Preliminary Results

1. **Structural study for the complex between DNMT1 and epigenetically modified nucleosome.** DNMT1 is a multi-domain protein, comprised of a large N-terminal regulatory region and a C-terminal methyltransferase (MTase) domain, linked via a conserved (GK)n dipeptide repeat (Fig. 1A). The regulatory region contains a replication-foci-targeting sequence (RFTS), a CXXC zinc finger domain and a pair of bromo-adjacent homology (BAH) domains. Previous structural and biochemical evidence from others and us has revealed that both the RFTS and CXXC domains regulate the activity of DNMT1 through autoinhibitory mechanisms: the RFTS domain directly interacts with the MTase domain to inhibit DNA binding whereas the CXXC domain specifically recognizes unmethylated CpG nucleotides, which in turn blocks the de novo methylation activity of DNMT1. Furthermore, the RFTS domain-mediated DNMT1 autoinhibition is subjected to regulation by histone ubiquitylation: During the S phase, E3 ubiquitin ligase UHRF1 is recruited to replicating heterochromatin through its association with both hemi-methylated CpG DNA and H3K9me3, where it stochastically catalyzes the mono-ubiquitylation of histone H3 at lysine 14 (H3K14Ub), lysine 18 (H3K18Ub) and/or lysine 23 (H3K23Ub), and PCNA-associated factor 15 (PAF15) at lysine 15 and 24. The DNMT1 RFTS domain recognizes all these modifications, with a preference for the two-mono-ubiquitin marks (i.e. H3K18Ub/H3K23Ub), leading to allosteric stimulation of DNMT1. Furthermore, our recent study has identified that the RFTS domain preferentially binds to H3K9me3 over H3K9me0, which serves to strengthen the enzymatic stimulation of DNMT1 by H3 ubiquitylation (H3Ub) (Fig. 1B). We determined the crystal structure of DNMT1 RFTS domain complexed with H3K9me3 peptide and two ubiquitins, providing the molecular basis for the H3K9me3 recognition (Fig. 1C). In addition, our cellular and genomic methylation analysis demonstrated that impairment of the RFTS-H3K9me3Ub recognition led to reduced co-localization of DNMT1 with H3K9me3, a global loss of DNA methylation patterns and genome instability in mouse embryonic stem (ES) cells. Together, these studies provide a basis for the proposed structural study of DNMT1 in complex with nucleosome.

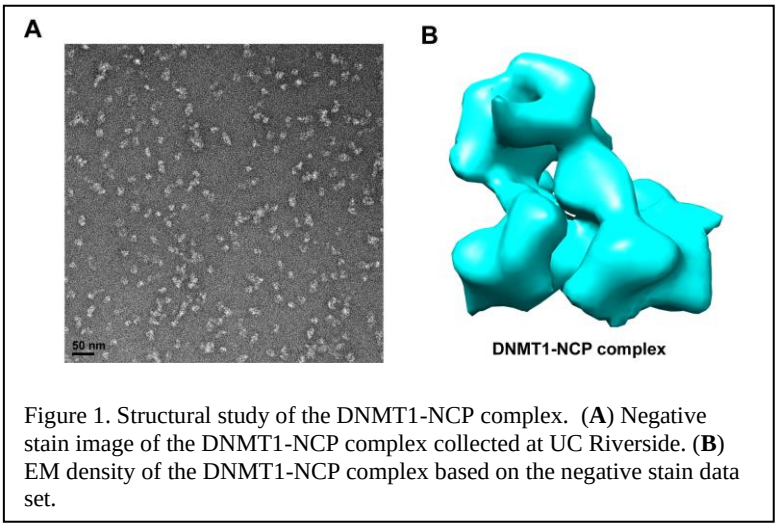

Figure 1. Structural study of the DNMT1-NCP complex. (**A**) Negative stain image of the DNMT1-NCP complex collected at UC Riverside. (**B**) EM density of the DNMT1-NCP complex based on the negative stain data set.

Currently, we have performed negative stain data collection on the DNMT1-NCP complex (Fig. 1A), which led to preliminary density map indicating close contact between DNMT1 and NCP (Fig. 1B). Currently, we are able to produce the DNMT1-NCP complex routinely and constantly improving the sample homogeneity.

2. **Structural study for the interaction between DNMT3A and epigenetically modified nucleosome.** We have also generated the covalent complex of DNMT3A-DNMT3L with H3K36me2-modified NCP (Fig. 2A), following the same approach previously used for generating the DNMT3A-DNMT3L-DNA complex[5]. Formation of the enzymatically crosslinked DNMT3A/3L-diNCP complex was confirmed by EMSA and purified via size exclusion chromatography (Fig. 2B). We have further performed negative stain EM analysis of the DNMT1-NCP complex (Fig. 2C), which indicated good sample homogeneity. Based on this progress, we plan to prepare the cryo-EM samples of the DNMT3A/3L-diNCP complex and perform cryo-EM data collection at PNCC. We expect that cryo-EM structural study of the DNMT3A-NCP complex will provide key insights into DNMT3A-mediated de novo DNA methylation under the chromatin environment.

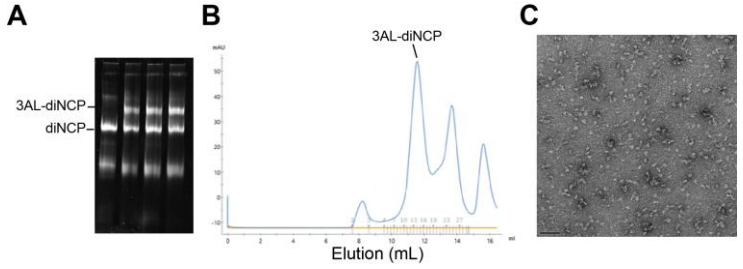

Figure 2. Structural study of the DNMT3A-NCP complex. (**A**) The electrophoretic mobility shift assay for the DNMT3A/3L-diNCP complex. (**B**) Size exclusion chromatography of DNMT3A/3L-diNCP complex. (**C**) Negative stain image of the DNMT3A/3L-NCP complex collected at UC Riverside.