Supporting Information

DNA Methylation Increases Nucleosome Compaction and Rigidity

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Methods

Nucleosome Reconstitution

X. laevis histone octamers were purified as described elsewhere [1,2]. *L. variegatus* 5S rDNA as described by (Siino et al., 2003) was generated by PCR using primers that contained biotin and Cy3 and a reverse primer containing Cy5 (Integrated DNA Technologies, Coraville IA). Primer sequences are Biotin-gga att cca acg aat(Cy3) aac ttc cag gga and Cy5-cat cca agt act aac cga gcc cta. His6-NAP1 was expressed and purified by nickel affinity as described in [3]. Mononucleosomes were assembled based on work by [4]. A series of reactions were set up to maximize nucleosome yield using varying amounts of 5S rDNA, histone octamers, and NAP1. Histone octamers and NAP1 were incubated at 30°C for 4h before adding 5S rDNA and incubating for an additional 4h at 30°C and then stored at 4°C before using. Reactions were analyzed by 5% native polyacrylamide gel electophoresis in 1X TBE.

Surface Immobilization

Quartz microscope slide is silanized and functionalized with polyethylene glycol (PEG, Laysan Bio, Arab AL). Some PEG molecules are biotinylated (Laysan Bio, Arab AL). 1/100 ratio of biotinylated/normal PEG was used to control the surface concentration of biotin. The surface was passivated by bovine serum albumine (BSA) before immobilizing nucleosomes on the surface through streptavidin-biotin conjugation.

SM FRET measurements

Single molecule fluroescence intensities were taken with a sensitive CCD camera (iXon+897, Andor Technology, Belfast UK) in a prism coupled total internal reflection (TIR) geometry based on a commercial microscope (TE2000, Nikon, Tokyo Japan) with modifications. The excitation is perpendicularly polarized along the TIR incidence. Imaging was done in a buffer with 10mM HEPES pH 7.6, 50mM NaCl, 5mM $MgCl_2$, 1mM DTT, 6% glycerol and 0.4% glucose. Glucose oxydase (0.08 U/uL, Sigma Aldrich) and Catalase (2.3 U/uL, Sigma Aldrich) mixture was used to elongate the dye photobleaching lifetime.

Methyltransferase treatment

M.SssI methyltransferase (New England Biolabs, Ipswich MA) was added (2.0 U/uL) to the immobilized nucleosomes. The reaction was supplemented with 0.16 mM S-adenosylmethionine (SAM). Other components of the reaction buffer is the same as described in the SM FRET measurements section. Incubation was done for 90 min followed by buffer exchange in the sample chamber.

Fluorescence Emission Polarization Measurements

A modified TIR based SM FRET imaging setup was used to measure the fluorescence polarization of the FRET acceptor in the nucleosome sample described in the above sections. Excitation and FRET donor imaging scheme is also the same as described above. The FRET acceptor emission is divided into two polarization components. The three spectrally separated emissions were focused on one CCD chip for simultaneous intensity recording.

