Conformational Properties of DNA revealed by CD Spectroscopy

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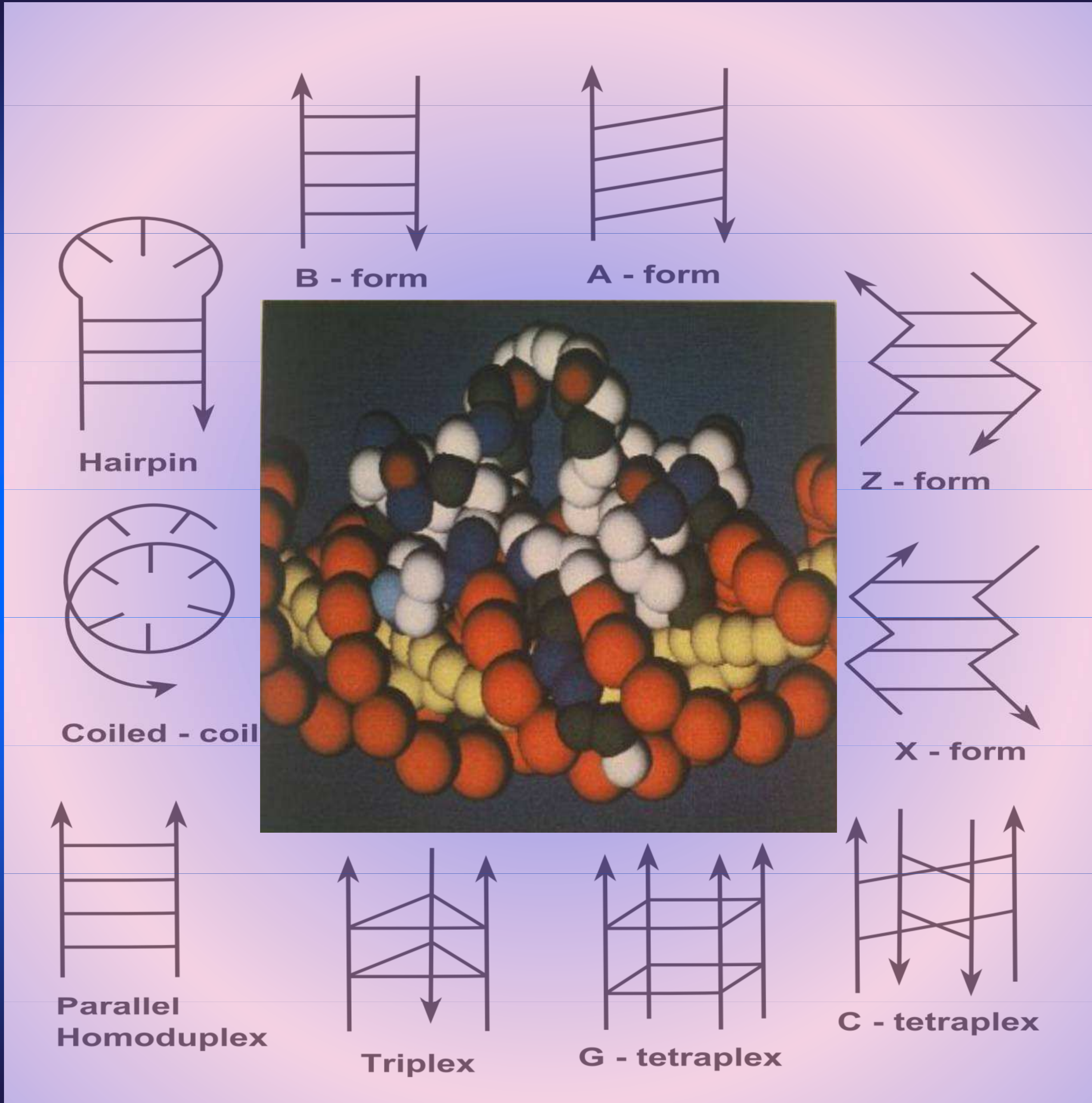
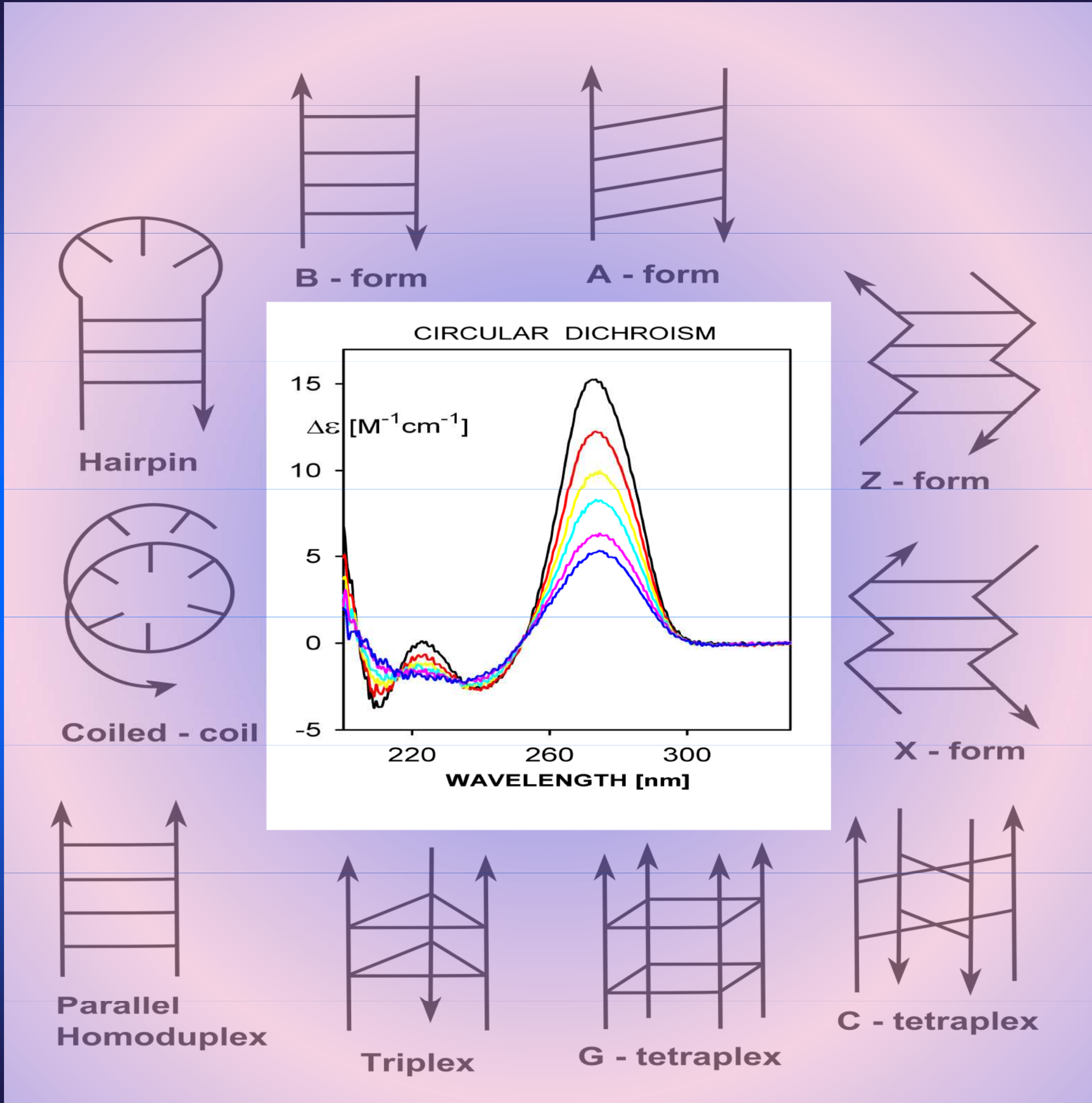
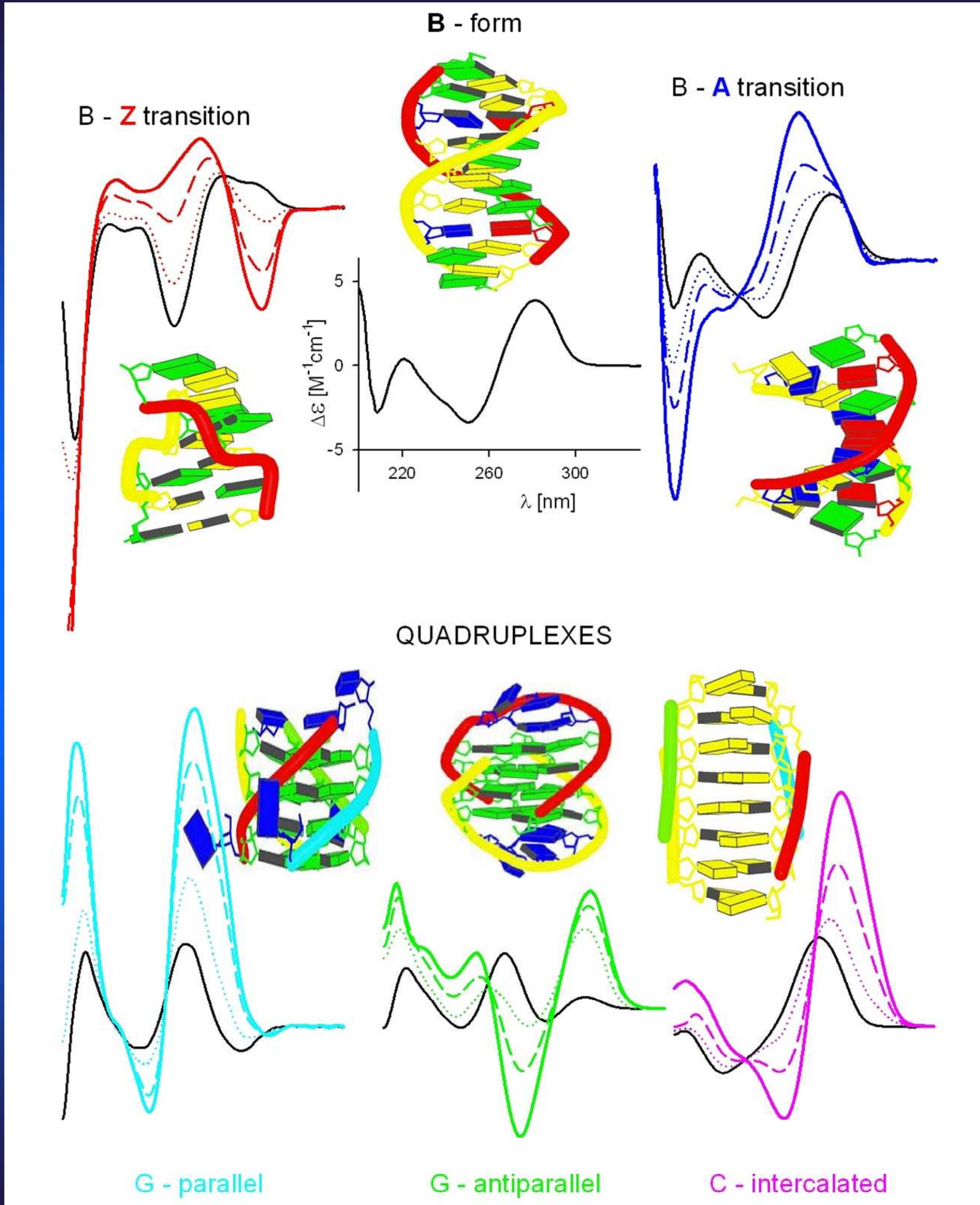
Laboratory of Biophysics of nucleic acids


B - form
A - form
Hairpin
Z - form
Coiled - coil
X - form
Parallel
Homoduplex
Triplex
G - tetraplex
C - tetraplex


B - form
A - form
Hairpin
Z - form
Coiled - coil
X - form
Parallel
Homoduplex
Triplex
G - tetraplex
C - tetraplex
CIRCULAR DICHROISM
Δε [M-1cm-1]
15
10
5
0
-5
220
260
300
WAVELENGTH [nm]



Kypr, J., Kejnovska, I., Renciuk, D., Vorlickova, M.: Nucleic Acids Res. **37** (2009) 1713-1725

Circular dichroism and optical activity of biopolymers

) CD – principle, quantities - ellipticity, ΔA, Δε, relation between ORD and CD

Optical activity property of a chiral molecule - the rotation of the plane of linearly polarized light traveling through chiral materials

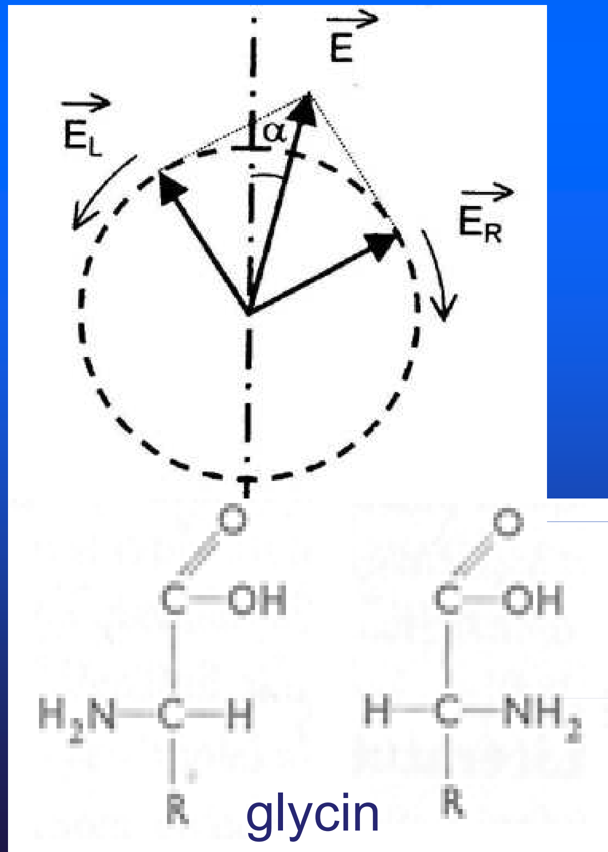
Chiral molecules (aminoacids, sugars) are those lacking mirror symmetry

Optical rotation of the plane of polarization (difference in refraction indexes –difference in propagation velocity)

may be either to the right (dextrorotatory -D)
or to the left (levorotatory –L) depending on the stereoisomer (enantiomer) present

Specific rotation – characteristic quantity

Optical rotatory dispersion - ORD
is the the variance of specific rotation with wavelength

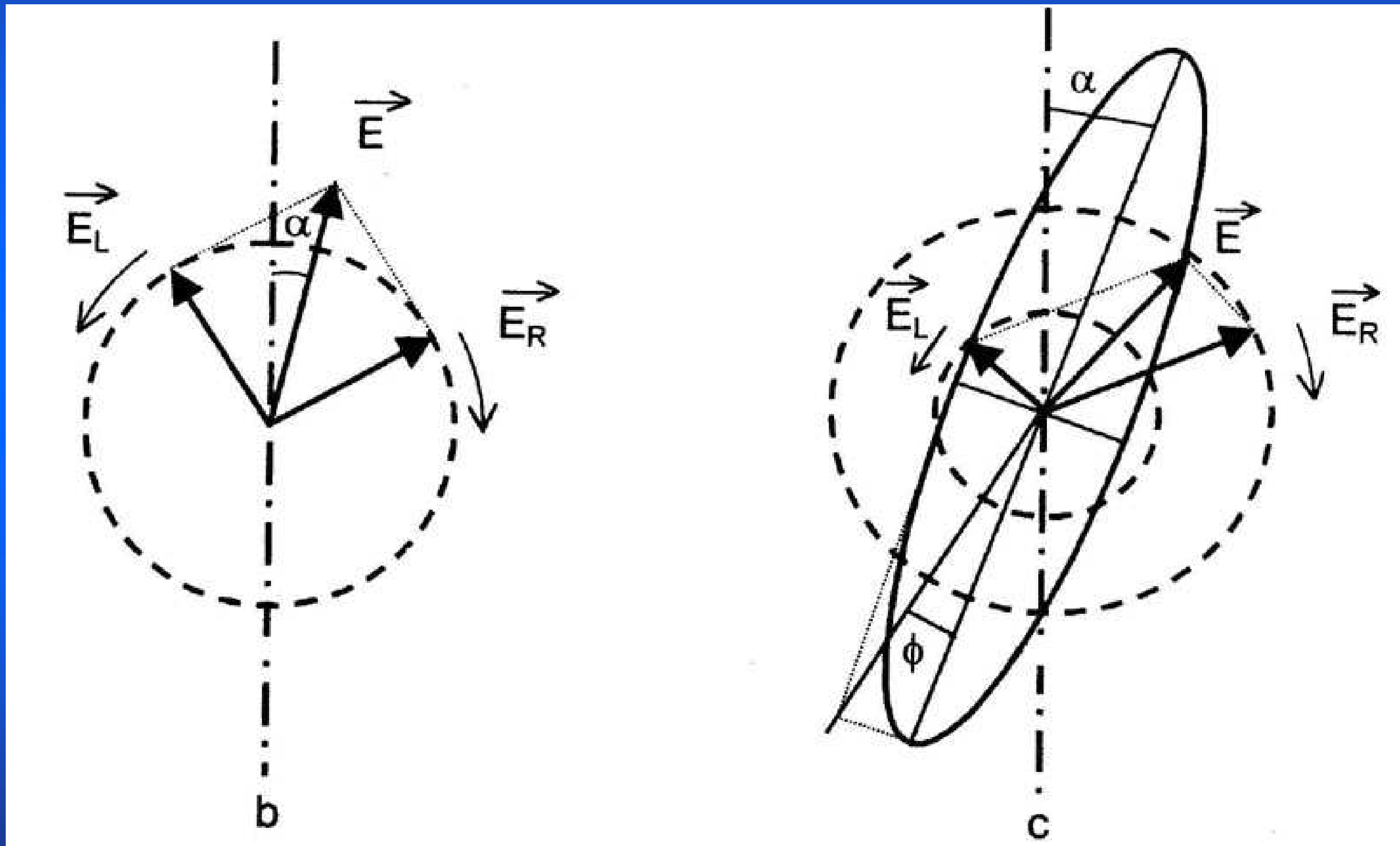


Specific rotation

$$[\alpha]^T_\lambda = \alpha/cl$$

Circular dichroism and optical activity of biopolymers

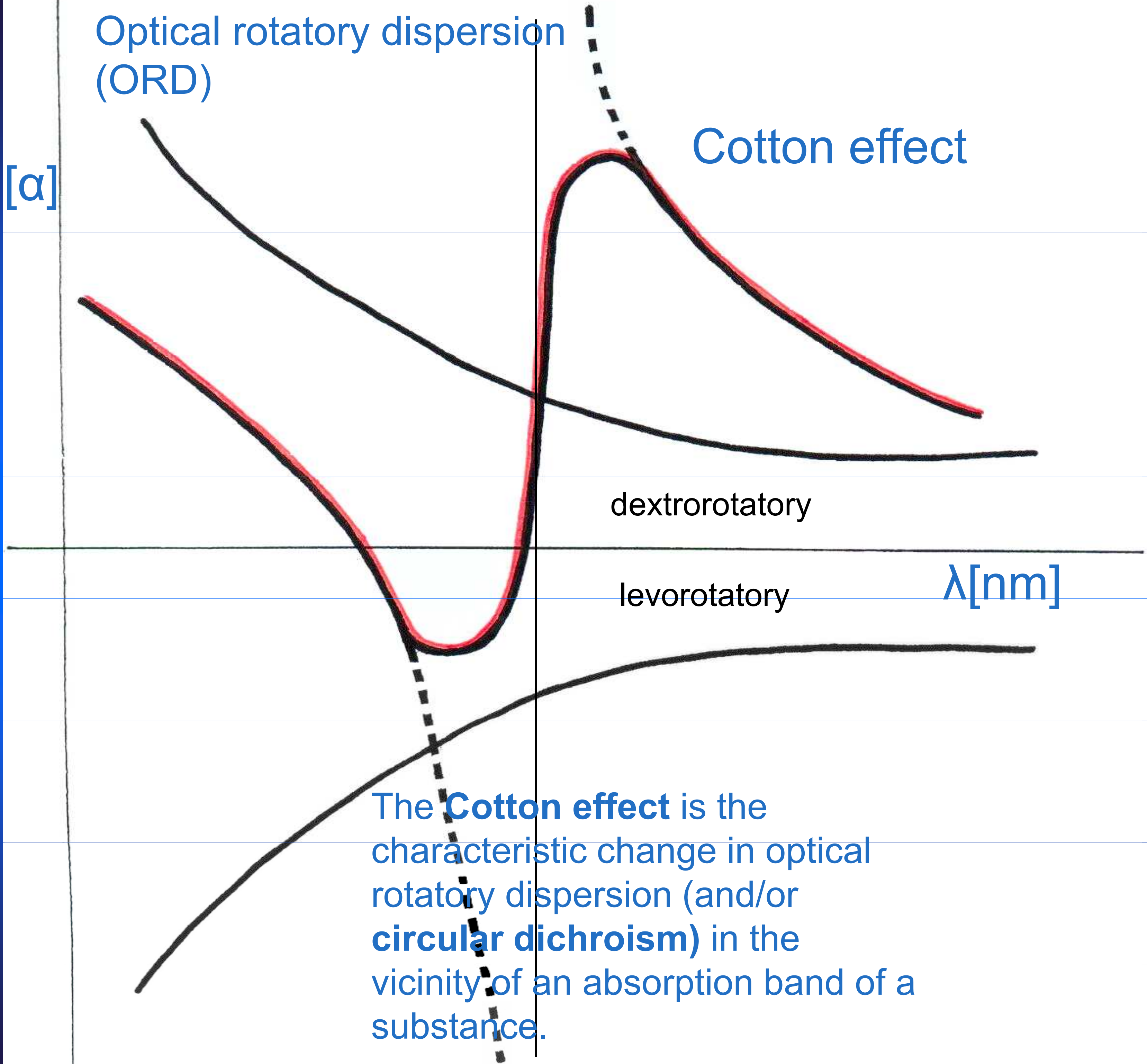
CD phenomenon – different absorption of the left-handed and right-haned circularly polarized light.



quantity- ellipticity φ [θ] $tg\ \theta = b/a = \varepsilon_L - \varepsilon_{R/}\ \varepsilon_L + \varepsilon_R$ = difference/sum

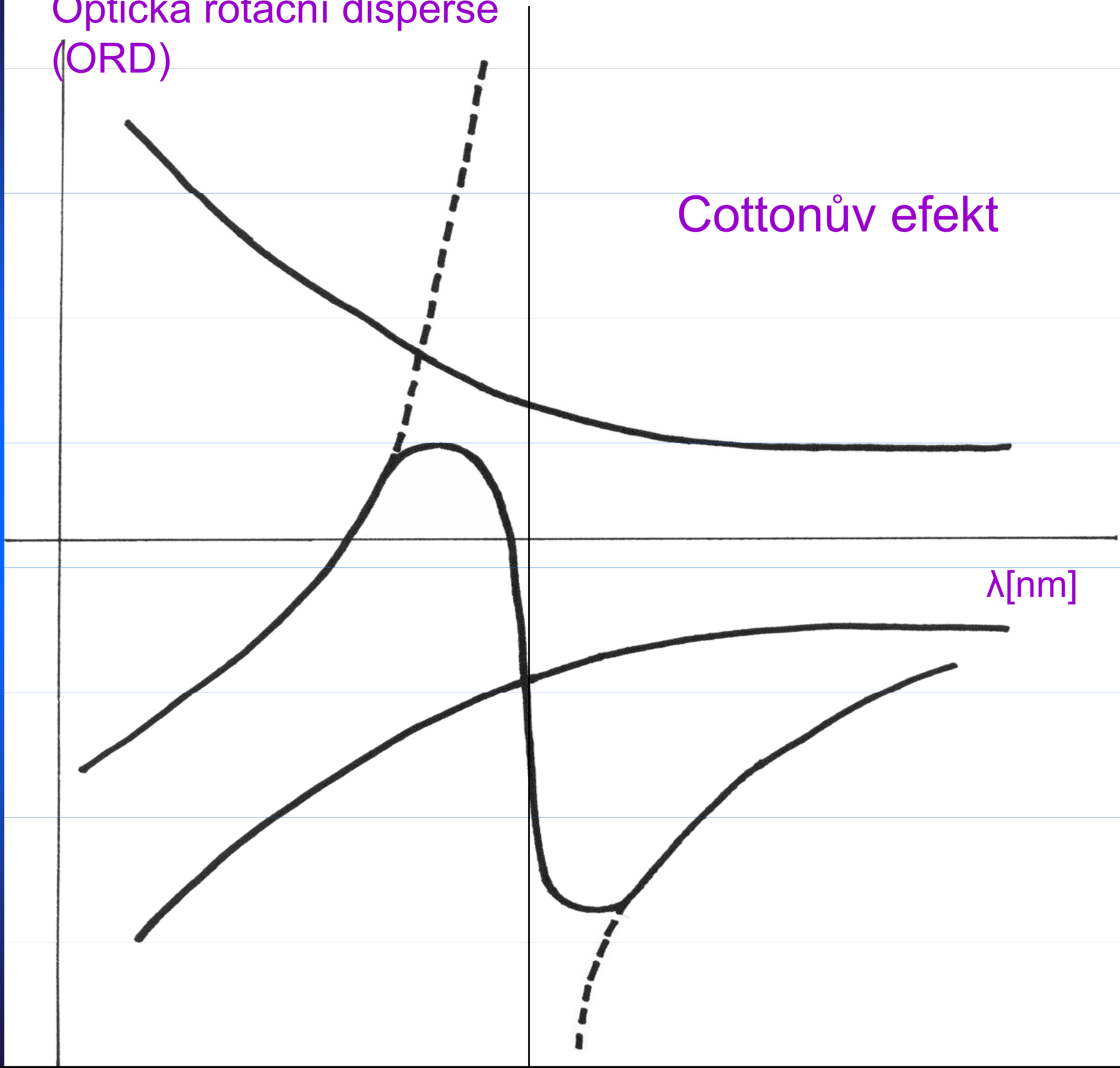
Circular dichroism $\Delta\varepsilon$ $\Delta\varepsilon = \varepsilon_L - \varepsilon_R = \Delta A/cl$, $\theta=3300.\ \Delta\varepsilon$

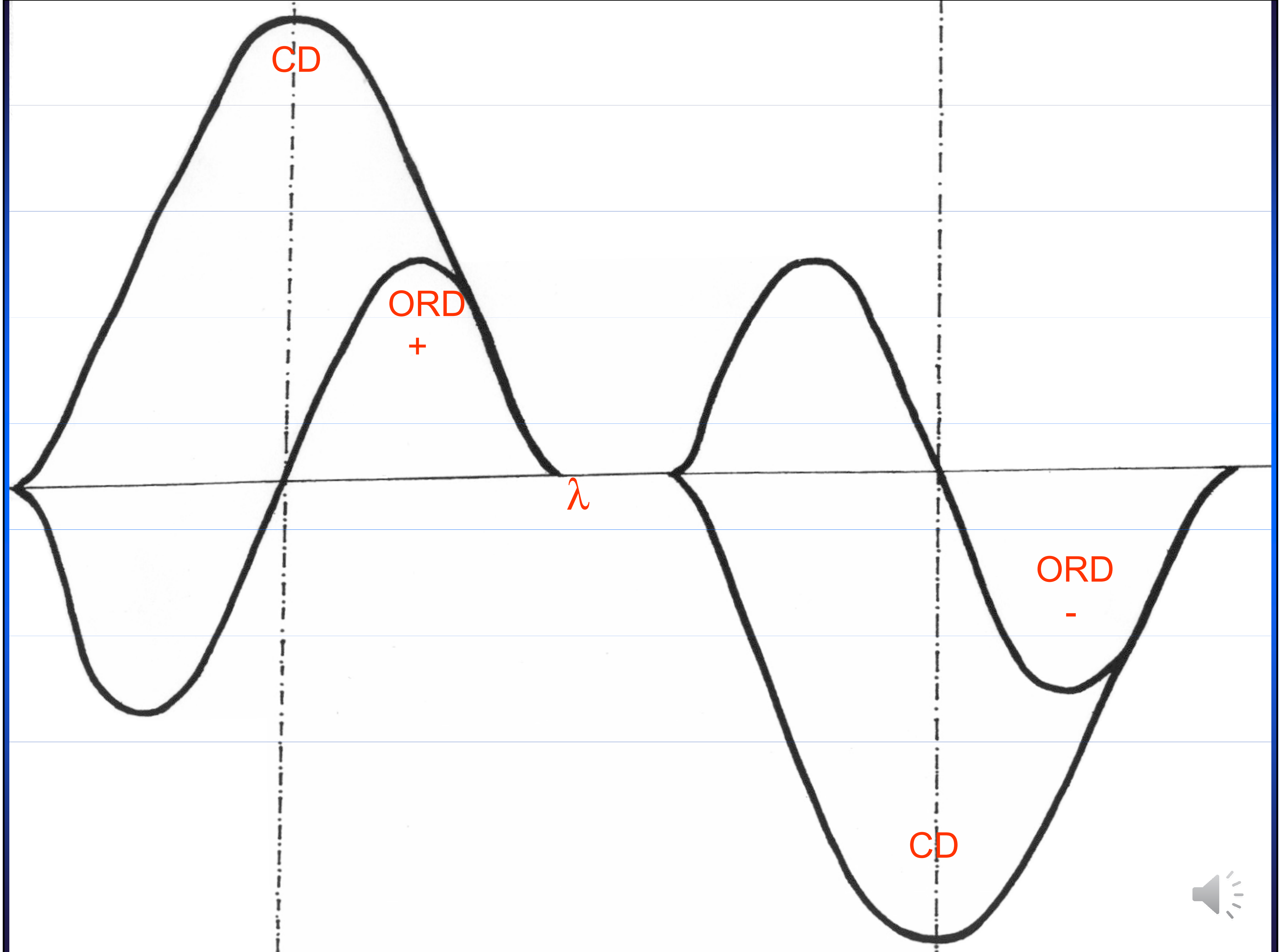
Optical rotatory dispersion (ORD)



