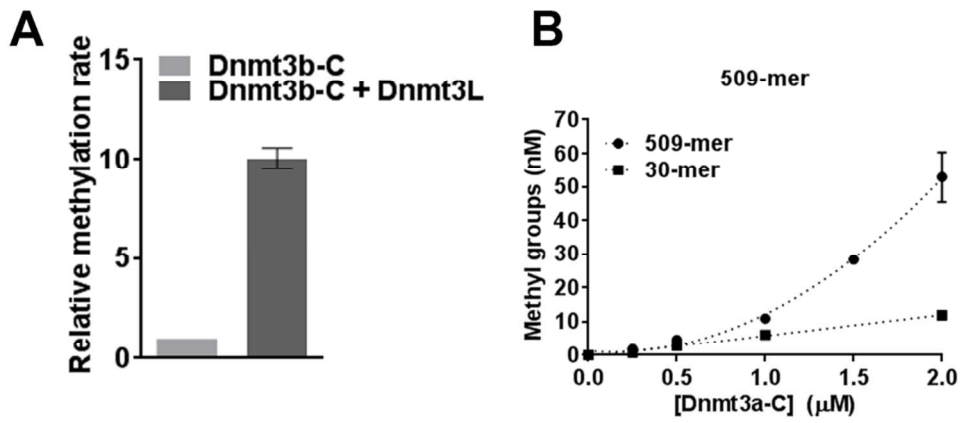
# Dnmt3b methylates DNA by a non-cooperative mechanism and its activity is unaffected by manipulations at the predicted dimer interface

**Allison B. Norvil[1], Christopher J. Petell[1], Lama Alabdi, Lanchen Wu, Sandra Rossie and Humaira Gowher**

Department of Biochemistry, Purdue University Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907

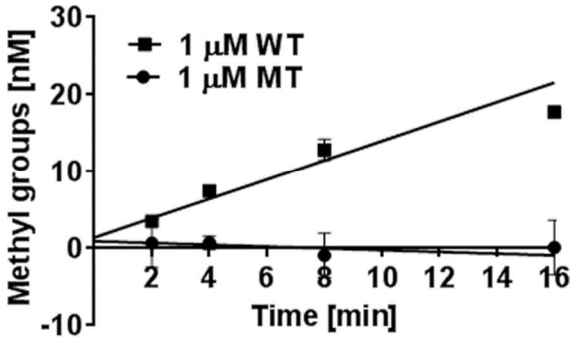
Running title: Dnmt3b catalytic mechanism is non-cooperative

