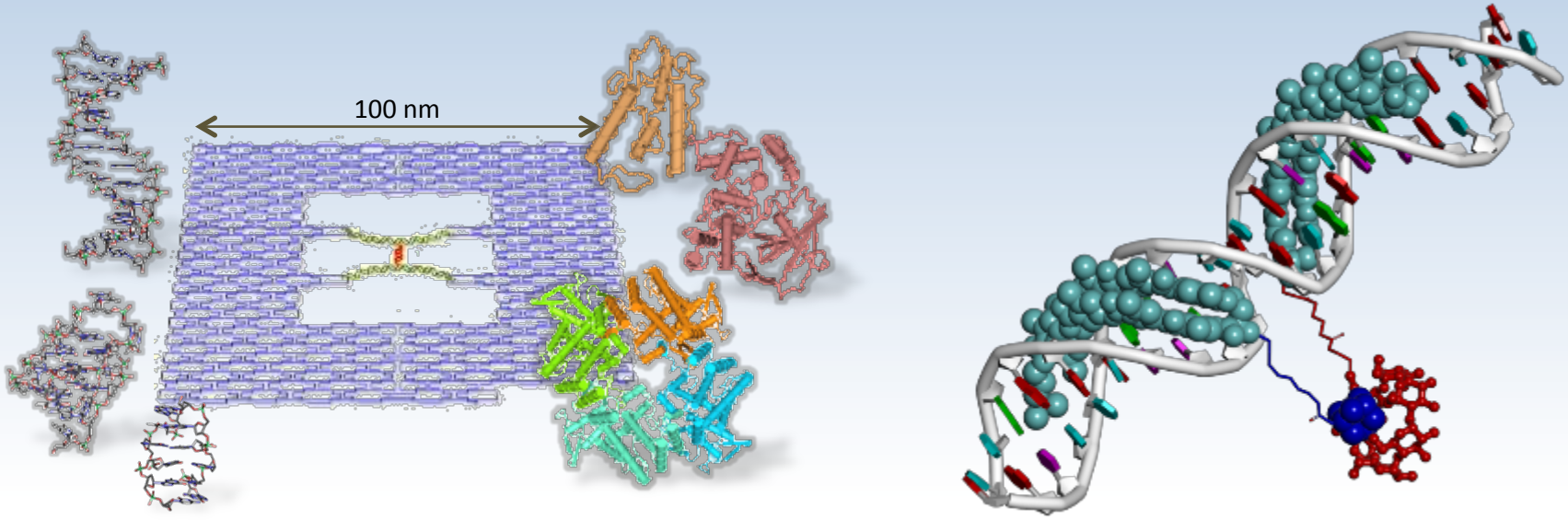
生体分子機能論 2018-3
3次元構造

Advanced Course in Molecular Biology and Biochemistry



Hiroshi Sugiyama
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Institute for Integrated Cell-Material Sciences (iCeMS)
Kyoto University

Basics

1 Basic elements of nucleic acids and their synthesis
2 Sequencing of DNA
3 3D structure of DNA 1
4 3D structure of DNA 2

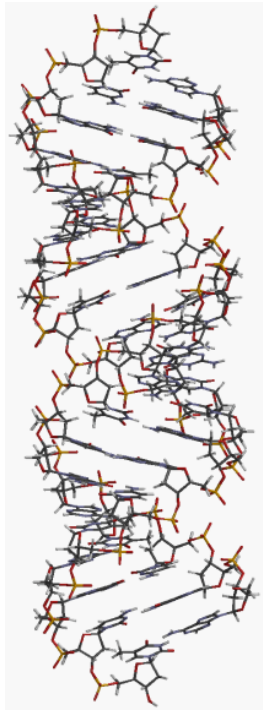
Chemistry

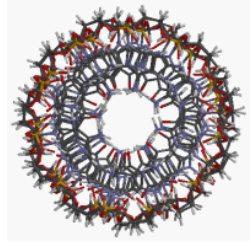
5) DNA alkylation
6) Hydrogen abstraction 1
7) Hydrogen abstraction 2
8) Charge transfer

Biology

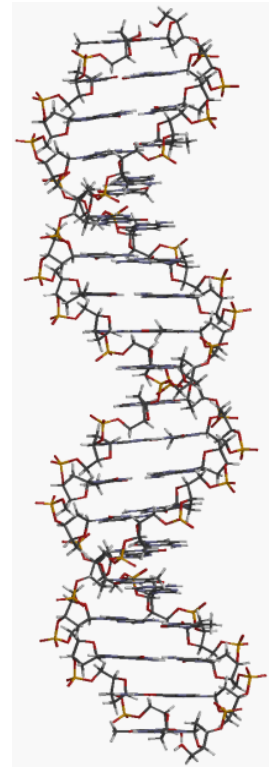
9) Epigenetics 1
10) Epigenetics 2
11) ATRX

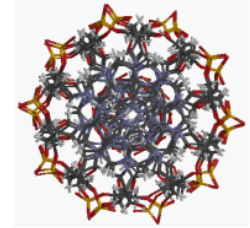
DNA Structures



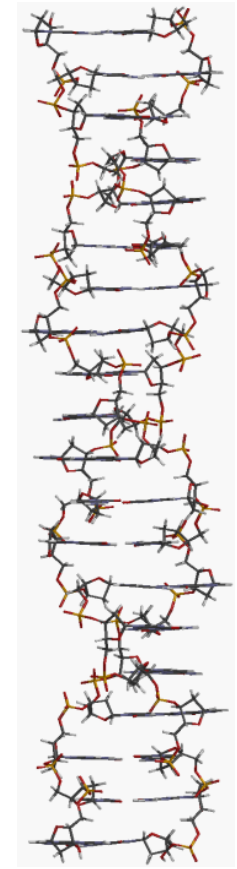


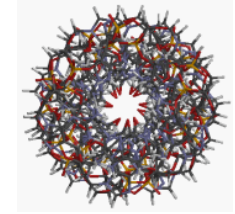
A-DNA





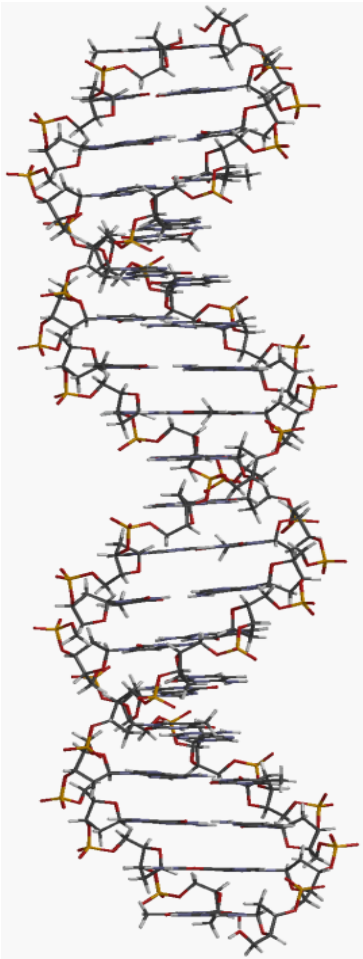
B-DNA

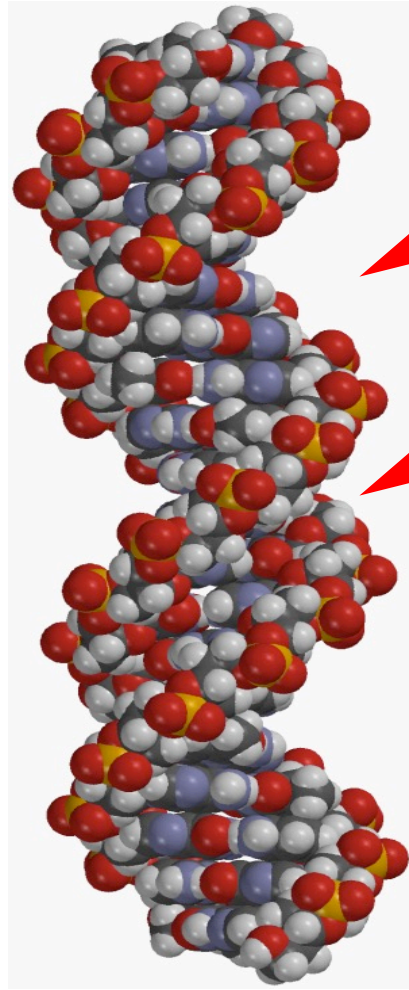




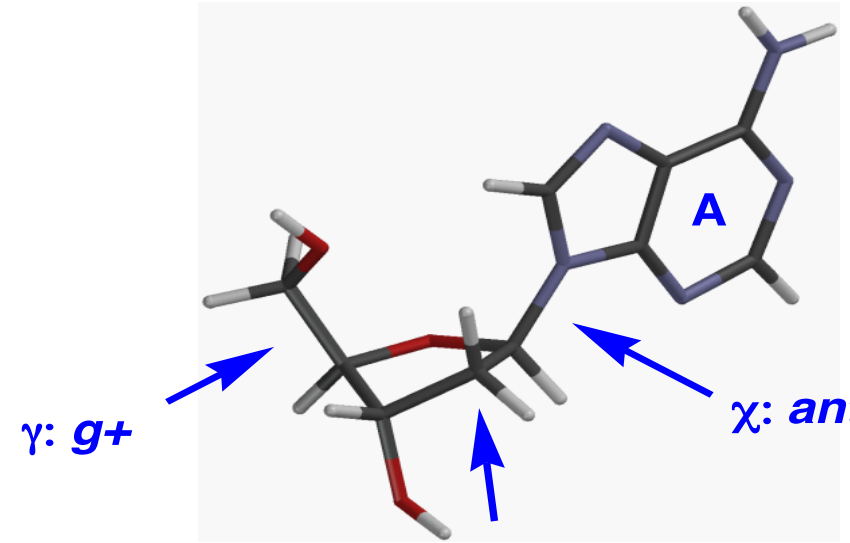
Z-DNA

B-DNA



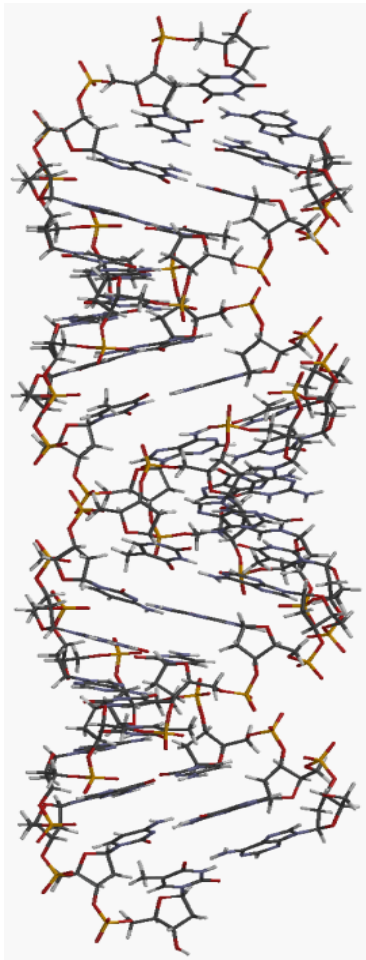


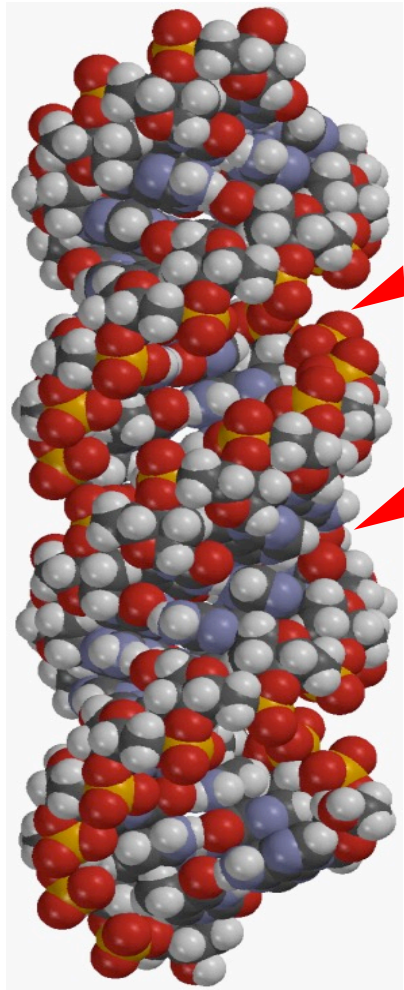
	Groove width (Å)	Groove depth (Å)
Major groove	11.7	8.8
Minor groove	5.7	7.5



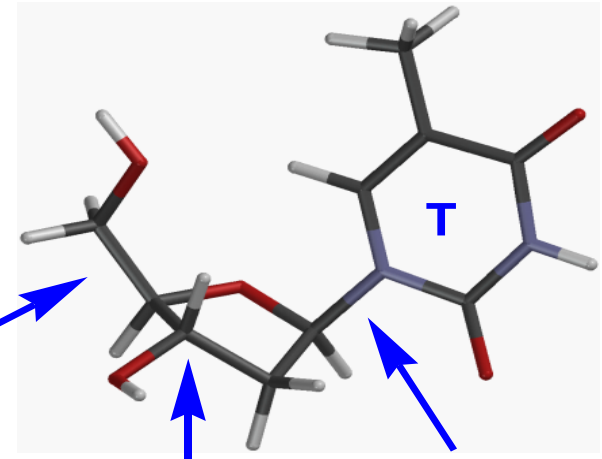
Structure sense: Right-handed
Residues per turn: 10

A-DNA





	Groove width (Å)	Groove depth (Å)
Major groove	2.7	13.5
Minor groove	11.0	2.8



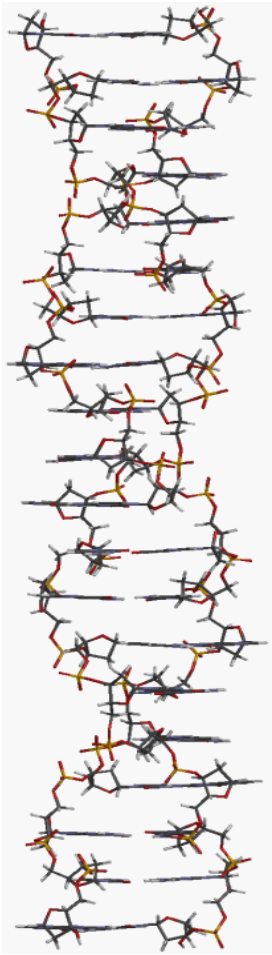
γ: *g+*

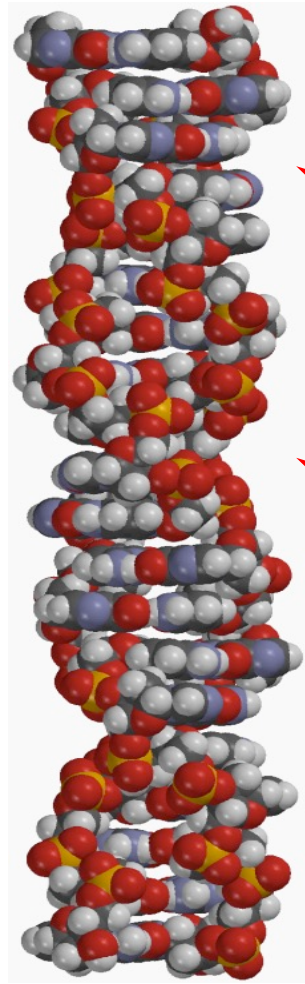
χ: *anti*

C3'-endo

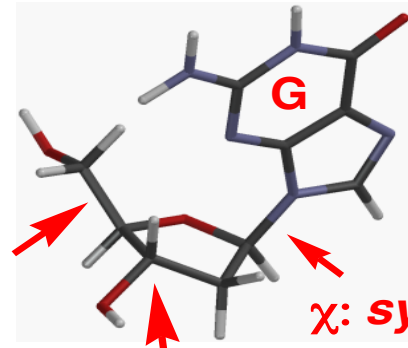
Structure sense: Right-handed
Residues per turn: 11

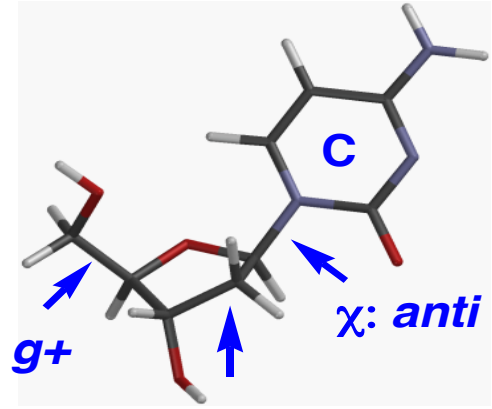
Z-DNA





	Groove width (Å)	Groove depth (Å)
Minor groove	2.0	13.8
Major groove	8.8	3.7





Structure sense: Left-handed
Residues per turn: 12

Nature 1979, 282, 680

Molecular structure of a left-handed
Double helical DNA fragment at atomic resolution

Wang AH, Quigley GJ, Kolpak FJ, Crawford JL, van Boom JH, van der Marel G, Rich A

Nature Review Genetics 2003, 4, 566

Z-DNA: the long road to biological function

Alexander Rich and Shuguang Zhang

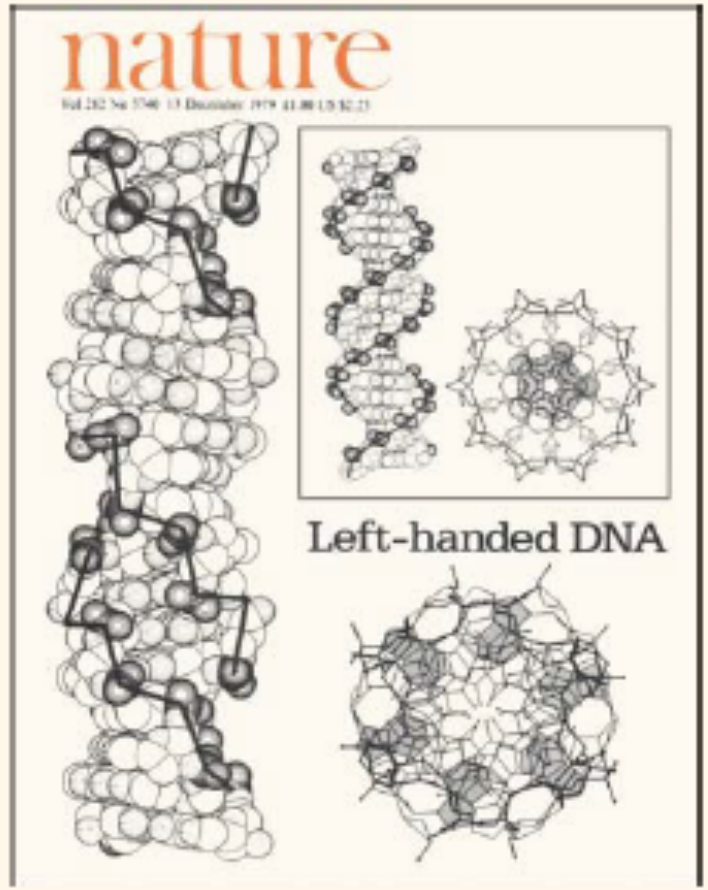
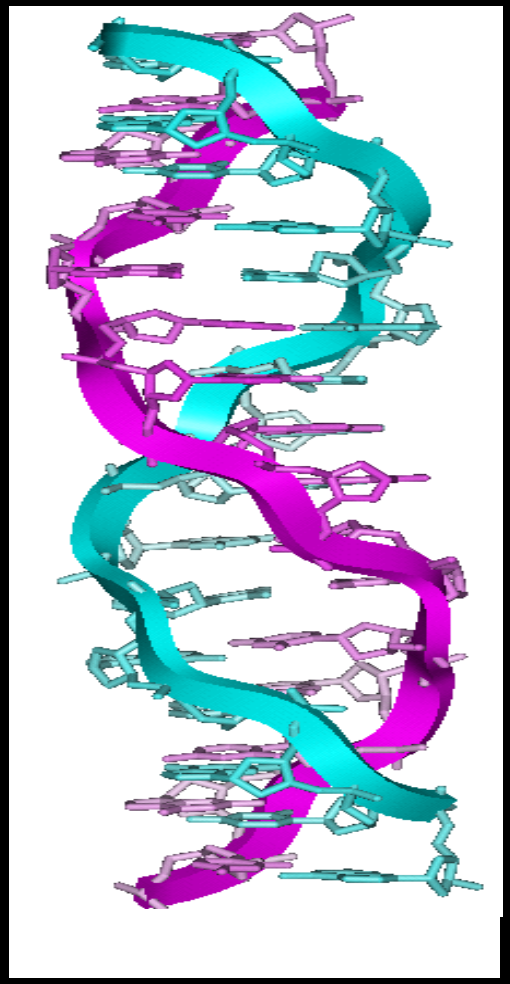
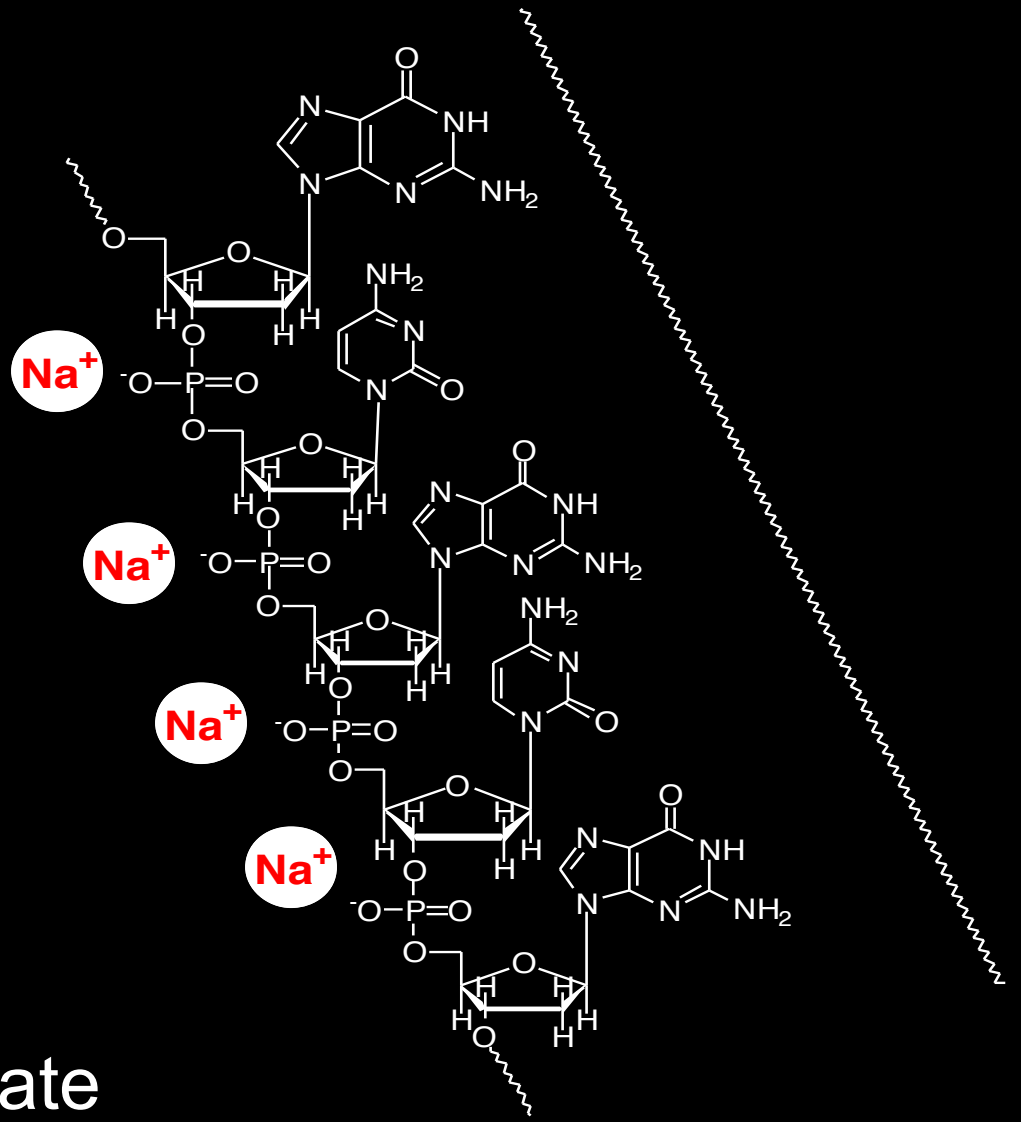


Figure 1 | **Discovery of Z-DNA.** Cover of *Nature*, showing the discovery of left-handed Z-DNA in side and end views, with the right-handed B-DNA in the enclosed box[1]. The shaded guanine residues in Z-DNA are near the outside of the helix in the end view, compared with their central location in B-DNA.