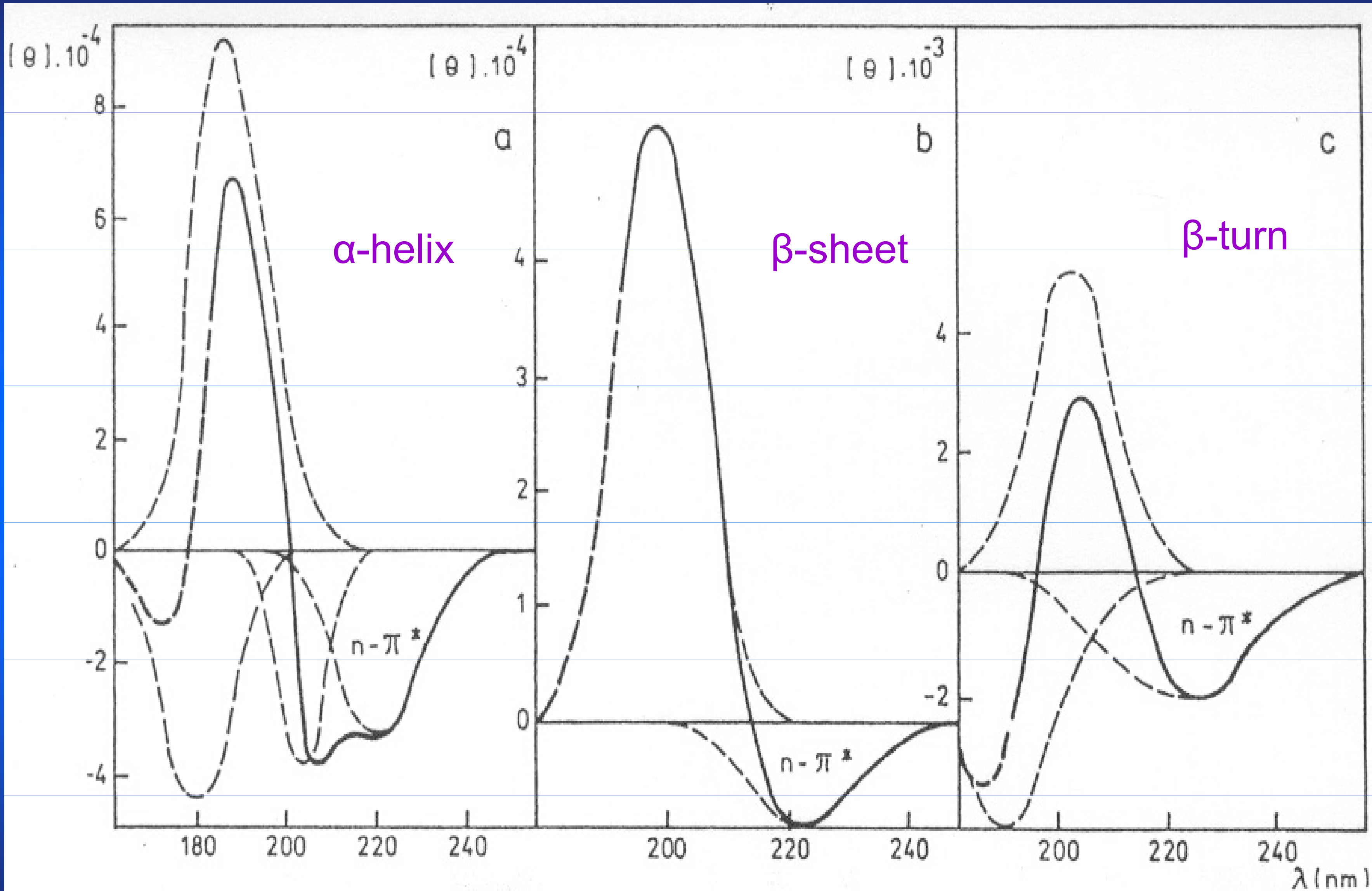
The **Cotton effect** is the characteristic change in optical rotatory dispersion (and/or **circular dichroism)** in the vicinity of an absorption band of a substance.

Optická rotační disperse (ORD)

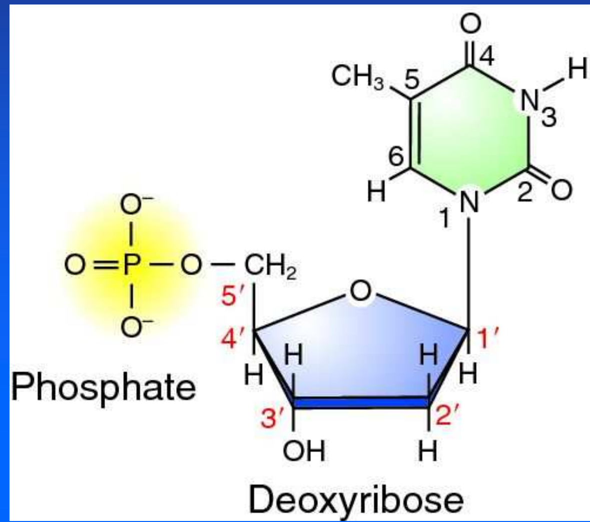



CD
ORD
+
λ
ORD
-
CD

CD of proteins



Preconditions of the origin of CD



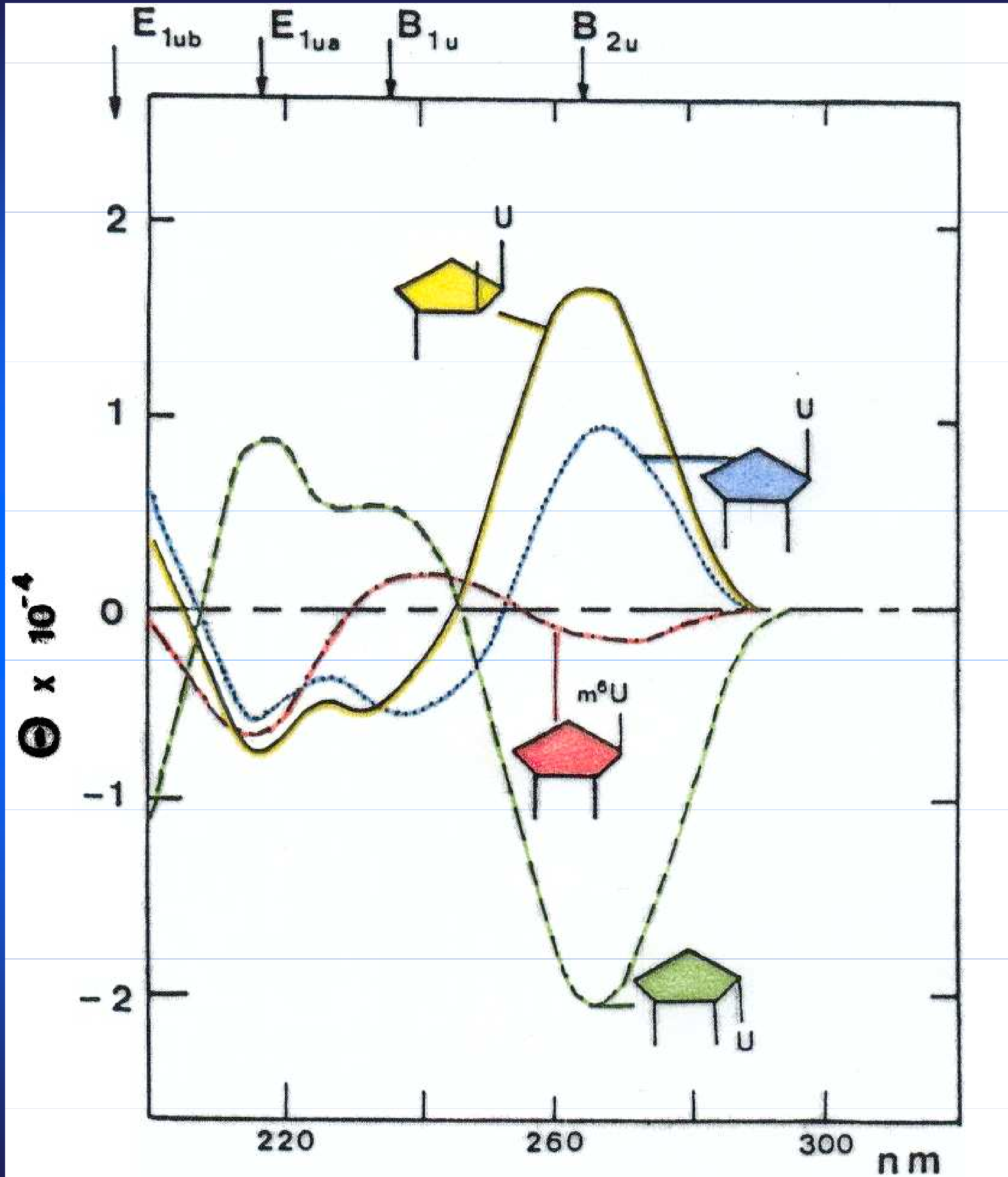
ABSORBCE

\+ CD

CHIRALITY

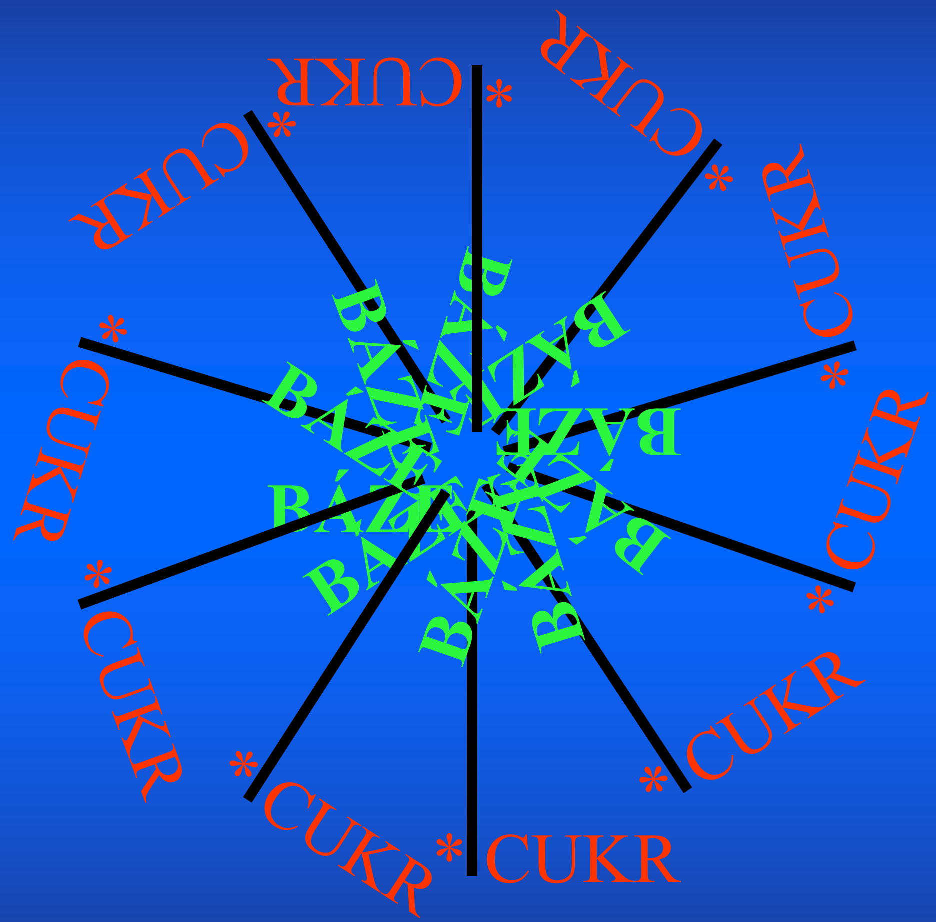
BASE

SUGAR



E1ub
E1ua
B1u
B2u
2
1
0
−1
− 2
Θ x 10^-4
U
U
m^6U
U
220
260
300
nm

Conditions of the origin of CD



Circular dichroism and optical activity of biopolymers

) CD – principle, quantities - ellipticity, Δε, relation between ORD and CD

) **Advantages and disadvantages of CD spectroscopy**

Advantages

Enormous sensitivity - low concecntration of studied substances
easy solubility
even in extreme conditions

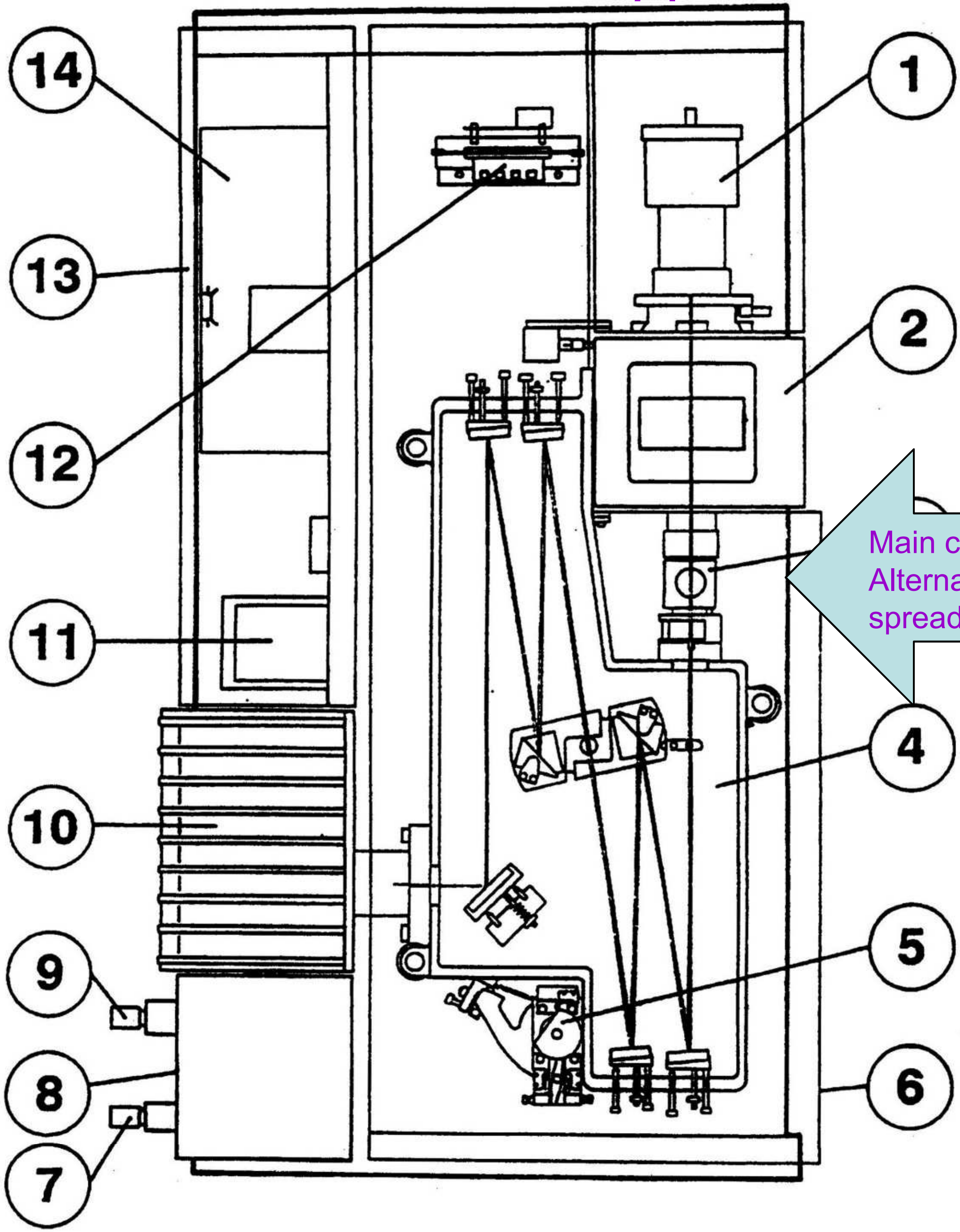
Easy manipulation - titraton
transition between different structures
whole conformational space

Discrimination between cooperative and non-cooperative changes

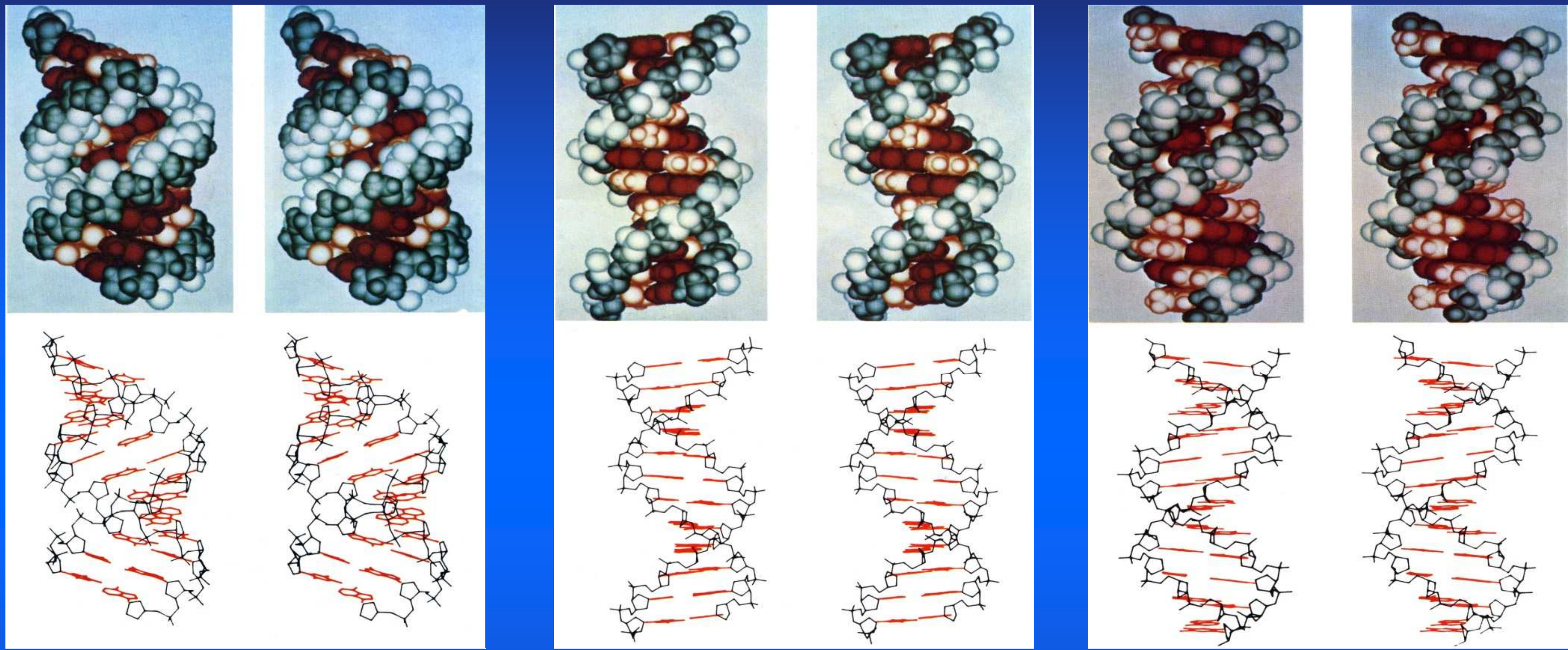
Disadvantages

no explicit relation between CD spectrum and structure of complex molecules
experience

Interior of the CD apparatus



Main component
Alternately depressed and spread out



A

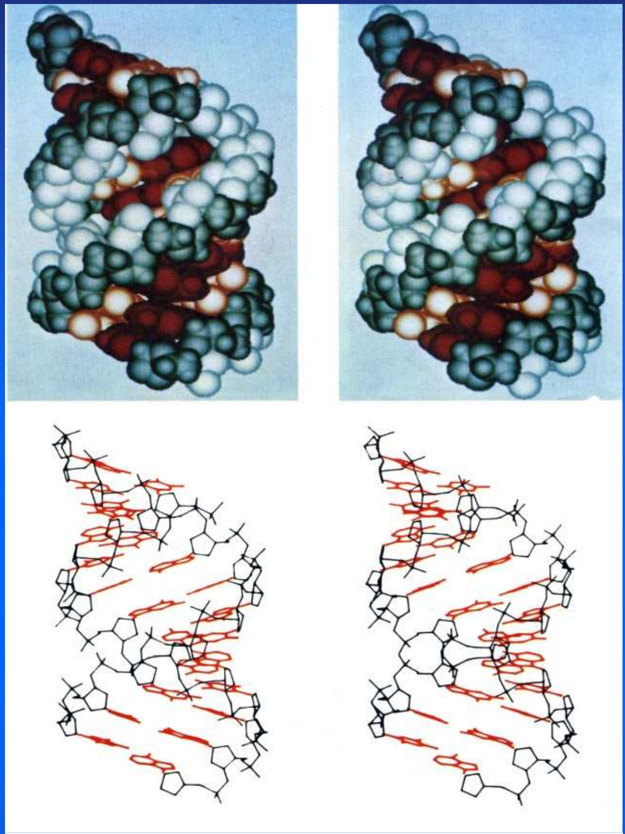
corpulent

B

C,D,E,T

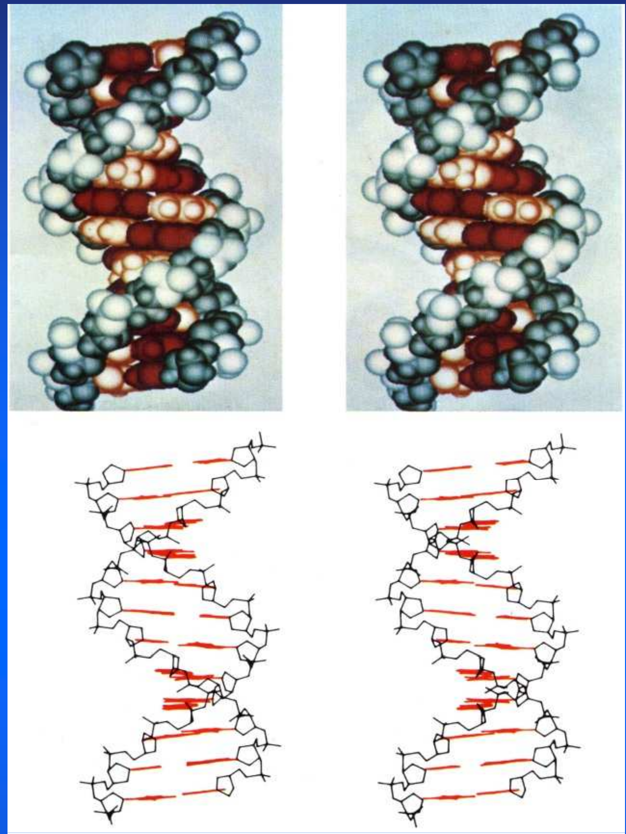
Long DNA molecules can be oriented by mechanical stroking. X ray diffraction pattern obtained on these semicrystaline matter enables to determine some periodicities of the DNA arrangement

M. Wilkins, R. Franklin, W+C

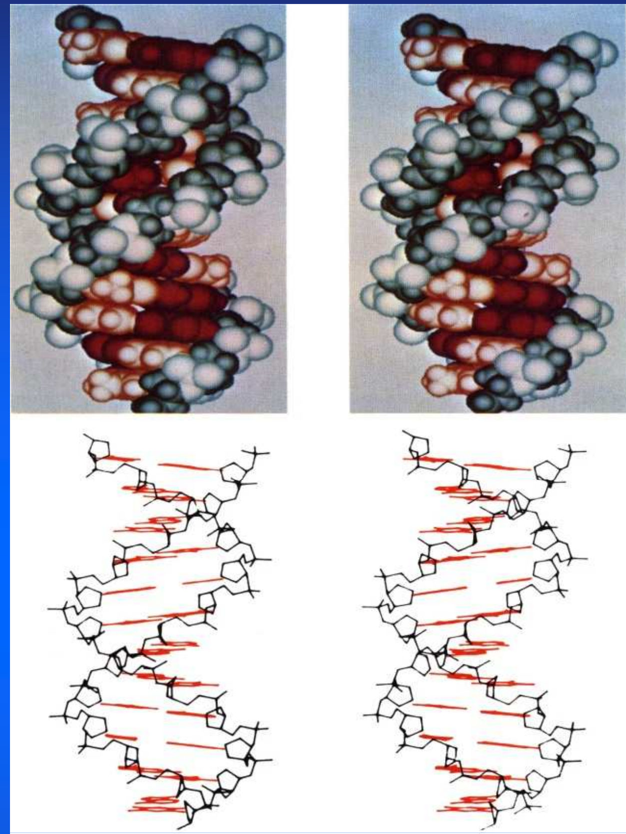


A

corpulent



B



C,D,E,T

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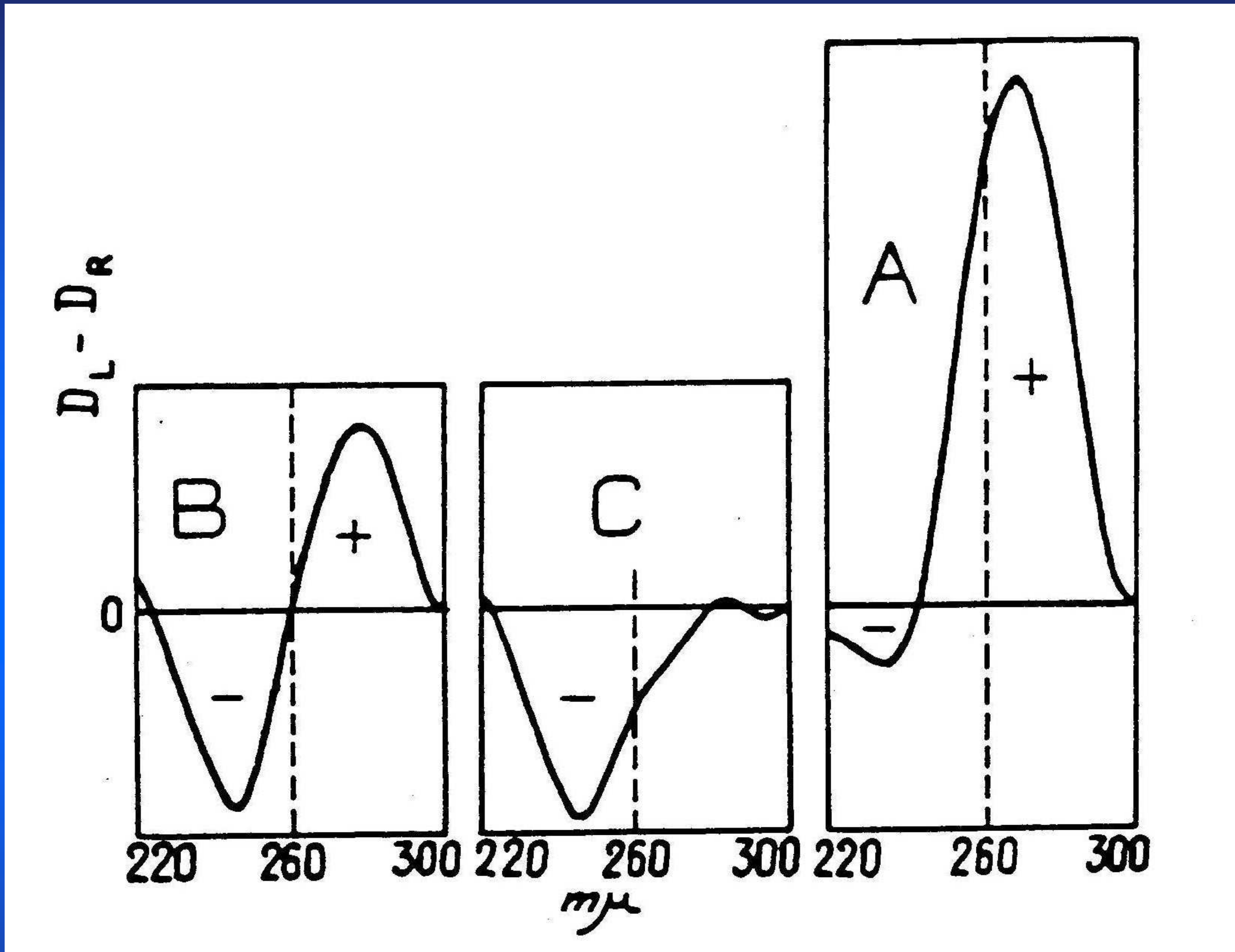
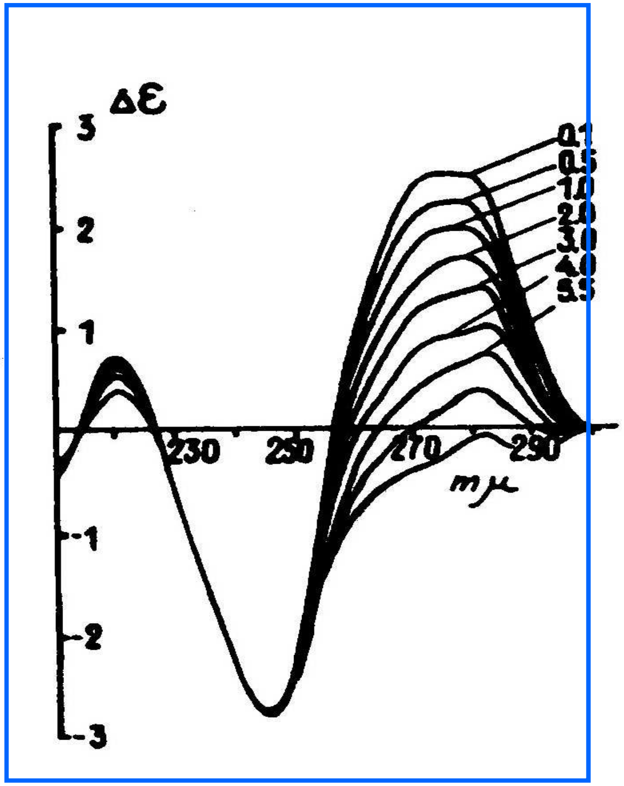
M. Wilkins, R. Franklin, W+C

