The Abdus Salam
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2038-3

Conference: From DNA-Inspired Physics to Physics-Inspired Biology

1 - 5 June 2009

Nanomechanics of single and double stranded DNA

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Nanomechanics of single and double stranded DNA

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Stretching molecules (DNA) by AFM allows exploring their high-energy conformations that are not accessible to X-ray crystallography, NMR and other mode of spectroscopy which investigate molecules near their equilibrium state

DNA-Inspired Physics

Nanomechanics of ssDNA

Base-stacking interactions

Measurement Errors in

Force Spectroscopy by AFM

Physics -Inspired Biology

Nanomechanics

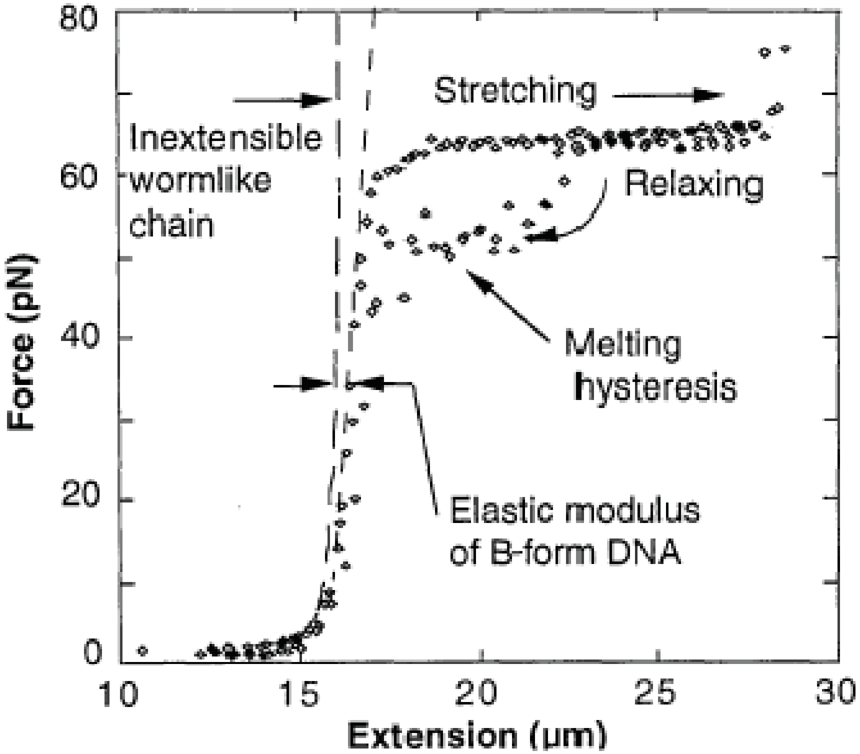
of intact and damaged dsDNA

UV-damage to DNA by AFM imaging

Overstretching B-DNA: The Elastic Response of Individual Double-Stranded and Single-Stranded DNA Molecules

Steven B. Smith, Yujia Cui, Carlos Bustamante*

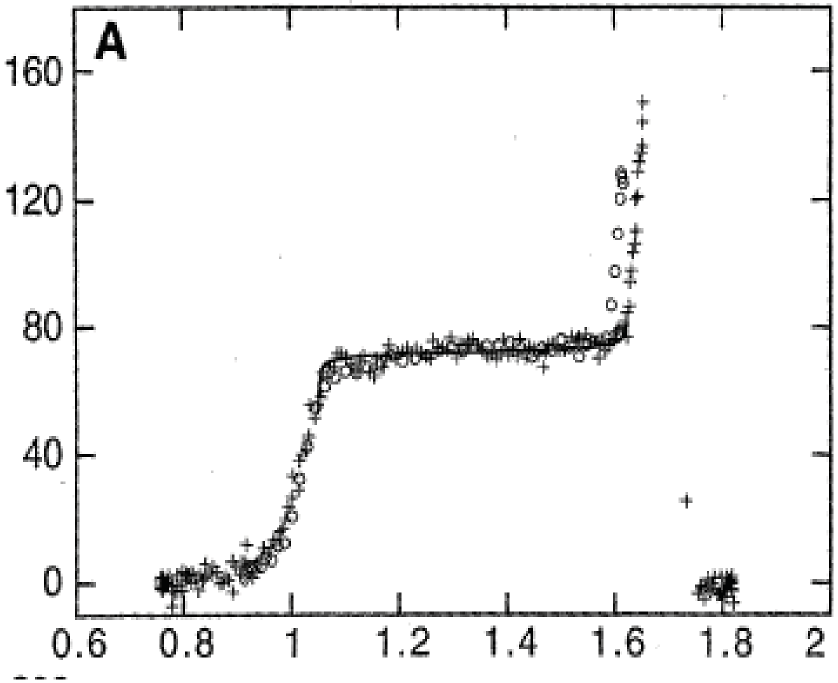
Single molecules of double-stranded DNA (dsDNA) were stretched with force-measuring laser tweezers. Under a longitudinal stress of ~65 piconewtons (pN), dsDNA molecules in aqueous buffer undergo a highly cooperative transition into a stable form with 5.8 angstroms rise per base pair, that is, 70% longer than B-form dsDNA. When the stress was relaxed below 65 pN, the molecules rapidly and reversibly contracted to their normal contour lengths. This transition was affected by changes in the ionic strength of the medium and the water activity or by cross-linking of the two strands of dsDNA. Individual molecules of single-stranded DNA were also stretched giving a persistence length of 7.5 angstroms and a stretch modulus of 800 pN. The overstretched form may play a significant role in the energetics of DNA recombination.



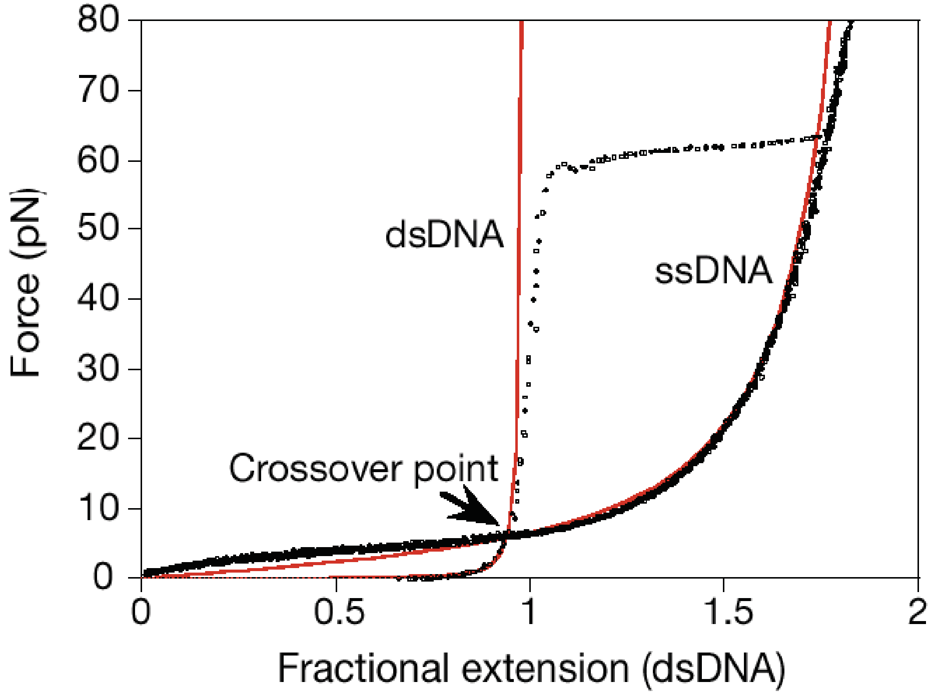
DNA: An Extensible Molecule

Philippe Cluzel, Anne Lebrun, Christoph Heller,*
Richard Lavery, Jean-Louis Viovy, Didier Chatenay,†
François Caron‡

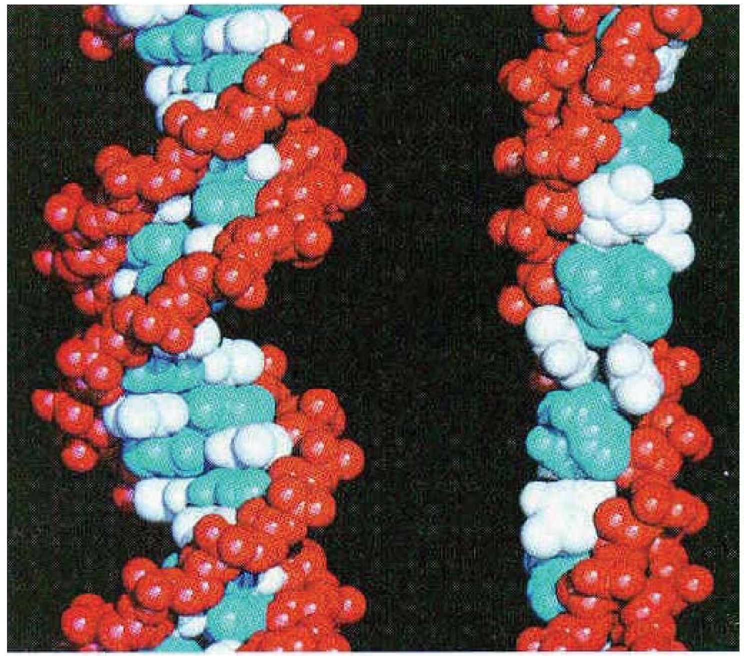
The force-displacement response of a single duplex DNA molecule was measured. The force saturates at a plateau around 70 piconewtons, which ends when the DNA has been stretched about 1.7 times its contour length. This behavior reveals a highly cooperative transition to a state here termed S-DNA. Addition of an intercalator suppresses this transition. Molecular modeling of the process also yields a force plateau and suggests a structure for the extended form. These results may shed light on biological processes involving DNA extension and open the route for mechanical studies on individual molecules in a previously unexplored range.



Mechanochemistry of DNA damage and repair



From: Wuite et al, (2000). *Nature* ***404***, 103.

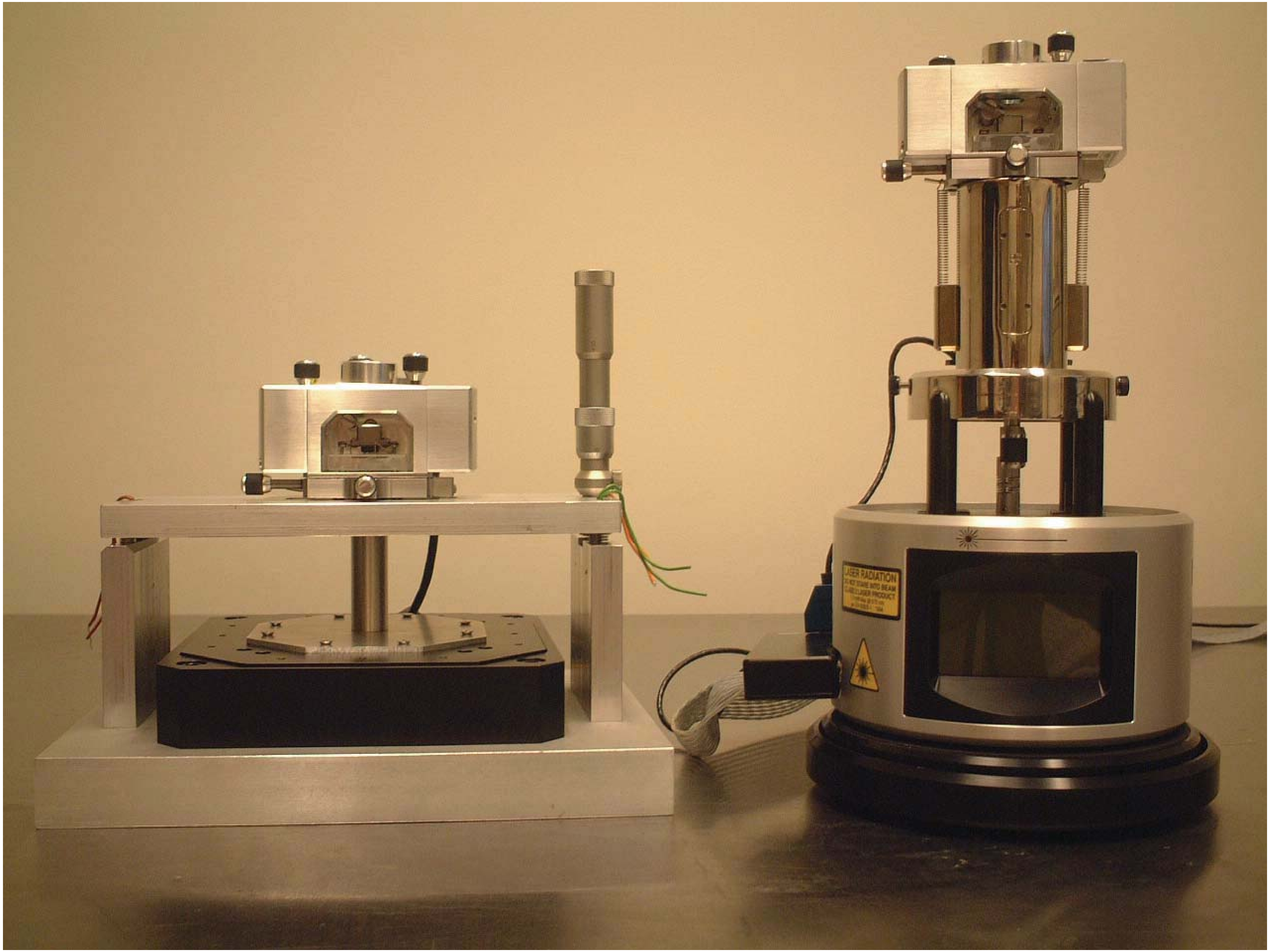


Cluzel, et al. Science, 271 792-794 (1996)

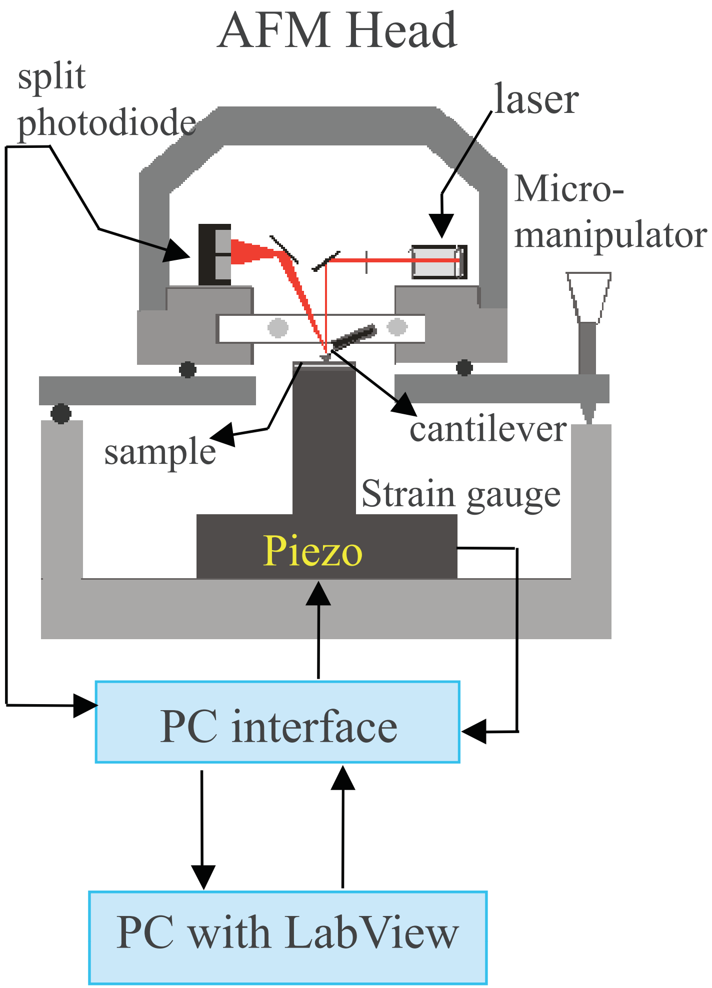
Atomic force microscopy: air and fluid imaging

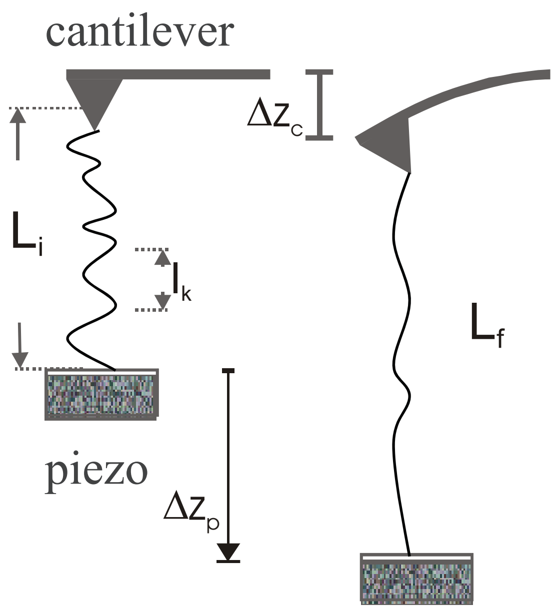
Optical microscope - made for King George III (second half of 18th century)

AFM - made by DI (end of 20th century)


LASER RADIATION
DO NOT STARE INTO BEAM
CLASS 2 LASER PRODUCT

Atomic Force Microscope





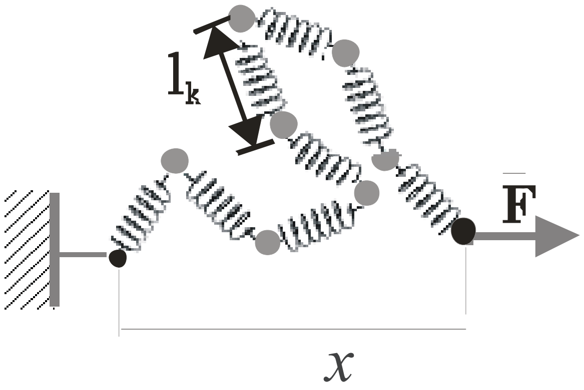
$$X = L_f - L_i$$

$$F = K_c \Delta Z_c$$

(K_c, spring constant)

Freely jointed chain with segment elasticity

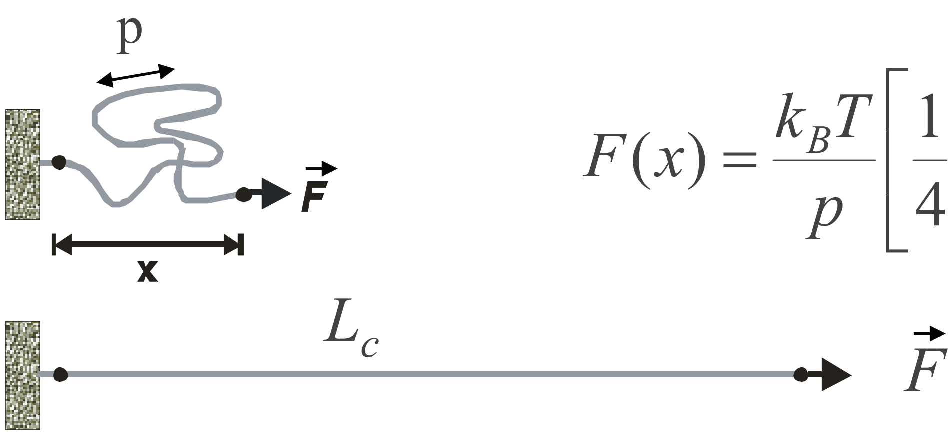
ssDNA polysaccharides



$$x(F) = \left(\coth\left(\frac{F l_K}{k_B T} \right) - \frac{k_B T}{F l_K} \right) \left(L_{con} + \frac{nF}{k_{segment}} \right)$$