Z DNA in Physiological Salt Condition is Difficult to Obtain

For example: $d(CGCGCG)_2$ Retains Typical B form at 2 M NaCl

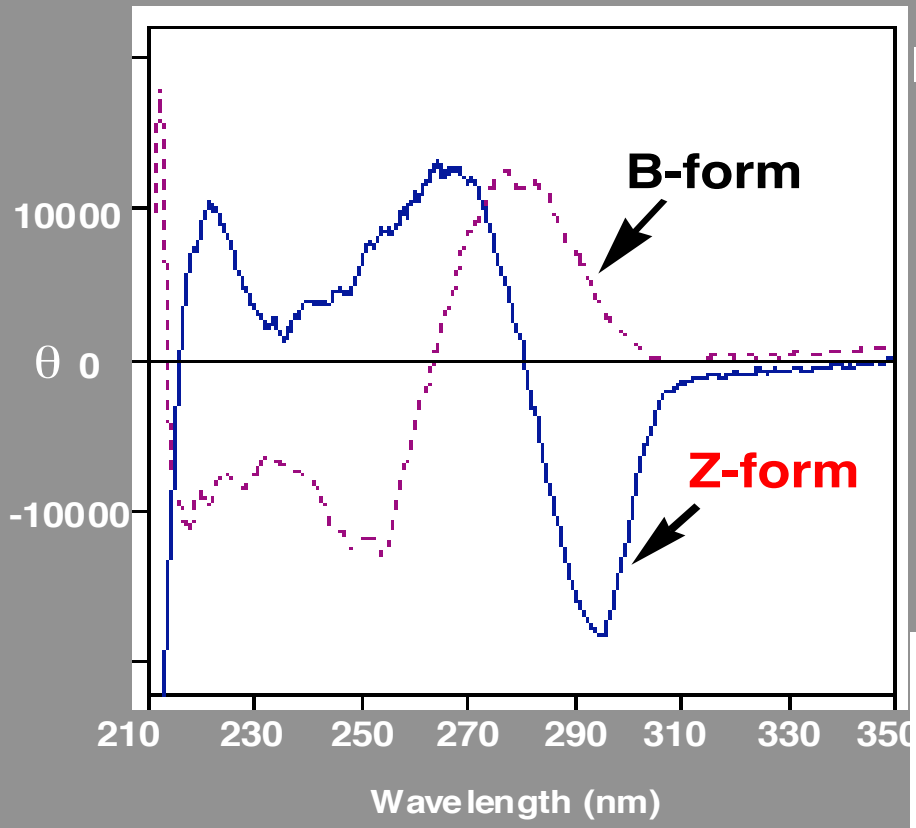




Zigzag structure of phosphate

Z-DNA 12.5 Å B-DNA 17.5 Å

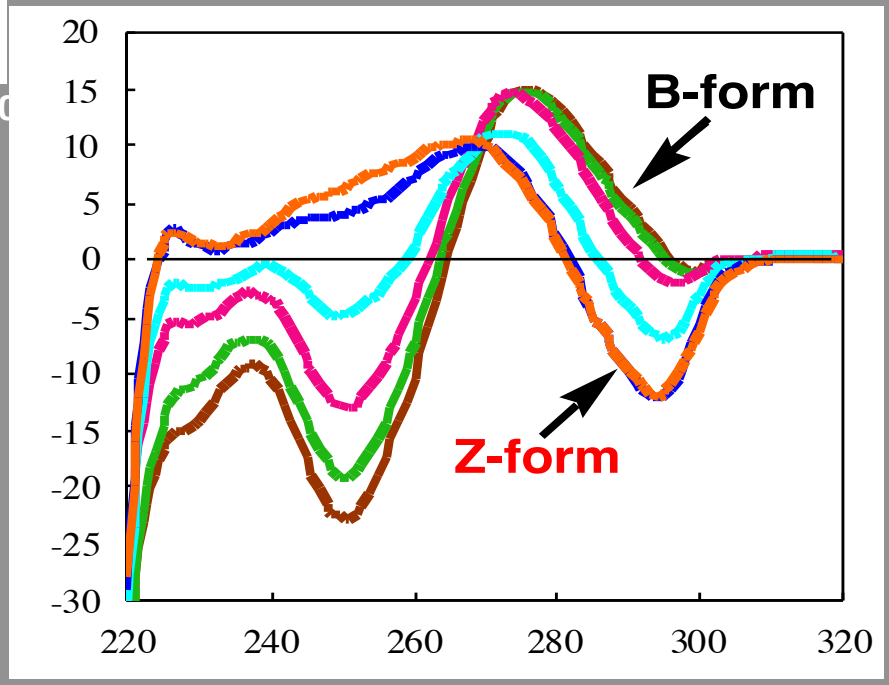
CD spectra of B-DNA and Z-DNA



In B-DNA: a positive peak at 270-280 nm
a negative peak at about 250 nm
(低塩濃度)

A "reversal"

In Z-DNA: a positive peak at about 265 nm
a negative peak at about 290 nm
(高塩濃度)



B—Z転移中点: NaCl濃度を増加させることにより、
DNAがB型からZ型に変化する。

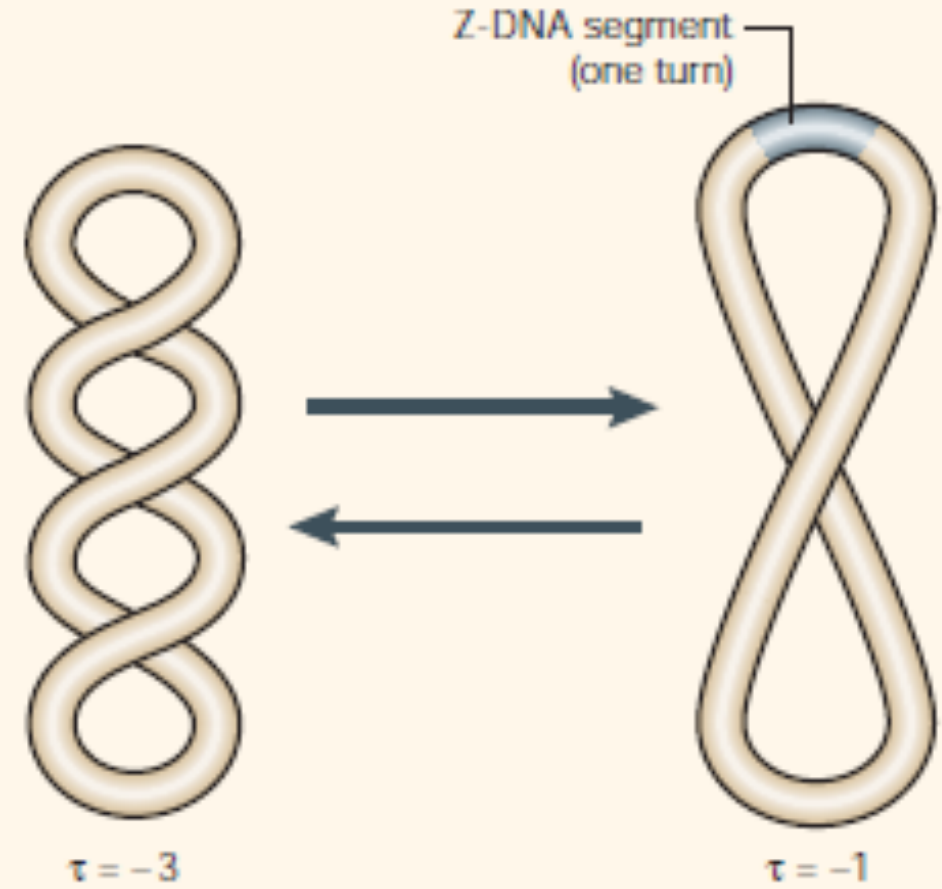


Figure 2 | **Negative supercoiling stabilizes Z-DNA.** A double helical plasmid with three negative supercoils (τ) is shown on the left. The energy of supercoiling is proportional to the square of the number of supercoils. On the right, one turn switches the DNA from right- to left-handed, and two negative supercoils are removed. The change of negative supercoiling energy stabilizes Z-DNA formation.

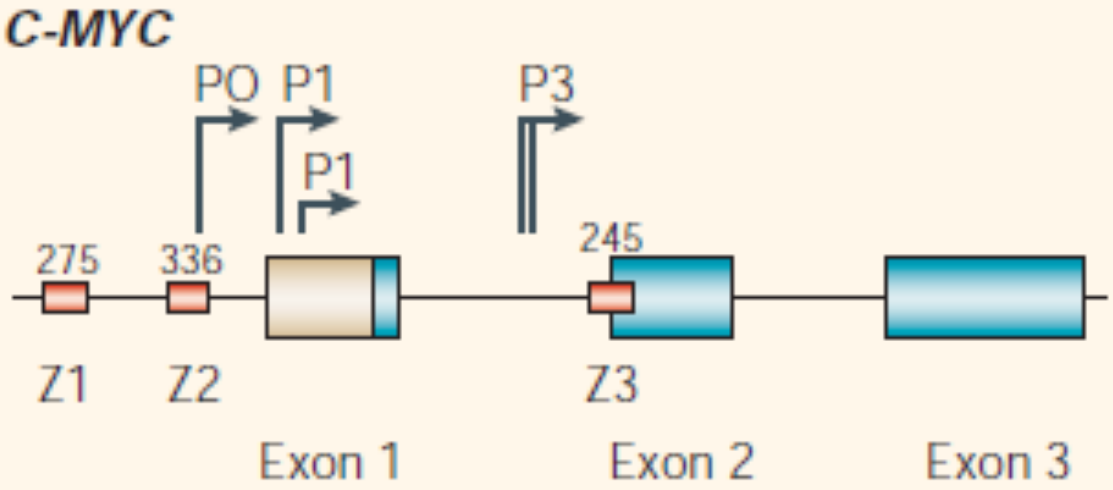
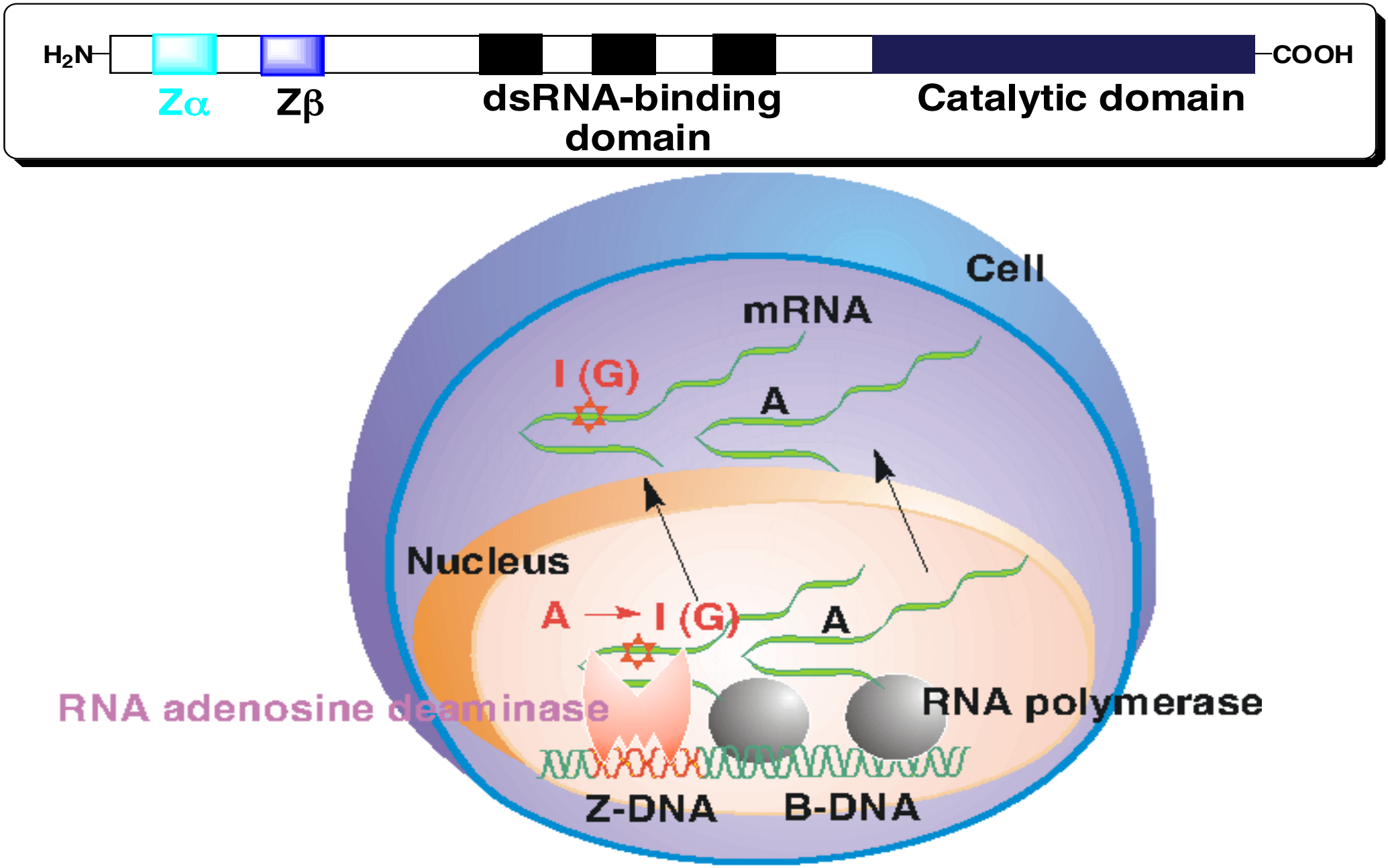
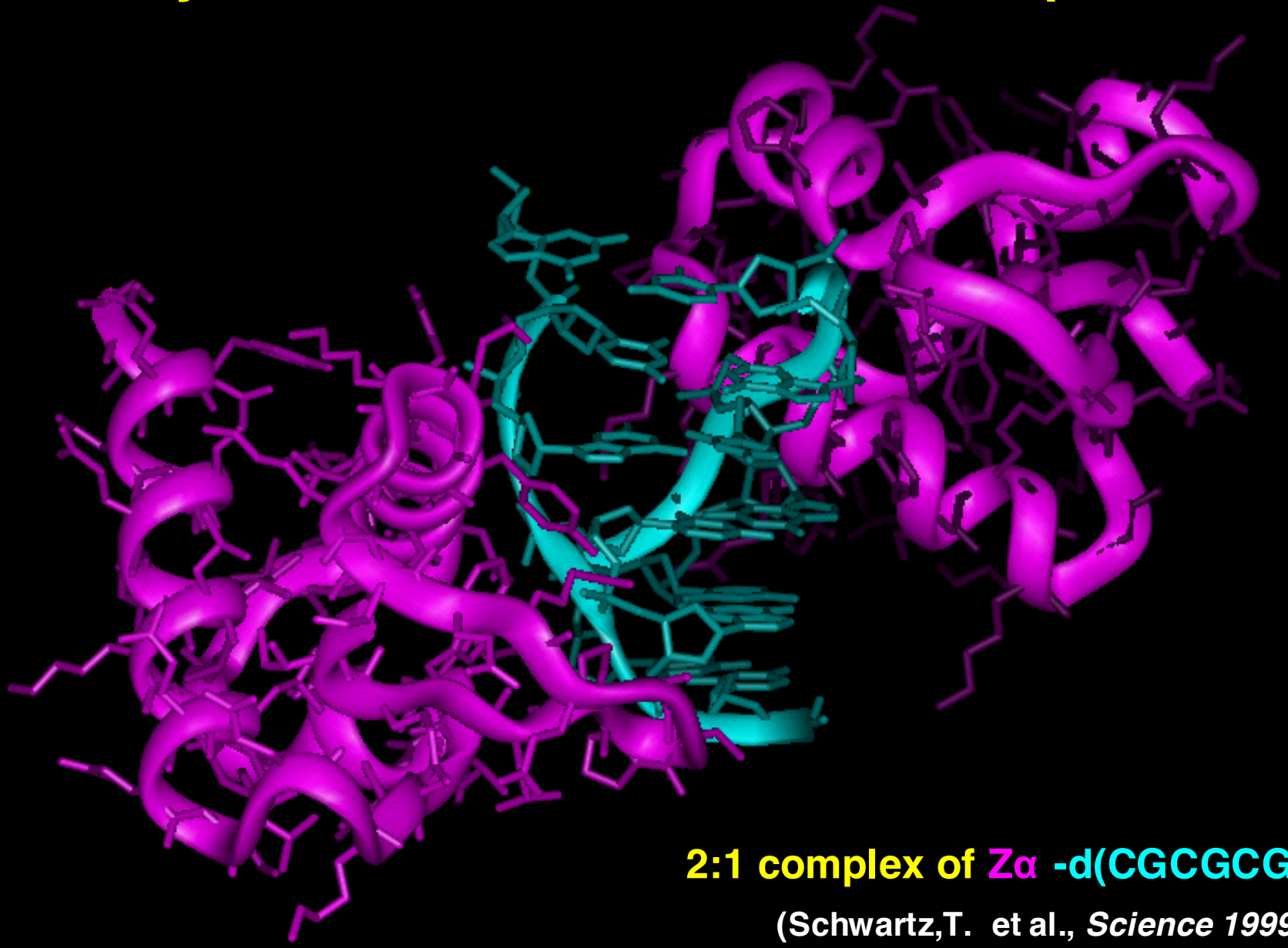


Figure 3 | **Transcription stabilizes Z-DNA.** Metabolically active permeabilized nuclei were used to show that *C-MYC* transcription is associated with the formation of Z-DNA in three restriction fragments, which are labelled Z1–Z3 (numbers above the boxes show the size of the fragments). These are all found near the *C-MYC* promoters, which are labelled P0–P3 (REF. 26). When transcription is turned off, the continued action of topoisomerases relaxes the Z-DNA, which disappears entirely after ~15 minutes. Therefore, Z-DNA is formed transiently in association with transcription.

Human Double-Stranded RNA Adenosine Deaminase (ADAR1)



Crystal Structure of Zα -DNA Complex

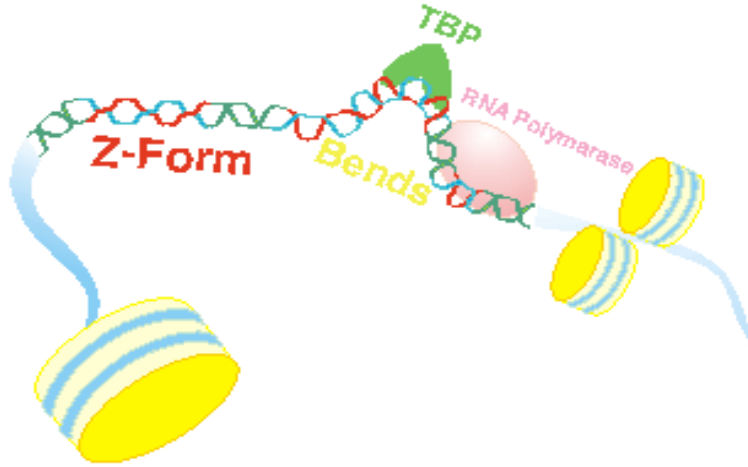


2:1 complex of Zα -d(CGCGCG)$_2$

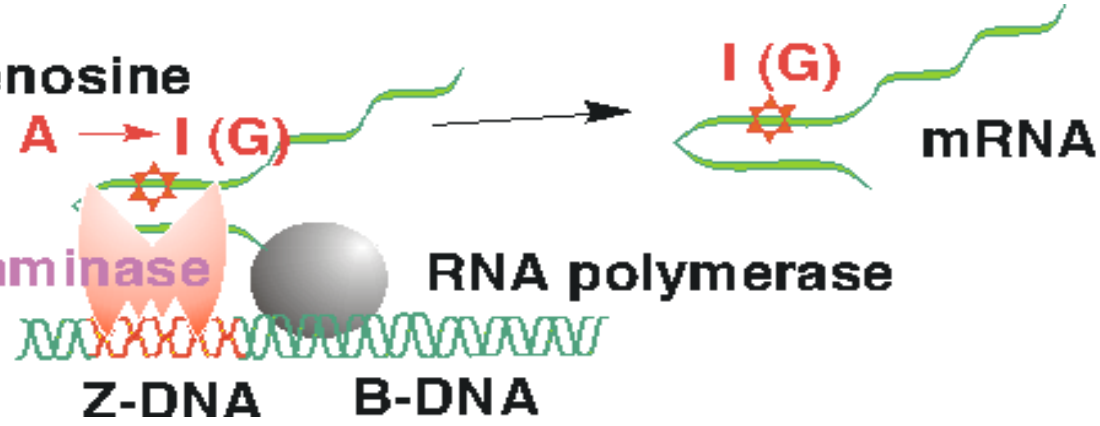
(Schwartz,T. et al., *Science 1999*.)