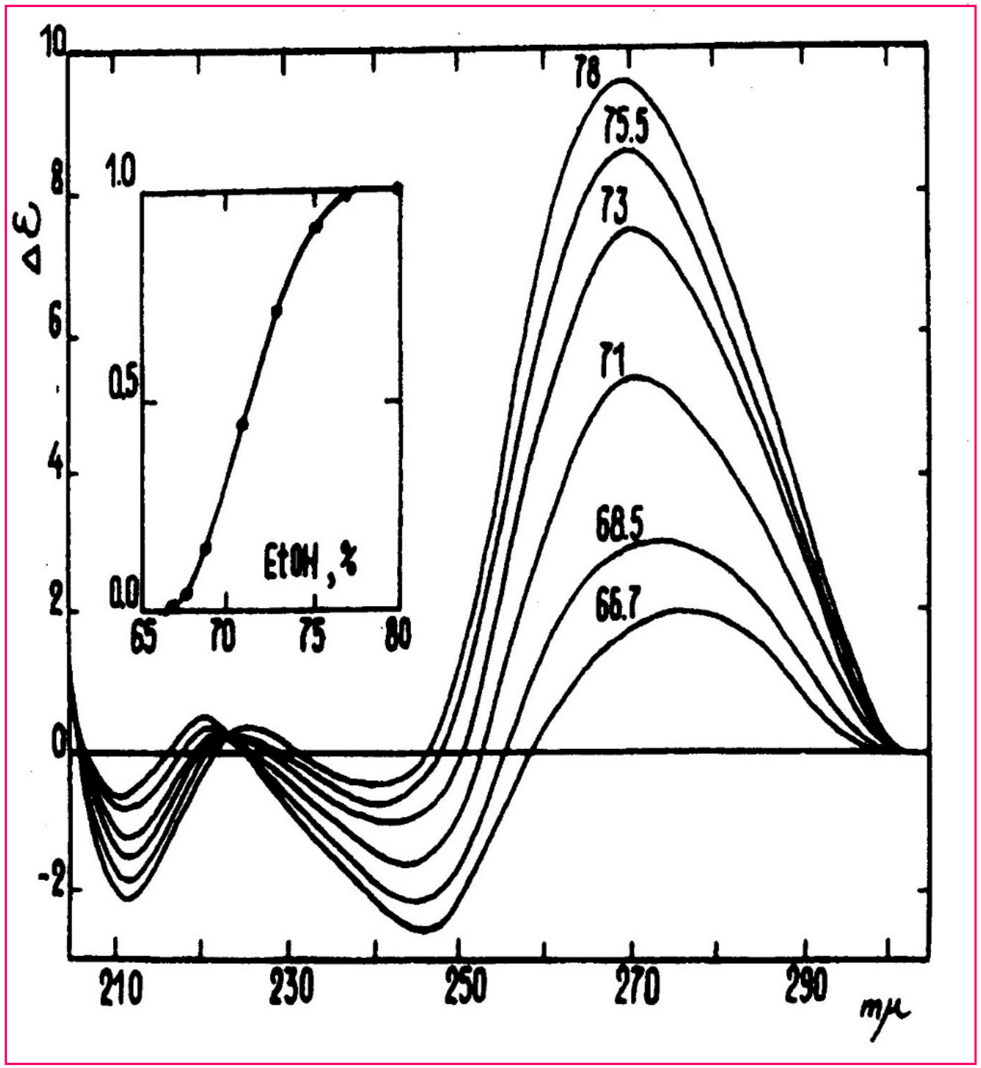


Tunis-Schneider, M.J.B. + Maestre, M.F.

Examples of cooperative and non-cooperative structural changes

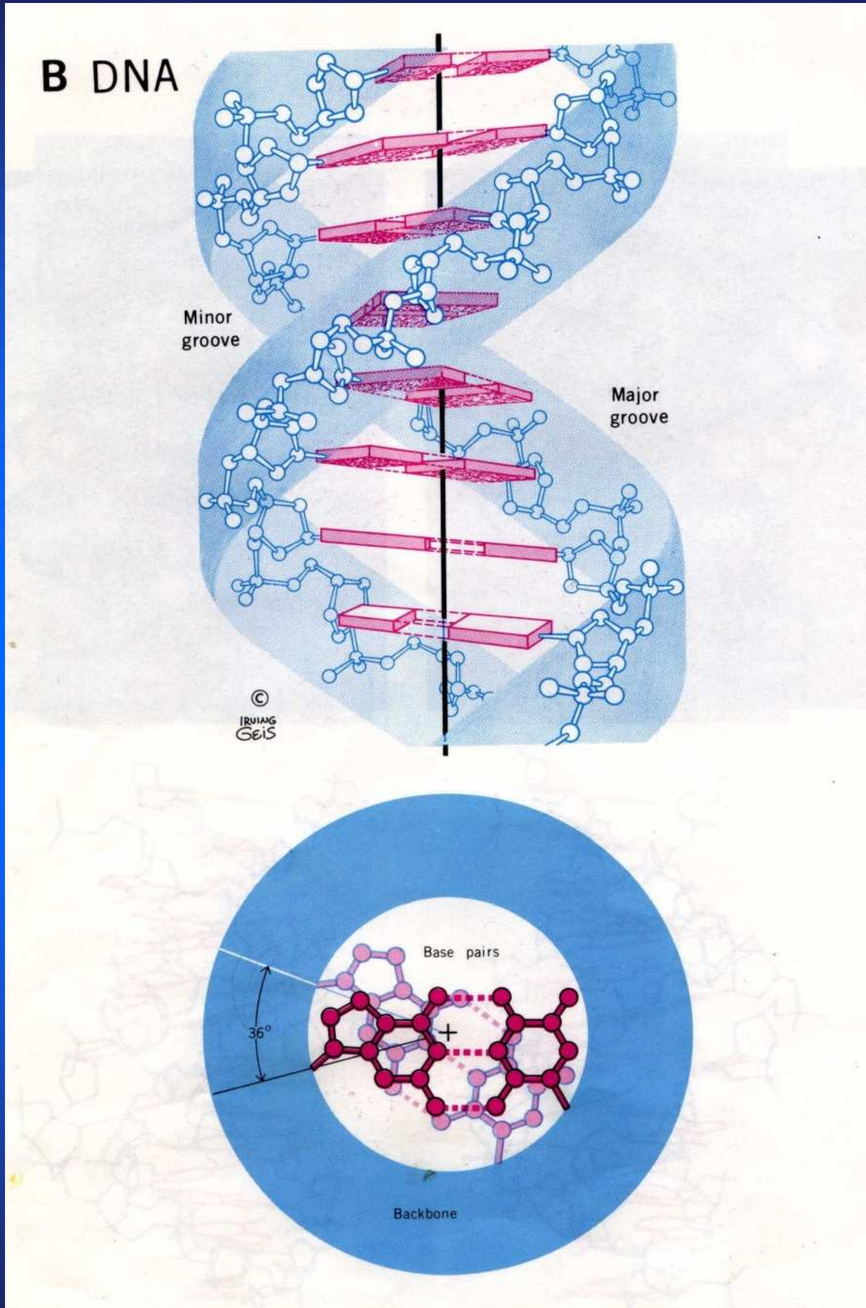
Non-cooperative changes within the same structure

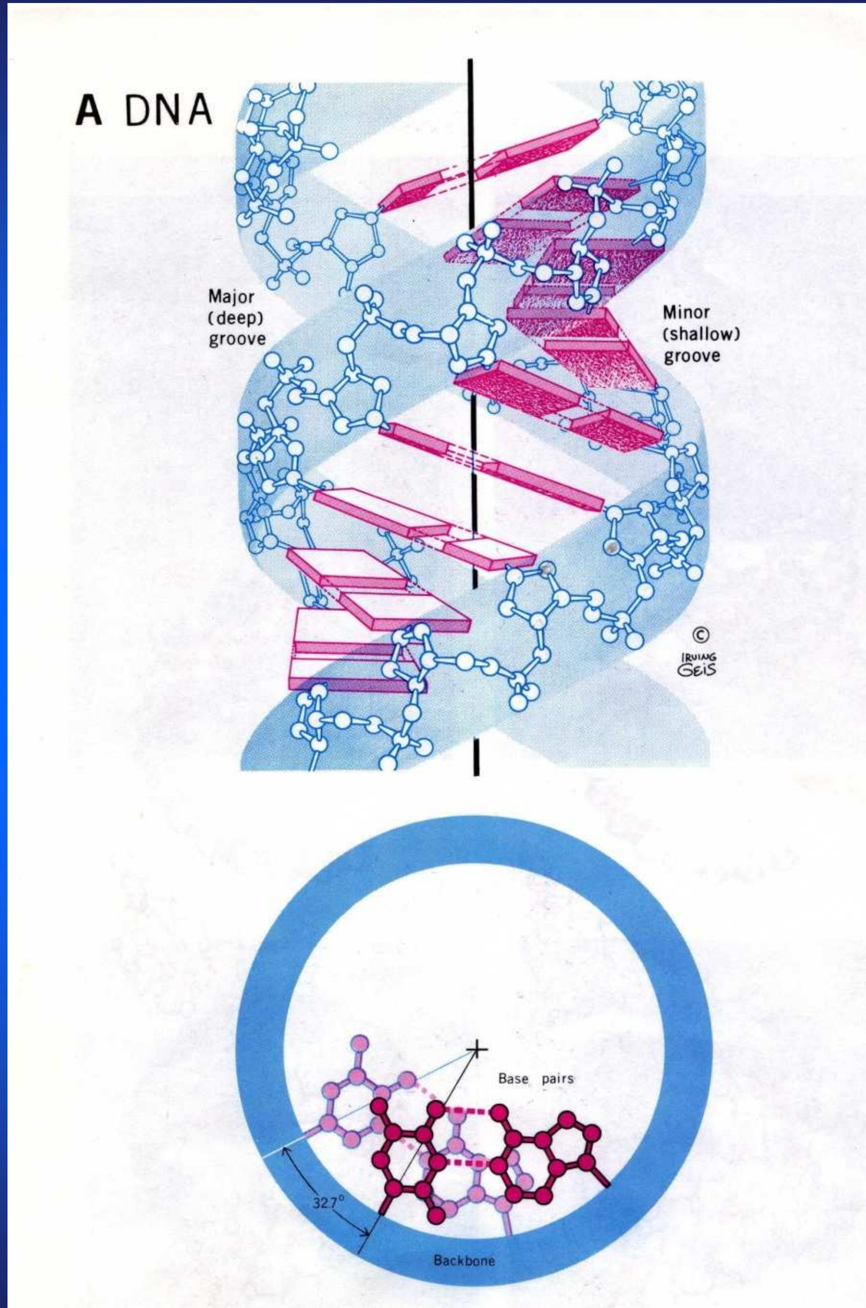


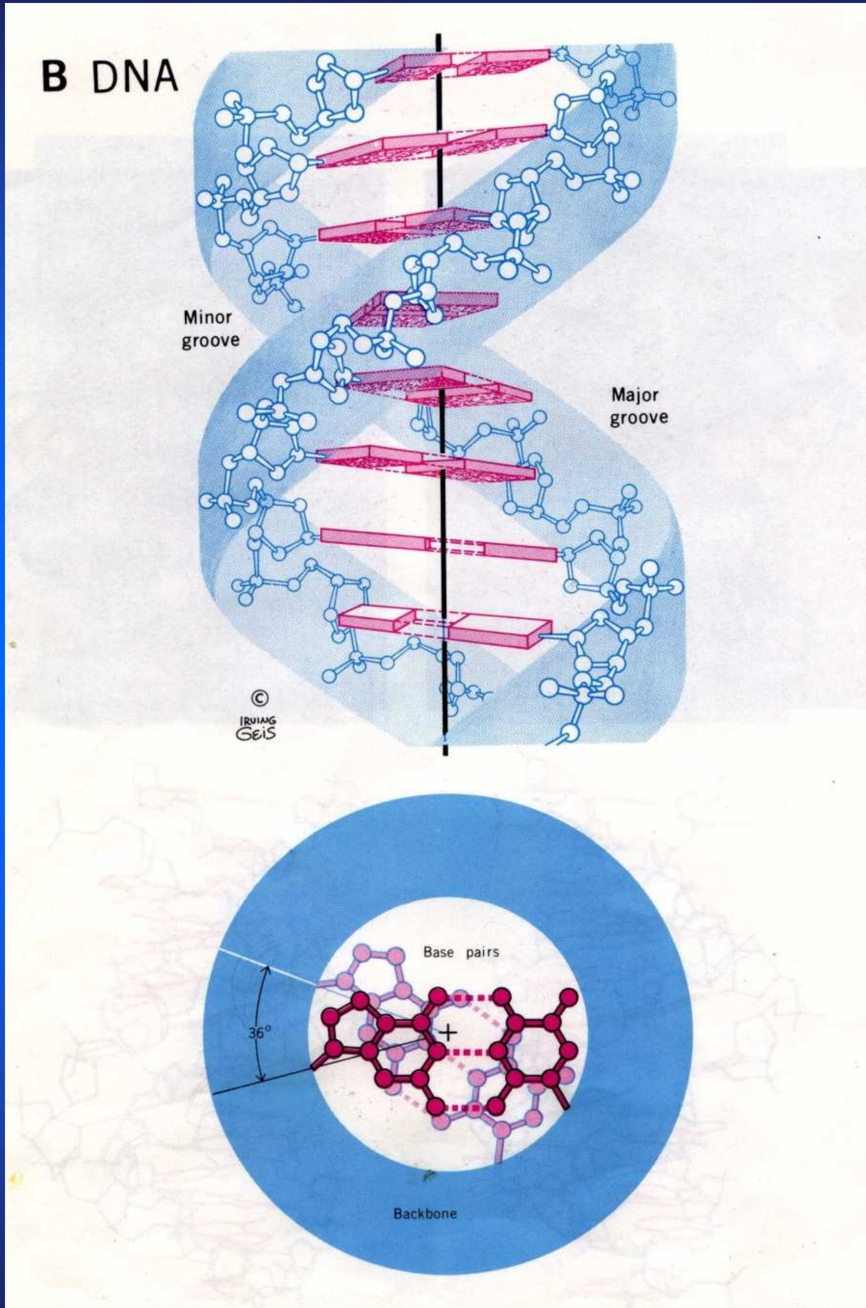


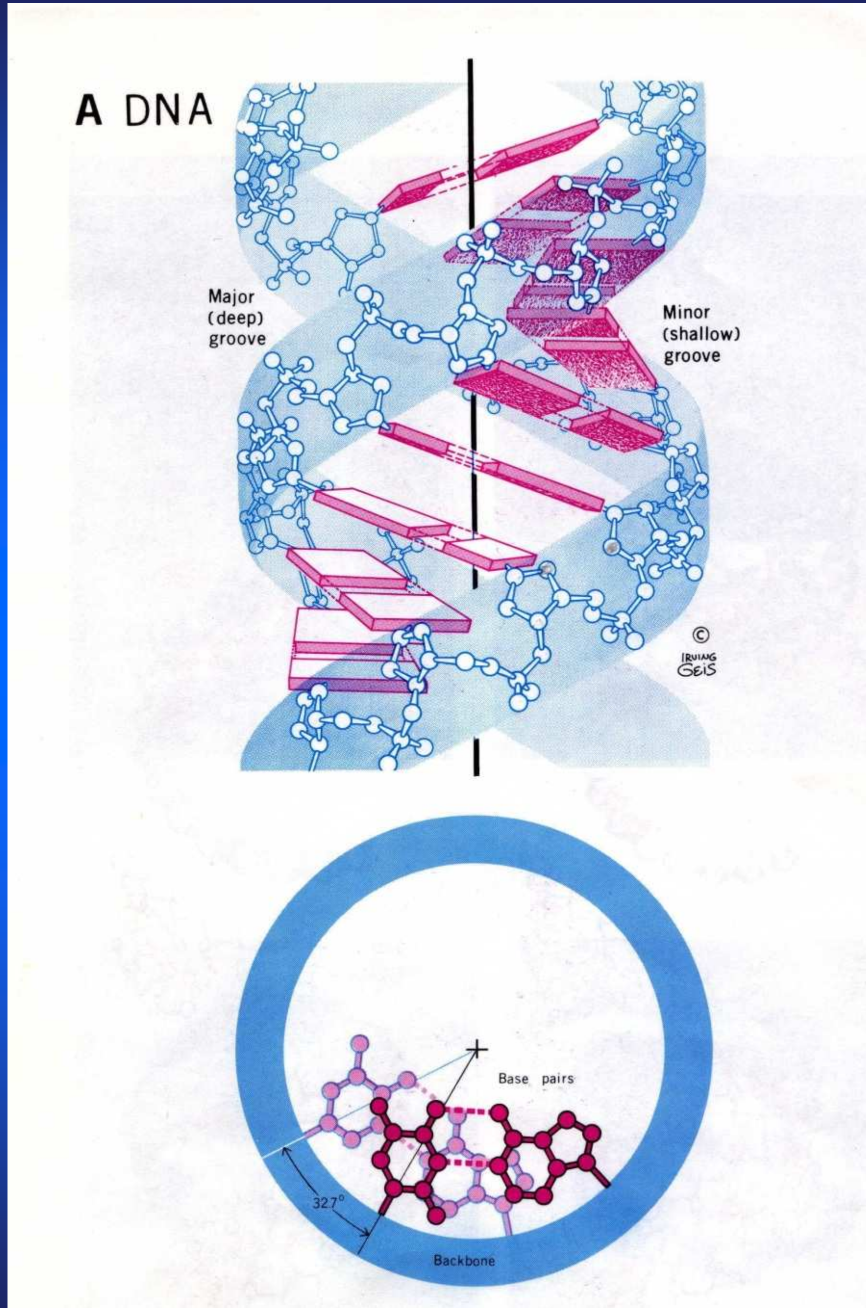
Cooperative changes between discrete structures

