SUPPORTING INFORMATION

Effects of CpG methylation on the structure of nucleosomes

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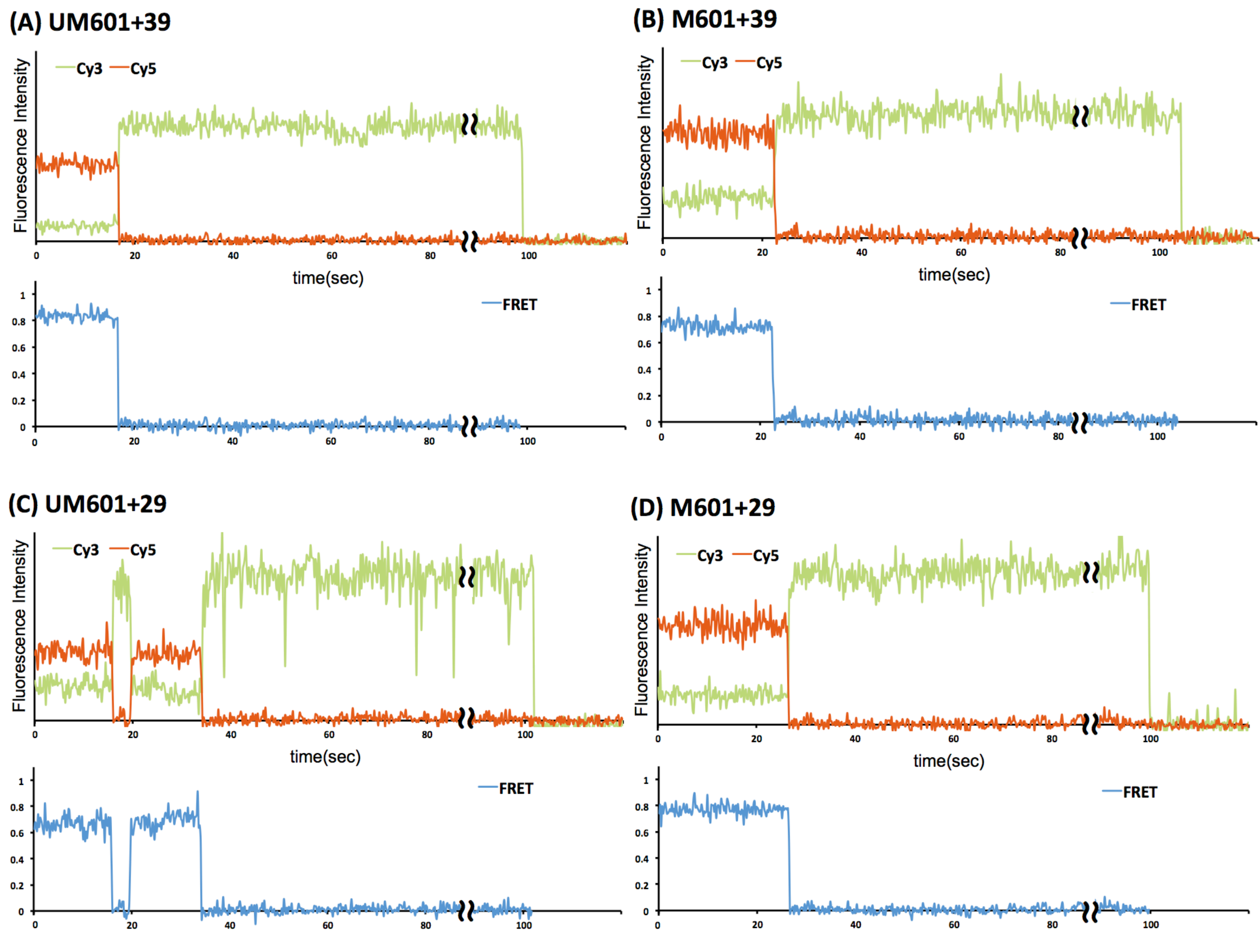
FIGURE S1. Sample FRET traces for (A) UM601+39, (B) M601+39, (C) UM601+29 and (D) M601+29 nucleosomes. Traces show mainly one stable FRET state. Trace (C) shows blinking of Cy5.