The Proposed Biological Role of Z-DNA

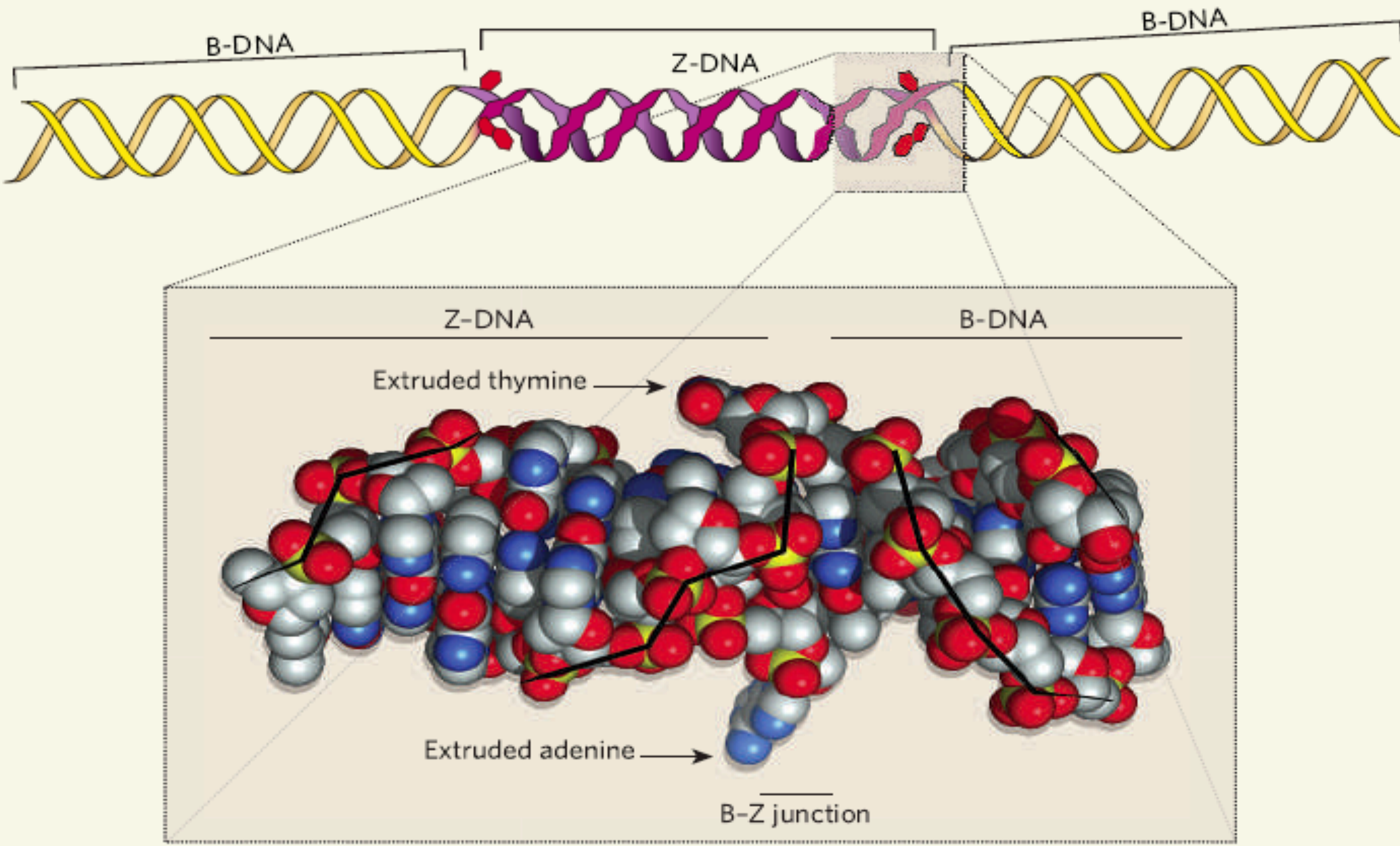
(1)Z-DNA is stabilized by an RNA polymerase moving through a gene.



(2)Double-stranded RNA adenosine deaminase. ADAR1



(3)Tumor-associated protein DLM-1



Rich A., Kim KK. et al. Nature 2005, 437, 1183.

1272–1278 *Nucleic Acids Research, 1996, Vol. 24, No. 7* *© 1996 Oxford University Press*

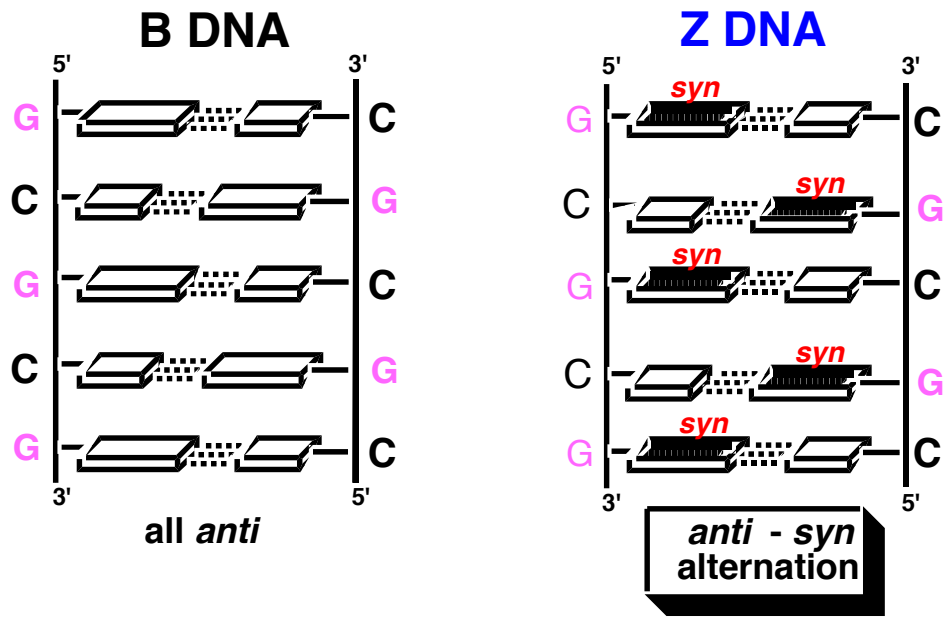
Synthesis, structure and thermodynamic properties of 8-methylguanine-containing oligonucleotides: Z-DNA under physiological salt conditions

Hiroshi Sugiyama, Kiyohiko Kawai, Atsushi Matsunaga, Kenzo Fujimoto, Isao Saito, Howard Robinson and Andrew H.-J. Wang[1,*]

Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606-01, Japan and [1]Department of Cell and Structural Biology, 506 Morrill Hall, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Z DNA in Physiological Salt Condition is Difficult to Obtain

ex. 18mer $d(G^mC)_4A^{Br}U(G^mC)_4$ Retains Typical B form at 4 M NaCl



$d(G^mC)_4A^{Br}U(G^mC)_4$

$d(^mCG)_4{}^{Br}UA(^mCG)_4$

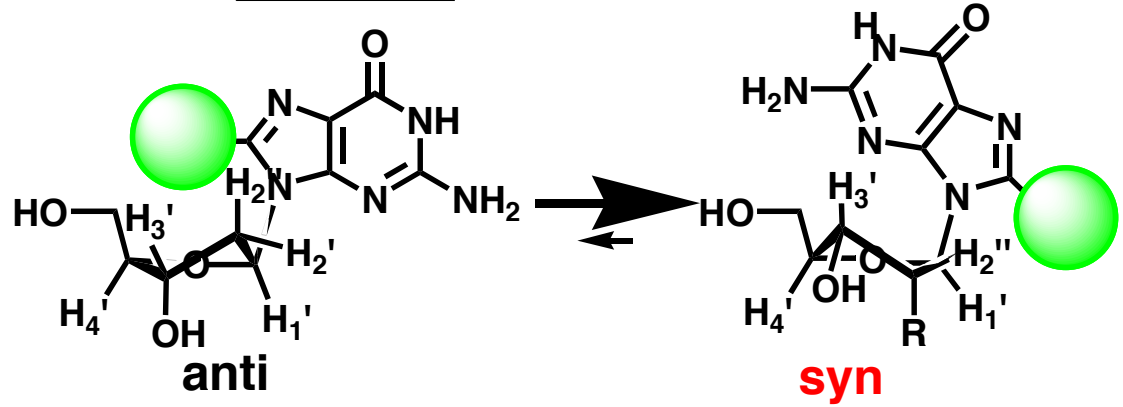
Introduction of Me at dG H8

▽

***syn* conformation**

▽

Stabilize Z-DNA



R = H (*Nucleic Acids Res.* 24, 1272, 1996)

R = OH (*J. Am. Chem. Soc.* 125, 13519, 2003)

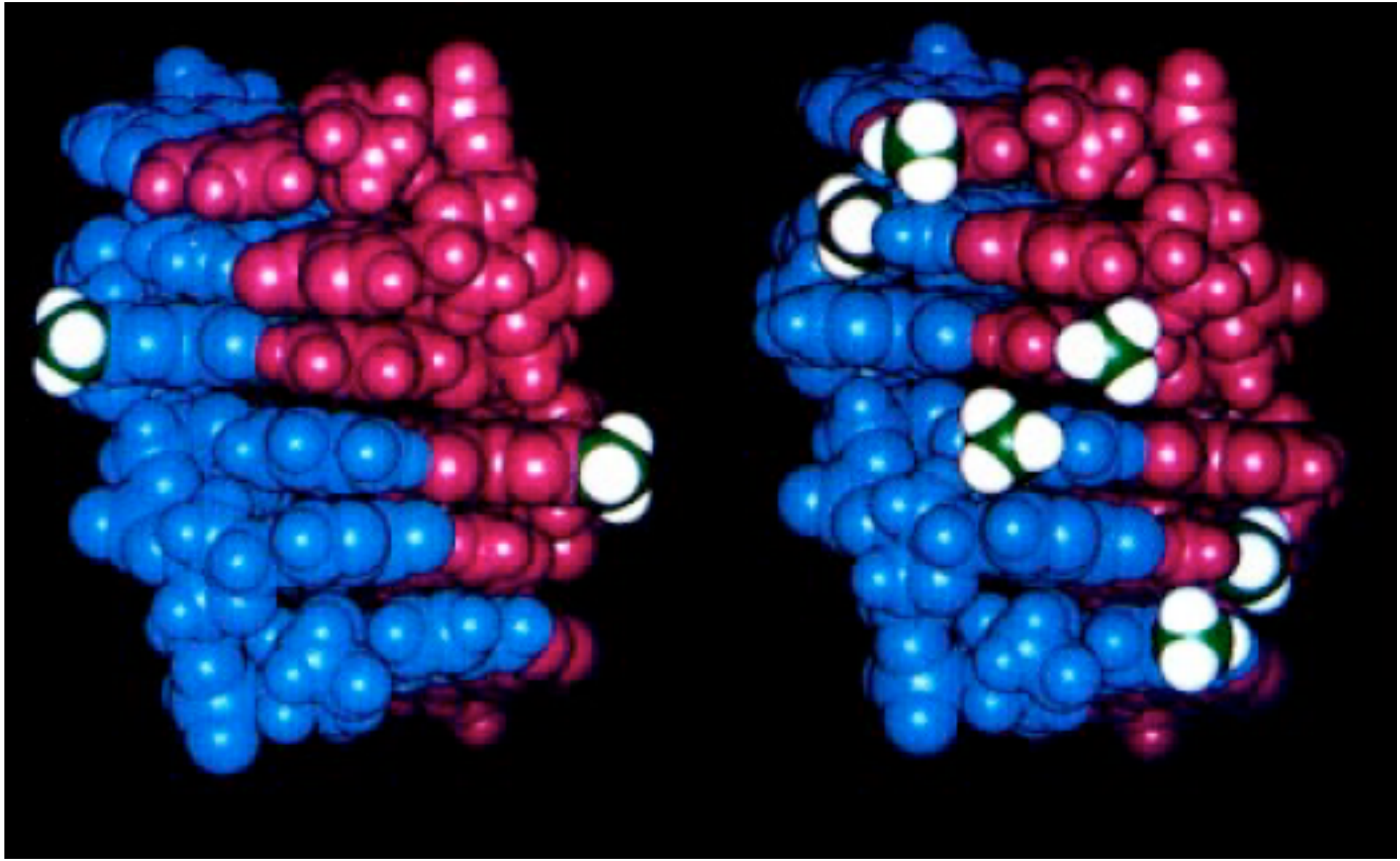
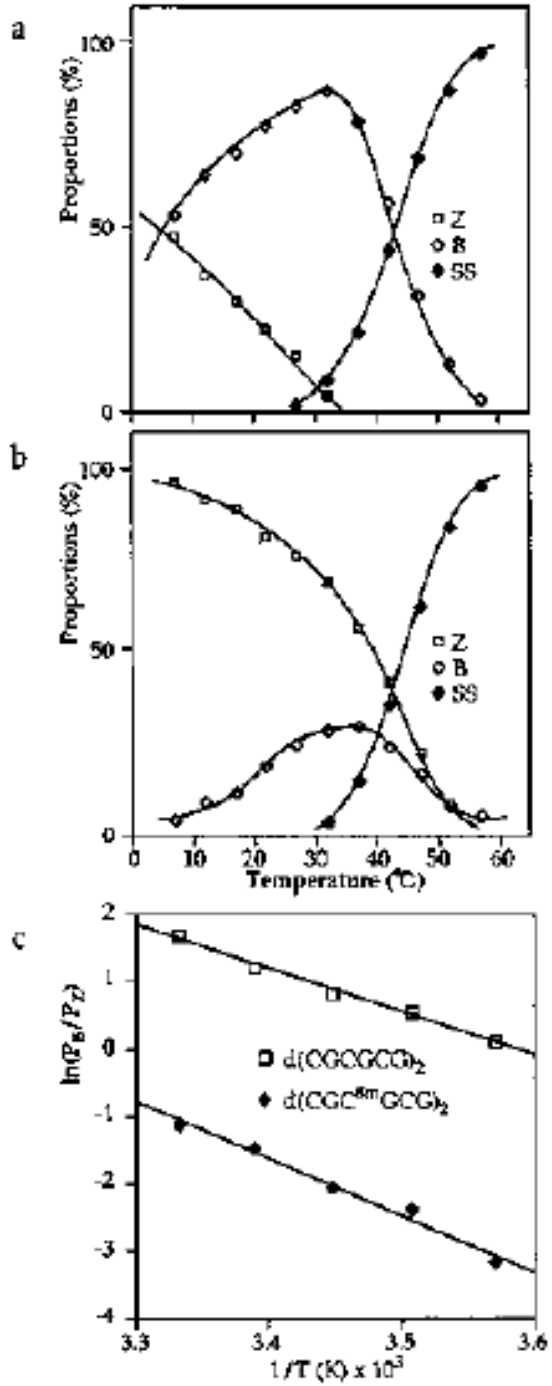


Figure 3. The refined model for d(CGC[m^8G]CG)$_2$ Z-DNA structure (left) and the model of d(m^5C-G)$_3$ (right).



$$\Delta G = \Delta H - T\Delta S$$

$$\Delta G = -RT\ln K$$

Figure 6. Proportions of Z, B and SS conformations of (a) d(CGCGCG) and (b) d(CGC[m^{8}G]CG) (**2**) as a function of temperature. Sample solutions contained 0.15 mM hexanucleotide (base concentration) in 2.6 M NaCl, 5 mM Na cacodylate buffer, pH 7.0. Proportions of Z, B and SS were obtained by a combination of UV and CD spectroscopy. (c) van't Hoff plot for the Z–B conformational transition of d(CGCGCG) and d(CGC[m^{8}G]CG) (**2**) obtained from the optical data and the NMR data from Figure 5.

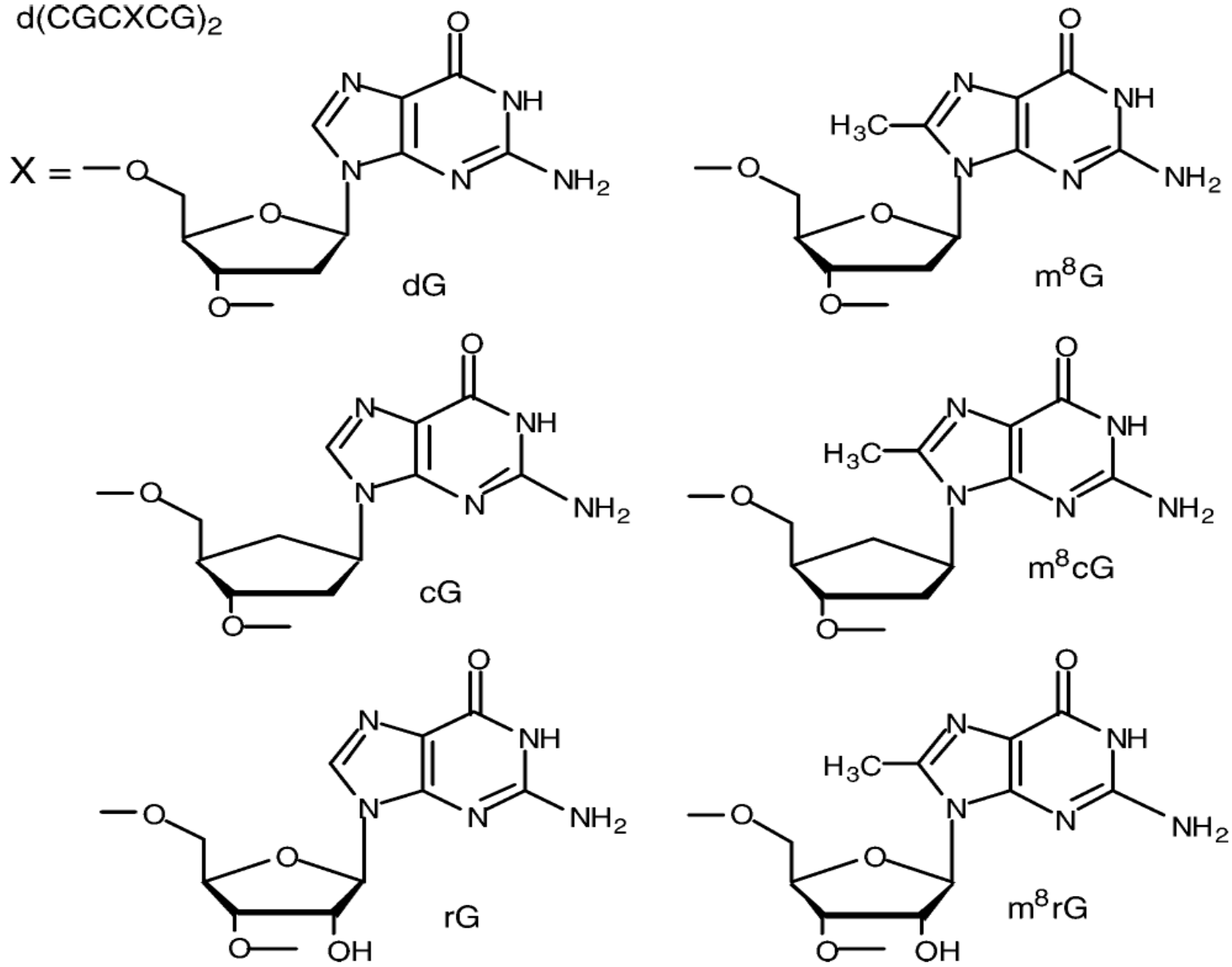
J. AM. CHEM. SOC. 2003, *125*, 13519−13524

8-Methylguanosine: A Powerful Z-DNA Stabilizer

Yan Xu,[†] Reiko Ikeda,[†] and Hiroshi Sugiyama*[,†,‡]

Contribution from the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda, Tokyo 101-0062, Japan, and Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8501, Japan

Scheme 1



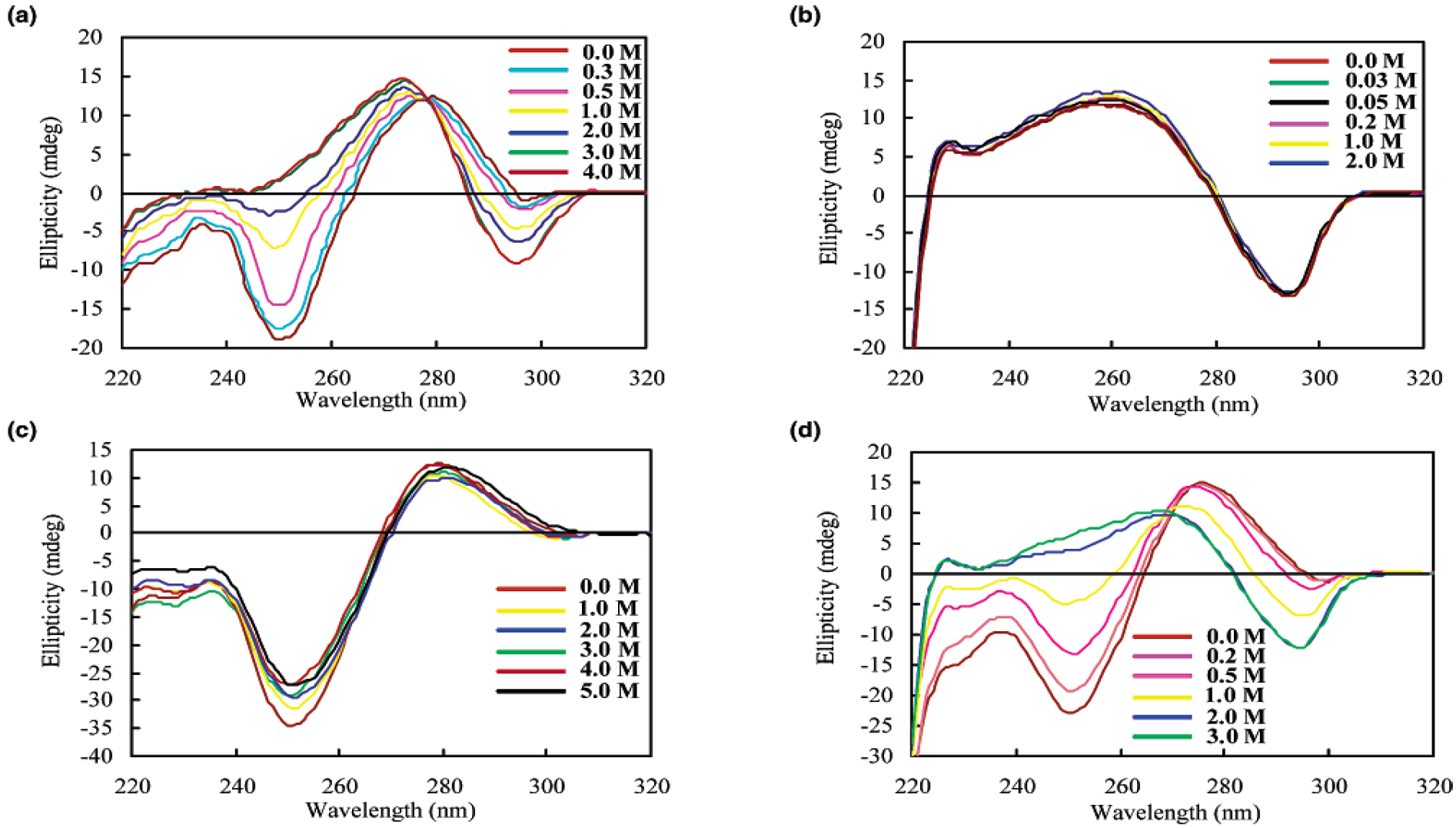


Figure 1. CD spectra of d(CGCm^{8}cGCG)$_2$ (a), d(CGCm^{8}rGCG)$_2$ (b), d(CGCcGCG)$_2$ (c), and d(CGCrGCG)$_2$ (d) in 5 mM Na cacodylate buffer, pH 7.0, at 10 °C at various NaCl concentrations (all oligomers, 0.15 mM base concentration).