Ivanov, V.I

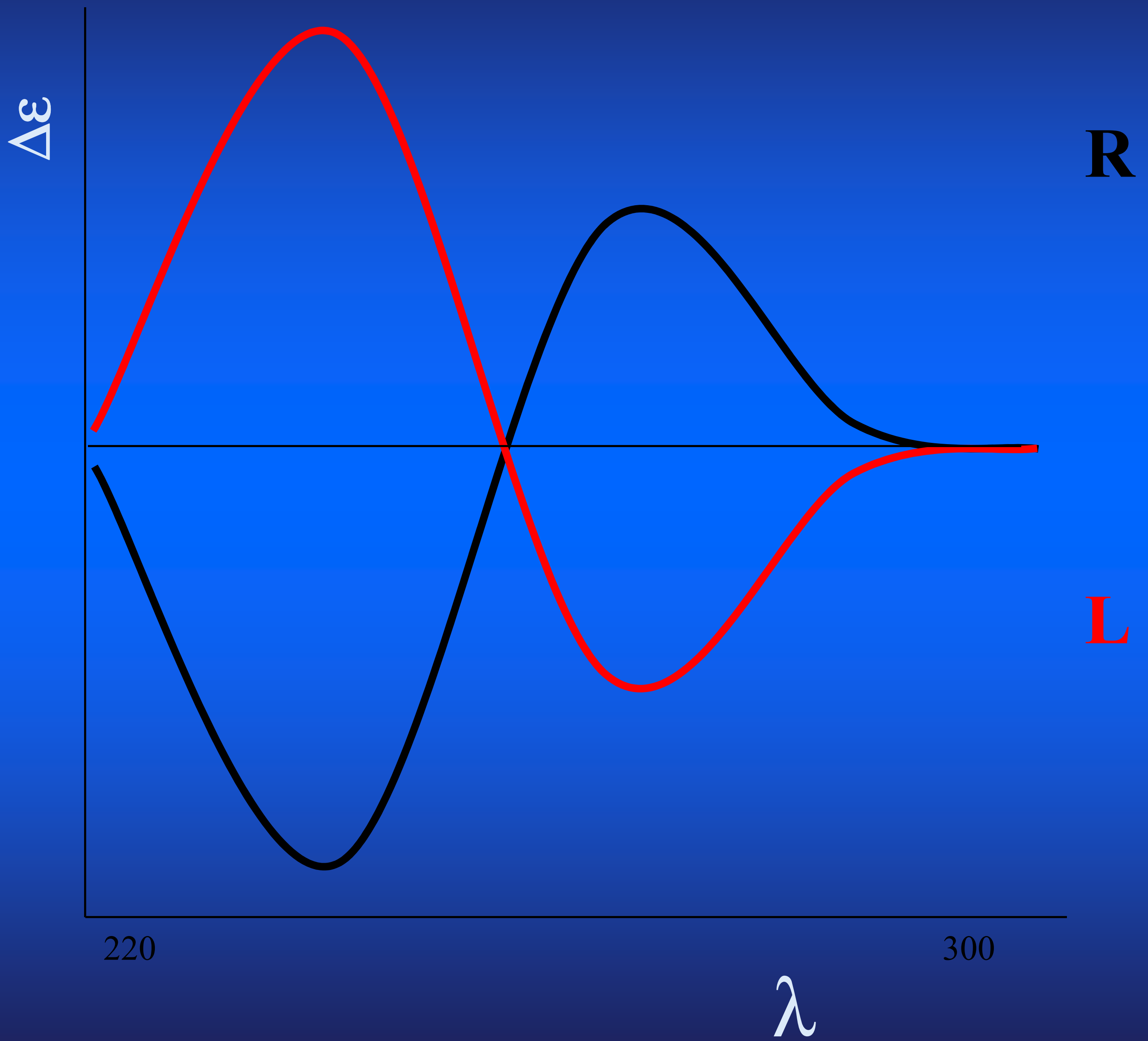

B DNA
Minor groove
Major groove
Base pairs
36°
Backbone


A DNA
Major (deep) groove
Minor (shallow) groove
Base pairs
32.7°
Backbone


B DNA
Minor groove
Major groove
Base pairs
36°
Backbone

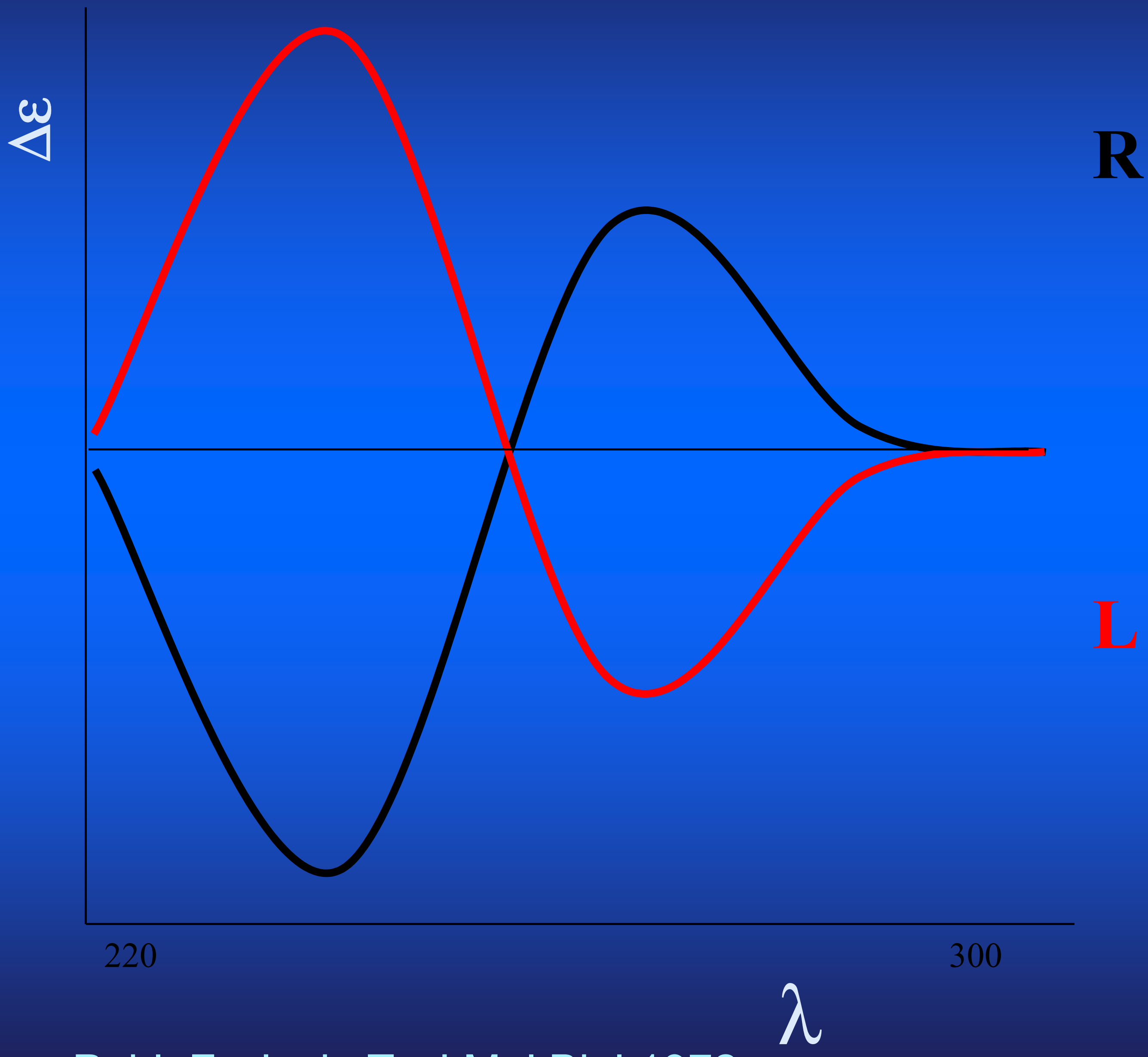

A DNA
Major (deep) groove
Minor (shallow) groove
Base pairs
32.7°
Backbone

GCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC

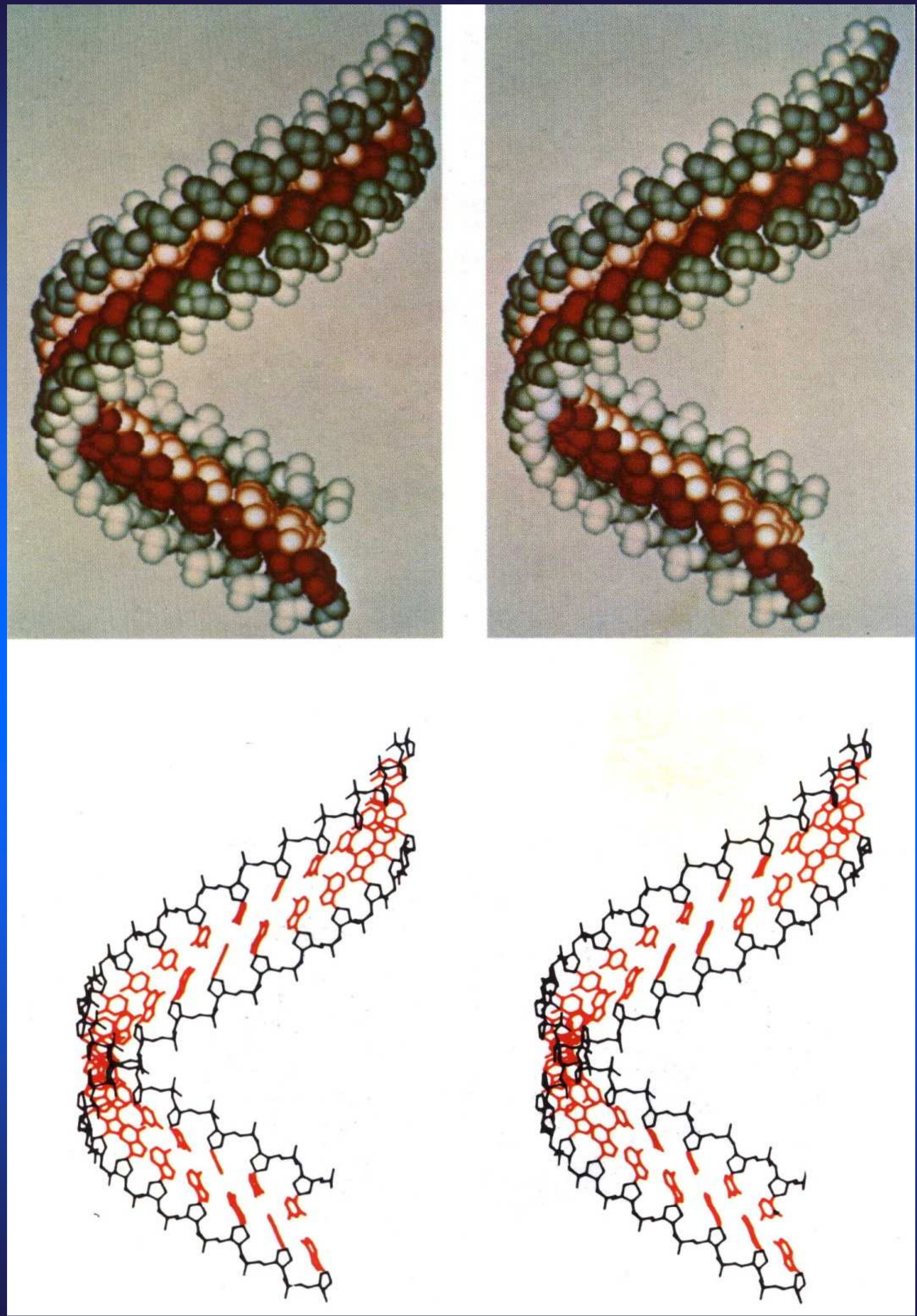


Pohl, F,. Jovin T.: J.Mol Biol 1972

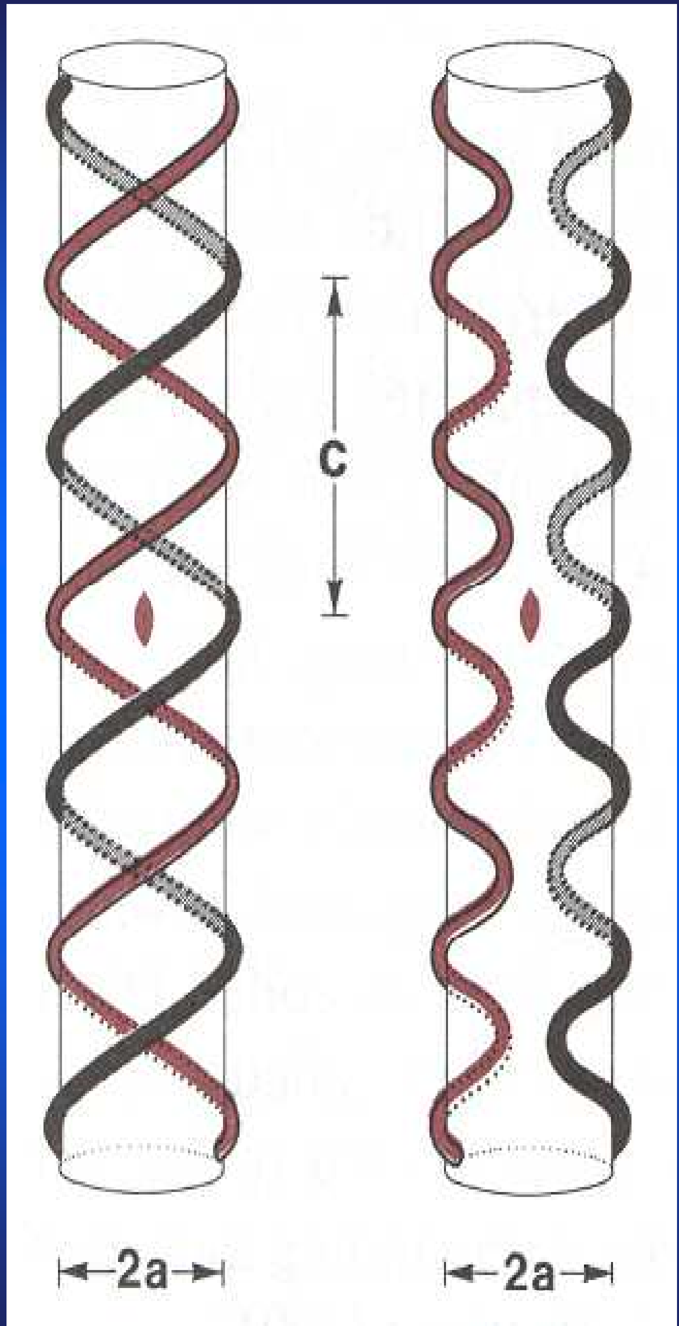
GCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC

