Detecting DNA Methylation through Changes in Transverse Proton Relaxation

Hamsa Jaganathan,[a] Pengfei Wang[a], Jonathon Klein[b], Amy C. Lossie[b], and Albena Ivanisevic*[,ac]

Electronic Supplementary Information

Experimental Section:

1. Design of Nanoparticle – DNA System with Restriction Enzymes

In general, DNA was coated with (5 nm) iron oxide NPs at a weight ratio of 1:1 Fe_2O_3 NP:DNA. Gold NPs (~ 5 nm) coated with poly-L-lysine were purchased from Ted Pella Inc. Iron oxide NPs were synthesized and characterized by lab. The 1:1 weight ratio has studied to be an optimal ratio for double-stranded DNA and for enzyme reaction. Structures formed with ratios, such as 1:5 DNA: NP or greater, were studied by AFM and circular dichroism. These structures were unstable due to the mechanical loading of NPs on dsDNA and denaturation of DNA occurred.

1.1. Eco*RI and* Bam*HI Enzymes Experiments*

Unmethylated λ-DNA (Promega) was used to monitor the cutting activity of *Eco*RI and *Bam*HI enzymes. Both *Eco*RI and *Bam*HI (20 u) were added to separate samples of NP coated unmethylated λ-DNA (0.05 $\mu g \cdot \mu L^{-1}$). The reaction was performed at room temperature and relaxation times were obtained immediately at time points: 0, 0.5, 1, 2, 3, and 4 h after terminating the enzyme reaction at 65 C for 15 mins. Unmethylated λ-DNA was methylated using the CpG methyltransferase (20 u), *M.Sss*I (New England Biolabs). S-adenosylmethionine was added to the buffered solution of NP coated DNA strands and the enzyme reaction were performed for 1 hour at 37ºC. The reaction was terminated at 65ºC for 20 minutes. Similar restriction enzyme reactions were performed and relaxation times were measured by NMR for NP coated methylated DNA.

1.2. Dpn*I Enzyme Experiments:*

Methylated (48,502bp) and unmethylated (48,502bp) λ-DNA, purchased from Promega, was used for *Dpn*I experiments, which is specific to *Dam* methylation. A total concentration of both types of DNA was 10 $ng \cdot \mu L^{-1}$ to coat Fe_2O_3 NP. *Dpn*I (10 u) from Promega was added to each sample, incubated at 37 C for 0.5, 1, 2, 3, 4 h and terminated at 65 C for 15 mins. The samples were then immediately measured for changes in relaxation times.

1.3. Hpa*II Enzyme Experiments:*

Human DKO methylated and unmethylated DNA (Zymo Research Corp. Irvine, CA) was used for *Hpa*II experiments. After coating NPs at a 1:1 DNA:NP ratio to DNA concentration of 1 $ng.\mu L^{-1}$, *Hpa*II (10 u) was added to each sample, incubated at 37 C for 0.5 h and terminated at 65 C for 15 mins.

1.3.1 Cultured DNA Isolation:

Clonal cultures of BACs were grown overnight in 500mL LB+12.5 chloramphenicol at 37°C. DNA was isolated from the cultures using the Nucleobond BAC 100 kit (Clontech, Mountain View, CA, USA) following the manufacturer's protocol. The resulting pellet was resuspended in 500uL of 1X TE buffer.

250uL of each BAC was methylated using *M.Sss*I methylase. Samples were methylated following NEB's large-scale methylation protocol. DNA was then cleaned and concentrated from the reaction mixture using a Zymo DNA Clean and Concentrator 5 kit (Zymo Research, Irvine, CA, USA). Samples were stored overnight at 4°C. Each of the four samples was then split and digested by either *Hpa*II or *Msp*I. This resulted in eight samples total:

1. BAC 130 unmethylated *Hpa*II (130-U-*Hpa*II)
2. BAC 130 unmethylated *Msp*I
3. BAC 130 methylated *Hpa*II
4. BAC 130 methylated *Msp*I
5. BAC 330 unmethylated *Hpa*II
6. BAC 330 unmethylated *Msp*I
7. BAC 330 methylated *Hpa*II
8. BAC 330 methylated *Msp*I

The samples were then coated with iron oxide NPs and the relaxation times were measured by NMR.

2. Gel Electrophoresis Experiments

Gel electrophoresis experiments were performed on 0.5% agarose gels in 1×Tris Acetate EDTA (TAE) electrophoresis buffer. Gels were pre-stained with ethidium bromide before running. DNA extension of 1 kb ladder (Invitrogen) with a volume of 1 µg was loaded as the marker. Samples containing loading buffer (20 µL) were loaded after the DNA marker. The experiments with *Dpn*I on NP coated λ-DNA and *Hpa*II on NP coated human DNA were run for 3 h with a constant voltage set at 75 V.

Gel electrophoresis experiments on cultured DNA were also performed. All eight samples were run out on a 1% agarose gels in 1X TAE buffer. 2uL of Orange G was mixed with 10uL of DNA (undigested DNA is in TE buffer, digested samples are in digestion mixtures). The gel was run at 35V for 2 hours in a 10cm gel box. 5uL of Hyperladder I (Bioline) was used as a marker.

