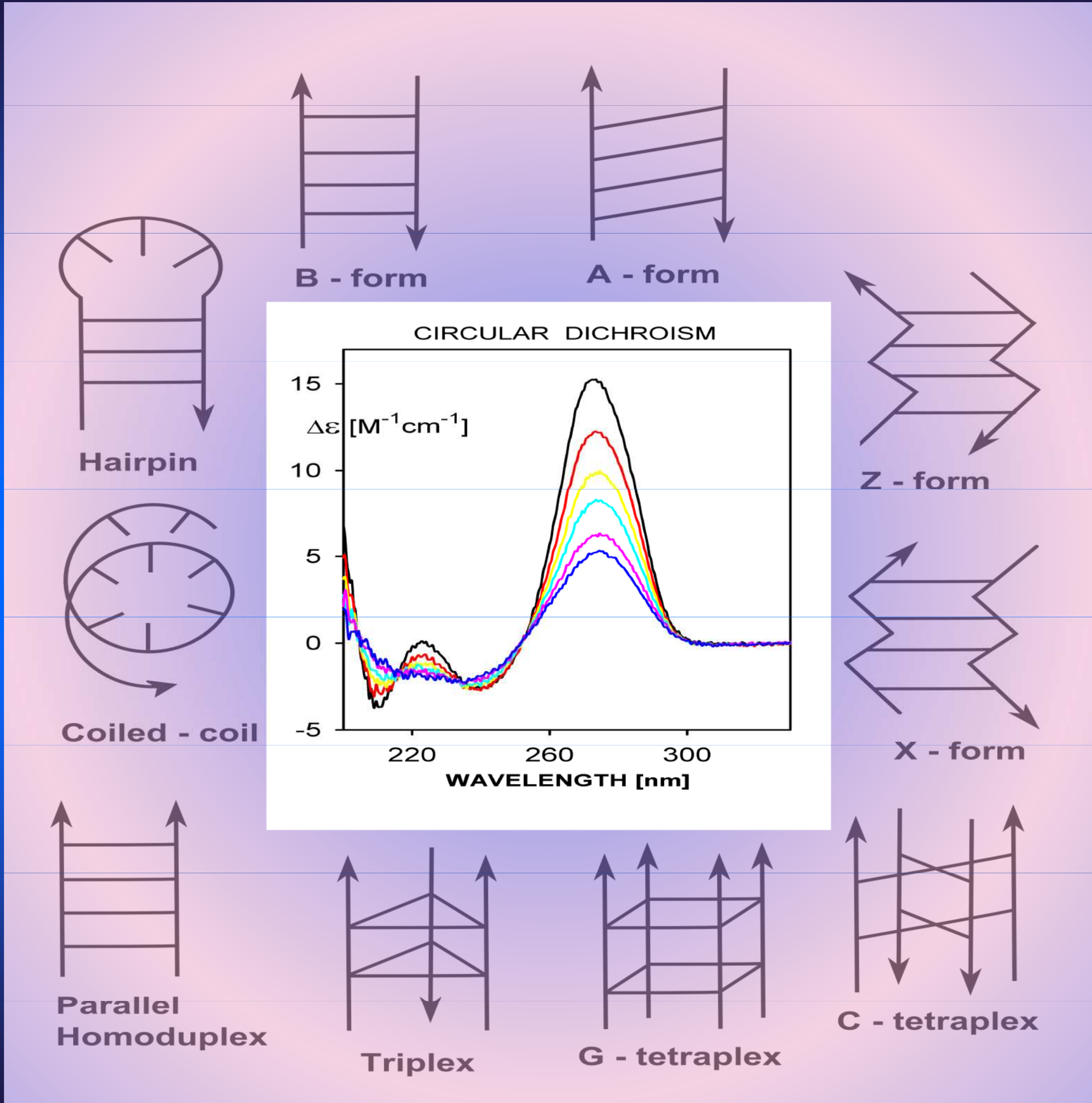
CD Spectroscopy and its Role in the History of our Knowledge of DNA Conformation

Michaela Vorlíčková mifi@ibp.cz
Institute of Biophysics
Czech Academy of Sciences Brno
Czech Republic
Laboratory of Biophysics of Nucleic Acids



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]

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

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)

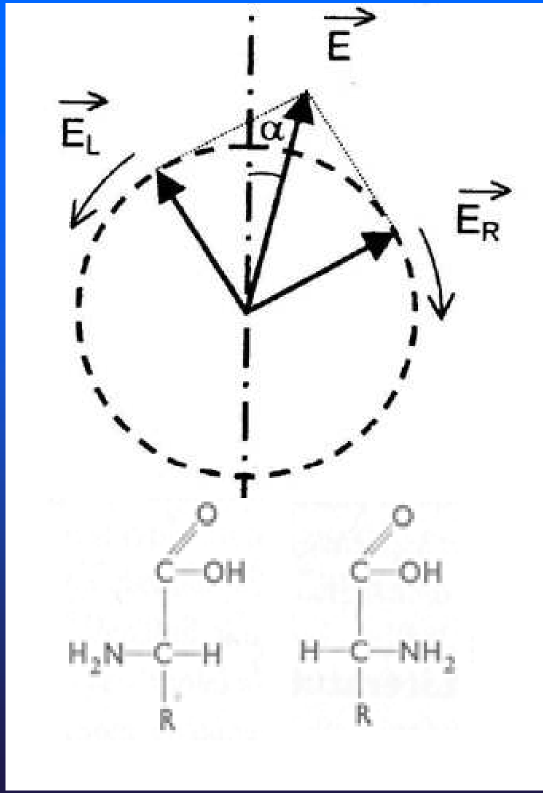
or to the left (levorotatory –L) depending on the stereoisomer (enantiomer) present

Specific rotation – characteristic quantity

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

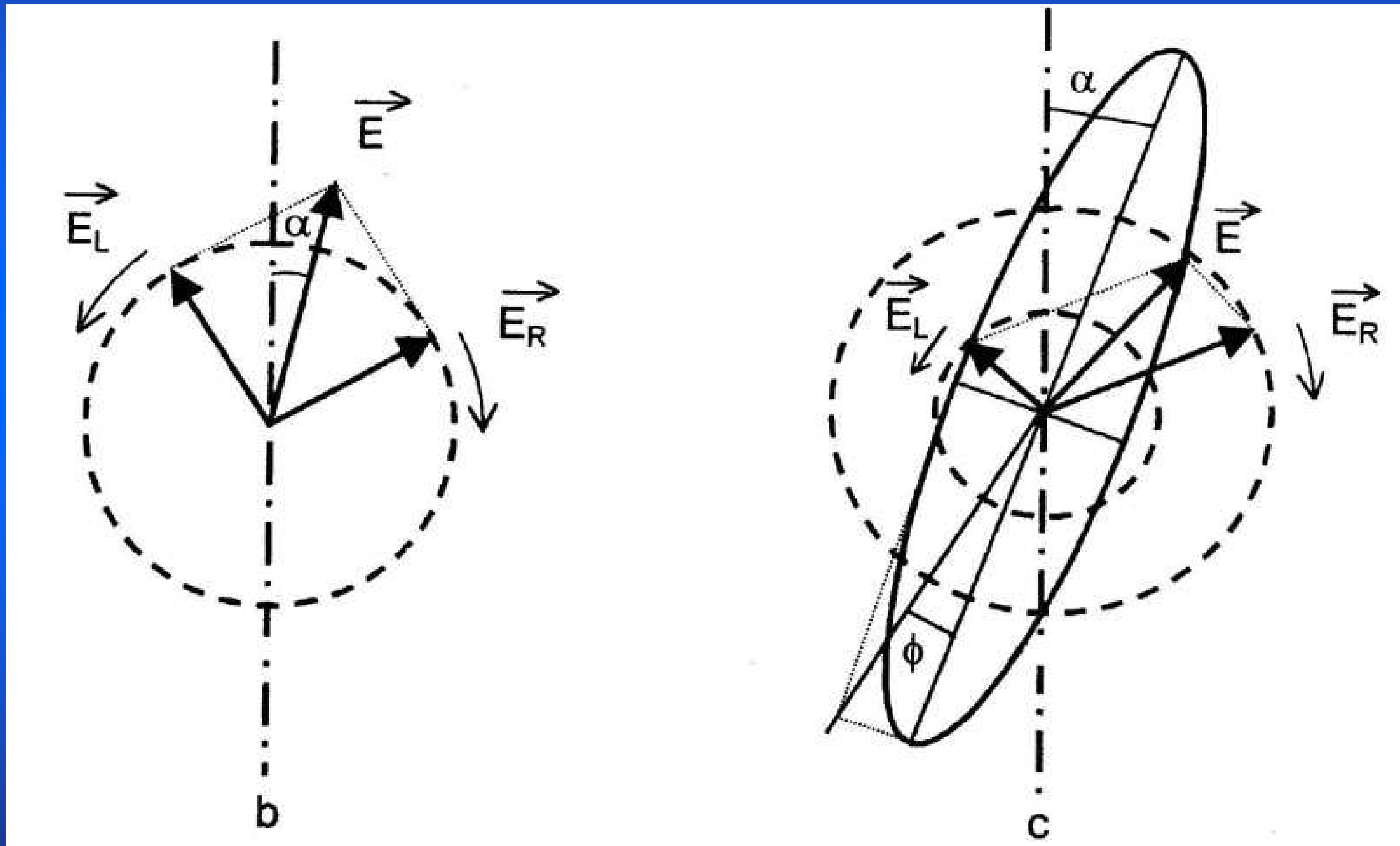
Optical rotatory dispersion - ORD

is the dependednce of specific rotation on the wavelength



Circular dichroism and optical activity of biopolymers

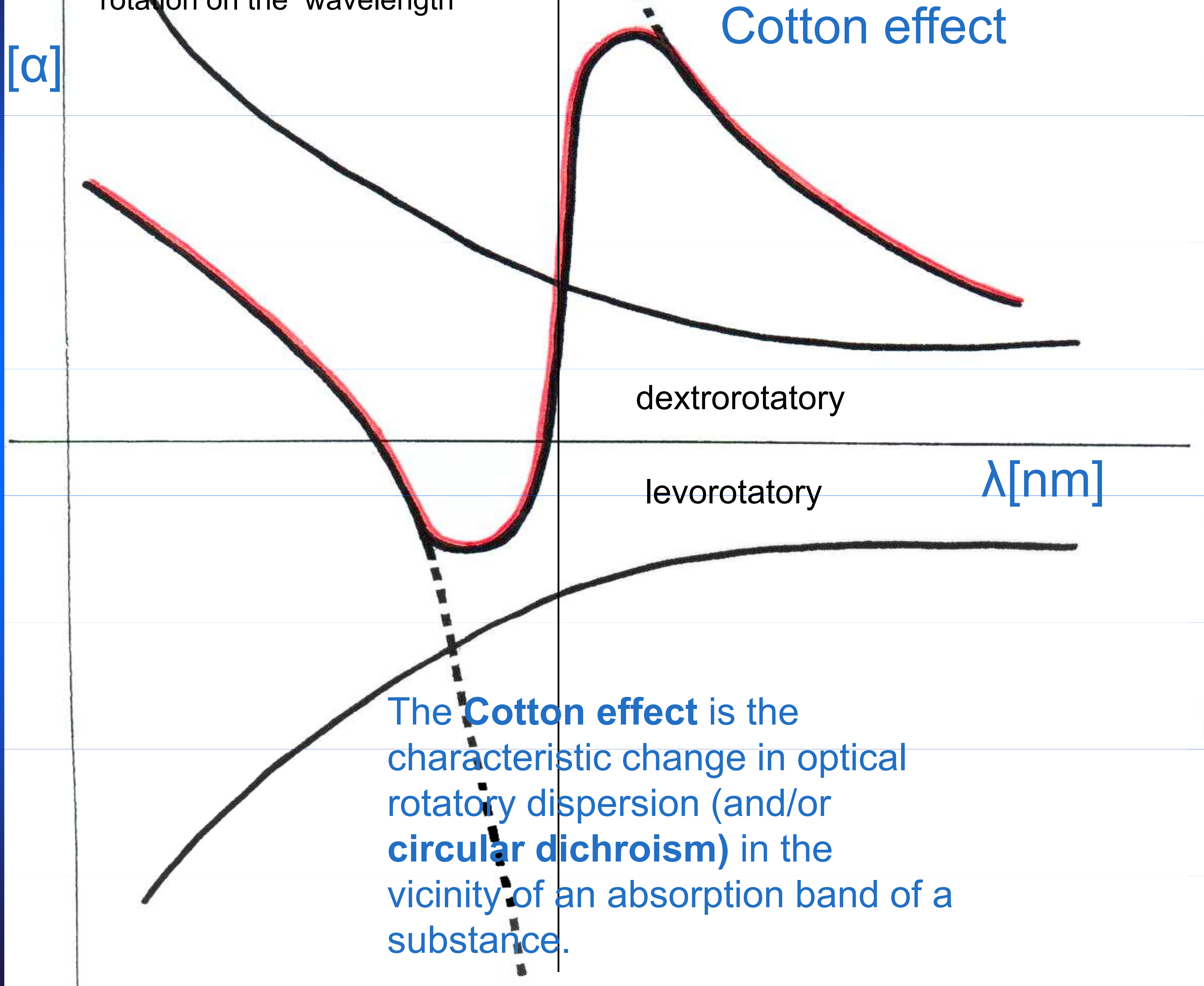
CD phenomenon – different absorption of the left-handed and right-haned circularly polarized light.



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

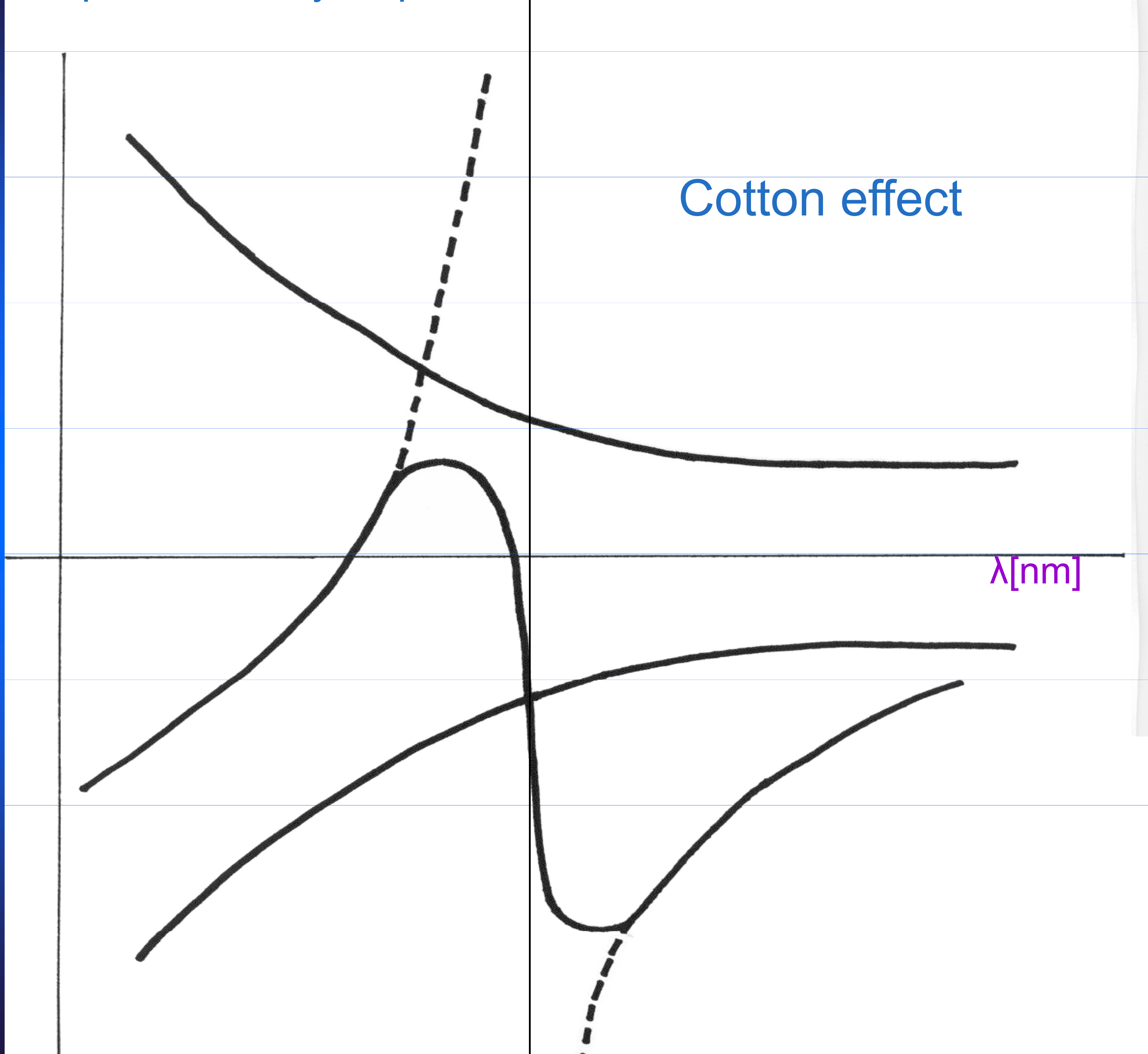
Circular dichroism Δε $\Delta\varepsilon = \varepsilon_L - \varepsilon_R = \Delta A/cl$, θ=3300. Δε

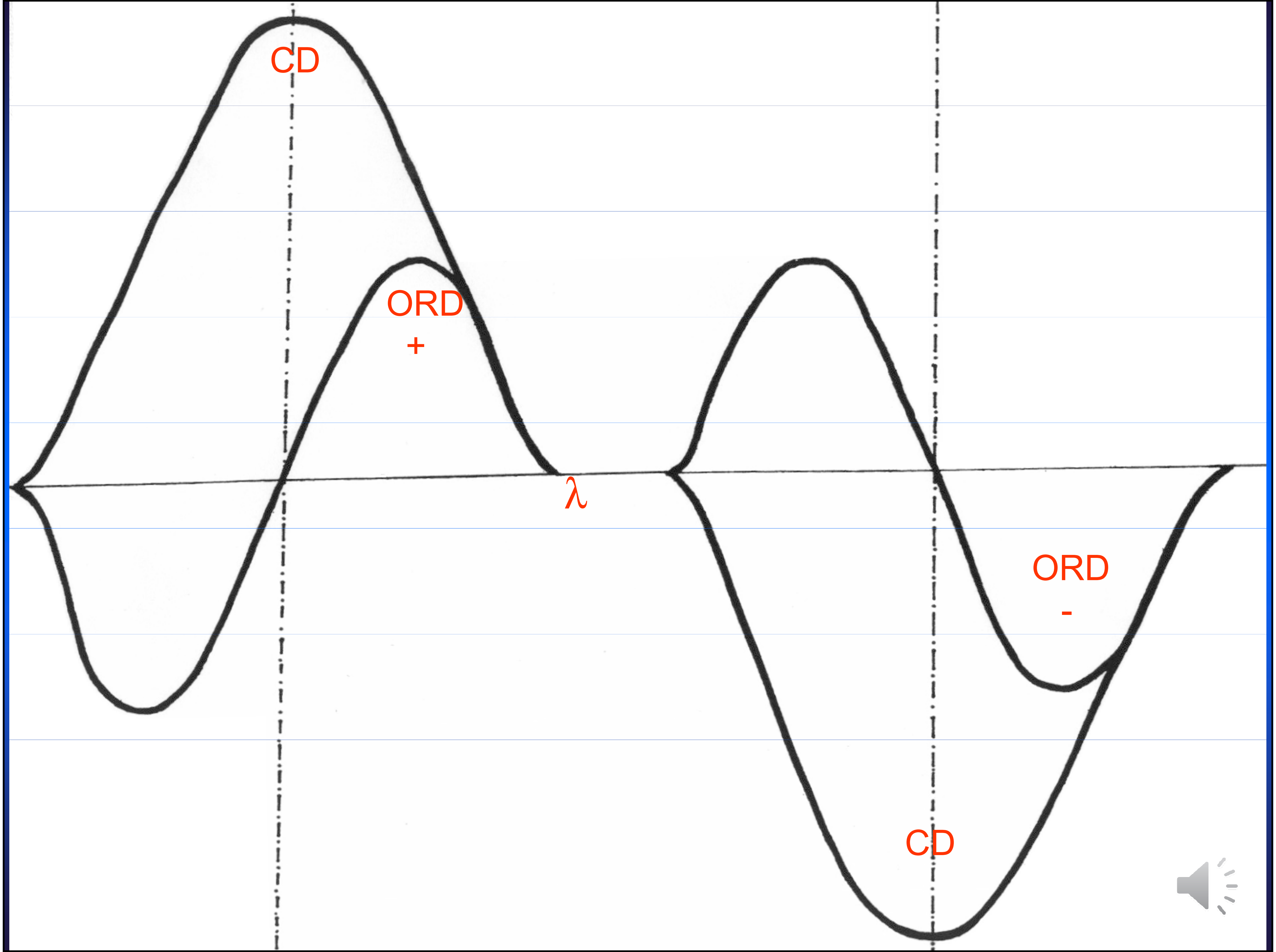
Optical rotatory dispersion (ORD) is the dependednce of specific rotation on the wavelength



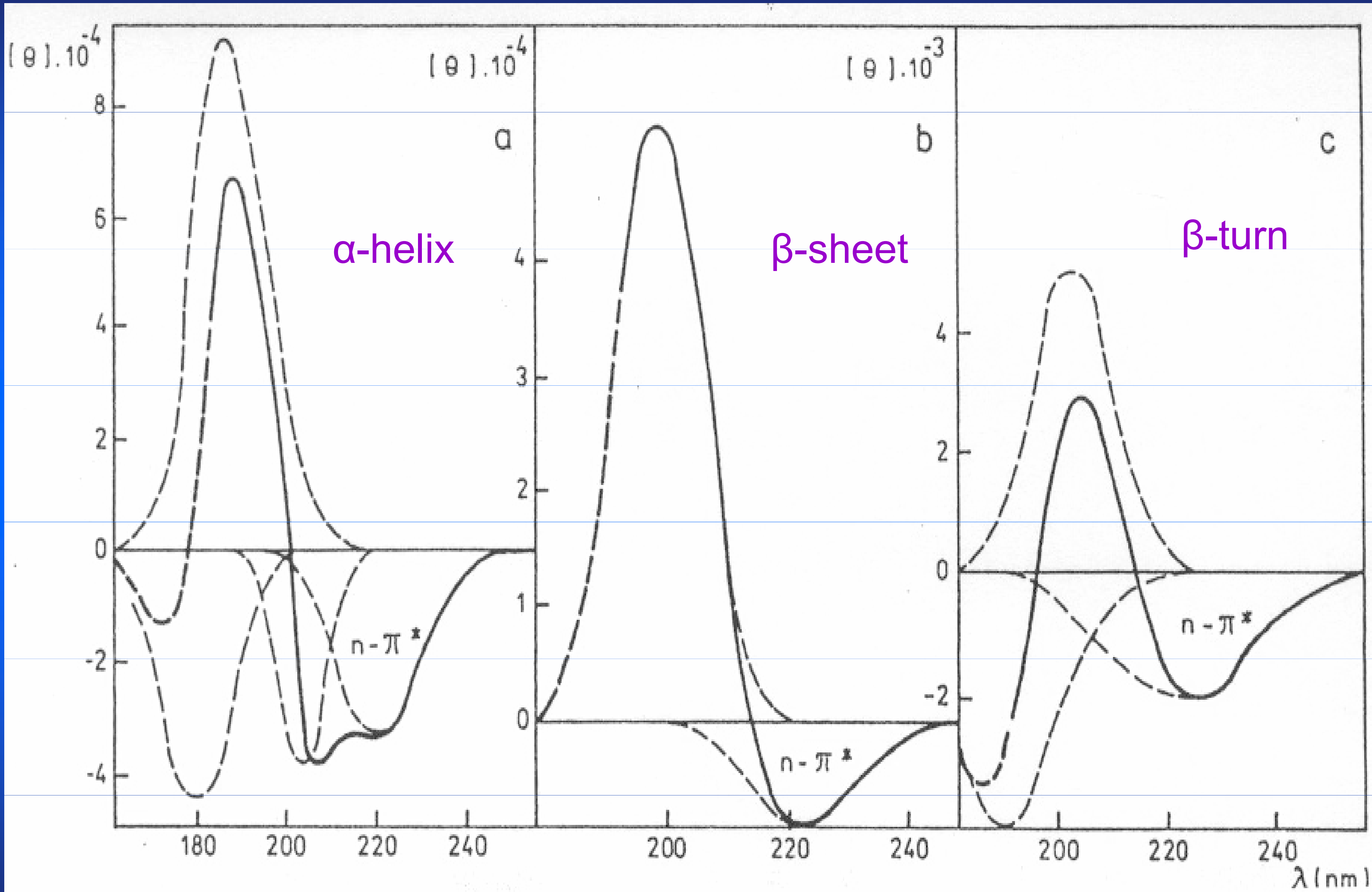
The **Cotton effect** is the characteristic change in optical rotatory dispersion (and/or **circular dichroism)** in the vicinity of an absorption band of a substance.