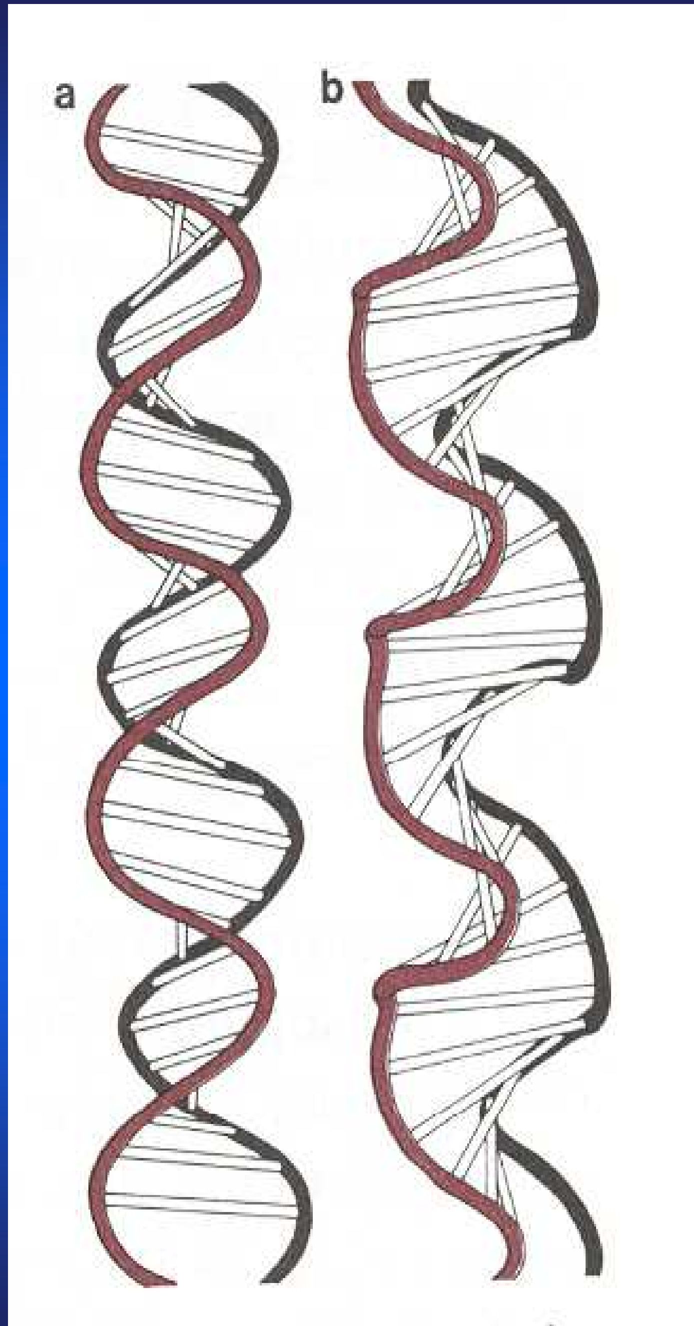


Pohl, F,. Jovin T.: J.Mol Biol 1972

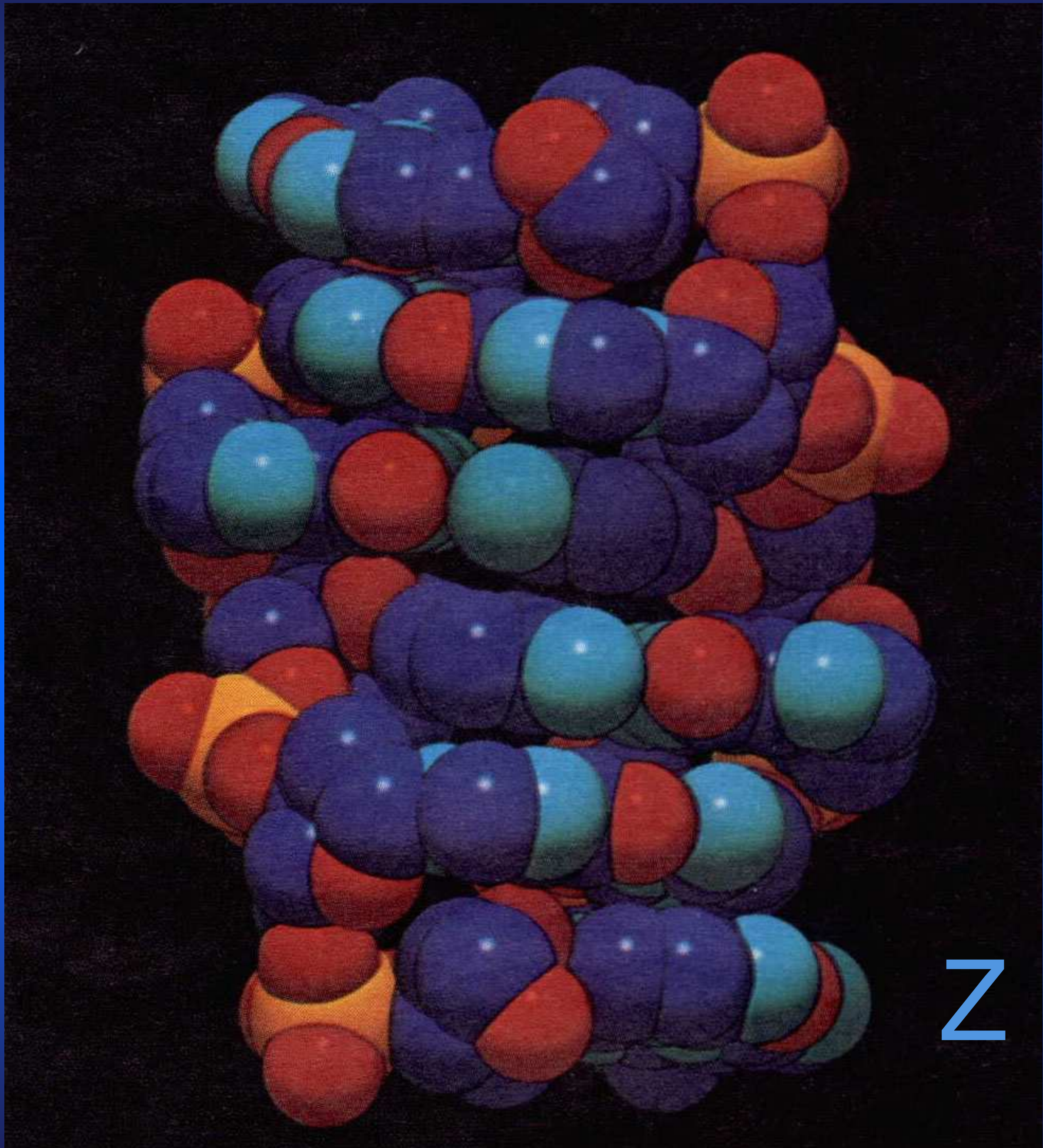


Vilma Olson

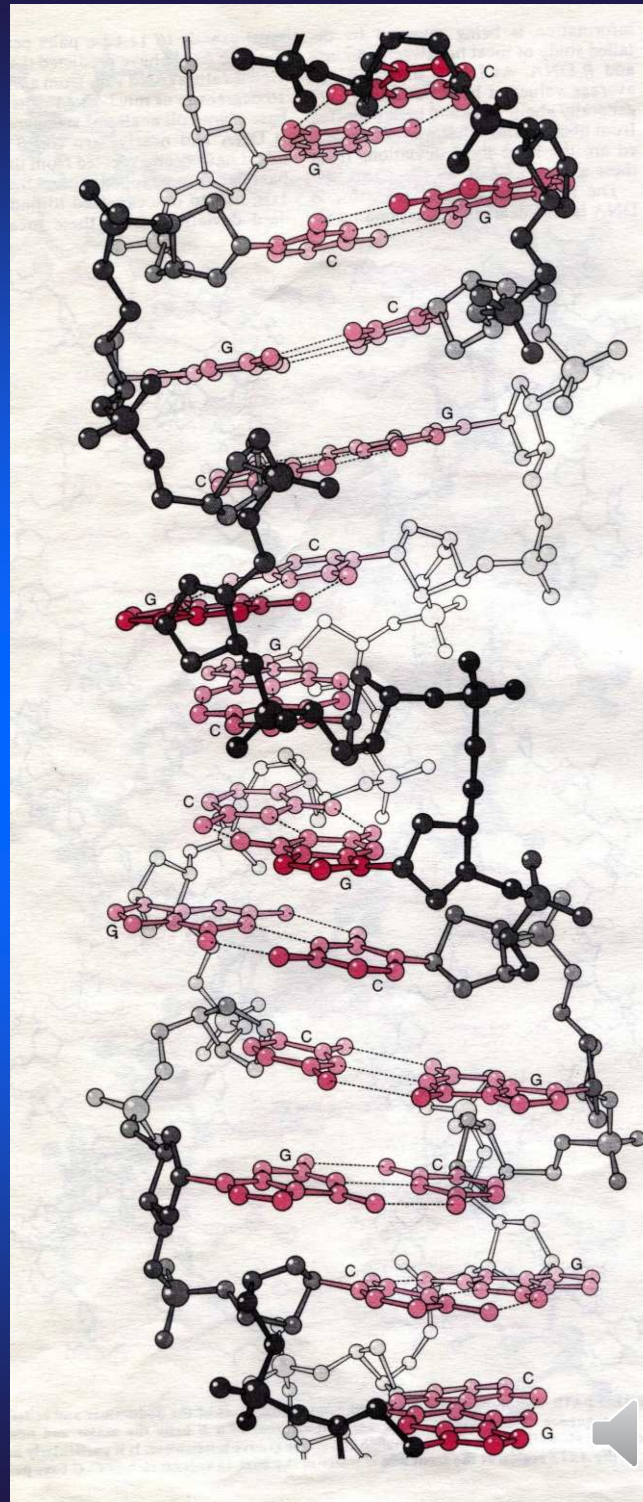


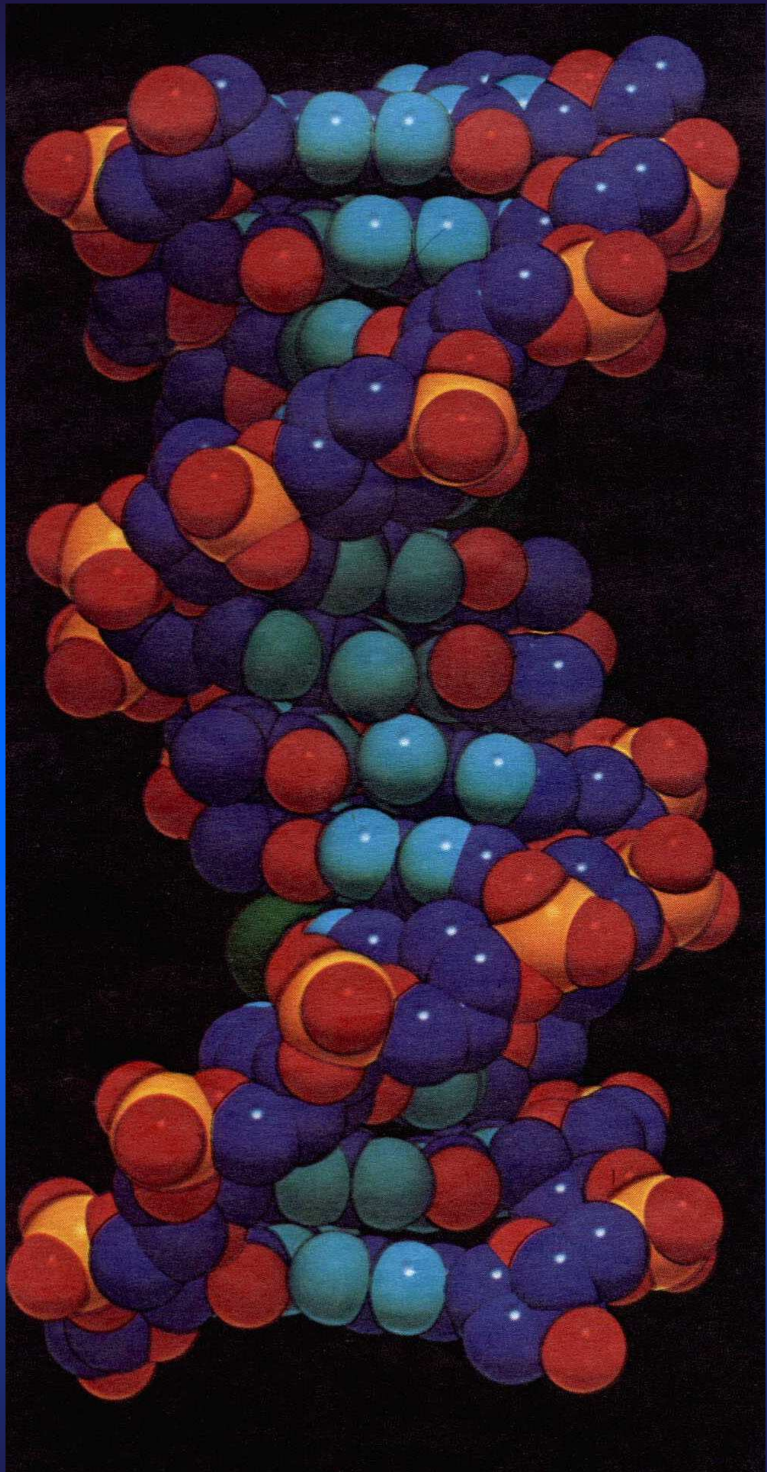


Sasisekharan

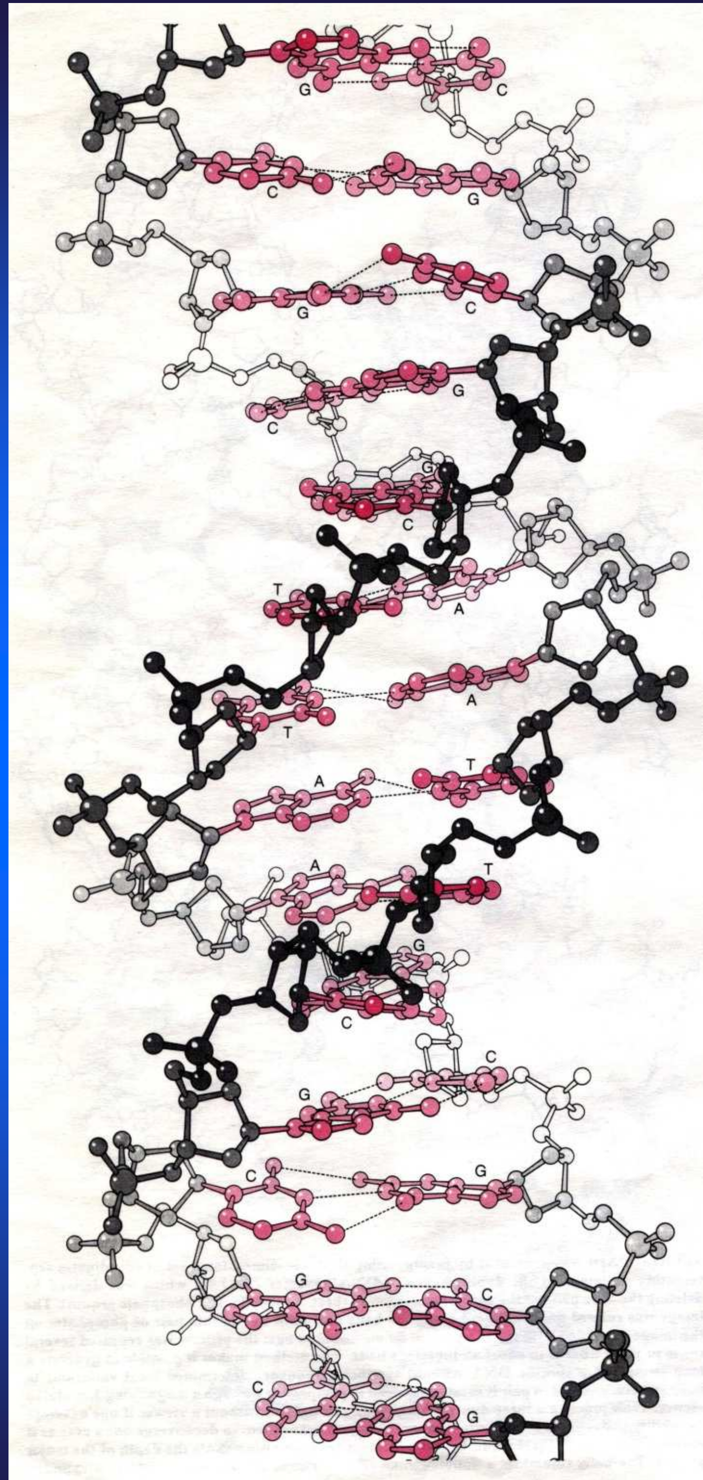


Dickerson

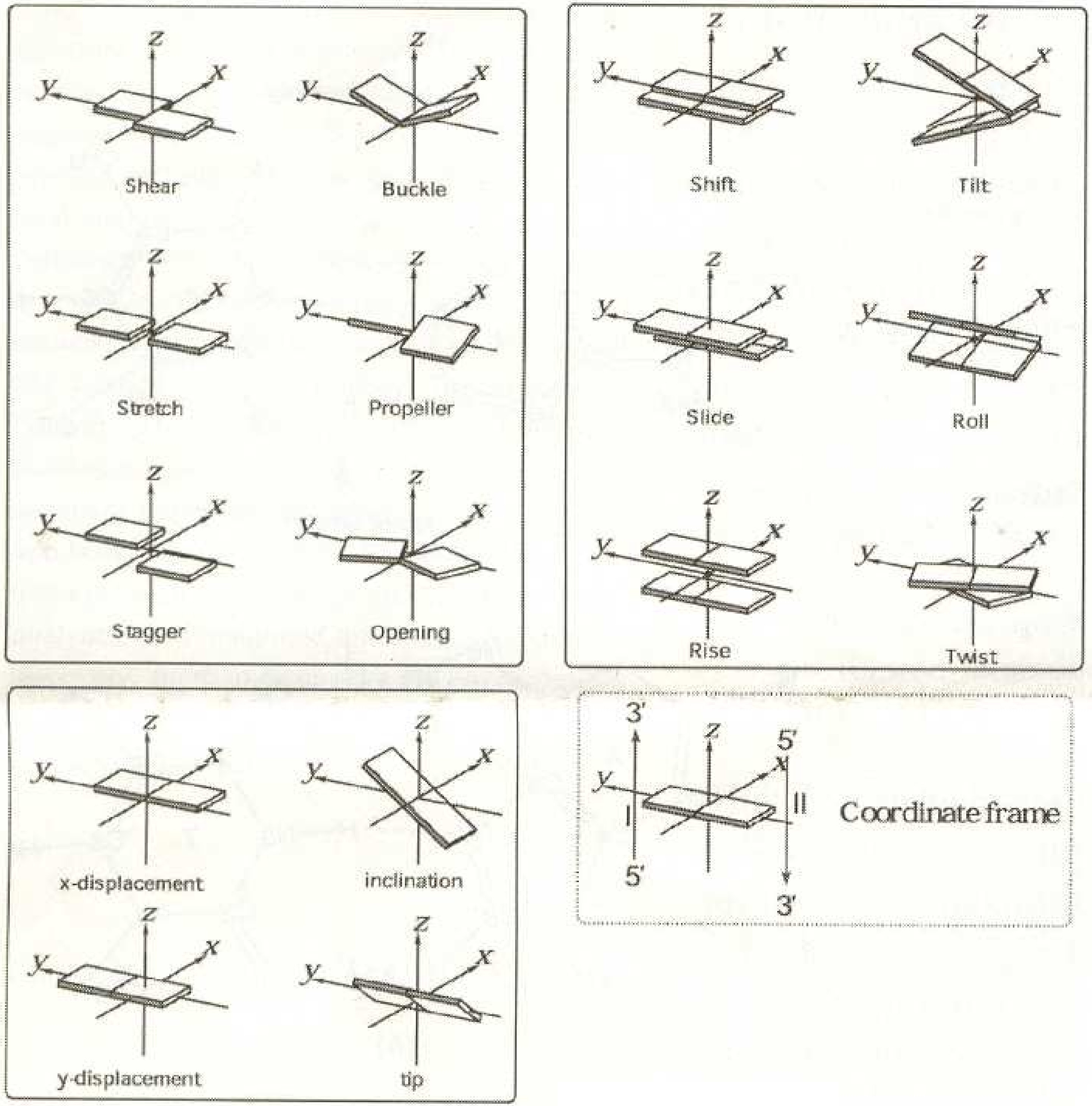




B

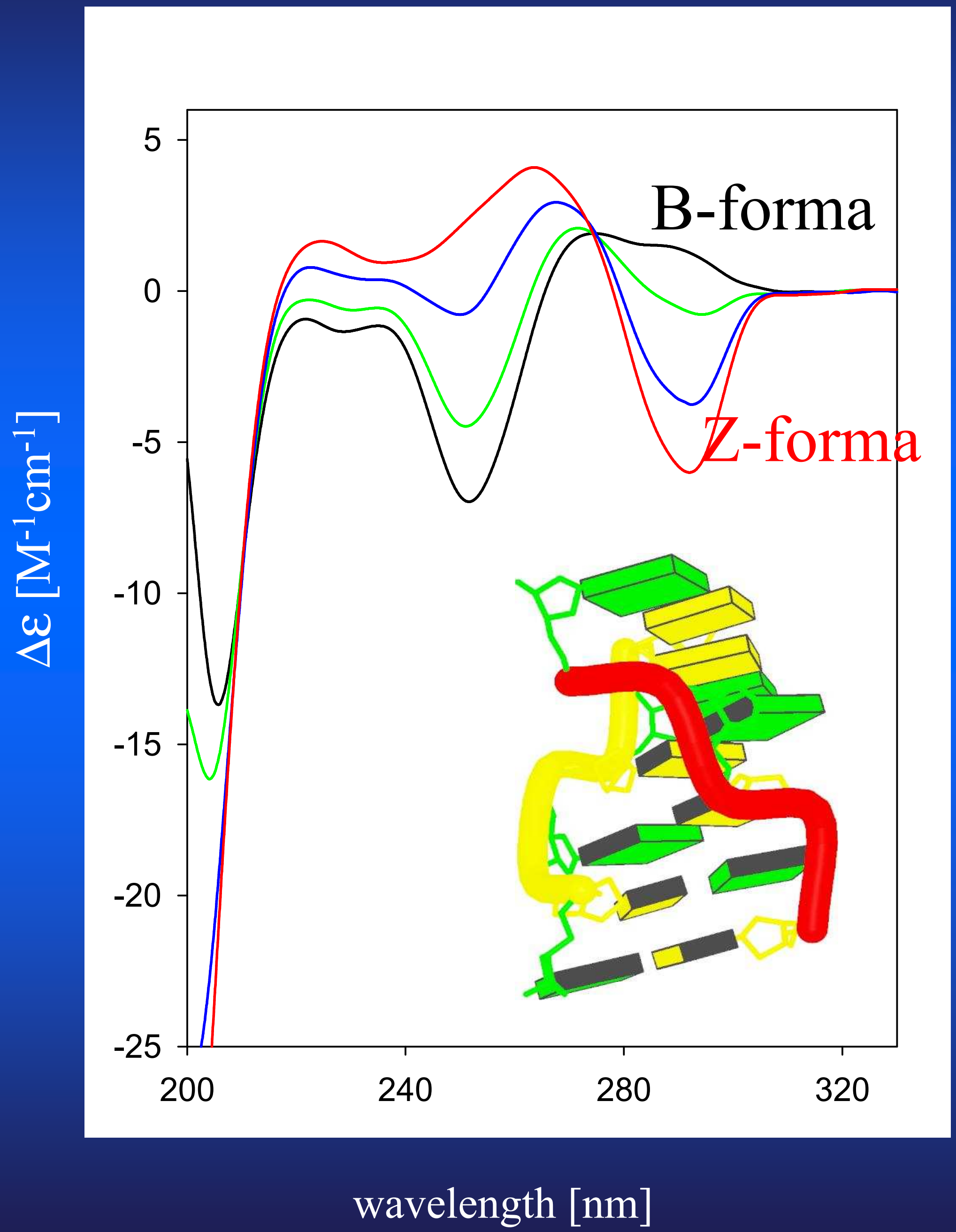


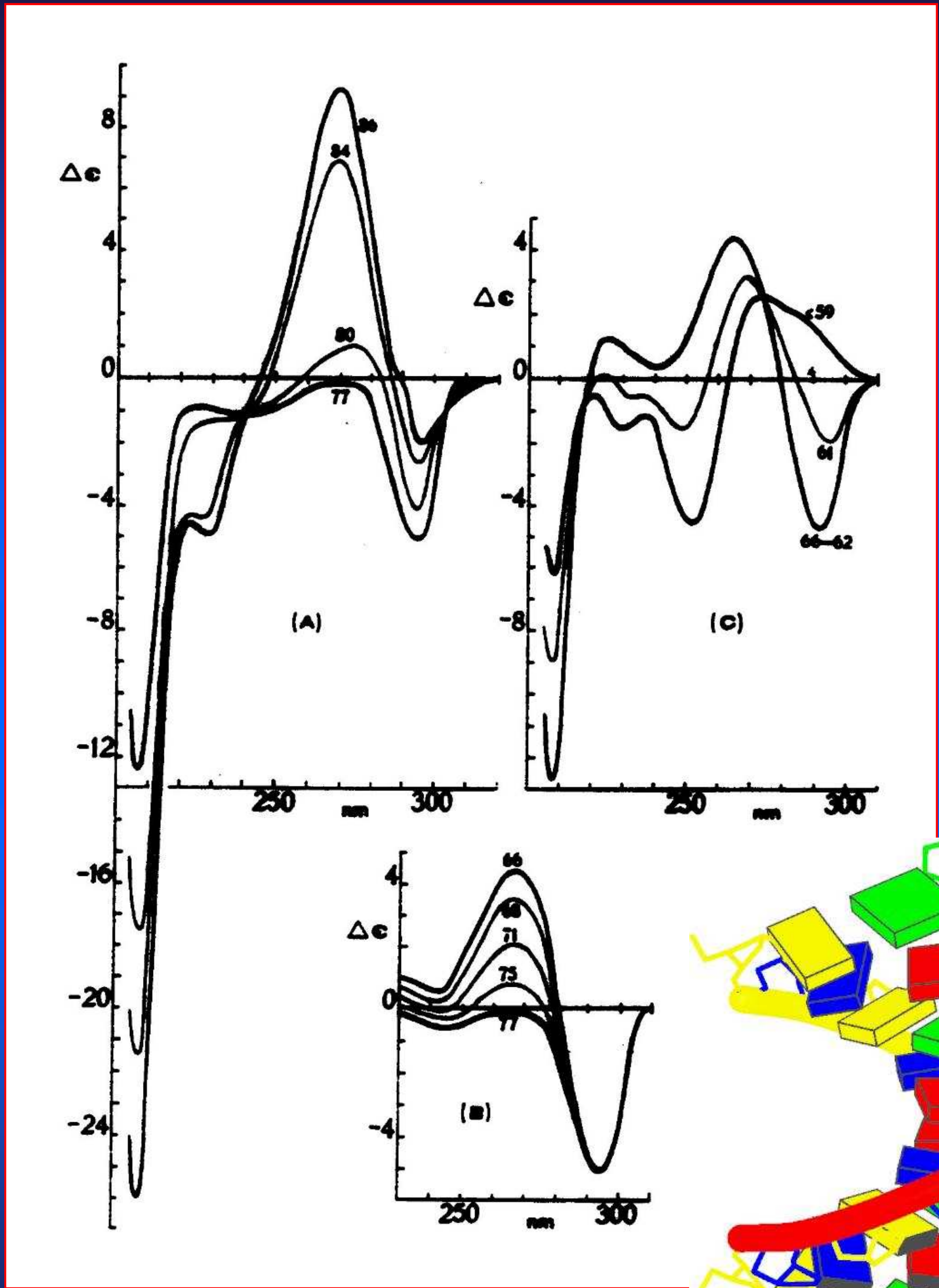
Dickerson



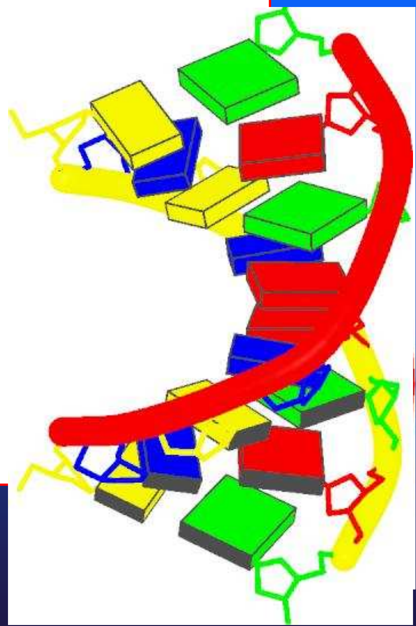
Shear
Buckle
Stretch
Propeller
Stagger
Opening
Shift
Tilt
Slide
Roll
Rise
Twist
x-displacement
inclination
y-displacement
tip
Coordinate frame

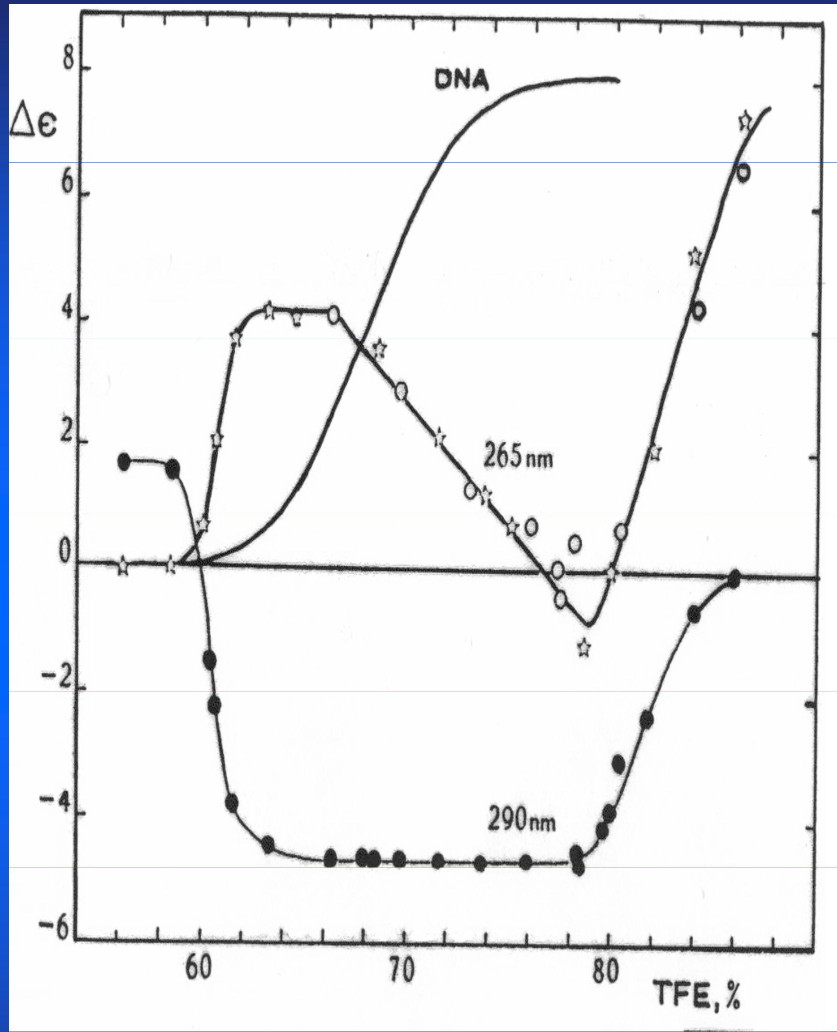
CD spectral changes accompanying B-Z transition of poly(dG-dC)





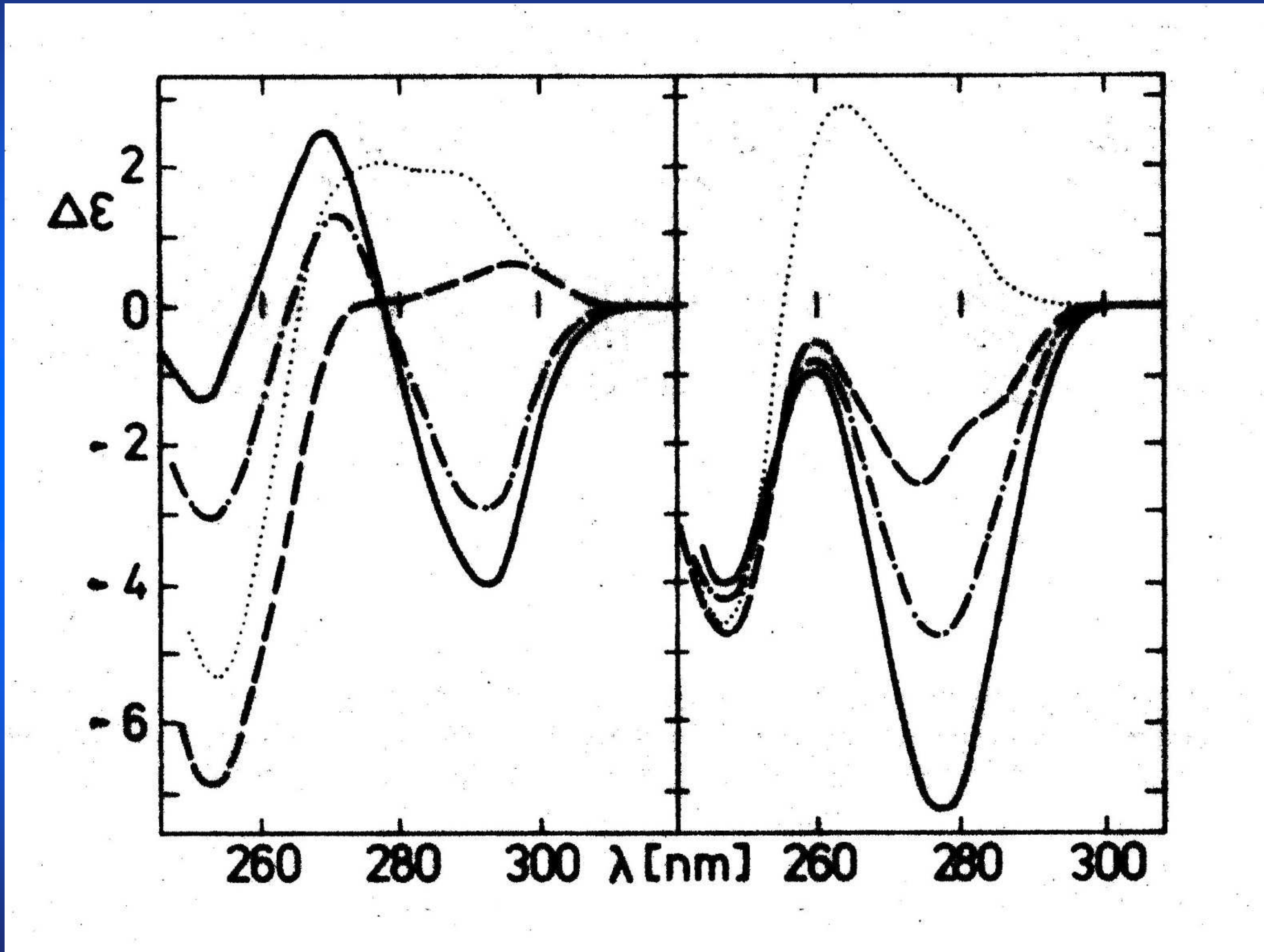
Δε
8
4
0
-4
-8
-12
-16
-20
-24
84
80
77
(A)
(C)
c59
61
66–62
250
300
nm
66
71
75
(B)





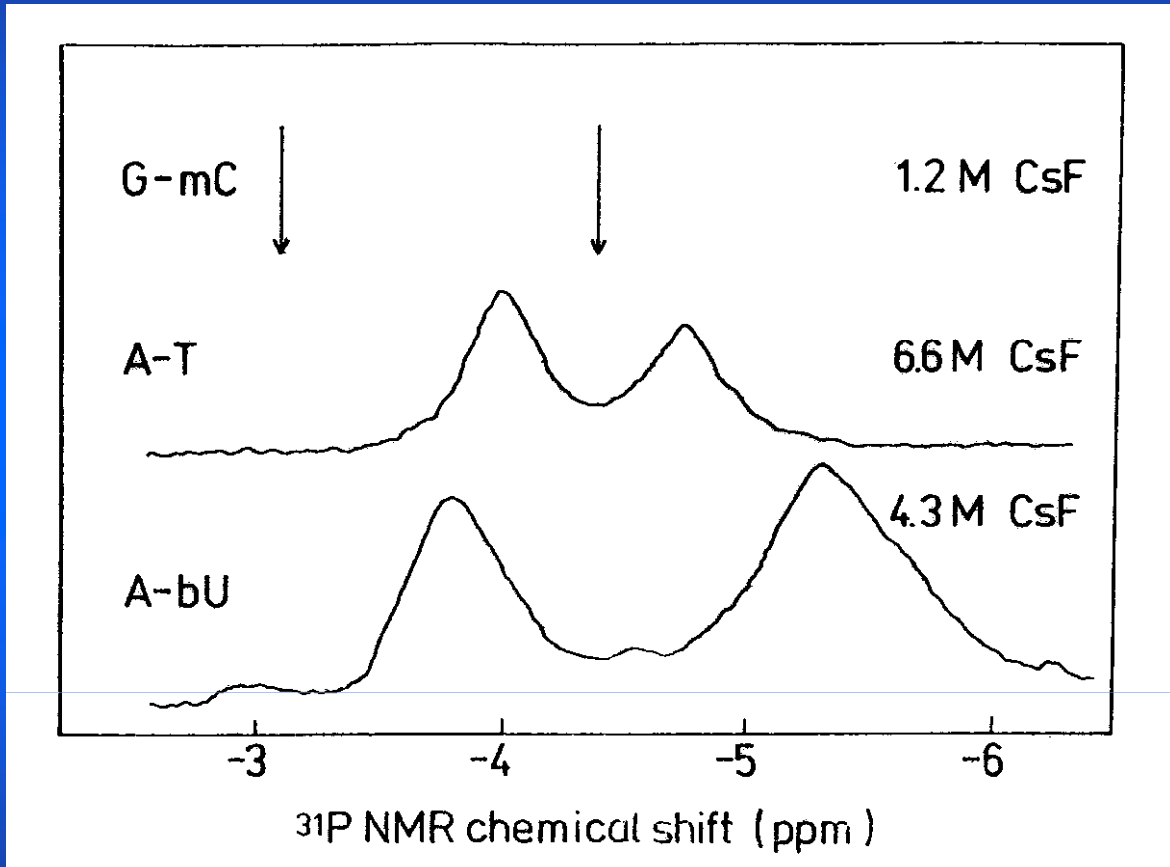
Δε
DNA
265 nm
290 nm
8
6
4
2
0
-2
-4
-6
60
70
80
TFE, %

ATATATATATATATATATATATATATATATATATATATATA



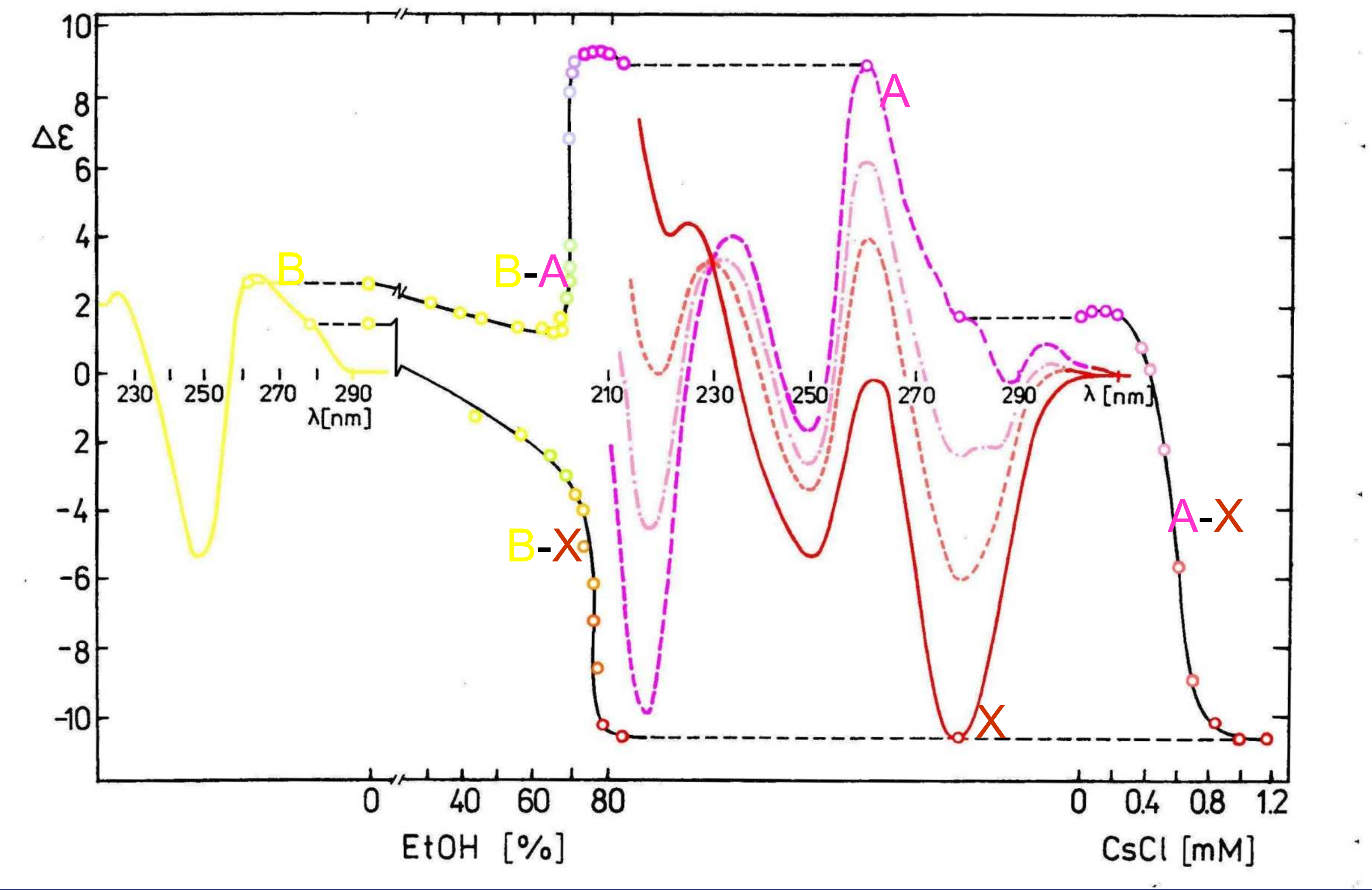
Vorlíčková, M., Sklenář, V., Kypr, J.: *J. Mol. Biol.* **166** (1983) 85-92

ATATATATATATATATATATATATATATATATATATA



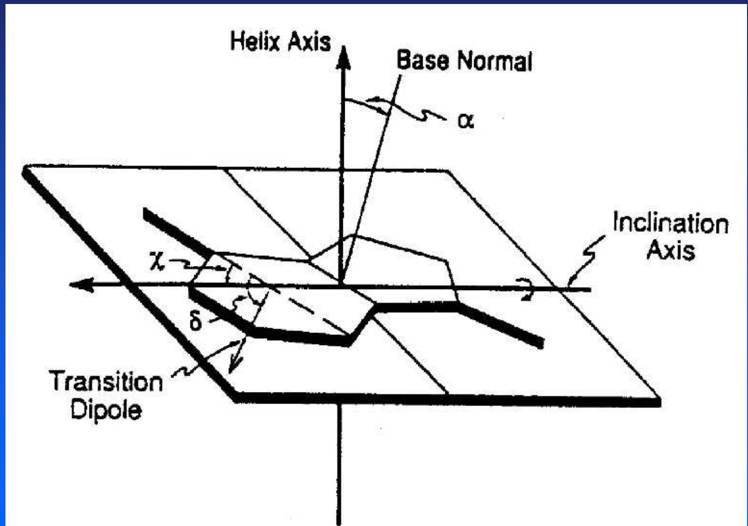
X-DNA

Vorlíčková, M., Sklenář, V., Kypr, J.: *J. Mol. Biol.* **166** (1983) 85-92

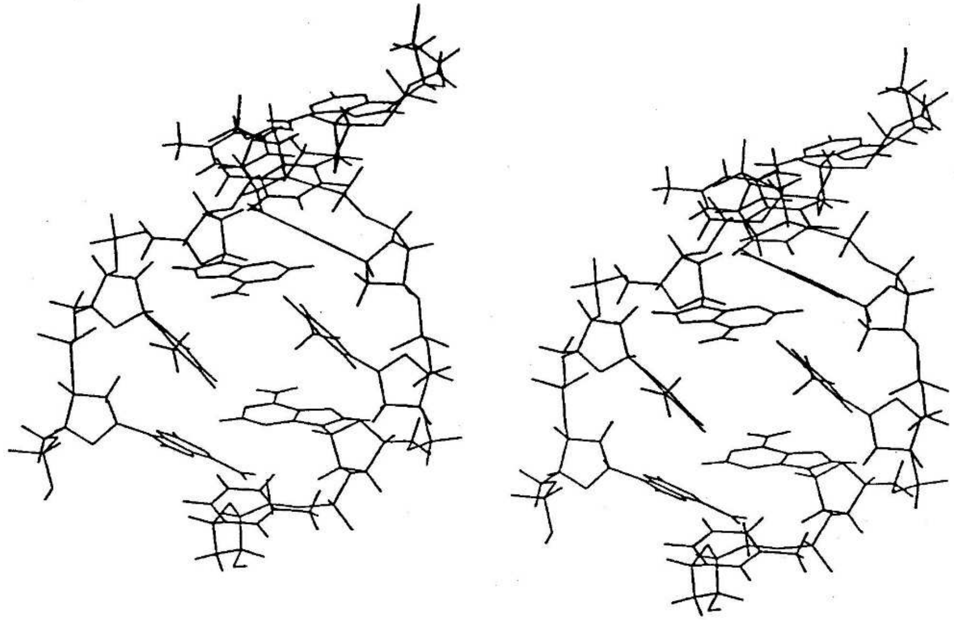


ΔE
10
8
6
4
2
0
2
-4
-6
-8
-10
230
250
270
290
λ[nm]
210
230
250
270
290
λ [nm]
B
B-A
B-X
A
X
A-X
0
40
60
80
EtOH [%]
0
0.4
0.8
1.2
CsCl [mM]

X - DNA



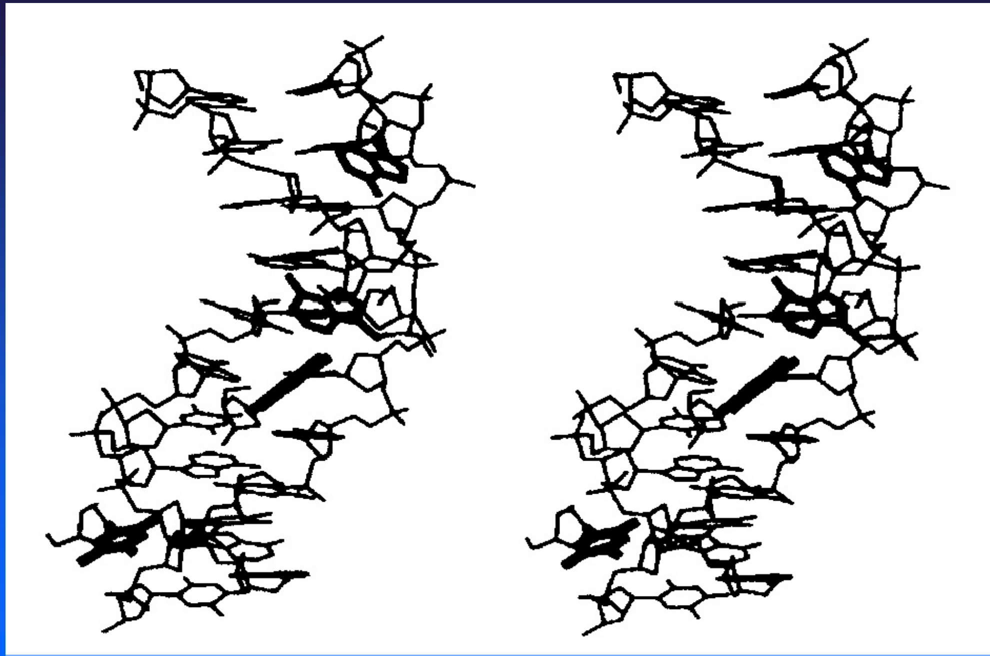
	α [deg]	χ [deg]
dA	20.9	95.2
dT	39.7	60.8

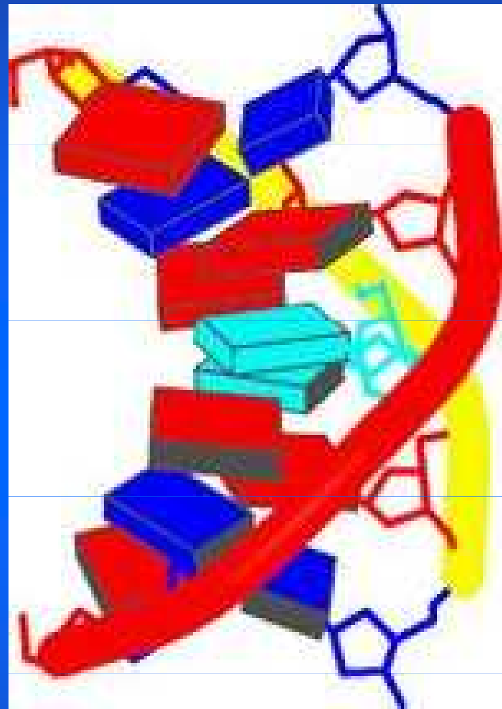


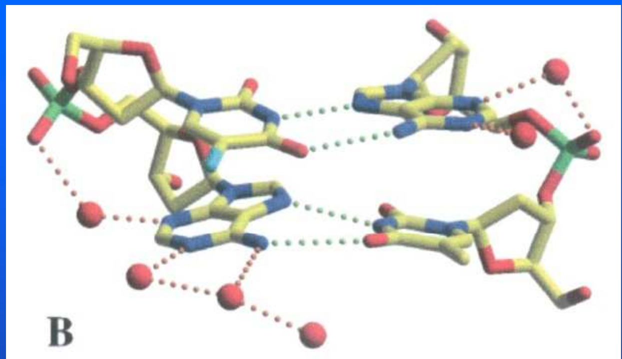
α - inclination of the base normal from the helix axis

χ - orientation of the inclination axis relative to the $C_4 - C_5$ bond of the purine base or the $C_6 - C_5$ bond for the pyrimidine base

Inclin.	Buckle	Propeller	Helical Twist	
20°	31°	40°	36° ApT	7 bp / turn
			66° TpA	







ALTERNATING A-T FRAGMENT WITH HOOGSTEEN BASE PAIRING

Subirana, J. *Proc.Nat.Acad.Sci.USA* , **99**, pp. 2806, 2002.
Biochemistry , **43**, pp. 4092 - 4100, 2004.

