Preliminary Results (PI: Jikui Song, University of California, Riverside)

Structure and regulation of DNMT1-mediated maintenance DNA methylation. To provide the molecular basis for DNMT1-mediated DNA methylation in chromatin (Fig. 1A), we seek to solve the cryoEM structures of DNMT1 in complex with epigenetically modified nucleosome core particle (NCP) and other chromatin factors (e.g. USP7). In preliminary studies, we prepared the DNMT1 protein enzymatically crosslinked with various NCP samples for Cryo-EM data collection (Fig. 1B). Our preliminary data led to 3.6-3.8 Å-resolution density maps that are currently subject to refinement and structural modeling (Fig. 1C-F). Through adjusting the linker DNA sequence, our study successfully captured several functional states of DNMT1, including a productive, processing DNA methylation complex (Complex1 in Fig. 1C,D) and an initial substrate search complex (Complex 2 in Fig. 1E,F)). Structural analyses of these complexes reveal multiple protein-protein and protein-DNA interactions underpinning the dynamic action of DNMT1 on NCP-templated substrates. We propose to investigate additional functional states of DNMT1-NCP complexes via adjusting linker DNAs and inclusion of additional regulatory factors, which will provide critical insights into DNMT1-mediated maintenance DNA methylation in the chromatin environment.

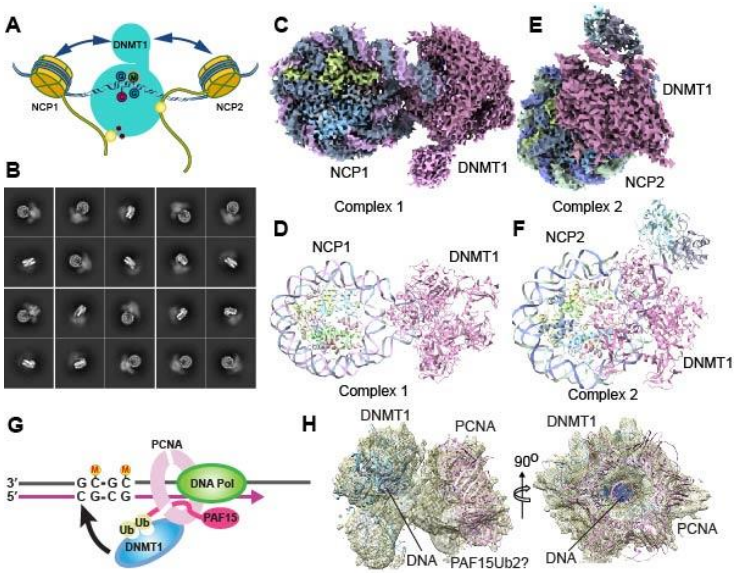

Figure 1. Structural study of the DNMT1 complexes. (**A**) Model for the crosstalk between DNMT1 and epigenetically modified NCPs in regulating maintenance DNA methylation. Unmodified and methylated cytosines are denoted as "C" and "M", respectively. (**B**) 2D classification of a DNMT1-NCP complex. (**C,D**) Cryo-EM density map (C) and ribbon representation (D) of DNMT1 bound to the NCP with linker sequence 1 at 3.6 Å-resolution. (**E,F**) Cryo-EM density map (E) and ribbon representation (F) of DNMT1 bound to the NCP with linker sequence 2 at 3.8 Å-resolution. (**G**) Model for the replication-dependent DNA methylation by DNMT1. (H) Two orthogonal views of the density map for the DNMT1 in complex with DNA, PCNA and two-mono-ubiquitylated PAF15 (PAF15Ub2) at ~6 Å resolution. No sharpening was applied to the map.

DNMT1-mediated DNA methylation is further regulated by PCNA and PCNA-associated factor 15 ubiquitylated at lysine 15 and 24 (PAF15Ub2) (Fig. 1G). To provide the molecular basis for this regulation, we have obtained a ~6 Å-resolution density map corresponding to the DNMT1-PCNA complex (Fig. 1H). It is apparent that DNMT1 is packed against the outer surface of the PCNA trimer, creating a continuous DNA-binding site. Building on this progress, we are optimizing the DNA constructs and sample preparation conditions to stabilize the entire into a more compact conformation for cyro-EM study.