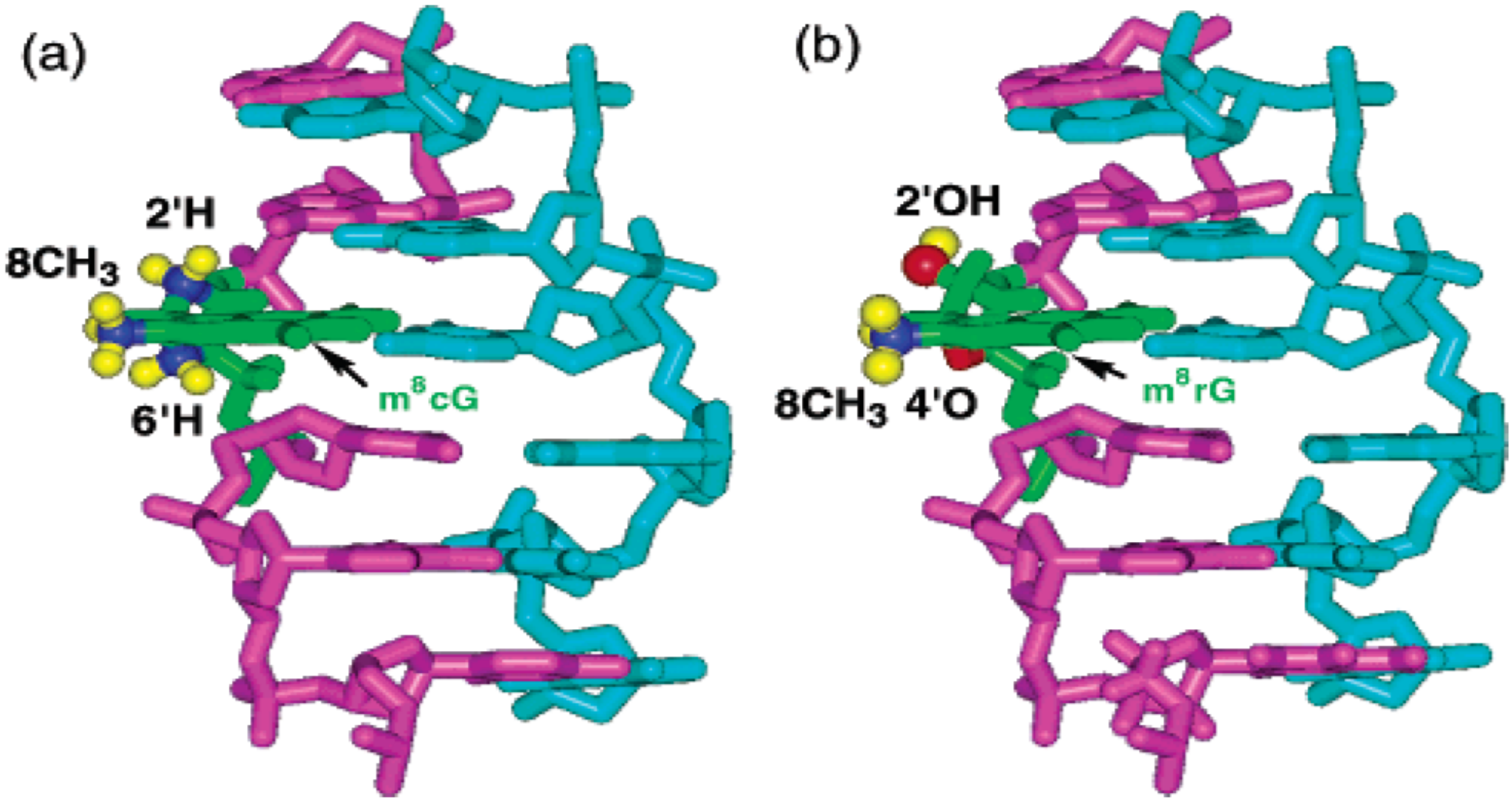


Figure 3. Molecular models of (a) d(CGCm^{8}cGCG)$_2$ and (b) d(CGCm^{8}rGCG)$_2$.

Table 2. Midpoint NaCl Concentrations for B−Z Transitions of Various Deoxyoctanucleotides Derivatives[a]

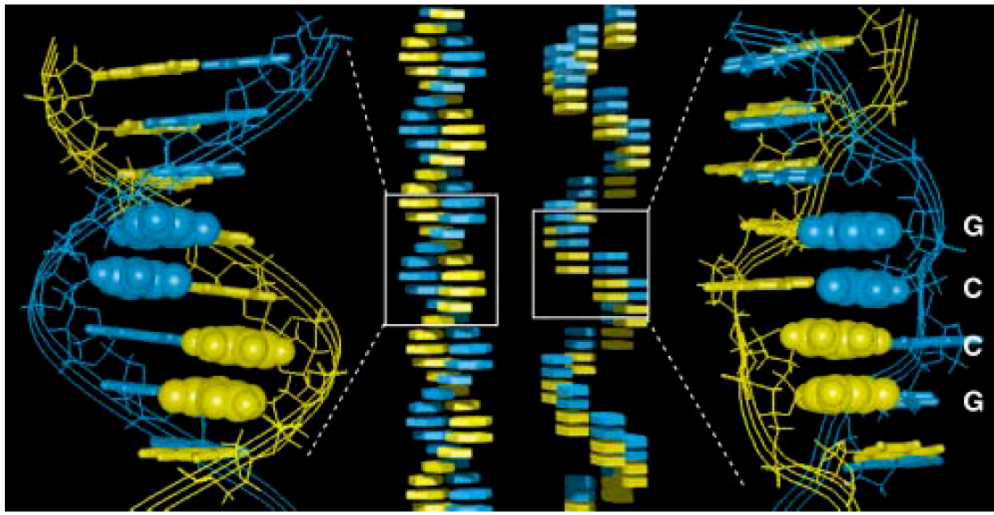
	duplexes	NaCl
a	d(CGCG^{I}UGCG)/d(Cm^{8}GCACm^{8}GCG)	800
b	d(CGCrGUGCG)/d(Cm^{8}GCACm^{8}GCG)	420
c	d(CGCG^{I}UCGC)/d(Cm^{8}rGCACm^{8}rGCG)	0
d	d(CACATGCG)/d(Cm^{8}GCATm^{8}GTG)	2450
e	d(CACATGCG)/d(Cm^{8}rGCATm^{8}rGTG)	200
f	d(Cm^{8}GCACm^{8}GCG)/d(CGCGTG)	1100
g	d(Cm^{8}rGCACm^{8}rGCG)/d(CGCGTG)	210

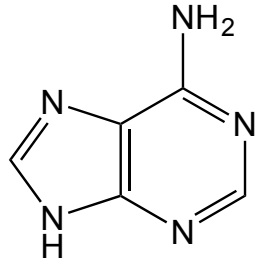
[a] Experiments were carried out as described in the footnote to Table 1.

Angew. Chem. Int. Ed. **2003**, *42*, 6018 –6020

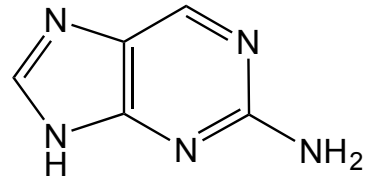
A Nanothermometer Based on the Different π Stackings of B- and Z-DNA

*Ryu Tashiro and Hiroshi Sugiyama**

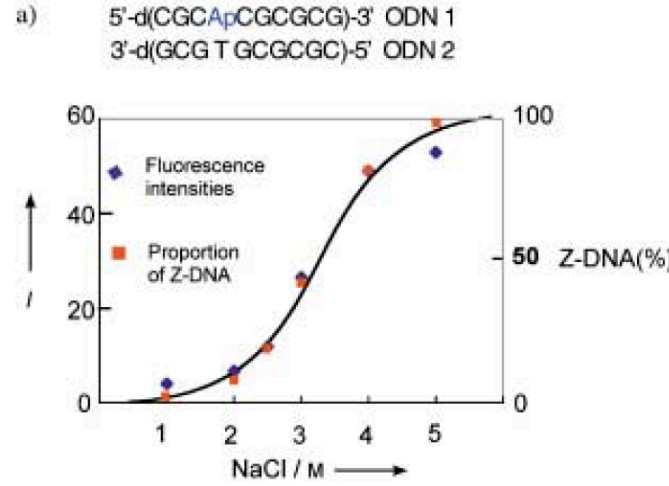


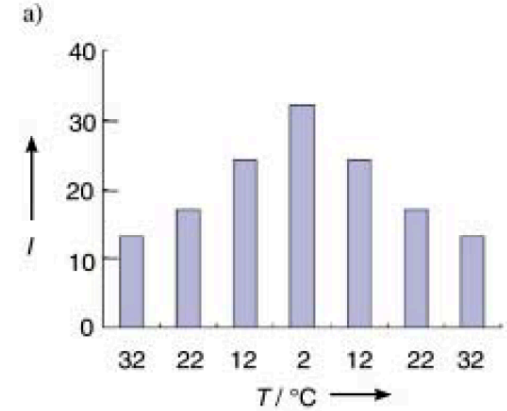


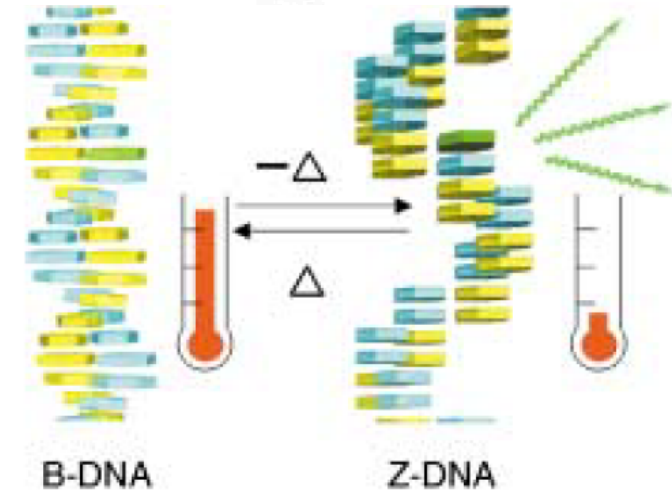
adenine



2-aminopurine





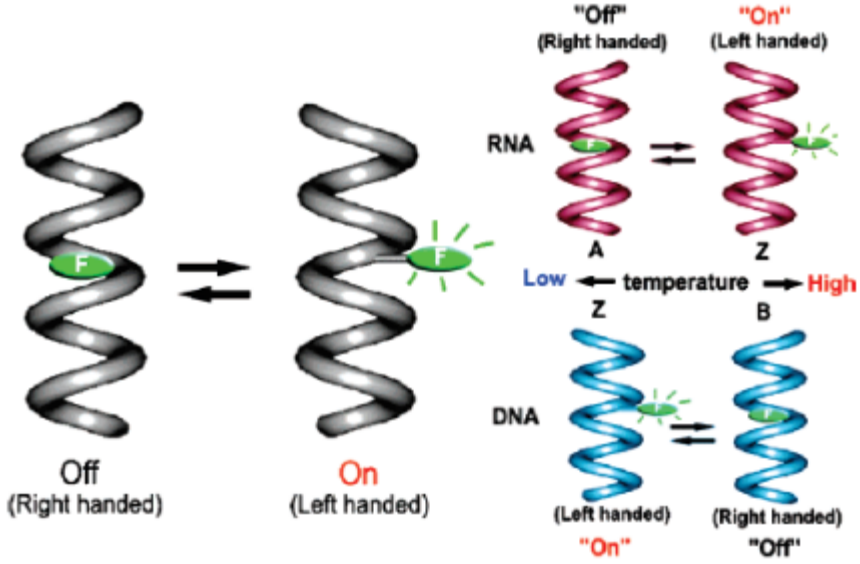


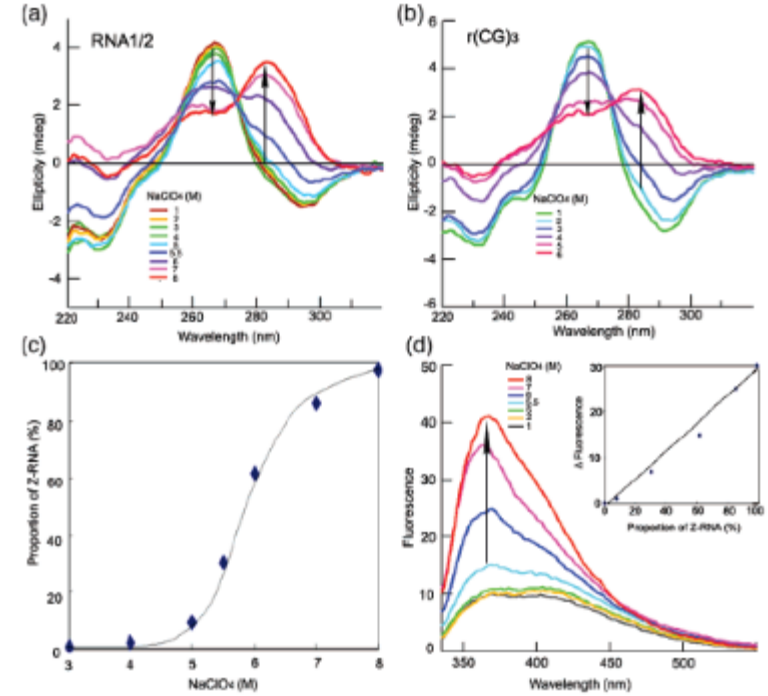
J. AM. CHEM. SOC. 2005, *127*, 2094−2097

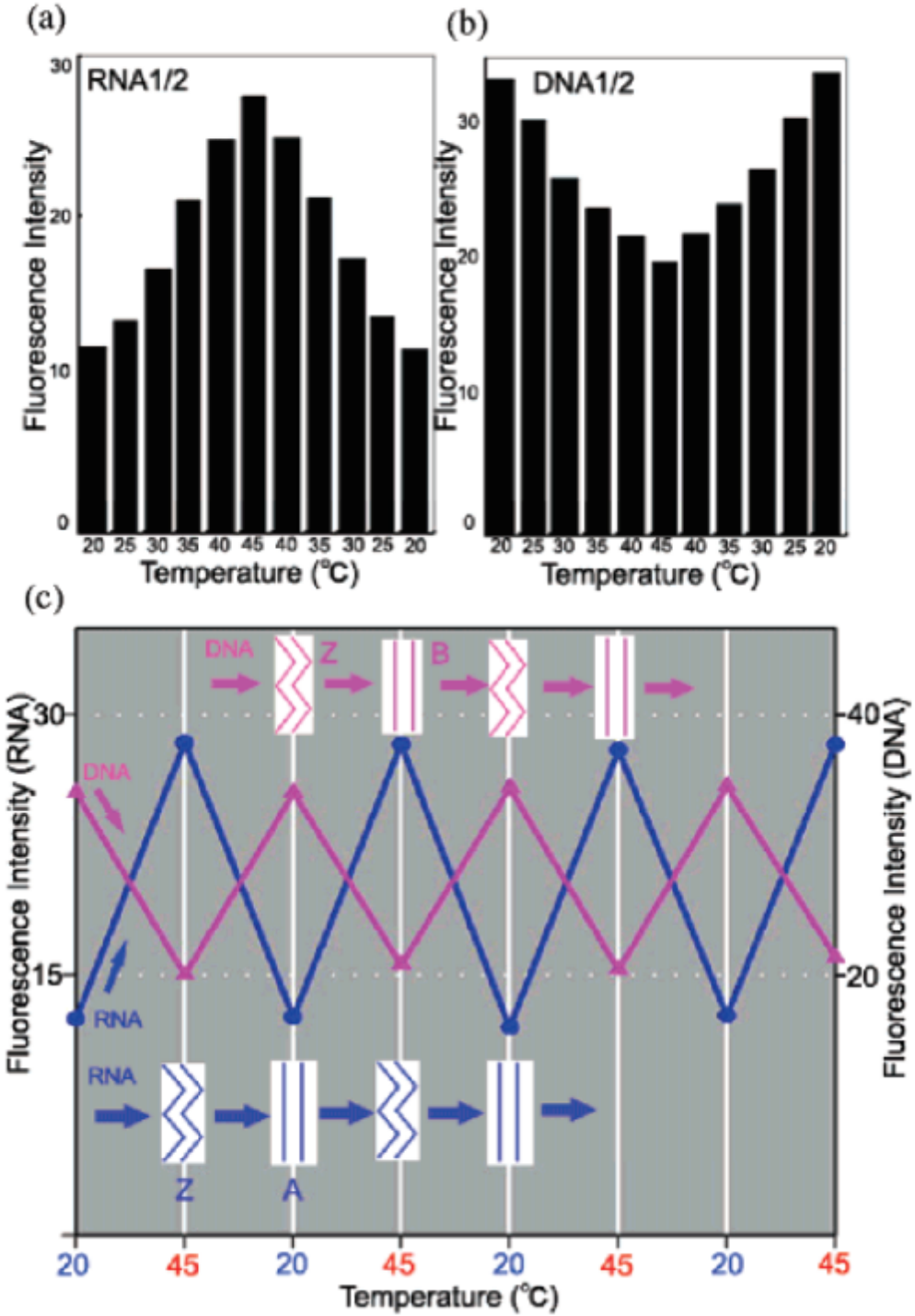
Biomolecule-Based Switching Devices that Respond Inversely to Thermal Stimuli

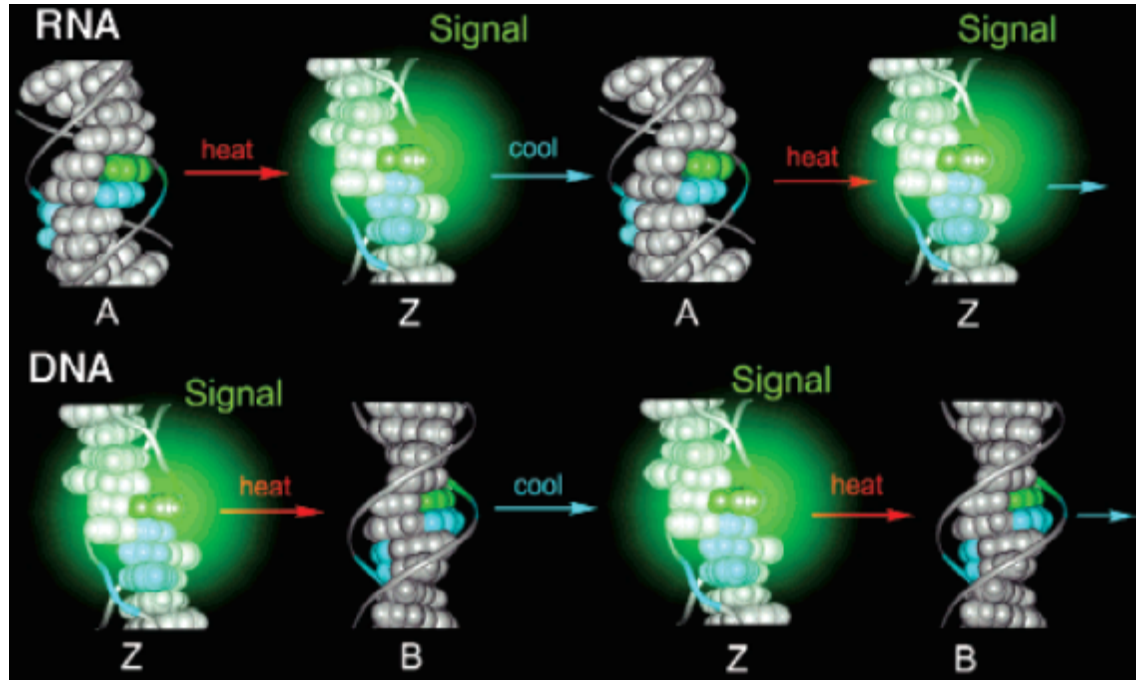
Ryu Tashiro† and Hiroshi Sugiyama*,‡

Division of Biofunctional Molecules, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Surugadai, Kanda, Chiyoda, Tokyo 101-0062, Japan, and Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo, Kyoto 606-8502, Japan