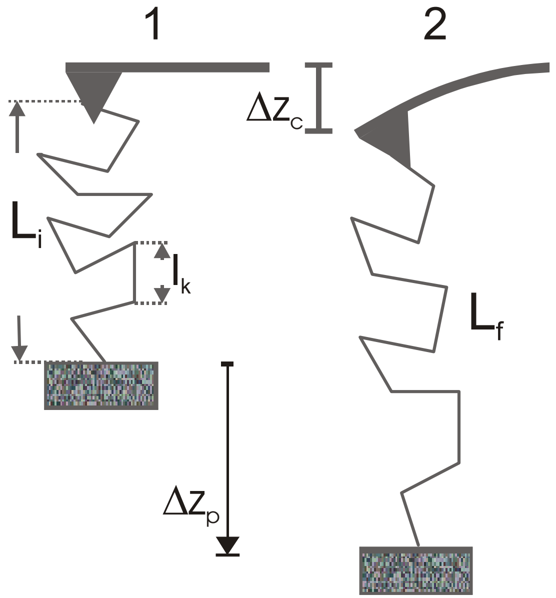
Worm-like chain

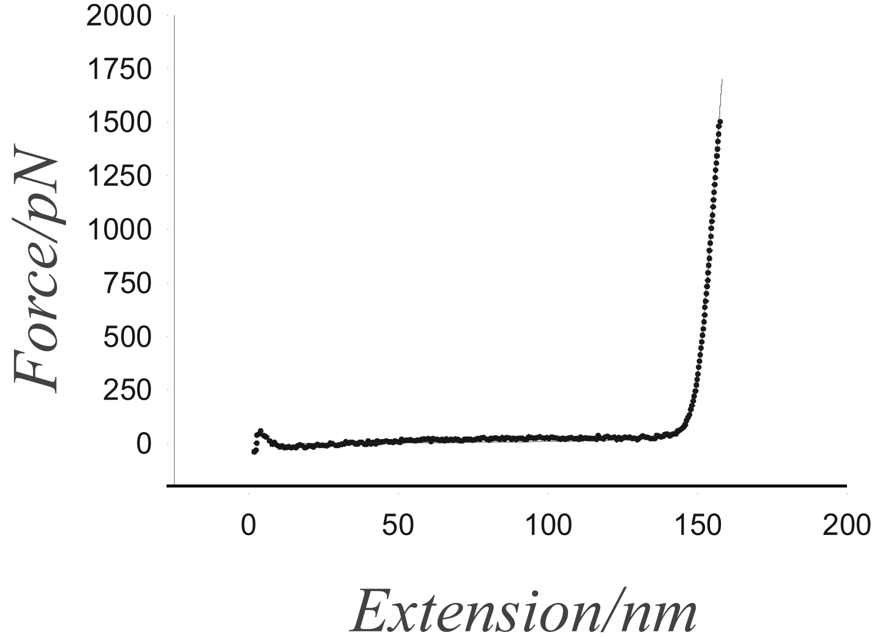
ds DNA modular proteins



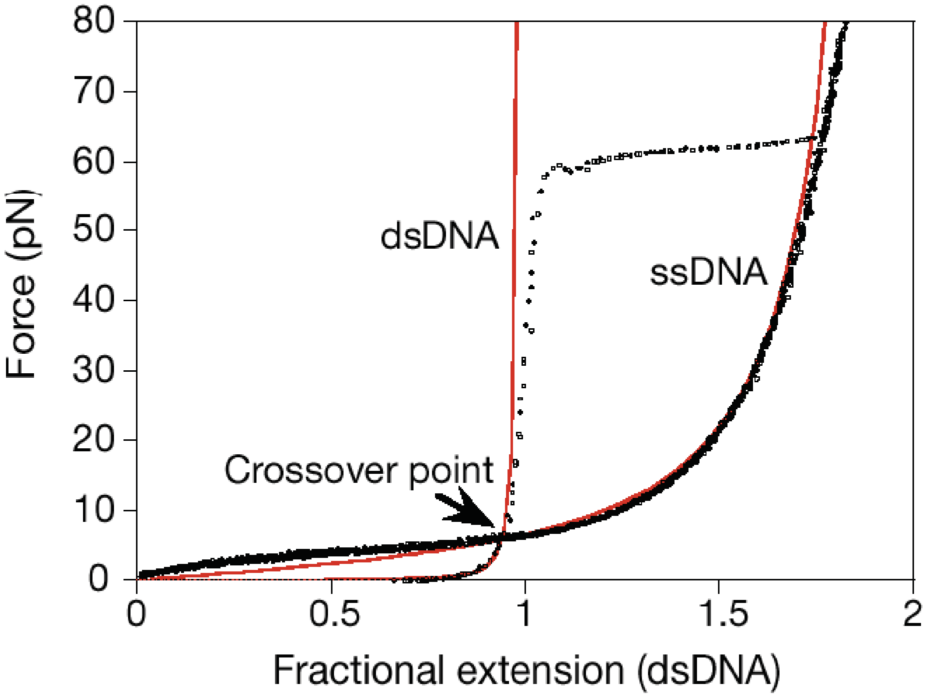
$$F(x) = \frac{k_B T}{p} \left[\frac{1}{4} \left(1 - \frac{x}{L_{con}} \right)^{-2} - \frac{1}{4} + \frac{x}{L_{con}} \right]$$

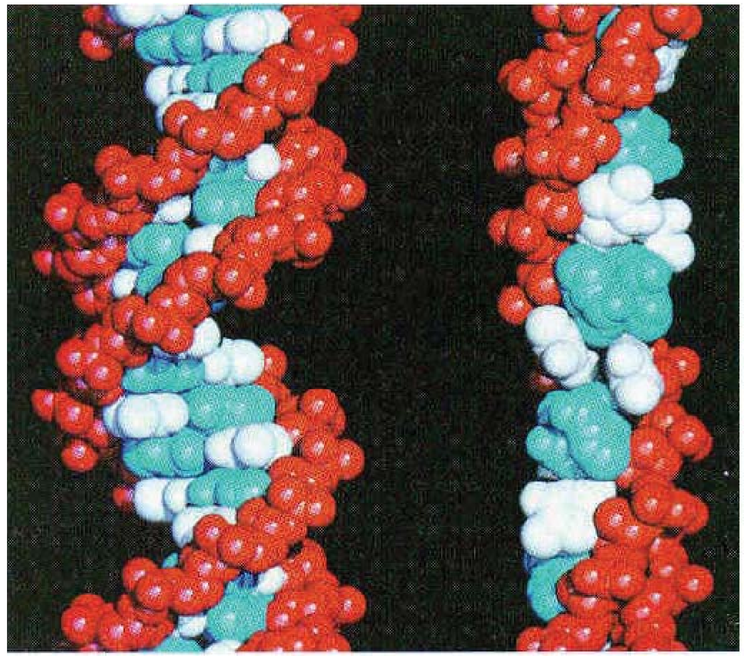
Entropic elasticity





Mechanochemistry of DNA damage and repair

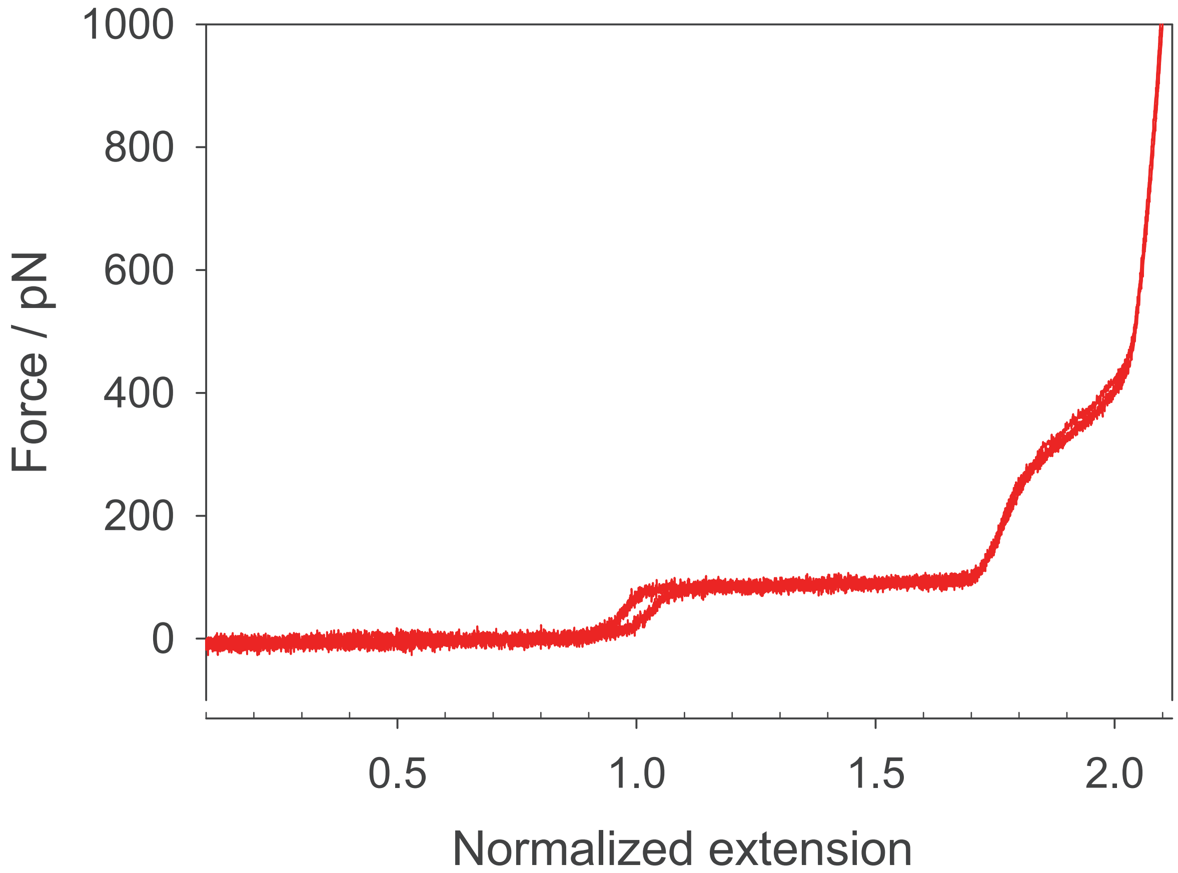




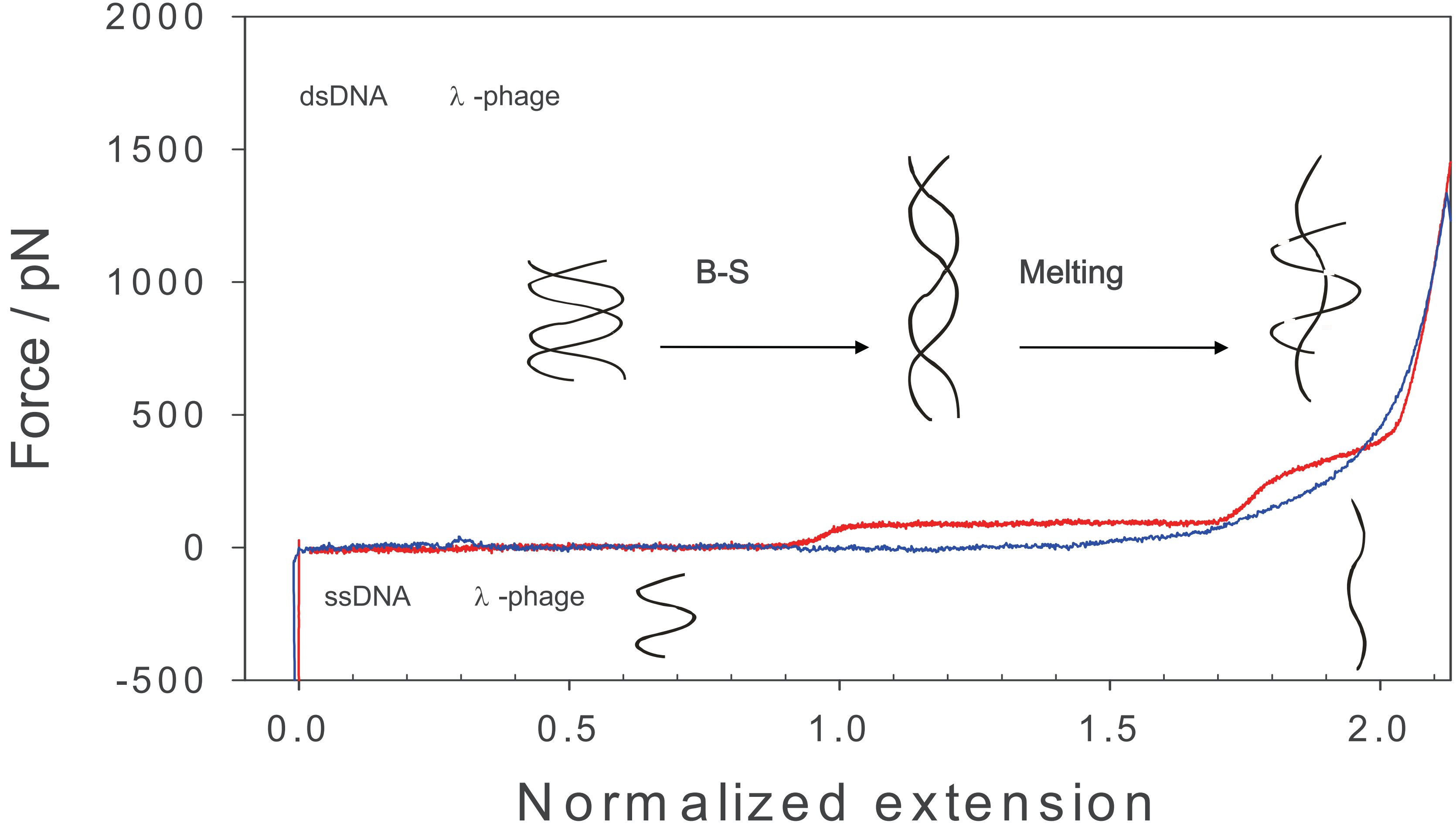
From: Wuite et al, (2000). *Nature* ***404**,* 103
Smth, Bustamante et al, Science, 1996.

Cluzel, et al. Science, 271 792-794 (1996)

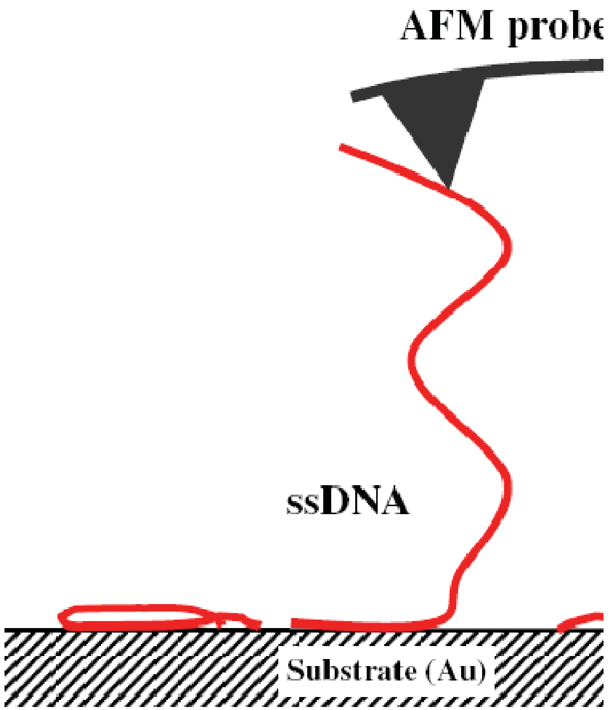
Force spectrograms of dsDNA measured by AFM

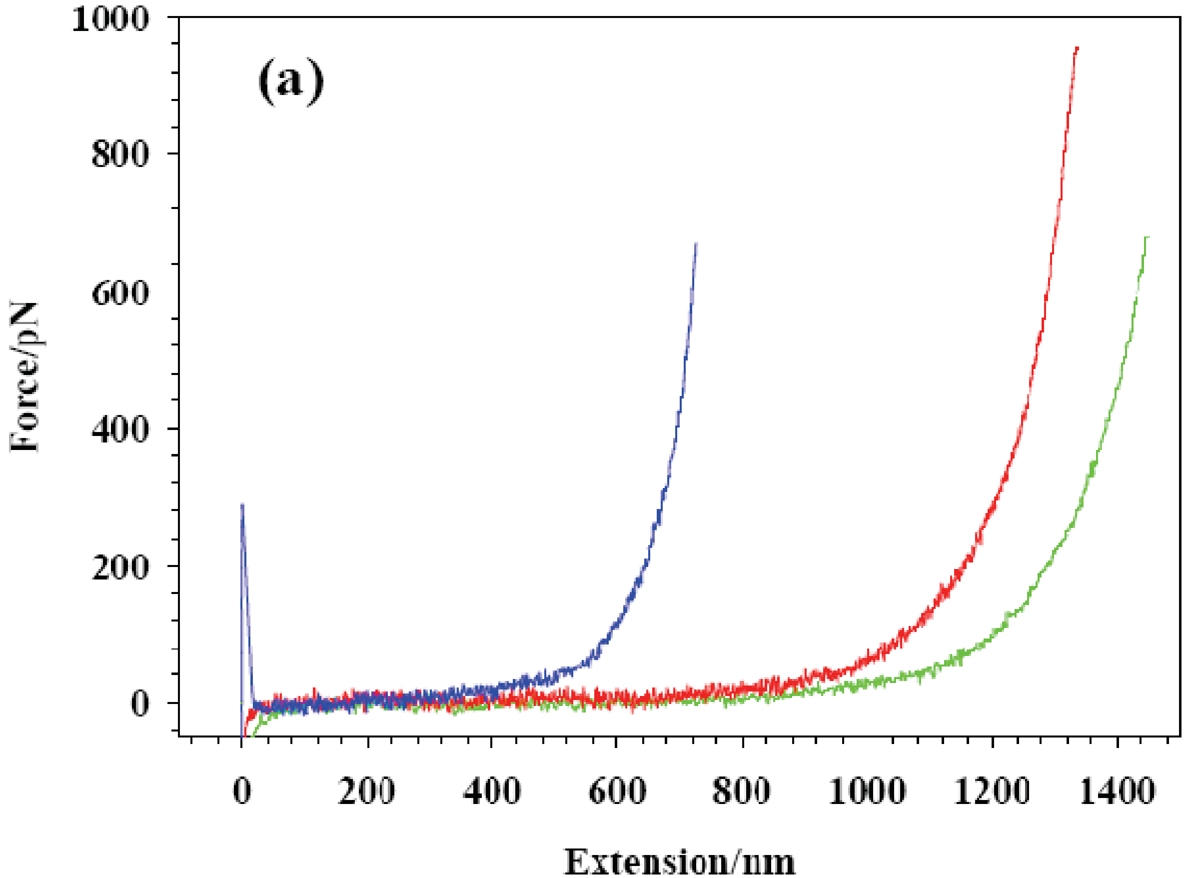


Force-extension curves of λ phage DNA



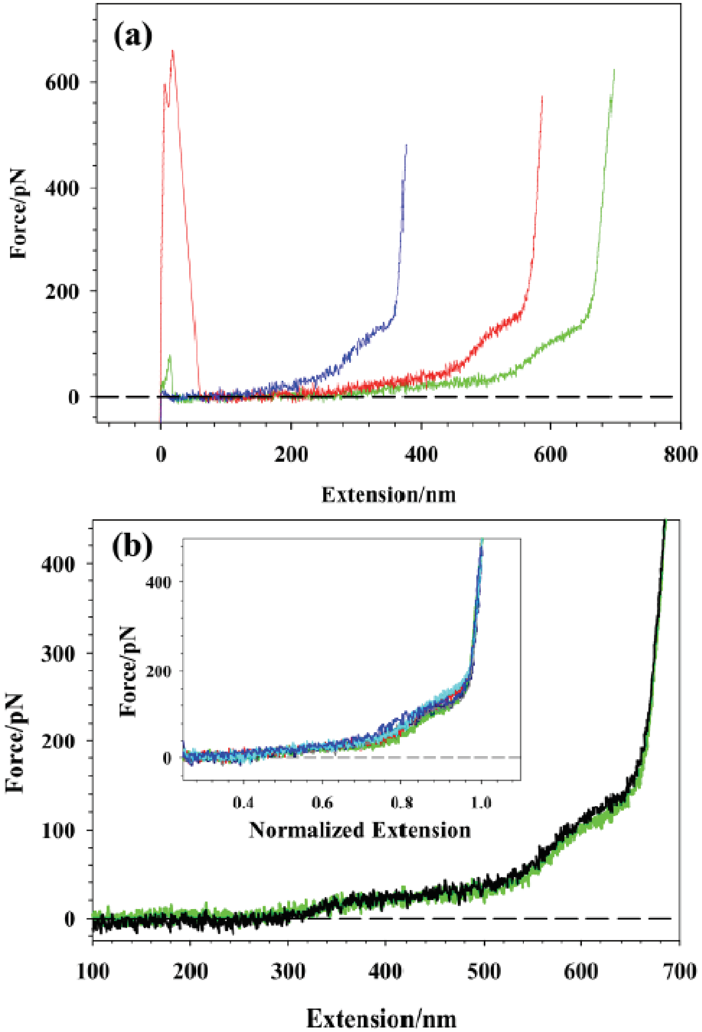
The Elasticity of poly(dT)