Table of Contents



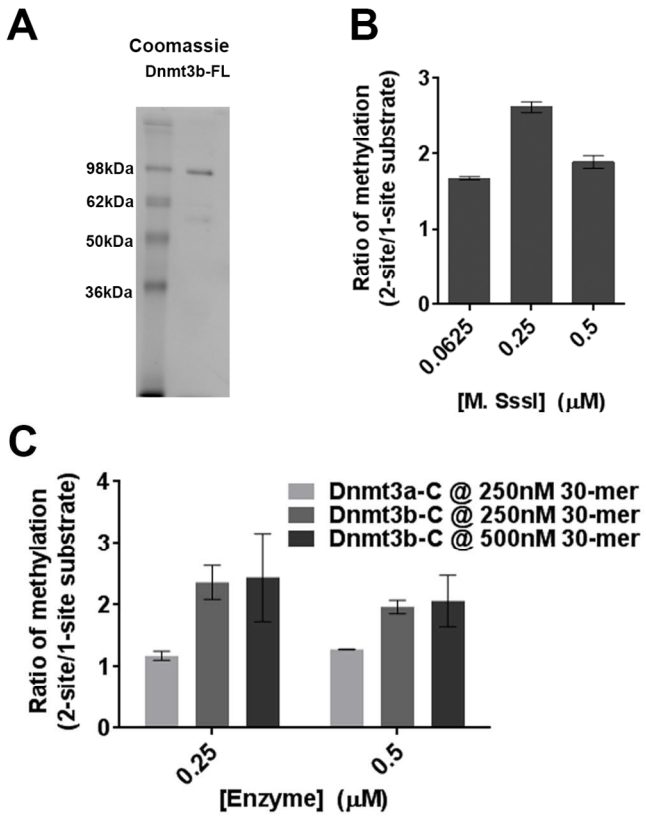

**SI FIGURE 1**

Dnmt3b-C is stimulated by Dnmt3L and Dnmt3a-C is cooperative. (A) Allosteric stimulation of Dnmt3b-C by Dnmt3L. DNA methylation activity of Dnmt3b-C (1 μM) was measured using a 30-mer DNA (500 nM) substrate in presence or absence of Dnmt3L (1 μM). Average +/- standard error of the mean (n = 3). (B) Cooperative mechanism of Dnmt3a-C. DNA methylation activity of Dnmt3a-C was measured at various enzyme concentrations using a short 30-mer and a long 509-mer DNA substrate. Data were fit to linear regression (30-mer) or second order polynomial (509-mer).



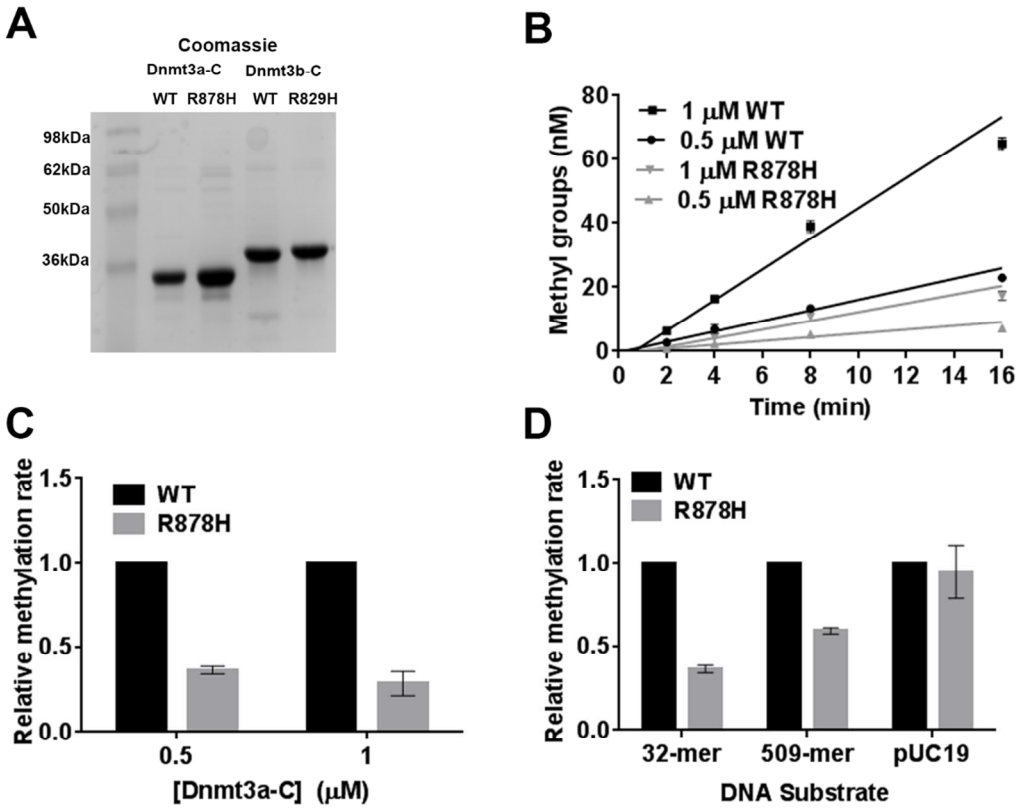

**SI FIGURE 2**

Dnmt3b-C E703A is catalytically inactive. Catalytic activity of WT Dnmt3b-C and Dnmt3b-C E703A. Methylation assays were performed as described in Materials and Methods using 250 nM of the 30-mer substrate and data were fit to linear regression. Average +/- standard error of the mean (n = 4).



