Supporting Online Information for

Hydrophobicity of Methylated DNA as a Mechanism for Gene Silencing

Parminder Kaur[1,2], Birgit Plochberger[5], Peter Costa[2], Stephanie M. Cope[2,3], Sara M. Vaiana[2,3] and Stuart Lindsay[1,2,3,4]

[1]Biodesign Institute, [2]Department of Physics and [3]Center for Biological Physics, [4]Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287, USA, [5]Institute of Applied Physics, Vienna University of Technology, Wiedner Hauptstraße 8-10/E134 A 1040 Wien, Austria.

1. Sequence of the DNA Template

The hTERT promoter is shown in black, the 601 positioning sequence in red and the 30 bp linker in green.

gcactcgggccaccagctccttcaggcaggacacctgcgggggaagcgccctgagtcgcctgcgctgctctccgcatgtc
gctggttccccccggccgccctcaaccccagccggacgccgaccccggggaggcccacctggcggaaggaggggcgg
cgggggcggccgtgcgtcccagggcacgcacaccaggcactgggccaccagcgcgcggaaagccgccgggtccccg
cgctgcaccagccgccagccctggggccccaggcgccgcacgaacgtggccagcggcagcacctcgcggtagtggctg
cgcagcagggagcgcacggctcggcagcggggagcgcgcggcatcgcgggggtggccggggccagggcttcccacgt
gcgcagcaggacgcagcgctgcctgaaactcgcgccgcgaggagagggcggggccgcggaaaggaaggggaggggc
tgggagggcccggaggggctgggccggggacccgggaggggtcgggacggggcggggtccgcgcggaggaggcgg
agctggaaggtgaaggggcaggacgggtgcccgggtccccagtccctccgccacgtgggaagcgcggtcctgggcgtct
gtgcccgcgaatccactgggagcccggcctggccccgacagcgcagctgctccgggcggacccgggggtctgggccgc
gcttccccgcccgcgcgccgctcgcgctcccagggtgcagggacgccagcgagggccccagcggagagaggtcgaatc
ggcctaggctgtggggtaacccgagggaggggccatgatgtggaggccctgggaacaggtgcgtgcggcgacccctttgg
ccgctggcctgatccggagacccagggctgcctccaggtccggacgcggggcgtcgggctccgggcaccacgaatgccg
gacgtgaaggggaggacggaggcgcgtagacgcggctggggacgaacccgaggacgcattgctccctggacgggcac
gcgggacctcccggagtgcctccctgcaacacttccccgcgacttgggctccttgacacaggcccgtcatttctctttgcag
gttctcaggcggcgaggggtccccaccatgagcaaaccaccccaaatctgttaatcacccaccggggcggtcccgtcga
gaaagggtgggaaatggagccaggcgctcctgctggccgcgcaccgggcgcctcacaccagccacaacggccttgacc
ctgggccccggcactctgtctggcagatgaggccaacatctggtcacatcccgcccgcacagggtggagggcaacctcg
gggtccaggcacctggctccaagcctcgtggatccgcttgatcgaacgtacgcgctgtcccccgcgttttaaacgccaagg
ggattactccctagtctccaggcacgtgtcagatatatacatcctgtcggaccgagctcctcgggatgcatcccgccctgg
agaatcttggtgccgaagccgctcaattggtcgtagcaagctctaccaccgcttaaacgcacgtaagggctgtcccccgc
gttttaaccgccaagaggattactccccagtctccaggcacgcgtcagatatatacatcctgtgcatgtattgaactcggga

tgcatcccgcccctggagaatcttggtgccgaagccgctcaattggtcgtagcaagctctaccaccgcttaaacgcacgtaa
gggctgtcccccgcgttttaaccgccaagaggattactccccagtctccaggcacgcgtcagatatatacatcctgtgcat
gtattgaactcgggatgcatcccgcccctggagaatcttggtgccgaagccgctcaattggtcgtagcaagctctaccaccg
cttaaacgcacgtaagggctgtcccccgcgttttaaccgccaagaggattactccccagtctccaggcacgcgtcagatat
atacatcctgtgcatgtattgaactcgggatgcatcccgcccctggagaatcttggtgccgaagccgctcaattggtcgtagc
aagctctaccaccgcttaaacgcacgtaagggctgtcccccgcgttttaaccgccaagaggattactccccagtctccagg
cacgcgtcagatatatacatcctgtgcatgtattgaactcgggatgcatcccgcccctggagaatcttggtgccgaagccgc
tcaattggtcgtagcaagctctaccaccgcttaaacgcacgtaagggctgtcccccgcgttttaaccgccaagaggattac
tccccagtctccaggcacgcgtcagatatatacatcctgtgcatgtattgaactcgggatgcatcccgcccctggagaatctt
ggtgccgaagccgctcaattggtcgtagcaagctctaccaccgcttaaacgcacgtaagggctgtcccccgcgttttaacc
gccaagaggattactccccagtctccaggcacgcgtcagatatatacatcctgtgcatgtattgaactcgggatgcatccc
gcccctggagaatcttggtgccgaagccgctcaattggtcgtagcaagctctaccaccgcttaaacgcacgtaagggctgtc
ccccgcgttttaaccgccaagaggattactccccagtctccaggcacgcgtcagatatatacatcctgtgcatgtattgaac
tcgggatgcatcccgcccctggagaatcttggtgccgaagccgctcaattggtcgtagcaagctctaccaccgcttaaacgc
acgtaagggctgtcccccgcgttttaaccgccaagaggattactccccagtctccaggcacgcgtcagatatatacatcct
gtgcatgtattgaactcgggagatctgcatgca

