Preliminary Results

Structural study for the complex between DNMT1 and chromatin factors. DNMT1 undergoes dynamic conformational transition under different chromatin conditions. To elucidate the regulatory mechanism for the DNMT1-mediated DNA methylation, we plan to determine the cryoEM structures of DNMT1 under different functional states, including apo form, DNA-bound and nucleosome-bound states (Fig. 1A). In the last cycle, we were awward one-day access to Titan Krios at NCCAT, which allowed us to have the data collection for full-length DNMT1 under apo state. In our preliminary data processing, we have already obtained a map at 3.8 Å resolution, which allowed us to model a number structural domains (Fig. 1B). In the coming cycle, we plan to collect the cryoEM data for the full-length DNMT1 protein in complex with DNA molecules, which promises to reveal the structural basis for the regulatory domains. These preliminary studies have prepared us to perform the proposed research. We will prepare the DNMT1 samples in a similar manner to that for the apo form DNMT1.

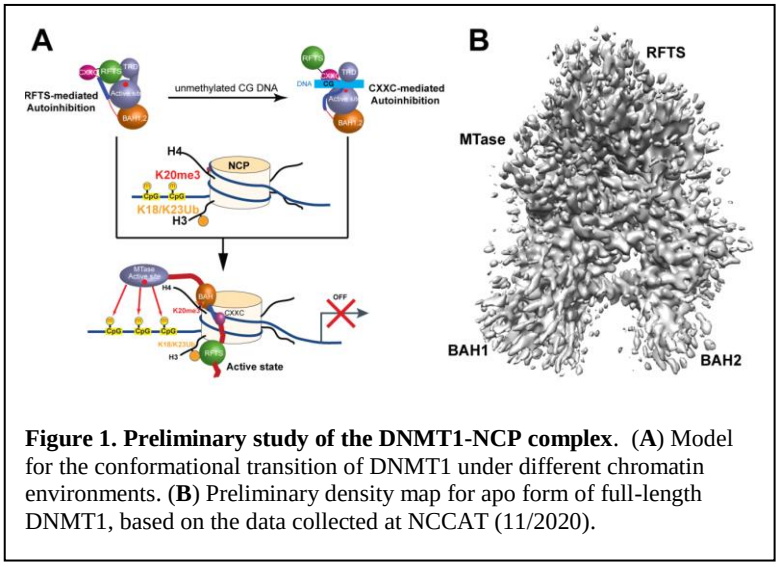

Figure 1. Preliminary study of the DNMT1-NCP complex. (**A**) Model for the conformational transition of DNMT1 under different chromatin environments. (**B**) Preliminary density map for apo form of full-length DNMT1, based on the data collected at NCCAT (11/2020).

Structural study for the interaction between AMT complex and DNA substrates. The AMT complex is comprised of four subunits, proper assembly of which is essential for efficient DNA methylation (Fig. 2A). In preliminary studies, we have successfully reconstituted the enzymatically active AMT complex that is ready for structure determination. The negative stain images collected for this complex sample indicates high homogeneity (Fig. 2A). Furthermore, our initial cryoEM sample preparation also appears promising. We are currently optimizing the cryo-EM samples for data collection and structure determination in the near future. The cryo-EM structural study of the AMT-DNA complex will provide key mechanistic insights into m6A DNA methylation in eukaryotes and its implication in health and disease.

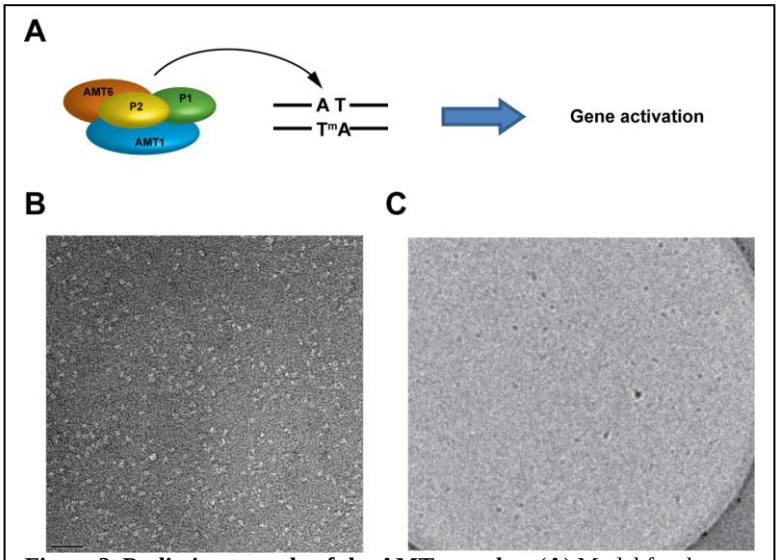

Figure 2. Preliminary study of the AMT complex. (**A**) Model for the assembly and activity of the AMT complex, which is formed by AMT1, AMT7, P1 and P2 proteins. (**B**) Negative stain image of the AMT complex. (B) CryoEM image of the AMT complex.