SUPPLEMENTARY METHODS

Preparation of 147 bp DNA constructs and reconstitution of nucleosomes using NAP1

A fluorescently labeled 147 bp double stranded DNA (dsDNA) construct with an additional 10 base linker oligonucleotide was generated by ligation with T4 DNA ligase as previously reported[1]. Briefly, we purchased several fragments of oligonucleotides some of which contain biotin, Cy3 or Cy5 (Integrated DNA Technologies, Coraville IA). The same set of

oligonucleotide fragments with methylated 5'-CpG-3' dinucleotides was also purchased from the same vendor. The oligonucleotides were annealed to dsDNA by heating and cooling down from 95 °C to 5 °C for 18 min followed by purification using a PCR purification kit (QIAGEN). The annealed dsDNA was treated with T4 DNA ligase (New England Biolabs) for 16 hrs at 16 °C. The ligated 147 bp DNA construct after purification with a PCR purification kit was used to assemble nucleosomes. His6-yeast Nap1 histone chaperone was expressed and purified with nickel-NTA affinity chromatography[2] and *X. laevis* histone octamer core particles were provided by Dr. Song Tan (Penn State University). Histone octamers and Nap1 were incubated at 30 °C for 4 hrs before nucleosome assembly reactions. After incubating the reaction mixture for additional 4 hrs at 30 °C, reactions were analyzed with 5% native polyacrylamide gel electrophoresis (PAGE) in 1x TBE buffer.

Fluorescence anisotropy measurements

Fluorescence anisotropy was measured on the reconstituted unmethylated or methylated 601+39 nucleosomes (~40 nM) in a buffer containing 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.5mM $MgCl_2$, 1mM DTT and 3% glycerol at excitation/emission 532nm/570nm for Cy3, 600/670 for Cy5, and 532/670 for Cy5 excited via FRET from Cy3 using a commercial fluorometer (Fluoromax4, HORIBA). The anisotropy $r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH})$ was calculated from the parallelly (I_{VV}) and perpendicularly (I_{VH}) polarized fluorescence intensities with the correction factor G for the setup.

Single molecule FRET measurements and analysis

The surface of a quartz microscope slide was silanized and subsequently coated with polyethylene glycol (PEG, Laysan Bio, Arab AL), which contains one biotinylated PEG (Laysan Bio, Arab AL) out of hundred. We passivated the surface with bovine serum albumine (BSA) before immobilizing DNA on the surface via biotin-streptavidin conjugation. To monitor the process of nucleosome assembly, a FRET pair (Cy3-Cy5) labeled unmethylated or methylated DNA was immobilized on the surface treated slide and hisone-Nap1 complexes were injected onto the slide. Histone octamers and Nap1 were incubated at 30 °C for 4 hrs in a buffer containing 10 mM Tris-HCl (pH 7.5), 400 mM NaCl, 0.5mM $MgCl_2$, 1mM DTT and 3% glycerol and diluted to a low salt condition (150 mM NaCl) before the injection. The fluorescence signals were recorded from the point of histone injection until most of the fluorophores in the field of view photobleaches. A protocatechuate dioxygenase (Sigma Aldrich) and protocatechuic aicd (Sigma Aldrich) mixture was used to elongate the fluorophore photobleaching lifetime and stabilize the emission.

Fluorescence signals from individual Cy3 and Cy5 fluorophores were imaged on an electron multiplying CCD camera (EMCCD, 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 customization. The excitation of the FRET donor Cy3 was achieved with a laser beam at 532 nm (Laser Quantum, UK) that is perpendicularly polarized along the TIR incidence. Fluorescence emission from FRET pairs (Cy3 and Cy5) were spectrally separated into two regions (550-645nm and 645-750nm) with a dichroic mirror and the two spectrally separated images of Cy3 and Cy5 were projected on a single EMCCD chip in order to simultaneously collect fluorescence signals from the two fluorophores in a time resolved manner.

A series of fluorescence images at a frame rate of 1/25ms was recorded until most of the fluorophores in the field view photobleaches.

Calculations of the angle between dipoles of a FRET pair (β)

Fluorescent labels in the DNA constructs are along the phosphate backbone, which is why we observe a very high level of anisotropy (0.33~0.34). Therefore, the depolarization of the fluorescence emission from the acceptor is mainly due to i) the tumbling of the nucleosome and ii) poor alignment between the dipoles of the FRET pair.