2. Isolation of the DNA template

E.Coli were transformed to express the required htert-601 strain in their genetic material .The transformed bacteria were cultured in LB (lysogeny) broth on a large scale to yield the plasmid. Amplicillin antibiotic was added to the LB broth while culturing to prevent the growth of other strains of the bacteria. Plasmid (6991 bp) was purified out of the bacterial pellet using Qiagen Giga prep kit and was further subjected to double digestion by the restriction enzymes BamH1 and Ecor1, releasing out 2905 bp of the plasmid DNA containing htert- 601 sequences. For the methylation studies the purified plasmid was treated with CpG Methyltransferase (M.SssI) and S-Adenosine methionine (SAM) as a cofactor for the reaction. The methylation reaction was carried out overnight and the products were further purified to give methylated DNA. Changing the amount of SAM produced various levels of methylated DNA. Incubating the mDNA and the normal DNA with the

restriction enzyme Ava1 for 1 hr tested methylation. DNA were purified by running them through gel electrophoresis and further purifying and extracting DNA out of these gels and was further dissolved in 15mM NaCl and 1mM phosphate solution. The purified fractions of the DNA were imaged by AFM.

3. Quantification of the degree of methylation

A Matlab program (listed below) was written to randomly cut some fraction of the total Ava1 sites The lengths corresponding to each cut are loaded into the data array AvaCutSites. A fraction (MaxSites-MaxProt) of these lengths were selected randomly using the function Randperm, and the process repeated 40,000 times. In the example below, the number of protected sites (MaxProt) is set to zero. The final distribution of lengths was multiplied by length to reflect the fact that the intensity of the gel band will be proportional to the length of each frgmant as well as the number of fragments.

```
MaxSites=17;
MaxProt=0;
Iterations=40000;%stable statistics need 40,000
nbins=40;
B=[];
y=[];
n=[];
Cumulative=[];
for k=1:Iterations
    AvaCutSites2=AvaCutSites;
    A=randperm(MaxSites);
    for p=1:MaxProt
        B(p)=A(p)+1;
    end
    AvaCutSites2(B)=[];
    for q=1:(MaxSites-MaxProt+1)
        y(q)=AvaCutSites2(q+1)-AvaCutSites2(q);
```

```
    end
    Cumulative=[Cumulative y];
end
[n,xout]=hist(Cumulative,nbins);%Histogrammed Y.X data
n2=n.*xout; %Gel intensity is proportional to length
%bar(xout,n)
bar(xout,n2)
OutPut=[xout' n' n2'];
csvwrite('0_sites.csv',OutPut);
```

An example of the outputs for 4 values of MaxProt is shown in Figure S1 below, together with the distributions matched on Figure 1B

