EXTENDED EXPERIMENTAL PROCEDURES

DNA sequences: Oligonucleotides (synthesized by IDT) for PCR amplification of templates. The name of the oligonucleotide corresponds to the labeling strand and position. Biosg and ibio refer to biotin, idSp refers to abasic site, iAmMC6T refers to amino-modified C6 dT linker for labeling with Cy3 or Cy5, 5Cy5 and 5Cy3 refer to end-labeled positions generated through phosporamidite chemistry. Other abbreviations include: LH (left half), RH (right half), MF (middle fragment) RRH (right right handle), LL(left left), RTA (right TA (dinucleotide), A (for immobilization scheme A), B (for immobilization scheme B). The primer sequences are color-coded according to the sections of the 601 template (see below): extra-chromosomal handles (black); left outer quarter (magenta); left inner quarter (orange); right inner quarter (green); right outer quarter (blue).

(1) I77 5'- /5Biosg/TATA CGCGG CCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTG/iAmMC6T/CCC

(2) J-12 5'- GG GCGGCGACCT /idSp/GGTCGCTG/iAmMC6T/T CAATACATGC ACAGGAT GTATATATC

(3) I68 5'-/5Biosg/TATA CGCGG CCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACG/iAmMC6T/AC G

(4) J-1 5'- GG GCGGCGACCT /idSp/GGTCGCTGTT CAATACATG/iAmMC6T/ ACAGGAT GTATATA

(5) J7 5'- GG GCGGCGACCT /idSp/GGTCGCTGTT CAATACATGC ACAGGA/iAmMC6T/ GTATATA

(6) I57 5'-/5Biosg/TATA CGCGG CCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGTAG ACAGCTCTAG CACCGC/iAmMC6T/TAA

(7) J15 5'- GG GCGGCGACCT /idSp/GGTCGCTGTT CAATACATGC ACAGGAT GTATATA/iAmMC6T/CT G

(8) I46 5'-/5Biosg/TATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGTAG ACAGC/iAmMC6T/CTA

(9) J24 5'- GG GCGGCGACCT /idSp/GGTCGCTGTT CAATACATGC ACAGGAT GTATATATCT GACACG/iAmMC6T/GCC TGGA

(10) I38 5'-/5Biosg/TATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCG/iAmMC6T/AG A

(11) J28 5'-GG GCGGCGACCT /idSp/GGTCGCTGTT CAATACATGC ACAGGAT GTATATATCT GACACGTGCC /iAmMC6T/GGA

(12) I27 5' GG GCGGCGACCT /idSp/GGA CCCTATA CGCGG CCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCG/iAmMC6T/AG A

(13) J45 5'- /5Biosg/TGTT CAATACATGC ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG GGAGTAA/iAmMC6T/CC C

(14) I15 5'- GG GCGGCGACCT /idSp/ GGA CCC TATA CGCGG CCGCC CTGGAGAATC CCGG/iAmMC6T/GCC

(15) J57 5'- /5Biosg/TGTT CAATACATGC ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG GGAGTAATCC CCTTGGCGG/iAmMC6T/ TAA

(16) I9-A 5'-/5Biosg/TATA CGCGG CCGCC CTGGAGAA/iAmMC6T/C CC

(17) J58-A 5'- GG GCGGCGACCT /idSp/TGTT CAATACATGC ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG GGAGTAATCC CCTTGGCGGT /iAmMC6T/AAA

(18) I9   5'- GG GCGGCGACCT /idSp/ TATA CGCGGCCGCC CTGGAGAA/iAmMC6T/C CC

(19) J58   5'- /5Biosg/TGTT CAATACATGC ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG GGAGTAATCC CCTTGGCGGT /iAmMC6T/AAA

(20) I-1   5'-/5Biosg/TATA CGCGG CCGC/iAmMC6T/ CTGGAGAA/iAmMC6T/C CCGGT

(21) J79   5'- GG GCGGCGACCT /idSp/GGTCGCTGTT CAATACATGC ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG GGAGTAATCC CCTTGGCGGT TAAAACGCGG GGGACAGCGC G/iAmMC6T/AC G

(22) I-12   5'-/5Biosg/ GGACCCTA/iAmMC6T/A CGCGGCCGCC CTGGAGAA/iAmMC6T/C CCGGT

(23) I58-MF   5'- /5Biosg/TATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGGGA GTAATCCCCT TGGCGGT/iAmMC6T/AA A