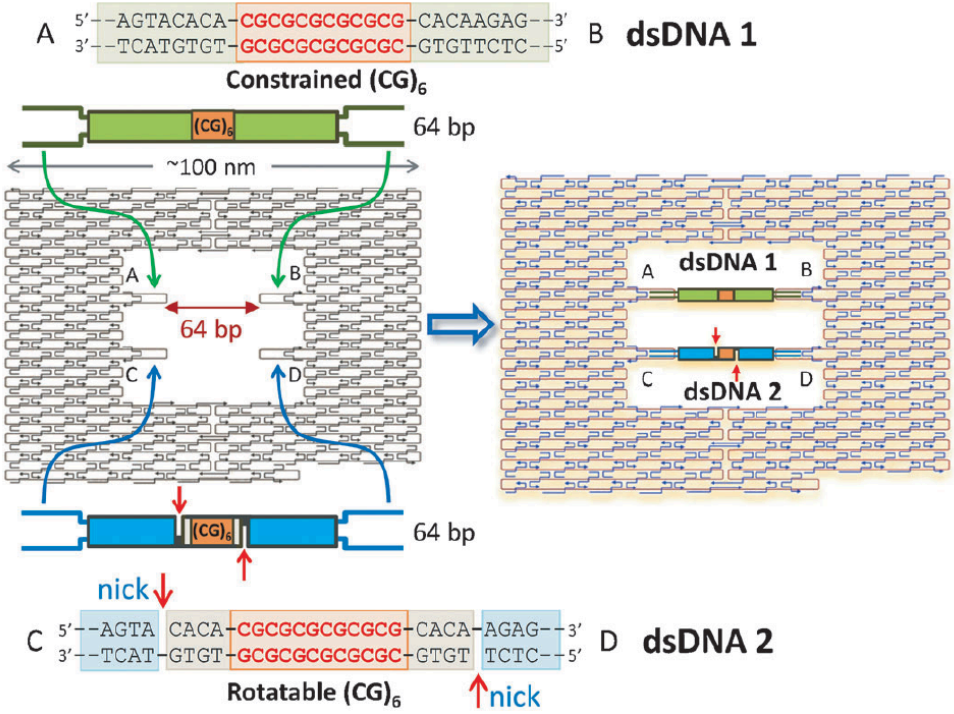

(a)
RNA1/2
Fluorescence Intensity
Temperature (°C)
(b)
DNA1/2
Fluorescence Intensity
Temperature (°C)
(c)
DNA
Z
B
RNA
Z
A
Fluorescence Intensity (RNA)
Fluorescence Intensity (DNA)
Temperature (°C)


RNA
Signal
heat
cool
heat
Signal
A
Z
A
Z
DNA
Signal
heat
cool
Signal
heat
Z
B
Z
B

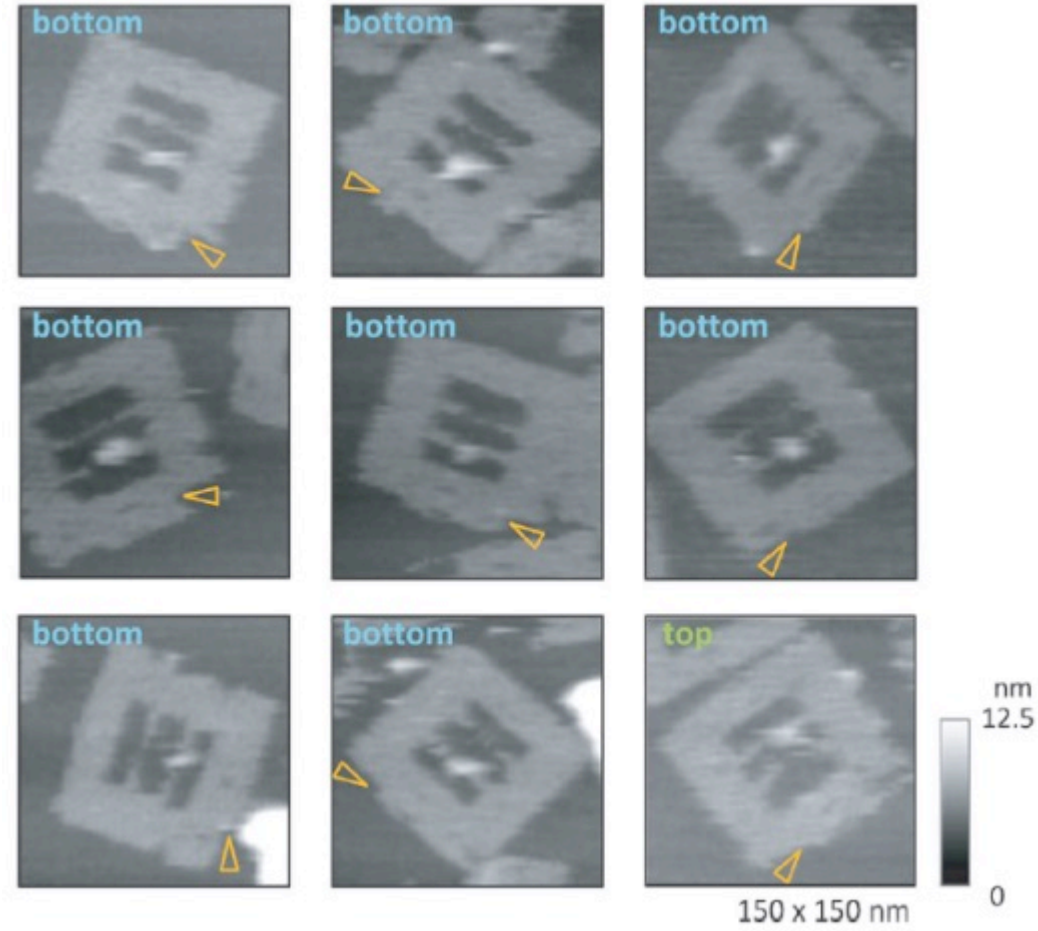
Chem. Eur. J. **2013**, *19*, 16887 – 16890

Regulation of B–Z Conformational Transition and Complex Formation with a Z-Form Binding Protein by Introduction of Constraint to Double-Stranded DNA by using a DNA Nanoscaffold

Masayuki Endo,*[a, c] Masahiro Inoue,[b] Yuki Suzuki,[b, c] Chigusa Masui,[b] Hironobu Morinaga,[b] Kumi Hidaka,[a] and Hiroshi Sugiyama*[a, b, c]



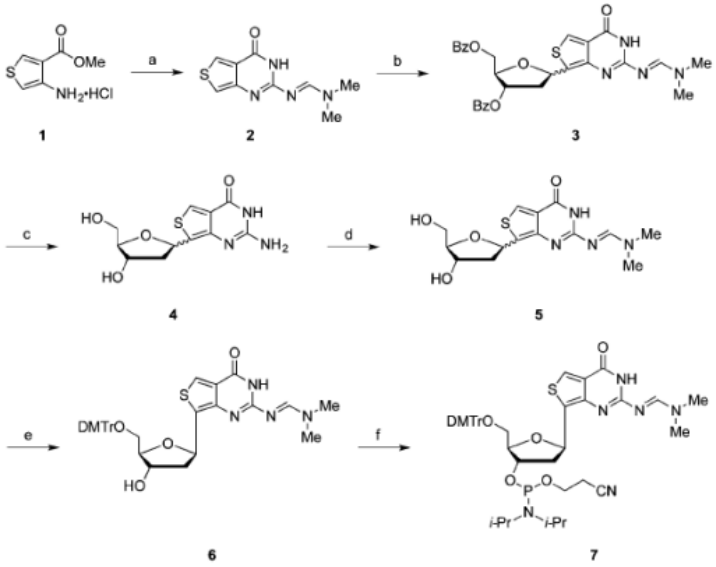
DNA frame type	dsDNA **1**	dsDNA **2**	dsDNAs **1** & **2** (both)
I[a]	7 (2.7 %)	74 (28.8 %)	5 (1.9 %)

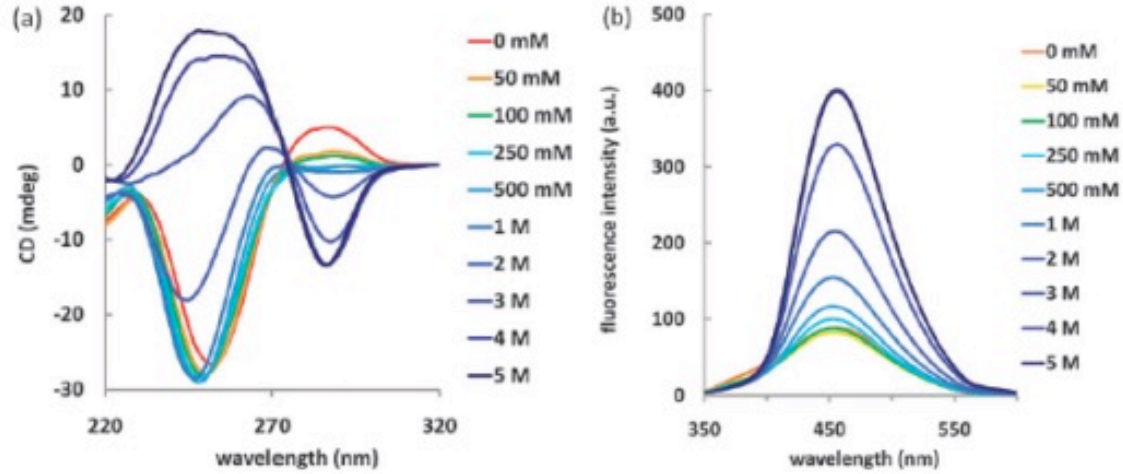


Chem. Commun., 2014, **50**, 1573–1575

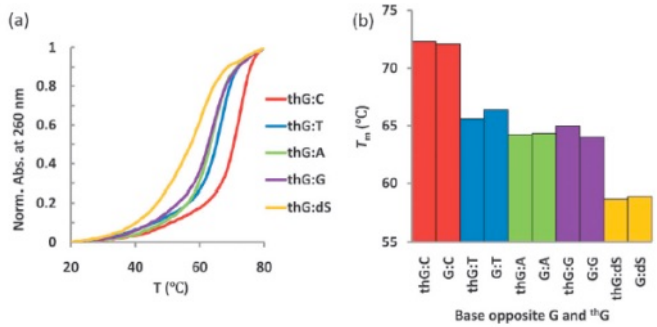
Highly emissive deoxyguanosine analogue capable of direct visualization of B–Z transition†‡

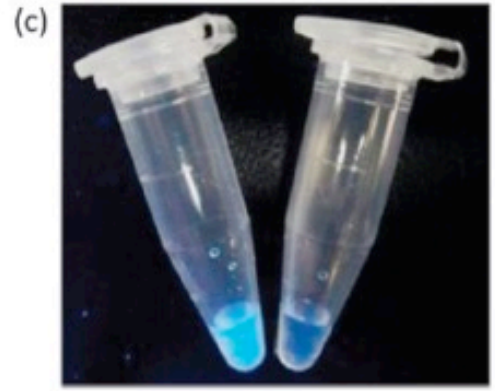
Soyoung Park,[a] Haruka Otomo,[a] Linjie Zheng[a] and Hiroshi Sugiyama*[abc]





ODN1·2:5′-CGTCCGTC**X**TACGCACGC-3′ **X**=th**dG** or **G**
ODN3·7:3′-GCAGGCAG**Y**ATGCGTGCG-5′ **Y**=C, T, A, G, or dSpacer





Artificial DNA: PNA & XNA, **2014**, 5, e28226-1-e28226-7.

Investigation of B-Z Transitions with DNA Oligonucleotides Containing 8-Methylguanine

Yu-Hsiao Chen[4], Soyoung Park*[1] Haruka Otomo[1], Sohei Sakashita[1], and Hiroshi Sugiyama*[1,2,3]

Table 1. Midpoint NaCl Concentrations for B-Z Transitions in Modified $d(CGCGCG)_2$ Derivatives

entry	Oligonucleotides	NaCl (mM)
1	$d(CGCGCG)_2$	2600[a]
2	$d(Cm^8GCGCG)_2$	115
3	$d(CGCm^8GCG)_2$	30[a]
4	$d(CGCGCm^8G)_2$	227
5	$d(Cm^8rGCGCG)_2$	19
6	$d(CGCm^8rGCG)_2$	0[a]
7	$d(CGCGCm^8rG)_2$	28

Table 2. Midpoint NaCl Concentrations for B-Z Transitions in Modified $d(GCGCGC)_2$ Derivatives

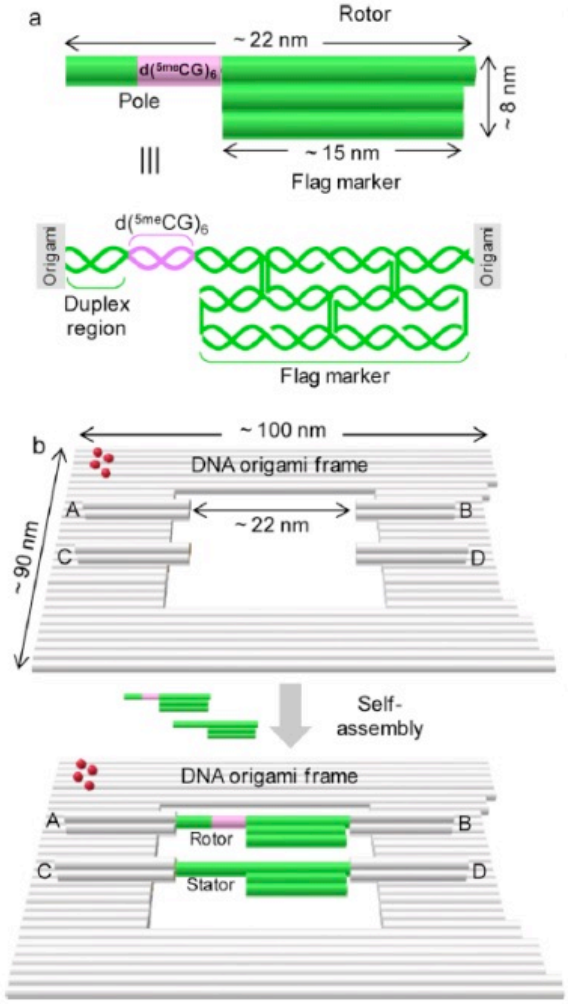
entry	Oligonucleotides	NaCl (mM)
1	$d(GCGCGC)_2$	undeterminable
2	$d(m^8GCGCGC)_2$	2500
3	$d(GCm^8GCGC)_2$	96
4	$d(GCGCm^8GC)_2$	1370
5	$d(m^8rGCGCGC)_2$	1320
6	$d(GCm^8rGCGC)_2$	59
7	$d(GCGCm^8rGC)_2$	1000

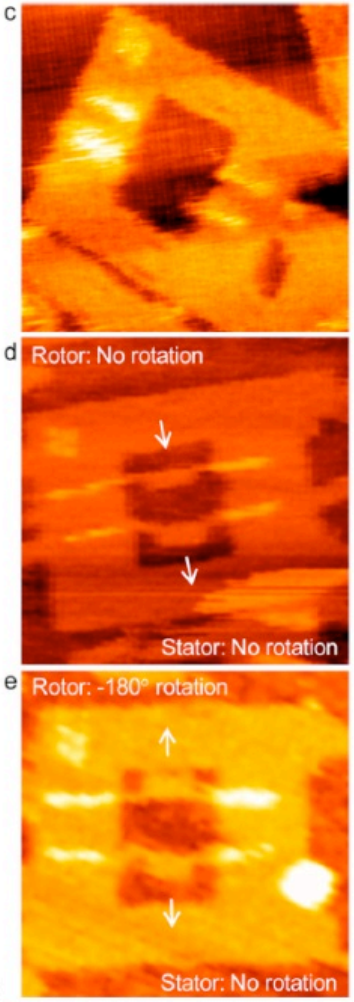
Midpoint NaCl concentration was determined from CD measurements at 10 °C at various NaCl concentrations.

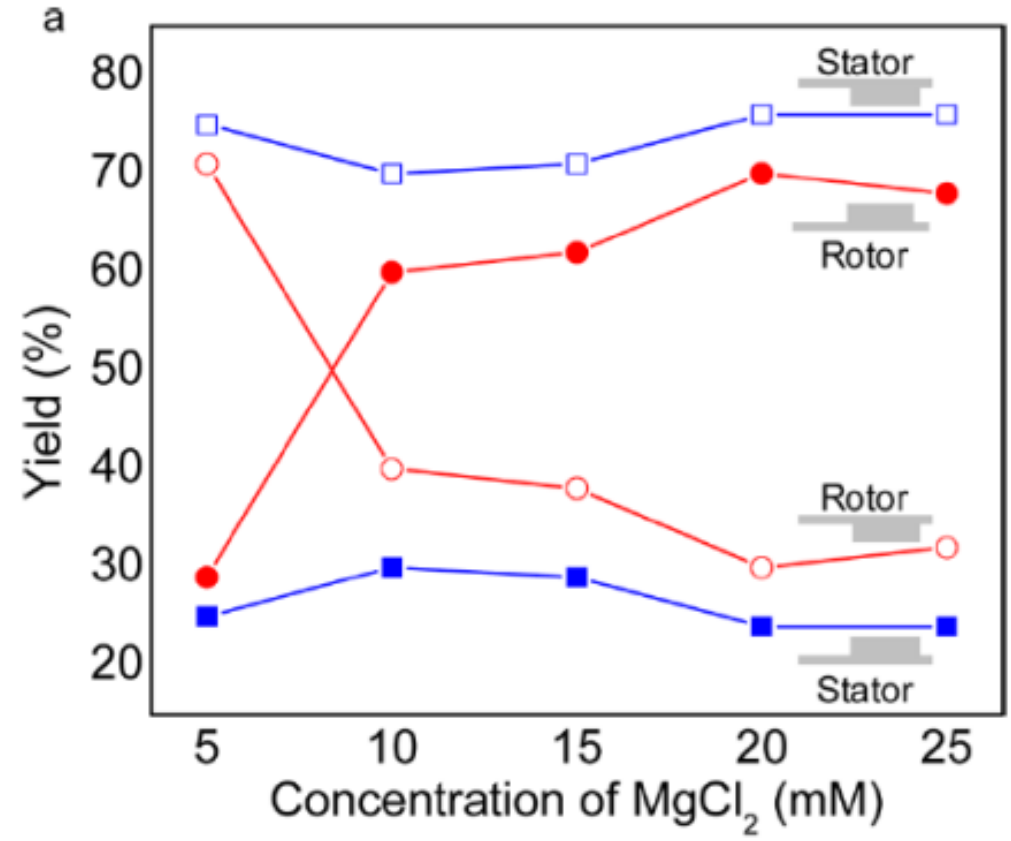
J. Am. Chem. Soc. 2013, 135, 1117−1123

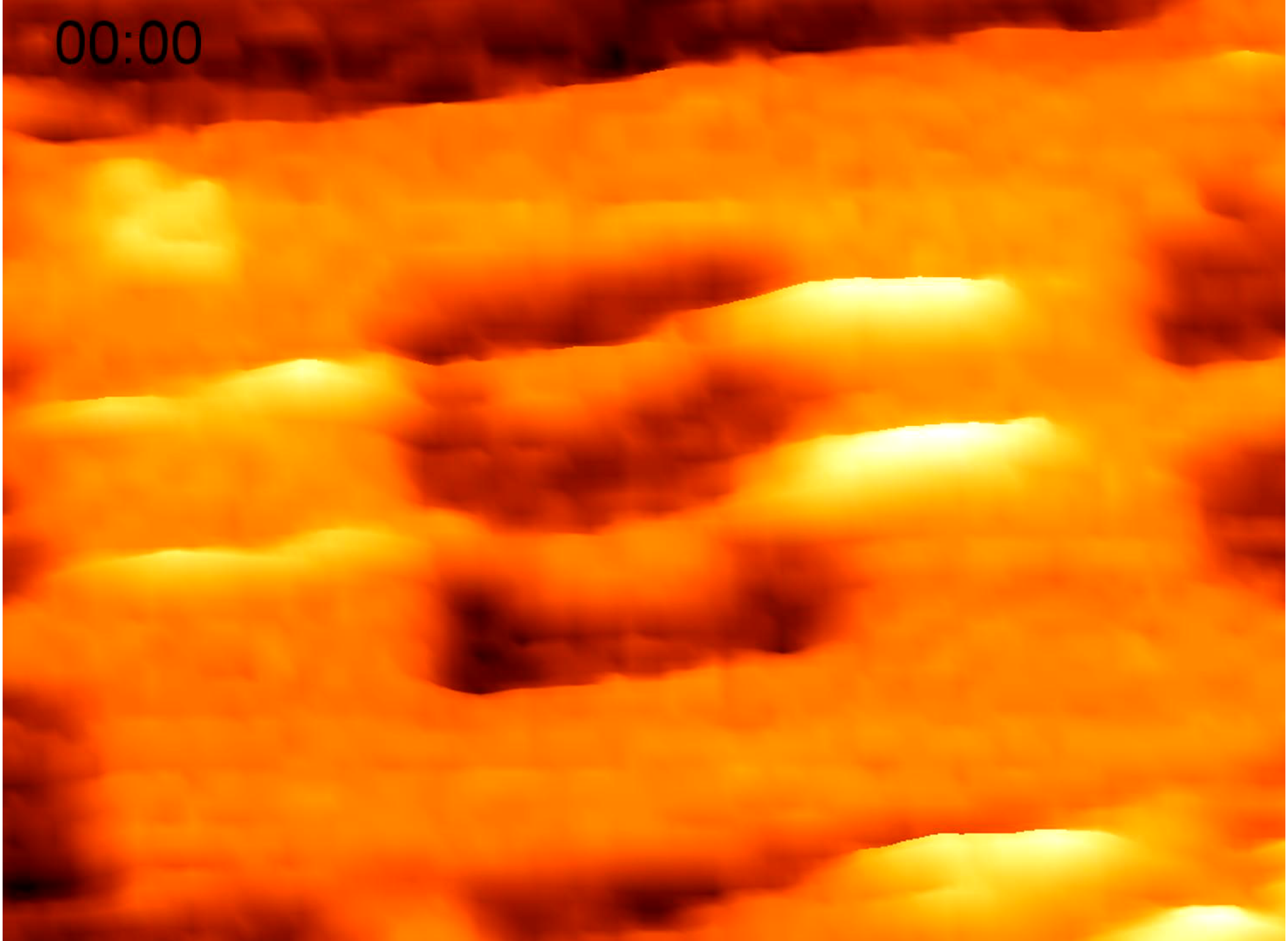
Direct and Real-Time Observation of Rotary Movement of a DNA Nanomechanical Device

