as the molecular mechanism of synaptic adhesion complex for synaptogenesis in nervous system[2]. These complementary studies clearly show how important the low-resolution information from negative stain EM is for addressing biological questions. Next, I will introduce an atomic resolution Cryo-EM structure of the engineered Ferritin in complex with anti-cancer drug, which can be utilized as a platform for non-toxic protein-based drug delivery[3]. Finally, I will introduce the Cryo-EM structure of bovine mitochondrial respirasome at 4.16 Å resolution, containing NADH-ubiquinone oxidoreductase (CI), two ubiquinol-cytochrome c oxidoreductase (CIII), cytochrome c oxidase (CIV). Sub-region refinement after masked 3D classification of CI and CIII can further improve the resolution of their density map in which we were able to build atomic models of entire CI and CIII subunits. These structural analysis can reveal two distinct, “active” and “inactive” conformations of CI. Furthermore, we could identify four different conformations by further 3D classification and refinement of the entire respirasome, suggesting rotations around the pivot between CI and $CIII_2$ in the inner mitochondrial membrane would be implicated in the efficient electron transfer processes. Our respirasome structures provide insights into the electron transport from NADH to cytochrome c in cellular respiration.

Reference

1. Reconstruction of LPS transfer cascade reveals structural determinants within LBP, CD14 and TLR4-MD2 for efficient LPS recognition and transfer. **Immunity (2017).**
2. Structural Insights into Modulation of Neurexin-Neuroligin Trans-synaptic Adhesion by MDGA1 /Neuroligin-2 Complex. **Neuron (2017).**
3. Structural basis for LAR-RPTP/Slitrk complex-mediated synaptic adhesion. **Nat Commun. (2014).**
4. Four-fold Channel-Nicked Human Ferritin Nanocages for Active Drug Loading and pH-Responsive Drug Release. **Angew Chem Int Ed Engl. (2018).**