Optical rotatory dispersion

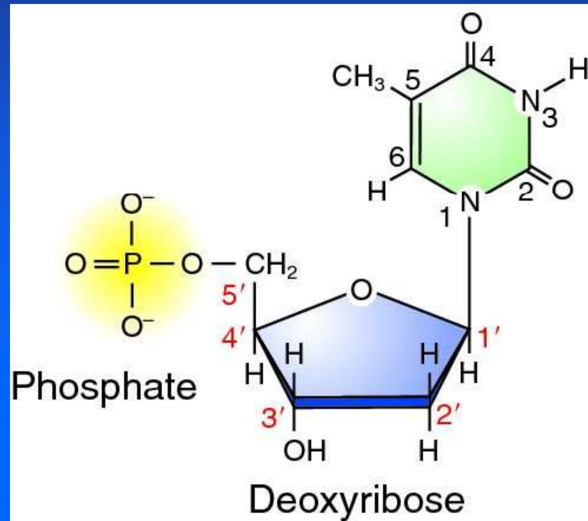



CD
ORD
+
λ
ORD
-
CD

CD of proteins



Preconditions for an appearance of CD of DNA



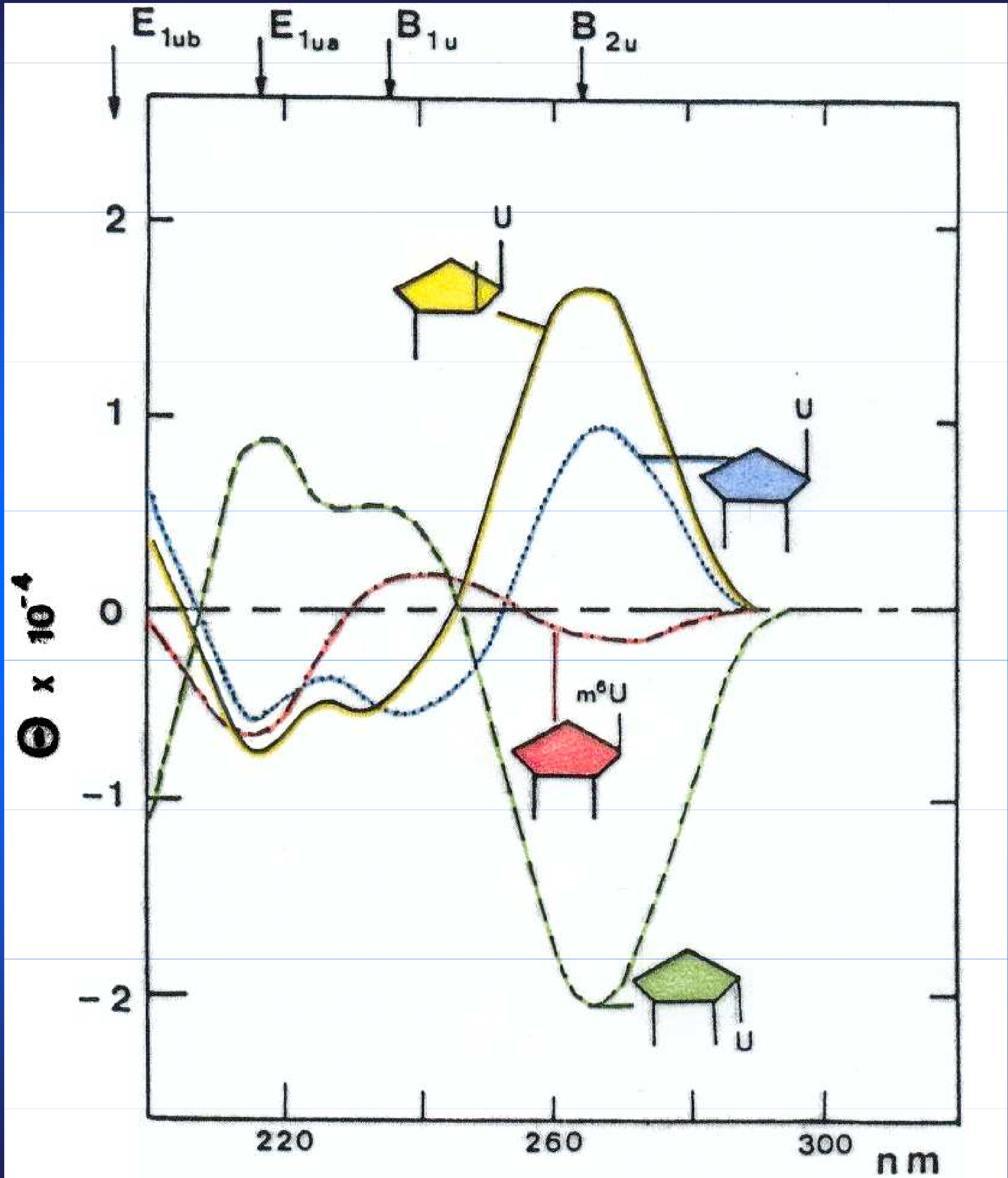
ABSORBANCE

\+ CD

CHIRALITY

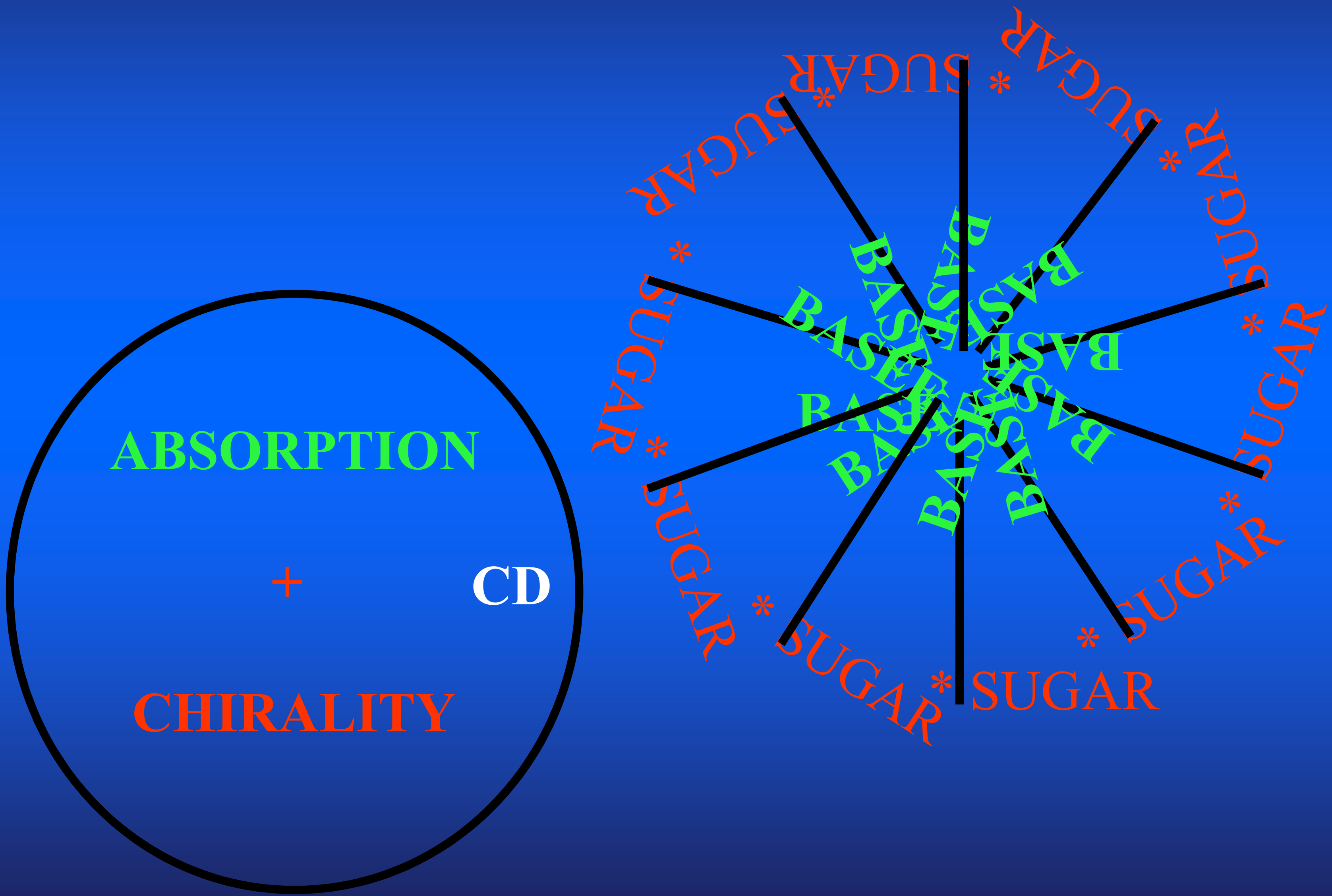
BASE

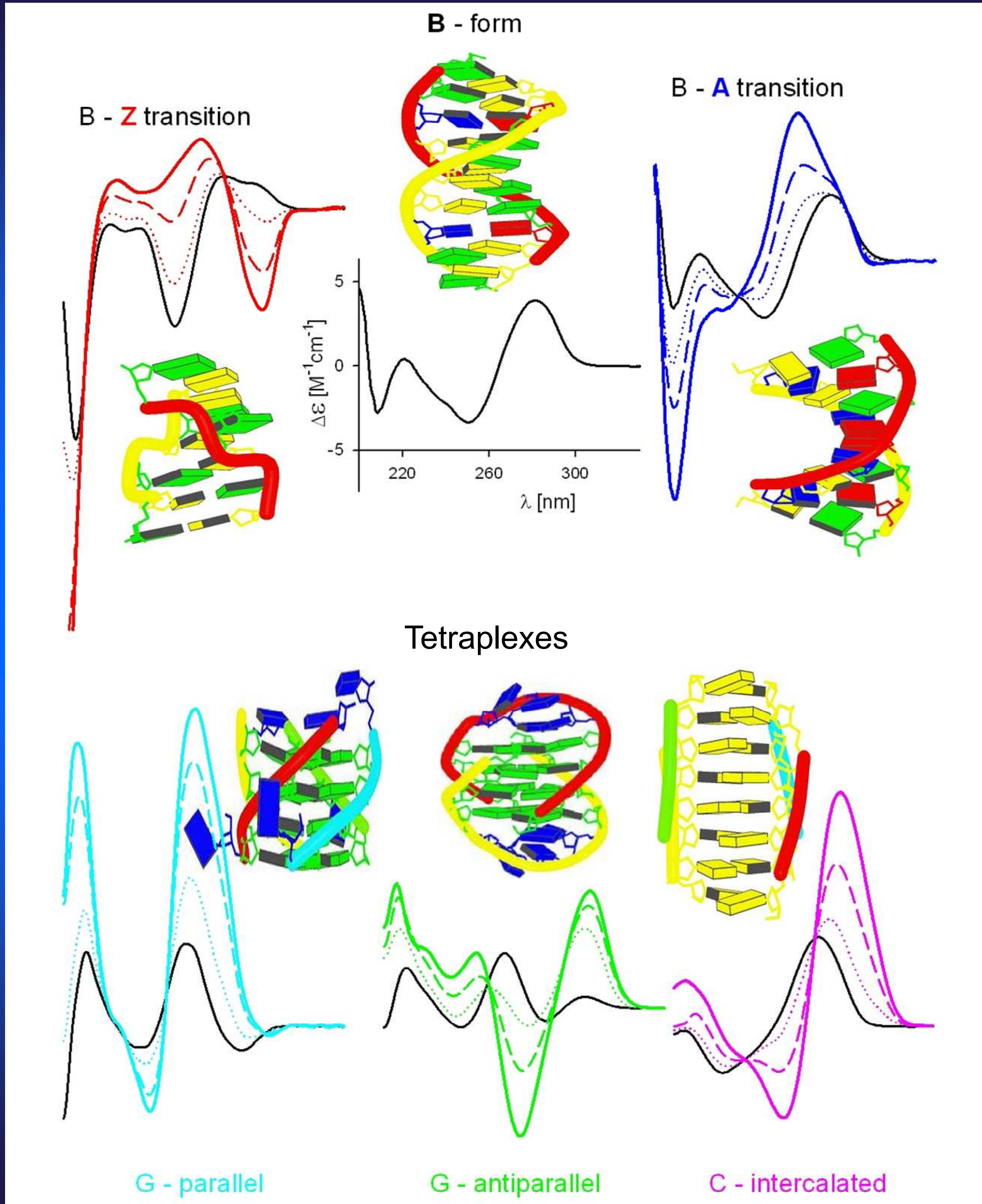
* SUGAR



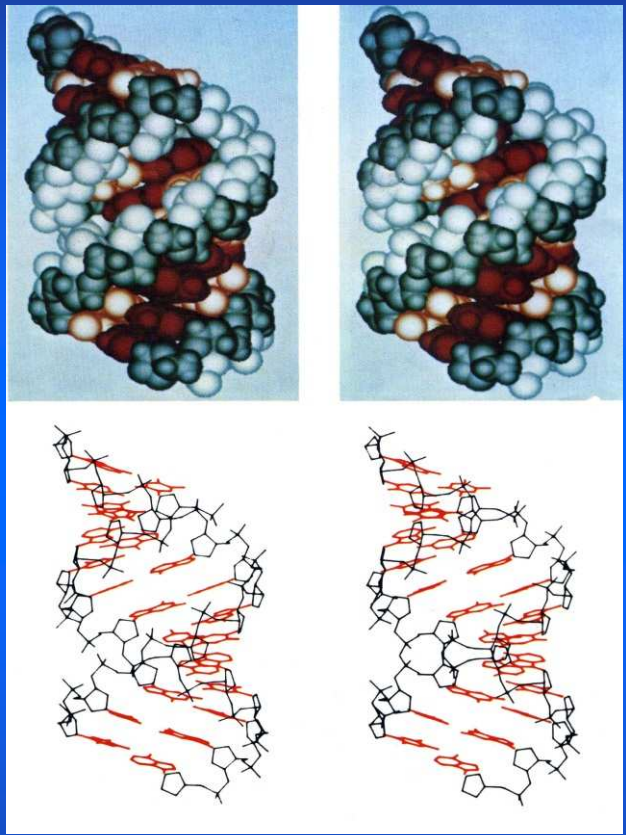
E1ub
E1ua
B1u
B2u
2
1
0
−1
−2
Θ x 10⁻⁴
U
U
m⁶U
U
220
260
300
nm

Conditions of the origin of CD

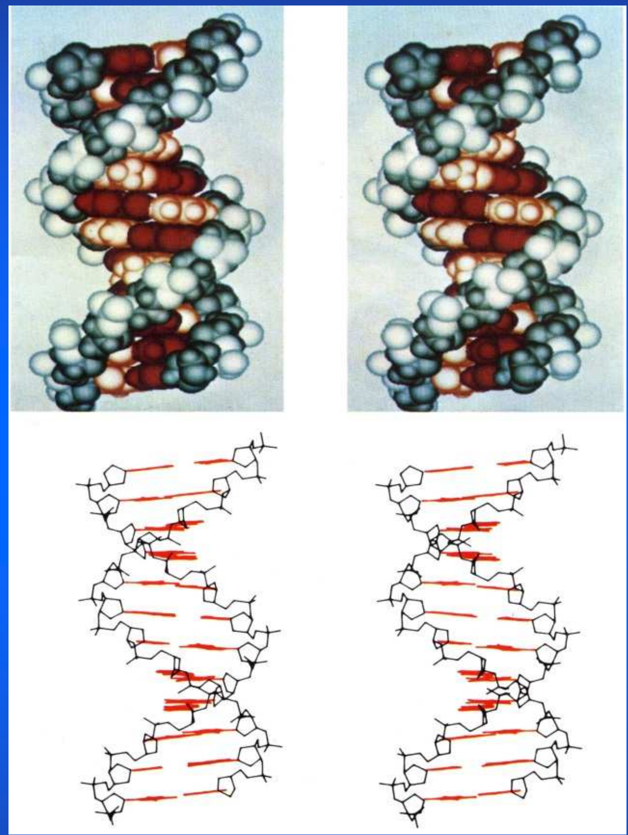




Kypr, J., Kejnovska, I., Renciuk, D., Vorlickova, M.:
Circular dichroism and conformational polymorphism of DNA. Nucleic Acids Res. **37** (2009) 1713-1725.



A



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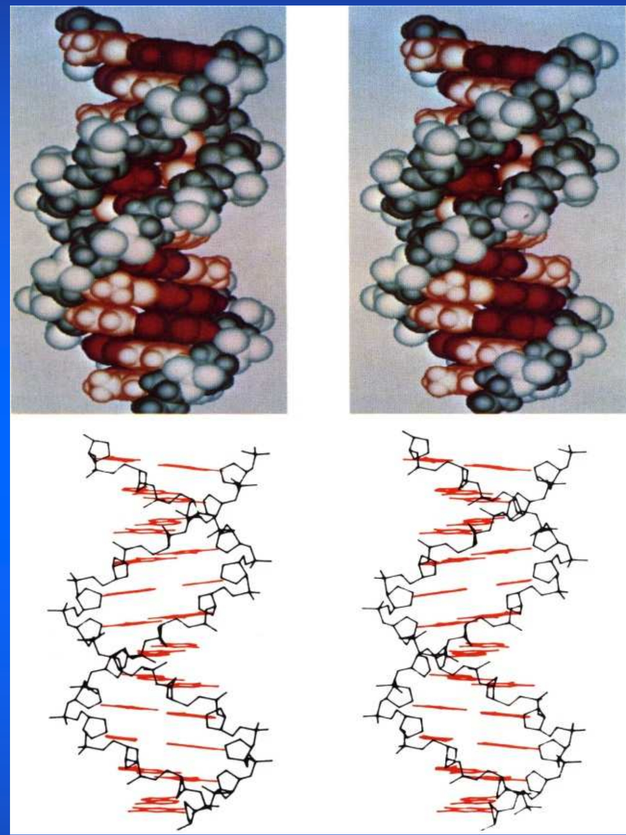
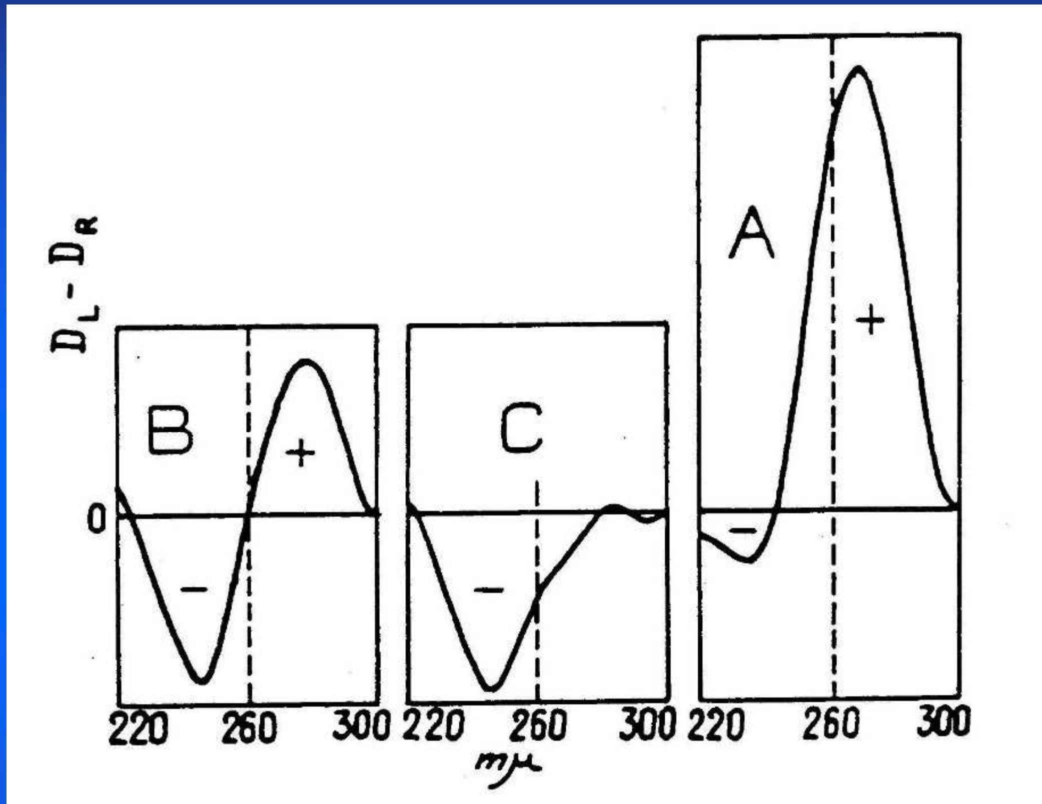


C,D,T…

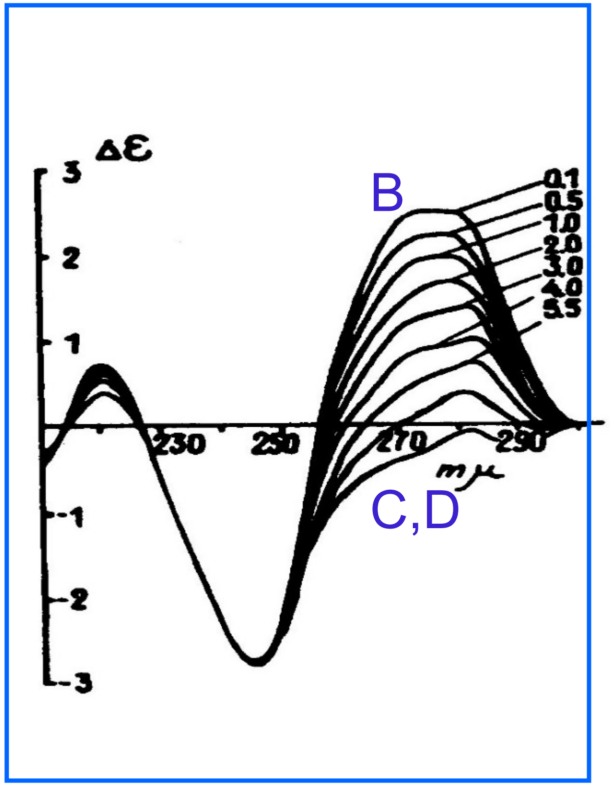
Long DNA molecules can be oriented by mechanical stroking. X ray diffraction pattern obtained on these semicrystaline matter enables to determine some periodicities of the DNA arrangement

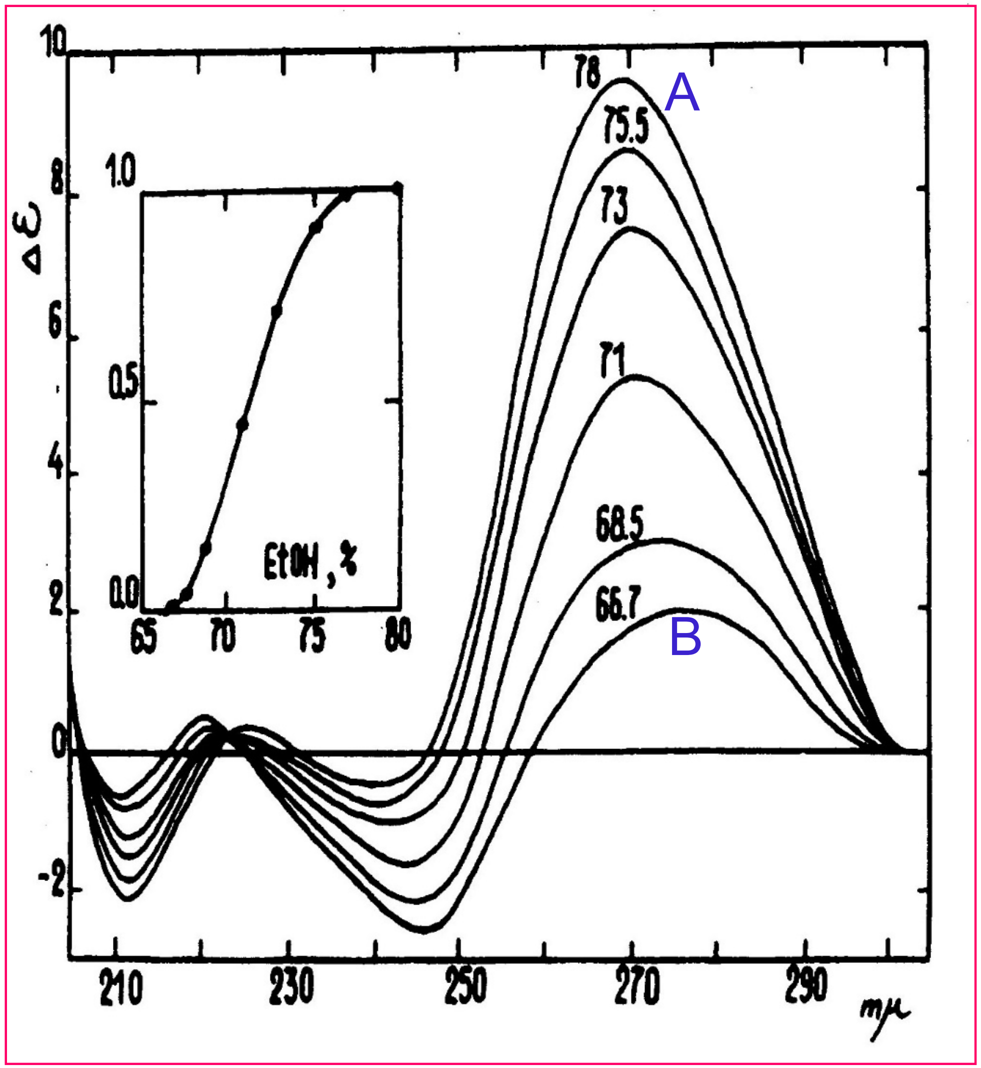
M. Wilkins, R. Franklin, W+C



Tunis-Schneider, M.J.B., Maestre, M.F.:
Circular dichroism spectra of oriented and unoriented deoxyribonucleic acid films - a preliminary study. J. Mol. Biol. 52 (1970) 521-541.