Arivazhagan Rajendran,[†] Masayuki Endo,*[,‡,§] Kumi Hidaka,[†] and Hiroshi Sugiyama*[,†,‡,§]





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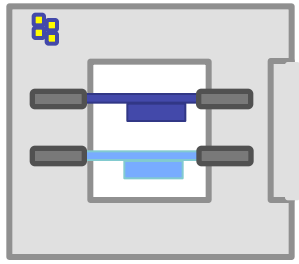
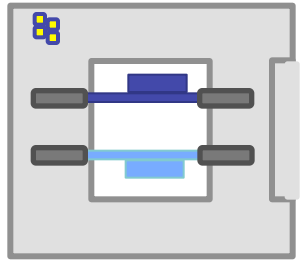
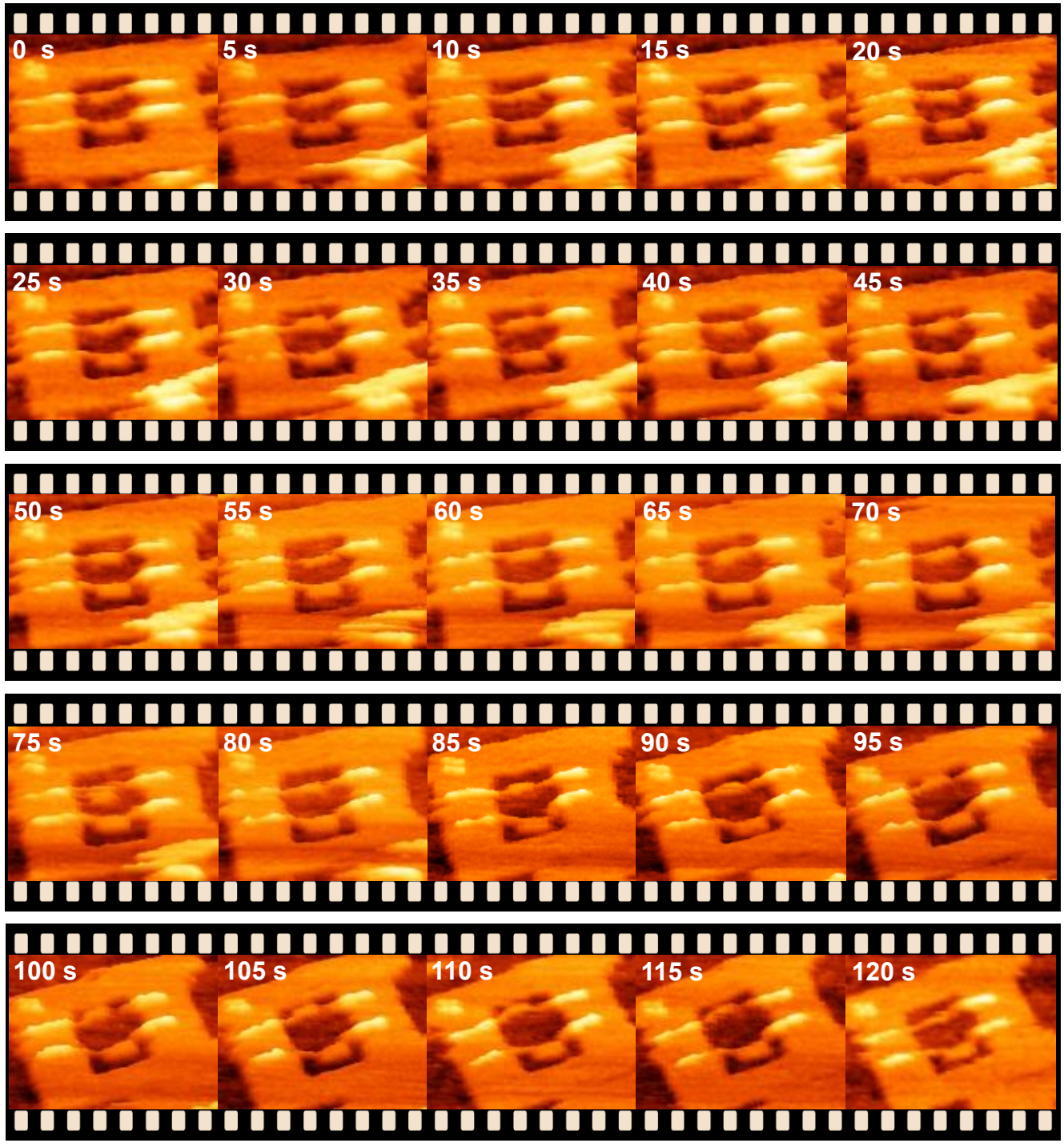
Start



Mg^{2+}





0.2 frame/sec

(5x speed)

150 × 110 nm

The End

J. AM. CHEM. SOC. 2004, *126*, 6566−6567

(*P*)-Helicene Displays Chiral Selection in Binding to Z-DNA

Yan Xu,[†] You Xin Zhang,[‡] Hiroshi Sugiyama,*[,†,§] Tomoko Umano,[⊥] Hideji Osuga,[⊥] and Kazuhiko Tanaka*[,⊥]

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda, Tokyo 101-0062, Japan, Jin Zhou Medical College, China, Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8501, Japan, and Department of Materials Science and Chemistry, Faculty of Systems Engineering, Wakayama University, Sakaedani 930 Wakayama 640-8510, Japan

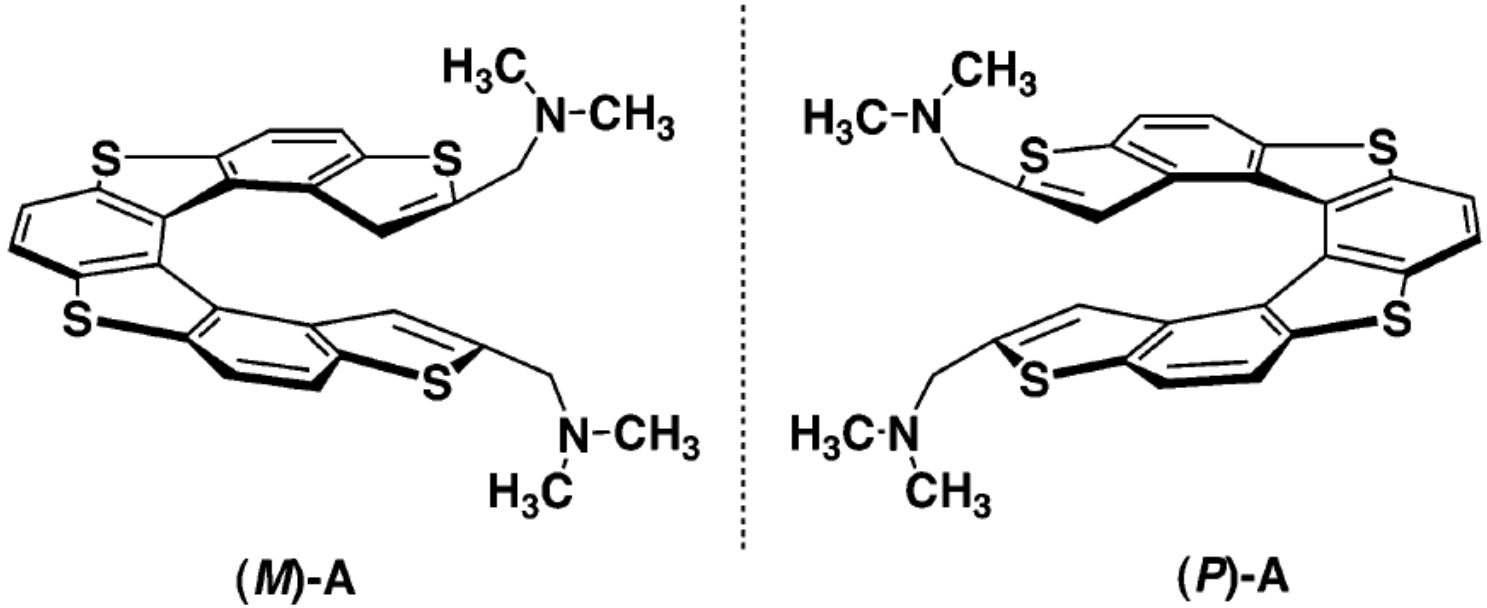
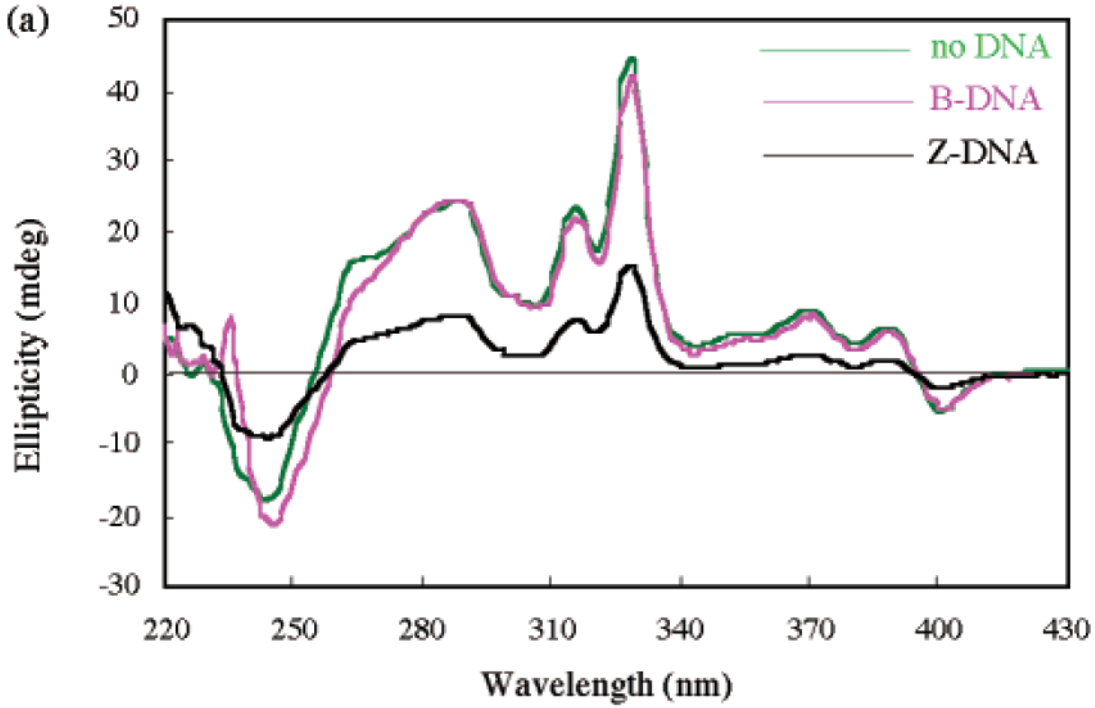
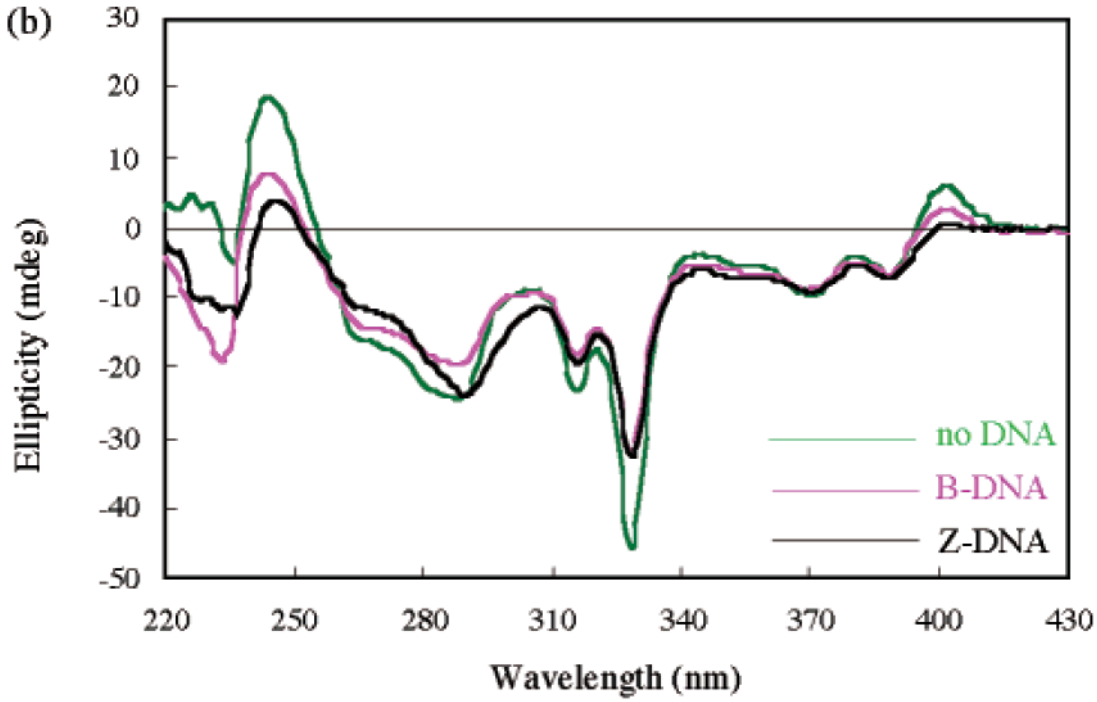


Figure 1. Structures of helicene (*P*)-A and (*M*)-A.


(a)
50
40
30
20
10
0
-10
-20
-30
Ellipticity (mdeg)
220
250
280
310
340
370
400
430
Wavelength (nm)
no DNA
B-DNA
Z-DNA


(b)
30
20
10
0
-10
-20
-30
-40
-50
Ellipticity (mdeg)
220
250
280
310
340
370
400
430
Wavelength (nm)
no DNA
B-DNA
Z-DNA

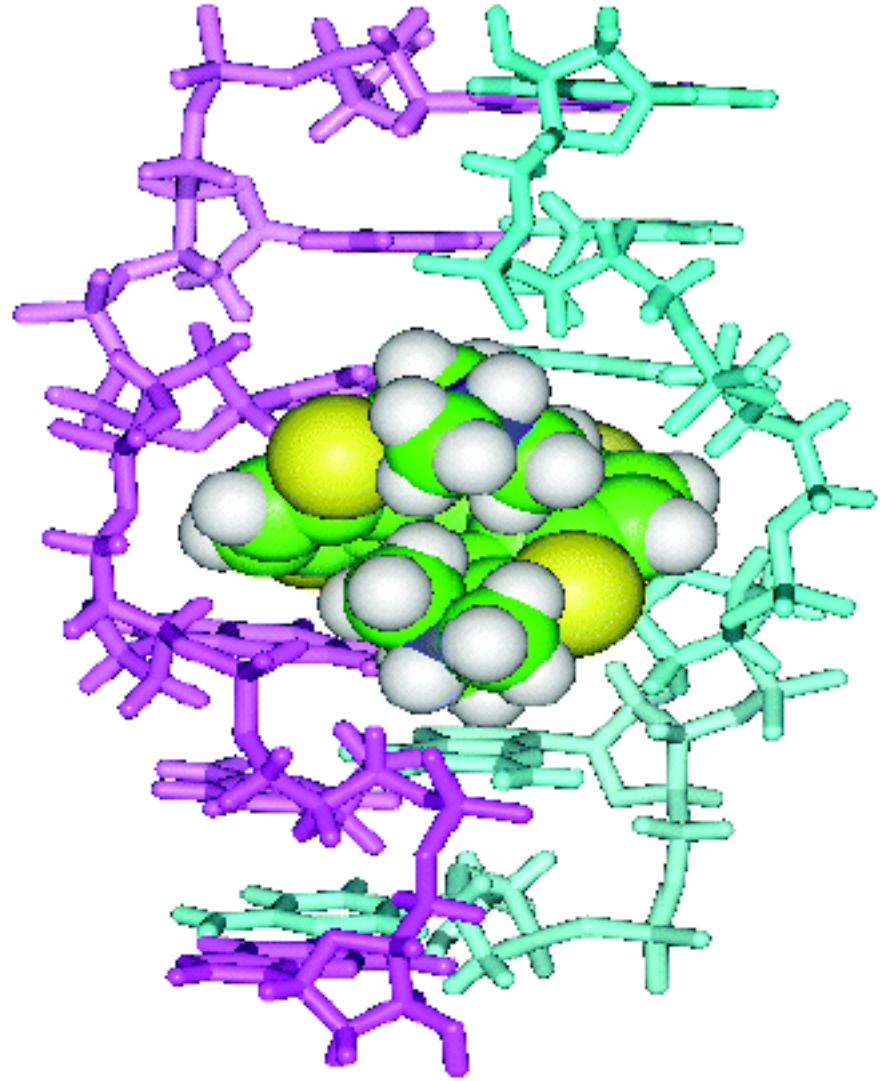
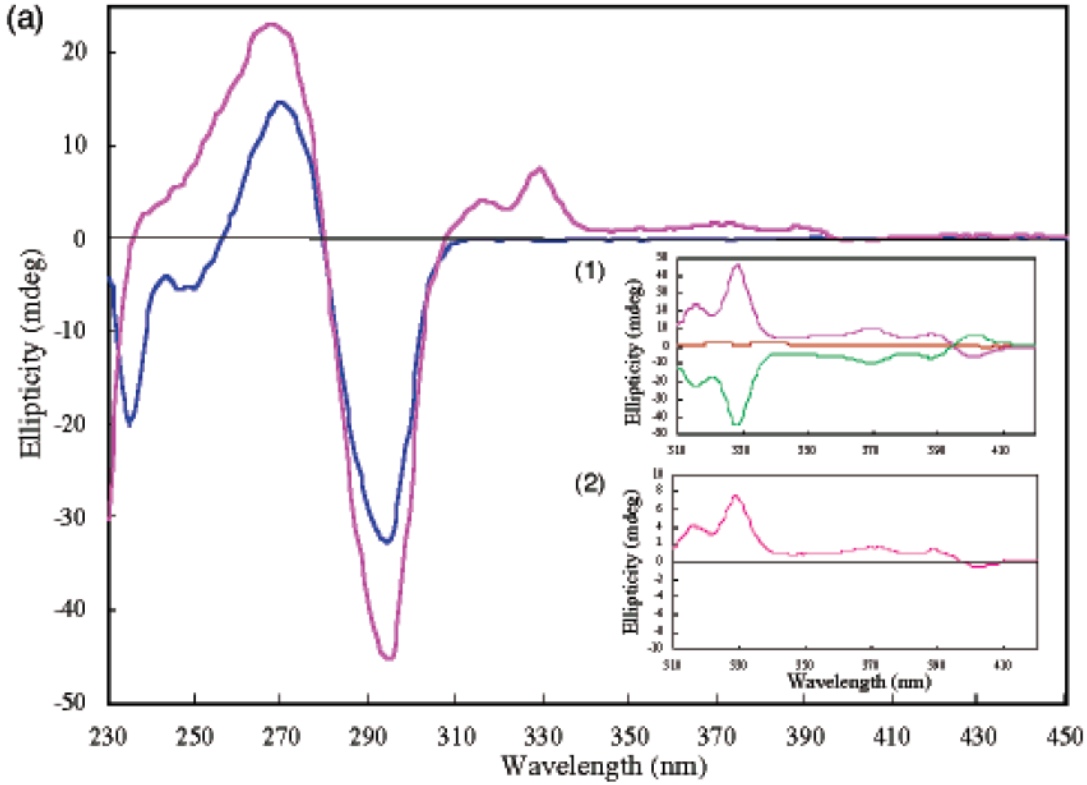
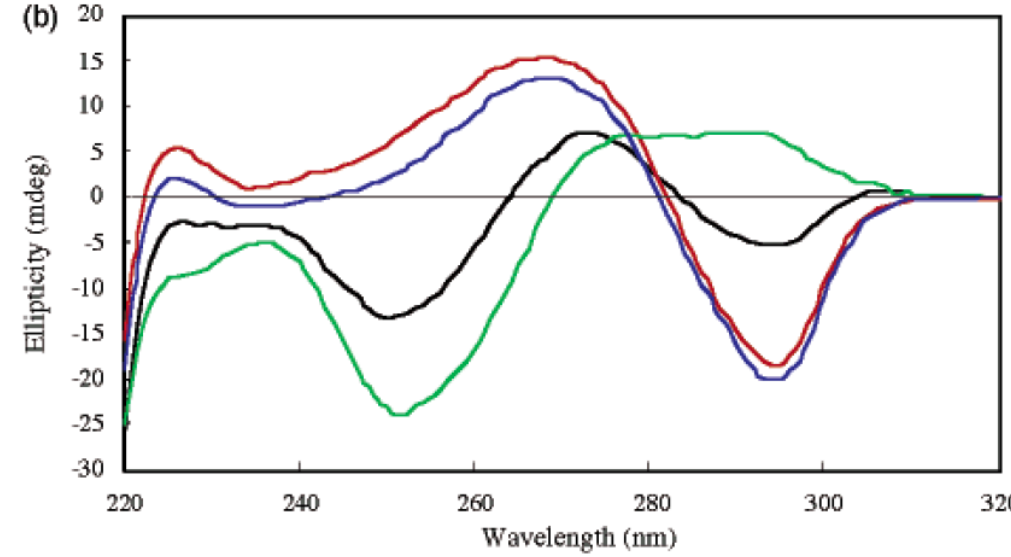


Table 1. Binding Constants for the Interactions of (*P*)-A and (*M*)-A with B-DNA and Z-DNA[a]