Instrumental setup for the FRET acceptor emission polarization measurements

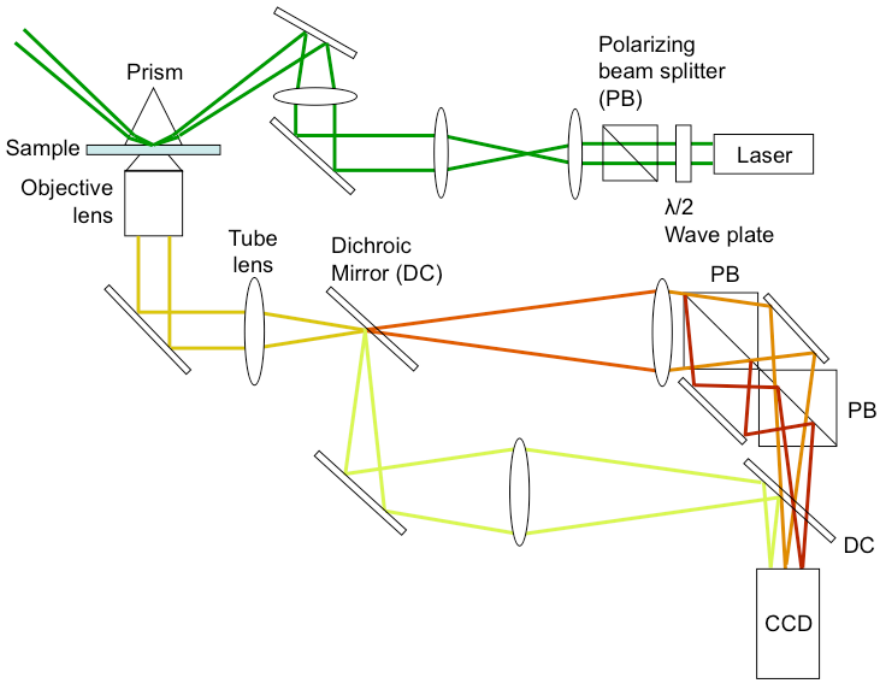

Fig. S1. Instrumental setup for the FRET acceptor emission aniotropy measurements. A microscope is constructed based on Nikon TE2000 (Tokyo, Japan). Choice of the microscope objective lens was a water immersion objective with 60x magnification (Plan Apo, Nikon, Tokyo, Japan). The single molecule fluorescence intensity time traces were recorded with an electron multiplying charge coupled device camera (EMCCD, iXon+ 897, Andor Technology, Belfast, UK). A total internal reflection geometry is employed for the excitation. 532nm Nd:YAG laser (Laser Quantum, Cheshire, UK) is used to excite

Cy3 or the FRET donor. The laser beam is parallel polarized to the optical axis. The fluorescence emission is divided into two spectral regions (550-645nm and 645-750nm). The 645-750nm emission is further divided into two polarization components. The EMCCD chip is divided into three different regions to separately image fluorescence from 550-645nm, 645-750nm parallel polarization component and 645-750nm perpendicular polarization component. FRET efficiency is calculated as $(I_{cy5,\parallel} + I_{cy5,\perp})/(I_{cy3} + I_{cy5,\parallel} + I_{cy5,\perp})$ and fluorescence polarization is calculated as $(I_{cy5,\parallel} - I_{cy5,\perp})/(I_{cy5,\parallel} + I_{cy5,\perp})$. Note that this figure and information has been submitted to another publisher as supplementary information of another manuscript[5].

Kinetic rates of the high FRET state upon DNA methylation

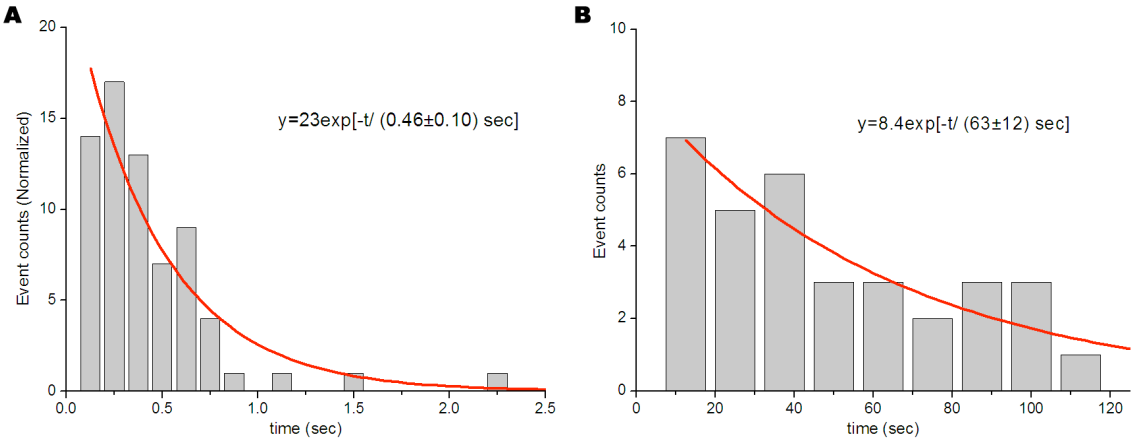

Fig. S2. Lifetime of the high FRET state and the rate of the high FRET state formation. (A) Histogram of the lifetime of the high FRET state from DNA methylated nucleosomes. (B) Histogram of the time interval between two high FRET states in DNA methylated nucleosome.

References

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