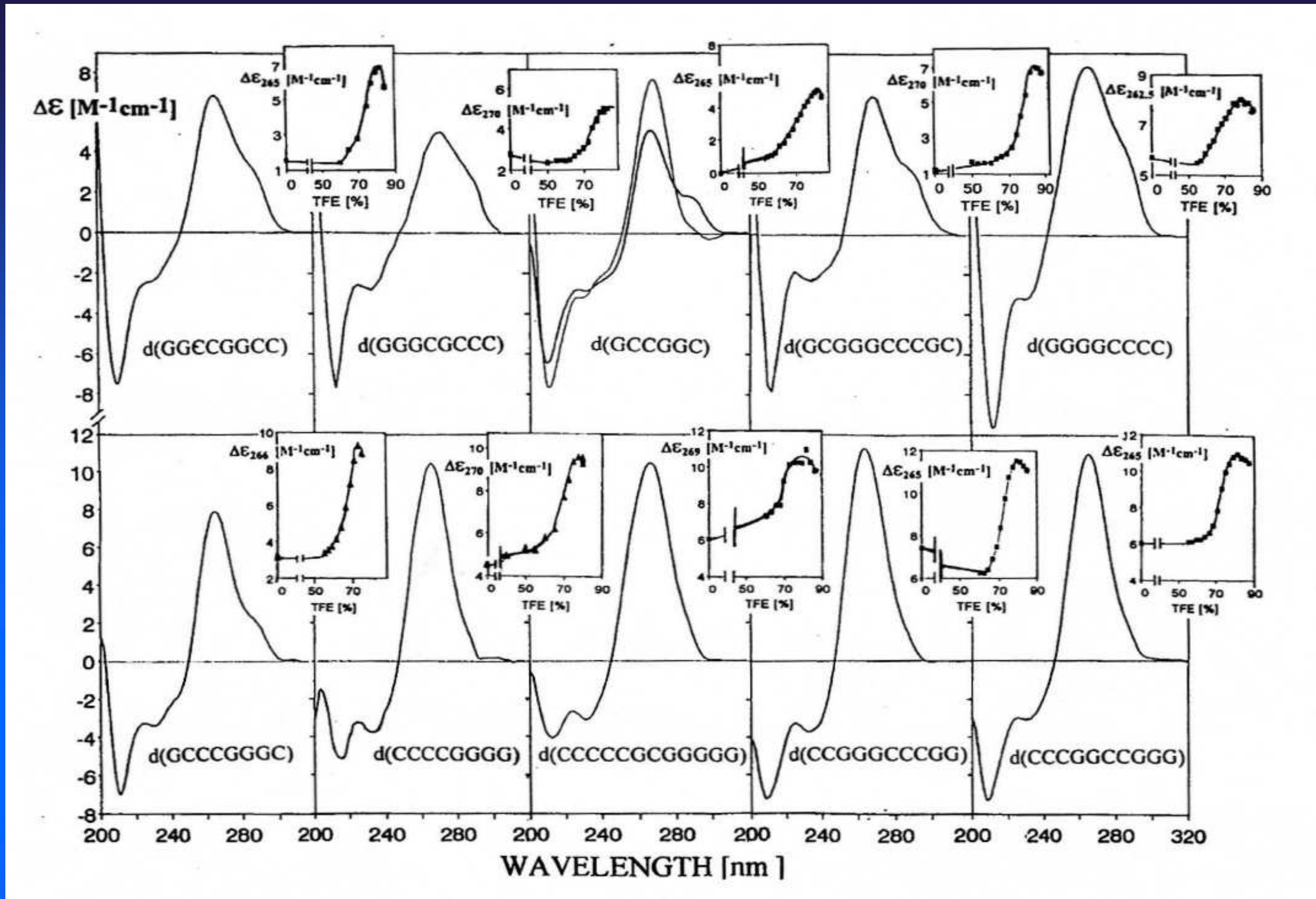
Non-cooperative changes
within the same global structure



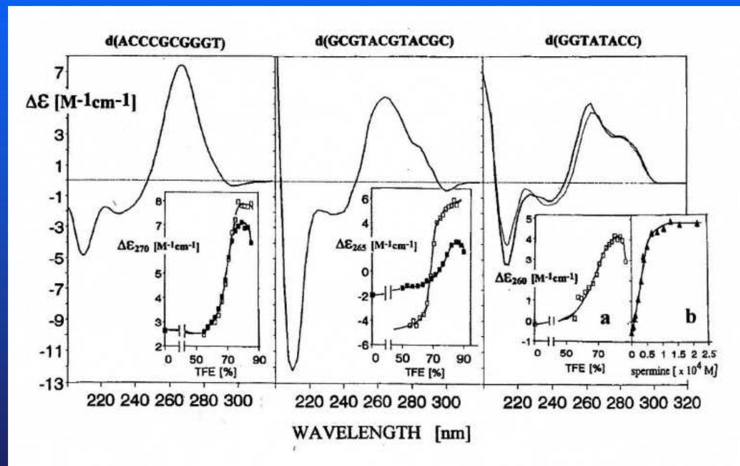


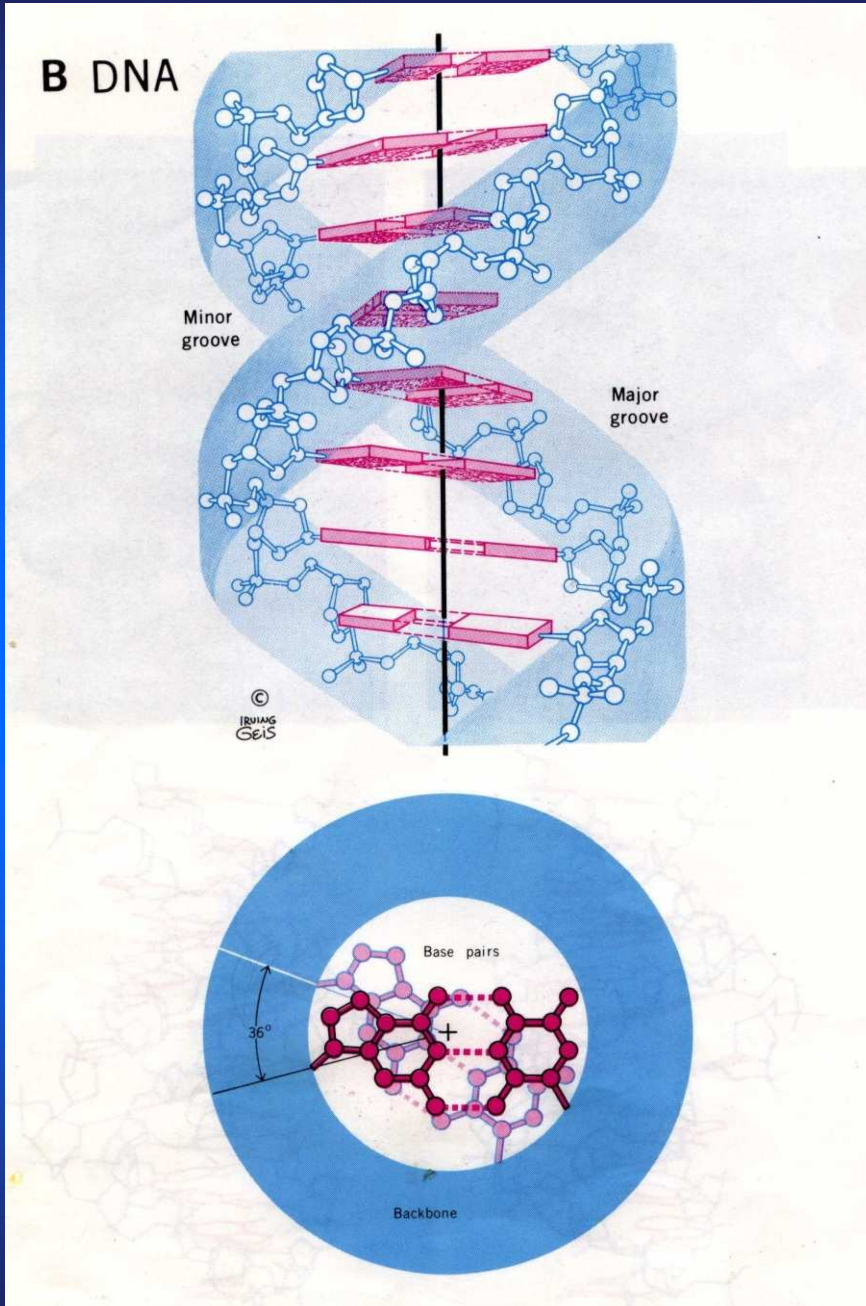
Cooperative changes between discrete structures

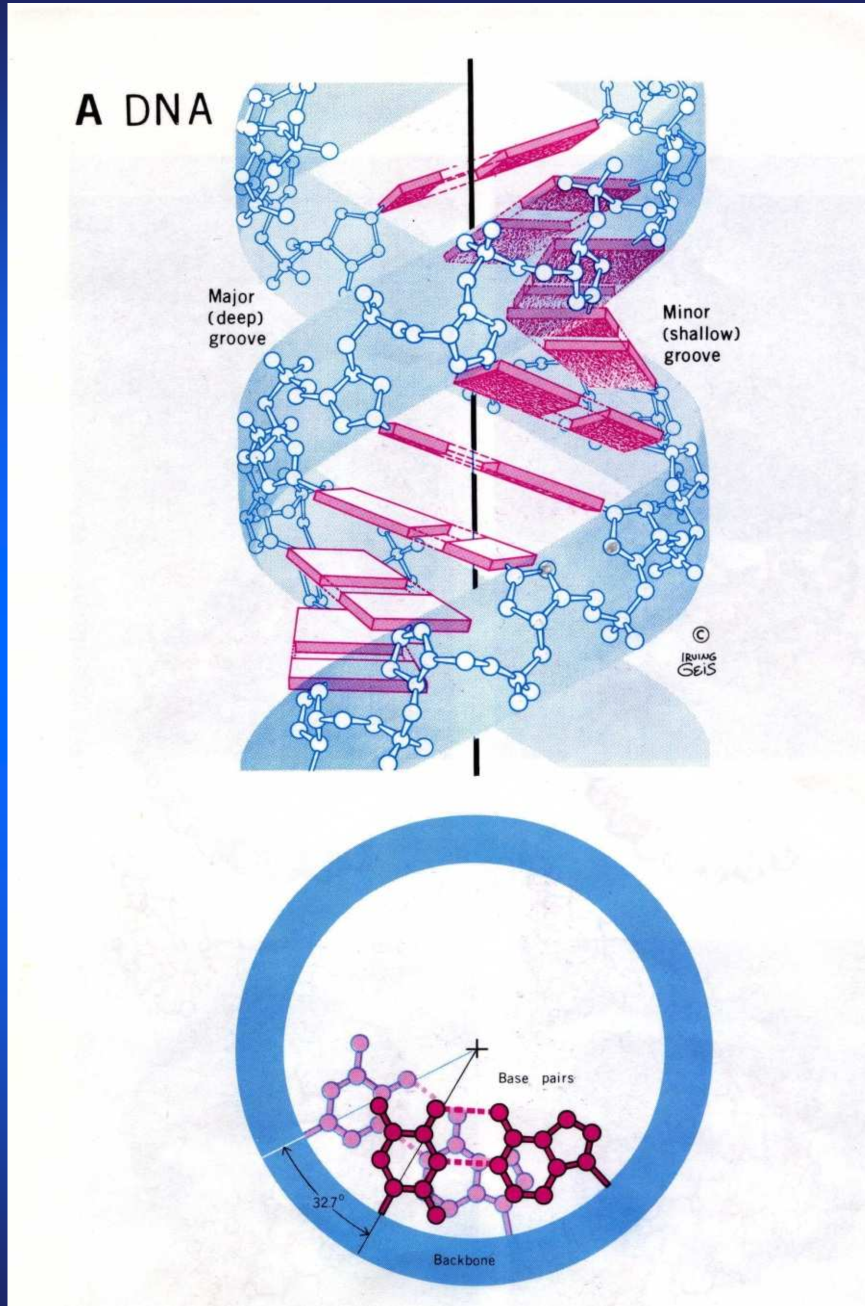
Ivanov, V. I., Minchenkova, L. E., Minyat, E. E, Frank-Kamenetskii, M. D., Schyolkina, A. K.:. The B to A transition of DNA in solution. J. Mol. Biol. 87 (1974) 817-833.



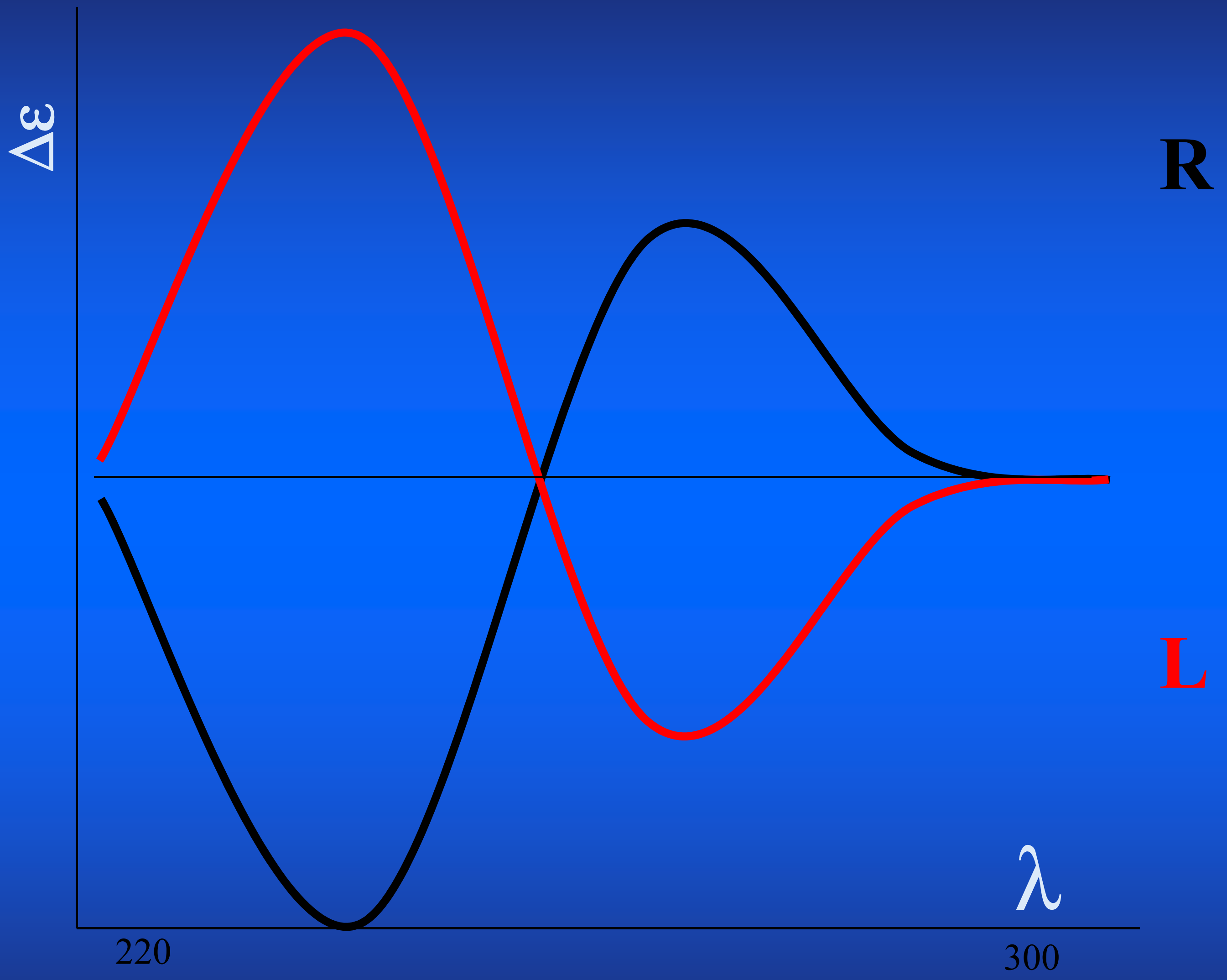
Kypr, J., Chladkova, J., Zimulova, M. Vorlickova, M.: Aqueous trifluoroethanol solutions simulate the environment of DNA in the crystaline state. Nucleic Acids Res. 27 (1999) 3466-3473.



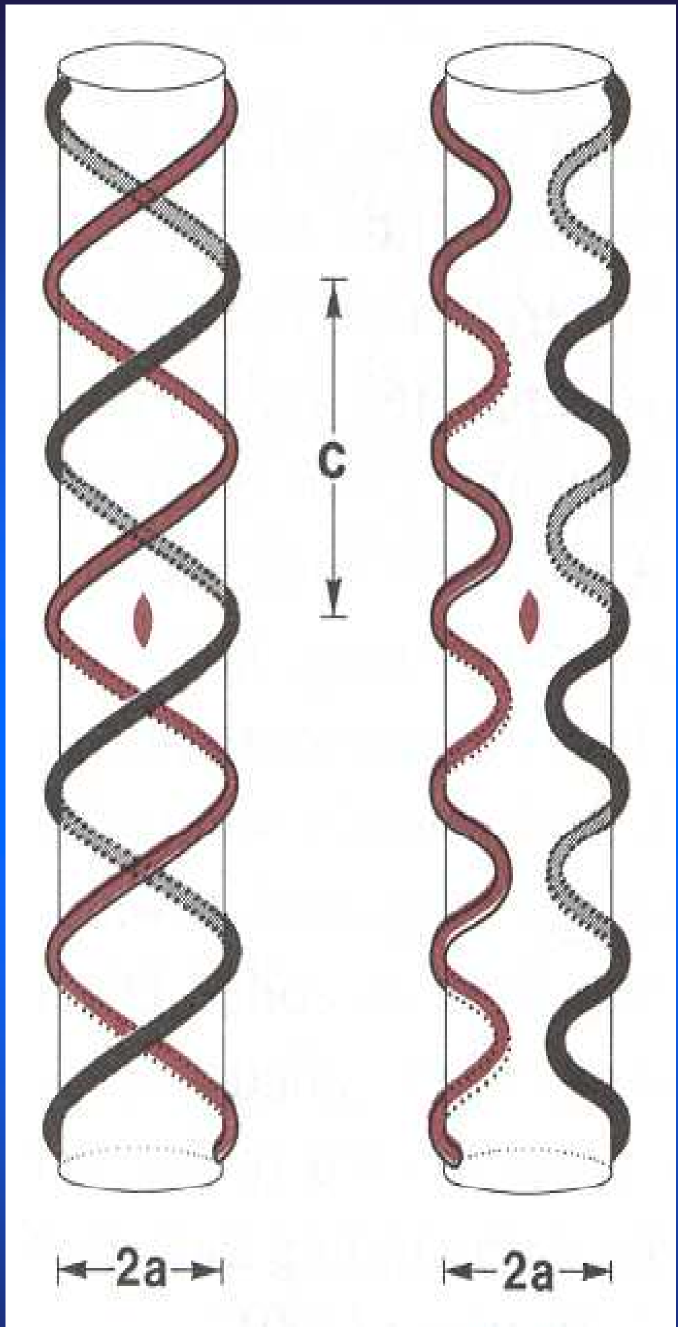

B DNA
Minor
groove
Major
groove
©
IRVING
GEIS
Base pairs
36°
Backbone

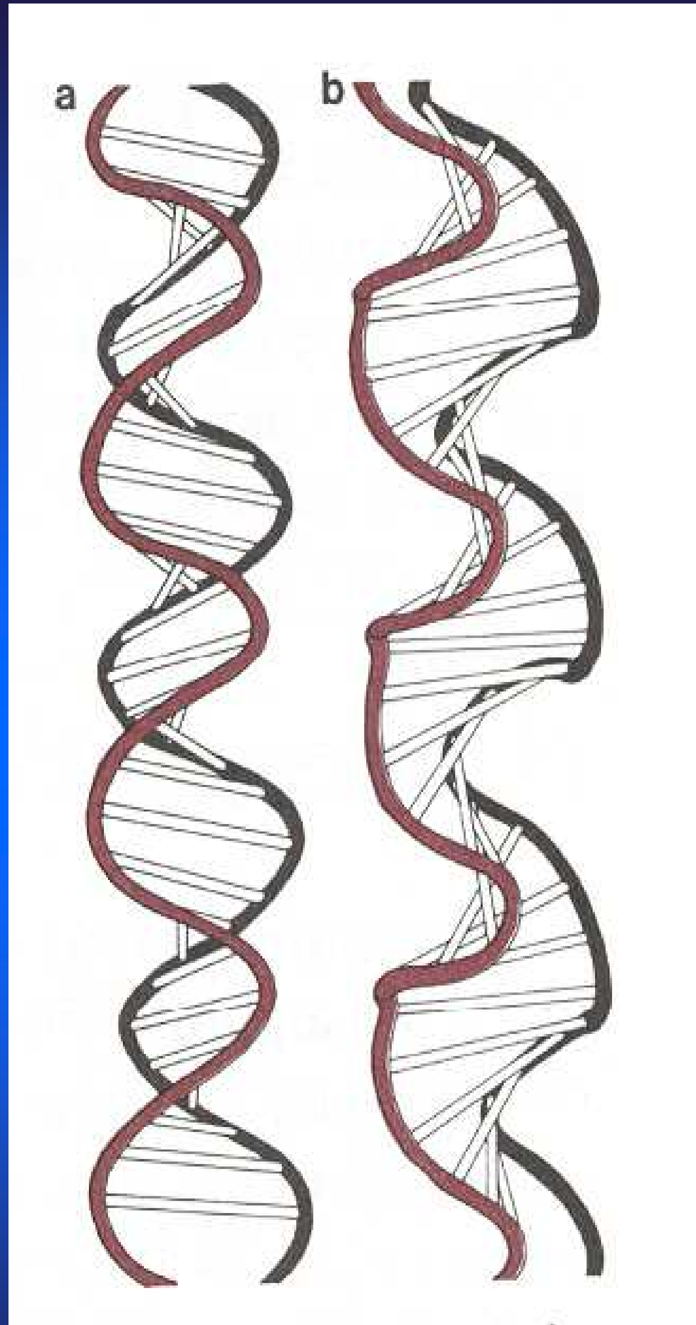

A DNA
Major
(deep)
groove
Minor
(shallow)
groove
©
IRVING
GEIS
Base pairs
32.7°
Backbone

GCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC

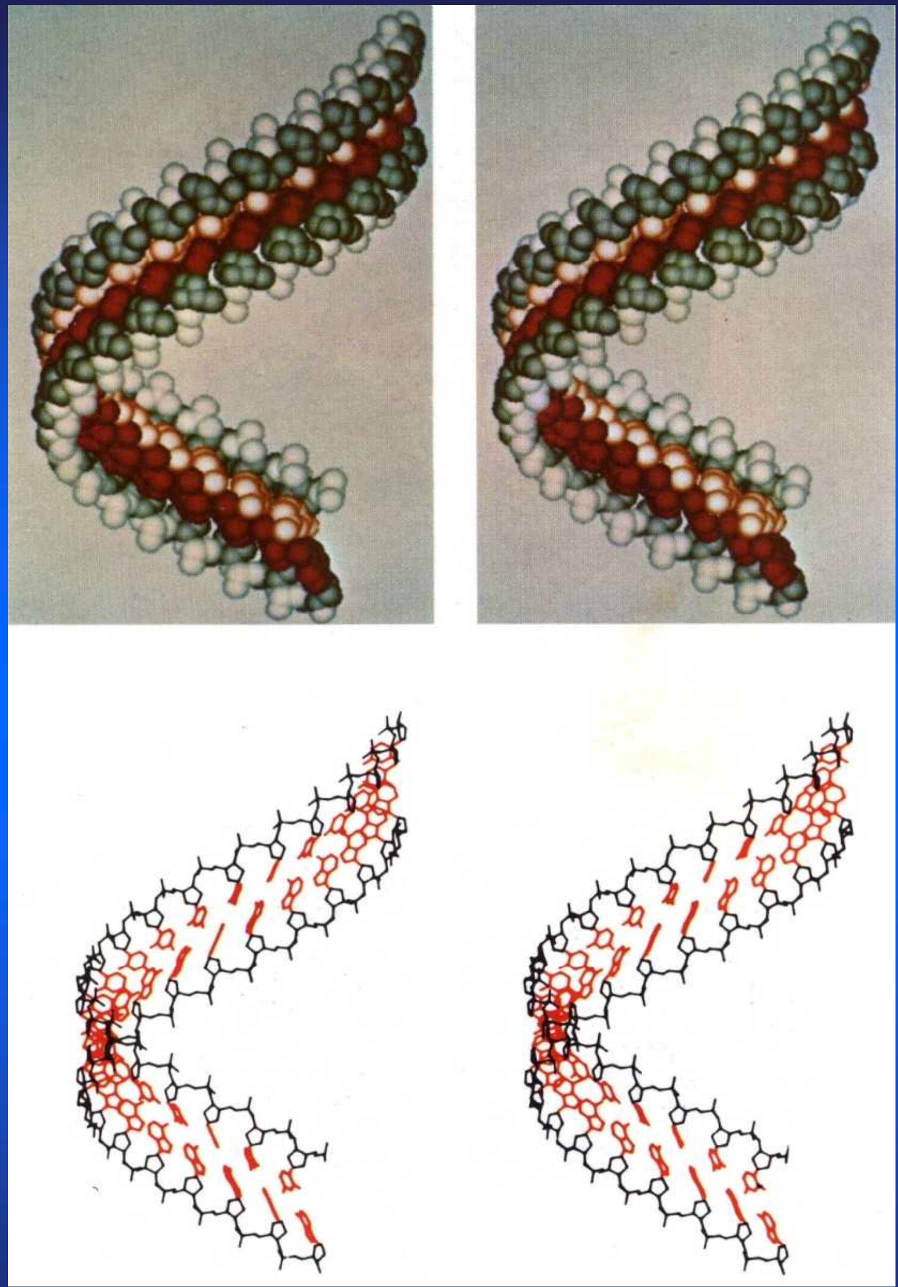


Pohl, F.M., Jovin T.M.: Salt-induced co-operative conformational change of a synthetic DNA: Equilibrium and kinetic studies with poly(dG-dC) J.Mol. Biol. 67 (1972) 375-396