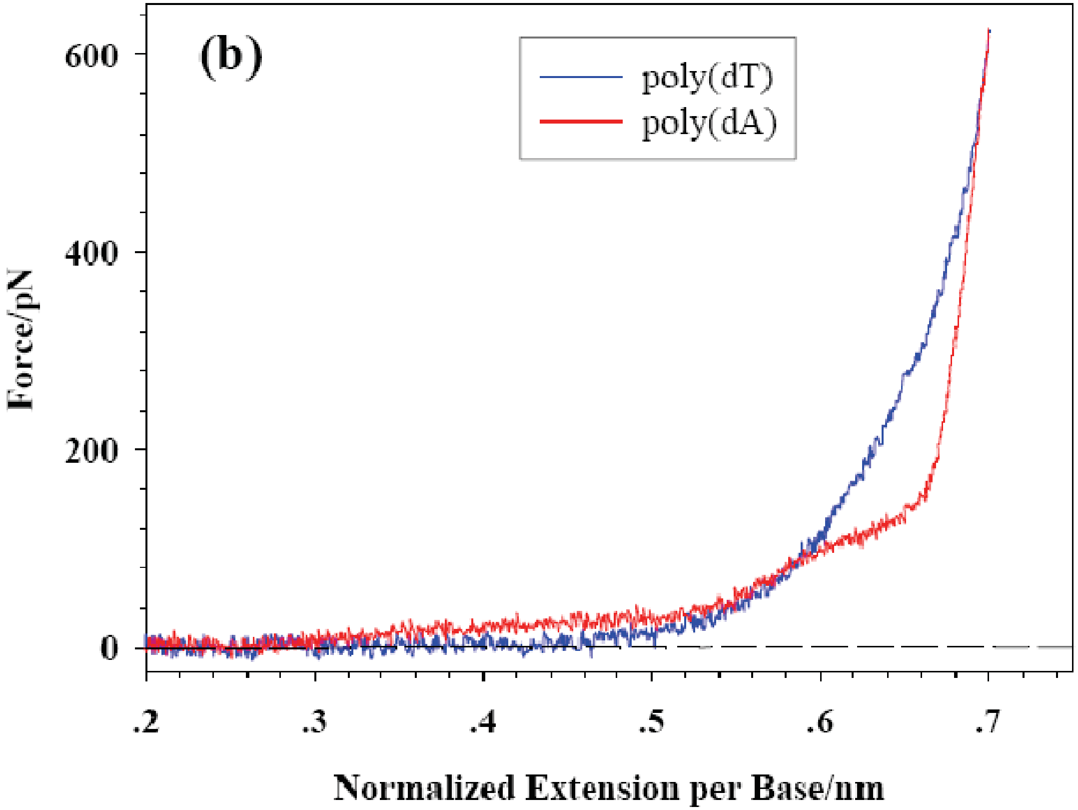




Direct Measurements of Base Stacking Interactions in DNA by Single-Molecule Atomic-Force Spectroscopy

Changhong Ke,[1,*] Michael Humeniuk,[1,*] Hanna S-Gracz,[2] and Piotr E. Marszalek[1,†]

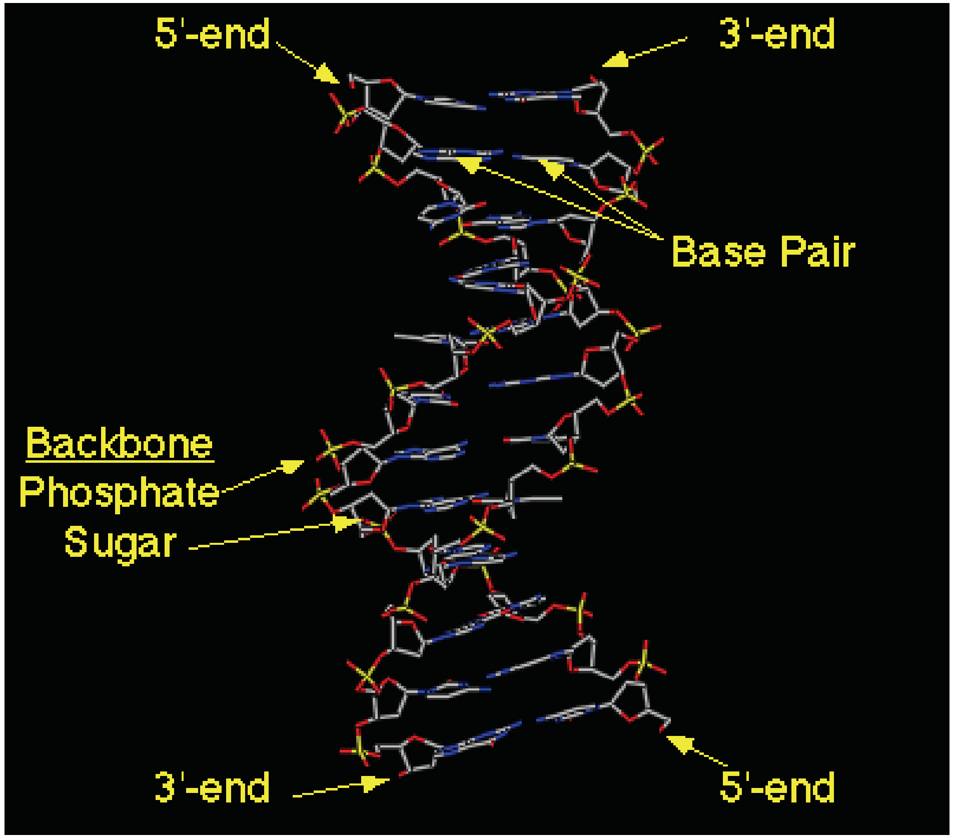


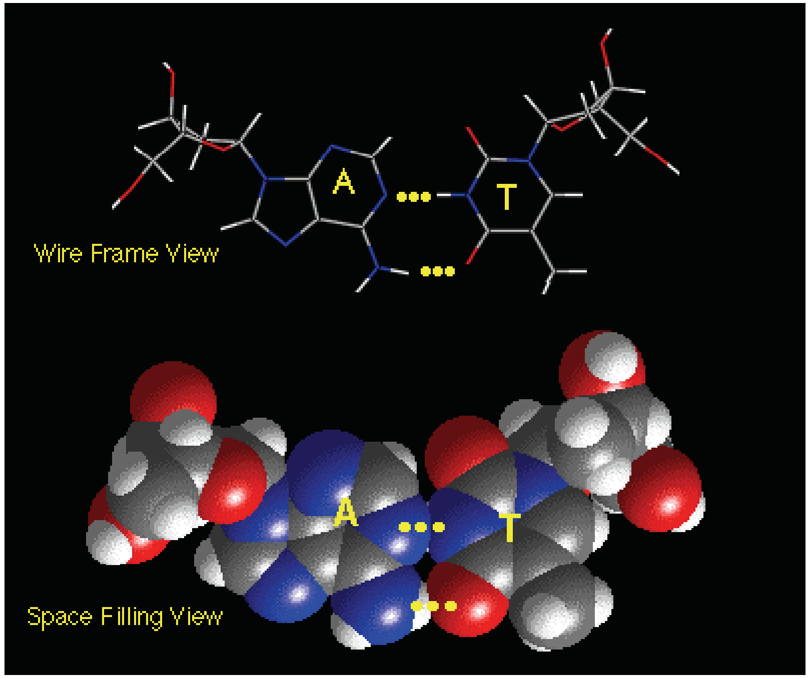


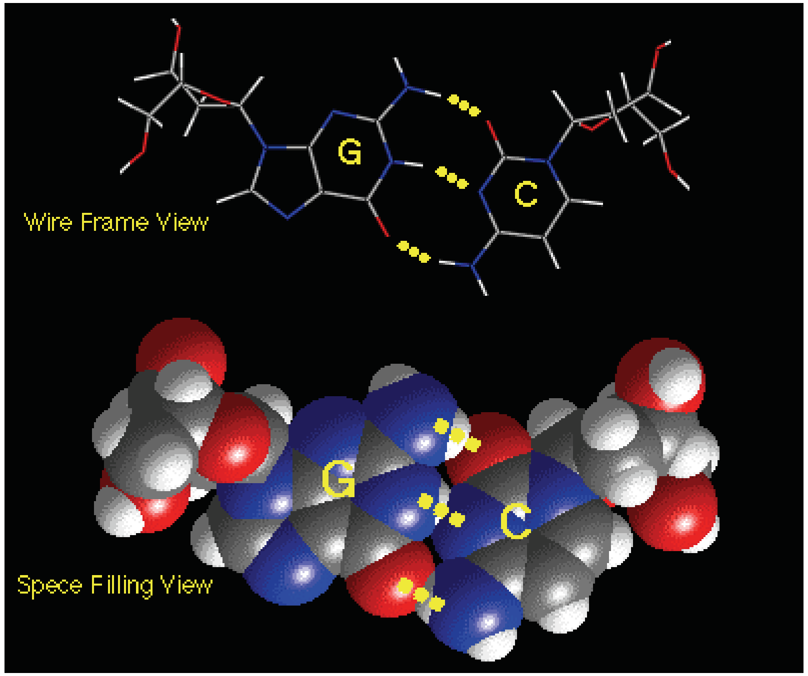
PRL **99,** 018302 (2007)