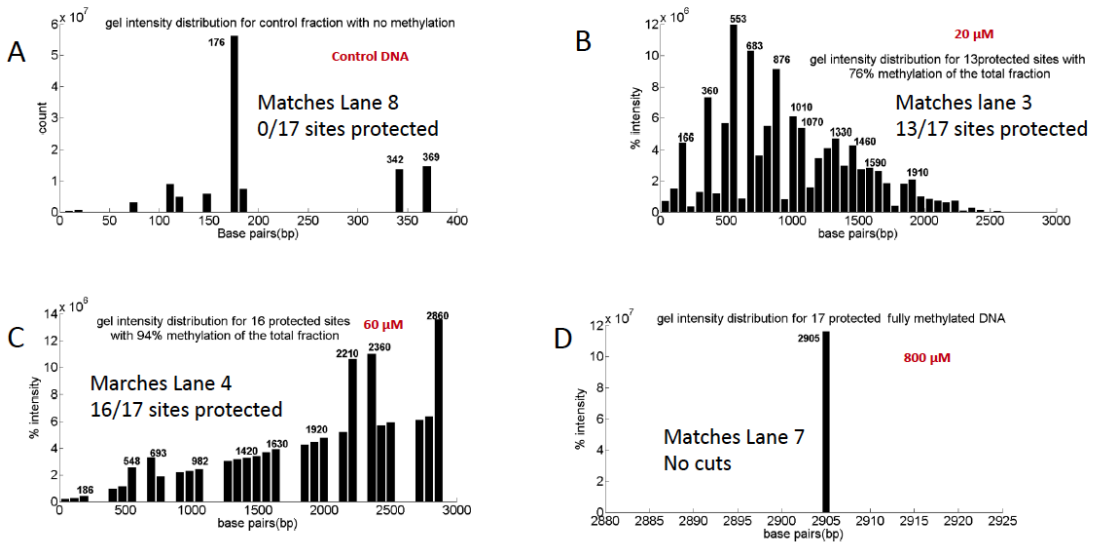

Figure S1: Simulated gel intensity distributions for no methylation (A) and full methylation (D). Intermediate amounts of methylation produce patterns that change rapidly with the fraction of protected sites (B and C). The corresponding lanes on Figure 1B are marked.

4. Volumes of methylated and control chromatin arrays

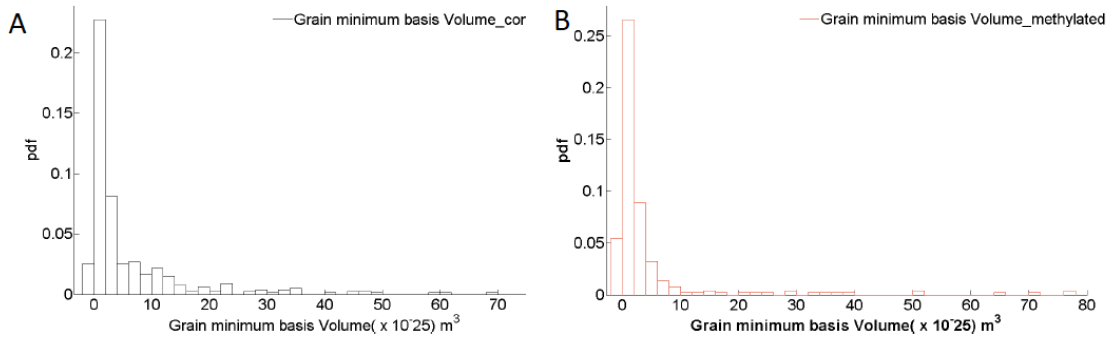

Figure S2: Volume distributions for the chromatin reconstituted on the control DNA (A) and the meDNA (B). Volumes are very nearly identically distributed, despite large differences in the area occupied by the images of the chromatin arrays (Figure 2 in the main text). Thus the amount of material (DNA, histones) in each preparation is similar.

5. Persistence lengths from AFM images

Image analysis

Image analysis was performed as previously described [1]. Briefly, an in-house program code implemented in Matlab was used to trace automatically and with human supervision DNA molecules. Thereby, the user determines the initial point and trial tangent direction. The trail tangent points are separated from each other by 2.5 nm. Each of these points is interpolated in height along a segment and weighted centered on the trail point normal to the trial tangent. This process is repeated until the end of the chain is reached by representing DNA contours as chains of xy pairs separated by 2.5 nm.

Data analysis

For persistence length determination we plotted the mean angle of pairs of points located at contour length s and s+L, averaging over s and over all observed DNA molecules. The persistence length ξ was determined by fitting equation (1) in the main text.

We subsequently measured the mean-square separation $\langle R(s), R(s+L) \rangle$ of pairs of points located at contour length *s* and *s+L*. To prove that the DNA molecule was in thermal equilibrium we compared this experimentally determined property by visual inspection with a previously found theoretical expression derived by Wiggins and Nelson [2] using the above calculated persistence length ξ:

$$\left\langle \left(R_{s,s+L}\right)^2 \right\rangle = 4\xi\left(L + 2\xi\left(\exp\left(\frac{-L}{2\xi}\right) - 1 \right)\right).$$

This formula was also used by Rivetti et al. who considered only the particular case of a worm-like chain.

[1] Paul A. Wiggins et.al; Nature Nanotechnology; Vol 1; 137-141; 2006

[2] Paul A. Wiggins and P.C. Nelson et.al; Phys.Rev., E73, 031906; 2006

[3] Rivetti et.al; J.Mol.Biol.; 264, 919-932; 1996