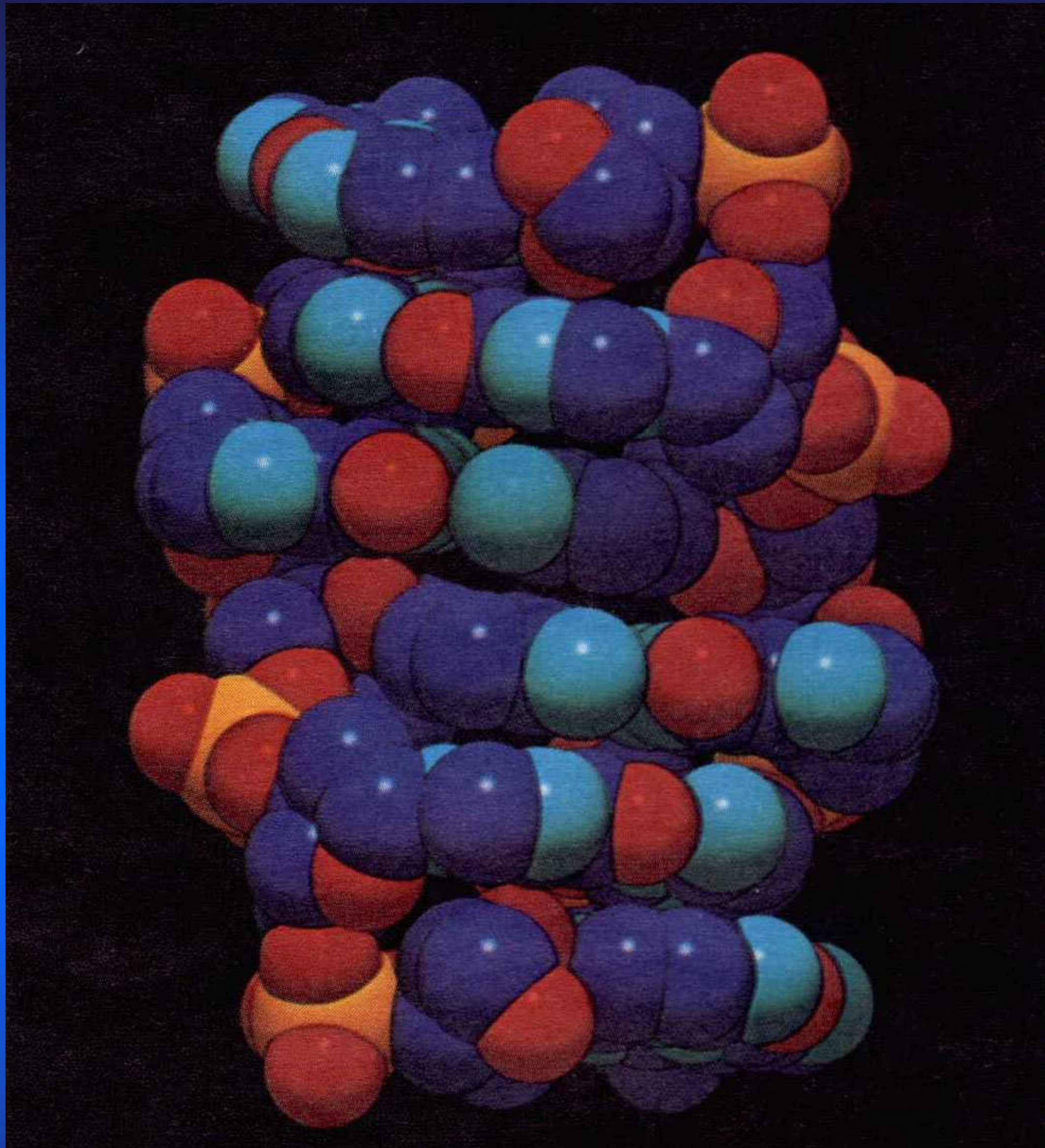




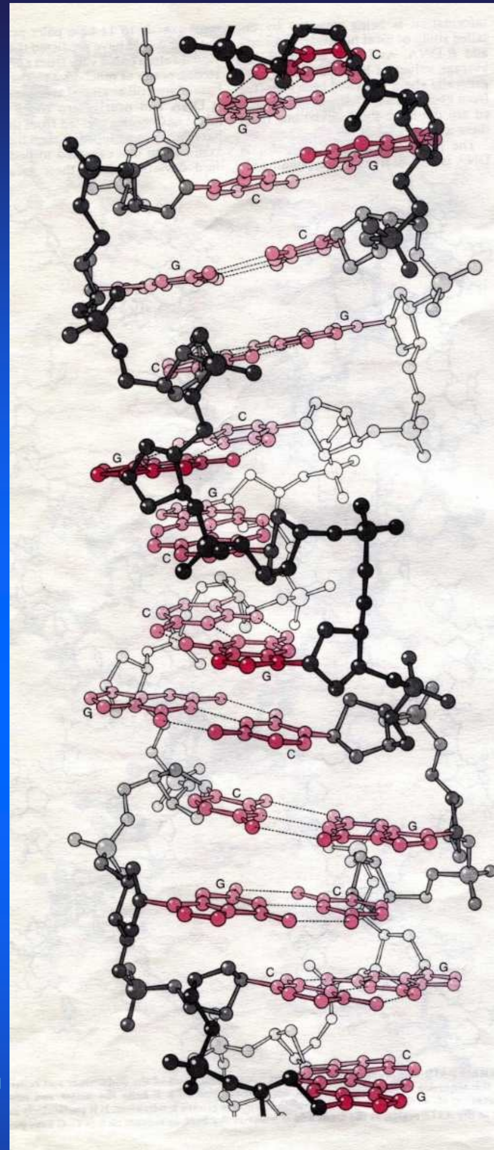
Rodley *et al.*, 1976; Sasisekharan and Pattabiraman, 1976, 1978; Bates *et al.*, 1977, 1980a; Albiser and Premilat, 1980, 1982; Millane and Rodley, 1981;



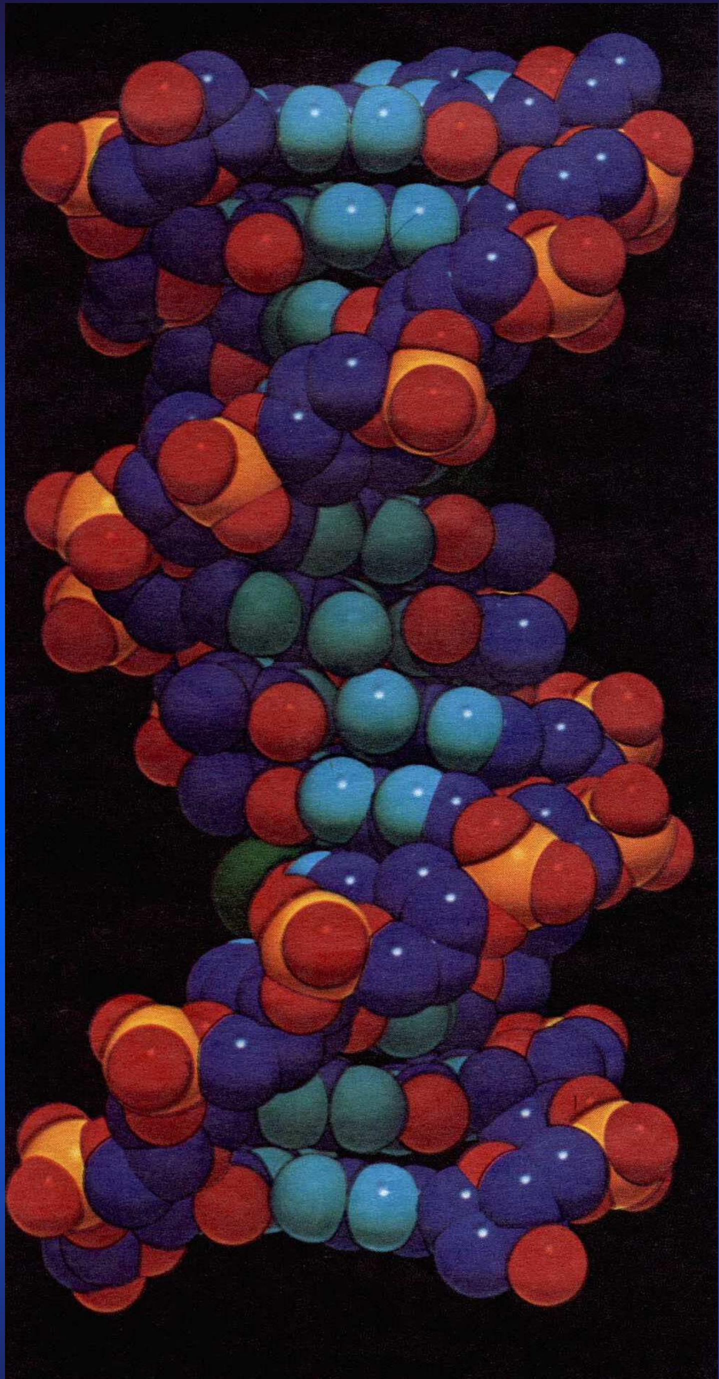
Vilma Olson:
Spatial configuration of ordered polynucleotide chains: A novel double helix.
Proc. Natl. Acad. Sci. USA 74 (1977) 1775-1779.



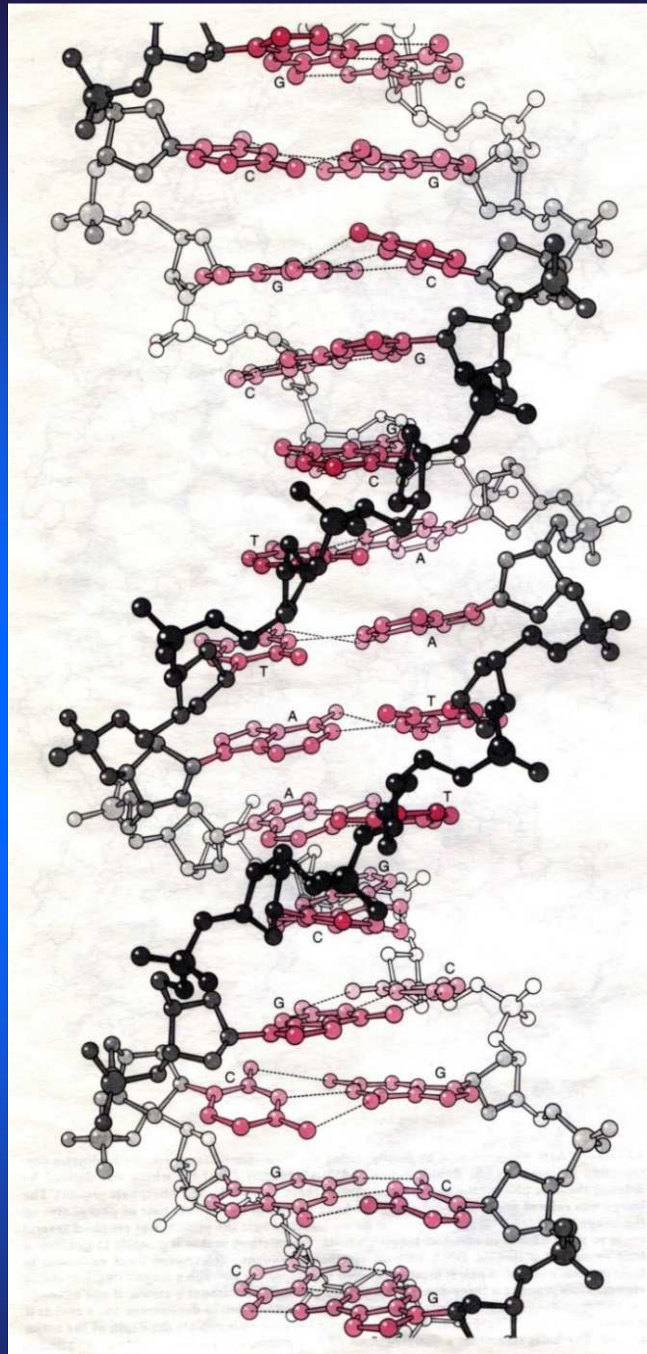
Z



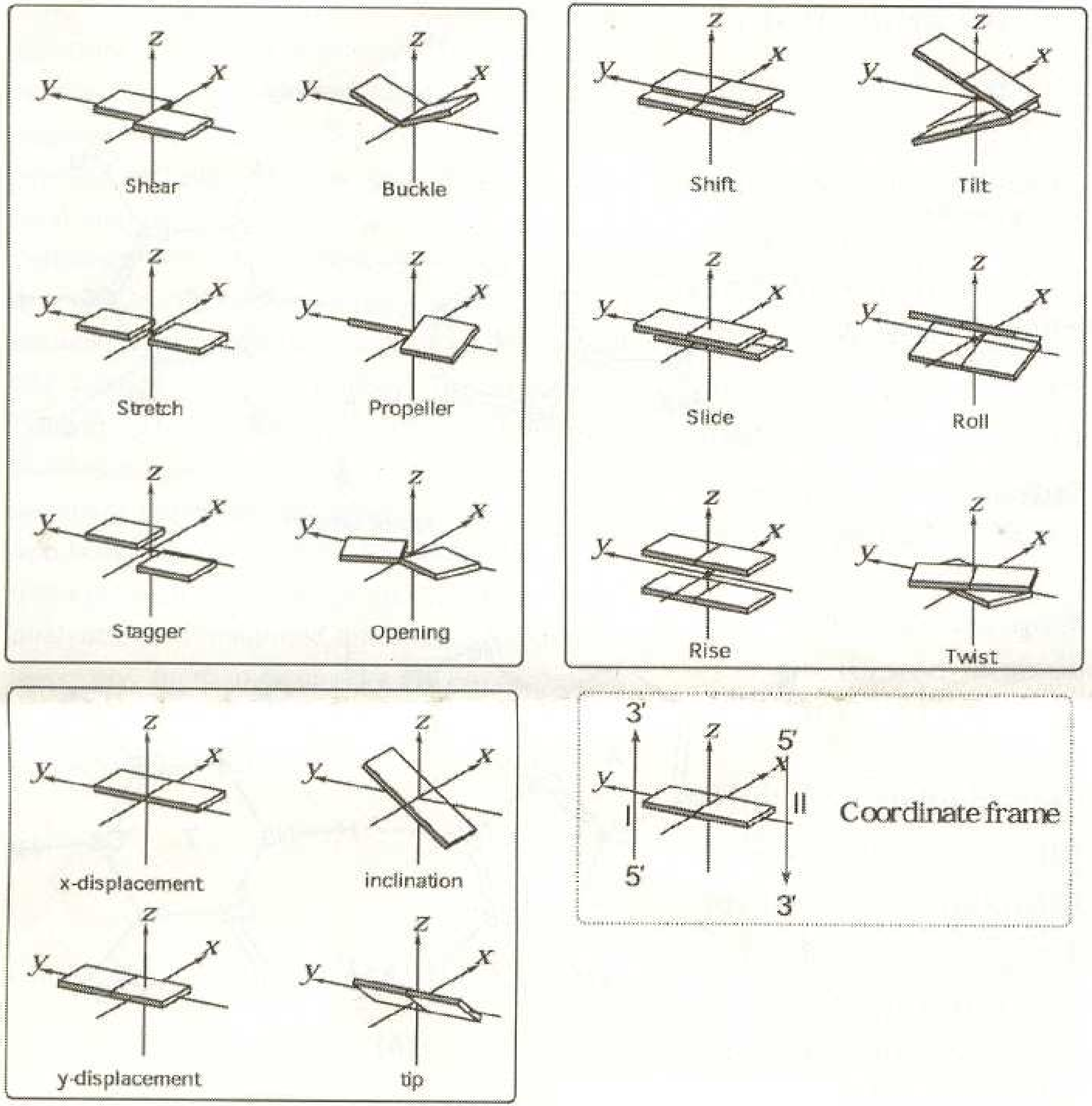
Wang, A. H.; Quigley, G. J.; Kolpak, F. J.; Crawford, J. L.; van Boom, J. H.; van der Marel, G.; Rich, A. Molecular structure of a left-handed double helical DNA fragment at atomic resolution. Nature. **282** (1979) 680–686.

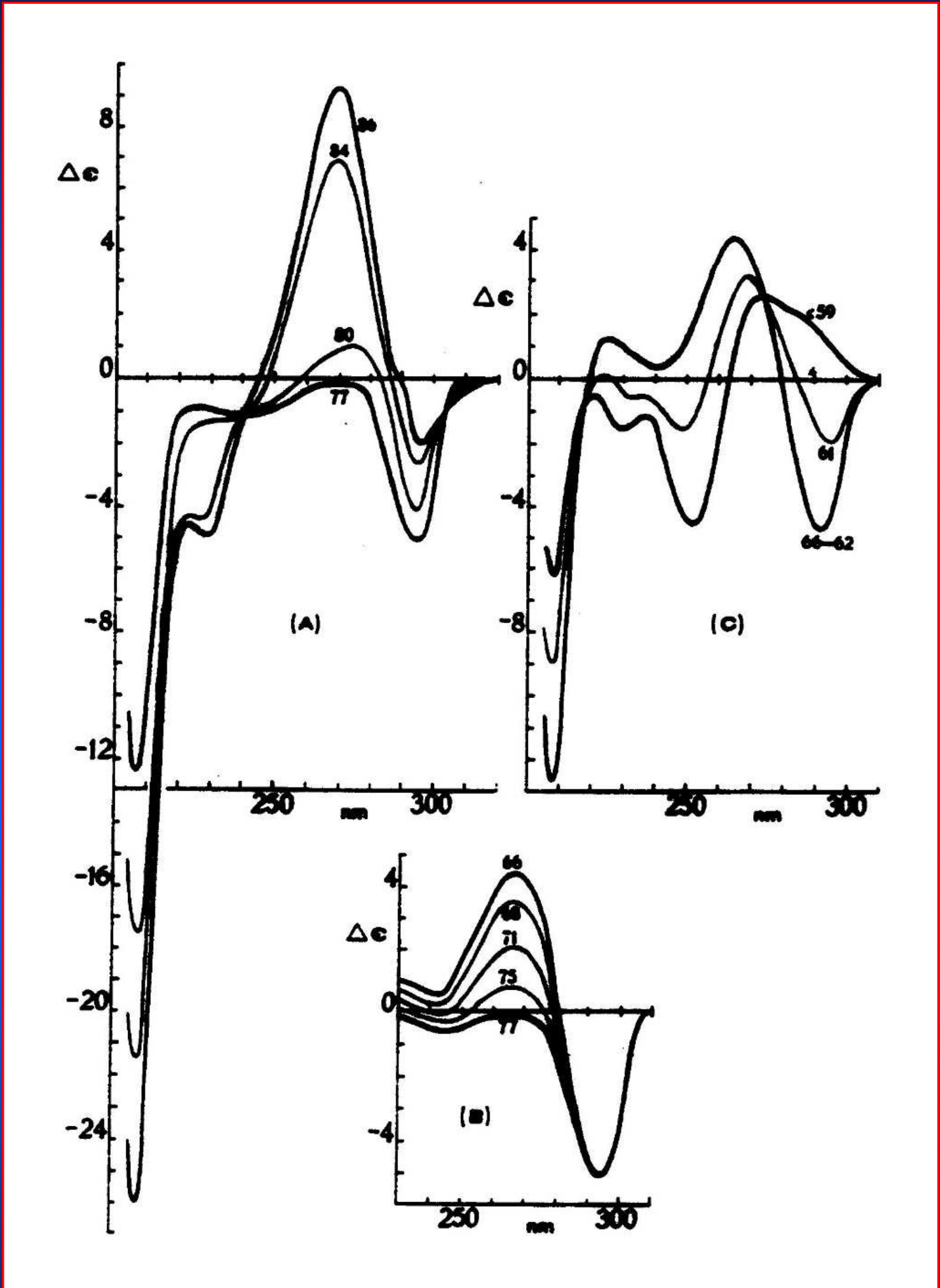


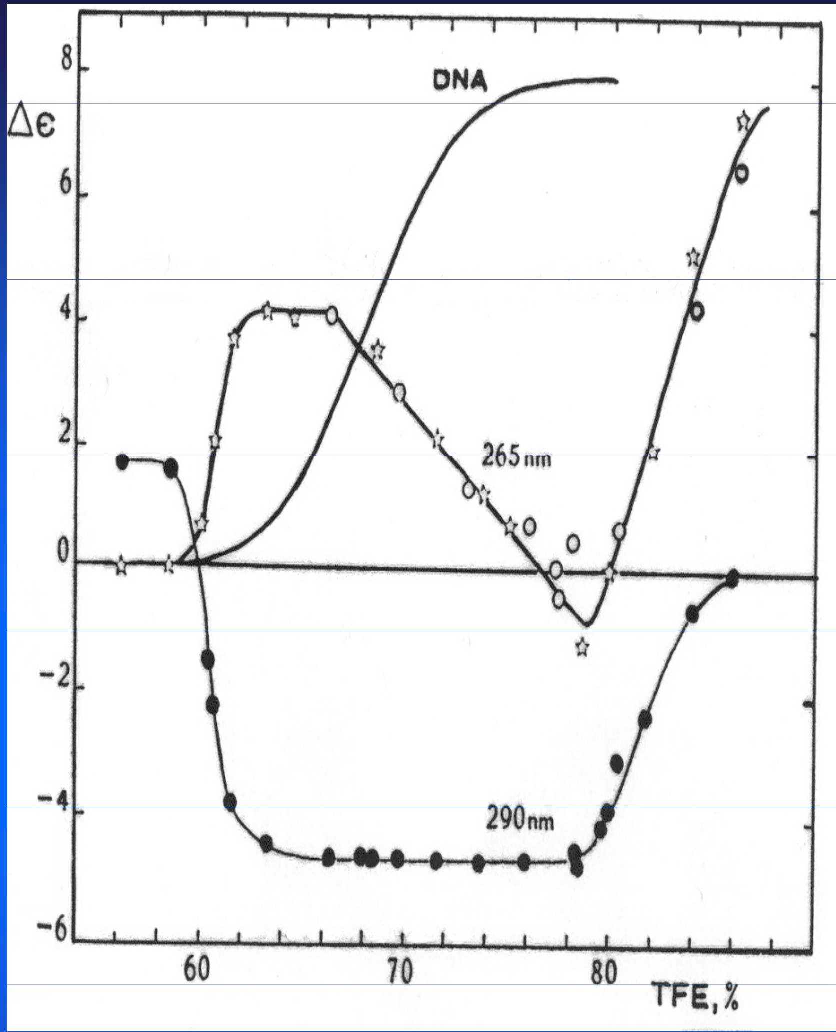
B



Wing, R., Drew, H., Takano, T., Broka, Ch., Tanaka, S., Itakura, K., Dickerson, R.E.:
Crystal structure analysis of a complete turn of B-DNA Nature 287 (1980) 755–758.

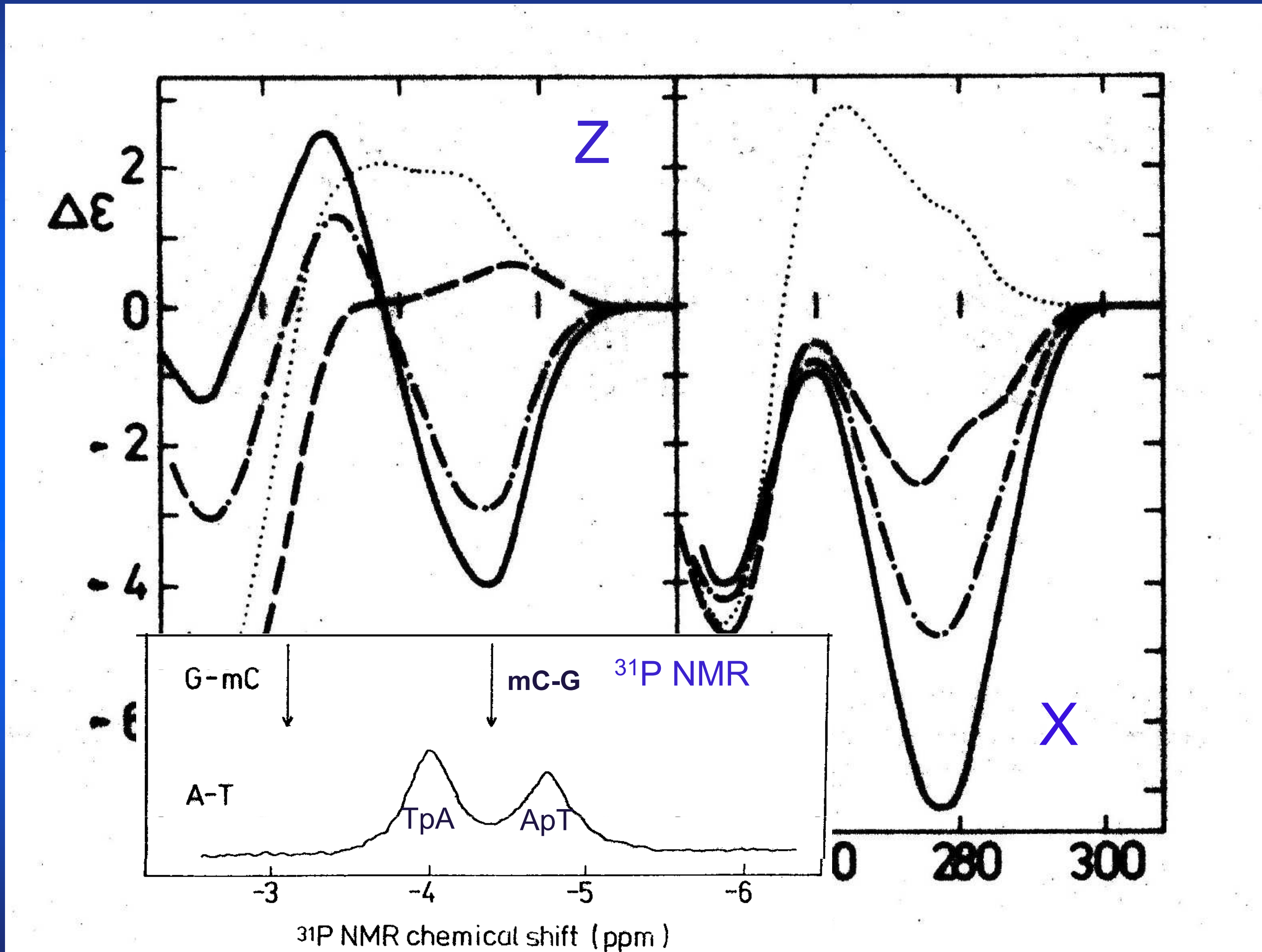

Shear
Buckle
Stretch
Propeller
Stagger
Opening
Shift
Tilt
Slide
Roll
Rise
Twist
x-displacement
inclination
y-displacement
tip
Coordinate frame