PHYSICAL REVIEW LETTERS

DNA structure: base pairing and stacking

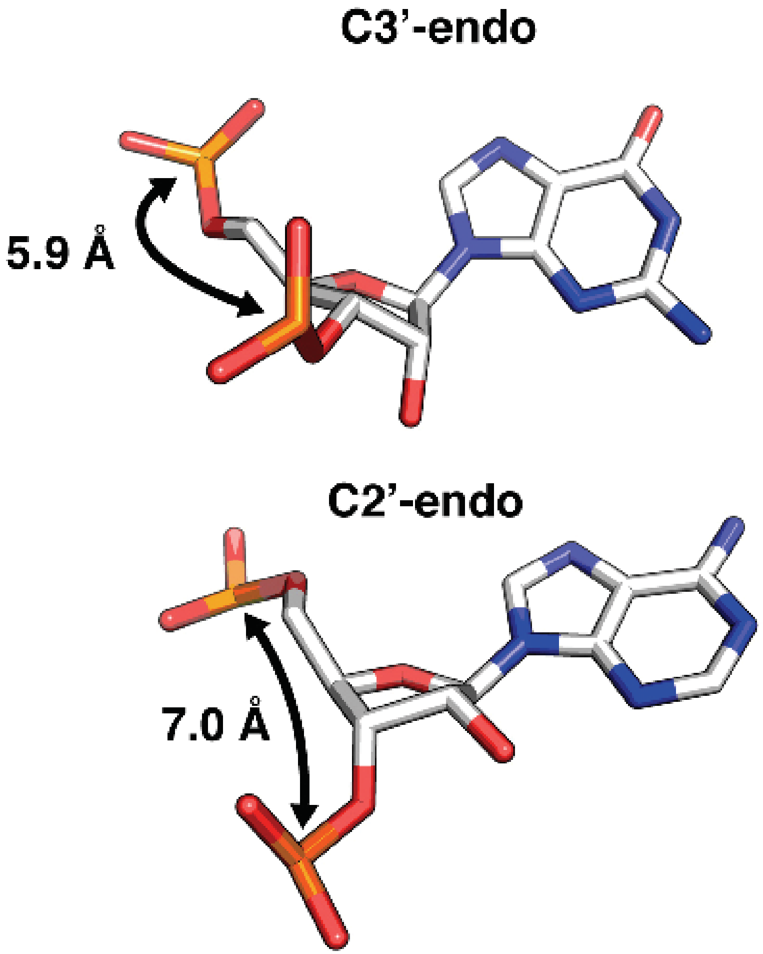




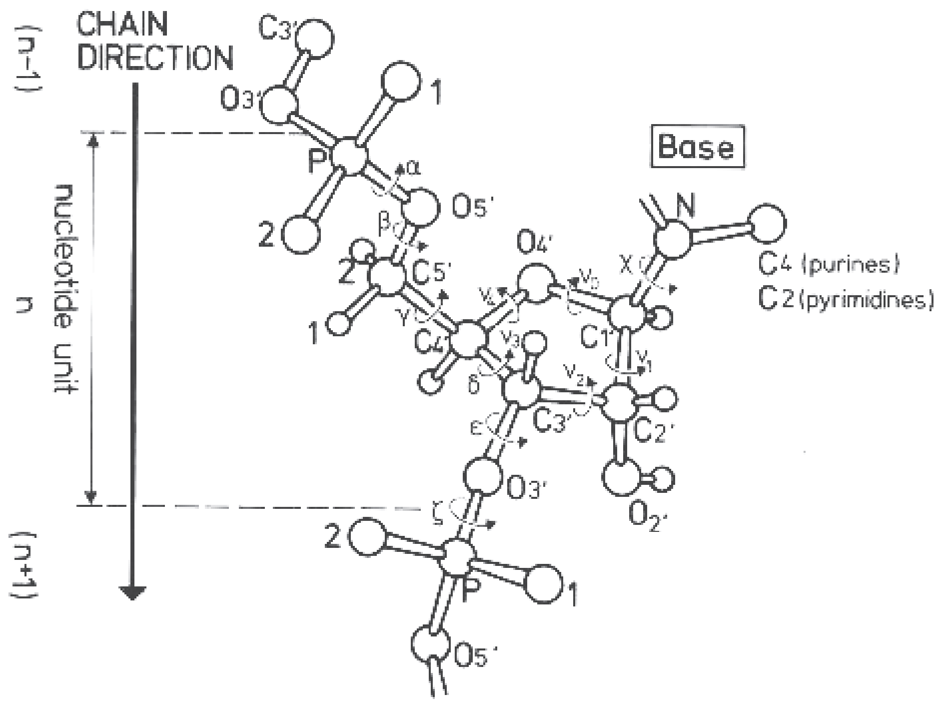


Nucleic Acid Structure

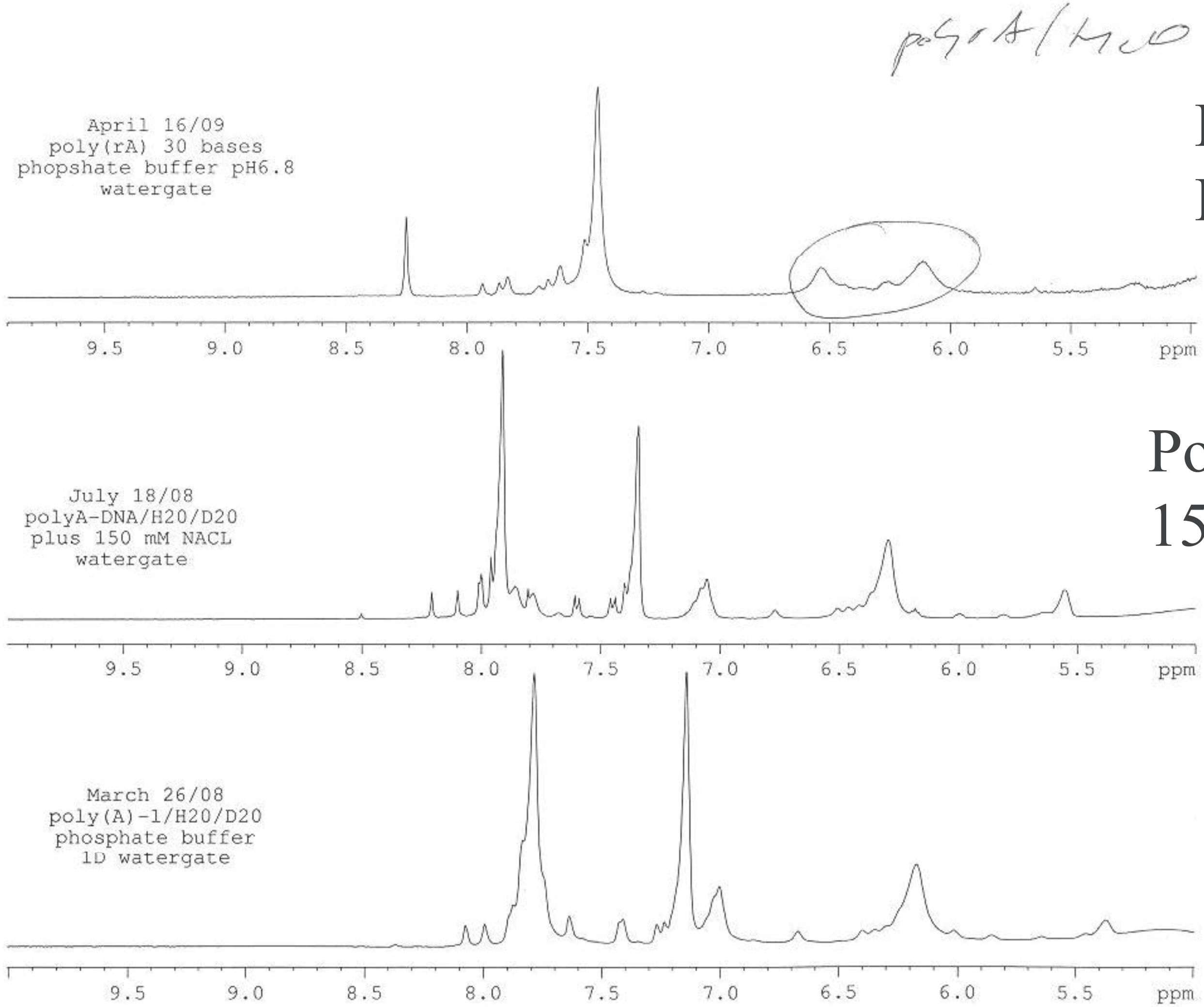
- Suger Pucker



- Torsion Angles



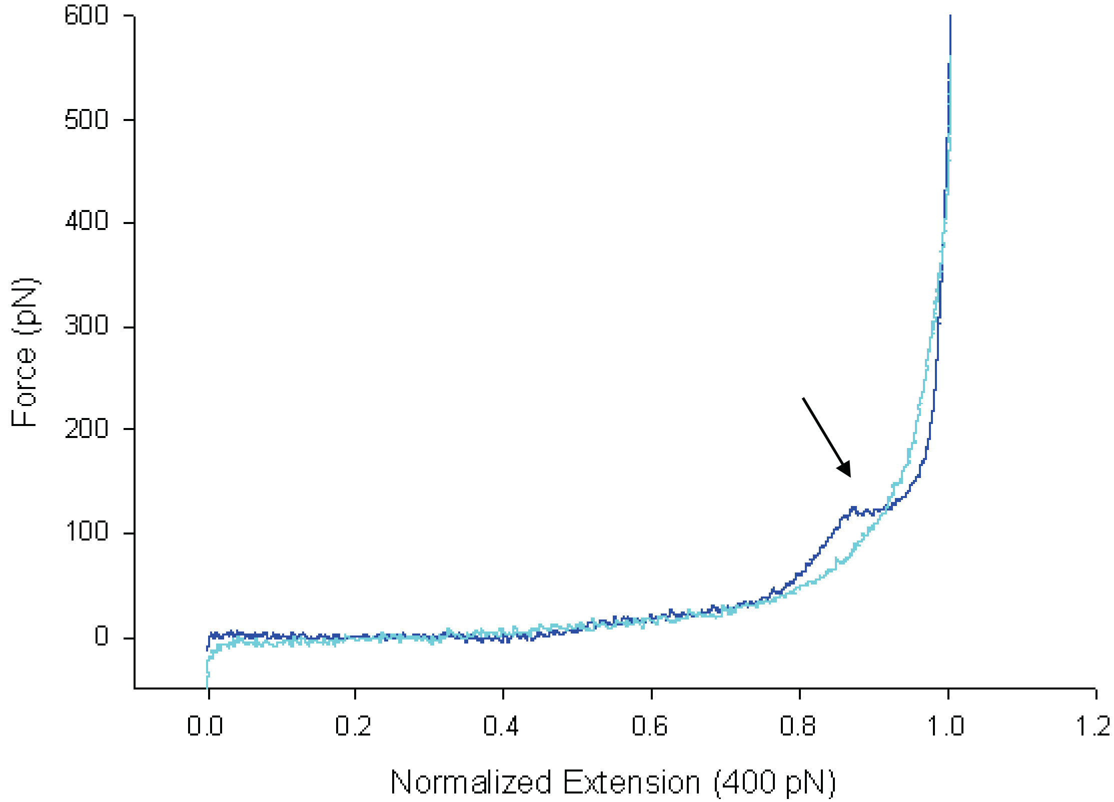
NMR 30 nt homopolynucleotides



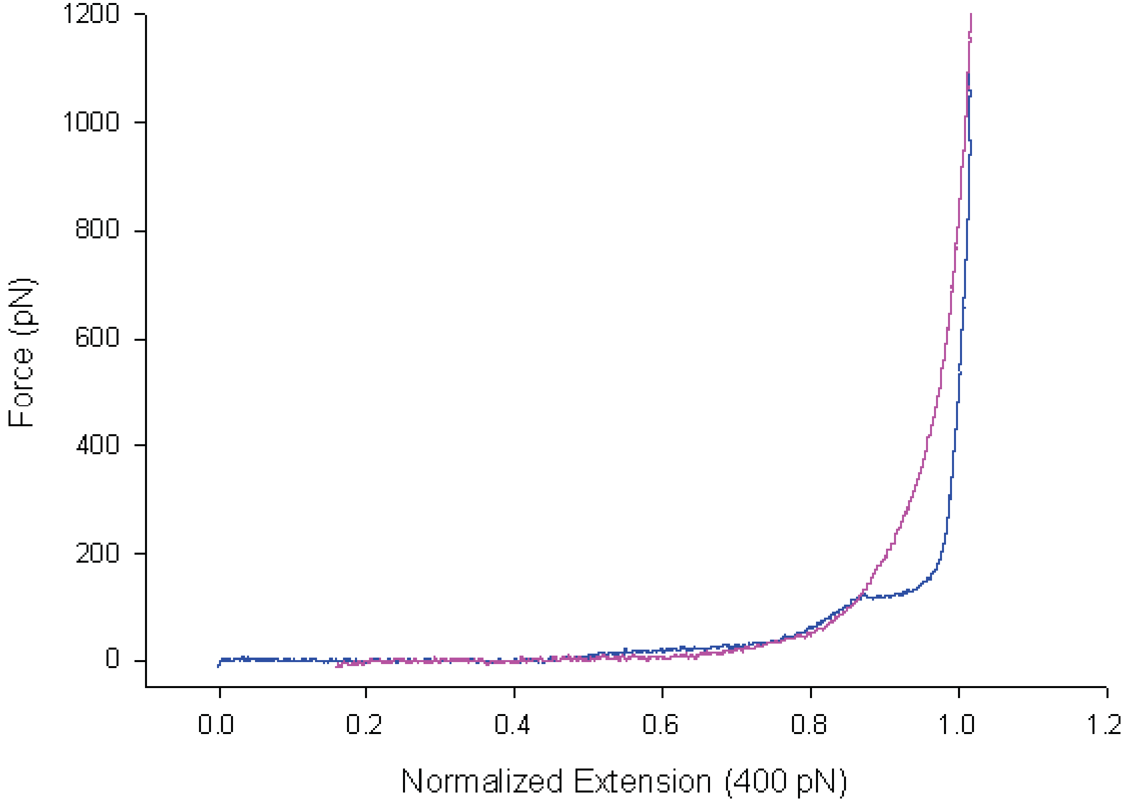
Poly(rA)
H_20

Poly(dA)
150 mM NaCl

Poly(rA) vs poly(dA)



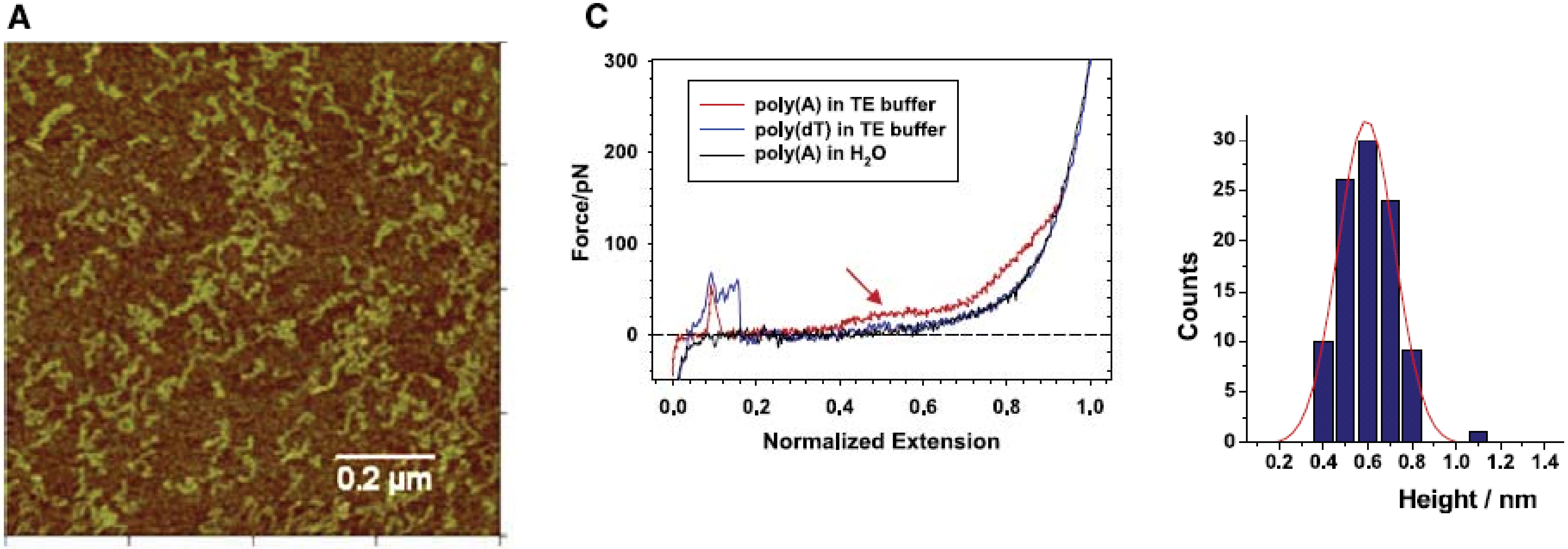
Poly(dA) TE Na150 vs dH2O



Biophysical Journal Volume 96 April 2009 2918–2925

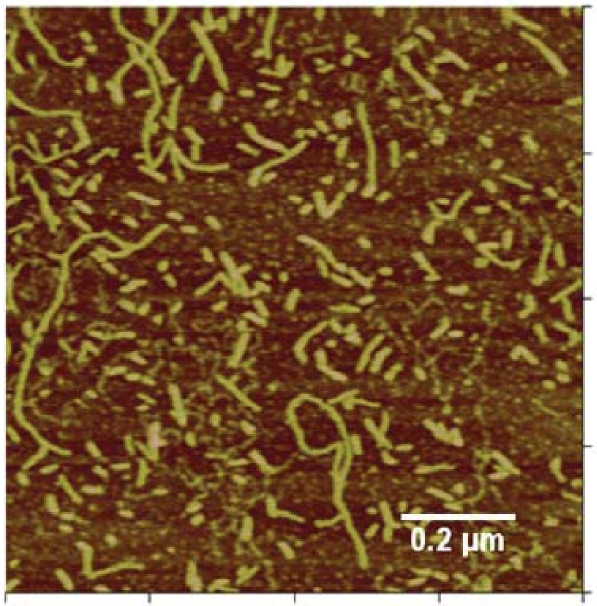
Detecting Solvent-Driven Transitions of poly(A) to Double-Stranded Conformations by Atomic Force Microscopy

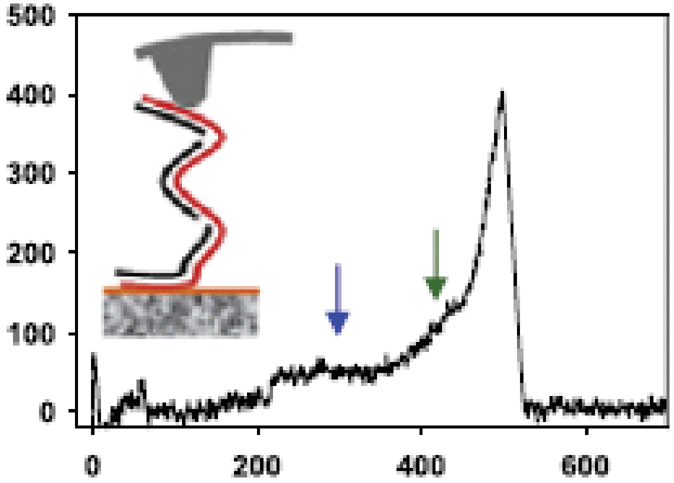
Changhong Ke,[†‡] Anna Loksztejn,[†§] Yong Jiang,[†] Minkyu Kim,[†] Michael Humeniuk,[†] Mahir Rabbi,[†] and Piotr E. Marszalek[†*]

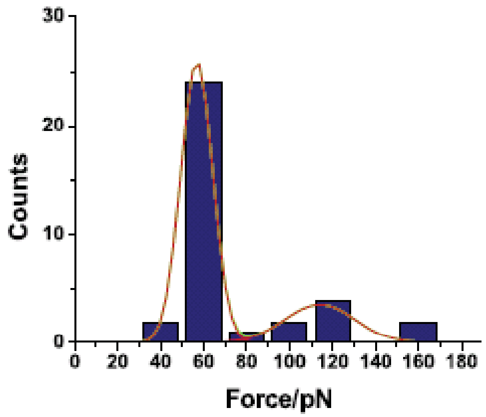


Poly(rA) at the pH of 5.5

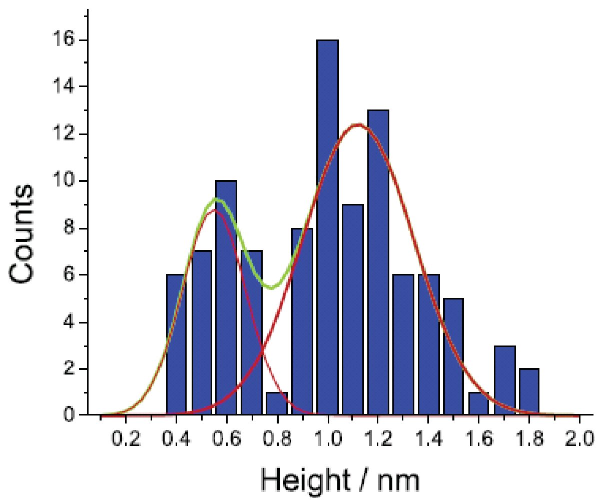
A

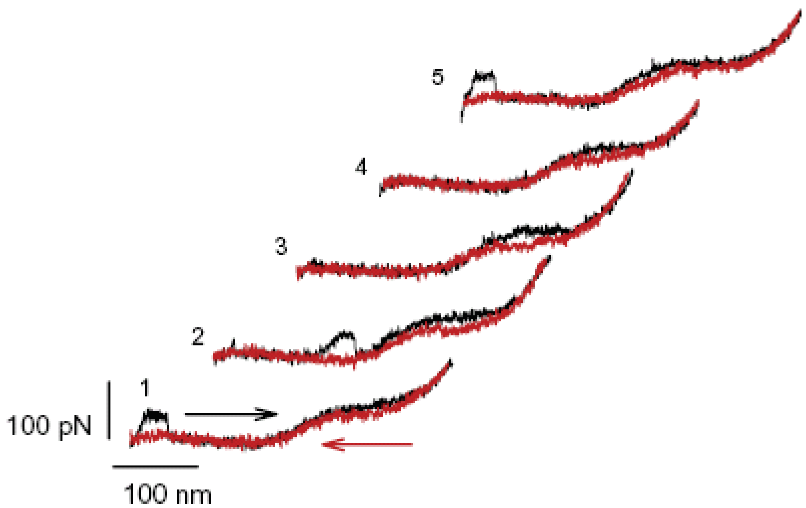






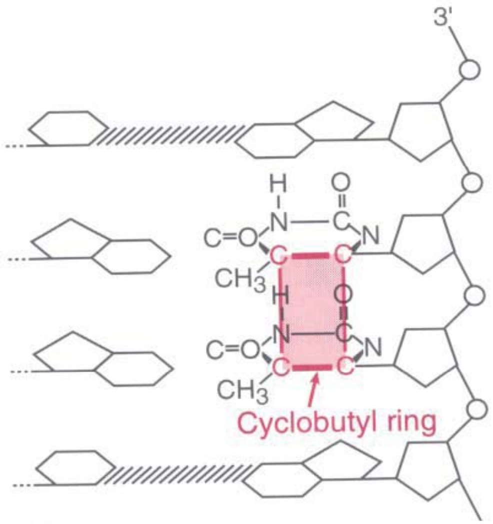
B

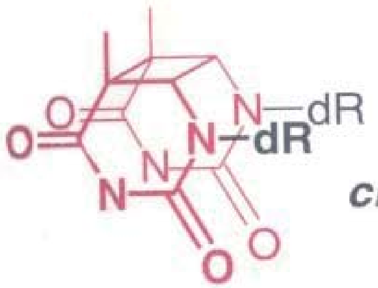




UV-induced DNAdamage

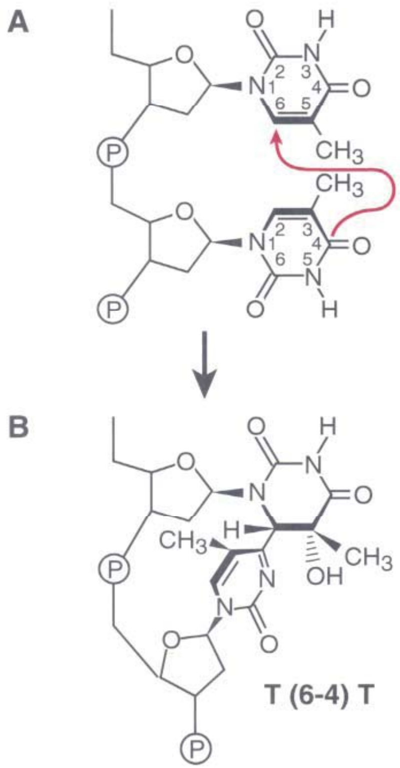
Cyclobutane pyrimidine dimers





cis-syn (T-T)

Pyrimidine-pyrimidone(6-4) lesions

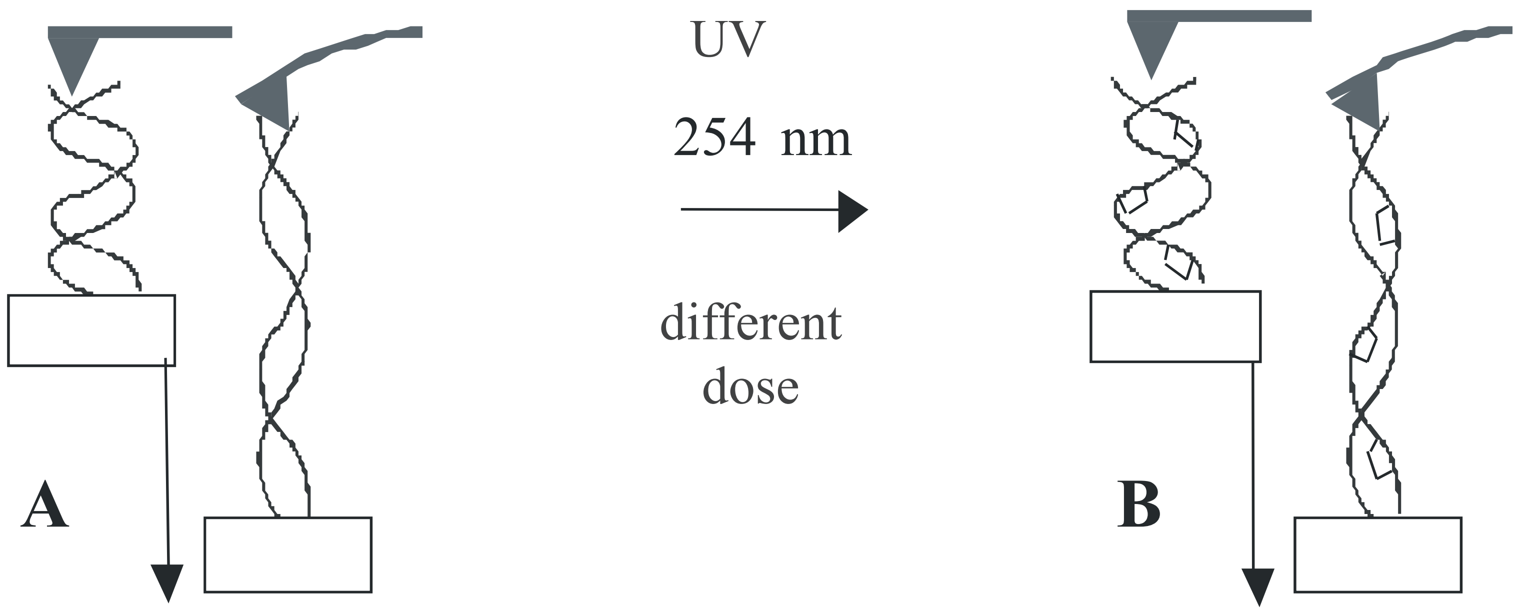


Population

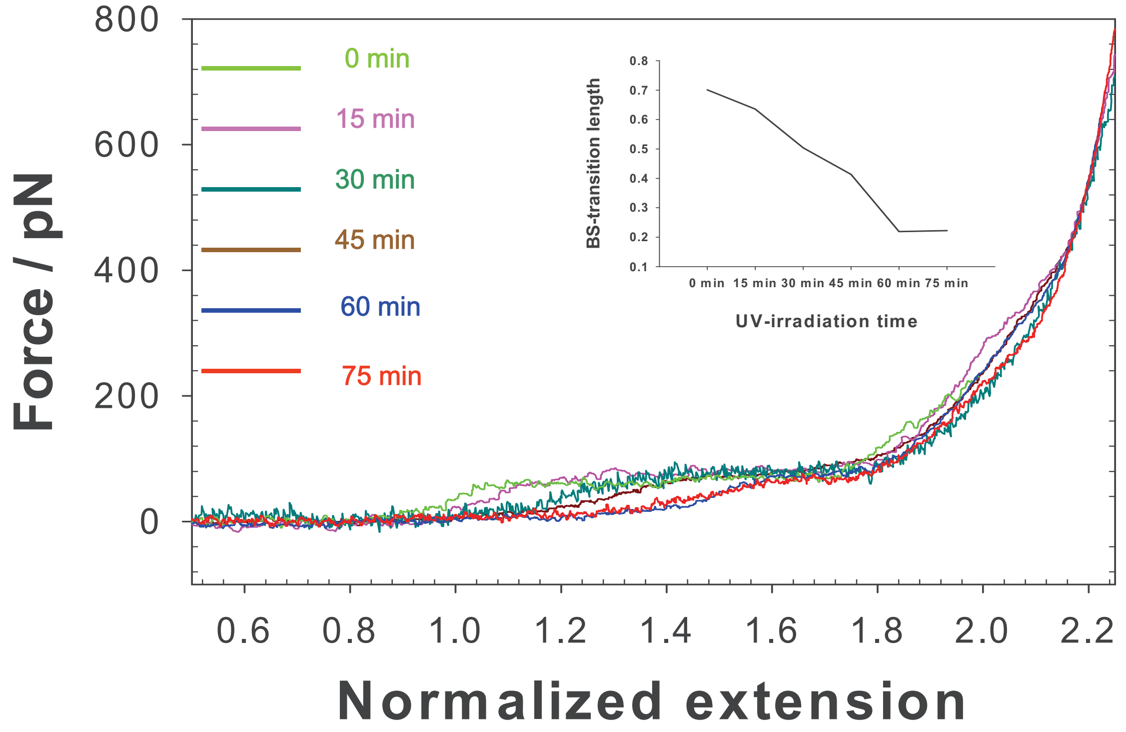
75% : 25%

(Adopted from Friedberg et al.)