In order to deconvolve the depolarization due to the poor alignment between the dipoles from nucleosome tumbling in the measured anisotropy, we used the following formula.

$$r_{\mathrm{rot+dipole}} = \frac{\int_0^\infty I_0 e^{-Ek_D t'} e^{-\theta t'} \int_0^\infty r_{dipole} e^{-k_A t} e^{-\theta t} dt dt'}{\int_0^\infty I_0 e^{-Ek_D t'} \int_0^\infty e^{-k_A t} dt dt'}$$

$$\Rightarrow r_{\mathrm{rot+dipole}} = \frac{Ek_D}{Ek_D + \theta} \frac{k_A}{k_A + \theta} r_{dipole}$$

$$\Rightarrow r_{dipole} = r_{\mathrm{rot+dipole}} \frac{Ek_D + \theta}{Ek_D} \frac{k_A + \theta}{k_A}$$

$$r_{\mathrm{rot,A}} = \frac{\int_0^\infty I_0 e^{-k_A t} r_0 e^{-\theta t} dt}{\int_0^\infty I_0 e^{-k_A t} dt} \quad and \quad r_{\mathrm{rot,D}} = \frac{\int_0^\infty I_0 e^{-(1-E)k_D \phi t} r_0 e^{-\theta t} dt}{\int_0^\infty I_0 e^{-(1-E)k_D \phi t} dt}$$

$$r_{\mathrm{rot,A}} = \frac{k_A}{k_A + \theta} r_0 \quad and \quad r_{\mathrm{rot,D}} = \frac{(1-E)k_D \phi}{(1-E)k_D \phi + \theta} r_0$$

$$\Rightarrow \frac{k_A + \theta}{k_A} = \frac{r_0}{r_{rot,A}} \quad and \quad \frac{\theta}{Ek_D} = (\frac{1}{E} - 1)\phi(\frac{r_0}{r_{rot,D}} - 1)$$

$$\Rightarrow r_{dipole} = \frac{Ek_D + \theta}{Ek_D} \frac{k_A + \theta}{k_A} r_{\mathrm{rot+dipole}}$$

$$= (1 + (\frac{1}{E} - 1)\phi(\frac{0.4}{r_{rot,D}} - 1)) \frac{0.4}{r_{rot,A}} r_{\mathrm{rot+dipole}}$$

, where $r_{\mathrm{rot+dipole}}$ is the measured anisotropy of Cy5 emission via FRET (i.e. the 532/670 column in table 2), $r_{\mathrm{rot,D}}$ and $r_{\mathrm{rot,A}}$ are respectively the average emission anisotropy of Cy3 and Cy5 labeled at a nucleosome with the direct excitations (i.e. 532/570 and 600/670 columns in table 2), r_{dipole} is the anisotropy due to the skewed dipole between Cy3 emission and Cy5 excitation, θ is the rotational decay rate for the tumbling of a nucleosome, k_D is the excitation decay rate of the donor, φ is the fluorescence quantum efficiency of the donor (0.15 was used for the calculations), k_A is the fluorescence decay rate of the acceptor, E is the FRET efficiency and I_o is the initial intensity of the fluorescence emission decay. When the FRET efficiency and anisotropy are

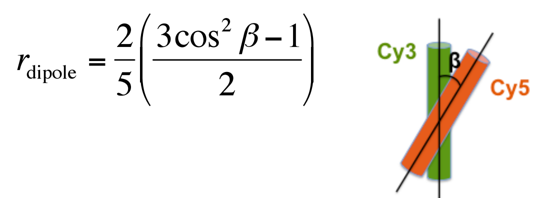
reasonably high and the quantum efficiency is low, this equation can be approximated as following[3].

$$r_{\text{dipole}} \approx \frac{0.4}{r_{rot,A}} r_{rot+dipole}$$

For instance, when the FRET efficiency is 0.7, $r_{\text{rot,D}}$ is 0.3, and the quantum efficiency is 0.15, the approximation yields only 2% error in the r_{dipole} value, which makes the approximated equation convenient to use under these conditions.

The fluorescence depolarization is due to the tumbling of the DNA construct and the angle between these two dipoles based on an assumption that the excitation and emission dipoles of these fluorophores are collinear which are strongly supported by reported anisotropy as high as 0.38 and 0.39 respectively for Cy3 and Cy5[4, 5]. Once we determine r_{dipole}, we calculated the angle between the dipoles of the FRET pair (β) based on the following relationship[6]. We used the unapproximated version for the r_{dipole} calculations.

$$r_{\text{dipole}} = \frac{2}{5}\left(\frac{3\cos^2\beta - 1}{2}\right)$$



SUPPLEMENTARY REFERENCES

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