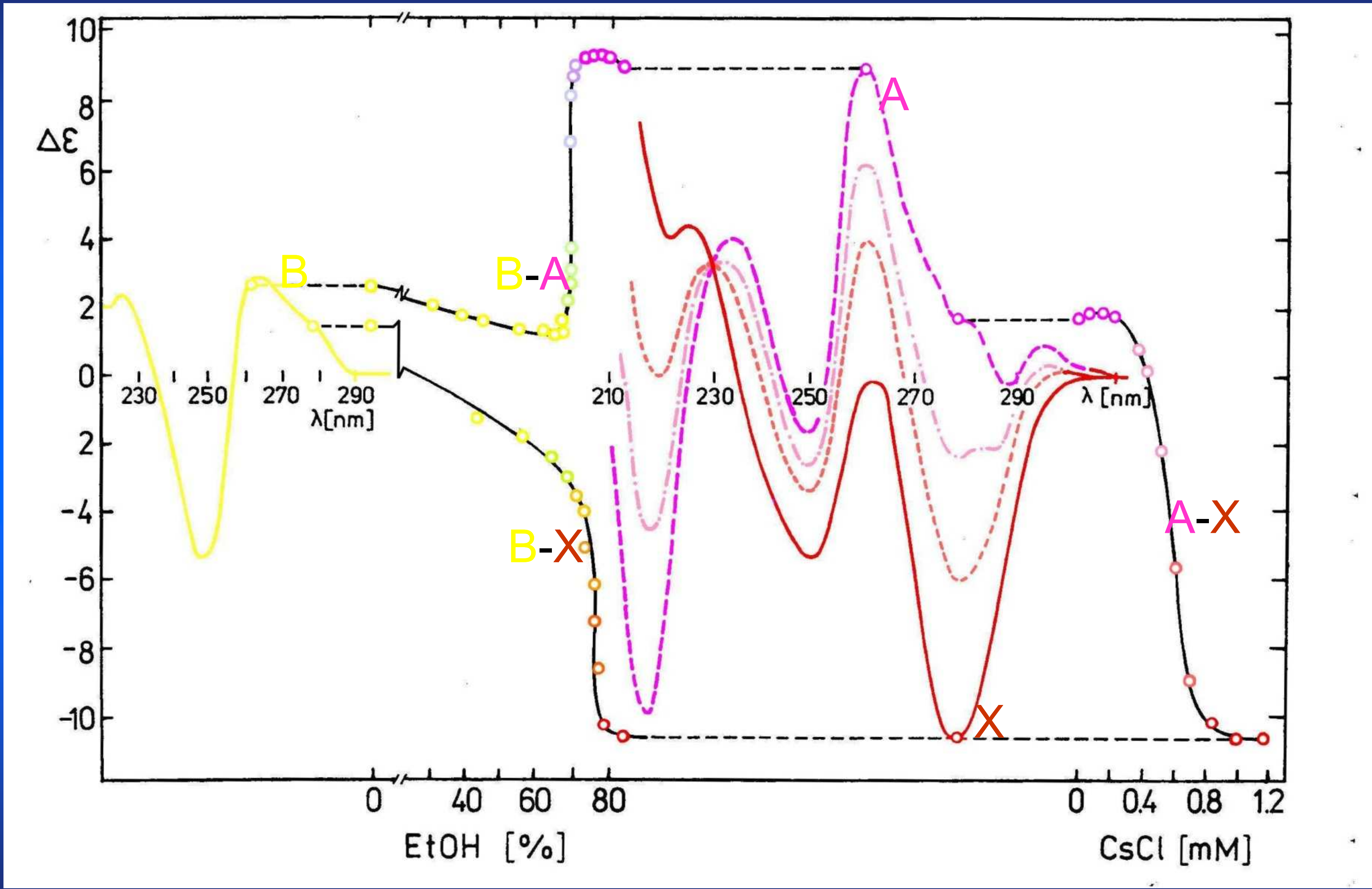
Valery I. Ivanov, Elvira E. Minyat
The transitions between left- and right-handed forms of poly(dG-dC)
Nucleic Acids Res. 9 (1981) 4783-4798

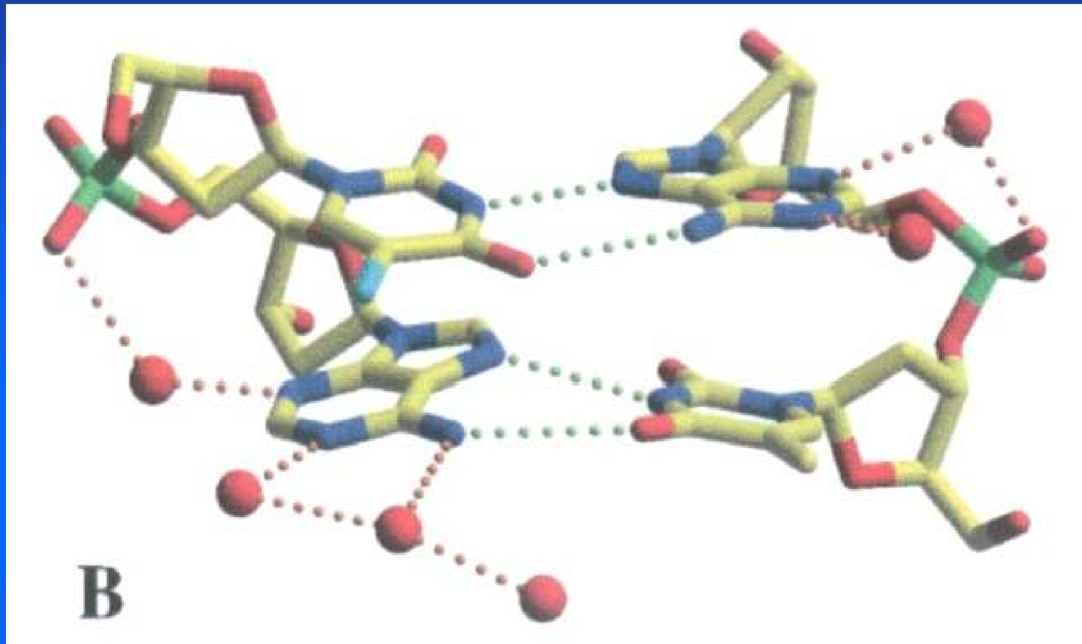
ATATATATATATATATATATATATATATATATATATATA

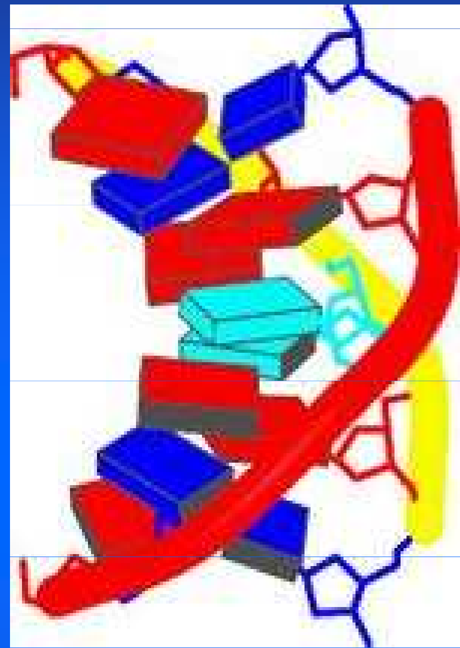


Vorlíčková, M., Sklenář, V., Kypr, J.: Salt-induced Conformational Transition of Poly[d(A-T)]
J. Mol. Biol. 166 (1983) 85-92

ATATATATATATATATATATATATATATATATATATATA

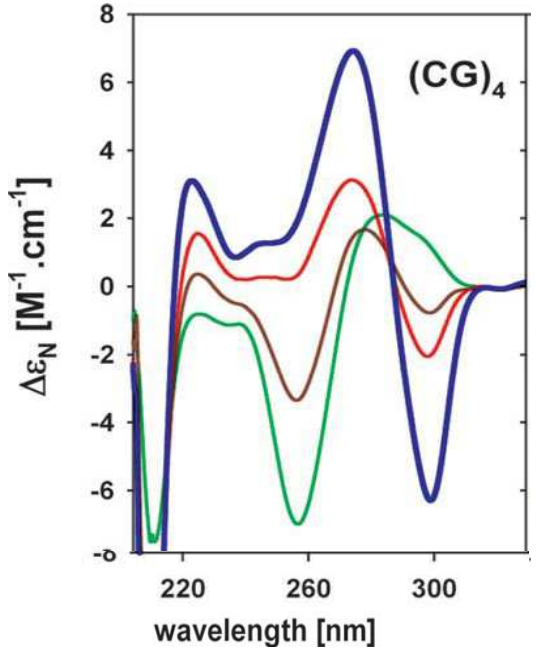


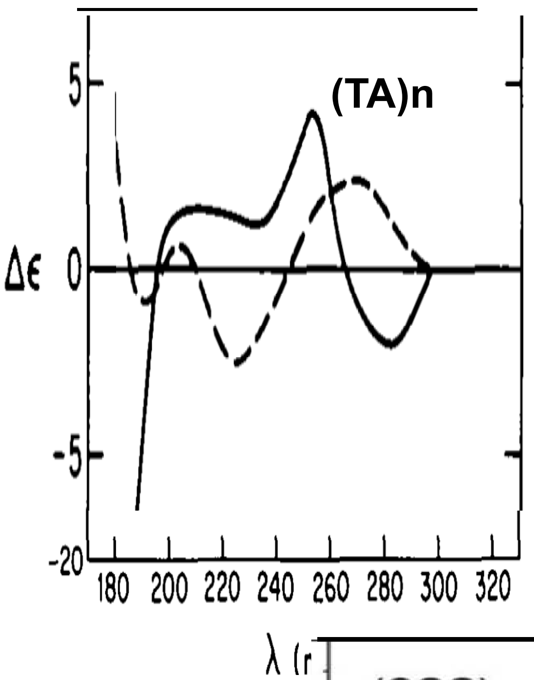


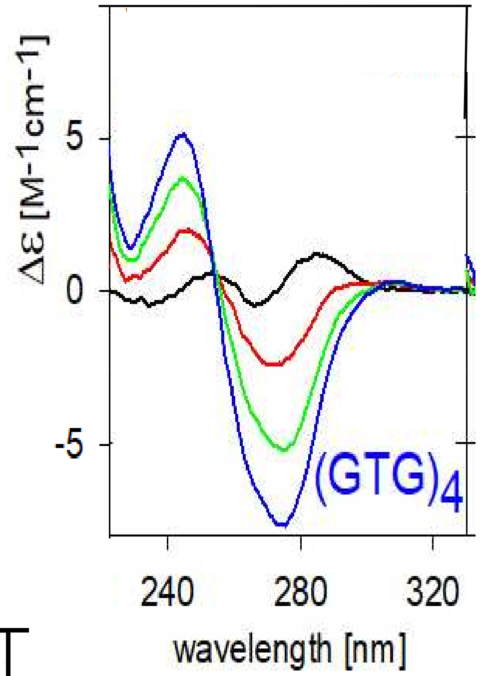


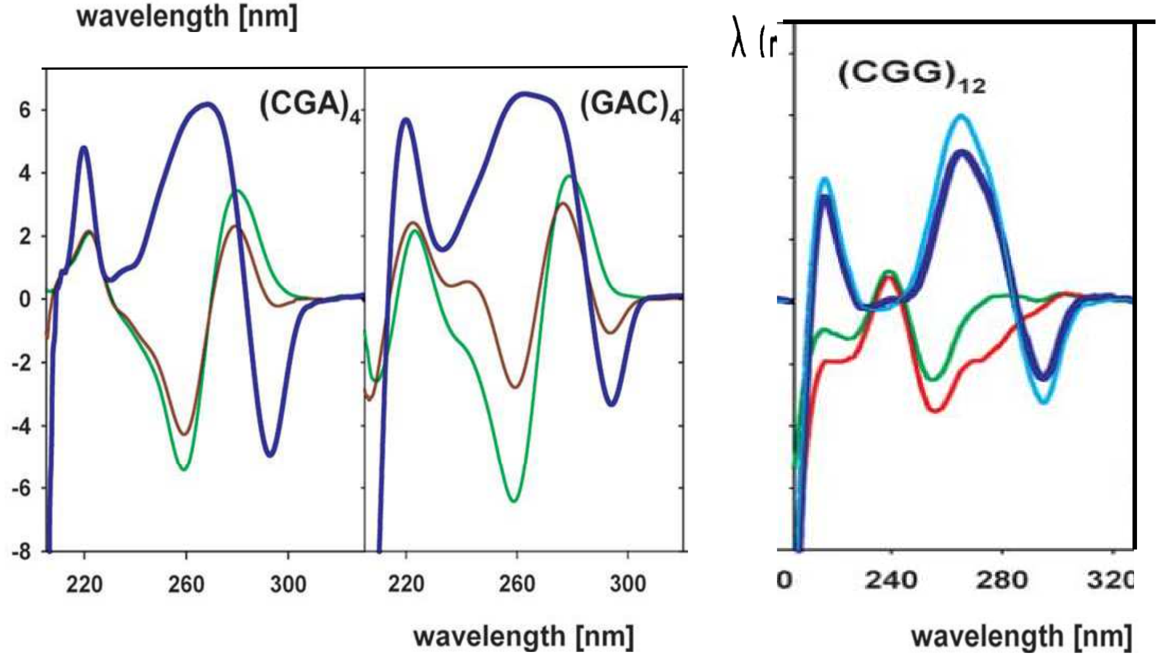
Antiparallel duplex of alternating A-T fragment with Hoogsteen base pairing.

Abrescia, N.G.A., Thompson, A., Huynh-Dinh, T., Subirana, J.A.: Alternating A-T fragment with Hoogsteen base pairing. Proc.Nat.Acad.Sci.USA , **99 (2002)** 2806 – 2811.


(CG)4
Δε_N [M^-1.cm^-1]
8
6
4
2
0
-2
-4
-6
220
260
300
wavelength [nm]


(TA)n
Δε
5
0
-5
-20
180
200
220
240
260
280
300
320
λ (r


Δε [M-1cm-1]
5
0
-5
(GTG)4
240
280
320
wavelength [nm]


(CGA)4
(GAC)4
(CGG)12
6
4
2
0
-2
-4
-6
-8
220
260
300
220
260
300
wavelength [nm]
0
240
280
320
wavelength [nm]

Z forms

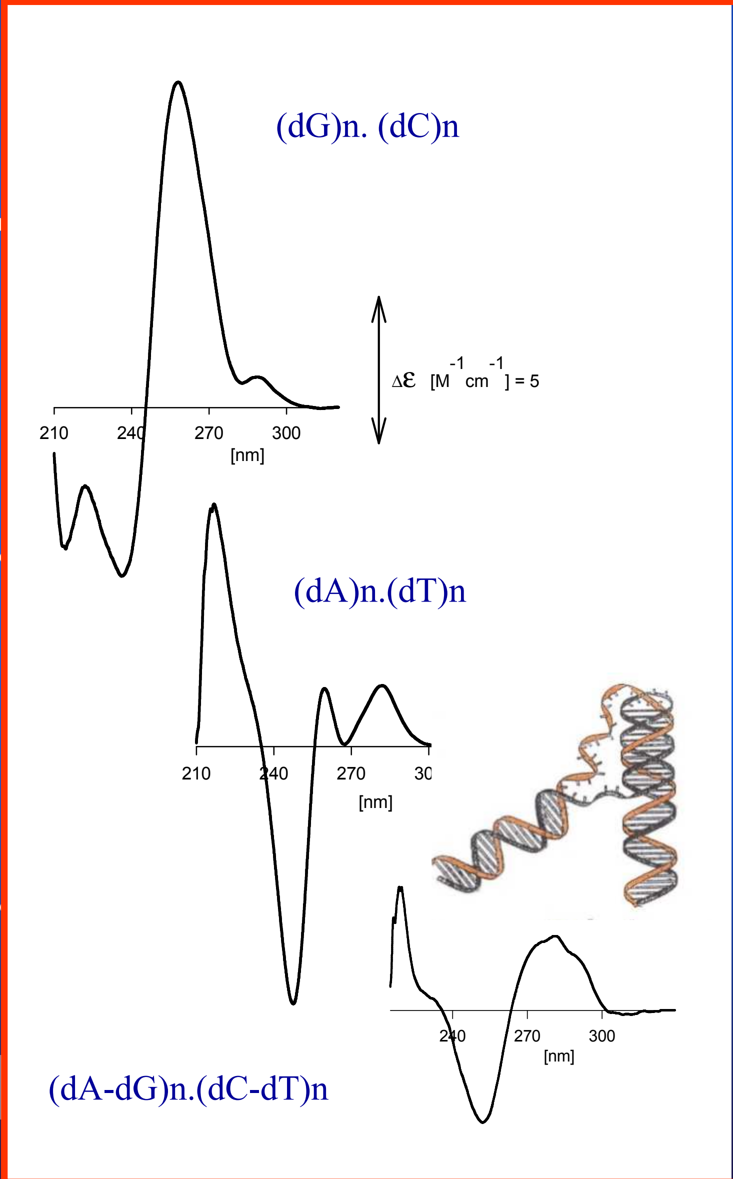
Alternating $(Pu\text{-}Py)_n$

$(Pu)_n \cdot (Py)_n$ complexes

… GCGCGC …
… CGCGCG …

… ATATAT …
… TATATA …

… ACACAC …
… TGTGTG …



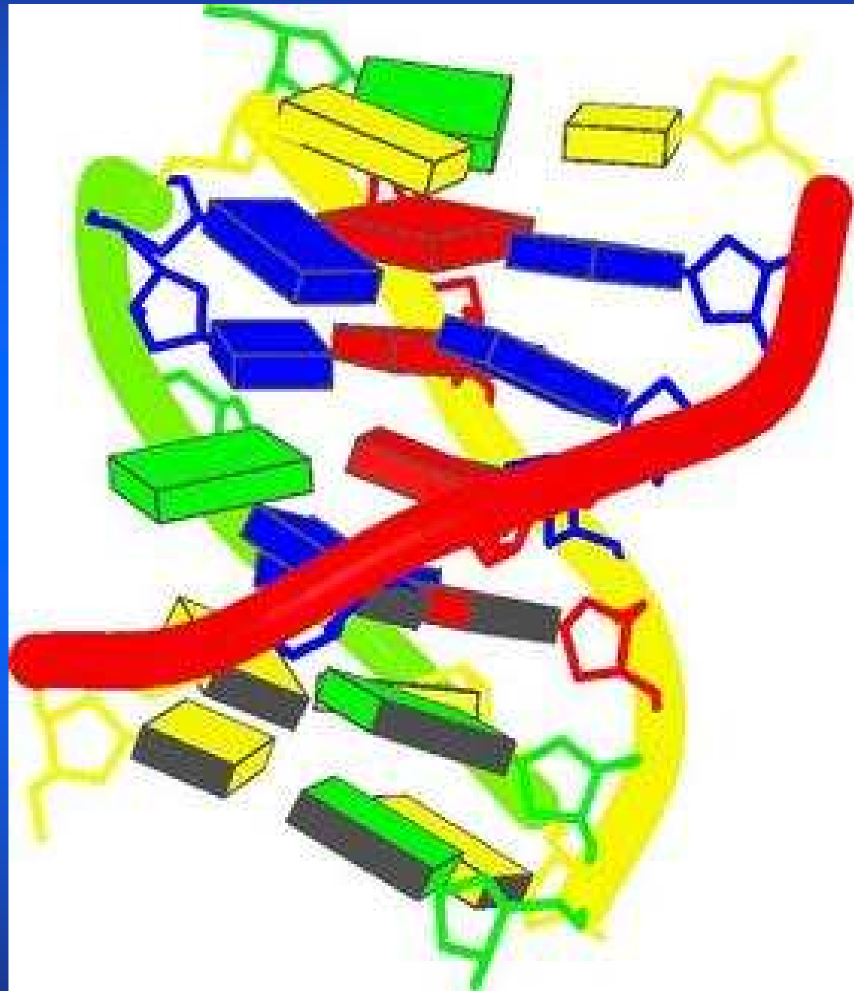
GGGGGG …
CCCCCC …

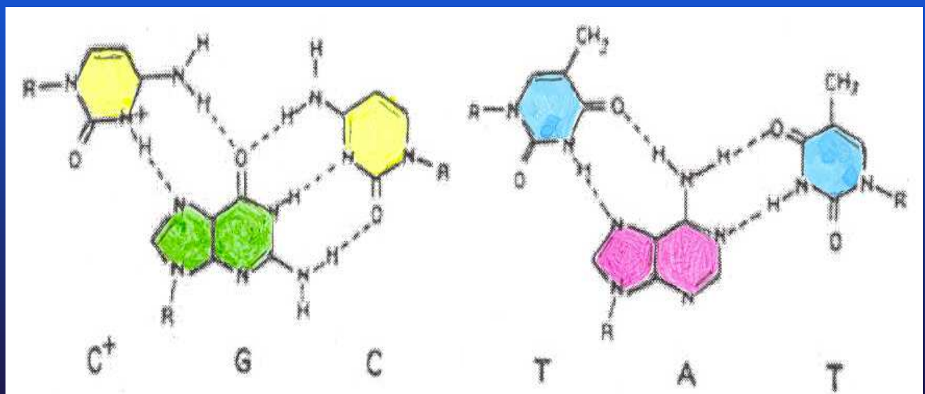
AAAAAA …
TTTTTT …

AGAGAG …
TCTCTC …

DNA Triplex

Pyrimidine. Purine. Pyrimidine

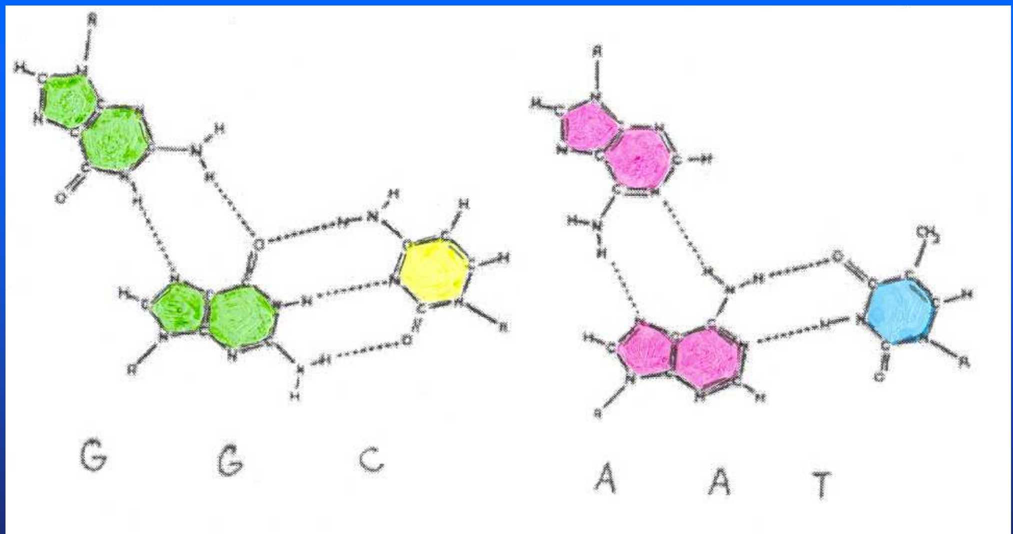


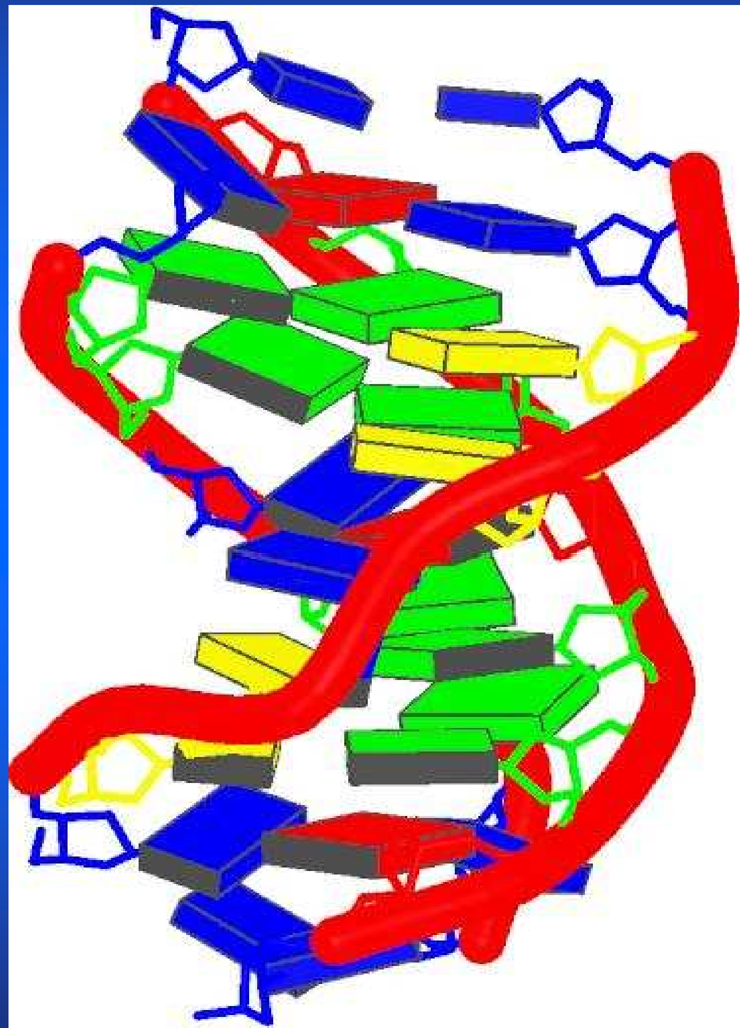


Radhakrishnan, I., Patel, D.J. (1994)

DNA TRIPLEX

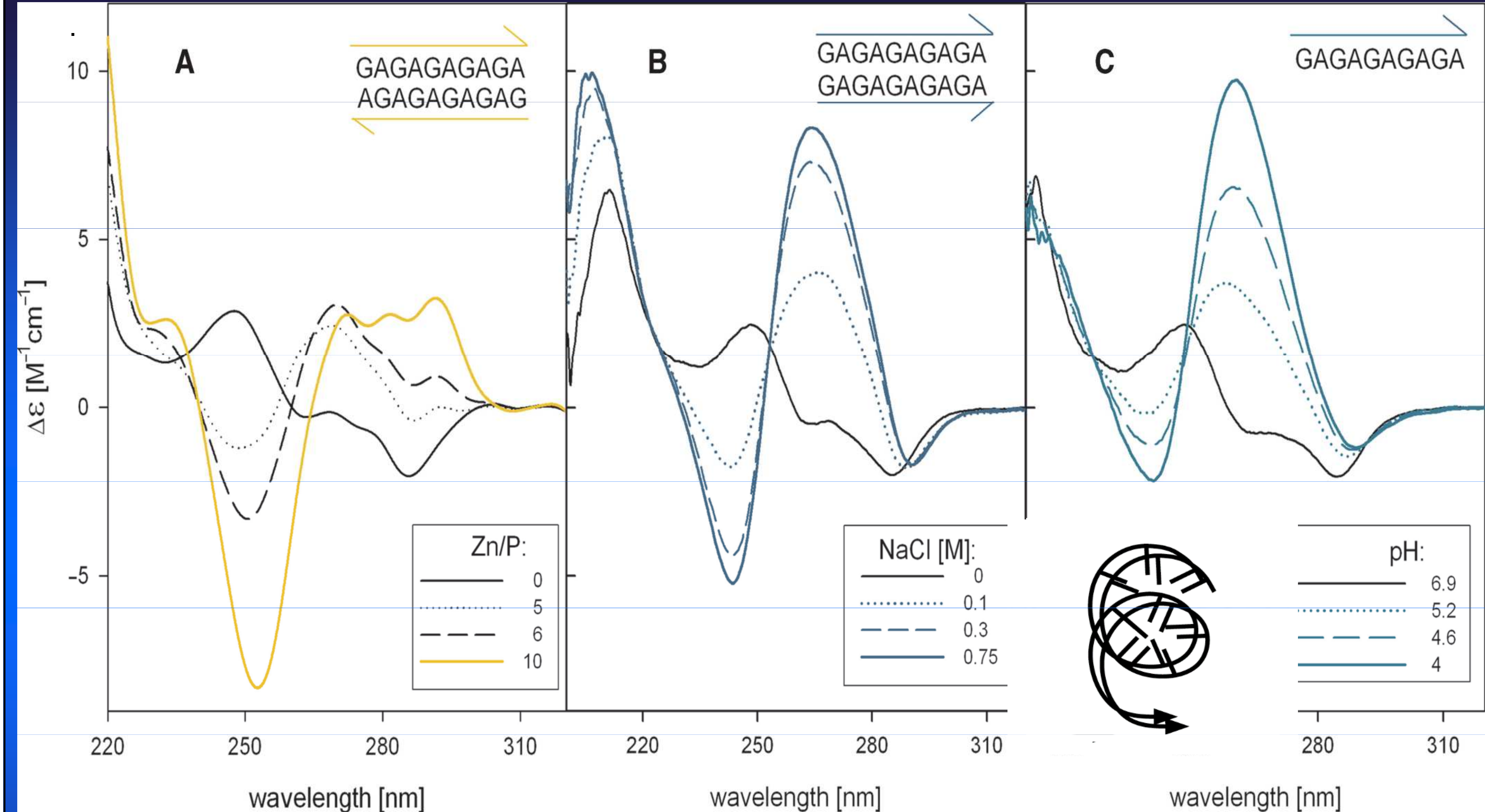
T C C T C C T T T T T T A G G A G G A T T T T T T G G T G G T





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

Pyrimidine. Purine. Purine



A Casasnovas, J.M., et al. Azorin, F.: Structural polymorphism of d(GA.TC)$_n$ DNA sequences. Intramolecular and intermolecular associations of the individual strands. J. Mol. Biol. 233 (1993) 671-6811.