3. Proton Relaxation Time Experiments

All samples were degassed by nitrogen flow for 1 minute to eliminate paramagnetic oxygen. Bruker Avance DPX300 (300 MHz) was used to measure the transverse T_2 relaxation times for each sample, using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. A total of eight scans per spectrum were measured with eight delay times to acquire T_2 times. All measurements were run under room temperature without sample spinning with a sample size of 3. STATA 10.0 (StataCorp LP, College Station, TX) was used to perform one-way analysis of variance with a sample size of 3 at a confidence interval of 95%.

Results:

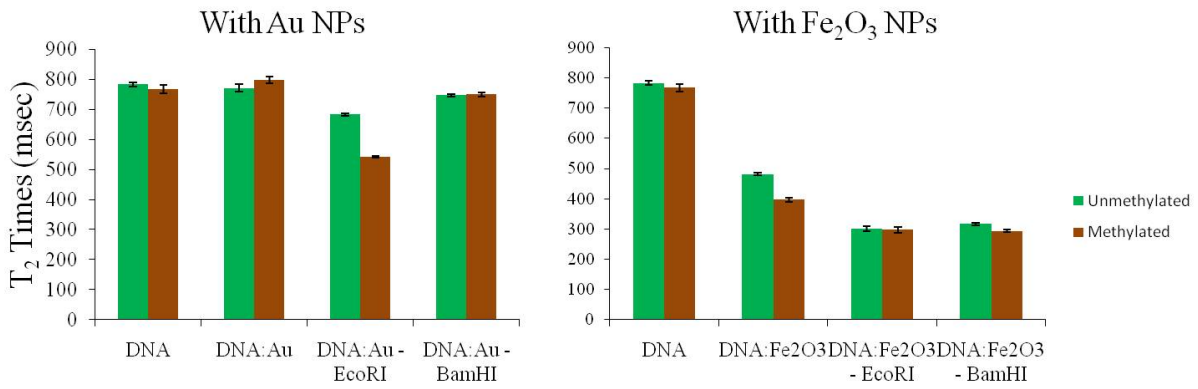


Figure S1. Plot of the transverse relaxation time (T_2) changes comparing between unmethylated and methylated λ-DNA for gold and iron oxide NPs cut by enzymes, EcoRI and BamHI, measured at room temperature. Error bars represent mean ± standard deviation for p-value = 0.05 and n = 3.

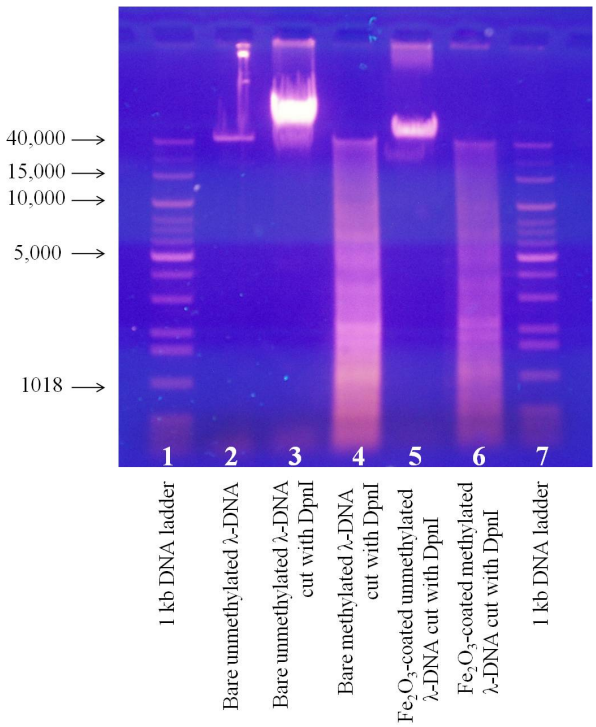


Figure S2. Image of gel verifying the cutting by DpnI enzyme on iron oxide NP-coated, methylated DNA strands.

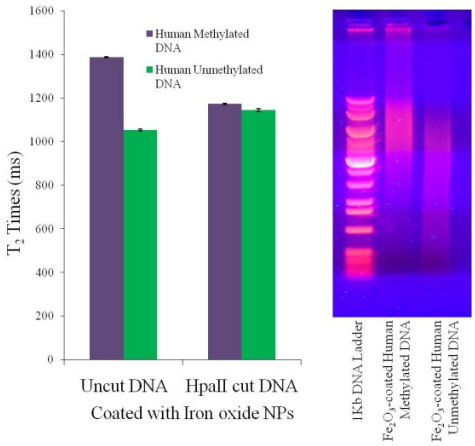


Figure S3. (Left) Plot of the transverse relaxation time (T_2) changes comparing between unmethylated and methylated human DNA for gold and iron oxide NPs cut by HpaII, measured at room temperature. Error bars represent mean ± standard deviation for p-value = 0.05 and n = 3. (R) Image of gel verifying the cutting by HpaII enzyme on iron oxide NP-coated, unmethylated and methylated Human DNA strands.

