Preliminary Results

Structure-function characterizations of mammalian DNA methyltransferases DNMT3A and DNMT3B. DNA methylation is one of the major epigenetic mechanisms that critically regulate gene expression and genome stability. Aberrant DNA methylation is associated with cancers and many other human diseases. Establishment of DNA methylation in mammals is achieved by de novo DNA methyltransferases DNMT3A and DNMT3B, which are closely related in sequence but possess similar but distinct functionalities. The specific aim of this project is to identify the structural features underlying the functional distinction between DNMT3A and DNMT3B.

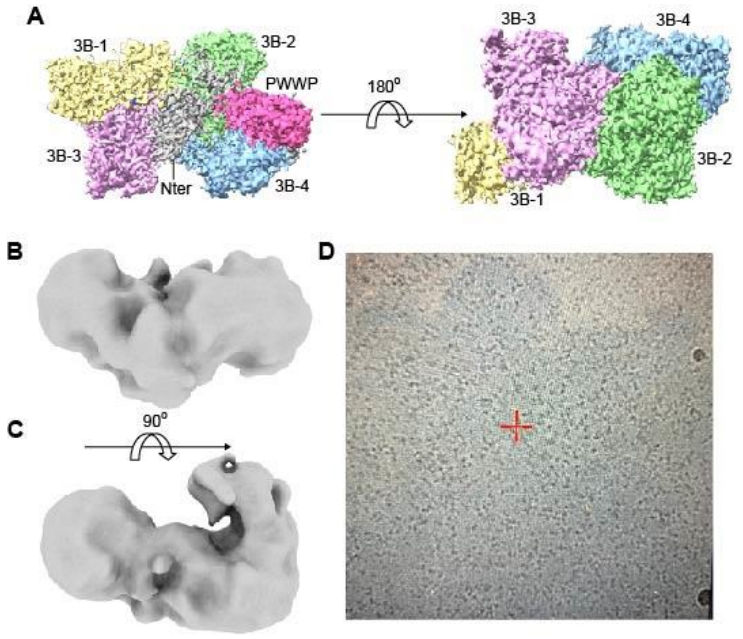

Figure 1. Structural study of the DNMT3A/DNMT3B homo-oligomer. (**A**) Cryo-EM density map of DNMT3B homotetramer at 3.1 Å resolution, with individual subunits labeled, including the N-terminal segment and PWWP domain from one of the subunits. (**B,C**) Negative stain map for the DNMT3A-DNMT3L heterotetramer. (**D**) Cryo-EM image of DNMT3A-DNMT3L from initial sample screening.

In preliminary studies, we have determined the cryoEM structures of DNMT3B at ~3.1 A resolution, providing molecular details for the intramolecular regulation (Fig. 1A). Importantly, DNMT3B forms a homotetrameric assembly, with the N-terminal ADD domains associated with the catalytic domains to block its substrate binding. Meanwhile, the PWWP domain from one of the DNMT3B molecules binds to the ADD domain to occupy its binding sites for histone H3. Together, these observations suggest an autoinhbitory mechanism underlying the site-specific DNA methylation by DNMT3B. Different from DNMT3B, the assembly form of DNMT3A in cells is dominated by the DNMT3A-DNMT3L complex, which acts on distinct chromatin regions than DNMT3B. To understand how DNMT3L regulates DNMT3A for its methylation specificity, we will determine the cryo-EM structure of full-length DNMT3A-DNMT3L complex. In the last four years, we have determined the crystal structures of the C-terminal domains of DNMT3A-DNMT3L in complex with various DNA substrates, which places us in an ideal position to pursue the proposed work. To date, we have already prepared the protein samples of the DNMT3A-DNMT3L complex and the cryoEM grids (Fig. 1B-D). Our preliminary analysis of the 2D classifications of the negative stain images reveals high sample homogeneity and a conformation that is similar but distinct from the DNMT3B homotetramer (Fig. 1A). We expect that the cryoEM data collection of the DNMT3A-DNM T3L sample at NCCAT will help us determine the structure at ~3A resolution. We expect that a 2-3 days of data collection at Titan Krios will be sufficient for the structural study.

Structure determination of the DNMT3A-DNMT3L complex by cryo-EM will provide important insights into the functional distinction between DNMT3A and DNMT3B, which is of great interest to the broad field of DNA methylation, epigenetics and cancer biology.