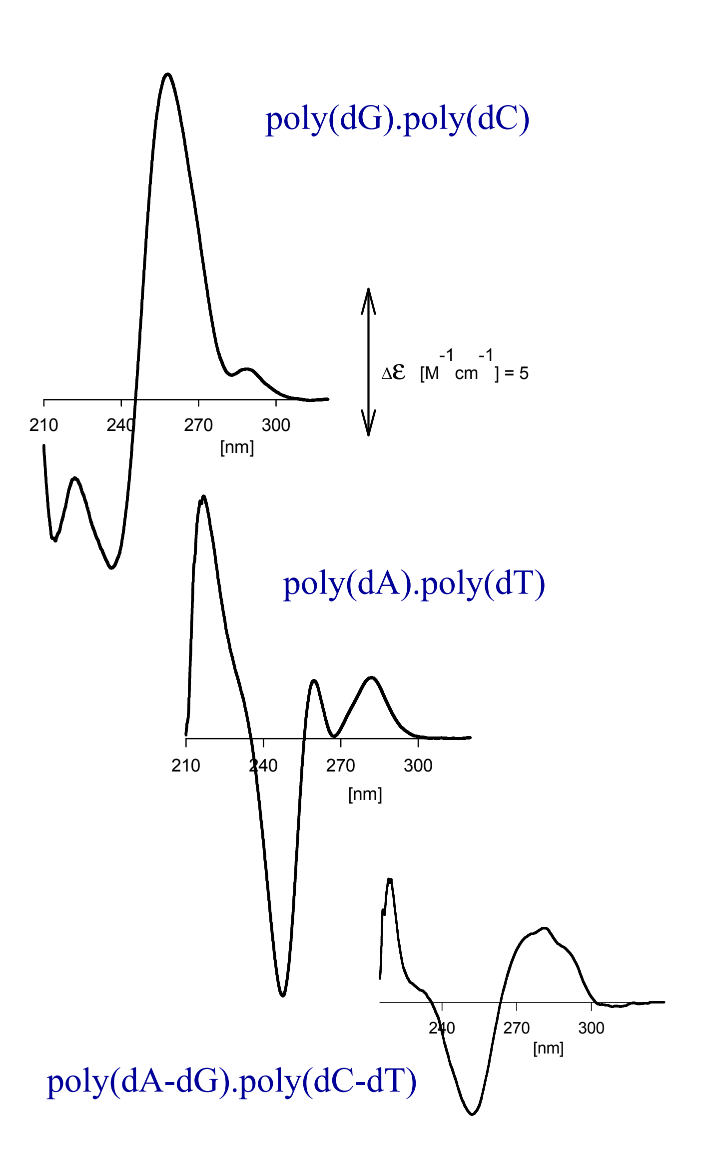
Alternating (Pu-Py)$_n$

(Pu)$_n$. (Py)$_n$ complexes

... [GCGCGC / CGCGCG] ...

... [ATATAT / TATATA] ...

... [ACACAC / TGTGTG] ...



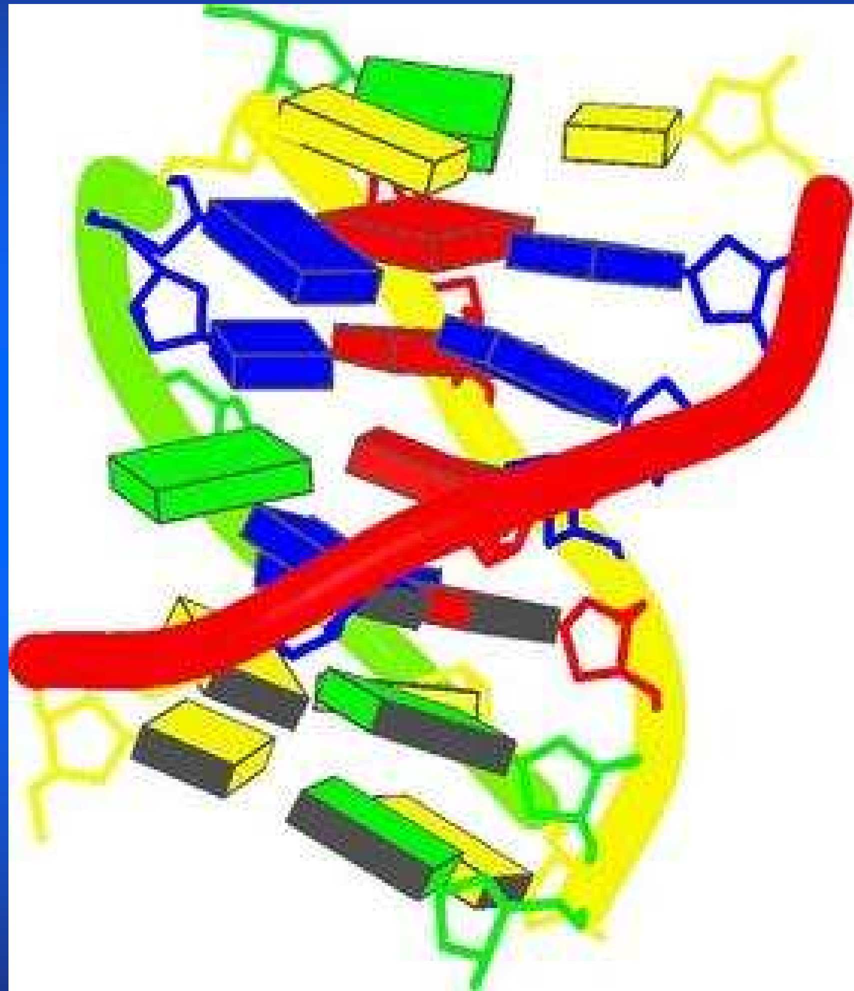
... [GGGGGG / CCCCCC] ...

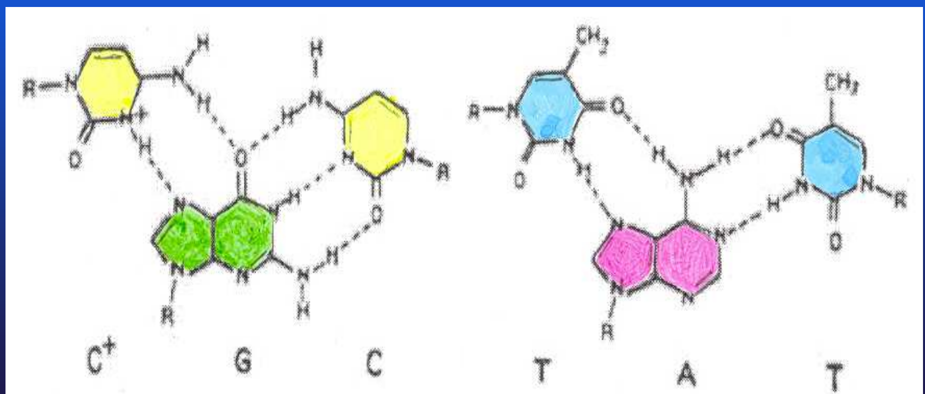
... [AAAAAA / TTTTTT] ...

... [AGAGAG / TCTCTC] ...

DNA Triplex

Pyrimidine. Purine. Pyrimidine

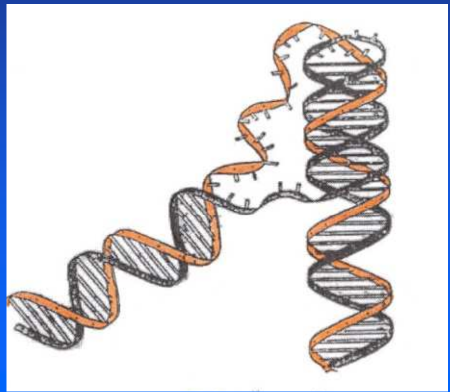


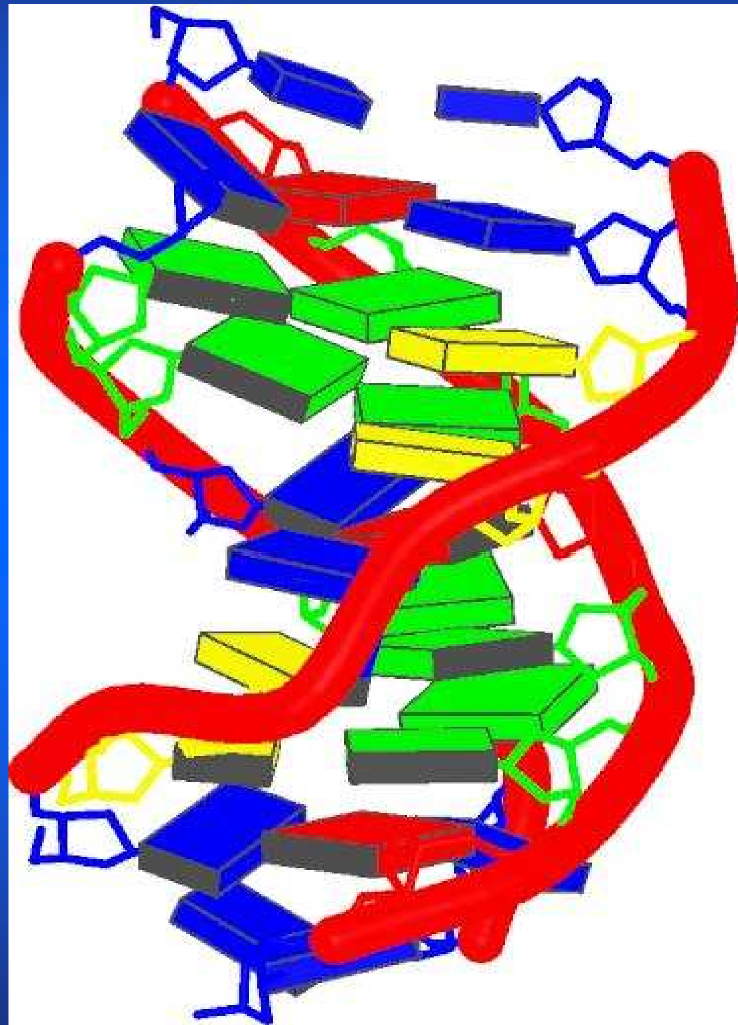


Radhakrishnan, I., Patel, D.J. (1994)

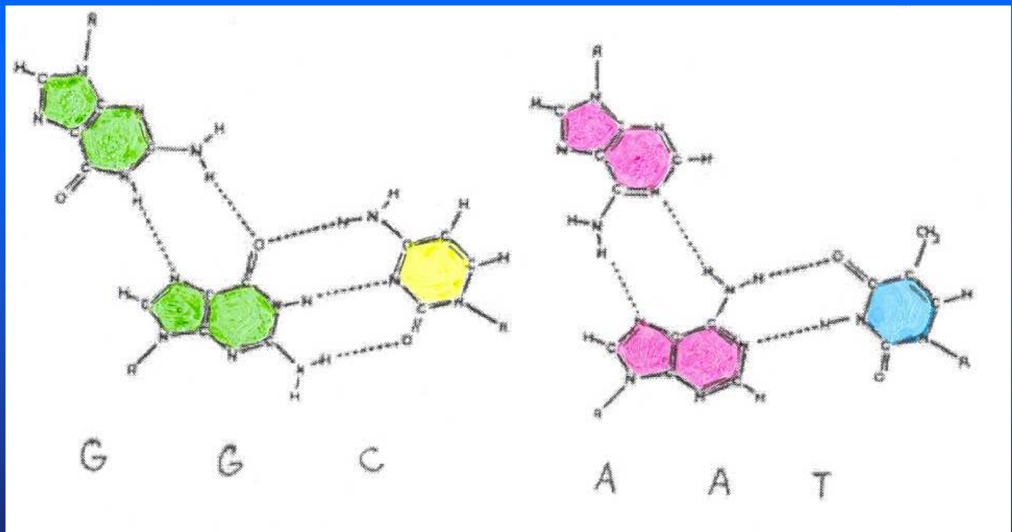
DNA TRIPLEX

T C C T C C T T T T T T A G G A G G A T T T T T T G G T G G T



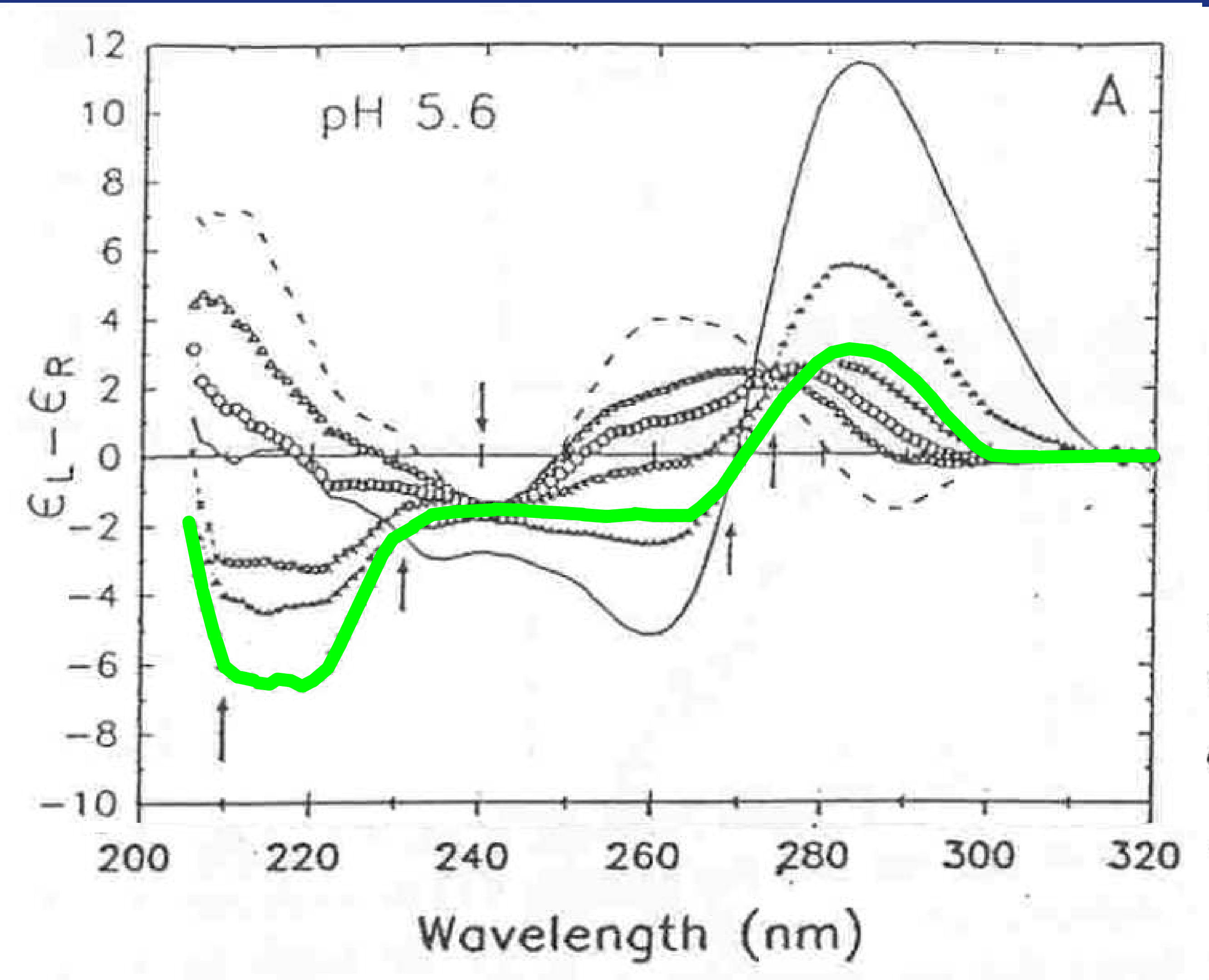


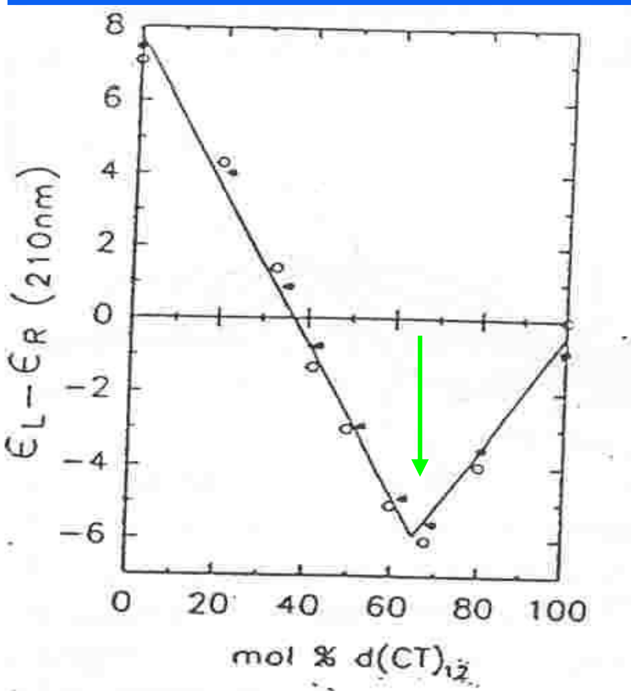
Radhakrishnan, I., Patel, D.J. (1993)



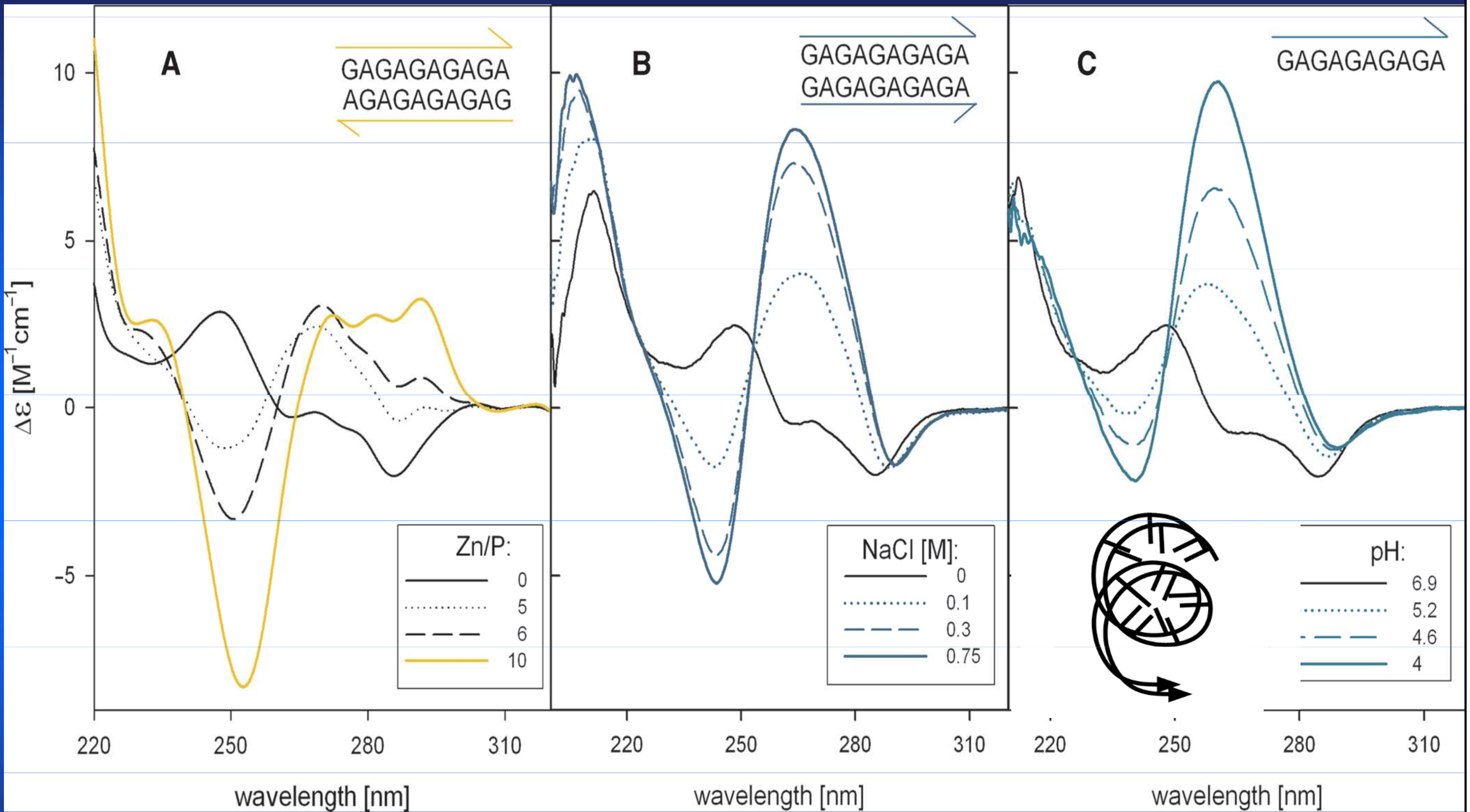
Pyrimidine. Purine. Purine

The triplex formation determined by mixing curves



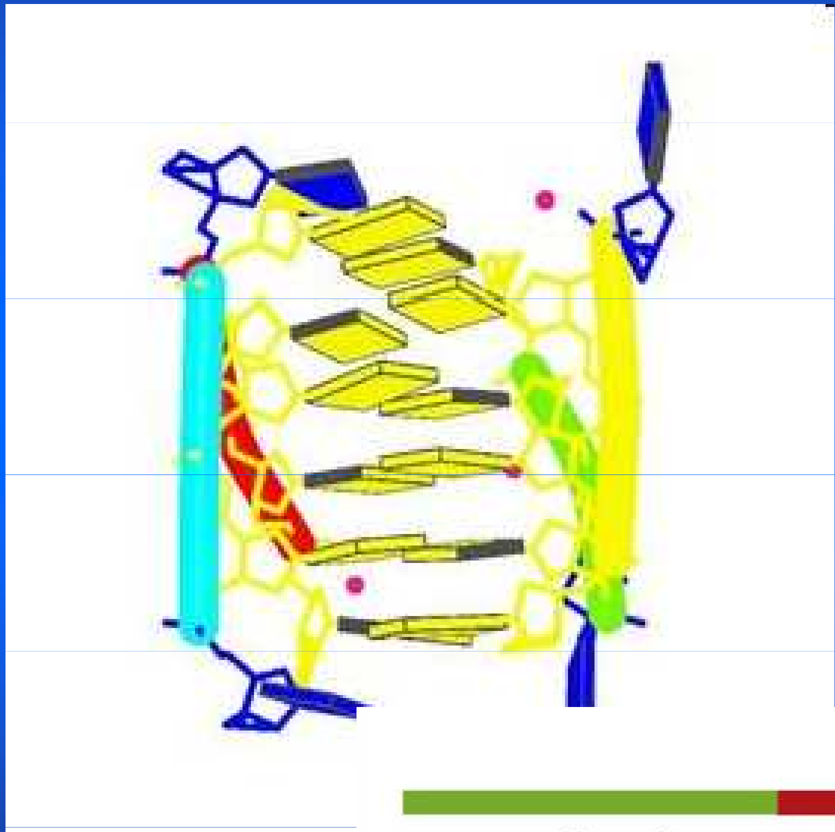


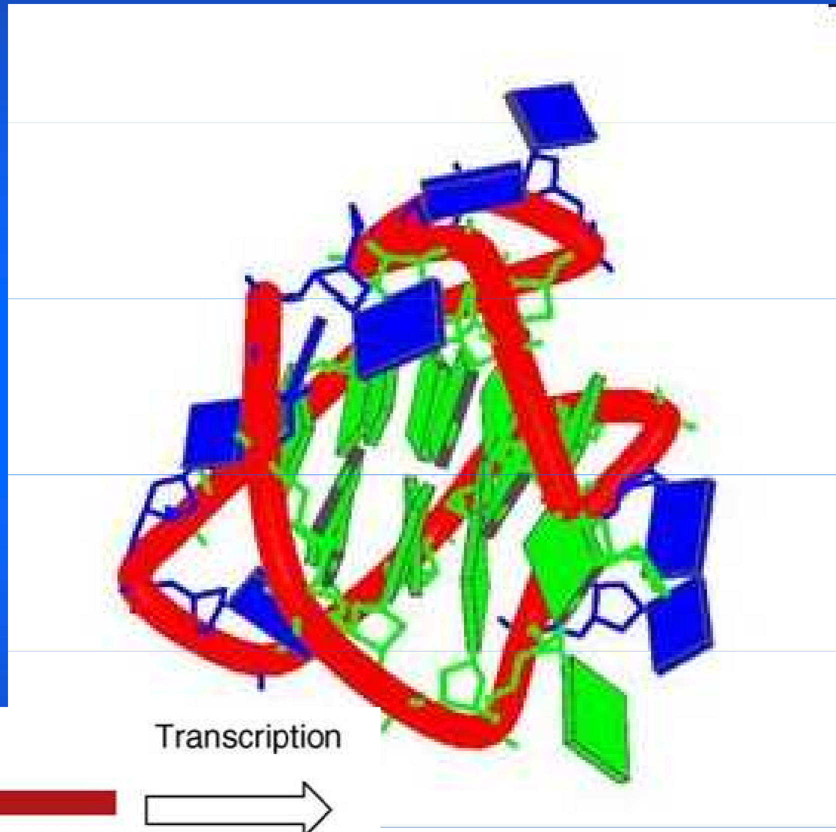
T Gray, D.M., Hung,S-H., Johnson, K.H.: Methods Enzymol. 246 (1995) 19-34.