Structure and mechanism of DNMT3A/3B-mediated *de novo* DNA methylation. DNMT3A and DNMT3B share a similar but distinct functionality in establishing DNA methylation patterns during development. Both proteins undergo homo-oligomerization and hetero-oligomerization and interact with specific histone modifications (e.g. H3K36me2/3) for foci-specific DNA methylation. To understand how DNMT3A/3B-mediated *de novo* DNA methylation is regulated by their oligomerization and interactions with NCPs, we have investigated the structures of DNMT3A and DNMT3B under various functional states. For instance, we have determined the cryo-EM structures of DNMT3B at 3.1 Å resolution, providing molecular details for its intramolecular regulation (Fig. 2A). 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 (Fig. 2A). Together, these observations suggest an autoinhibitory mechanism underlying the site-specific DNA methylation by DNMT3B. Furthermore, we have performed preliminary structural characterization of DNMT3A in complex with functional regulator DNMT3L, free and in complex with NCPs. Our initially negative stain analysis of the DNMT3A-DNMT3L complex reveals a conformation that is similar yet distinct from that of DNMT3B (Fig. 2B). Currently, we have generated the cryo-EM grids for the DNMT3A-DNMT3L complex, which indicate good particle homogeneity and density. In addition, our structural study of the DNMT3A-DNMT3L with epigenetically modified NCP demonstrated that the

DNMT3A-DNMT3L tetramer binds to the linker DNA bridging two neighboring NCPs. However, the relatively flexibility between the two NCPs confers a challenge in structure determination (Fig. 2D). To overcome this challenge, we will first focus on structure determination of the DNMT3A-DNMT3L complex with mono-NCP, following a similar sample preparation approach as that used for DNMT1-NCP complex (Fig. 1B). We expect that the cryoEM data collection of the DNMT3A-DNMT3L complexes will ultimately unravel the structural basis of *de novo* DNA methylation.

Structural study for Clr4-mediated H3K9 methylation. In preliminary studies, we have generated the sample for Clr4 bound to H3K14Ub-modified NCP. Furthermore, we have collected one cryoEM dataset for the complex sample from the NCI cryoEM facility and performed data processing using the Cryosparc program. Our preliminary analysis of the 2D and 3D classifications reveals the formation of the Clr4-NCP complex (Fig. 3A). In comparison with the free NCP particles, the population of the Clr4-NCP complex is relatively low, presumably due to the dynamic dissociation of the complex under the cryogenic condition. Nevertheless, these preliminary data led us to obtain a medium-resolution density map (~6 angstrom; Fig. 3B), providing initial insights into the interaction between Clr4 and NCP. Remarkably, the Clr4 protein covers one face of the NCP, prompting partial unwrapping the nucleosome particle. To further improve the structural resolution of the Clr4-NCP complex, we have improved the sample preparation through optimizing the complex formation and crosslinking conditions. SDS-PAGE analysis of our new samples indicates a higher population of the Clr4-NCP complex. We expect that additional data collection of the Clr4-NCP sample at NCCAT will help us refine the structure to a near-atomic resolution. Based on our preliminary results, we expect that a ~3-day data collection at Titan Krios will be sufficient for the structural study.

In summary, our proposed research promises to impact Our preliminary studies have placed us in an ideal position us to perform the proposed research, which is of interest to the broad field of epigenetics and cancer biology. The BAG mechanism at NCCAT provides an excellent opportunity in collectively moving these projects forward.