AFM experiments on UV irradiated DNA



Force-spectrograms of λ-phage DNA irradiated with different UV-doses

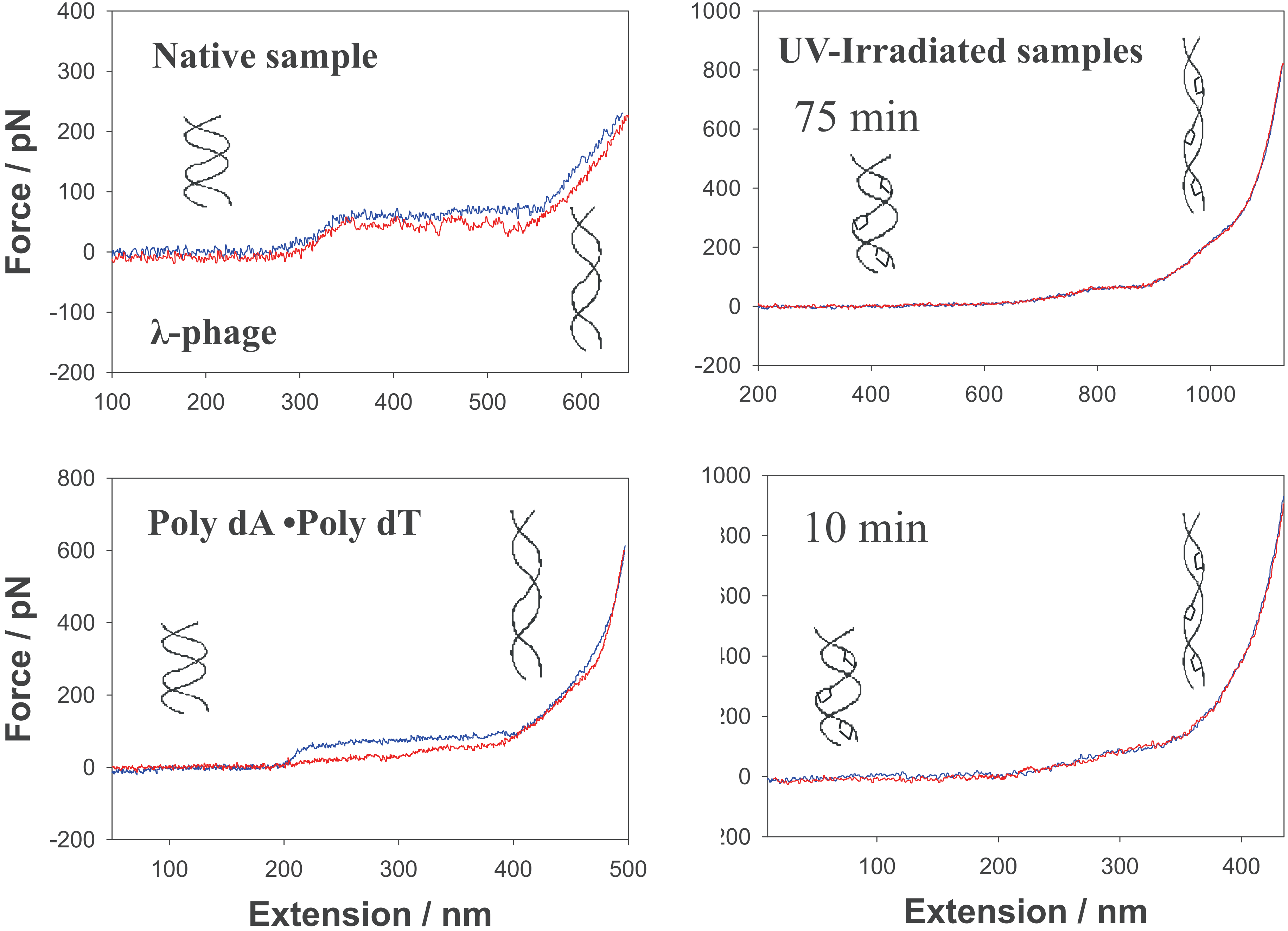


DNA irradiated with the UV light near ~260 nm can form ***pyrimidine dimers and 6-4 photoproducts***, which can change the elasticity of double stranded DNA(dsDNA).

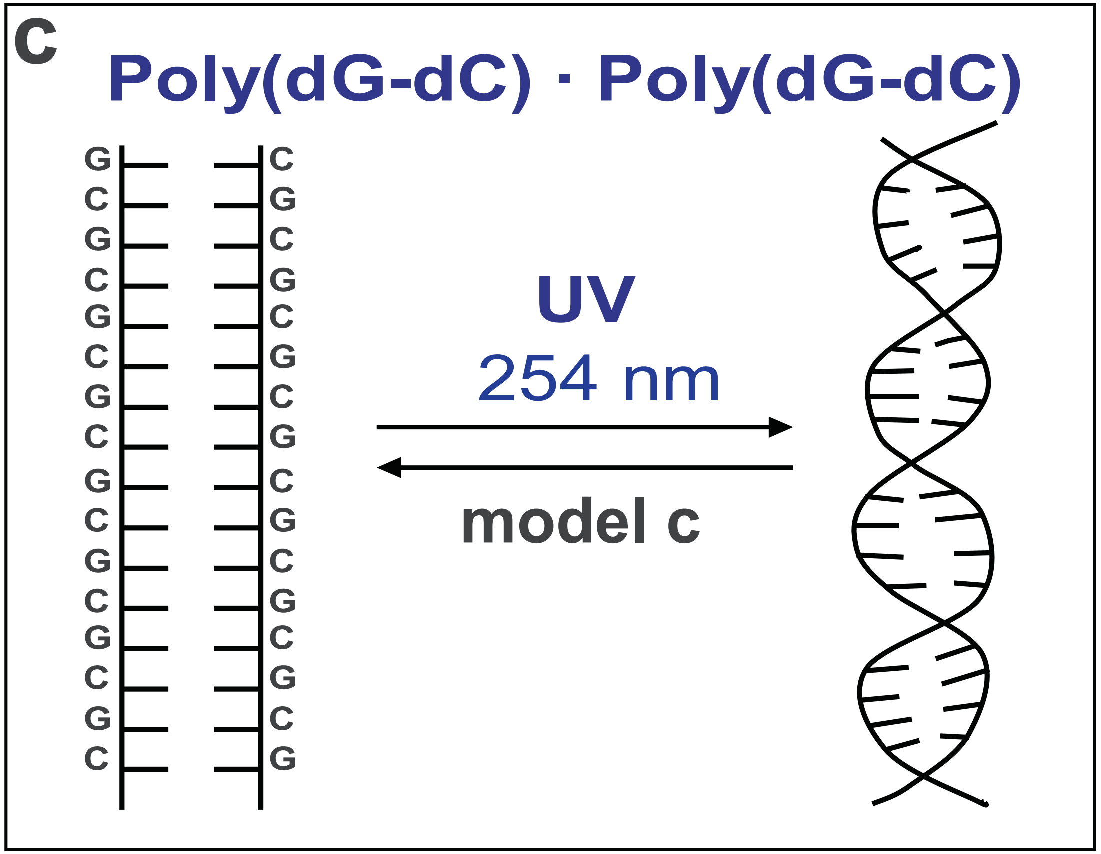
Nanomechanical Fingerprints of UV Damage To DNA**

*Gwangrog Lee, Mahir Rabbi, Robert L. Clark, and Piotr E. Marszalek**

small 2007, 3, No. 5, 809 – 813



Native sample
λ-phage
Force / pN
400
300
200
100
0
-100
-200
100
200
300
400
500
600
UV-Irradiated samples
75 min
1000
800
600
400
200
0
-200
200
400
600
800
1000
Poly dA •Poly dT
Force / pN
800
600
400
200
0
-200
100
200
300
400
500
Extension / nm
10 min
1000
0
100
200
300
400
Extension / nm

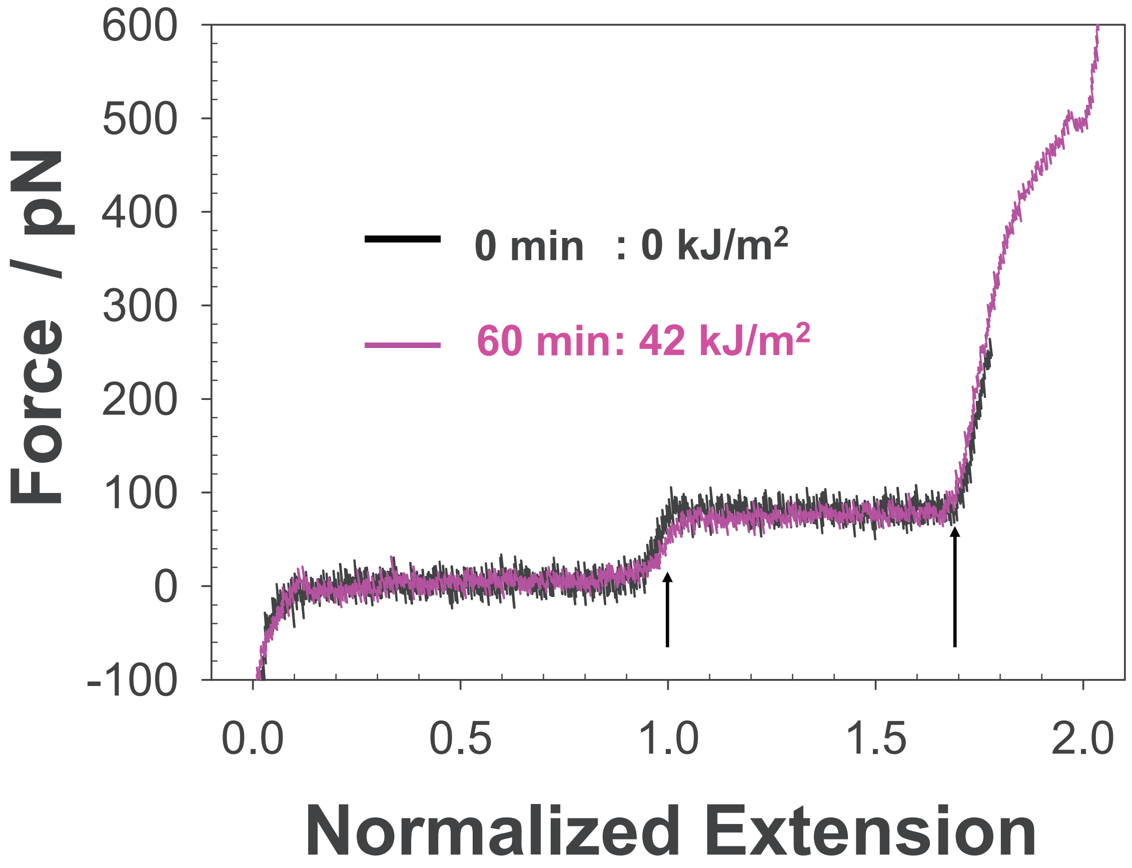


Nanomechanical Fingerprints of UV Damage To DNA**

small 2007, 3, No. 5, 809 – 813

*Gwangrog Lee, Mahir Rabbi, Robert L. Clark, and Piotr E. Marszalek**

Force-spectrograms of poly(dGdC)·poly(dGdC) irradiated by UV light at 256nm for 60 min



This observation support our hypothesis that the changes of B-S and melting transition comes from the extensive formation of CPDs and 6-4 lesions.

Pulling geometry induced errors in single molecule force spectroscopy measurements

Changhong Ke, Yong Jiang, Monica Rivera, Robert L. Clark, and Piotr E. Marszalek

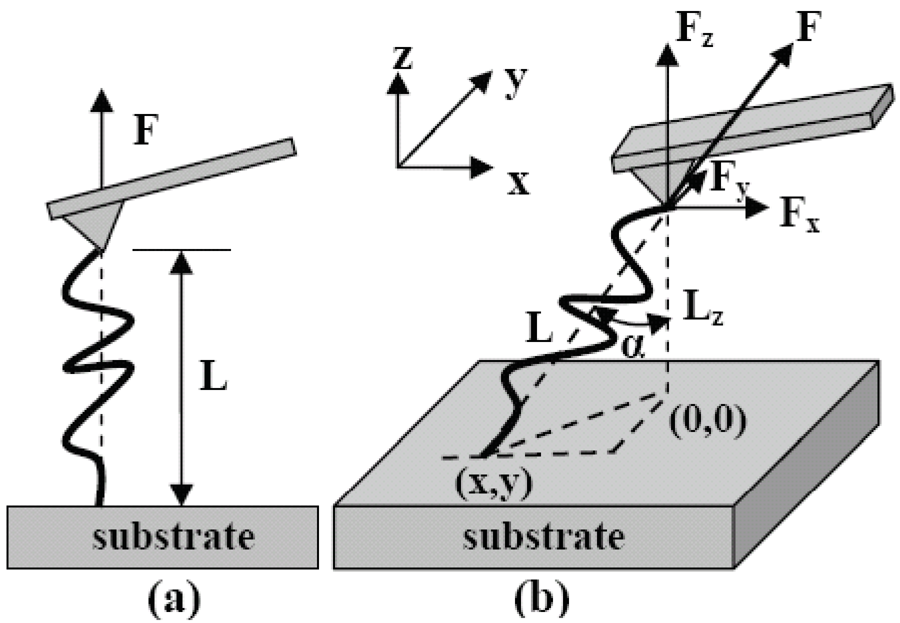
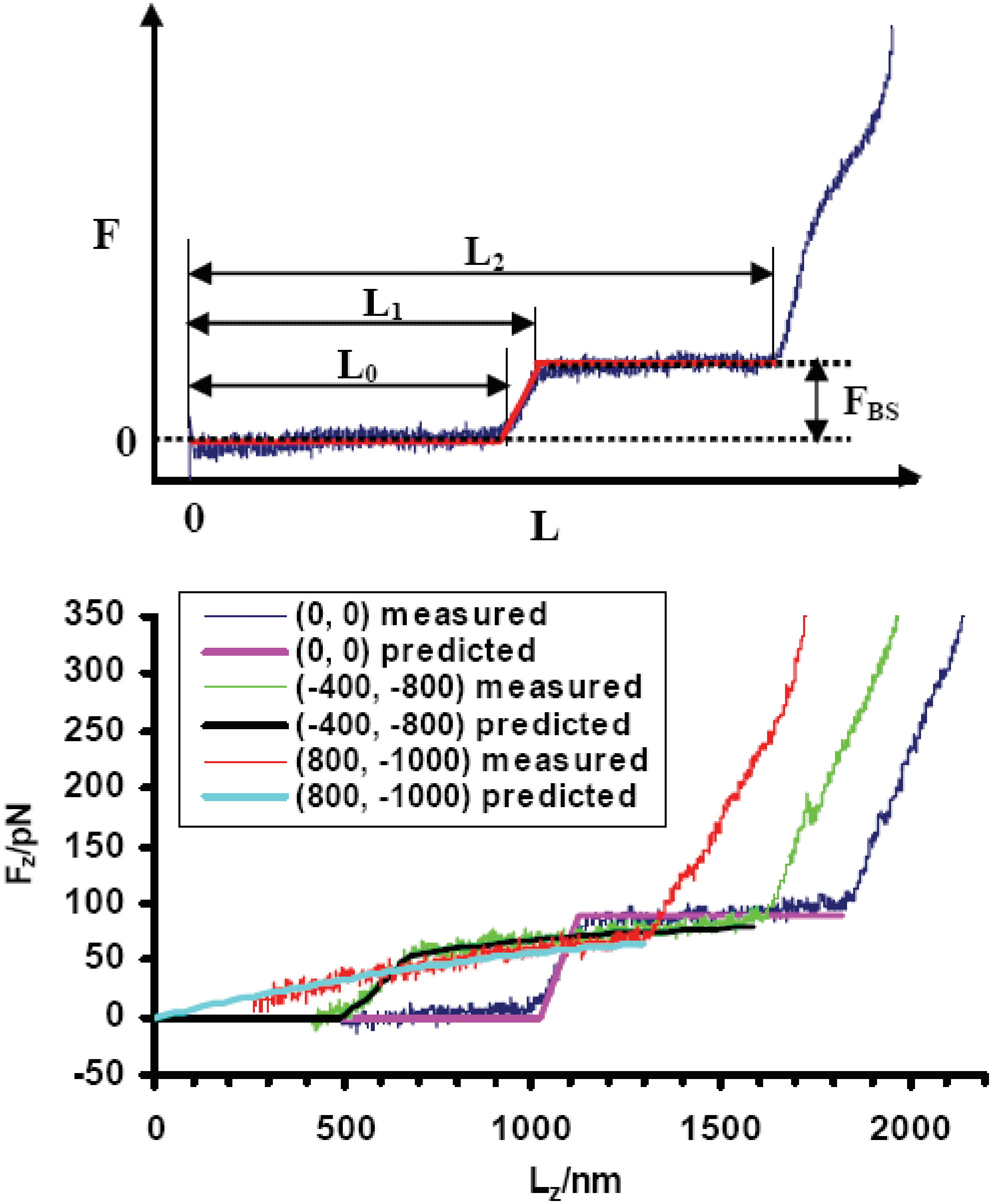
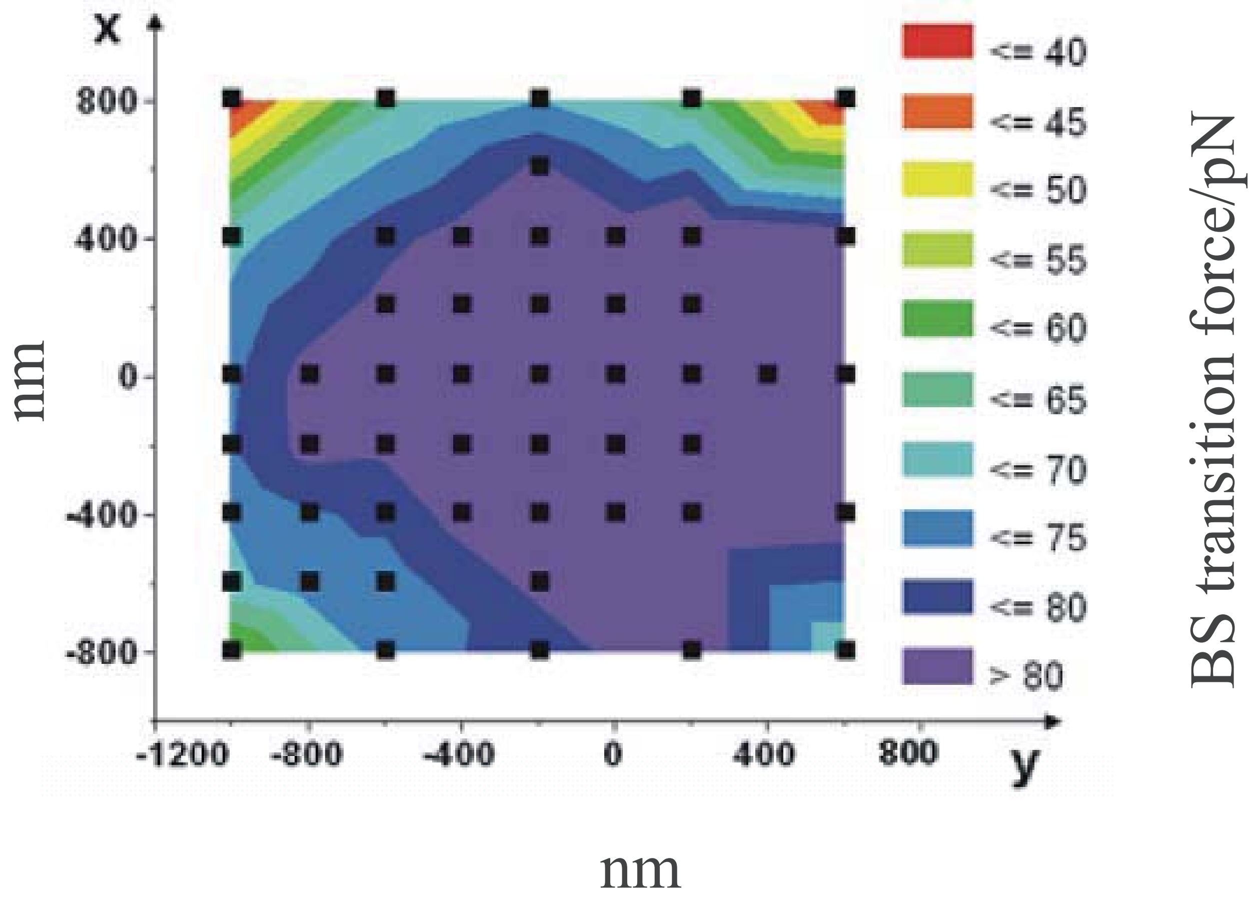