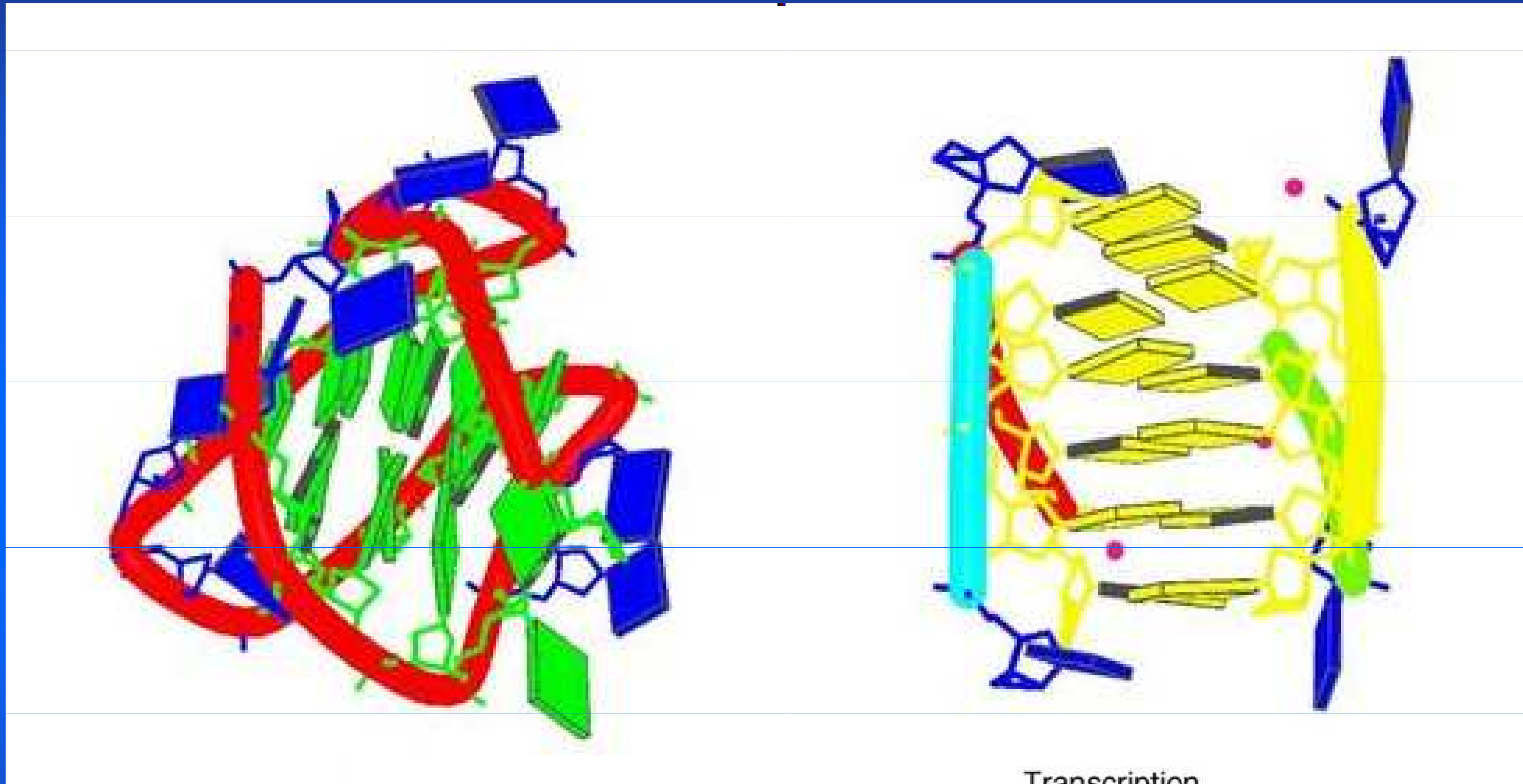
B Rippe, K., Fritch, V., Westhof, E., Jovin, T.M.: Alternating d(G-A) sequences form a parallel-stranded DNA homoduplex. EMBO J. 11 (1992) 3777-3786.

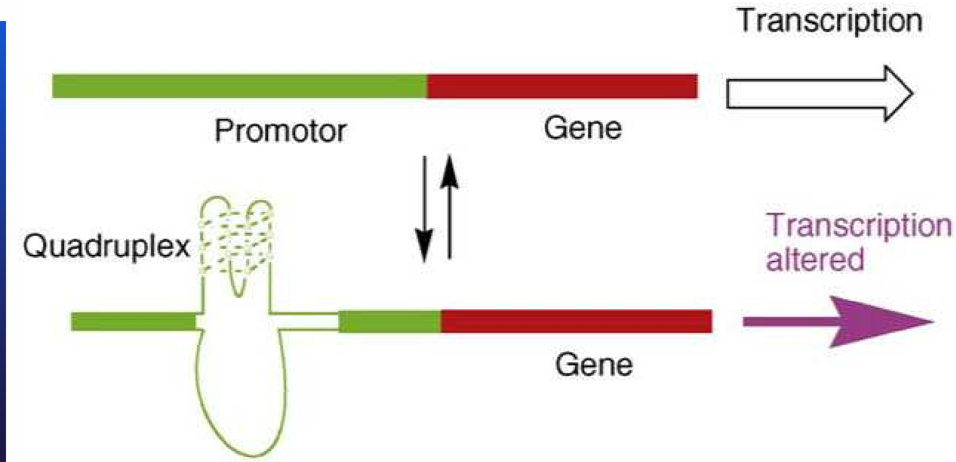
C Dolinnaya, N. G., Fresco, J. R: Single-stranded nucleic acid helical secondary structure stabilized by ionic bonds: d(A+-G)10. Proc. Natl. Acad. Sci. USA 89 (1992) 9242-9246.

C Vorlícková, M., Kejnovská, I., Kovanda, J., Kypr, J.: Dimerization of the guanine-adenine repeat strands of DNA. Nucleic Acids Research, 1999, Vol. 27, No. 2 581–586.

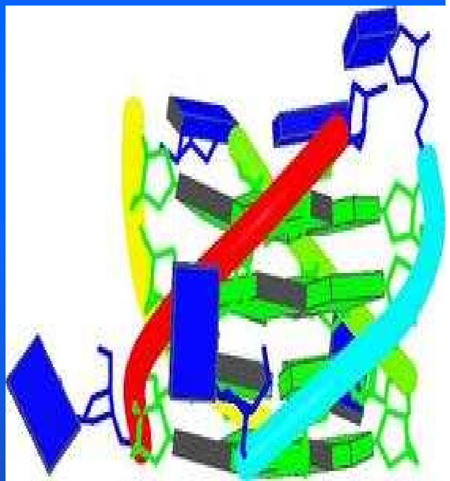
Quadruplexes

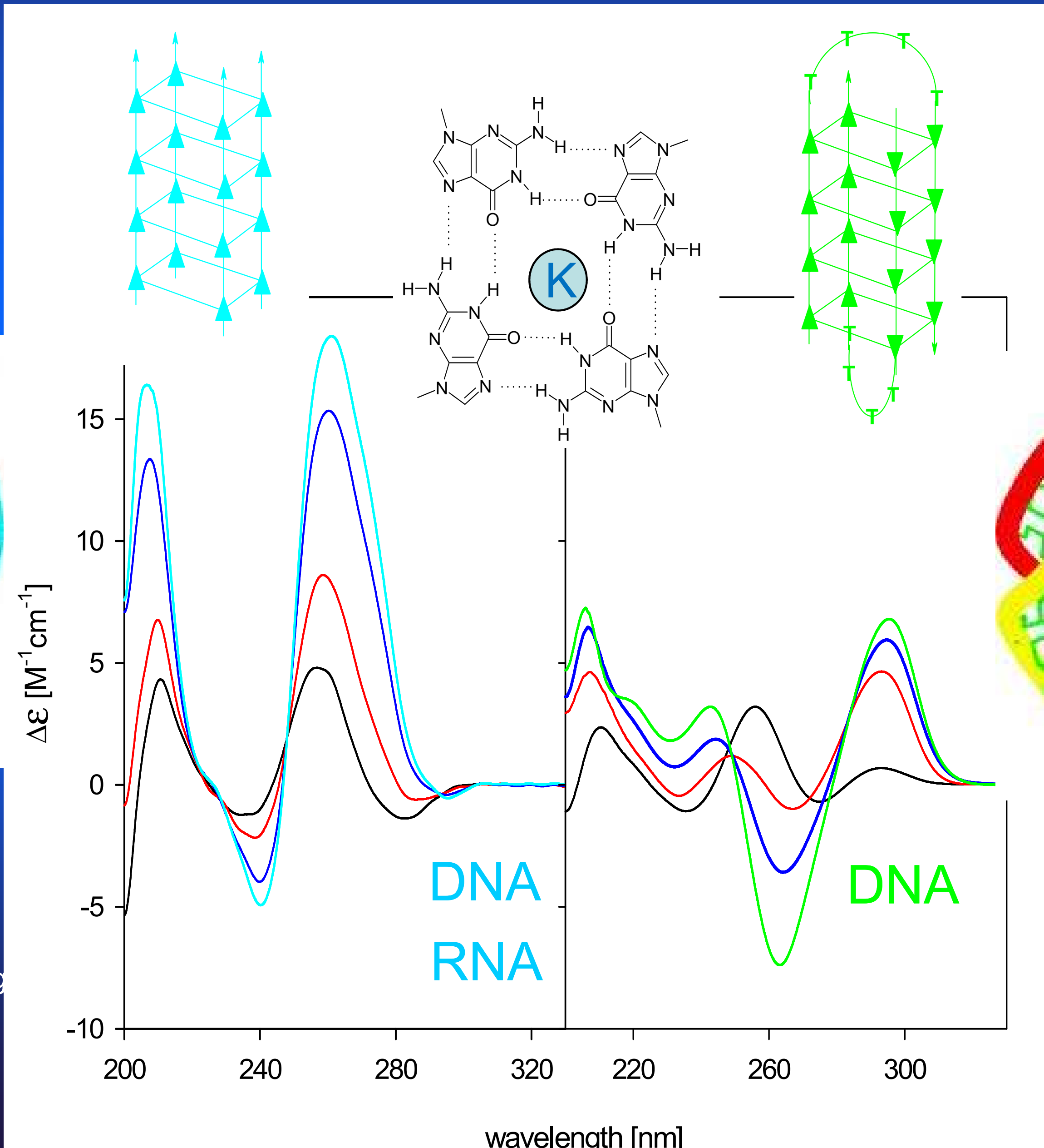
frequently occur in promoters of genes and were shown to control their expression.

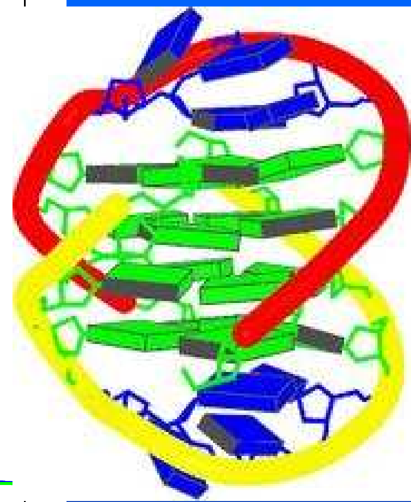




CD spectra reflecting formation of a parallel and antiparallel guanine quadruplex



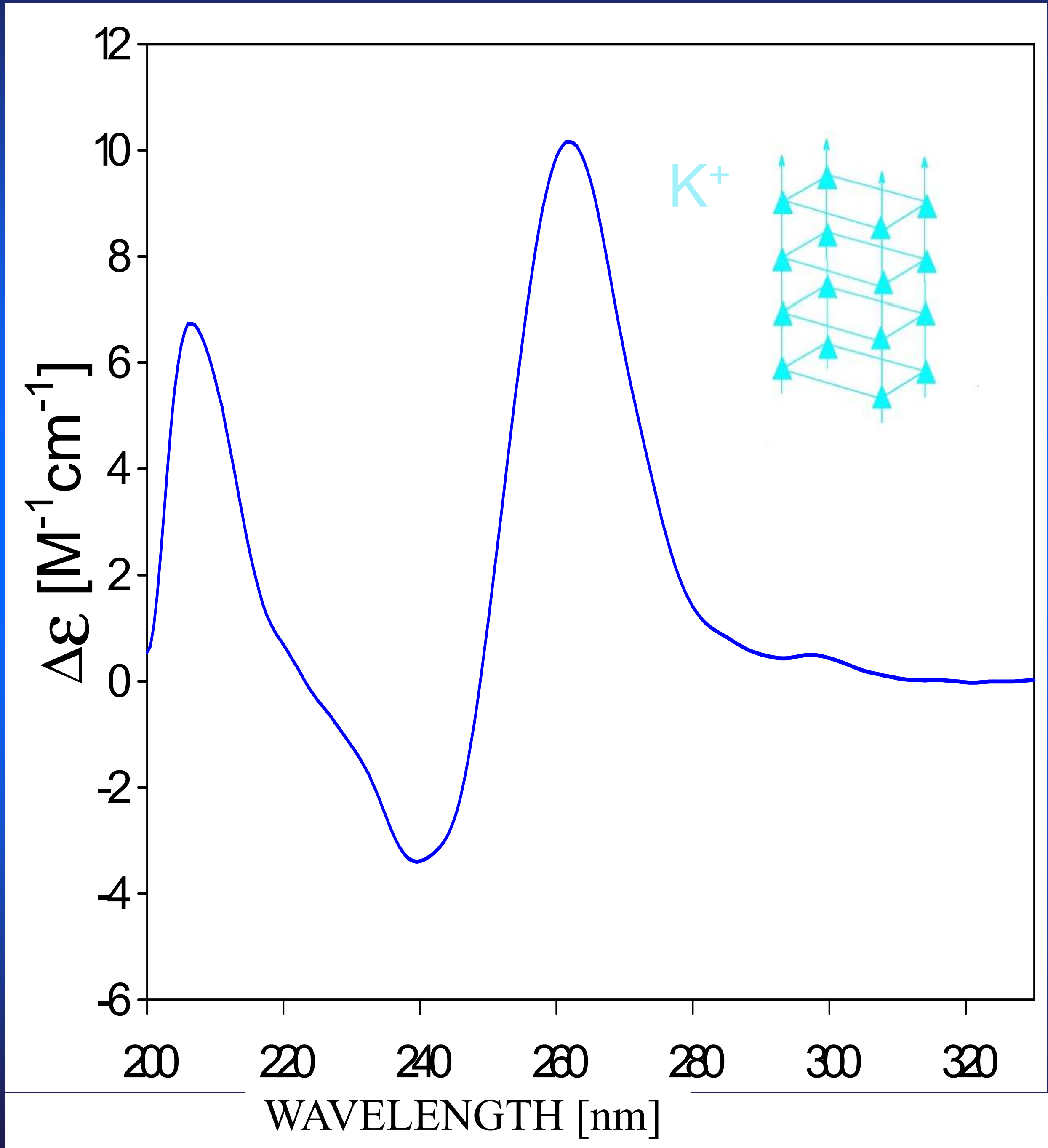


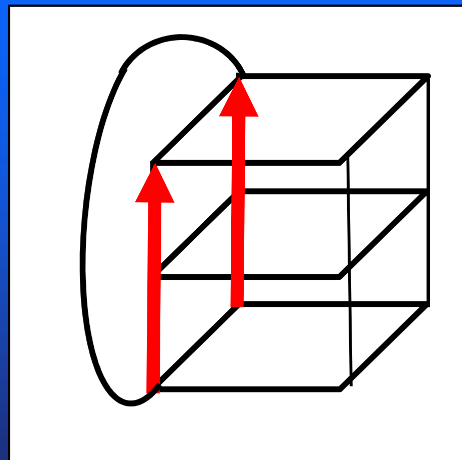


Penazova, H., Vorlickova, M. Guanine tetraplex formation by short DNA fragments containing runs of guanine and cytosine. Biophys. J. 73 (1997) 2054-2063

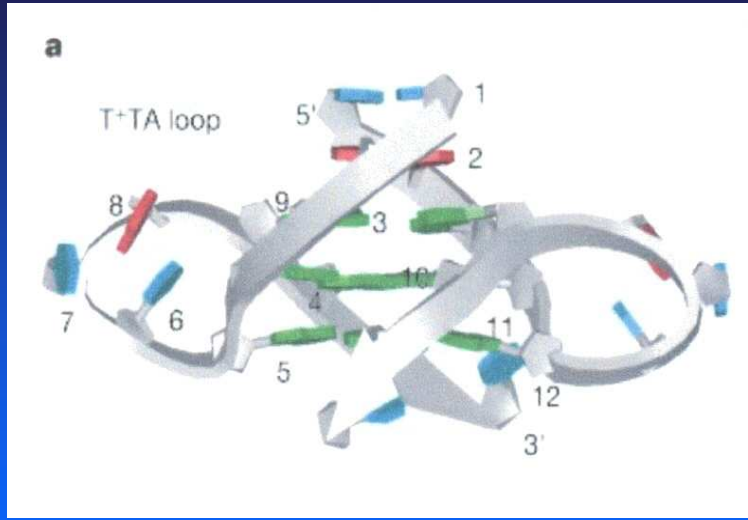
Fragment of Pu-27 promoter c-myc: (1998)

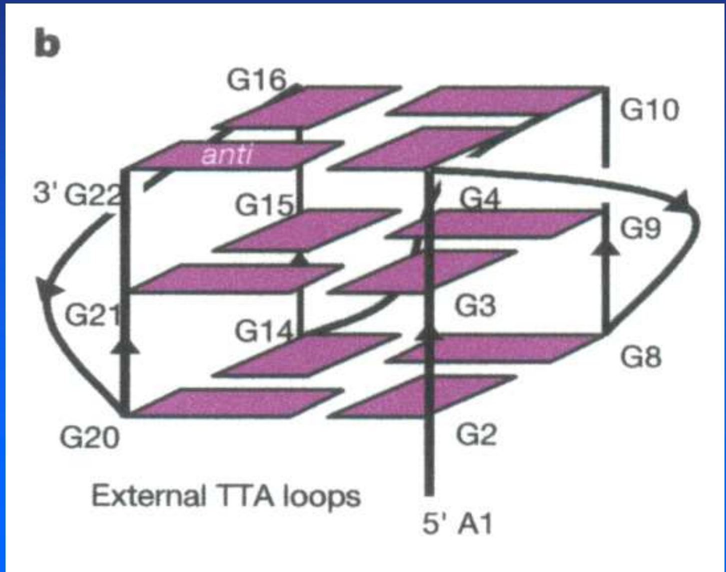
TGGGGAGGGTGGGGAGGGTGGGGAAGG

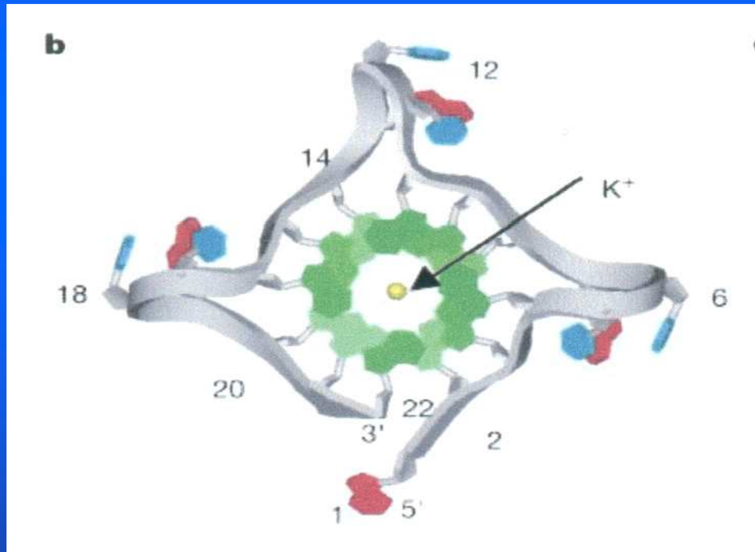




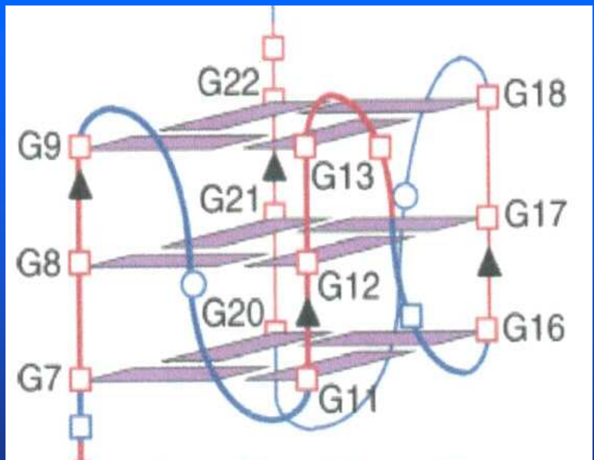
d(TAGGGTTAGGGT)







