NCCAT GUP1 Proposal Application Supplementary Information

Structure determination of 5-methylcytosine (5mC) DNA glycosylase DML3 in complex with DNA using single particle cryo-EM

Gundeep Kaur, Xing Zhang, and Xiaodong Cheng

[1]Departments of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

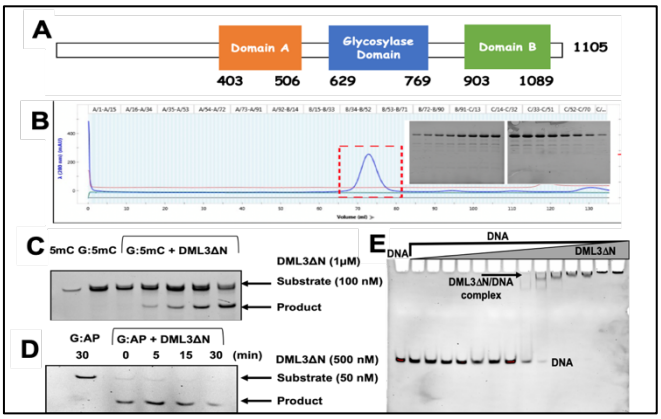

Figure 1: Schematic representation of A) domain organization of DEMETER family of enzymes. B) Size exclusion chromatography profile along with the SDS-PAGE profile showing the purified DML3ΔN present in the peak fractions. C) DML3ΔN has active DNA glycosylase activity on G:5mC DNA substrate. D) DML3ΔN has active lyase activity on G:AP substrate. E) DML3ΔN binds to G:T mismatched DNA in 1:1 stoichiometry.

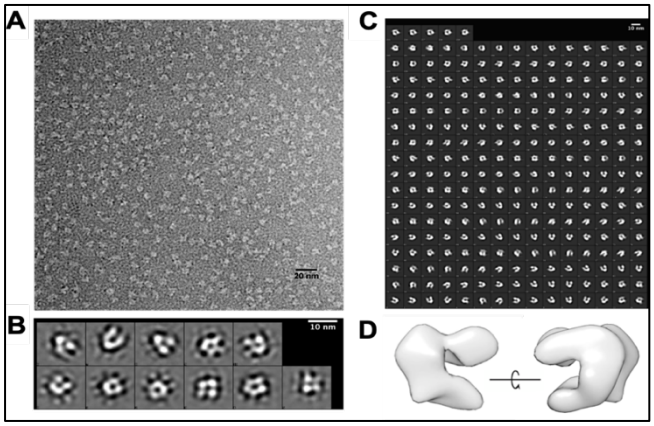

Figure 2: A) Representative EM micrograph of negatively stained DML3ΔN captured using JOEL 2100 transmission electron microscope. B) 2D class averages. C) 2D projections. D) Low resolution 3D EM model (shown in two different orientations) generated using EMAN2.

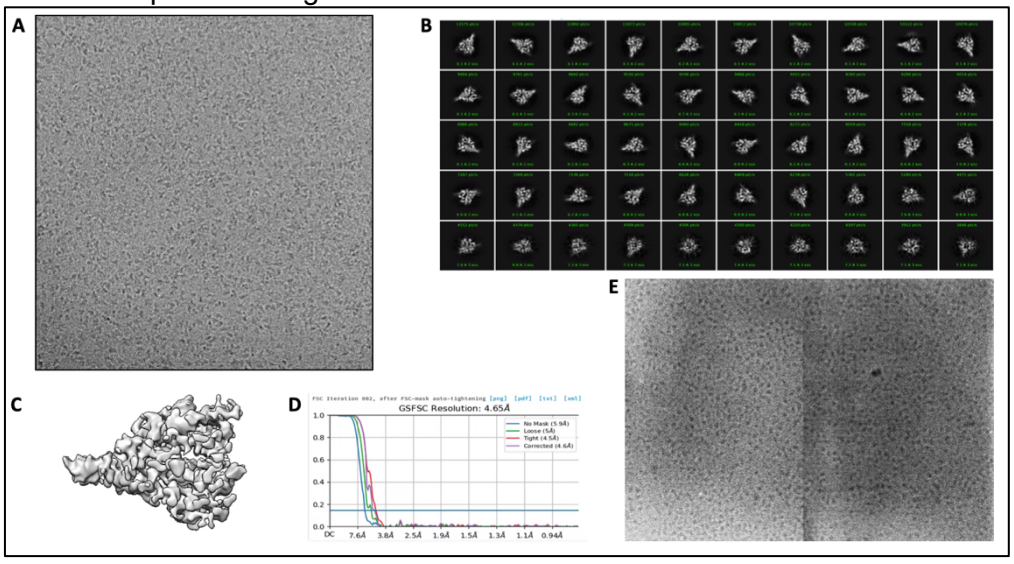

Figure 3: A) Representative cryo-EM micrographs of DML3ΔN captured using Krios. B) 2D class averages. C) cryo-EM 3D re construction of DML3ΔN alone. D) FSC curve showing the estimated resolution. E) Representative cryo-EM micrographs of DML3ΔN with DNA in the presence of detergent acquired on JOEL 2200 microscope.