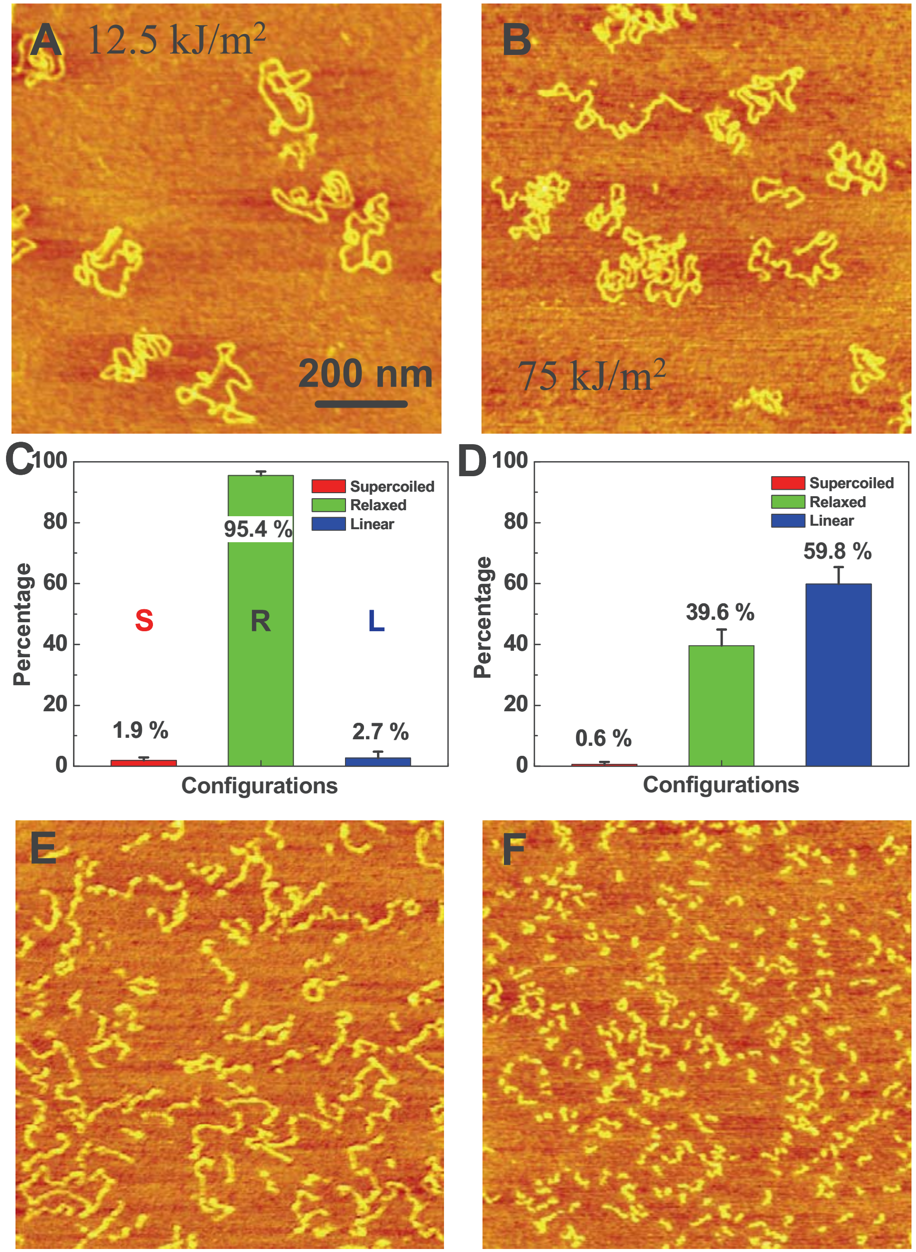
FIGURE 1 Schematic diagram of possible pulling situations in AFM-SMFS. (a) ideal situation; (b) general situation.


F
L2
L1
L0
0
FBS
0
L
(0, 0) measured
(0, 0) predicted
(-400, -800) measured
(-400, -800) predicted
(800, -1000) measured
(800, -1000) predicted
350
300
250
200
150
100
50
0
-50
Fz/pN
0
500
1000
1500
2000
Lz/nm



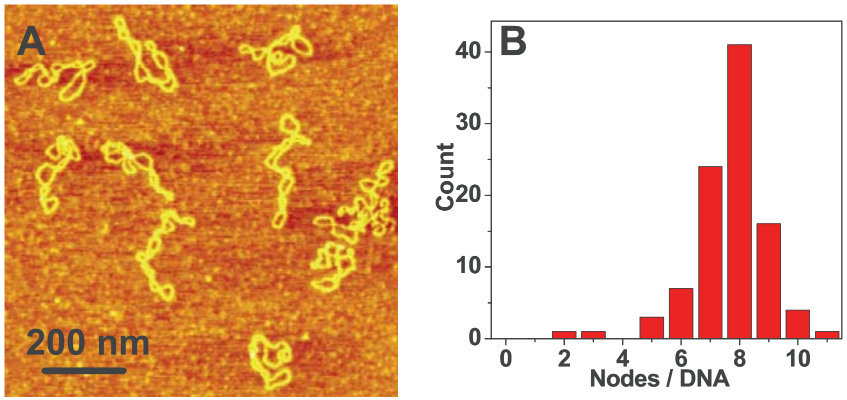
Biophys J BioFAST, published on February 26, 2007 as doi:10.1529/biophysj.107.104901

pUC18
Damaged by UVC



150 kJ/m2

600 kJ/m2



Intact DNA
(puc18)

T4 Endonuclease V

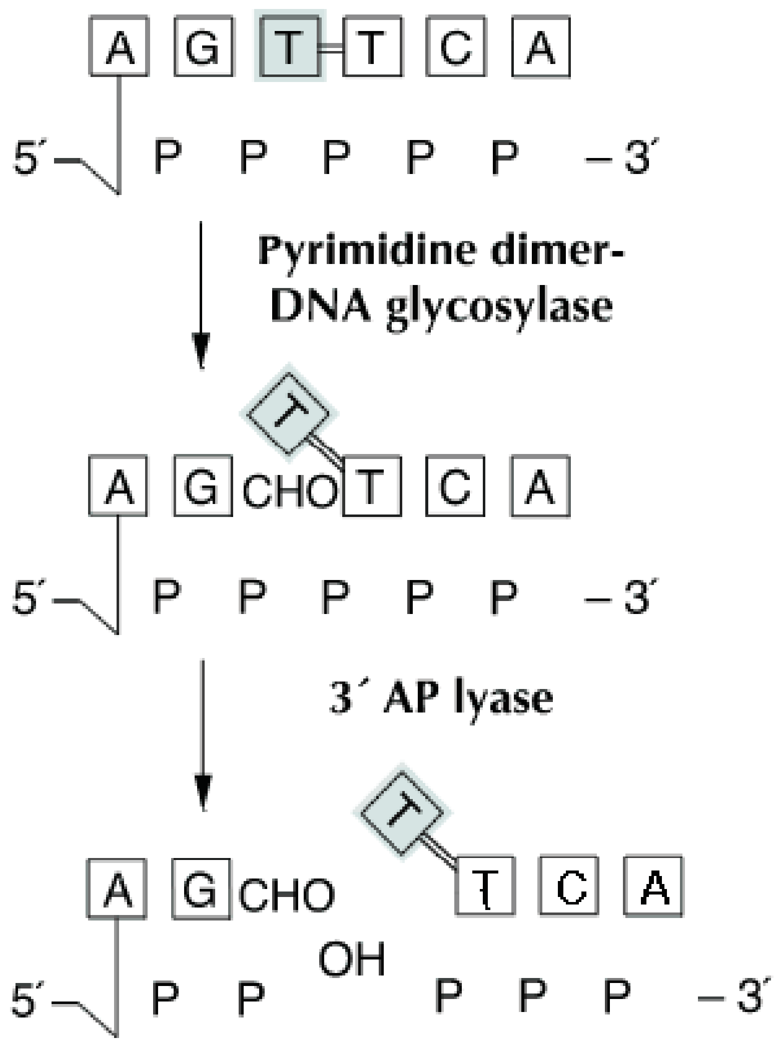
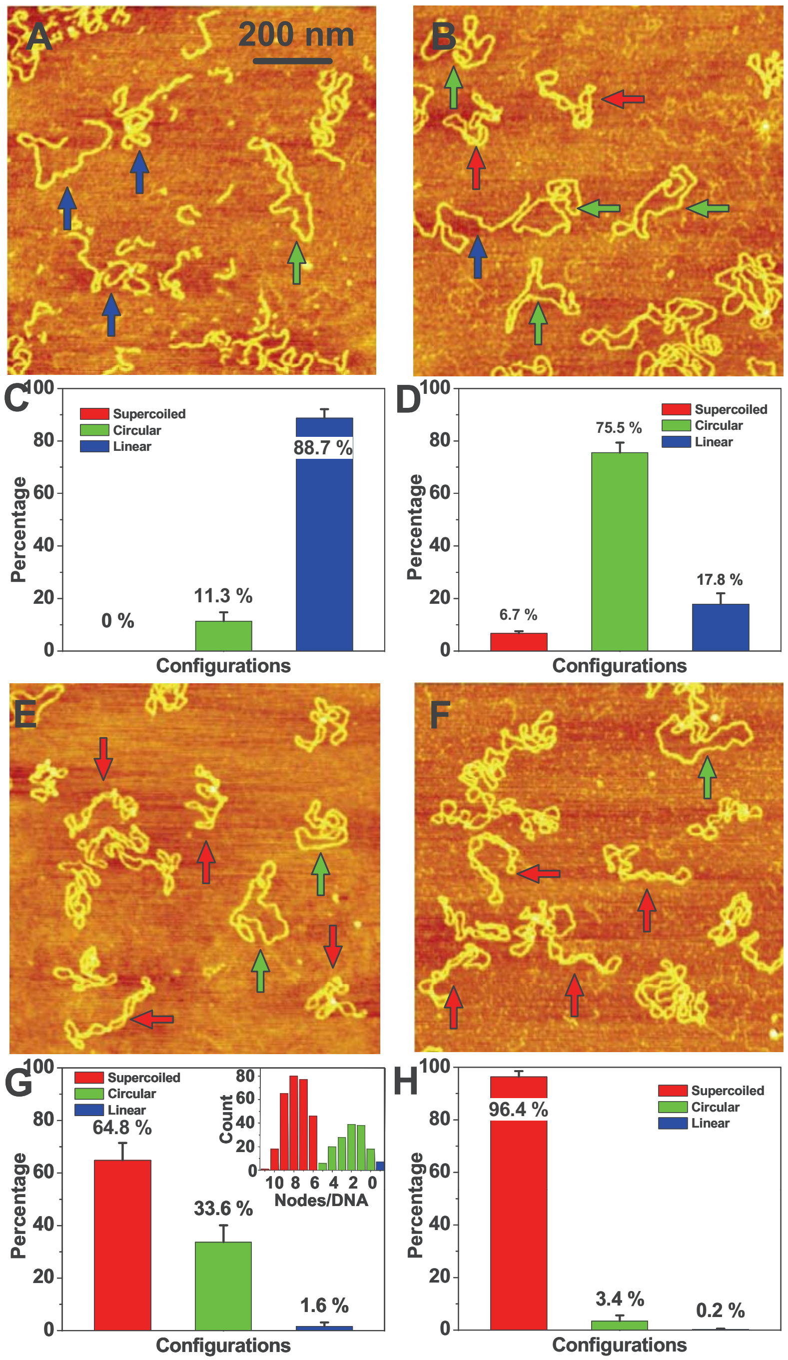


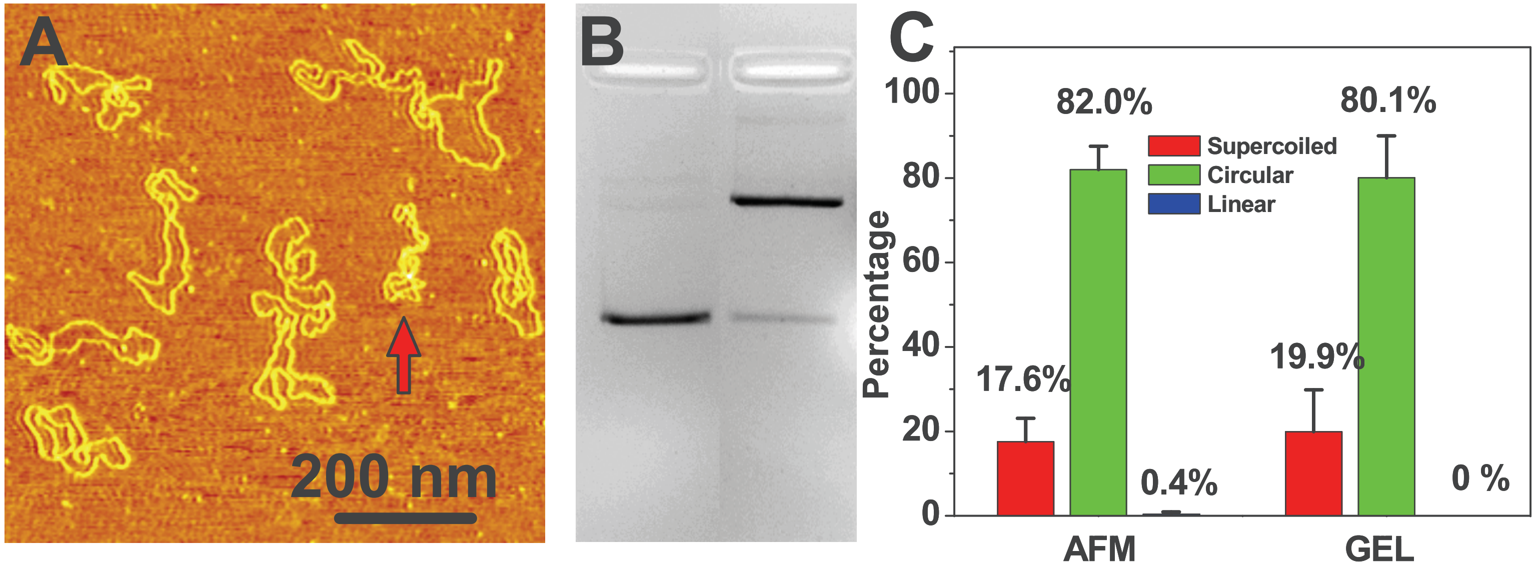
Fig. 1. Mechanism of pyrimidine dimer cleavage by T4 Endonuclease V. (Adapted with permission from Friedburg, E.C. et al. (1995) DNA Repair and Mutagenesis, ASM Press.)

pUC18+UVB+T4 EndoV



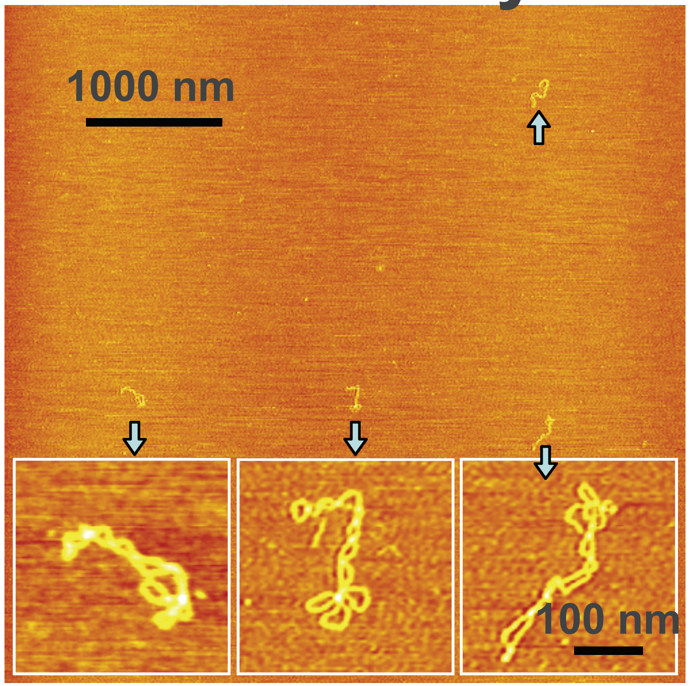
AFM images of pUC18 molecules irradiated with different doses of UVB radiation and incubated with T4 Endonuclease V. (A) 1.4 kJ/m^2-irradiated pUC18 (B) 229 J/m^2-irradiated pUC18 (E) 29 J/m^2-irradiated pUC18 (F) intact pUC18 (control experiment).

Comparison of AFM-based methodology with gel electrophoresis



UVB:135 J/m^2

Sensitivity



Detecting Ultraviolet Damage in Single DNA Molecules by Atomic Force Microscopy

Jiang, Ke, Mieczkowski, Marszalek
Biophysical Journal 93, 1758–1767 (2007).

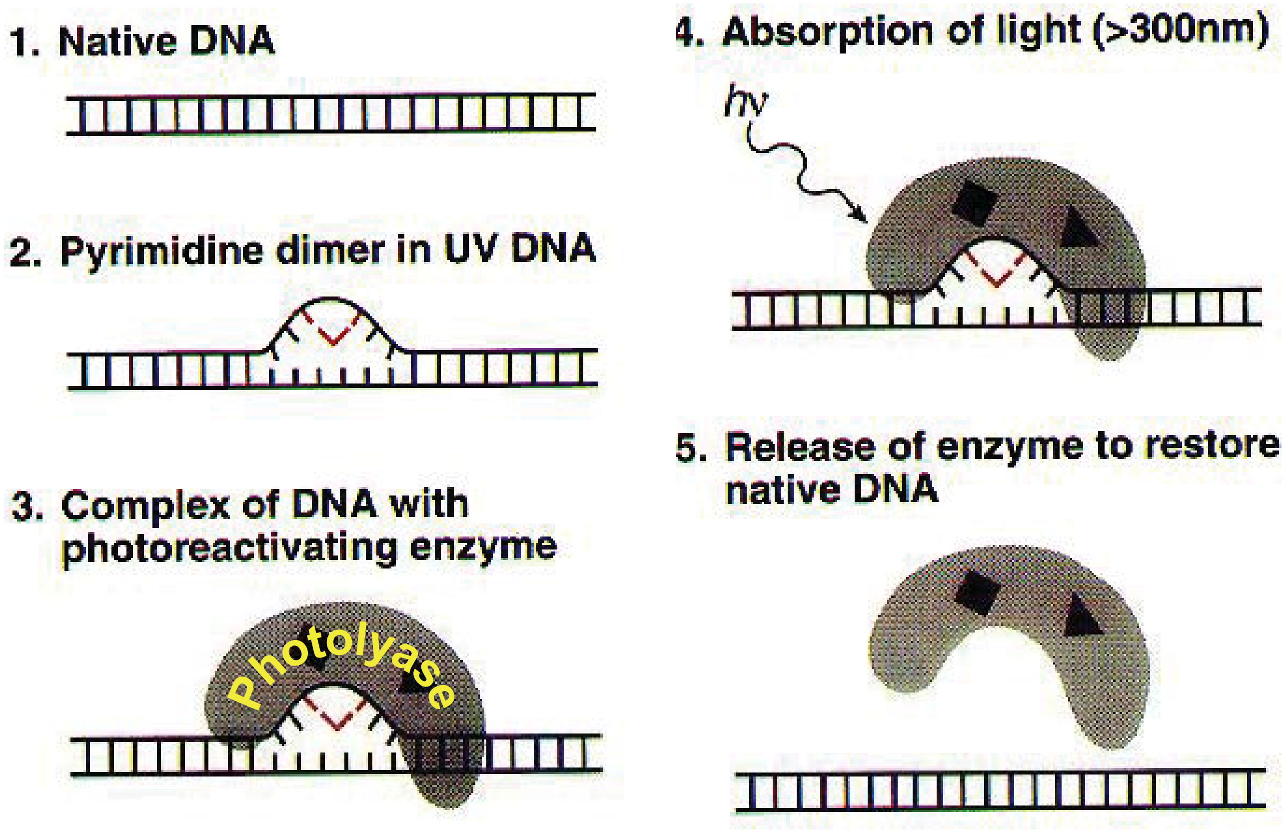
TABLE 1 Comparison of DNA detection sensitivity by gel electrophoresis and by AFM