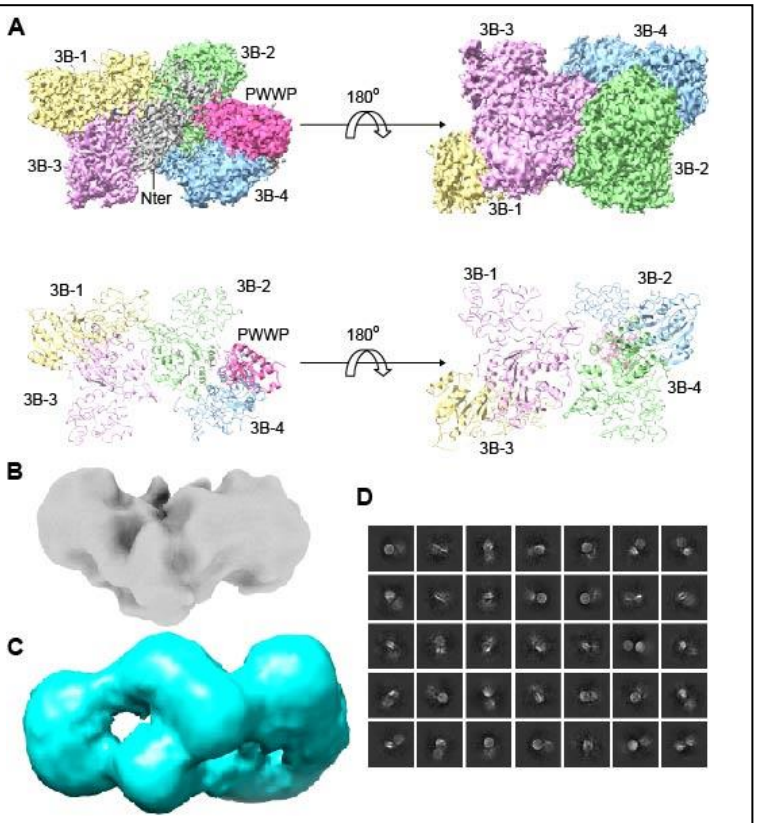

Figure 2. Structural study of the DNMT3A/3B-mediated de novo DNA methylation. (**A**) Cryo-EM density map (top) and ribbon representation (bottom) of the DNMT3B homo-oligomer, with individual subunits, as well as the PWWP domain from one of the subunits, color coded and labeled. (**B,C**) Negative stain density map of DNMT3A-DNMT3L complex, free (B) or bound to NCP (C). (**D**) 2D classification of the DNMT3A-DNMT3B-NCP complex, suggesting the formation of the complex and a need for sample optimization.

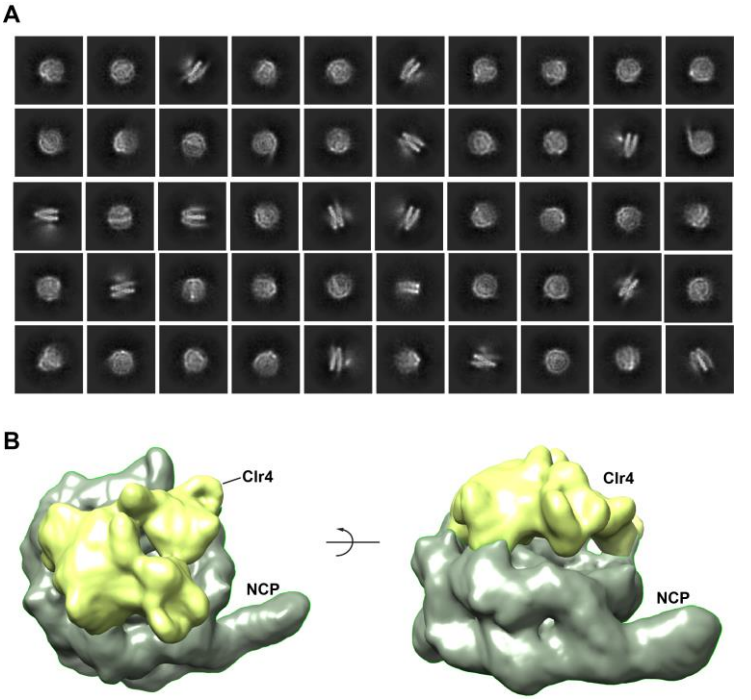

Figure 3. Structural study of the Clr4-NCP complex. (**A**) 2D classification of the DNMT1-NCP complex. (**B**) Preliminary density map (~6 Å) for the Clr4-NCP complex. No sharpening was applied to the map.