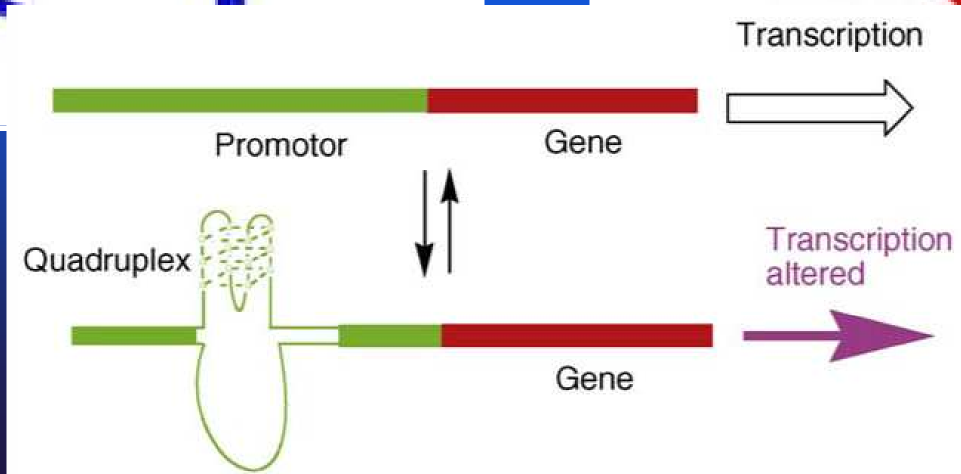

A
GAGAGAGAGA
AGAGAGAGAG
Zn/P:
0
5
6
10
B
GAGAGAGAGA
GAGAGAGAGA
NaCl [M]:
0
0.1
0.3
0.75
C
GAGAGAGAGA
pH:
6.9
5.2
4.6
4
Δε [M⁻¹cm⁻¹]
10
5
0
-5
220
250
280
310
wavelength [nm]
wavelength [nm]
wavelength [nm]

Quadruplexes

frequently occur in promoters of genes and were shown to control their expression.

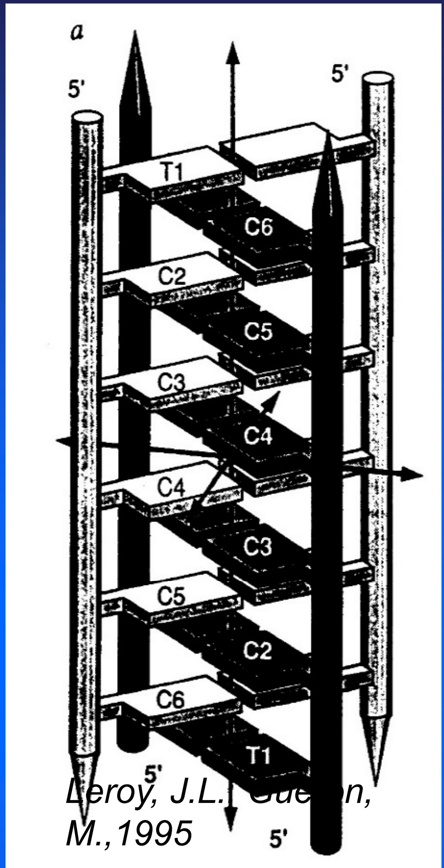


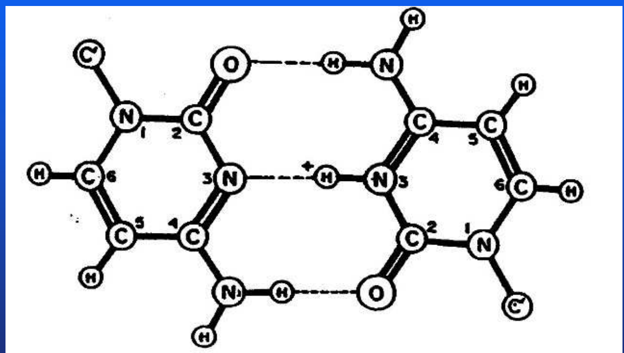




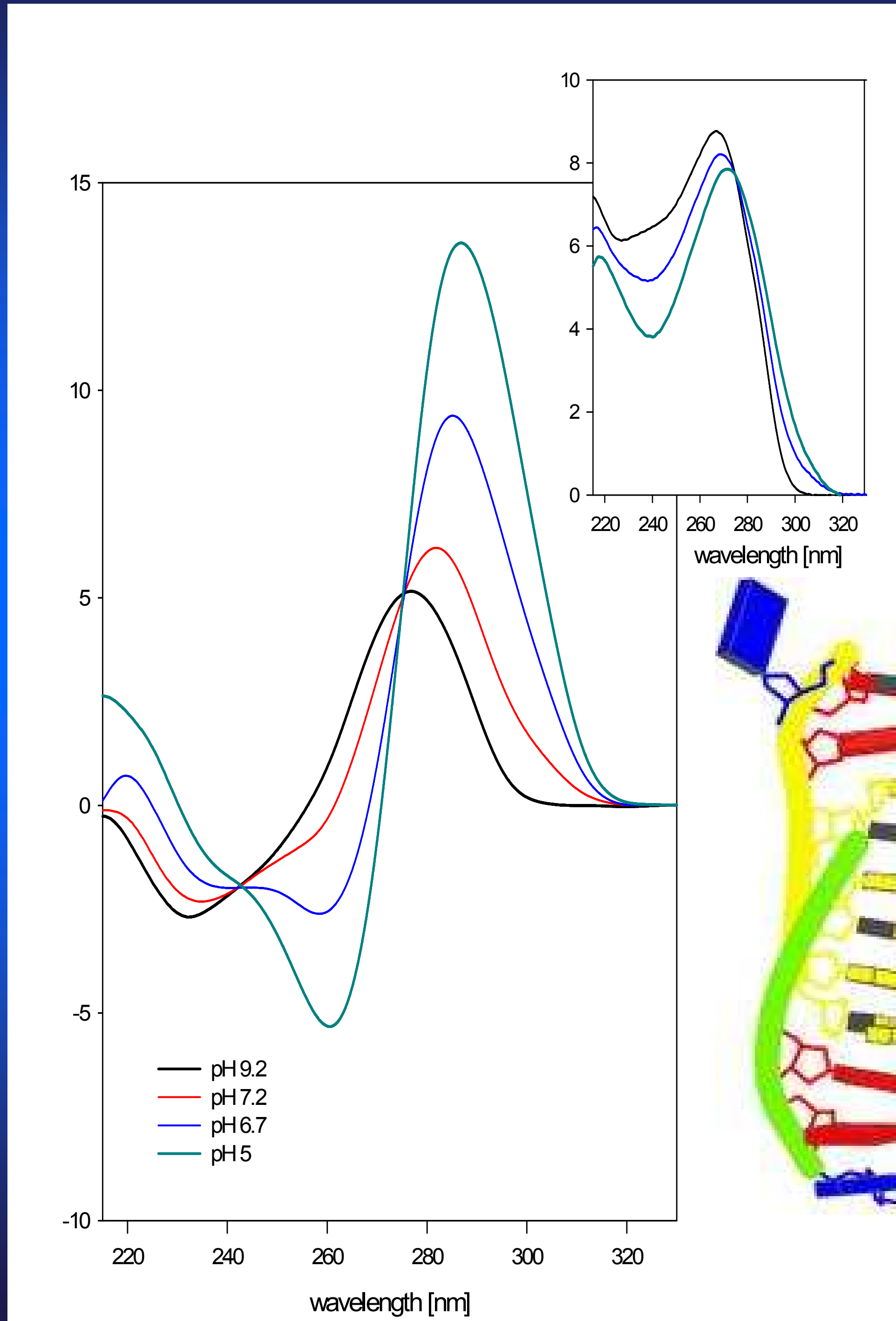
Wang, Y., Patel, D.J. (1994)

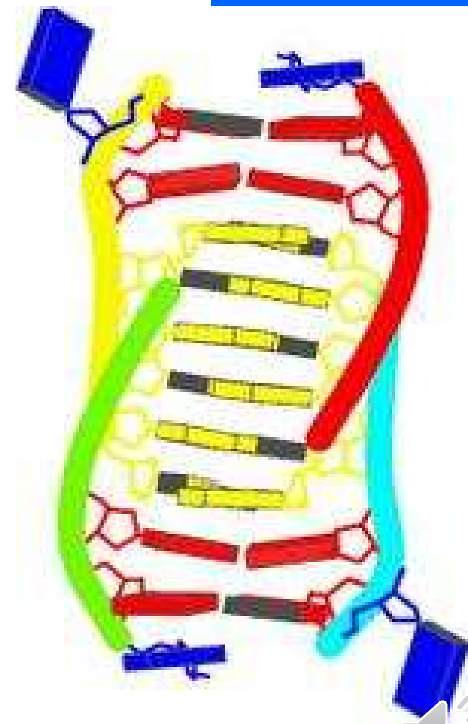
i - motif

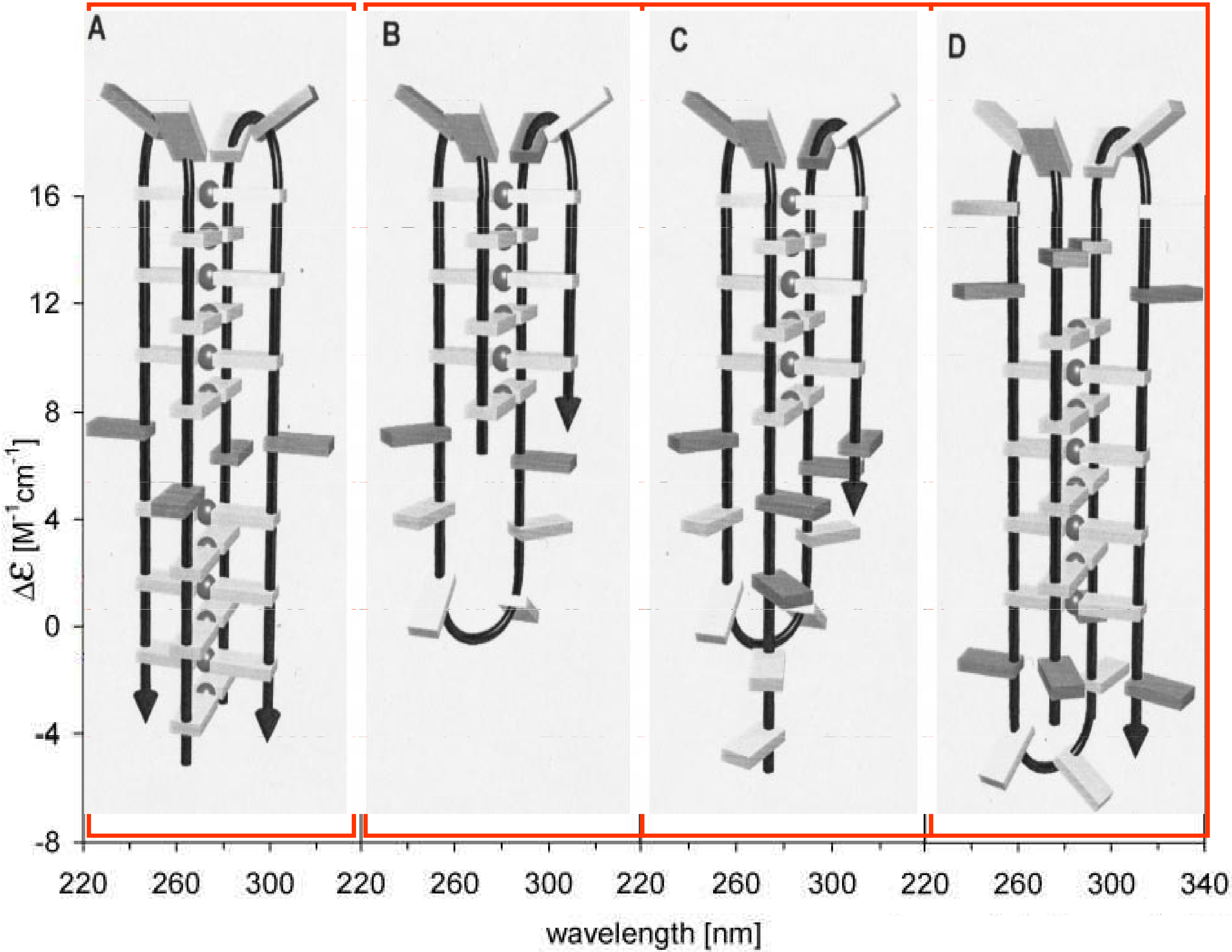




Two parallel-bonded duplexes are intercalated in the antiparallel fashion

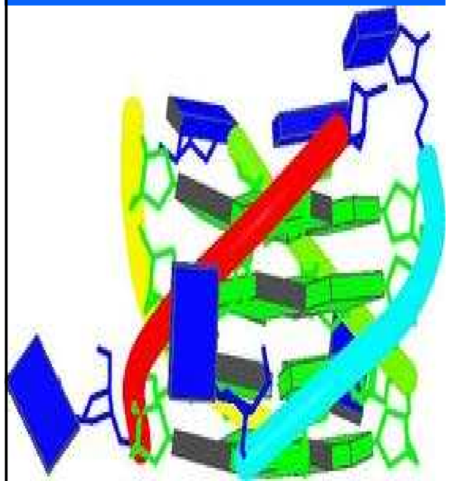


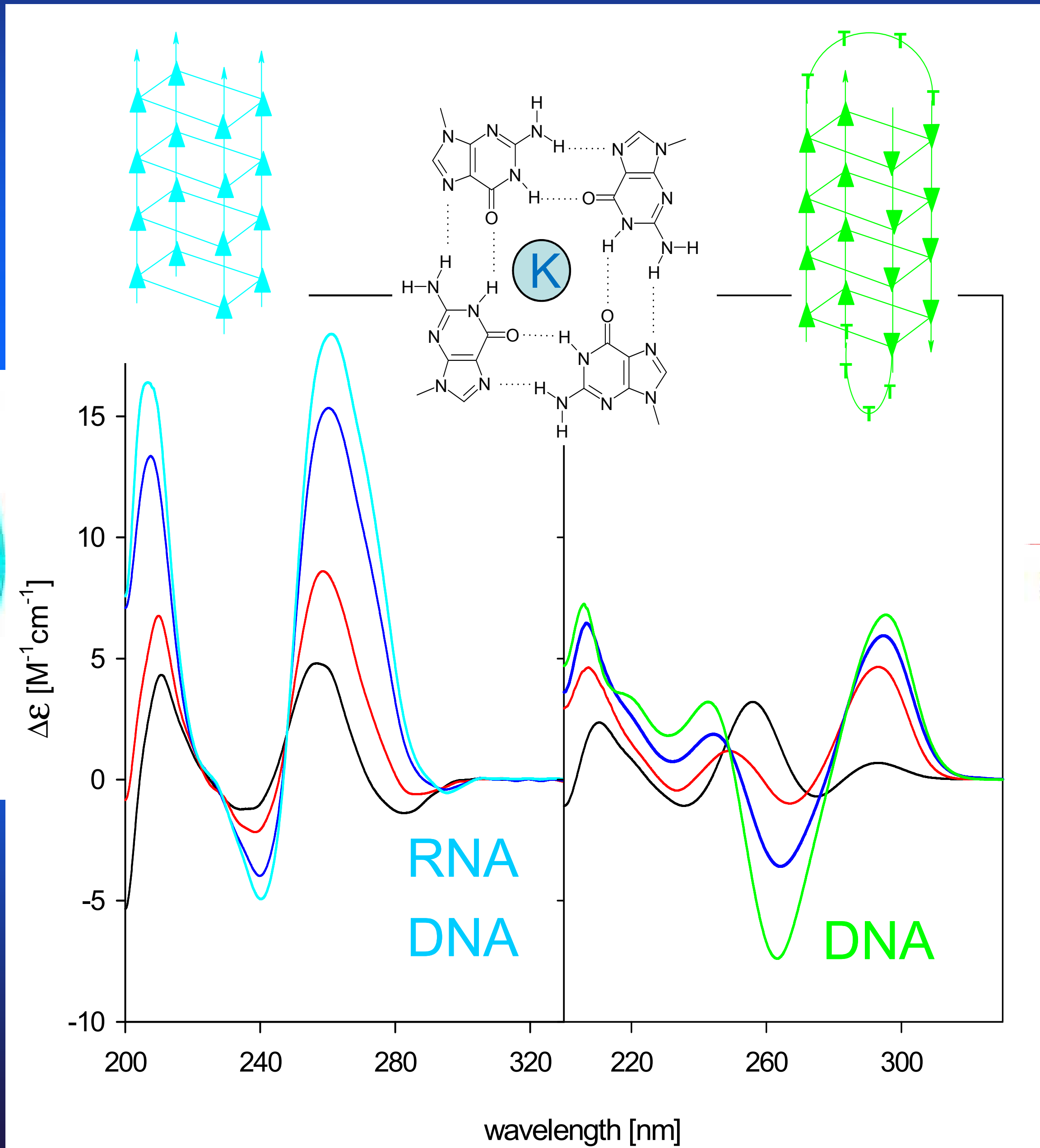


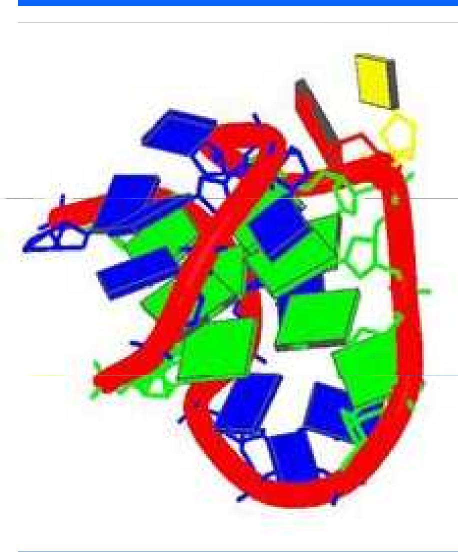


TCCCCACCTTCCCCACCCTCCCCACCCTCCCCCA

CD spectra reflecting formation of a parallel and antiparallel guanine quadruplex

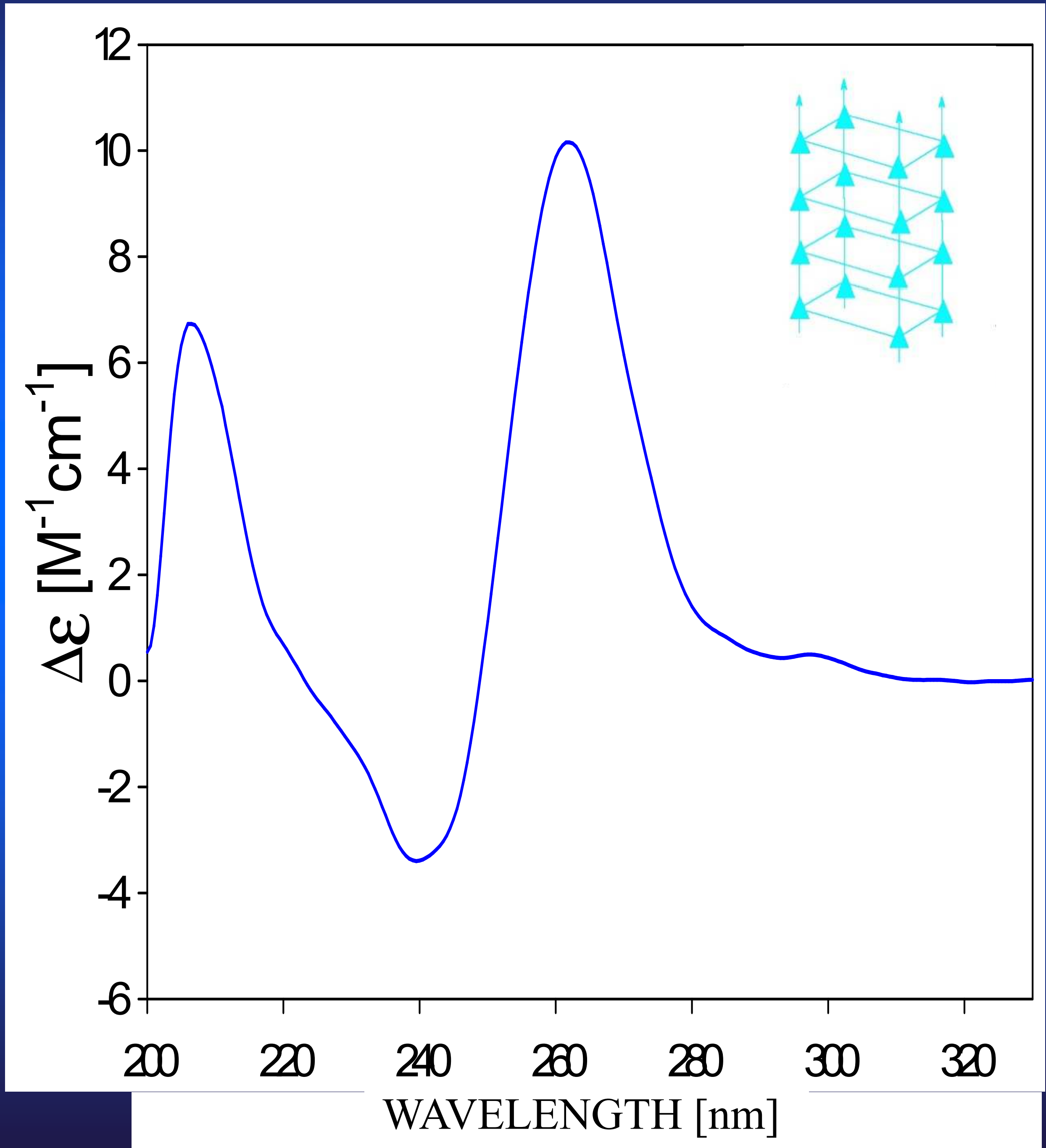


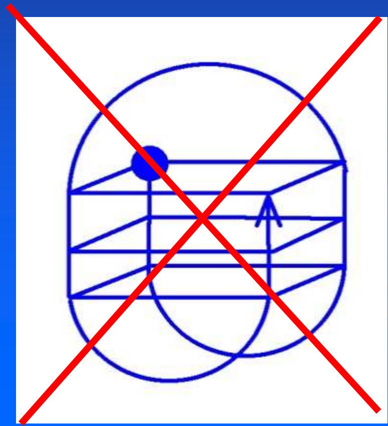


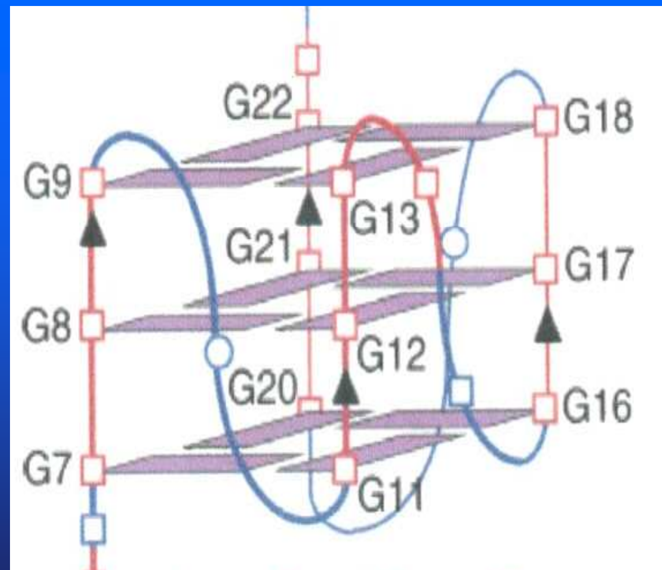


Fragment Pu-27 promotoru c-myc:

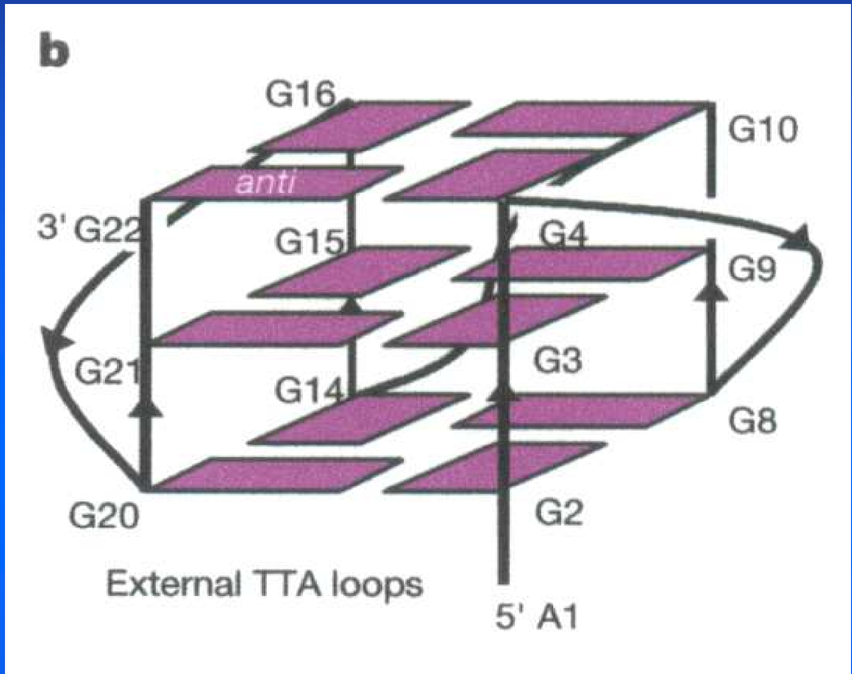
TGGGGAGGGTGGGGAGGGTGGGGAAGG

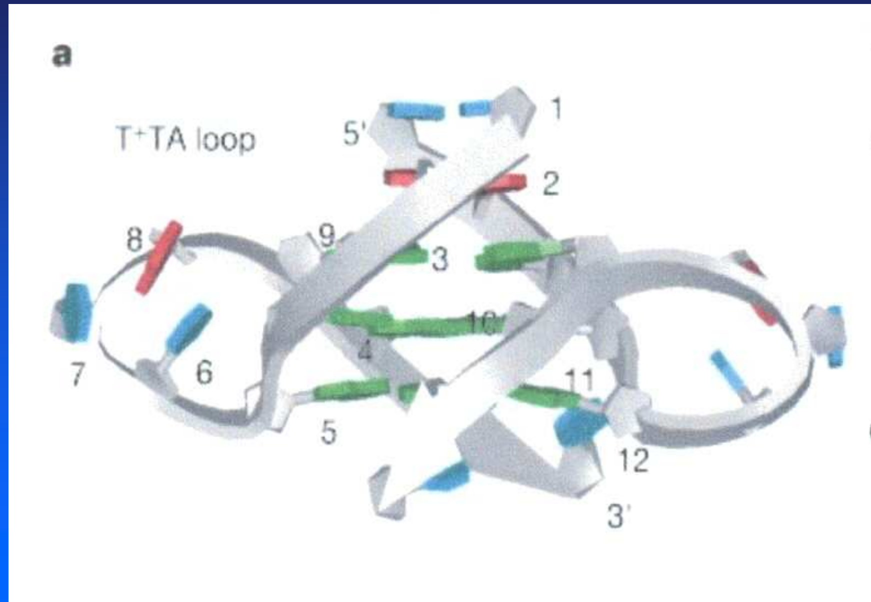


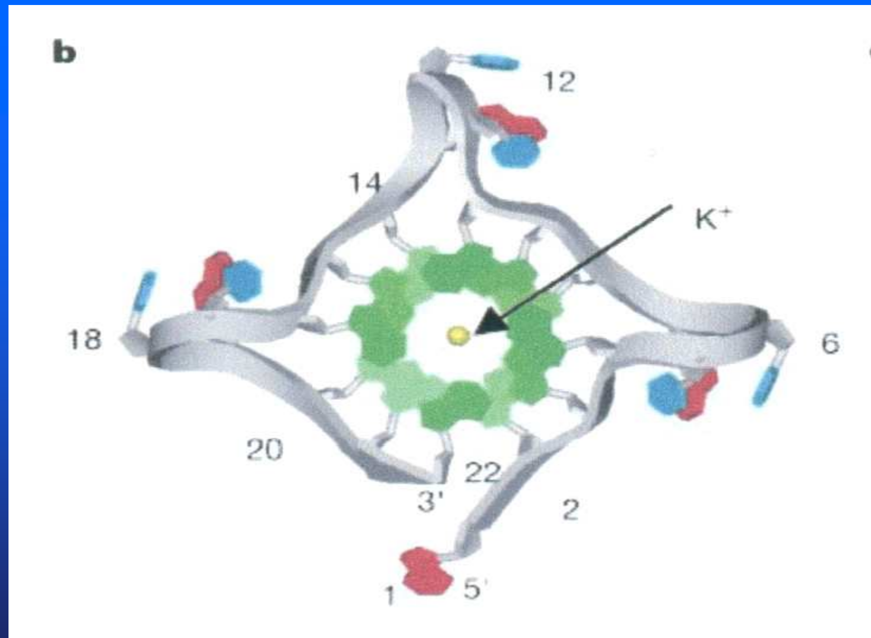




d(TAGGGTTAGGGT)