(24) J58-MF   5'- /5Biosg/TGTT CAATACATGC ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG TAGACAGCTC TAGCACCGCT /iAmMC6T/AAA

(25) J58-RTA   5'- /5Biosg/TGTT CAATACATGC ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG TAAGTAATCC TATTGGCGGT /iAmMC6T/AAA

(26) I9-RRH-1-10   5'- GG GCGGCGACCT /idSp/ TATA CAATACATGC CTGGAGAA/iAmMC6T/C CC

(27) J58-LL8-24   5'- /5Biosg/TGTT CAATACATGC ACAGGAT ATCCCGGTGC CGAGGCCGCC TGGAGACTAG GGAGTAATCC CCTTGGCGGT /iAmMC6T/AAA

(28) J7-LL8-24   5'- GG GCGGCGACCT /idSp/GGTCGCTGTT CAATACATGC ACAGGA/iAmMC6T/ ATCCCG

(29) DIG oligo   5′-AGGTCGCCGCCCT TT/digoxigenin/

(30) 601-LH top   /5Cy3/CAGAATCCGT CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCG

(31) 601-LH bottom   /5Cy5/ACGGATTCTG CGC GTACGTGCGT /iBiodT/TAAGCGGTG CTAGAGCTGT CTACGACCAA TTGAGCGGCC TCGGCACCGG GATTCTCCAG

(32) 601-RH top   /5Cy5/ACGGATTCTG TGTCCC CCGCGTT/iBiodT/TA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC AGATATATAC ATCCTGT

(33) 601-RH bottom   /5Cy3/CAGAATCCGT ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG GGAGTAATCC CCTTGGCGGT TAAAACGCGG GGGACA

(34) 601MF-LH top   /5Cy3/CAGAATCCGT CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGGGA GTAATCCCCT TGGCGGTTAA AACGCGGGGG ACA

(35) 601MF-LH bottom   /5Cy5/ACGGATTCTG TGT CCCCCGCGTT /iBiodT/TAACCGCCA AGGGGATTAC TCCCGACCAA TTGAGCGGCC TCGGCACCGG GATTCTCCAG

(36) 601MF-RH top   /5Cy5/ACGGATTCTG CGCGTA CGTGCGT/iBiodT/TA AGCGGTGCTA GAGCTGTCTA CTAGTCTCCA GGCACGTGTC AGATATATAC ATCCTGT

(37) 601MF-RH bottom   /5Cy3/CAGAATCCGT ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG TAGACAGCTC TAGCACCGCT TAAACGCACG TACGCG

(38) 601RTA-RH top	/5Cy5/ACGGATTCTG TGTCTA CCGCGTT/iBiodT/TA ACCGCCAATA GGATTACTTA CTAGTCTCCA GGCACGTGTC AGATATATAC ATCCTGT	
(39) 601RTA-RH bottom	/5Cy3/CAGAATCCGT ACAGGAT GTATATATCT GACACGTGCC TGGAGACTAG TAAGTAATCC TATTGGCGGT TAAAACGCGG TAGACA	
(40) LL8-24-RH top	/5Cy5/ACGGATTCTG TGTCCC CCGCGTT/iBiodT/TA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCGGCCTCG GCACCGGGAT ATCCTGT	
(41) LL8-24-RH bottom	/5Cy3/CAGAATCCGT ACAGGAT ATCCCGGTGC CGAGGCCGCC TGGAGACTAG GGAGTAATCC CCTTGGCGGT TAAAACGCGG GGGACA	

DNA templates and labeling schemes:

A: The templates of the 601 sequence and derivatives used in this investigation. The 601 template is Addgene Plasmid 26656: pGEM-3z/601 plasmid. The top and bottom strands of the 601 sequence are denoted by I and J strand, respectively (pdb file: 3MVD). The 'left' and 'right' sides in our templates correspond to the 5' and 3' ends of the I strand shown in the table. The templates 601 RRH1-10 and 601 LL8-24 were synthesized by PCR, while 601MF and 601RTA were synthesized by IDT. The 601 sequence is color coded: extra-chromosomal handles (black); left outer quarter (magenta); left inner quarter (orange); right inner quarter (green); right outer quarter (blue). The parts of the derivative sequences which are varied in comparison to the original 601 sequence are underlined and changed to the corresponding color portion of the 601 sequence.