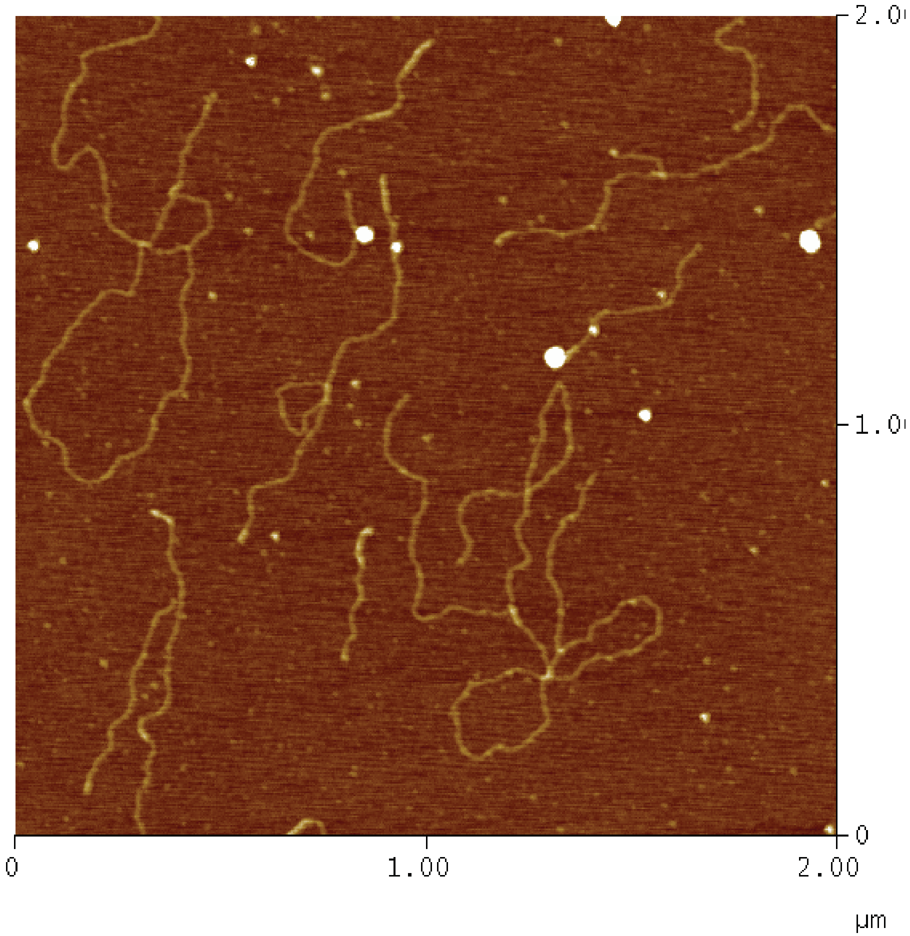
	Gel Electrophoresis	AFM
Minimum amount of DNA	~400 pg per lane (67)	~1 pg (total)
Sensitivity	~25 pg per band (67), (equivalent to 8.5×10^6 pUC18 molecules)	Single molecules

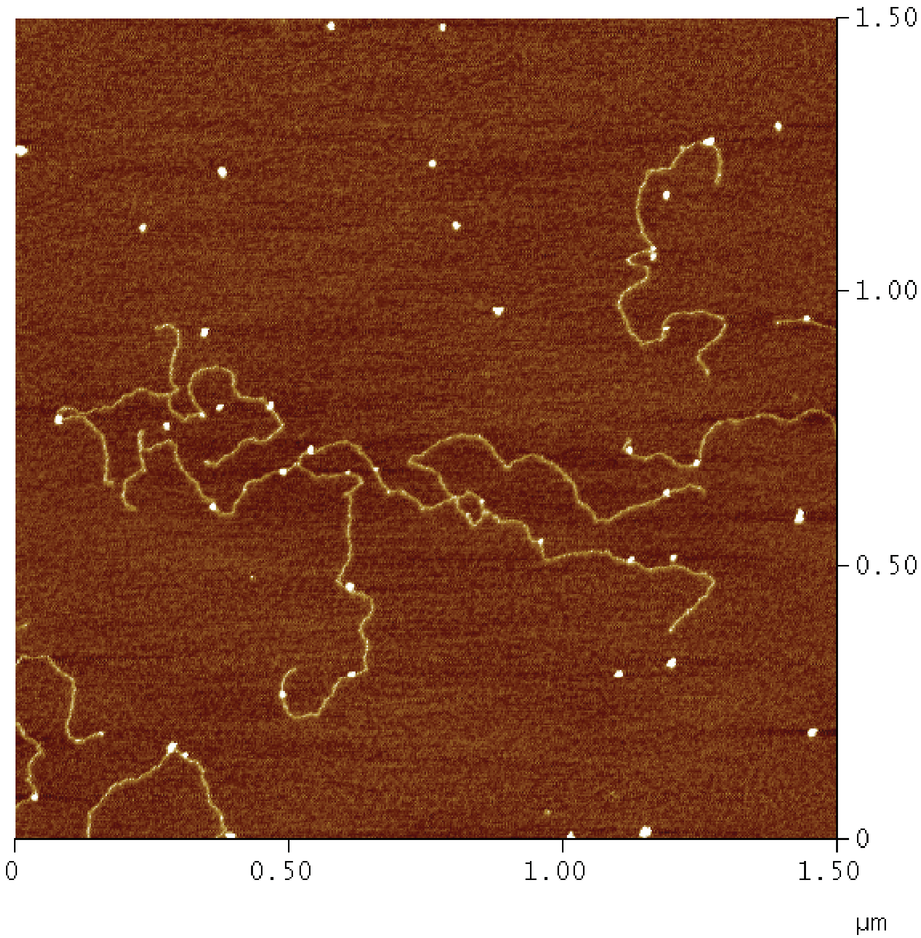
poly(dA)poly(dT)

……A-A-A-A-A-A-A-A-A……
……T-T-T-T-T-T-T-T-T-T……

Photolyase
54 kDa



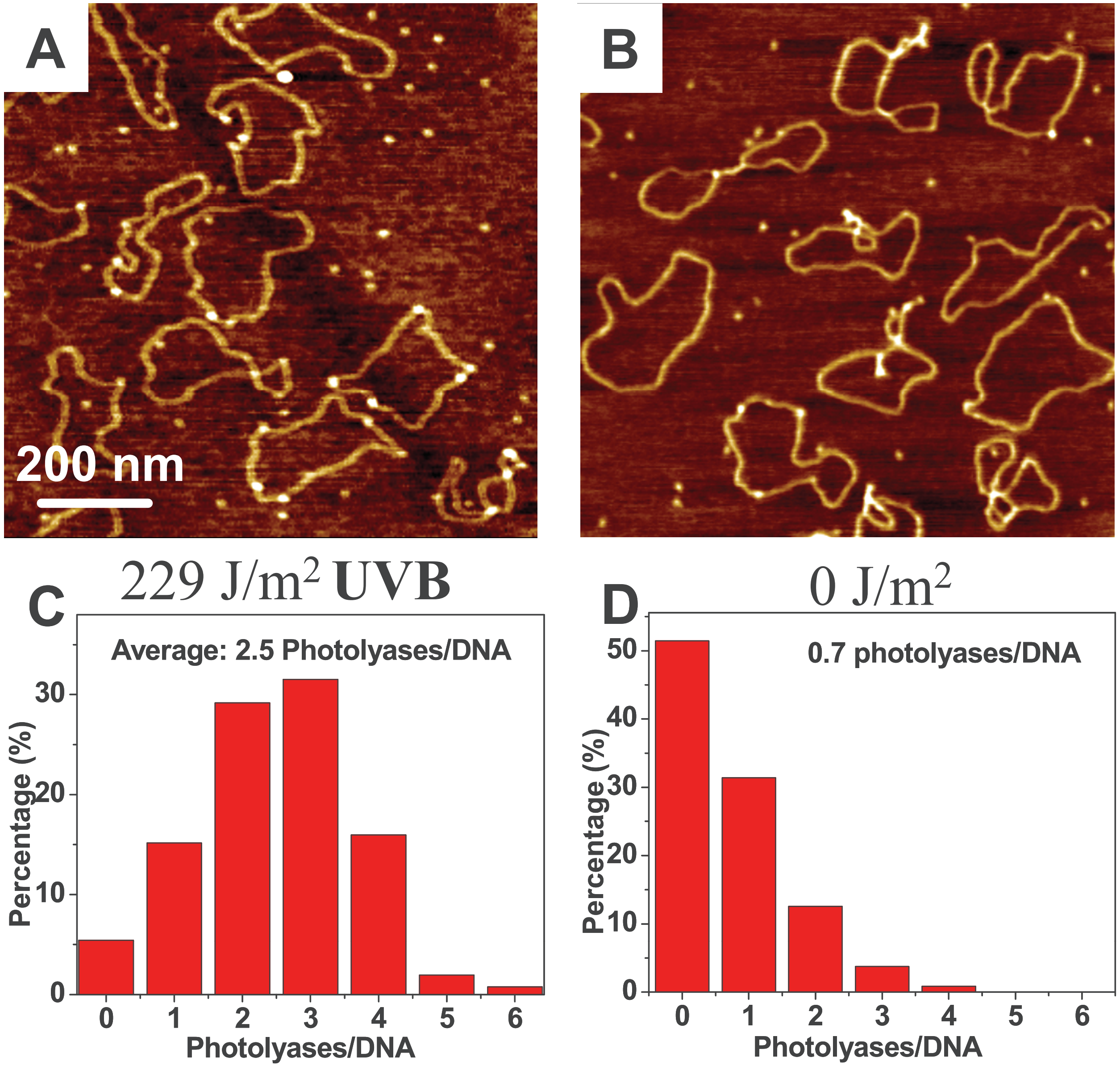


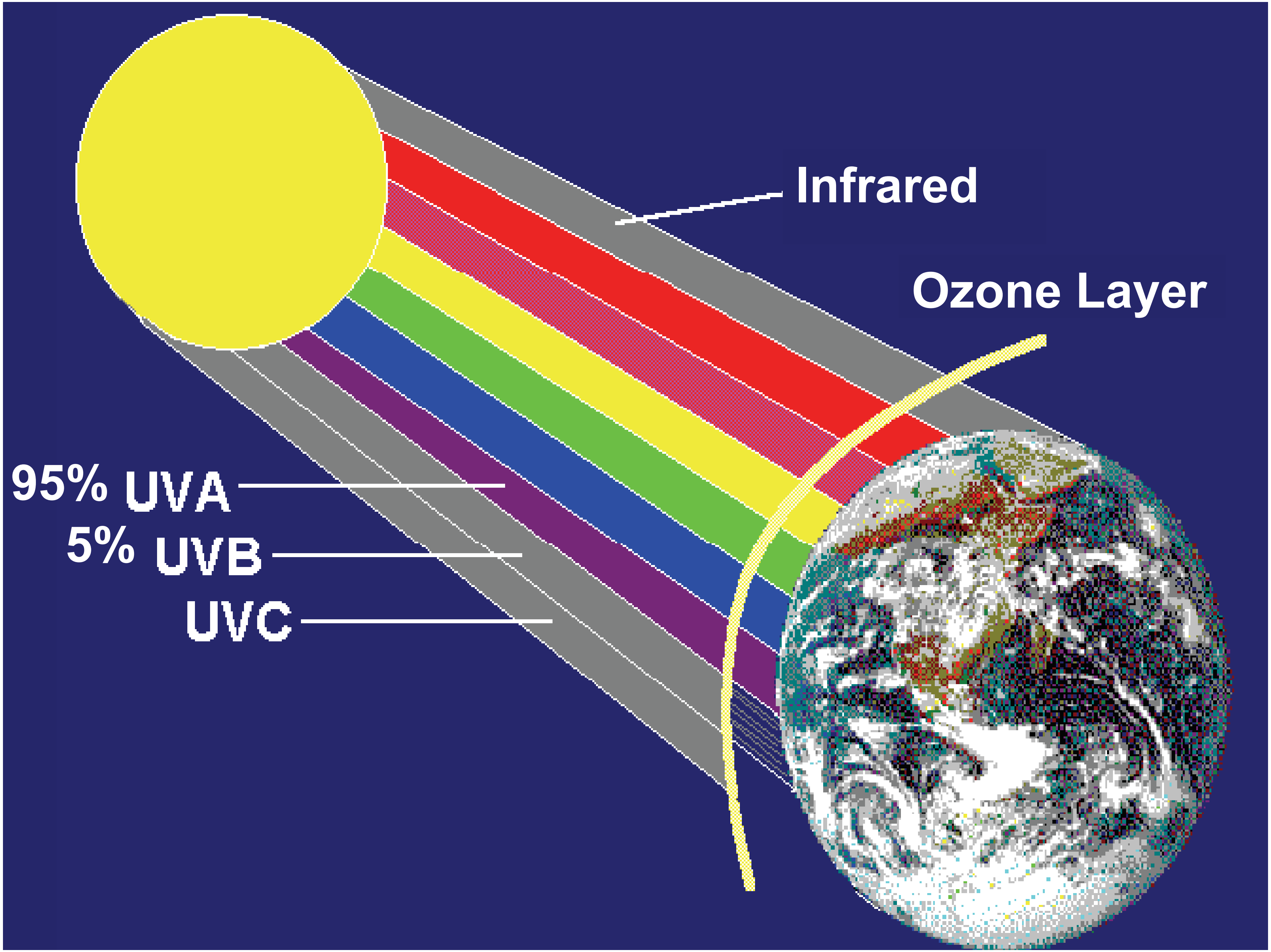


Poly(dA)poly(dT),NO UV with photolyase 0.6 enzyme/DNA

Poly(dA)poly(dT),10min UV with photolyase 1.8 enzyme/DNA

Photolyase binds to the CPD sites of pUC18




Infrared
Ozone Layer
95% UVA
5% UVB
UVC

Recent findings

Cyclobutane pyrimidine dimers are predominant DNA lesions in most cells exposed to UVA radiation

Donors	8-oxodGuo	T<>T	Ratio T<>T/ 8-oxodGuo
F	0.0085 ± 0.0064	0.057 ± 0.007	6.7
G	0.0080 ± 0.0030	0.077 ± 0.009	9.6
H	0.0087 ± 0.0039	0.061 ± 0.004	7.0
I	0.0066 ± 0.0024	0.050 ± 0.005	7.6
J	0.0081 ± 0.0040	0.040 ± 0.003	5.0
K	0.0023 ± 0.0036	0.112 ± 0.004	48.8
Mean	0.0071 ± 0.0025	0.066 ± 0.025	9.4

The results (expressed in lesions per 10^6 normal bases per Joules per centimeter squared) represent the slope ± SE of the linear regression for 8-oxodGuo or T<>T with respect to the applied UV dose for each donor. The mean is average ± SD.

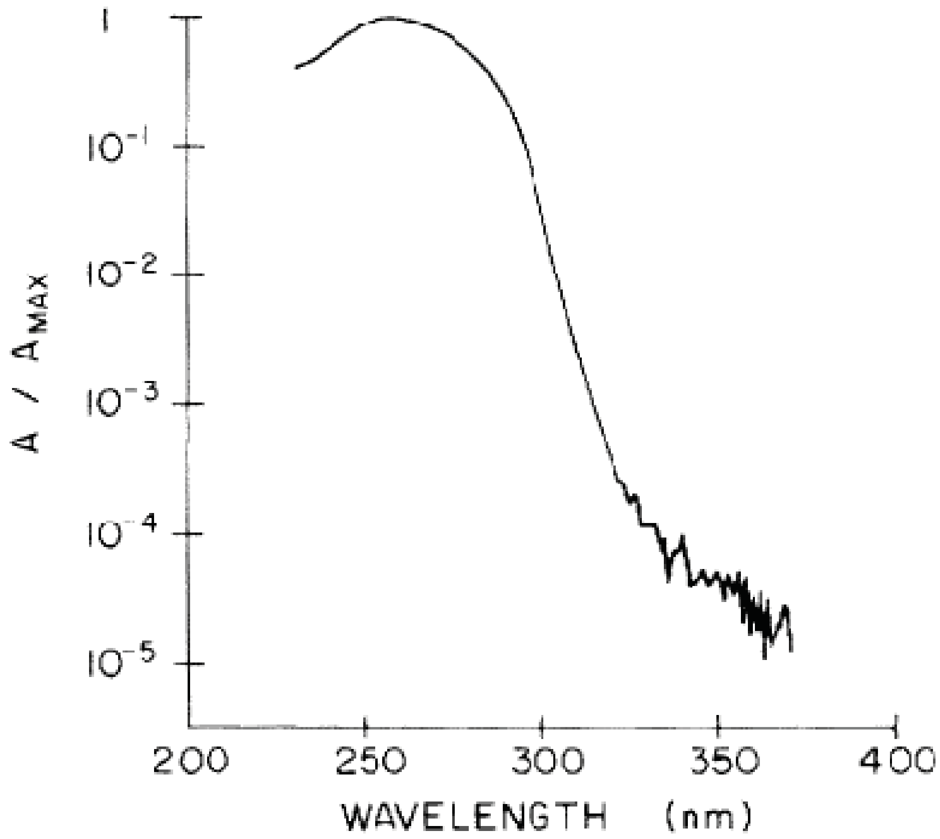
Mouret S, et al. (2006) PNAS 103, 13765

Absorption Spectrum of DNA for Wavelengths Greater than 300 nm

JOHN CLARK SUTHERLAND AND KATHLEEN PIETRUSZKA GRIFFIN

RADIATION RESEARCH 86, 399–409 (1981)

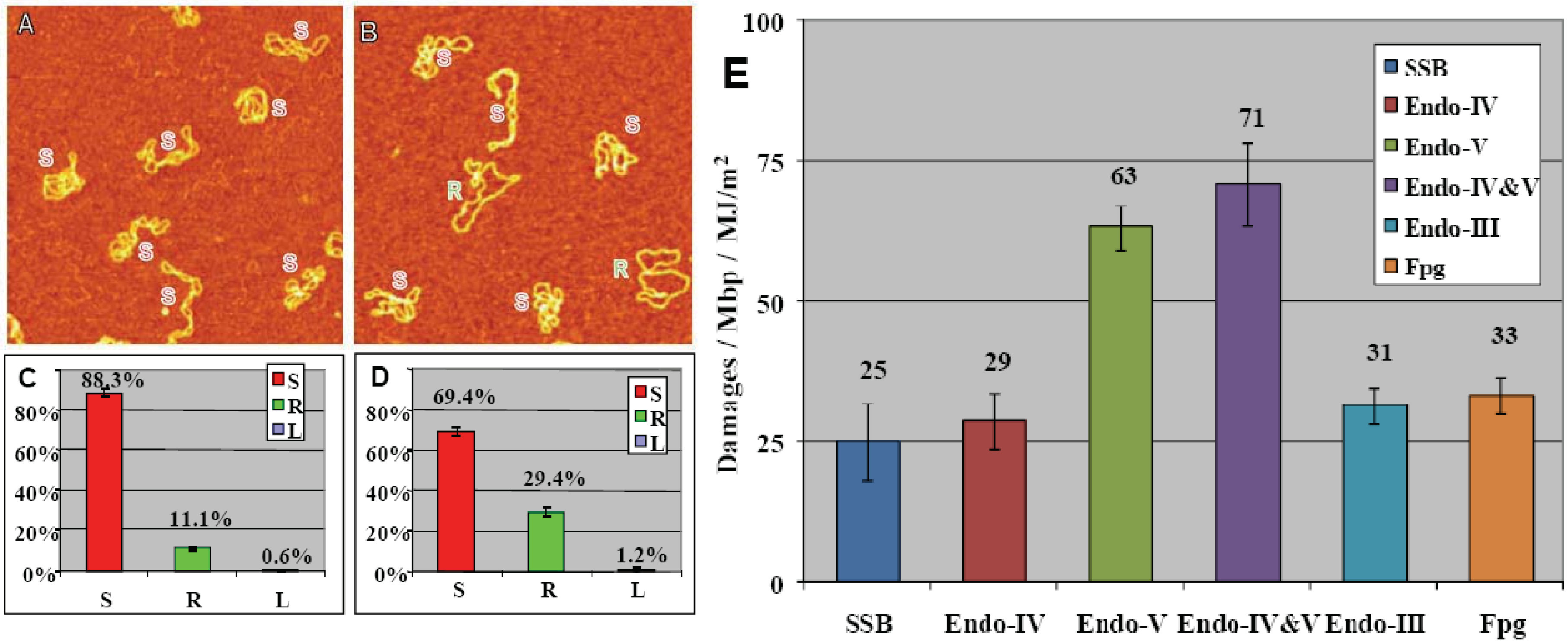
DNA ABSORPTION SPECTRUM ABOVE 300 nm



Damage-specific enzymes

Enzymes for damage detection	Supplier	Specific damages these enzymes can detected	Enzyme's activity to the damages
E. coli endonuclease IV	New England Biolabs	apurinic/apyrimidinic site base paired with adenine	100%
		5,6-dihydrothymine	<10%
T4 endonuclease V	New England Biolabs and Epicentre	cyclobutane pyrimidine dimers	100%
		apurinic/apyrimidinic sites	100%
E. coli endonuclease III	Trevigen	thymine glycol	100%
		apurinic/apyrimidinic sites	100%
		5,6-dihydrothymine	<10%
E. coli Fpg	Trevigen	8 oxoguanine base paired with a cytosine or guanine	100%
		apurinic/apyrimidinic site base paired with adenine	<10%
		5,6-dihydrothymine	<10%
E. coli Photolyase	Trevigen	cis-syn cyclobutane pyrimidine dimers	
Anti-Thymine Dimer Antibody, clone KTM53	Kamiya Biomedical Company	Thymine dimers	