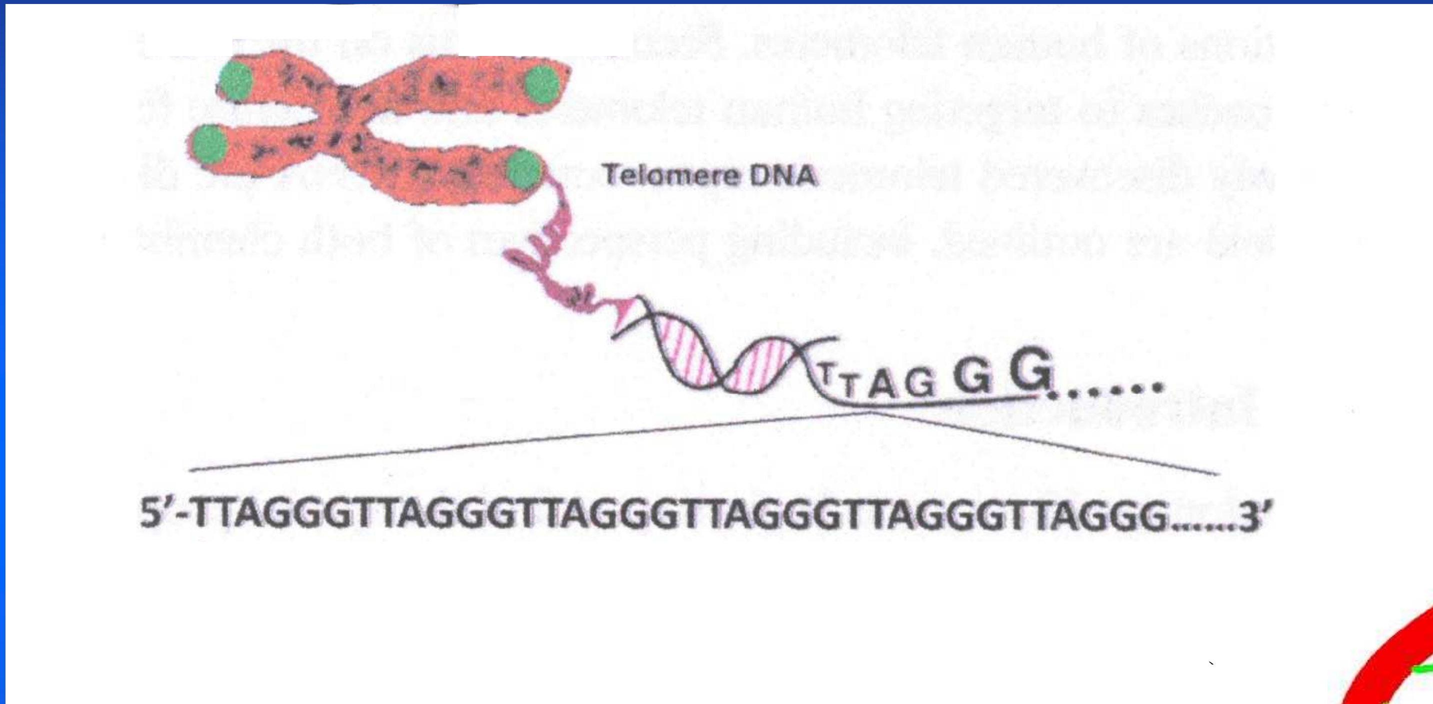
d[AGGG(TTAGGG)3]



Phan, A.T. et al.:
*J.Am.Chem.Soc.***126**(2004)8710

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

Human telomeric DNA forms quadruplex



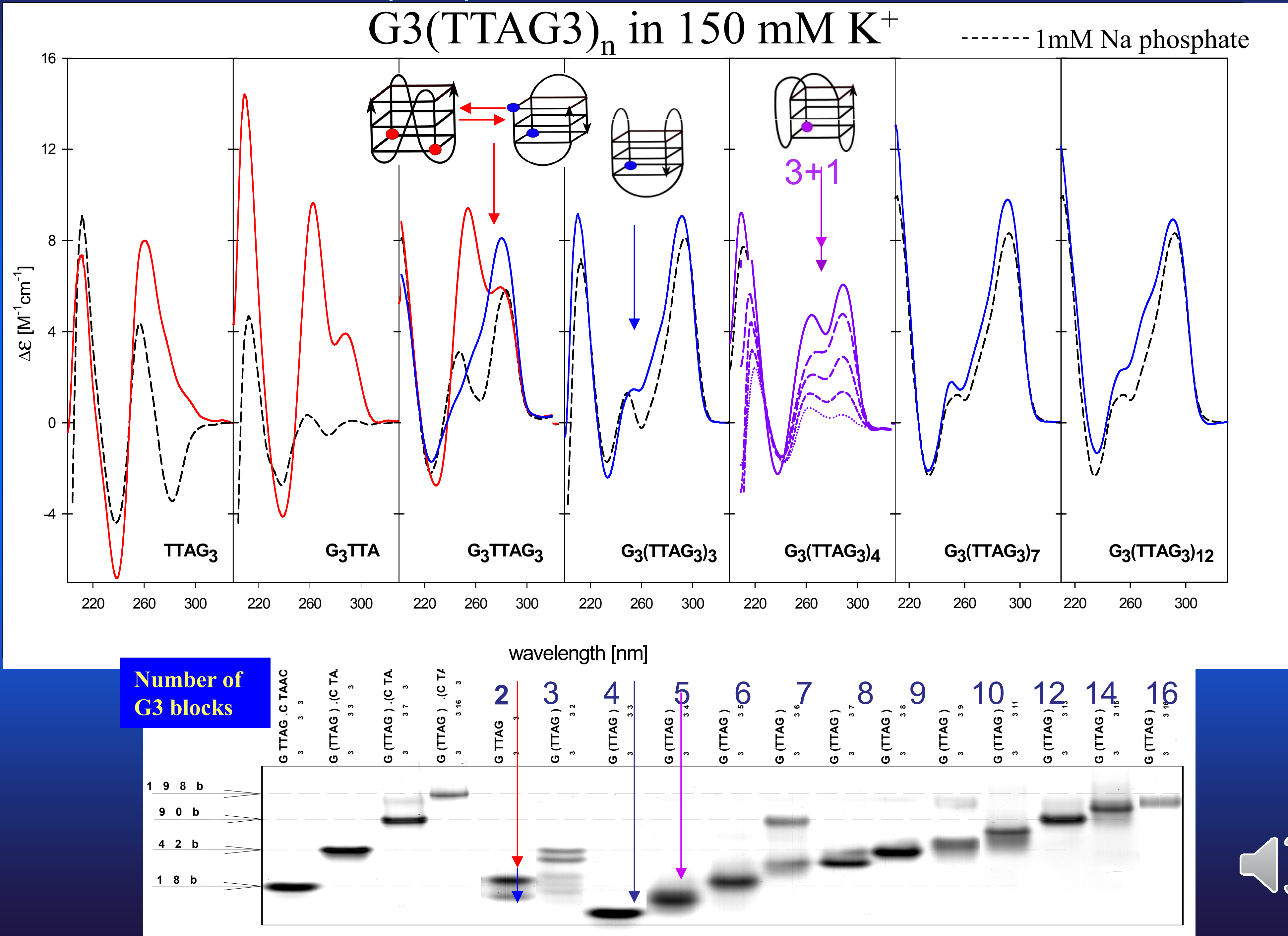
Telomeric DNA is associated with aging

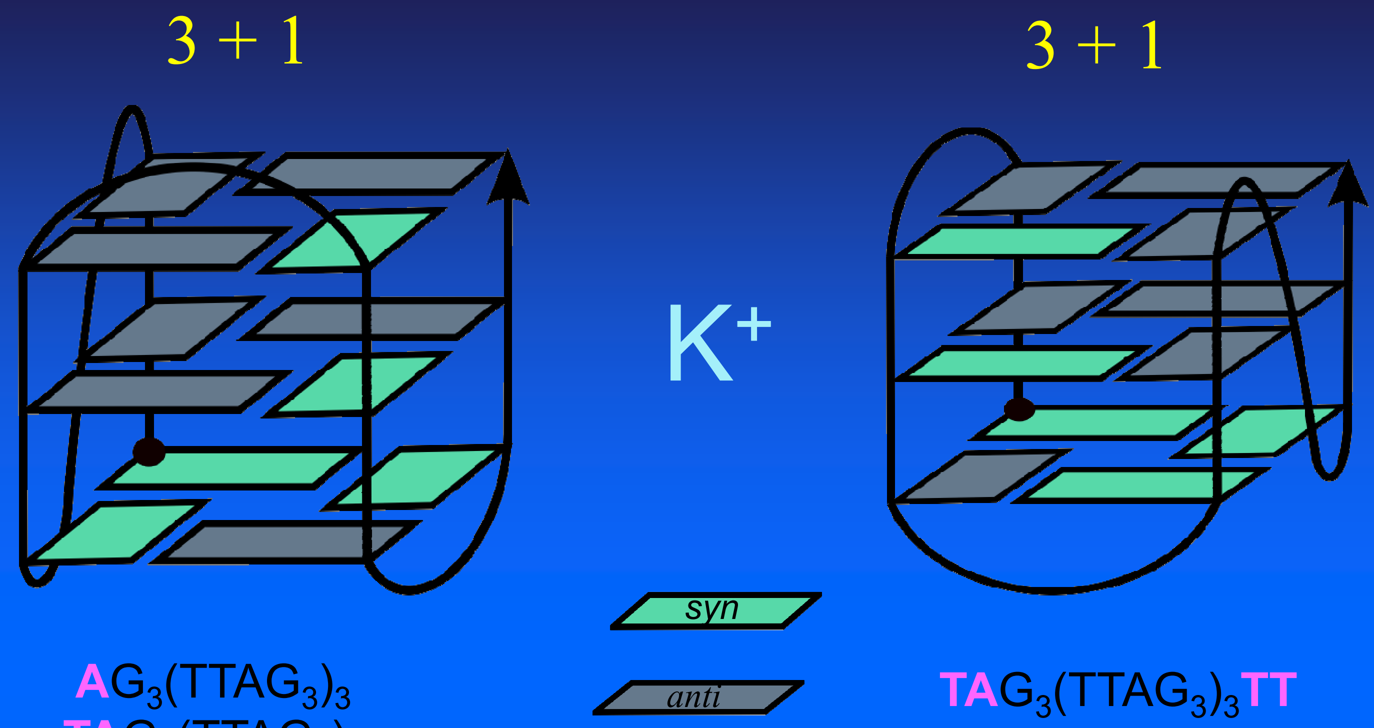
Telomerase – does not get older – ageless, immortal

Quadruplex does not allow telomerase to get on the sequence

The telomere quadruplex became a
target for developing anticancer drugs

Vorlíčková, M., Chládková, J., Kejnovská, I., Fialová, M., Kypr, J.:
Guanine quadruplex topology of human telomere DNA is governed by the number of (TTAGGG) repeats.
Nucleic Acids Res. **33 (2005)** 5851-5860.





AG_3(TTAG_3)$_3$
TAG_3(TTAG_3)$_3$
AAAG_3(TTAG_3)$_3$AA

TAG_3(TTAG_3)$_3$TT

- 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.

Vorlíčková, M., Chládková, J., Kejnovská, I., Fialová, M., Kypr, J.:
Guanine quadruplex topology of human telomere DNA is governed by the number of (TTAGGG) repeats.
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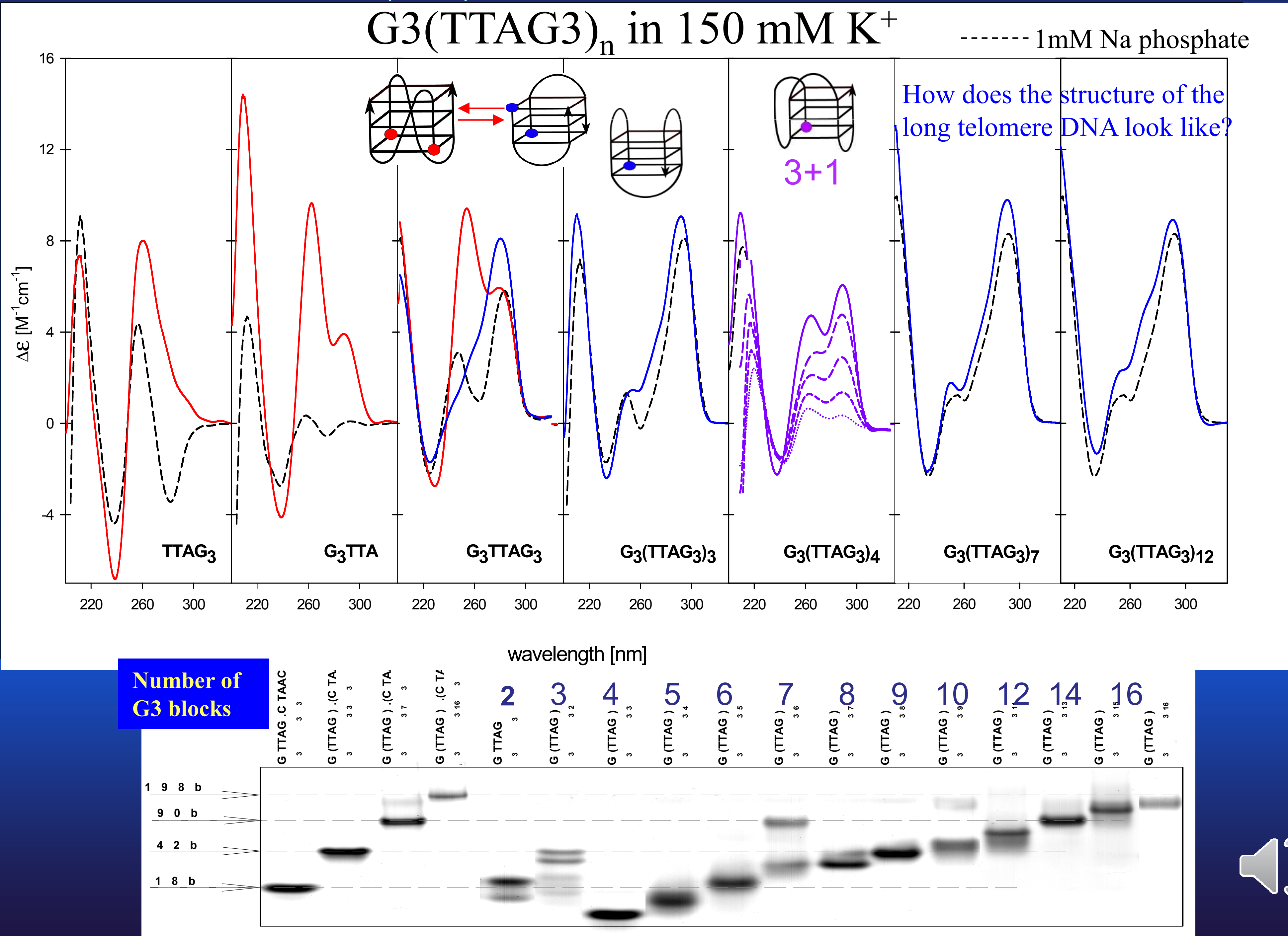
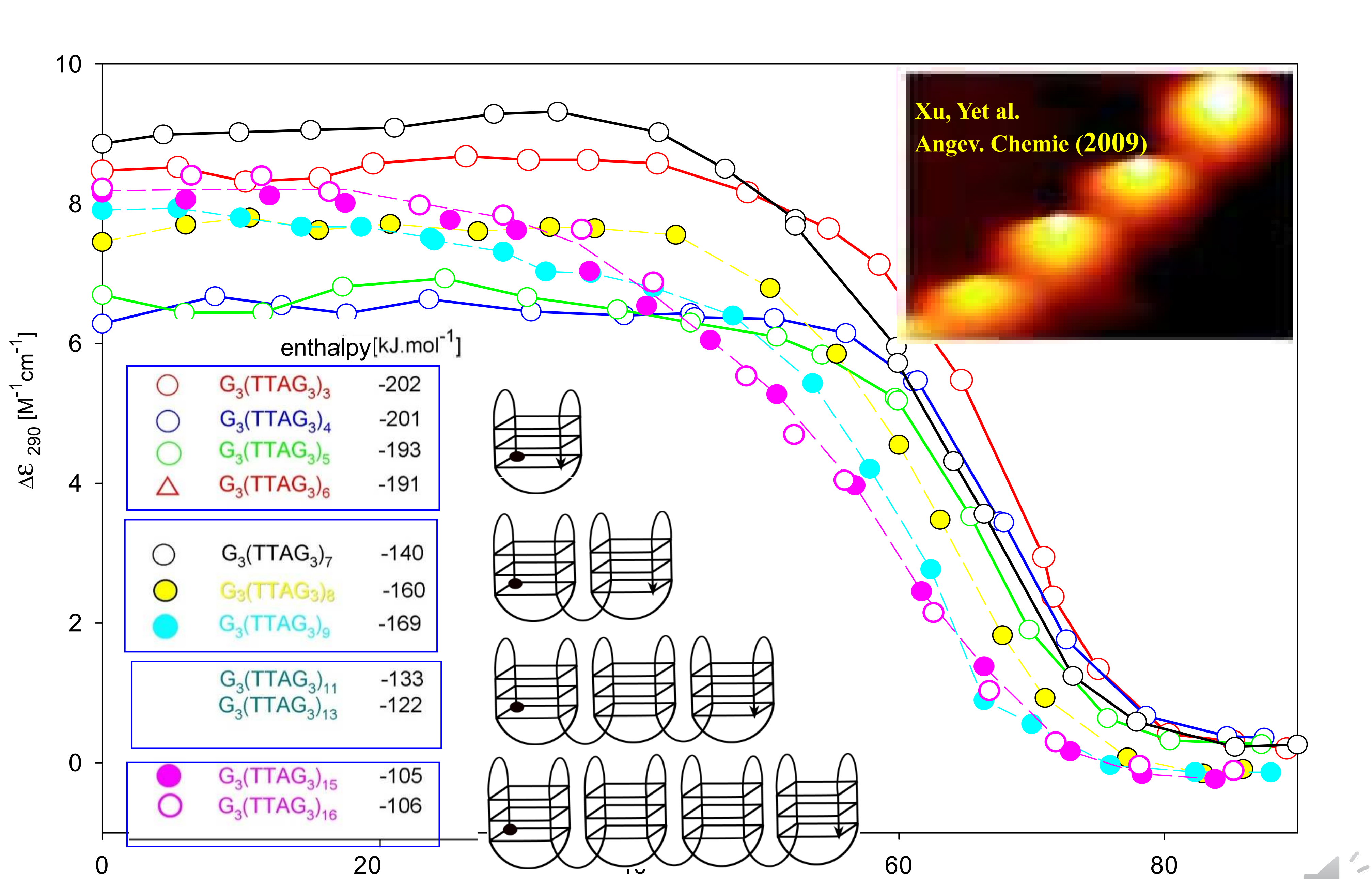
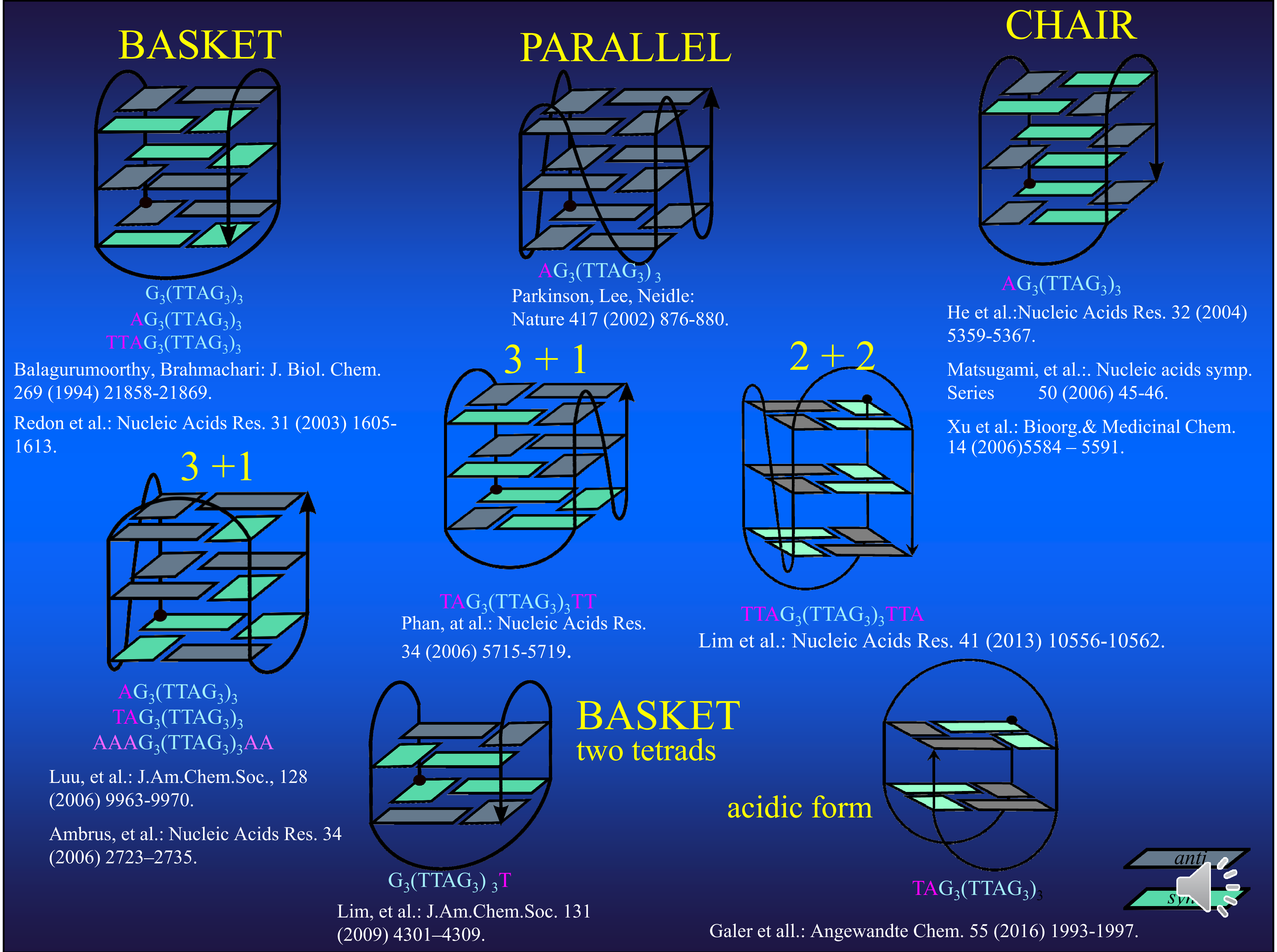


Vorlíčková, M., Chládková, J., Kejnovská, I., Fialová, M., Kypr, J.:
Guanine quadruplex topology of human telomere DNA is governed by the number of (TTAGGG) repeats.
Nucleic Acids Res. **33** (2005) 5851-5860.

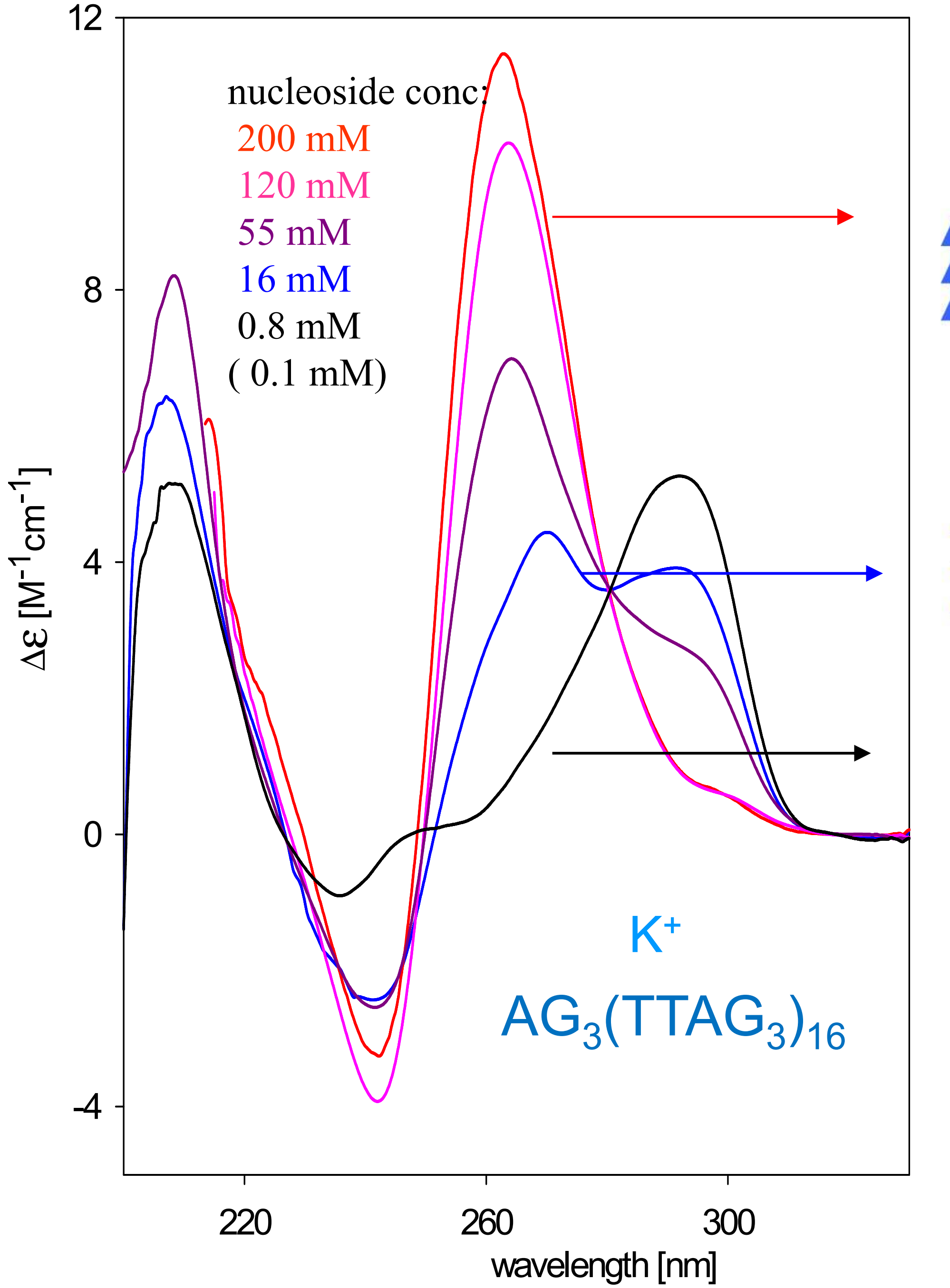


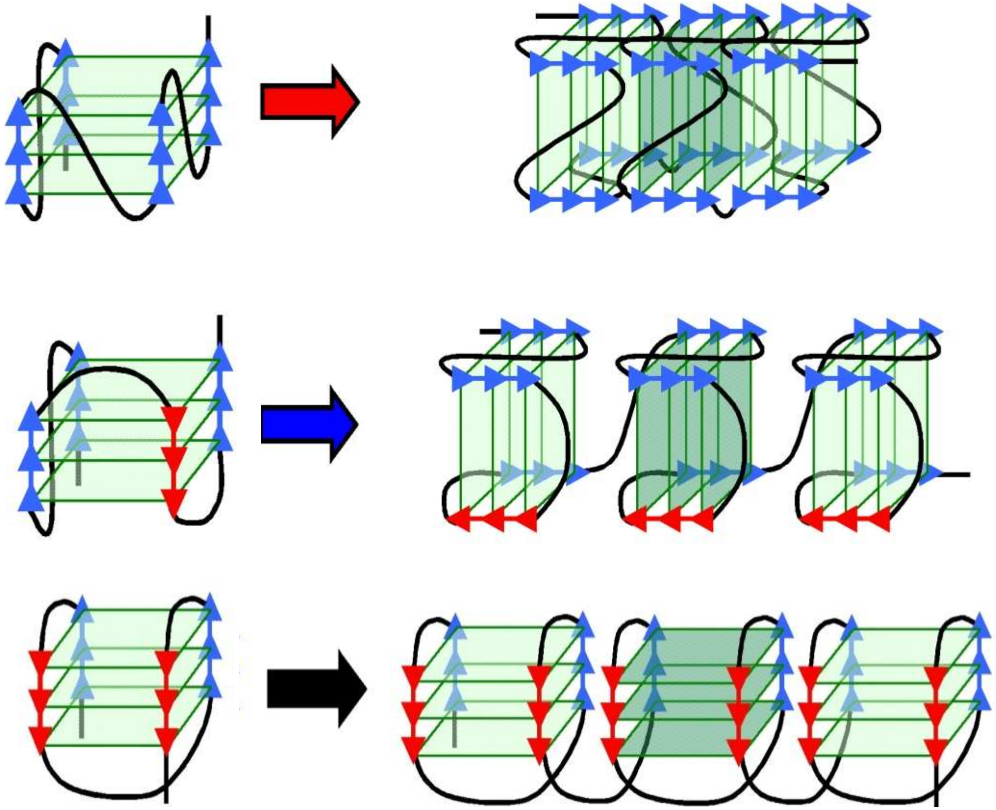
What is the structure of the bead?