B: Labeling schemes and names for all reconstituted nucleosomes using the original 601 sequence. The top and bottom strands of the 601 sequence are denoted by I and J strand, respectively. The I strand is shown in the table. The 601 sequence is color coded as described in panel A. Labeled positions are highlighted in red, and underlined. The sequence index starts from the 5' end of the 601 on each strand. The specific primers (listed above) used to generate each template are listed next to the template name.

A

601	GGACCCTATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
RRH1-10	GGACCCTATA CAATACATGC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
LL8-24	GGACCCTATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCGGCCTCG GCACCGGGAT ATCCTGT GCATGTATTG AACAGCGACC
601MF	GGACCCTATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGGGA GTAATCCCCT TGGCGGTTAA AACGCGGGGG ACACCGCGTA CGTGCGTTTA AGCGGTGCTA GAGCTGTCTA CTAGTCTCCA GGCACGTGTC AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
601RTA	GGACCCTATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCTA

```
CCGCGTTTTA ACCGCCAATA GGATTACTTA CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
```

B

ED1-12
(I77,J-12)
```
TATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
```

ED1-1
(I68,J-1)
```
TATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT ACATGTATTG AACAGCGACC
```

ED1
(I68,J7)
```
TATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
```

ED1.5
(I57,J15)
```
TATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
```

ED1.7
(I46,J24)
```
TATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
```

INT
(I38,J28)
```
TATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
```

ED2.8
(I27,J45)
```
GGACCCTATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACA
```

ED2.5
(I15,J57)
```
GGACCCTATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACA
```

ED2
(I9,J58)
```
GGACCCTATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACA
```

ED2-1 (I-1,J79)
```
GGACCCTATA CGCGGCCGCT CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACA
```

ED2-12 (I-12,J79
```
GGACCCTATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACA
```

IJ-12 (I-12,J-12)
```
GGACCCTATA CGCGGCCGCC CTGGAGAATC CCGGTGCCGA GGCCGCTCAA
TTGGTCGTAG ACAGCTCTAG CACCGCTTAA ACGCACGTAC GCGCTGTCCC
CCGCGTTTTA ACCGCCAAGG GGATTACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGT GCATGTATTG AACAGCGACC
```

Monte Carlo Simulation Procedures

We adopted a continuum model of symmetric nucleosomal DNA unwrapping developed by Sudhanshu *et. al.* (Sudhanshu et al., 2011) and extended it to a more general, asymmetric case. In this model, the nucleosomal DNA is modeled as a left handed helix with a radius of 4.18 nm and height per turn of 2.39 nm. The total free energy change ΔF upon binding of DNA to the nucleosome spool (the histone core) relative to the unbound state is calculated at forces ranging from 0 pN to 12 pN as was done in Figure 3 of Sudhanshu *et. al.* (Sudhanshu et al., 2011). In a more general case of asymmetric unwrapping, *m* and *n* base pairs can be unwrapped from the weak and strong side, respectively, where *m* can be different from *n*. In the model of Sudhanshu et al, *m*=*n*. In our two-dimensional model, in order to calculate the free energy change ΔF for (*m*, *n*) values, we assumed that the free energy change ΔF for (*m*, *n*) is equal to the (*m* + *n*) base pairs unwrapped case in the symmetric model. In addition, we reduced the binding energy of the inner quarter of the weak side to 1.5 pN nm / bp while all other quarters (two outer quarters and the inner quarter of the strong side) are assumed to have the binding energy of 3.5 pN nm / bp so that the average binding energy is 3 pN nm / bp as was used by Sudhanshu et al. With this energy function, we performed Monte Carlo simulations starting from 0.1 pN and increasing the force in 0.1 pN increments every 2000 time steps until 10 pN of force was reached. The initial position was *m*=1 and *n*=1.

Supplemental References

Sudhanshu, B., Mihardja, S., Koslover, E.F., Mehraen, S., Bustamante, C., and Spakowitz, A.J. (2011). Tension-dependent structural deformation alters single-molecule transition kinetics. Proceedings of the National Academy of Sciences of the United States of America *108*, 1885-1890.