DNA	(*P*)-A	(*M*)-A
B-DNA	$(1.4 \pm 0.3) \times 10^4 M^{-1}$	$(2.8 \pm 0.3) \times 10^4 M^{-1}$
Z-DNA	$(8.0 \pm 0.5) \times 10^4 M^{-1}$	$(2.4 \pm 0.2) \times 10^4 M^{-1}$

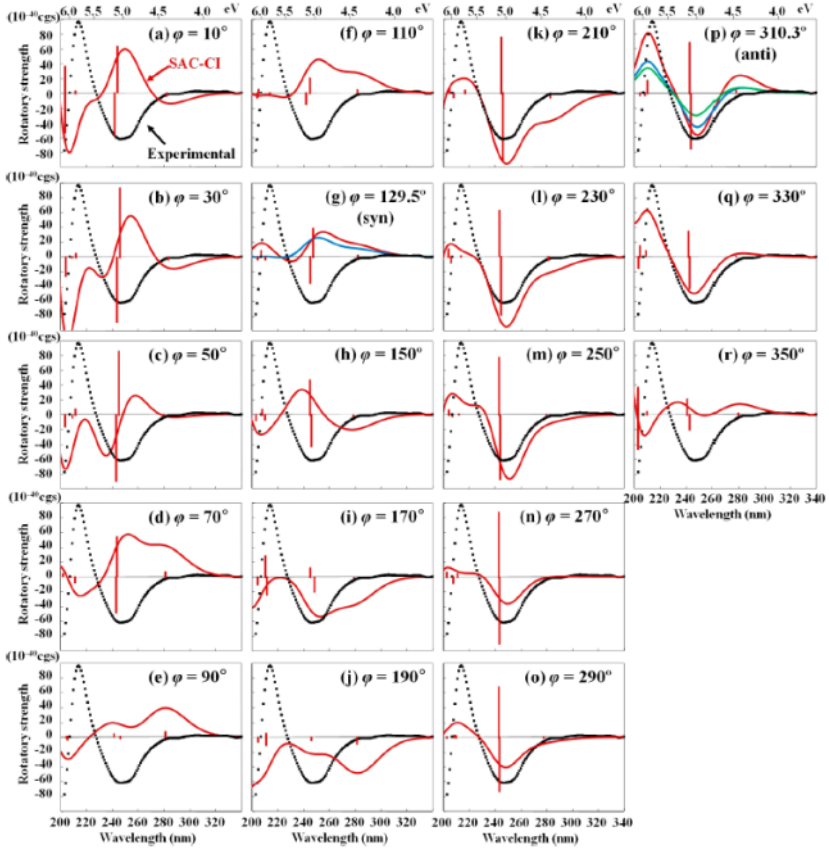


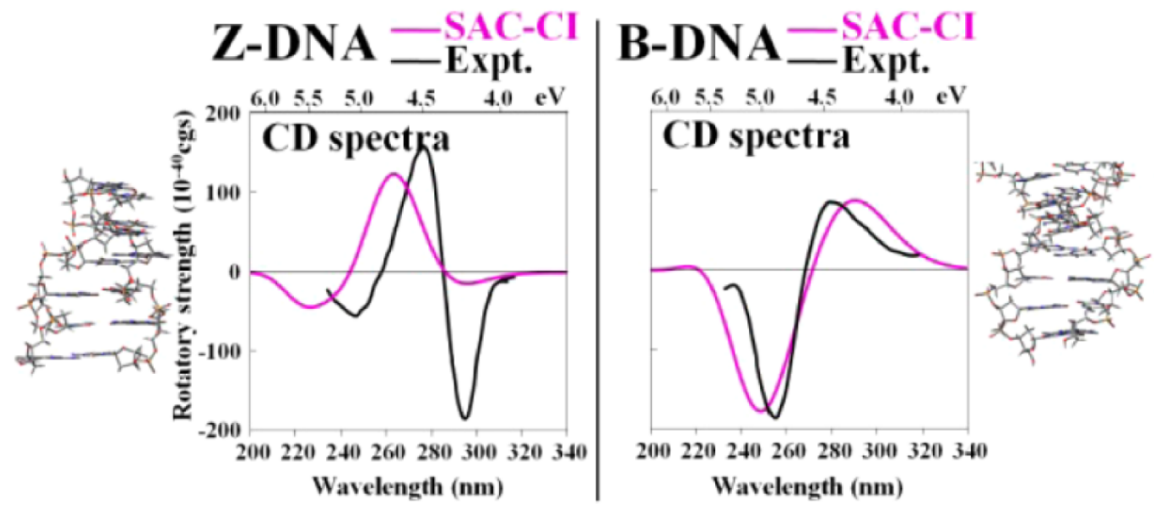


J. Phys. Chem. A 2013, 117, 42−55

Helical Structure and Circular Dichroism Spectra of DNA: A Theoretical Study

Tomoo Miyahara,[†] Hiroshi Nakatsuji,*[,†] and Hiroshi Sugiyama[‡]

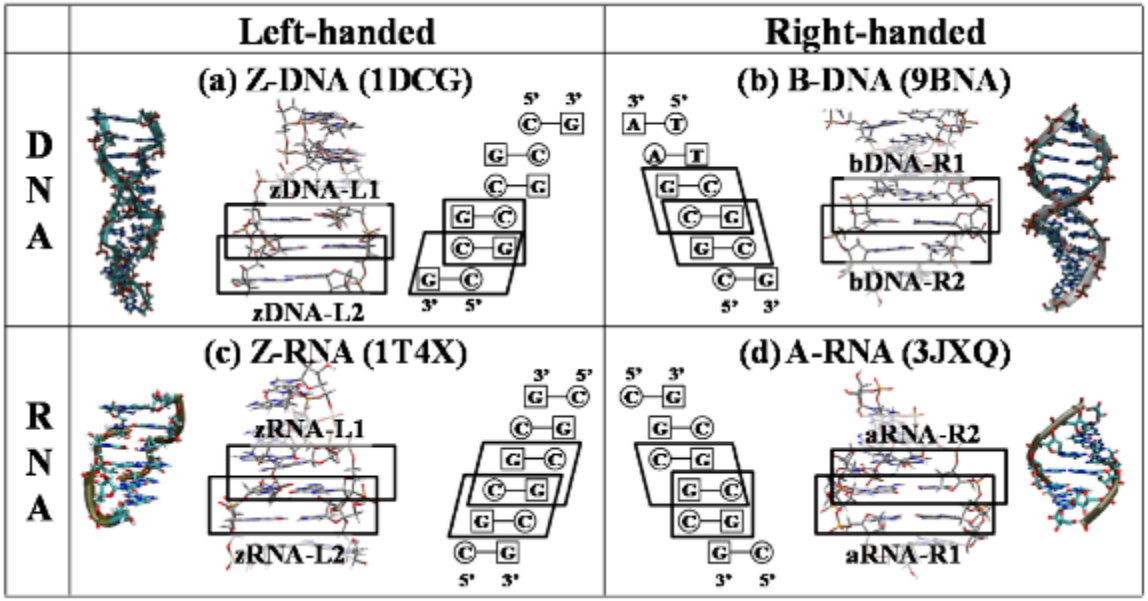


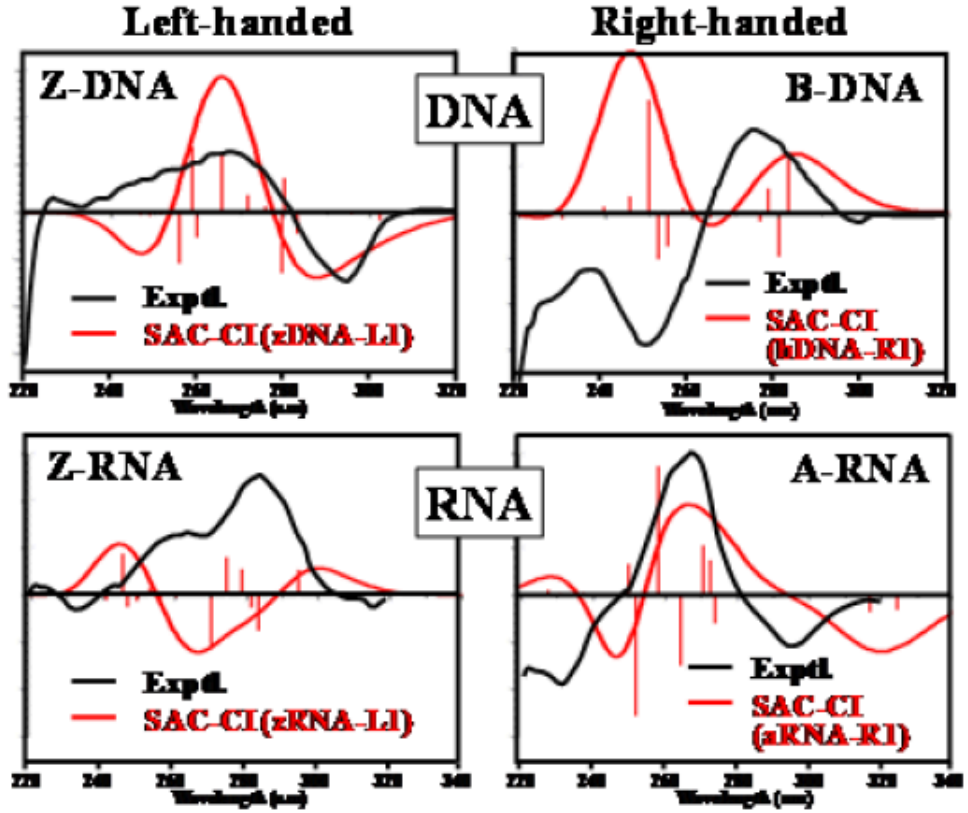


The Journal of Physical Chemistry in press

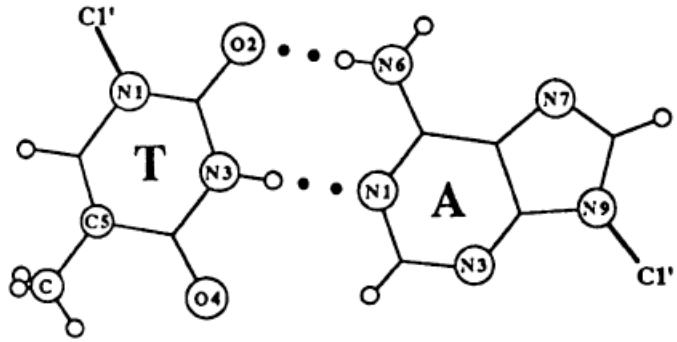
Similarity and Difference Between RNA and DNA Double-Helical Structures in Circular Dichroism Spectroscopy: A SAC-CI Study

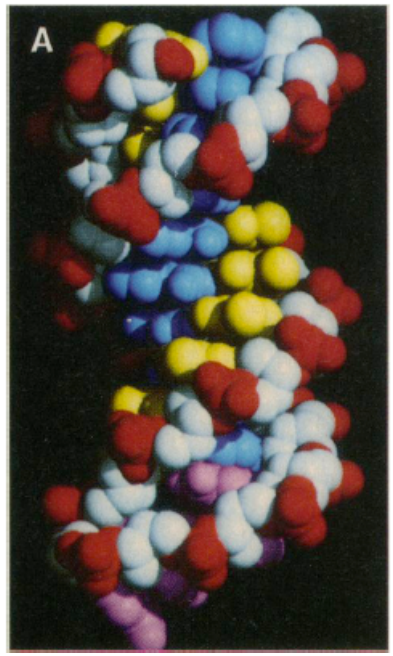
Miyahara, Tomoo; Quantum Chemistry Research Institute,
Nakatsuji, Hiroshi; Quantum Chemistry Research Institute (QCRI),
Sugiyama, Hiroshi; Kyoto University, Department of Chemistry, Graduate School of Science

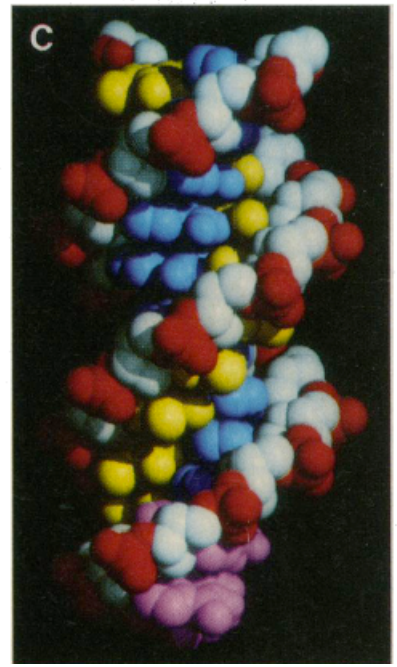


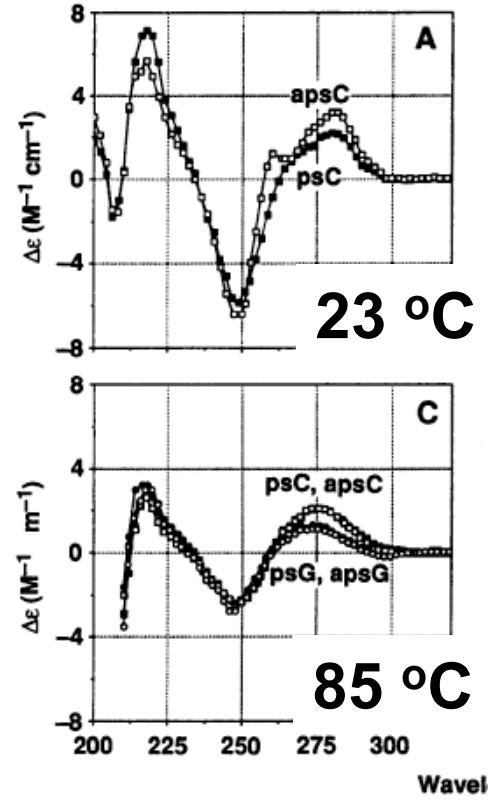


Parallel-stranded duplexes









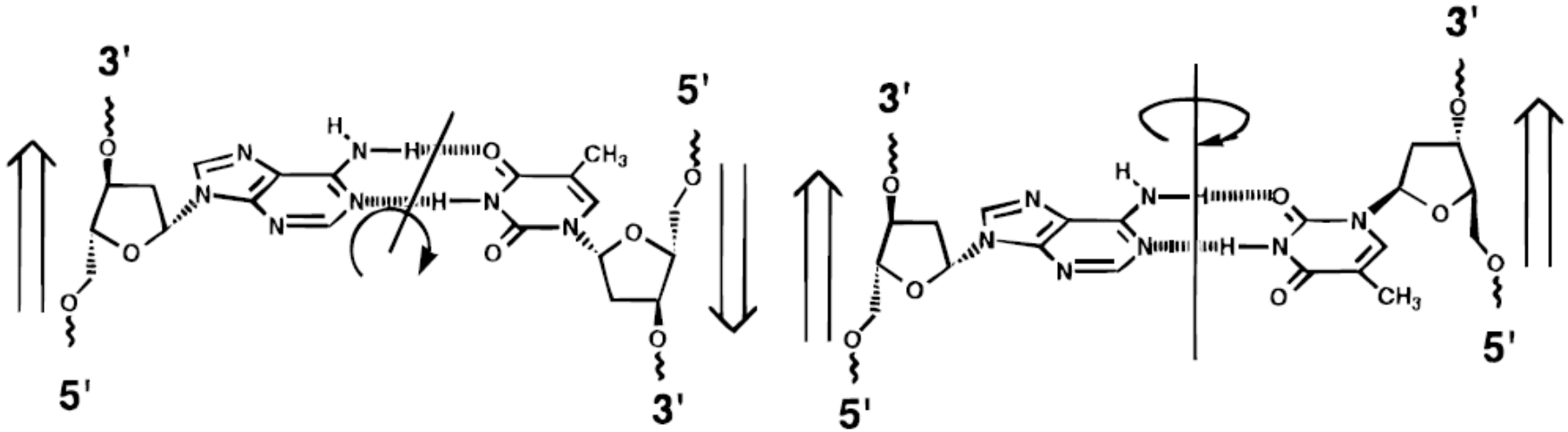
Van de Sande et al **Science** (1988) 241: 551

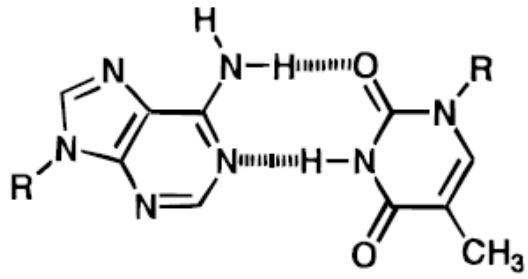
J. Am. Chem. Soc. **1996,** *118,* 9994−9995

Remarkably Stable Parallel-Stranded Oligonucleotides Containing 5-Methylisocytosine and Isoguanine

Hiroshi Sugiyama,* Shuji Ikeda, and Isao Saito*

Department of Synthetic Chemistry
and Biological Chemistry
Faculty of Engineering, Kyoto University
Kyoto 606-01, Japan





A T

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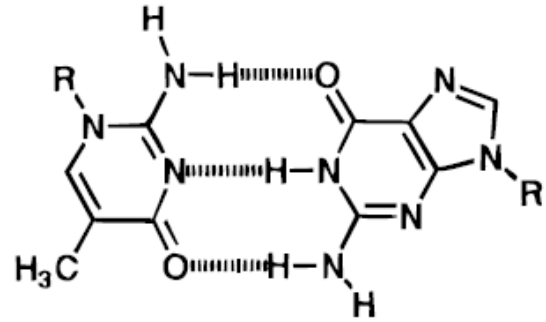
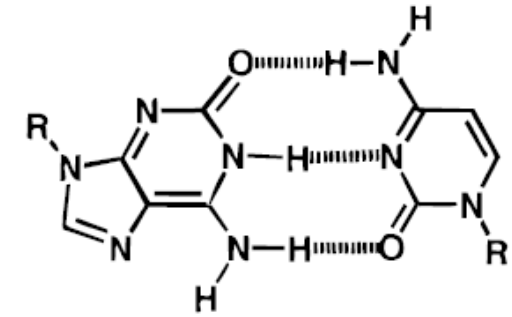


iC G

(-12.0 kcal)

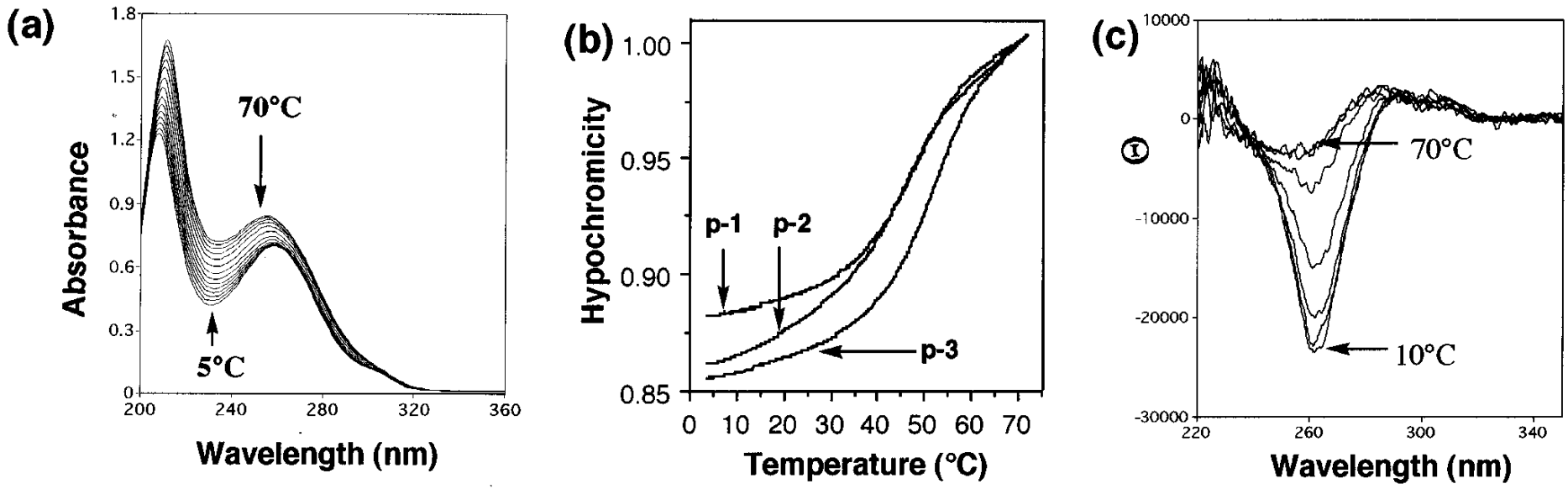


iG C

(-13.7 kcal)

Table 1. Melting Temperatures of Parallel- and Antiparallel Duplexes[a]

duplex	sequence	T_m (°C) at low salt[b]	T_m (°C) at 1.0 M NaCl
p-1	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT G C CT GA)-3′	25	46
ap-1	5′-d(ACGTGCCTGA)-3′ 3′-d(TGCACGGACT)-5′	25	56, 55[d]
c-1	5′-d(ACGTGCCTGA)-3′ 5′-d(TGCACGGACT)-3′	nd[c]	36[e]
ph-1	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-r(A C GU G C CU GA)-3′	24	50
aph-1	5′-r(ACGUGCCUGA)-3′ 3′-d(TGCACGGACT)-5′	23	53, 52[d]
pm-1	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT G A CT GA)-3′	nd[c]	37 (−9)[f]
pm-2	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT G G CT GA)-3′	nd[c]	38 (−8)[f]
pm-3	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT G T CT GA)-3′	nd[c]	33 (−13)[f]
pm-4	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT A C CT GA)-3′	nd[c]	26 (−20)[f]
pm-5	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT C C CT GA)-3′	nd[c]	22 (−24)[f]
pm-6	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT T C CT GA)-3′	nd[c]	32 (−14)[f]



