NCCAT BAG Proposal Application

Supplementary Information

Structure determination of epigenetics complexes using single particle cryo-EM

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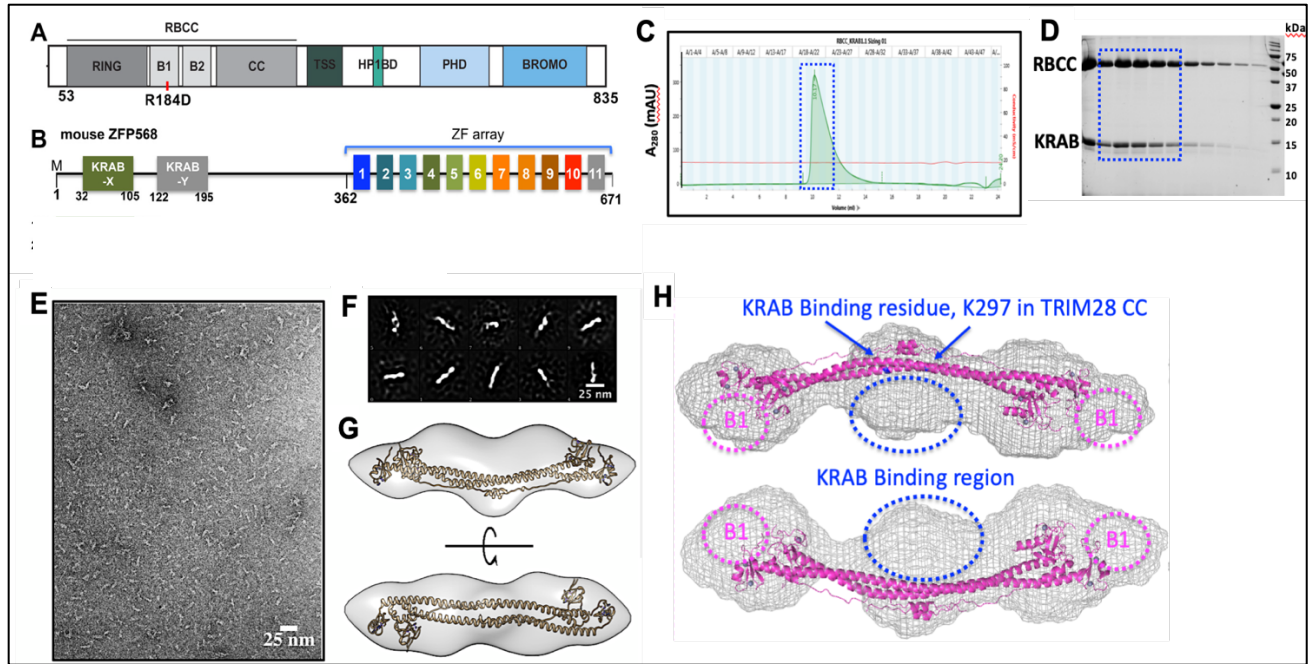
Figure 1: Schematic representation of A) human Trim8 and B) mouse ZFP568 used for expression. C) Size exclusion chromatography profile. D) SDS-PAGE profile showing the purified Trim28-RBCC/Krab-X complex present in the peak fractions (marked in blue). E) EM micrograph of negatively stained Trim28-RBCC/Krab-X complex at 25K magnification captured using JOEL 2100 transmission electron microscope. F) 2D class averages. G) 3D low resolution EM model (shown in two different orientations). H) The SAXS envelope of RBCC/Krab-X complex. The crystal structure lacking B1 box of RBCC is docked onto the EM map (G) and SAXS envelope (H) of RBCC/Krab-X complex.
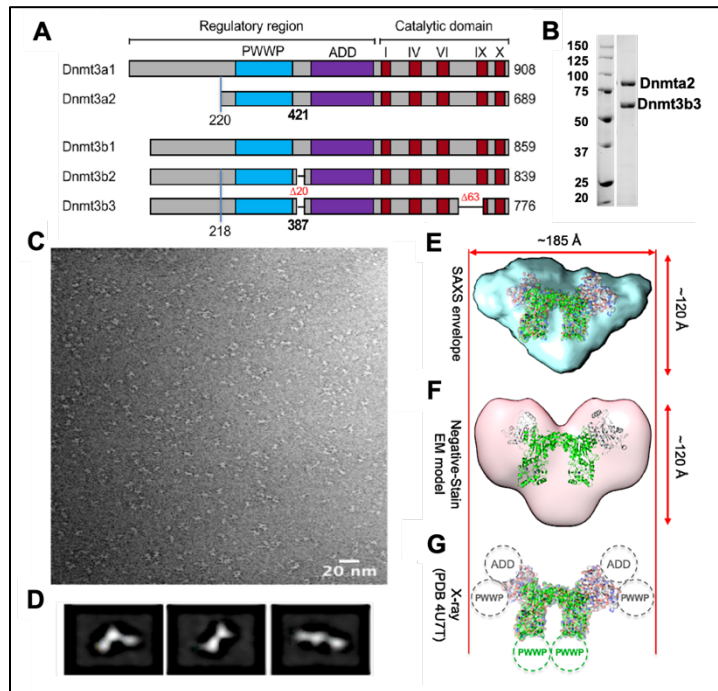
Figure 2: A) Domain organization of members of mouse Dnmt3 family. B) SDS-PAGE profile showing the purified full length Dnmt3a2/3b3 complex. C) EM micrograph of negatively stained Dnmt3a2/3b3 complex captured using JOEL 2100 transmission electron microscope. D) 2D class averages. E) Low resolution SAXS envelope of Dnmt3a2/3b3 complex. F) The negatively-stained 3D EM model. G) X-ray structure of Dnmt3a fragment containing ADD and C-terminal methyltransferase domain (in green) and Dnmt3L C-terminal methyltransferase-like domain (in grey) (PDB 4U7T). The additional domains used in the SAXS and EM studies are indicated by the dashed circles.

Figure 3: A) Size exclusion chromatography profile of PCIF1. B) SDS-PAGE profile showing the purified PCIF1 present in the peak fractions. C) EM micrograph of negatively stained PCIF1 captured using JOEL 2100 transmission electron microscope. D) 2D class averages. (C) The negative-stain EM model with docked X-ray structures of WW domain (PDB 2JX8) and MTase domain in complex with SAH (PDB 6IRW). (D) The SAXS envelope of PCIF1 docked with a homology model of full length PCIF1 (cyan). The flexible N-terminal residues and linker region between the two domains are indicated by the dashed red circle.

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