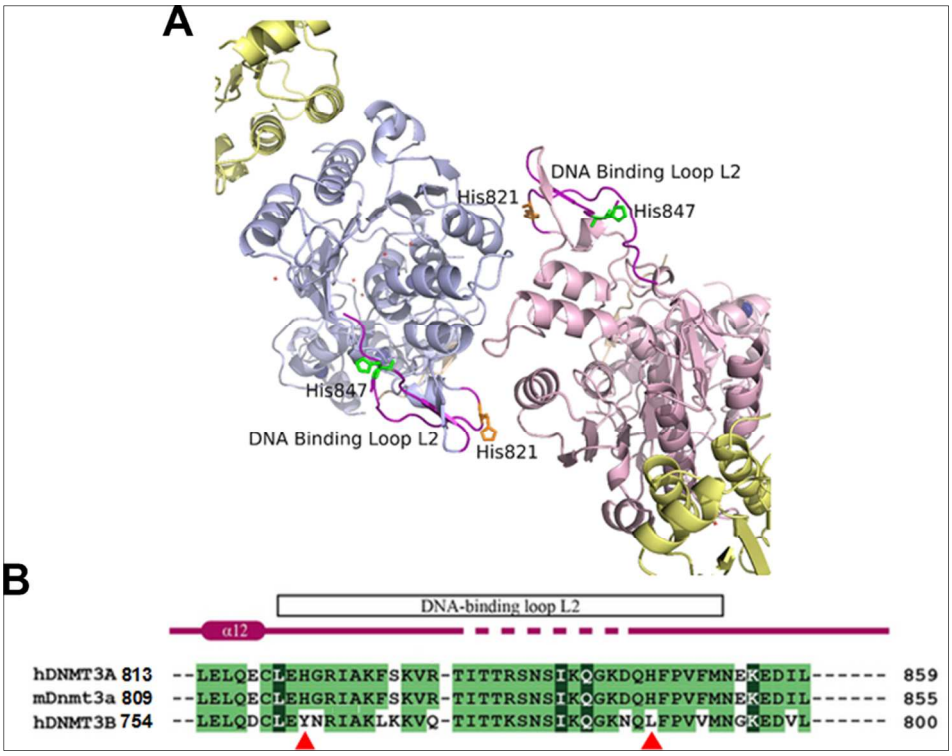

**SI FIGURE 3**

M. SssI and Dnmt3b-C display a processive mechanism. (A) WT: wild type; Coomassie stained SDS-PAGE gel showing purified His-tagged WT Dnmt3b-FL. (B) As a positive control for processivity, steady state DNA methylation levels were measured for 1 h at various concentrations of M. SssI using 1.5 μM 3H-labeled AdoMet (1:1 mixture of labeled and unlabeled) and either 1-site or 2-site substrates. For each enzyme concentration, the methylation of 2-site substrate was normalized to that of 1-site substrate to show the relative change in methylation level. (C) Steady state DNA methylation levels were measured after 500 nM of 1-site and 250 nM of 2-site substrate (equimolar CpG concentration) were incubated with Dnmt3b-C and 1.5 μM (0.75 μM of 3H-labeled and 0.75 μM of unlabeled) AdoMet. The methylation level for 2-site substrate was normalized to that of 1-site substrate to show the relative change. Average +/- standard error of the mean (n = 3).




**SI FIGURE 4**

Dnmt3a-C R878H loses activity and cooperativity. (A) WT: wild type; Coomassie stained SDS-PAGE gel showing purified His-tagged WT Dnmt3a-C, Dnmt3a-C R878H mutant, WT Dnmt3b-C, and Dnmt3b-C R829H mutant enzymes. B, Initial velocity of methylation was measured at early time points (2, 4, 8, and 16 min) to compare WT and R878H activity, in the presence of 0.5 or 1 μM of enzyme. The data were fit to linear regression which was weighted by 1/Y2. C, Methylation rate of the R878H mutant relative to WT enzyme at different enzyme concentrations. D, Methylation rates for the 0.5 μM of WT enzyme were set to 1 and the relative change in rate of methylation for the R878H mutant on different DNA substrates was plotted in the bar graph with normalized error. Average +/- standard deviation ($n \geq 2$).




**SI FIGURE 5**

Illustration of the histidines that are not conserved in Dnmt3b. (A) Dimer interface of the DNMT3A dimer in DNMT3A-DNMT3L heterotetramer based on the crystal structure (PDB, 2QRV). DNMT3A monomers are shown in purple and pink, while flanking DNMT3L is shown in yellow. Labeled histidines are located in the second DNA binding loop and are not conserved in DNMT3B. H821 is predicted to contribute to the pH sensitivity of DNMT3A (28). (B) Sequence alignment of Dnmt3a and Dnmt3b adapted from Guo. X, et al. 2015 (18). Red arrows mark Dnmt3a H821 and H847, showing these residues are not conserved in Dnmt3b.