BASKET
$G_3(TTAG_3)_3$
$AG_3(TTAG_3)_3$
$TTAG_3(TTAG_3)_3$
Balagurumoorthy, Brahmachari: J. Biol. Chem. 269 (1994) 21858-21869.
Redon et al.: Nucleic Acids Res. 31 (2003) 1605-1613.
PARALLEL
$AG_3(TTAG_3)_3$
Parkinson, Lee, Neidle: Nature 417 (2002) 876-880.
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.
3 + 1
$TAG_3(TTAG_3)_3TT$
Phan, at al.: Nucleic Acids Res. 34 (2006) 5715-5719.
2 + 2
$TTAG_3(TTAG_3)_3TTA$
Lim et al.: Nucleic Acids Res. 41 (2013) 10556-10562.
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.
BASKET
two tetrads
$G_3(TTAG_3)_3T$
Lim, et al.: J.Am.Chem.Soc. 131 (2009) 4301–4309.
acidic form
$TAG_3(TTAG_3)_3$
Galer et all.: Angewandte Chem. 55 (2016) 1993-1997.
anti

Different quadruplex structures observed for the same sequence at the same solvent conditions

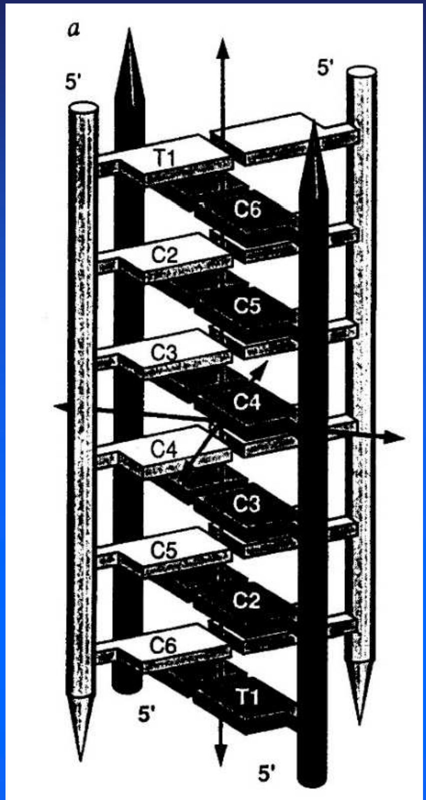




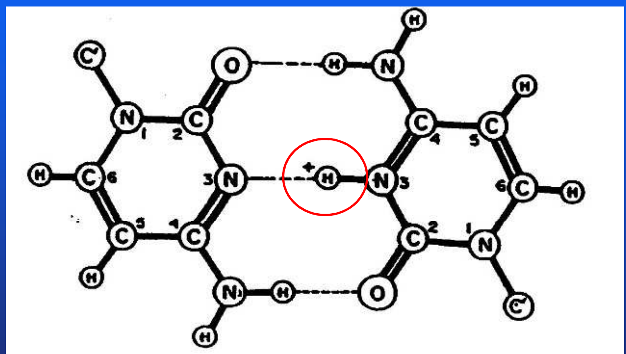
The arrangement of the human telomere quadruplex is polymorphic

Renciuk, D., Kejnovska, I., Skolakova, P., Bednarova, K., Vorlickova, M.:
Arrangements of human telomere DNA quadruplex in physiologically relevant K+ solutions
Nucleic Acids Research **37** (2009) 6625-6634

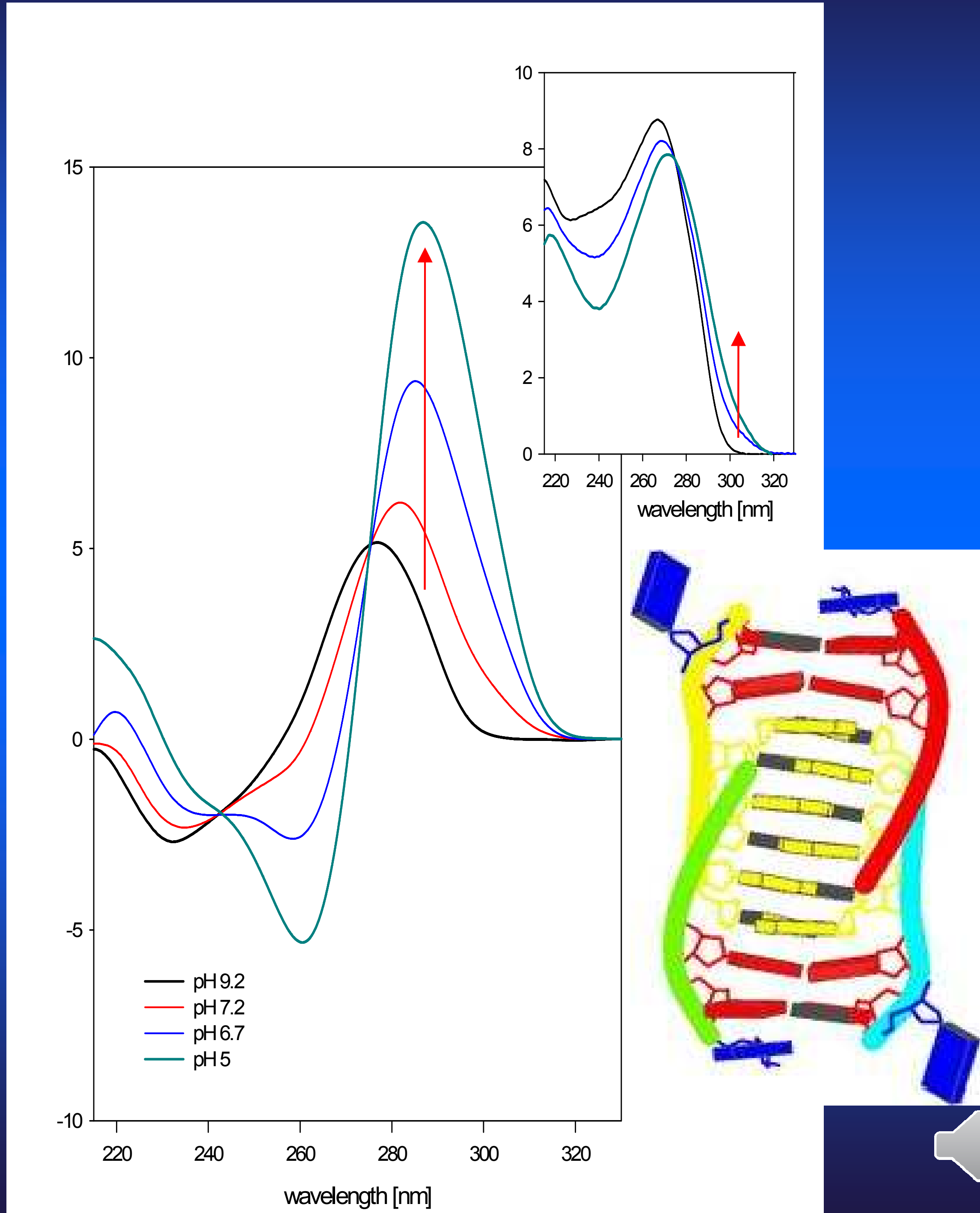
i - motif

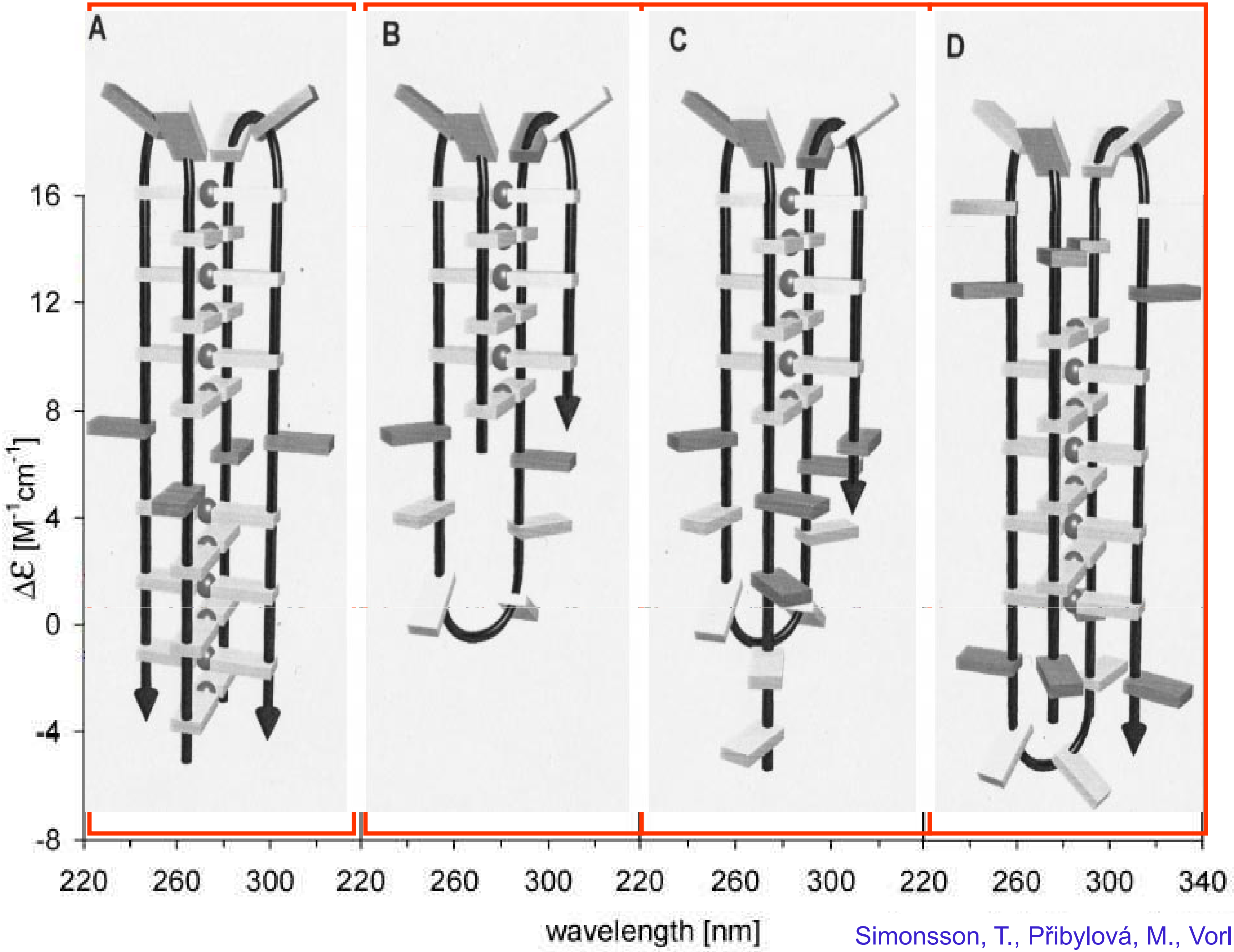


Gehring, K., Leroy, J.L., Gueron, M.: A tetrameric DNA structure with protonated cytosine.cytosine base pairs. Nature 363 (1993) 561-565.



Two parallel duplexes bound by C.C⁺ pairs are intercalated in the antiparallel fashion





Simonsson, T., Přibylová, M., Vorlíčková, M.: Biochem. Biophys. Res.Commun.278 (2000) 158–166

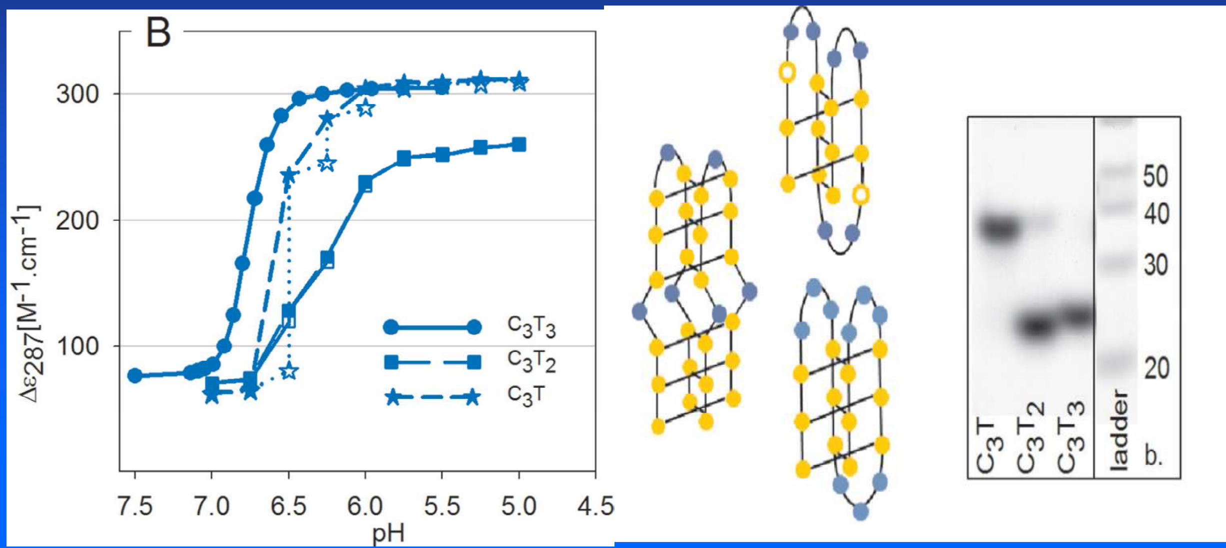
Human c-*myc* Promoter Fragment

TCCCCACCTTCCCCACCCTCCCCACCCTCCCCCA

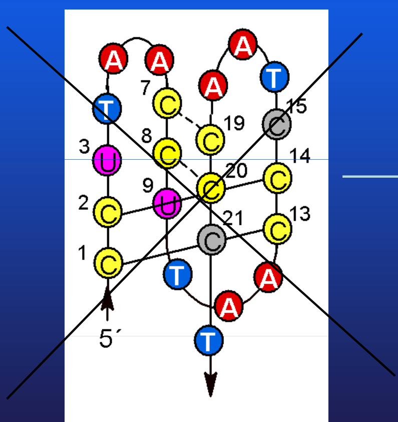
) Školáková, P., Renčiuk, D., Palacký, J., Krafčík, D., Dvořáková, Z., Kejnovská. I., Bednářová, K., Vorlíčková, M.: Systematic investigation of sequence requirements for DNA i-motif formation. Nucleic Acids Research 47 (2019) 2177–2189.

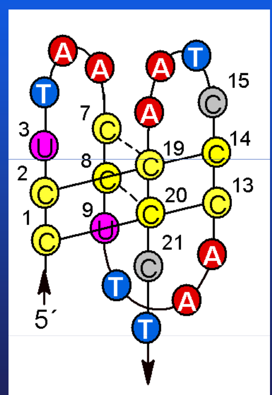
) Dvořáková, Z., Renčiuk, D., Kejnovská, I., Školáková, P., Bednářová, K., Sagi, J. Vorlíčková, M.: i-Motif of cytosine-rich human telomere DNA fragments containing natural base lesions. Nucleic Acids Research Nucleic Acids Research 46 (2018) 1624-1634.

(C3Tx)3Cn



Three nucleotides in the loop are optimal for intramolecular iM formationare
Single nucleotides in the loop result in bimolecular iM
One C is spent for loop in the case of two non-C nucleotides in the loop





Strictly alternating C.C+ pairing
Is required for iM stability

) Školáková, P., Renčiuk, D., Palacký, J., Krafčík, D., Dvořáková, Z., Kejnovská. I., Bednářová, K., Vorlíčková, M.: Systematic investigation of sequence requirements for DNA i-motif formation. Nucleic Acids Research 47 (2019) 2177–2189.

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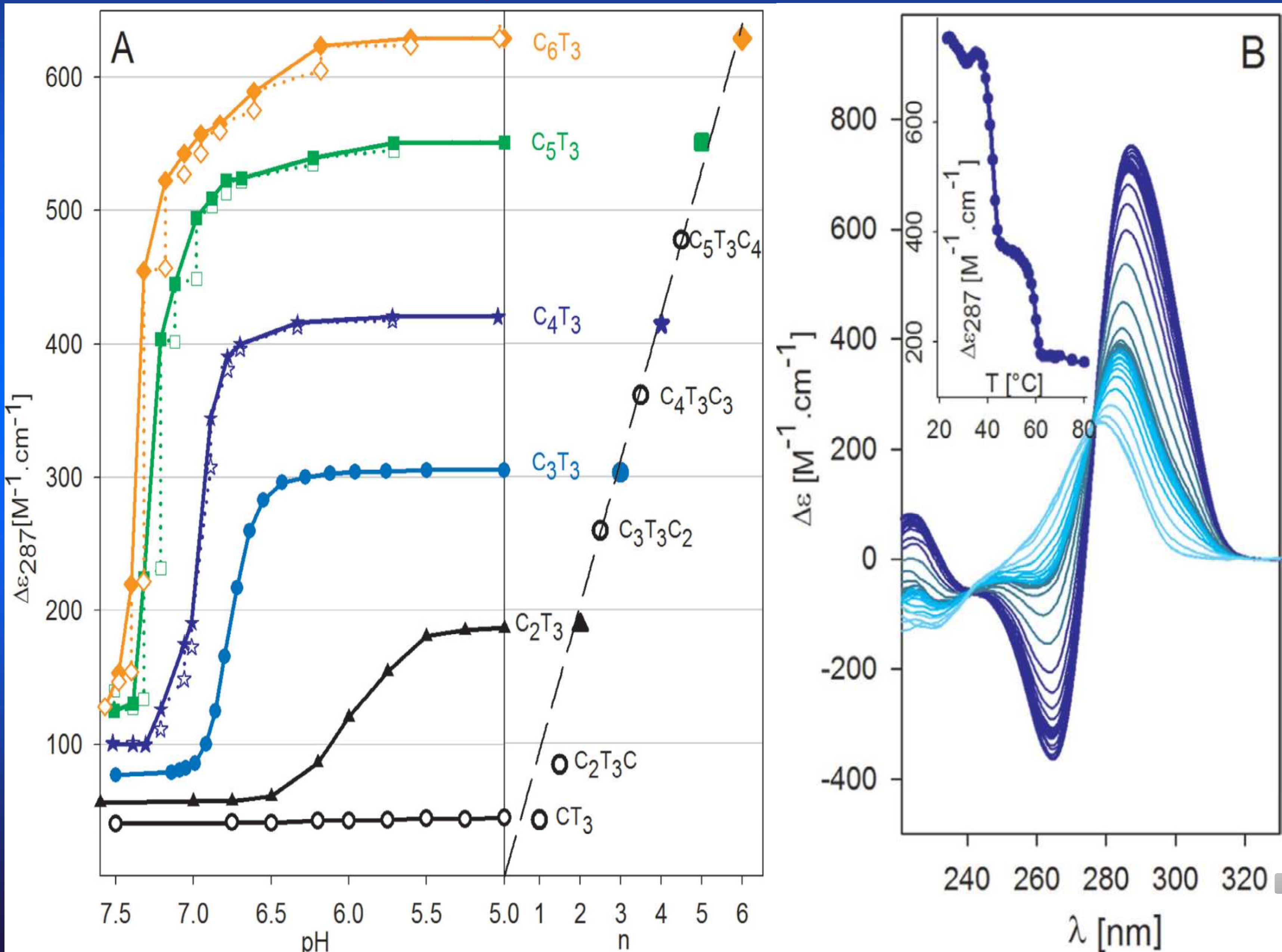
(CnT3)3Cn



) Školáková, P., Renčiuk, D., Palacký, J., Krafčík, D., Dvořáková, Z., Kejnovská. I., Bednářová, K., Vorlíčková, M.: Systematic investigation of sequence requirements for DNA i-motif formation. Nucleic Acids Research 47 (2019) 2177–2189.

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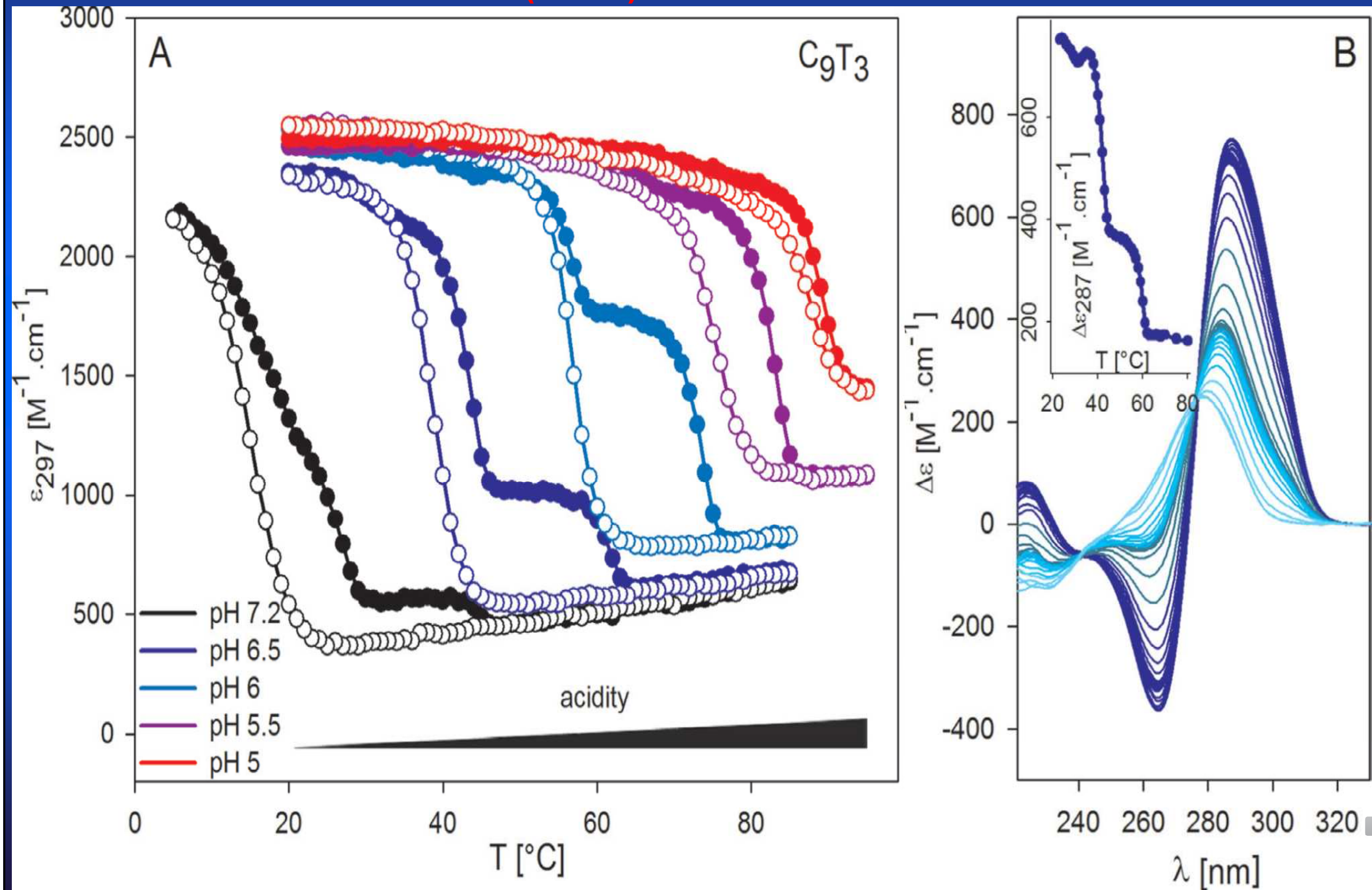
(CnT3)3Cn



Iva Kejnovská
Jan Palacký
Petra Školáková
Klára Bednářová
Zuzana Dvořáková
Daniel Renčiuk

Jaroslav Kypr

Jean-Louis Mergny
Jiří Šponer
Lukáš Trantírek
Jiří Fajkus
Miroslav Fojta

mifi@ibp.cz

CHIROPTICKÉ METODY

Optical Rotatory Dispersion - ORD

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

Circular Dichroism-CD

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

Infrared Circular Dichroism-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)

Fluorescence Detected circular Dichroism -FDCD

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

Circulary Polarized Luminiscence (emission) - CPL (CPE)

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

Circular Diferential Raman Dispersion - Raman CID

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