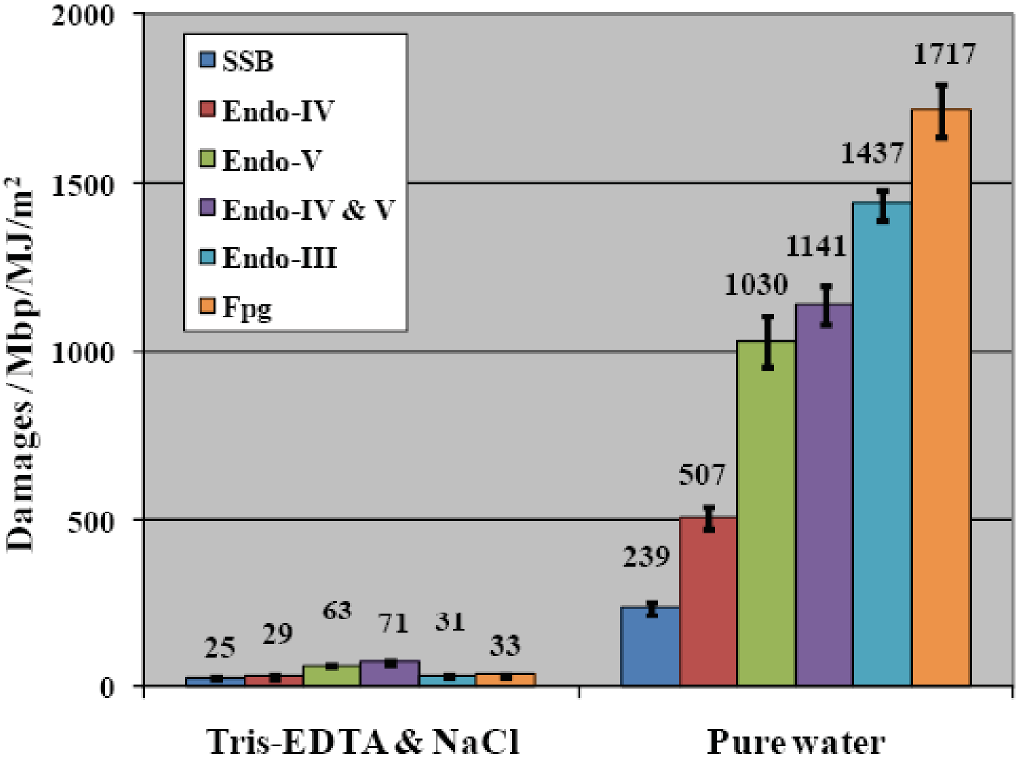
DNA dialyzed in Tris-HCl, buffer and irradiated in the same solution by UVA

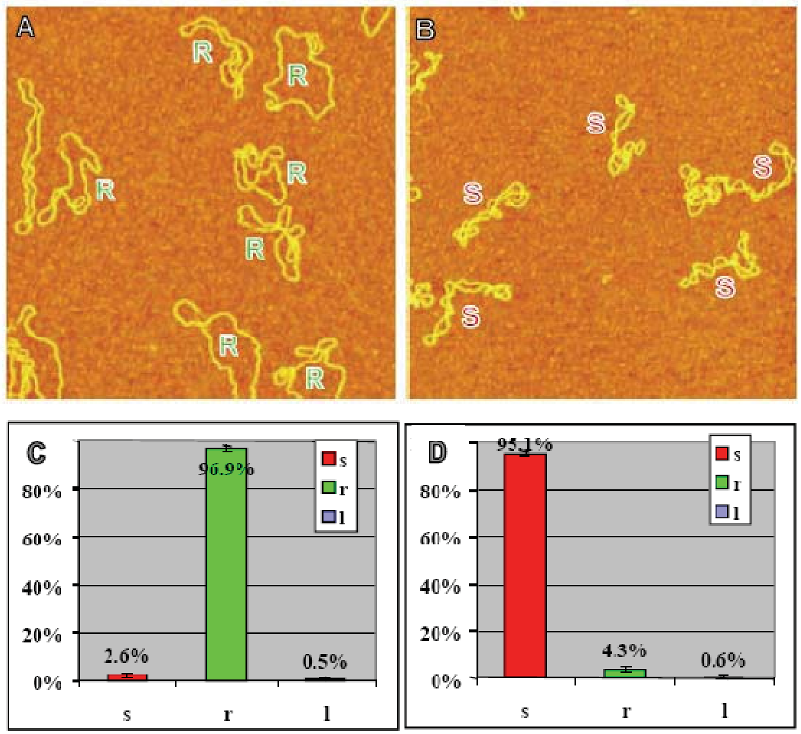


M1 Figure 1. AFM images on APS-mica (42) of different pUC18 DNAs that were subjected to 1.3 MJ/m2 UVA radiation and different enzyme treatments prior to imaging. DNA was dialyzed in 10 mM Tris-HCl, 1 mM EDTA and 100 mM NaCl buffer and irradiated in the same solution by UVA. After that the sample was diluted back to the suitable buffer for different enzyme incubation: (A) no enzyme treatment as control, (B) first E. coli endonuclease IV and then T4 endonuclease V. Scan size in all the images is 1 x 1 μm2. (C, D) are histograms of the occurrence of various configurations of pUC18 plasmids determined from the AFM images such as these shown in (A and B). Color code: red, supercoiled DNA (S); green, relaxed circular plasmids (R); blue, linear DNA (L). The error bars in the figures represent the standard deviation. Each histogram is based on 600-1000 DNA molecules from 30–36 AFM images. (E) Histogram summarizes the number of different damages per million base pairs per MJ/m2 after UVA irradiation and specific enzyme treatments. The values shown in the histogram represent averages from 2-5 separate experiments.

Monika, 5/2/2008

pUC18 dialyzed against Millipore water and irradiated in pure water by UVA

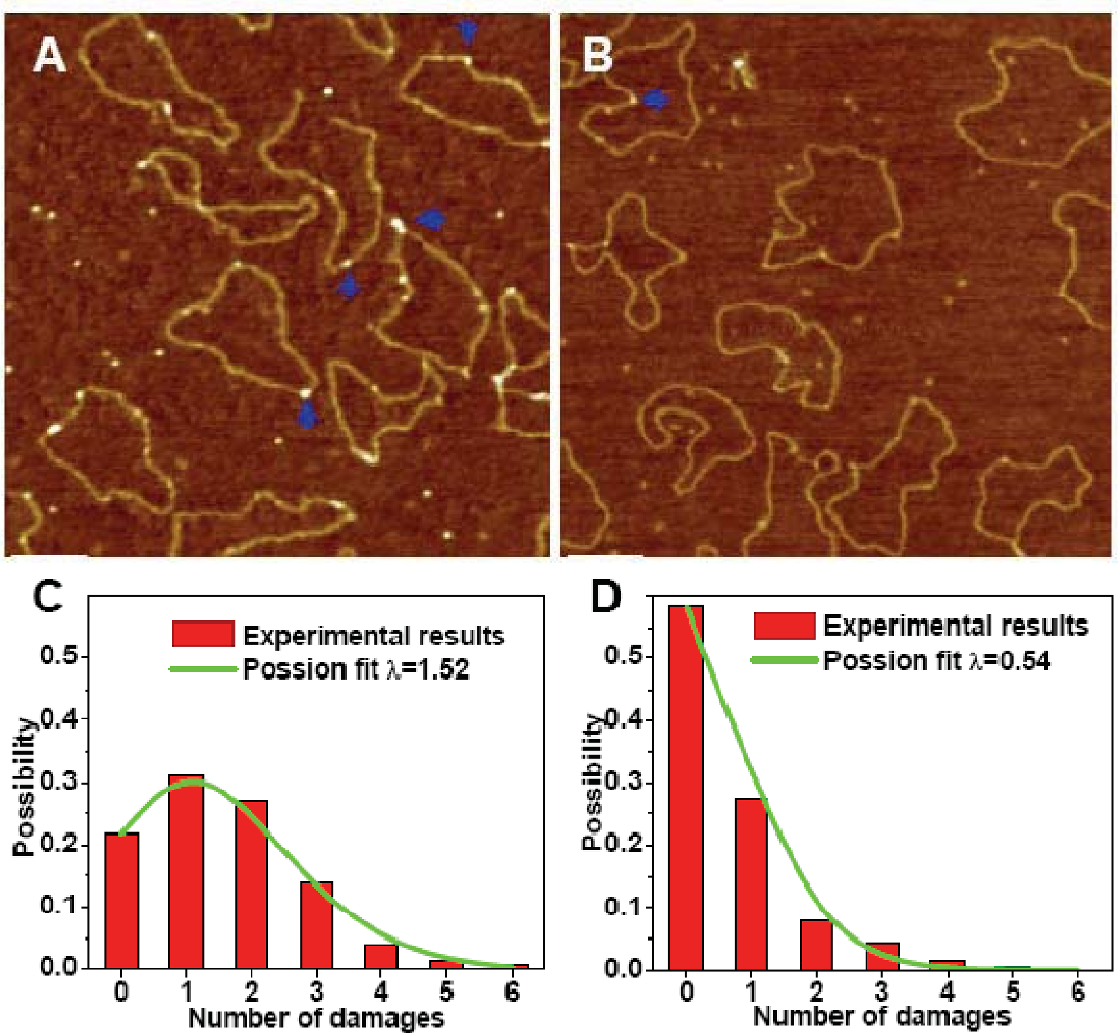




In pure water

Transferred back to Tris/NaCl

pUC18 irradiated in Millipore water and incubated with photolyase

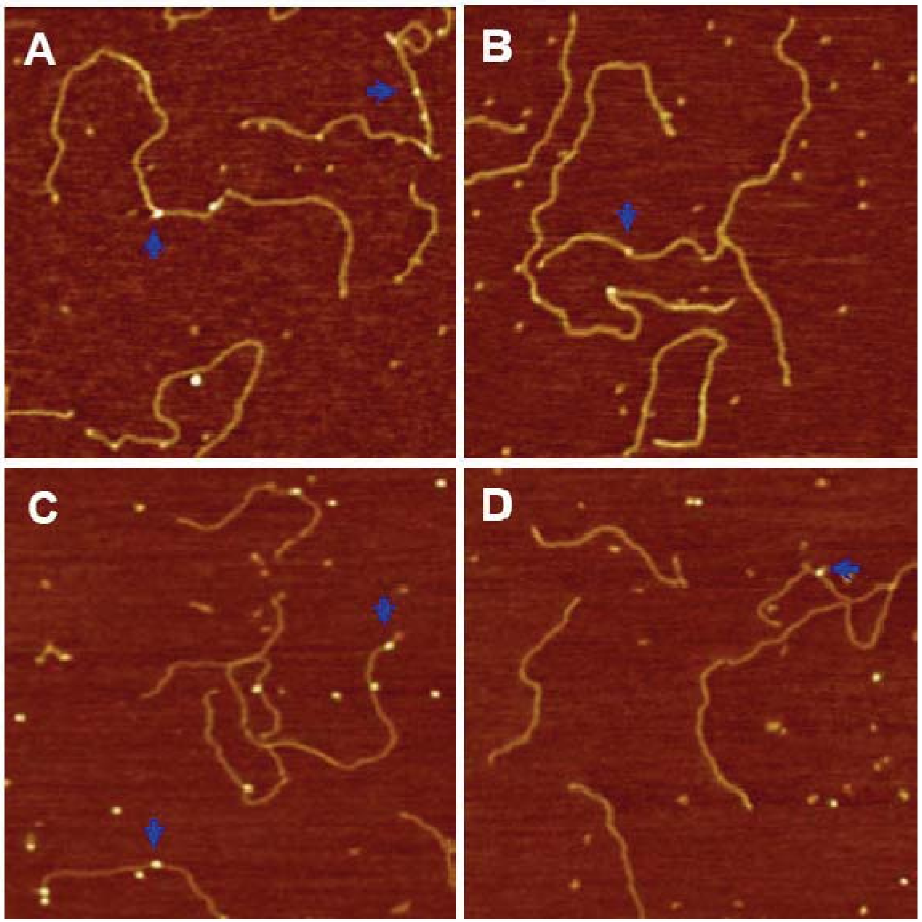


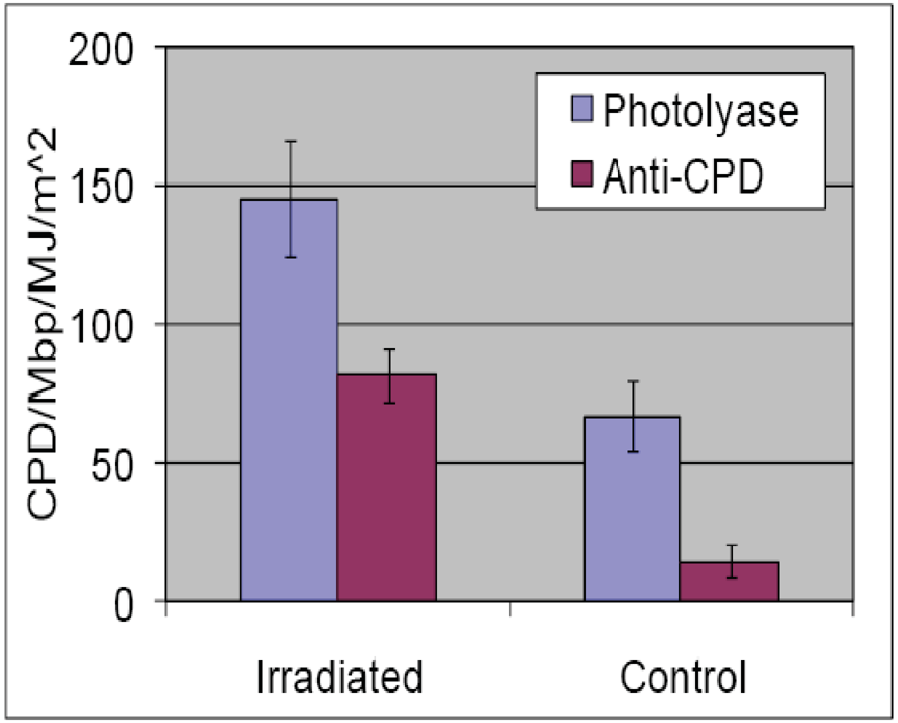
M2

M2 Figure 3. AFM images show photolyases binding to the CPD sites of pUC18 (some of them marked by blue arrows) with (A) 1 MJ/m2 UVA radiation and (B) no UVA radiation. Irradiation was performed on dialyzed plasmids suspended in pure water. Scan size in all the images is 1 x 1 µm2. (C-D) Histograms show the distribution of photolyase on pUC18 molecules as shown in A and B. The curves show the Poisson distribution fits which give the average damage l=1.52/plasmid for the UVA radiated DNA and 0.54 for control DNA, respectively.

Monika, 5/2/2008

UVA-irradiated poly(dA)Poly(dT) incubated with photolyase and with anti-CPD antibodies

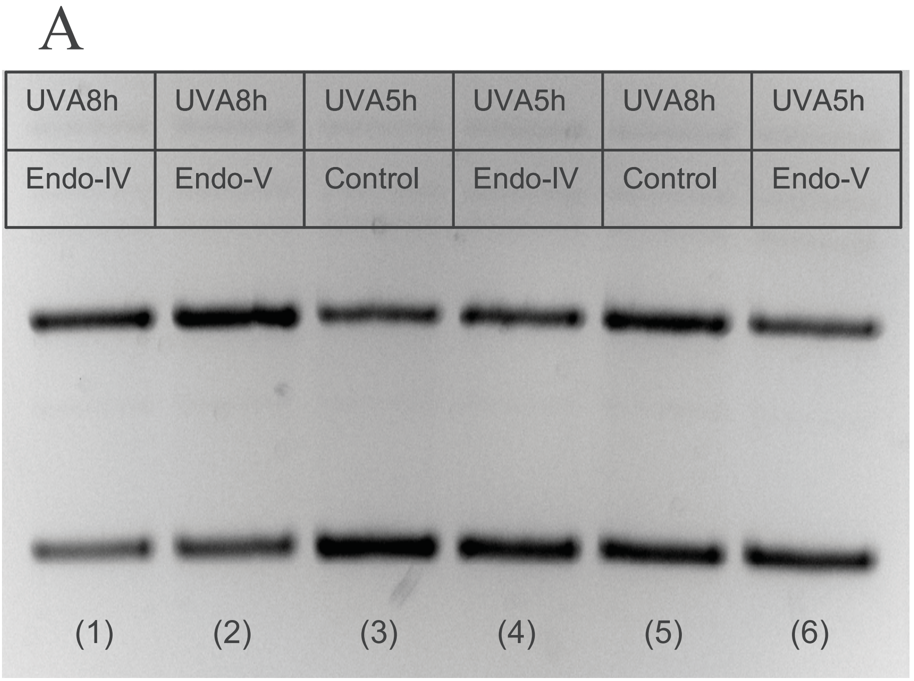


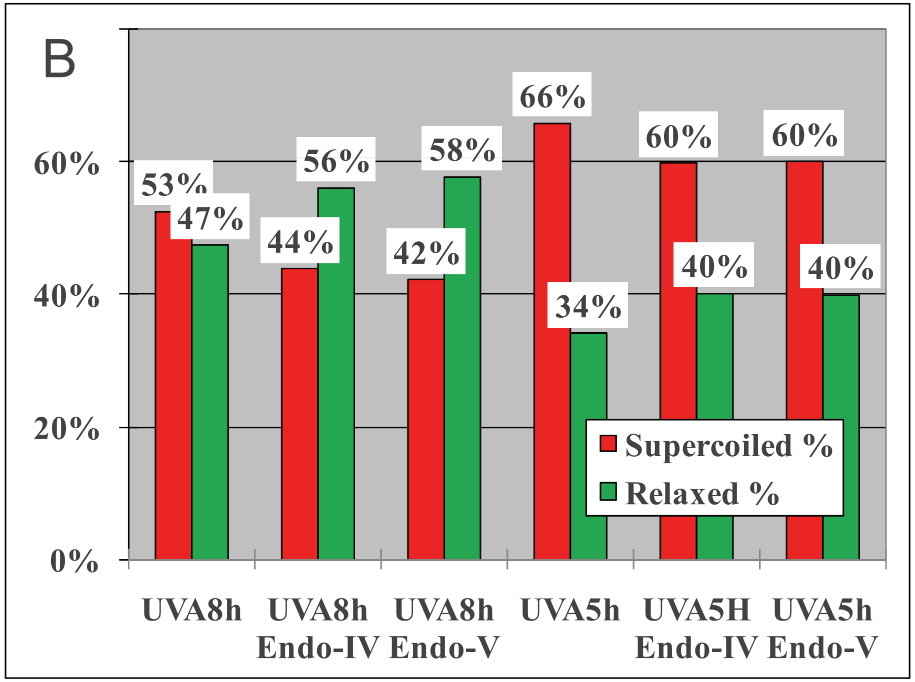


M3 Figure 4. (A-B) AFM images show photolyases binding to the CPD sites of poly(dA)-poly(dT) with (A) 6 MJ/m2 UVA radiation, and (B) no UVA radiation. (C-D) AFM images show anti-thymine dimer antibodies binding to the CPD sites of poly(dA)-poly(dT) with (C) 6 MJ/m2 UVA radiation, and (D) no UVA radiation as control. Scan size in all the images is 1 x 1 µm2. Histograms compare the damages detected by photolyase and anti-thymine dimer antibody on UVA irradiated poly(dA)-poly(dT) and intact poly(dA)-poly(dT).

Monika, 5/2/2008

Separating UV-damaged and enzyme-treated DNA by gel electrophoresis





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UVA Generates Pyrimidine Dimers in DNA Directly

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