

Seminar Announcement

Phase plate analysis of DNA and nucleosomes at near-atomic resolution

Date: 23 Sep 2016 Friday

Time: 4pm

Venue: Classroom 1, SBS



Speaker: Asst Prof. Sara Sandin
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Abstract

Our group works on chromatin structure and nuclear architecture, applying high-resolution Electron Microscopy (EM) methods to obtain mechanistic insight into how proteins regulate DNA folding. Cryo-EM is a powerful technique for structure determination of isolated macromolecular complexes at near-atomic resolution. Several important developments have contributed to the recent 'resolution revolution' in cryo-EM, including direct electron detection, correction of beam-induced motion, as well as improved classification and 3D reconstruction procedures. We have evaluated the potential of combining phase plate imaging and single particle analysis to determine the structure of a small protein–DNA complex [1]. To test the method, we made use of a 200 kDa Nucleosome Core Particle (NCP) reconstituted with 601 DNA for which a high-resolution X-ray crystal structure is known. We find that the phase plate provides a significant contrast enhancement that permits individual NCPs and DNA to be clearly identified in amorphous ice. The refined structure from 26,060 particles has an overall resolution of 3.9 Å and the density map exhibits structural features consistent with the estimated resolution, including clear density for amino acid side chains and DNA features such as the phosphate backbone. Our results demonstrate that phase-plate cryo-EM promises to become an important method to determine novel near-atomic resolution structures of small and challenging samples, such as nucleosomes in complex with nucleosome binding factors. In addition, I will discuss how phase-plate imaging can be applied to cell and tissue samples for correlative analysis of chromatin structure.

[1] Chua EY, Vogirala VK, Inian O, Wong AS, Nordenskiöld L, Plitzko JM, Danev R and Sandin S. (2016). 3.9 Å structure of the nucleosome core particle determined by phase-plate cryo-EM. *Nucleic Acids Res.* 2016 Aug 25. pii: gkw708. [Epub ahead of print]