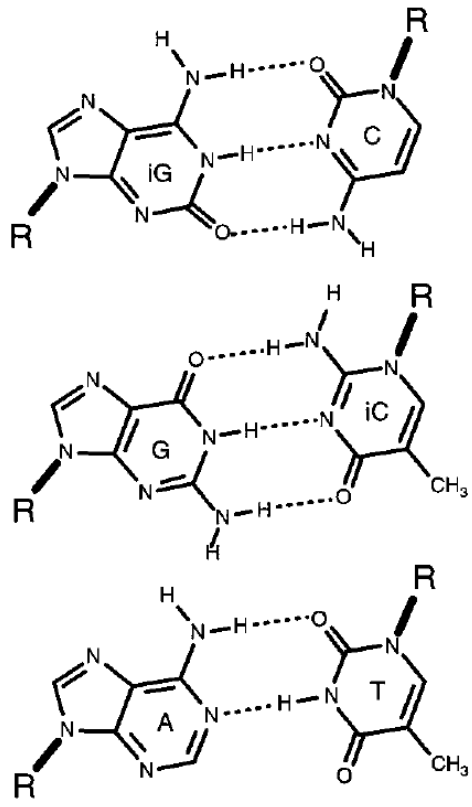
p-1	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT G C CT GA)-3′	25	46
p-2	5′-d(TAiCAiCiGiGAiCT)-3′ (ODN II) 5′-d(AT GT G C CT GA)-3′	14	45
p-3	5′-d(TiGAAiCiGiGAiCT)-3′ (ODN III) 5′-d(ACTT G C C T GA)-3′	18	50

Biophysical Journal Volume 75 September 1998 1163–1171

Structural Studies of a Stable Parallel-Stranded DNA Duplex Incorporating Isoguanine:Cytosine and Isocytosine:Guanine Basepairs by Nuclear Magnetic Resonance Spectroscopy

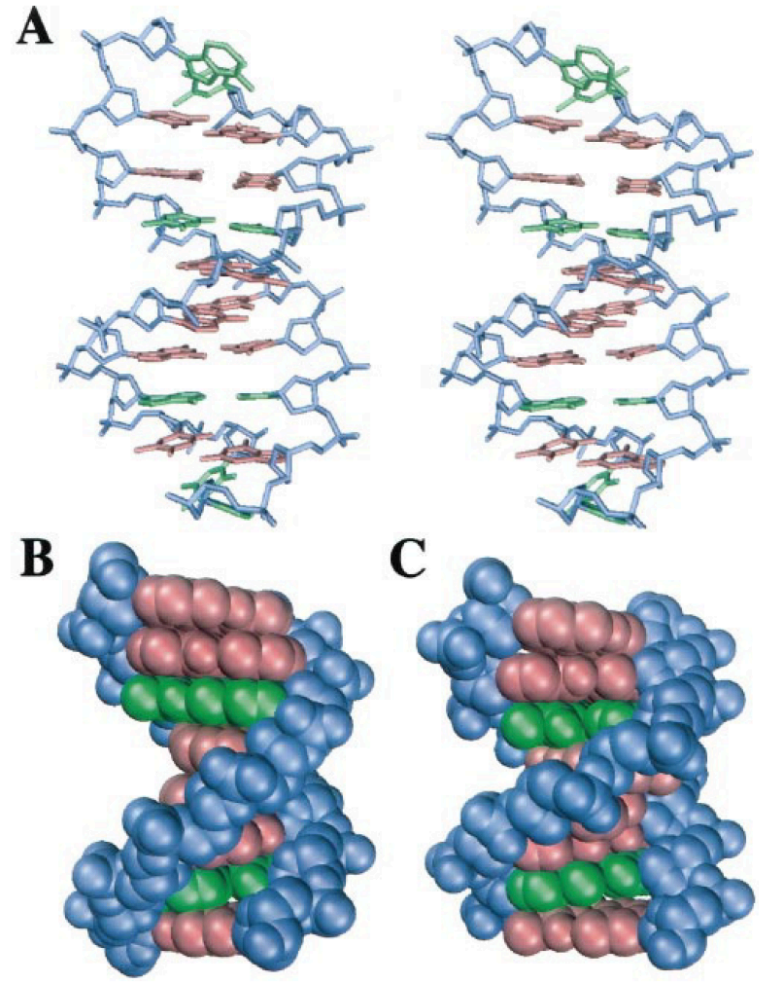
Xiang-Lei Yang,* Hiroshi Sugiyama,# Shuji Ikeda,§ Isao Saito,§ and Andrew H.-J. Wang*

A



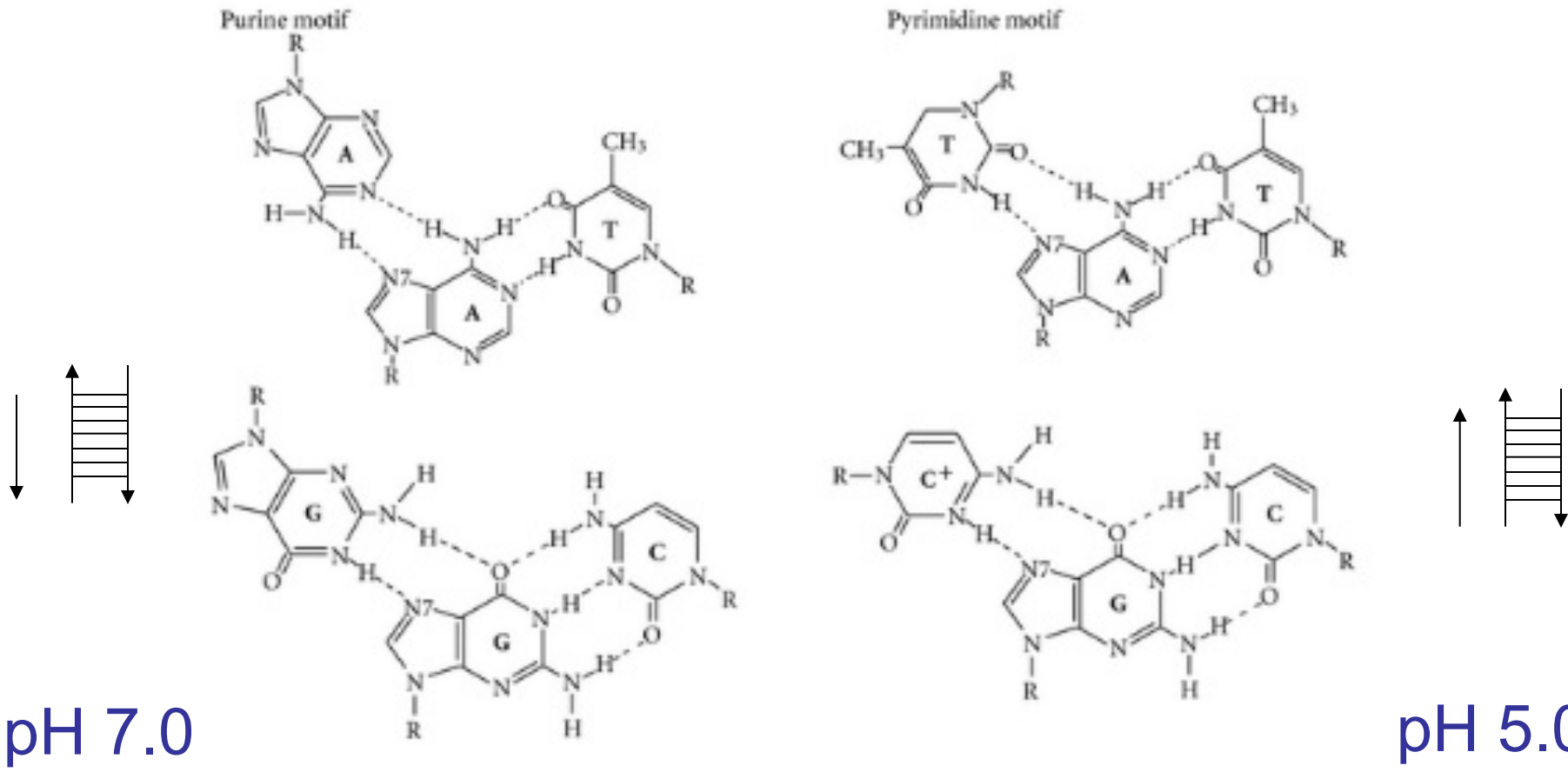
B

5'-T1 iG2 iC3 A4 iC5 iG6 iG7 A8 iC9 T10
5'-A11C12G13T14G15C16C17T18G19A20

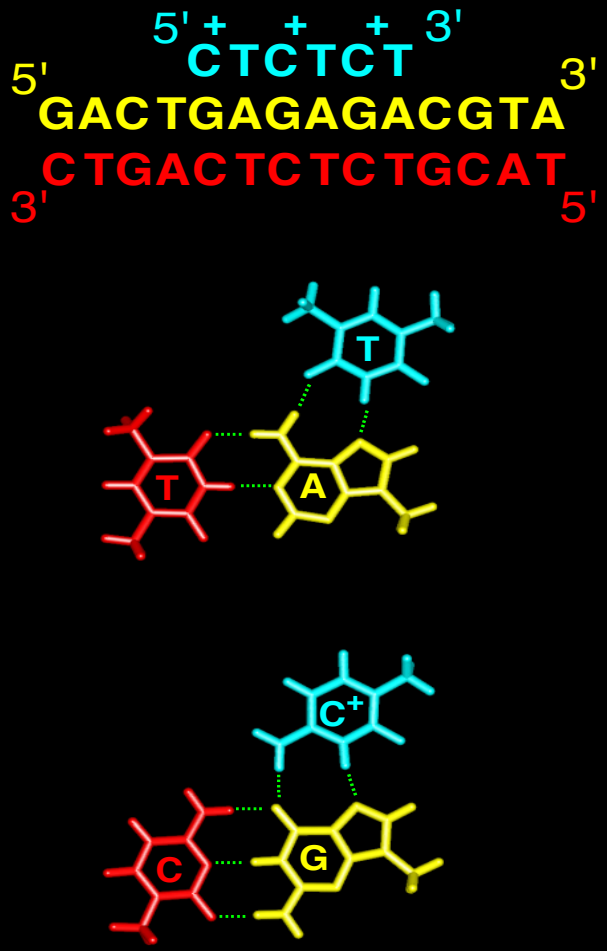


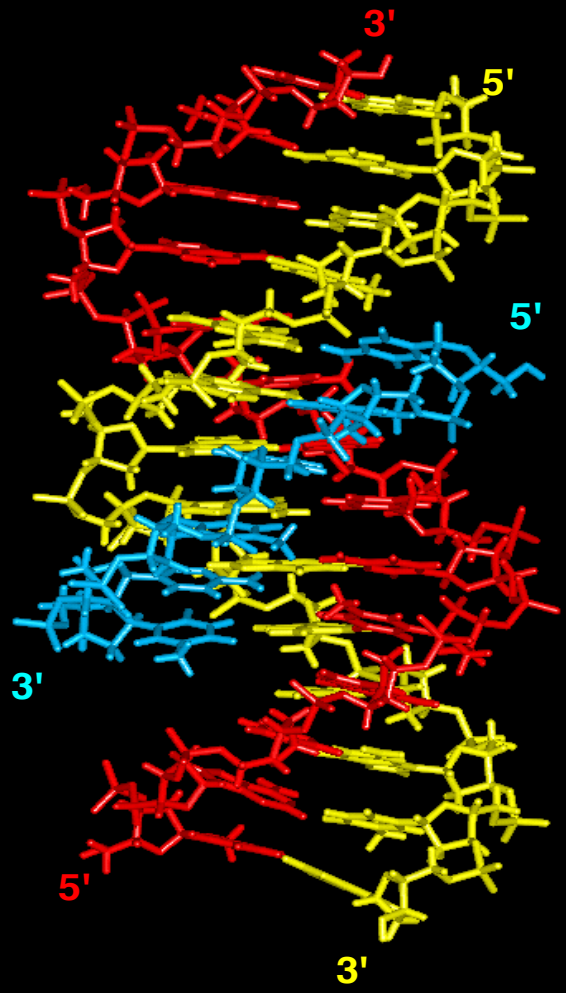
B-DNA Parallel duplex

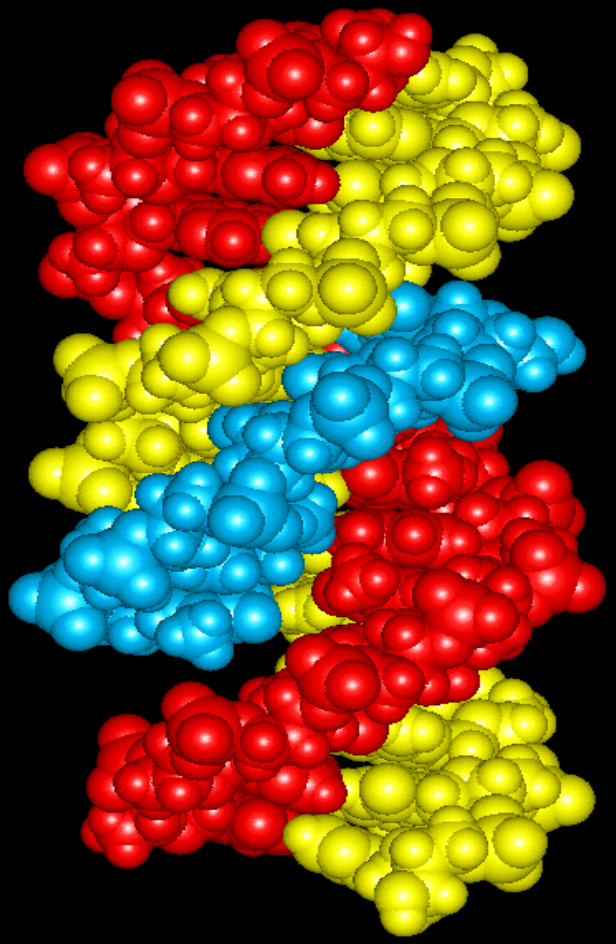
Triple helix



Triple helix structure







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RNA-DNA Triplex Formation by Long Noncoding RNAs

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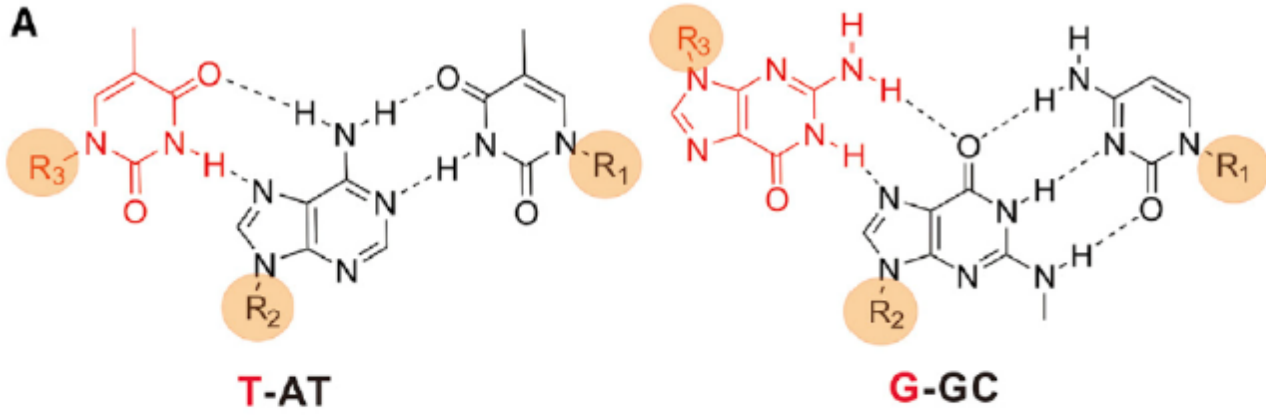

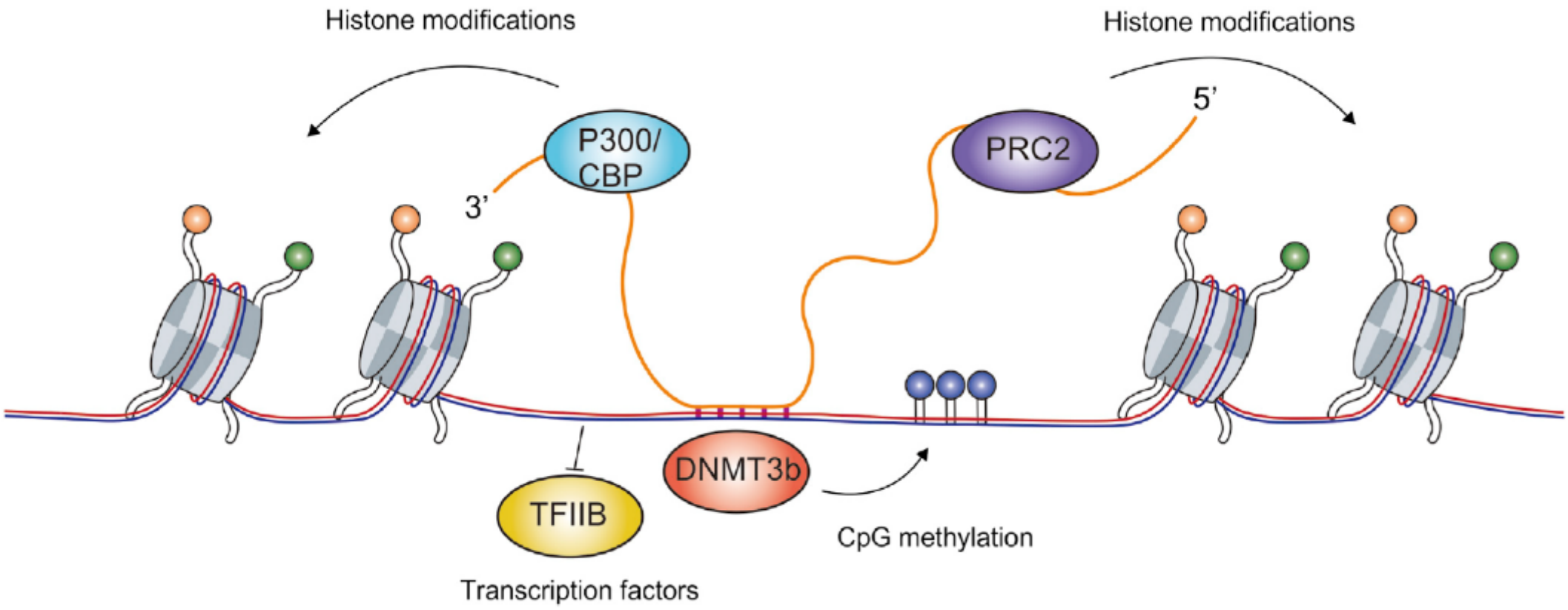

Figure 2. Examples of lncRNAs that Are Reported to Form RNA-DNA Triplexes

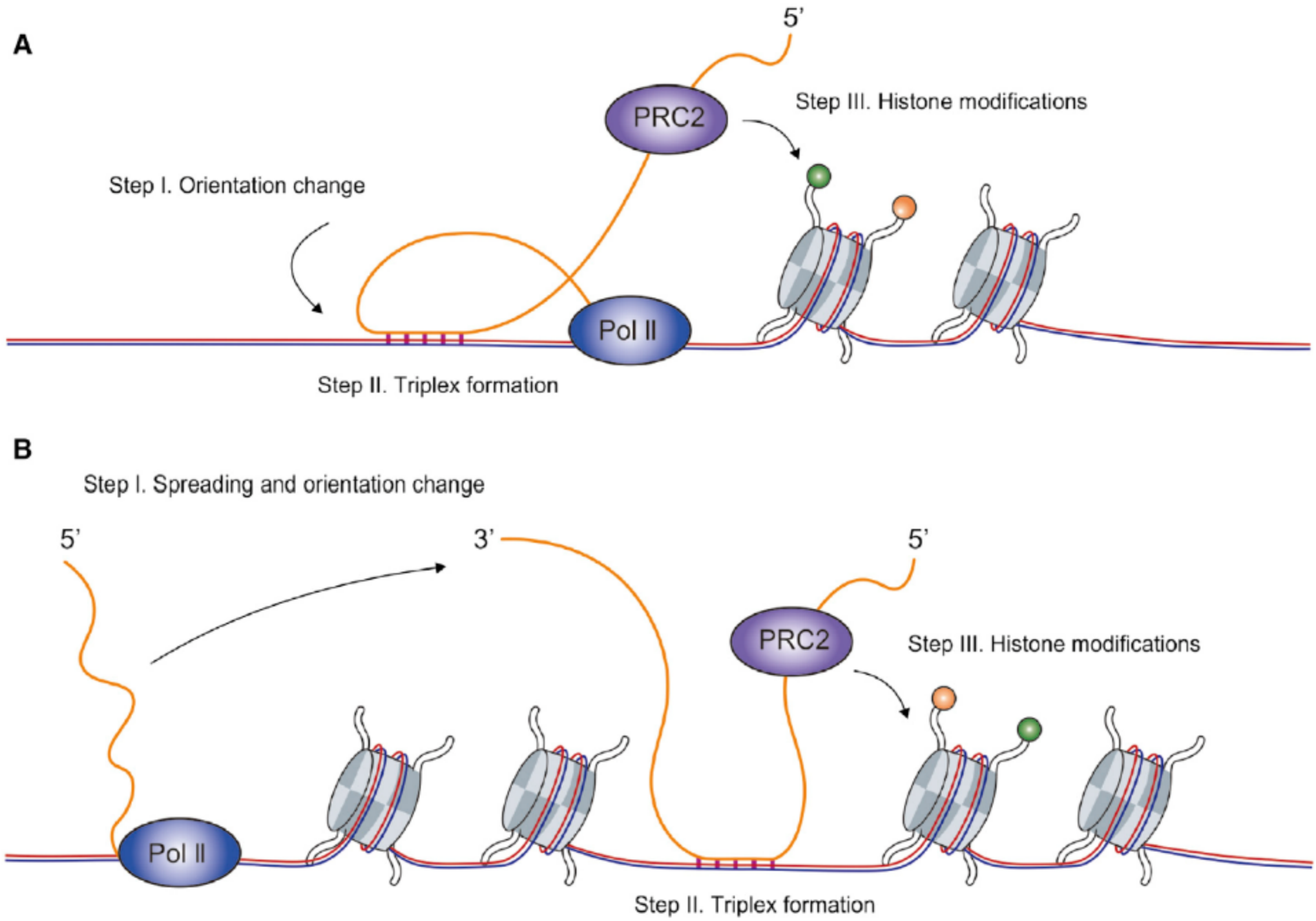

Figure 3. Orientation of Triplex Formation by lncRNAs