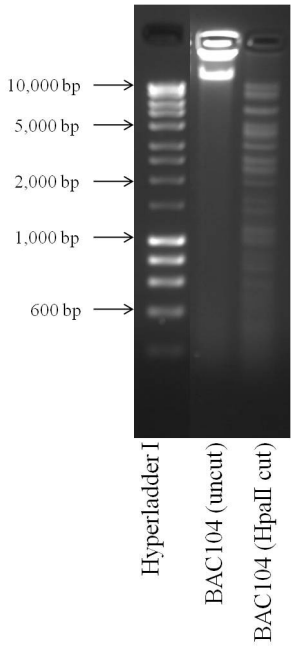


Figure S4. Image of gel verifying the cutting by HpaII on bare cultured DNA.

Table S1. Description of the different BACs tested.

	RP23-104G9	**RP24-130G7**	**RP23-330J7**
Short Name	BAC104	BAC130	BAC330
Chromosome	10	10	10
Location	86,832,172-87,061,791	86,850,974-87,025,024	86-757,984-86,970,365
Length (in bp)	229,620	174,051	212,382
G-C content (%)	42.31	42.66	41.79
CpG prevalence (%)	0.90	0.98	0.89

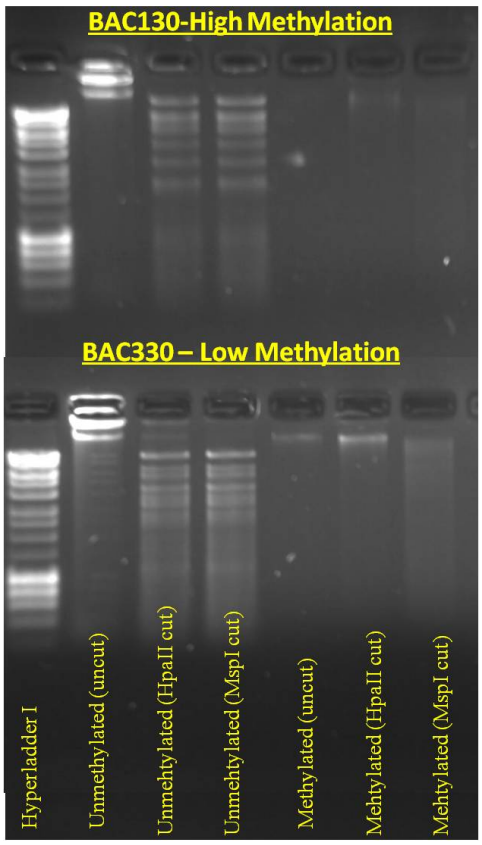


Figure S5. Image of gel verifying the cutting by HpaII and MspI enzymes on bare cultured DNA for high and low levels of methylation and CpG % content.

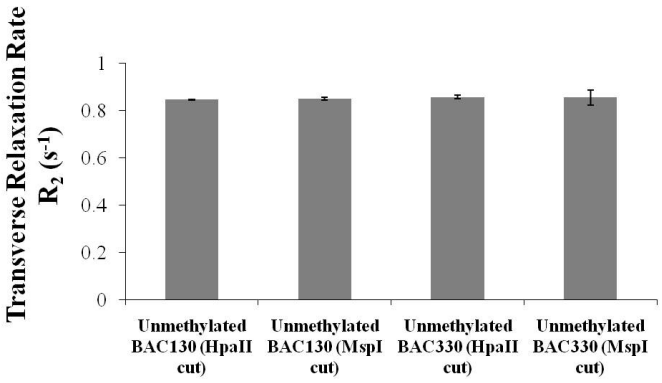
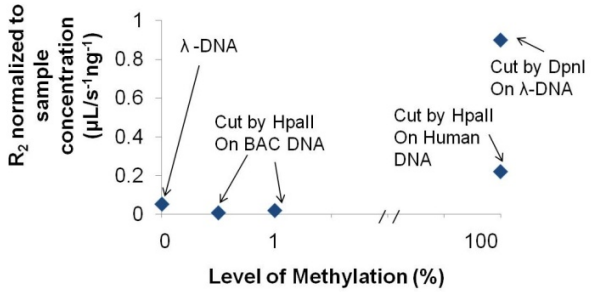


Figure S6. Plot of the transverse relaxation rate for iron oxide NP coated DNA cut by HpaII or MspI, comparing the influence of %CpG content.



Restriction Enzyme	Sensitivity to CpG or *dam* Methylation
EcoRI	Blocked by CpG overlapping
BamHI	0 %
DpnI	Cleaves *dam* methylation
HpaII	Completely blocked (~100%)
MspI	0 %

Figure S7. An estimated plot of the transverse relaxation rate normalized to sample concentration as a function DNA methylation level. Table displays general methylation sensitivity for restriction enzymes used in this study.