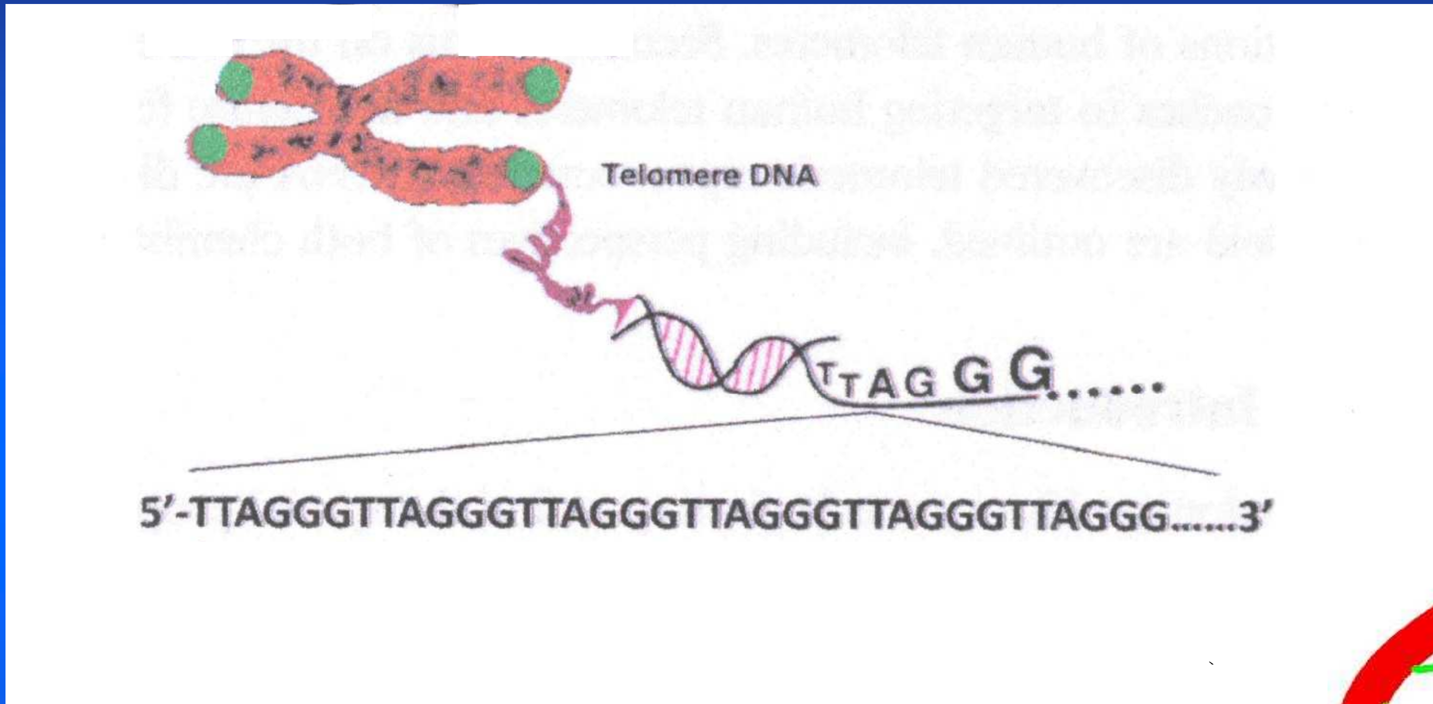




Parkinson, G.N., Lee, M.P.H, Neidle, S. *Nature* **417** (2002) 876-880.

d[AGGG(TTAGGG)3]

Human telomeric DNA forms quadruplex



Telomeric DNA is associated with aging

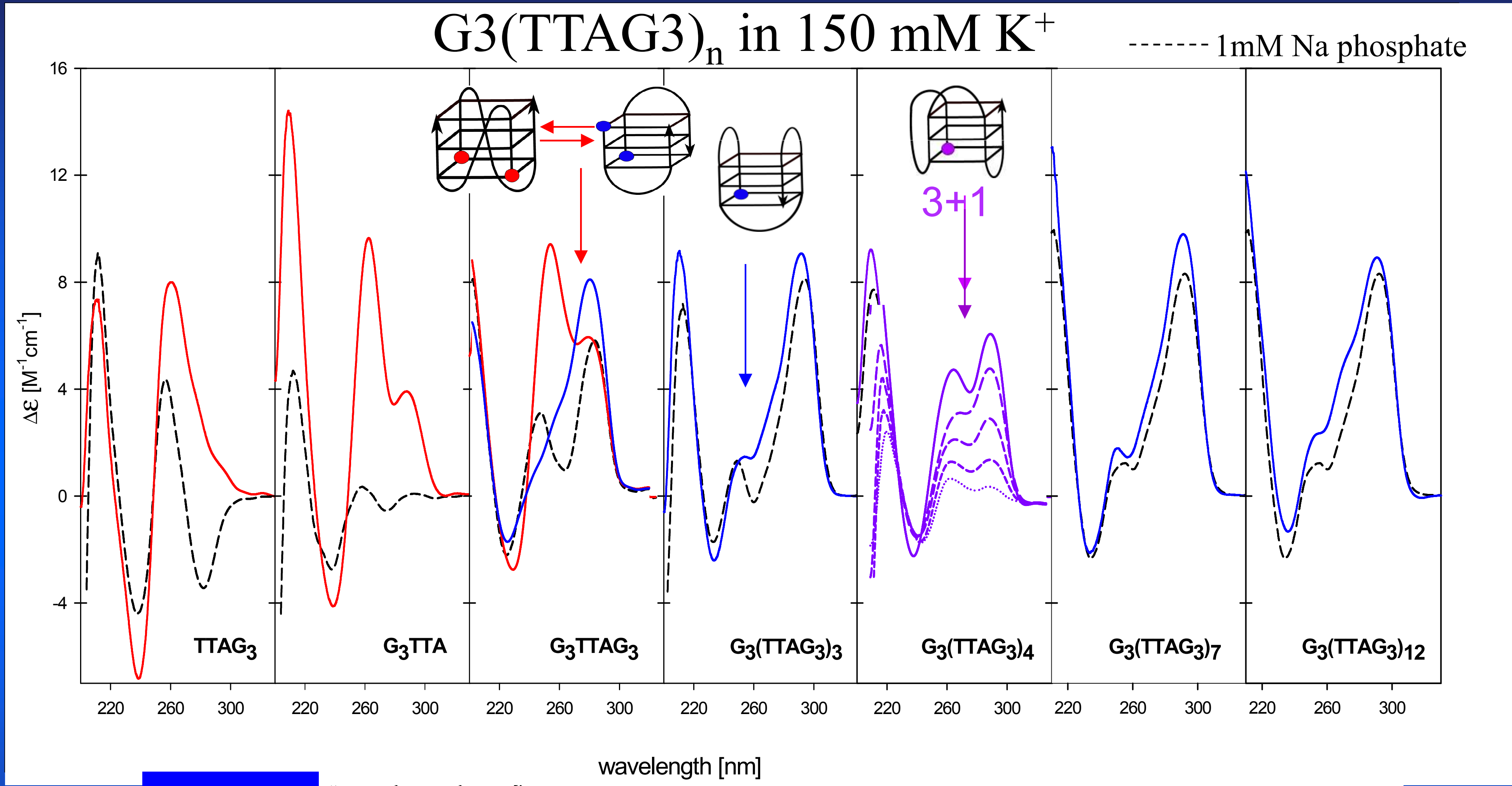
Telomerase – does not get older – ageless, immortal

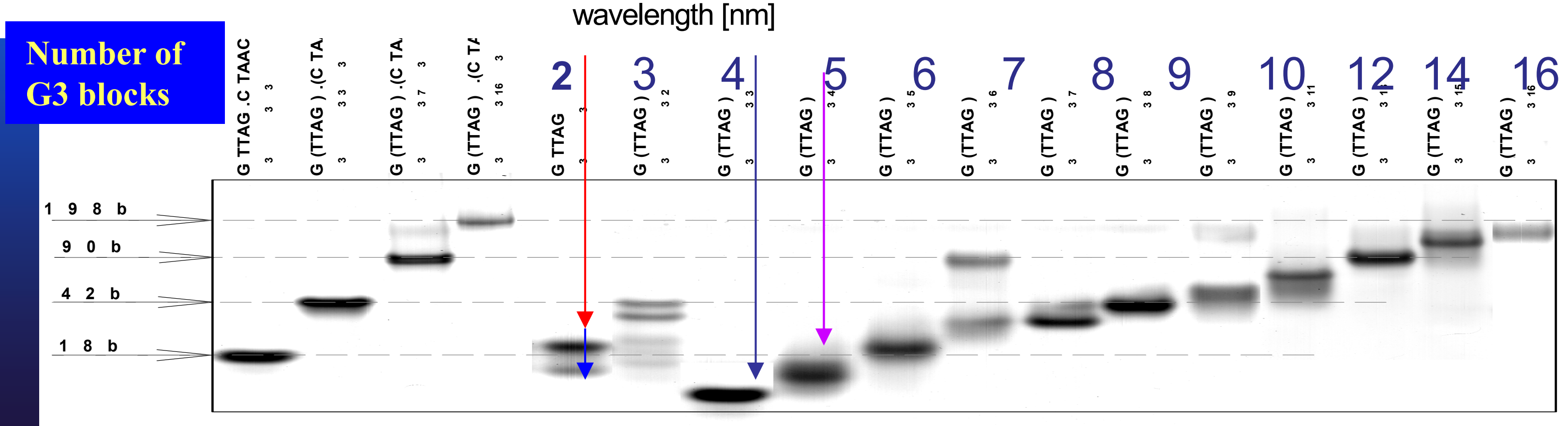
Quadruplex does not allow telomerase to get on the sequence

The telomere quadruplex became a target for developing anticancer drugs

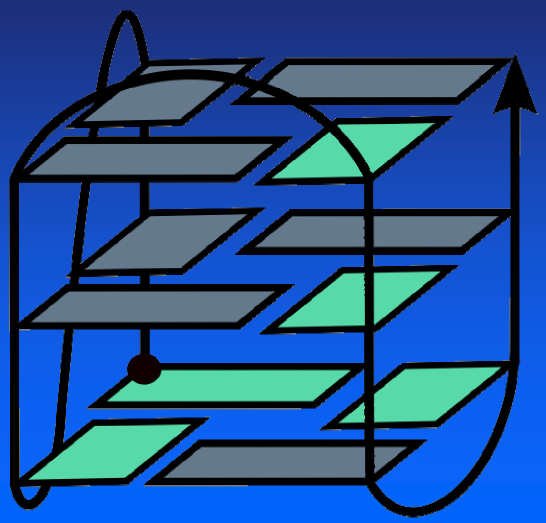
Guanine quadruplex topology of human telomere DNA is governed by the number of (TTAGGG) repeats.

Nucleic Acids Res. **33** (2005) 5851-5860.





3 + 1

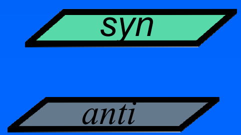


$AG_3(TTAG_3)_3$

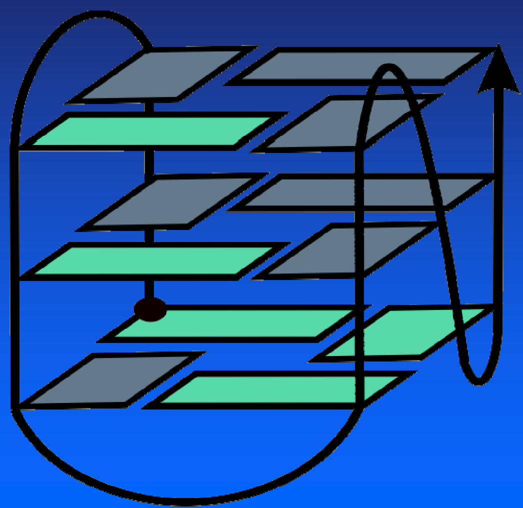
$TAG_3(TTAG_3)_3$

$AAAG_3(TTAG_3)_3AA$

K^+



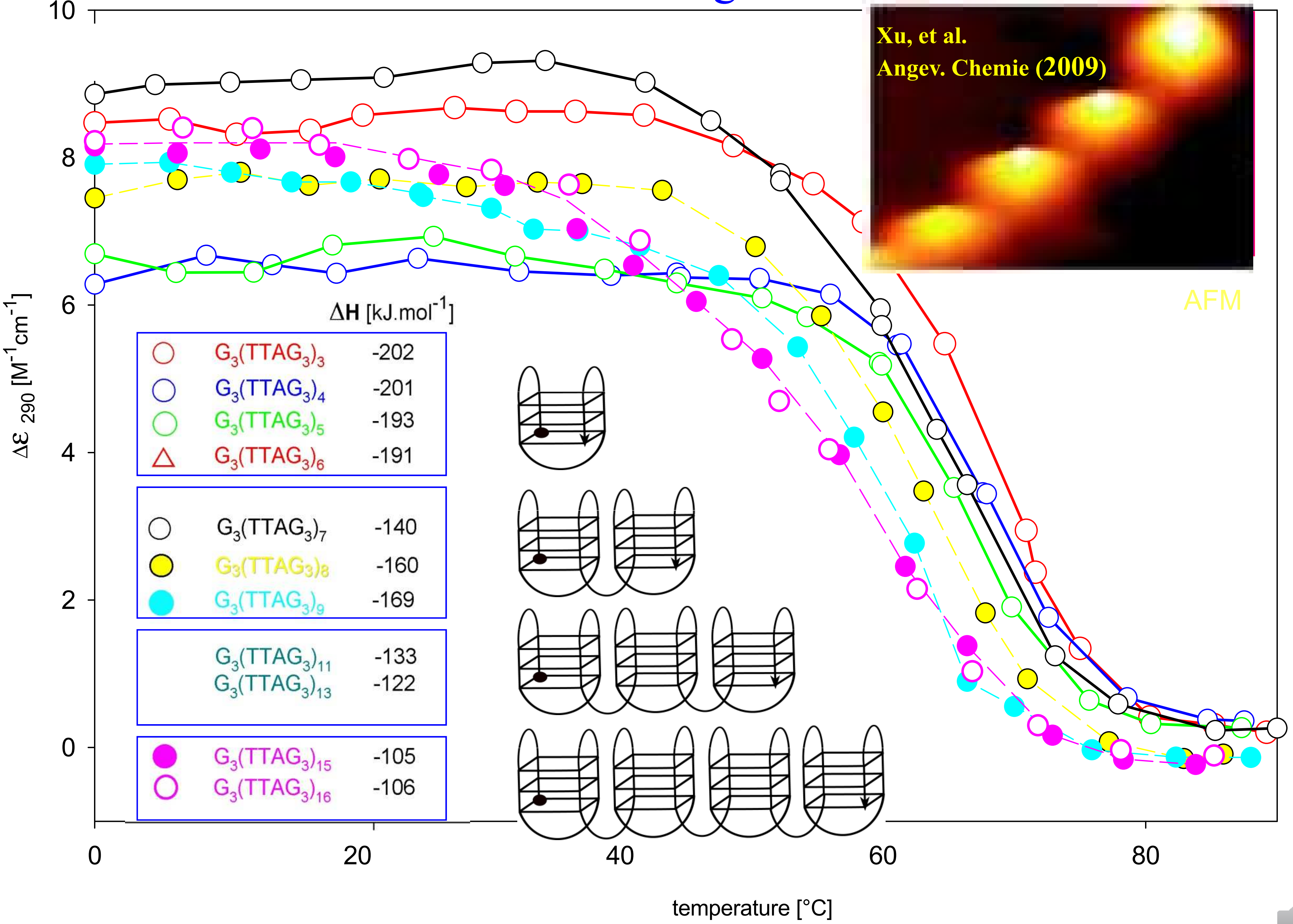
3 + 1



$TAG_3(TTAG_3)_3TT$

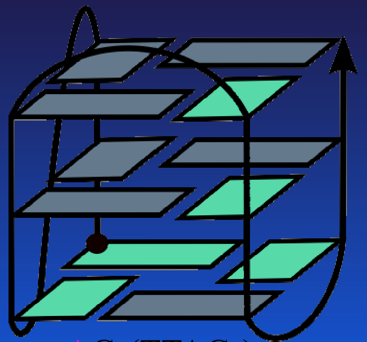
- Luu, K.N., Phan, A.T., Kuryavyi, V., Lacroix, L., Patel, D.J. (2006) J.Am.Chem.Soc., 128, 9963-9970.
- Ambrus, A., Chen, D., Dai, J., Bialis, T., Jones, R.A., Yang, D. (2006) Nucleic Acids Res. 34, 2723–2735.
- Phan, A. T., Luu, K.N., Patel, D.J. (2006) Nucleic Acids Res., 34, 5715-5719.

How does the structure of the long telomere DNA look like?



What is the structure of the bead?

3 + 1



$AG_3(TTAG_3)_3$

$TAG_3(TTAG_3)_3$

$AAAG_3(TTAG_3)_3AA$

Luu, et al.: J.Am.Chem.Soc., 128 (2006) 9963-9970.

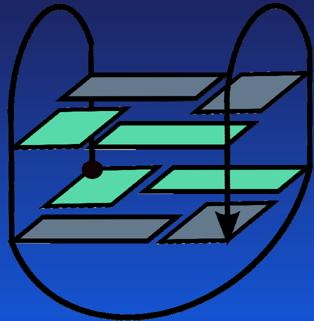
Ambrus, et al.: Nucleic Acids Res. 34 (2006) 2723–2735.

3 + 1

$TAG_3(TTAG_3)_3TT$

Phan, at al.: Nucleic Acids Res. 34 (2006) 5715-5719.

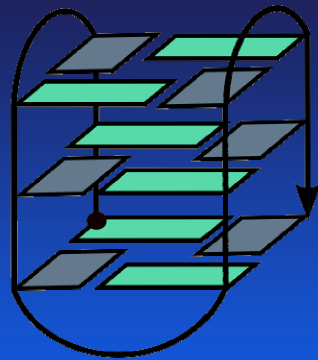
BASKET
two tetrads



$G_3(TTAG_3)_3T$

Lim, et al.: J.Am.Chem.Soc. 131 (2009) 4301–4309.

CHAIR



$AG_3(TTAG_3)_3$

He et al.:Nucleic Acids Res. 32 (2004) 5359-5367.

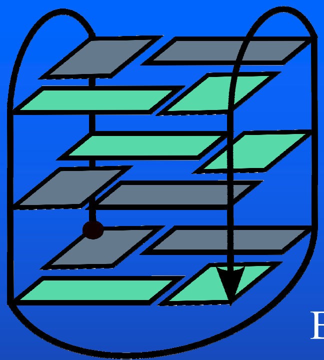
Matsugami, et al.:. Nucleic acids symp. series, 50 (2006) 45-46.

Xu et al.: Bioorg.& Medicinal Chem. 14 (2006)5584 – 5591.

K^+

0.2-5 mM strand concentration in NMR

3-50 µM strand concentration in CD



$G_3(TTAG_3)_3$

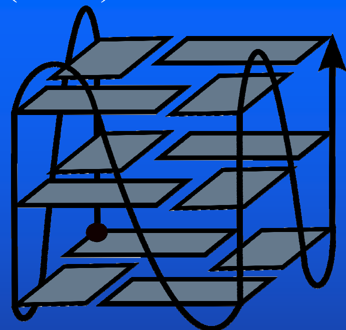
$AG_3(TTAG_3)_3$

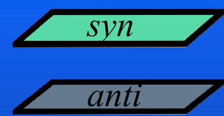
$TTAG_3(TTAG_3)_3$

BASKET

Balagurumoorthy, Brahmachari: J. Biol. Chem. 269 (1994) 21858-21869.

Redon et al.: Nucleic Acids Res. 31 (2003) 1605-1613.





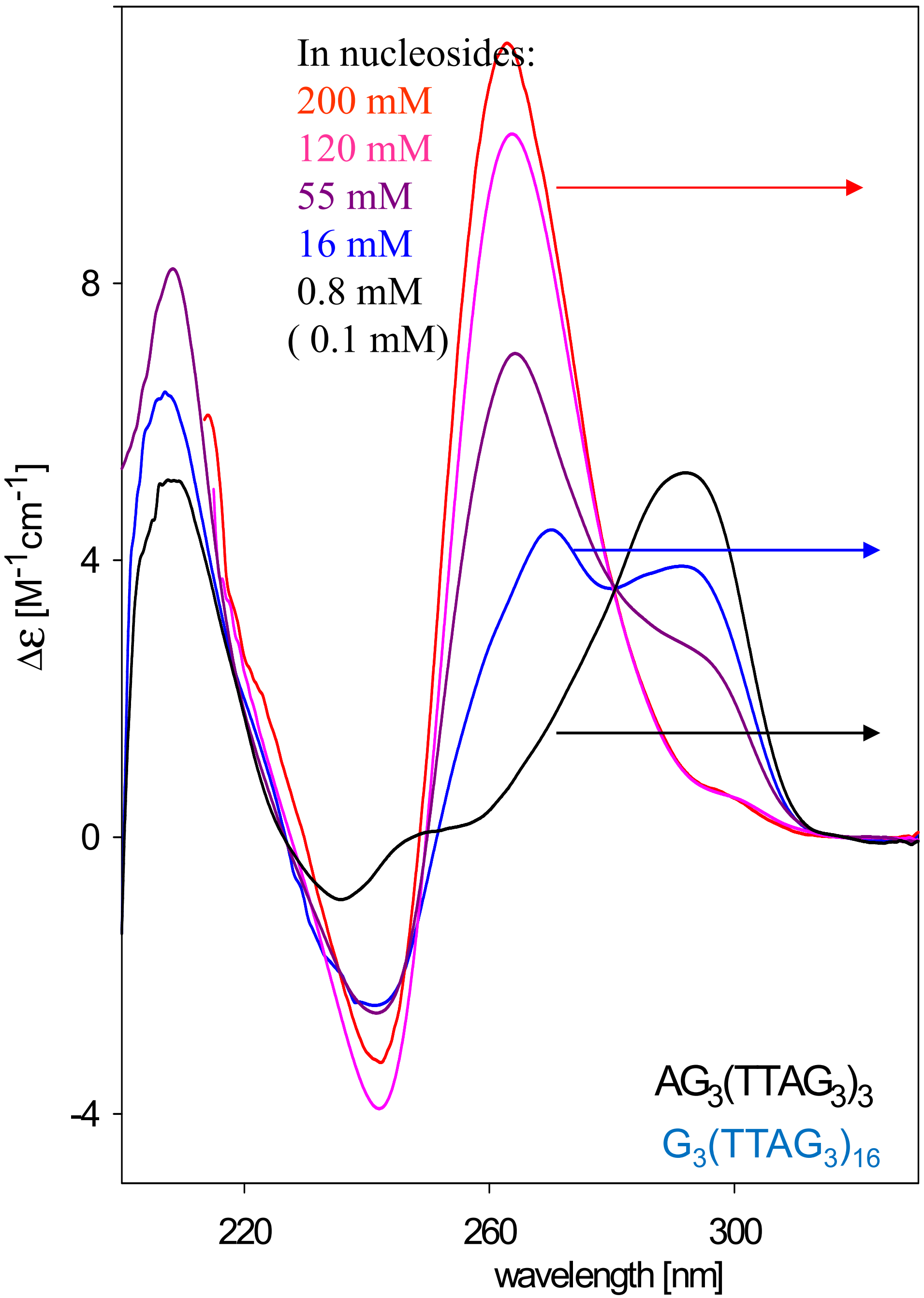
$AG_3(TTAG_3$

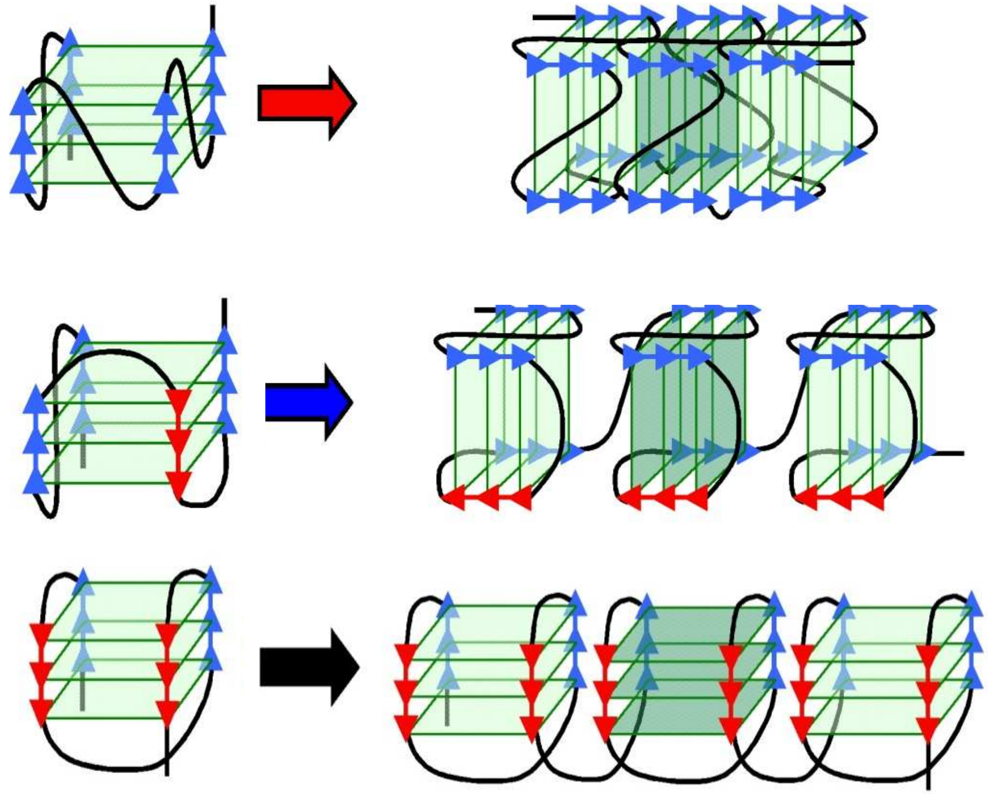
Parkinson, Lee, Neidle: Nature 417 (2002) 876-880.

PARALLEL

What may be the reason that different quadruplex structures were observed by various methods?

What may be the reason that different quadruplex structures were observed by various methods?





The arrangement of the human telomere quadruplex is polymorphic and depends on DNA concentration.

The particular structures may perform distinct functions.

Renčiuk et al.: Nucleic Acids Research ***37*** *(2009) 6625-6634*

CD spectroscopy and conformational properties of nucleic acids

) What is optical activity, chiral metters, optical rotation, circular dichroism

) Wat are the (two) conditions for the origin of CD effect

) What components are responsible for CD of nucleic acids and proteins

) What are the advantages of CD spectroscopy as compared with other methods of the structural studies of biopolymers

) What is the substance of the unique sensitivity of CD to structural changes in NA

) What is optical rotatory dispersion and Cotton effect

) What is the difference between cooperative and non-cooperative changes

) Global characteristics of the forms B, A and Z DNA (particulary the grooves, an inverse topology of base pairs in the case of the Z-form

) some examples of non-canonical forms of DNA

Types of four-stranded arrangements of NA

mifi@ibp.cz

CHIROPTICKÉ METODY

Optická rotační disperze-ORD

Závislost úhlu stočení roviny polarizace lineárně polarizovaného světla průchodem opticky aktivní látkou na vlnové délce procházejícího záření. (180-800 nm)

Cirkulární dichroismus-CD

Závislost rozdílu absorpce pro vlevo a vpravo kruhově polarizované světlo na vlnové délce absorbovaného záření v oblasti energií elektronových přechodů. (180-1000 nm)

Infračervený cirkulární dichroismus-IRCD (VCD)

Závislost rozdílu absorpce pro vlevo a vpravo kruhově polarizované světlo na vlnové délce absorbovaného záření v oblasti energií vibračních přechodů. (1-5 um)

Fluorescenčně detegovaný cirkulární dichroismus-FDCD

Závislost rozdílu intenzity fluorescence, excitované vlevo a vpravo kruhově polarizovaným světlem na vlnové délce excitačního záření. (~ 200 nm až vlnová délka emise)

Cirkulárně polarizovaná luminiscence (emise)-CPL (CPE)

Spektrální průběh rozdílu intenzit (spontánní) emise vlevo a vpravo cirkulárně polarizovaného světla. (Interval vlnových délek emise chromoforu)

Cirkulární diferenciální Ramanův rozptyl-Raman CID

Spektrální průběh rozdílů intenzit Ramanova rozptylu vlevo a vpravo kruhově polarizovaného dopadajícího záření. (Interval vlnových délek Ramanova jevu)