

CD spektroskopie a konformační vlastnosti DNA



Michaela Vorlíčková
Institute of Biophysics



Academy of Sciences of the Czech Republic, v.v.i. **Brno**

Laboratory of Biophysics of nucleic acids





Hairpin



Coiled - coil



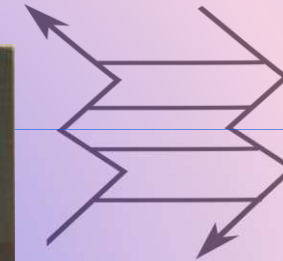
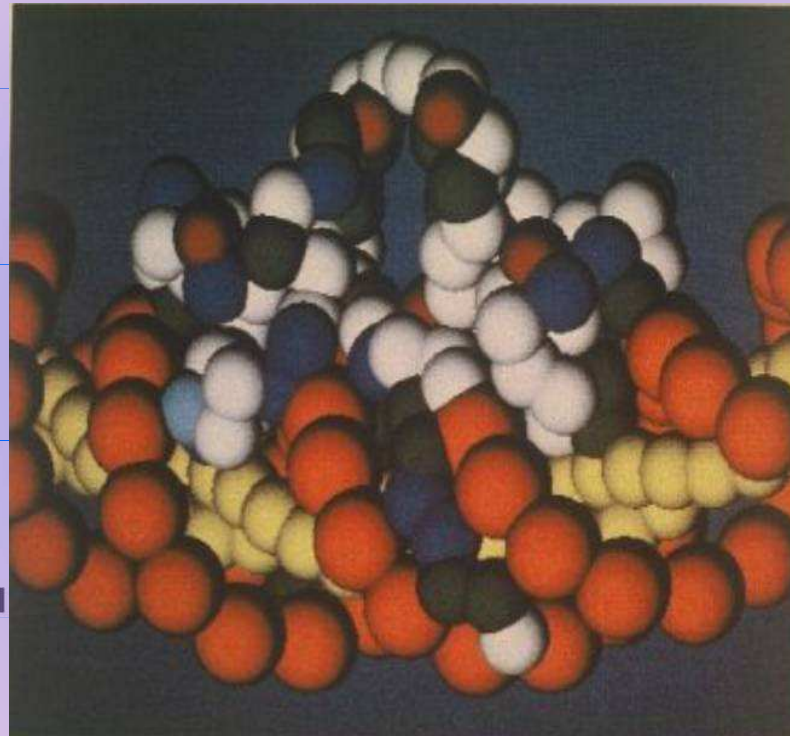
Parallel Homoduplex



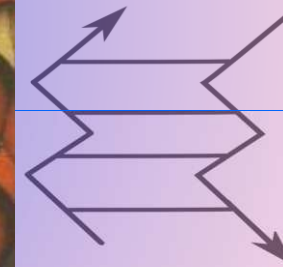
B - form



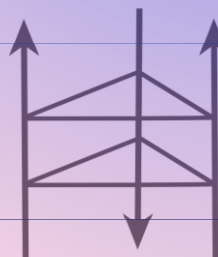
A - form



Z - form



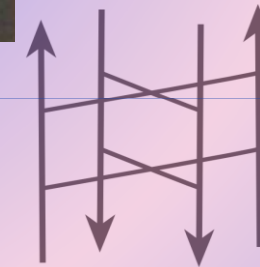
X - form



Triplex



G - tetraplex

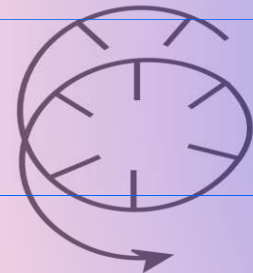


C - tetraplex





Hairpin



Coiled - coil



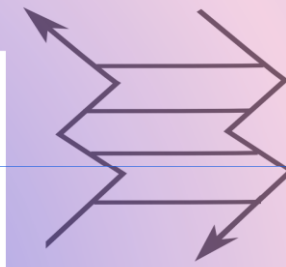
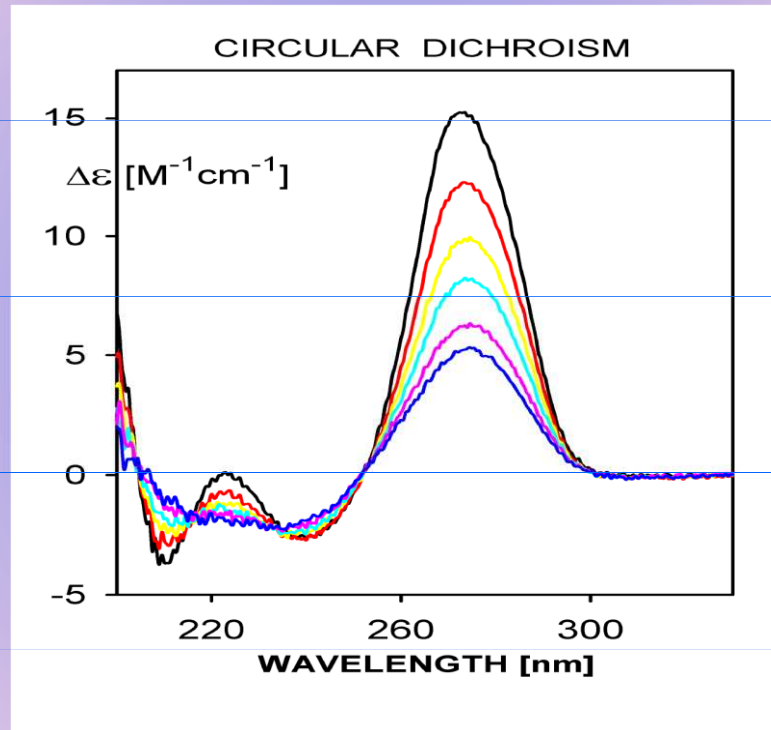
Parallel Homoduplex



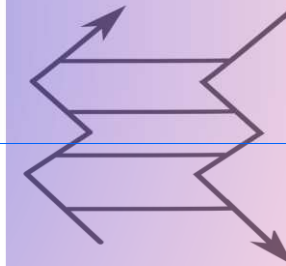
B - form



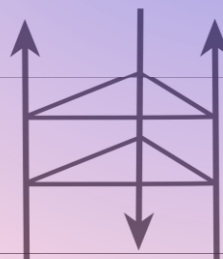
A - form



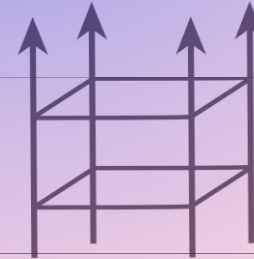
Z - form



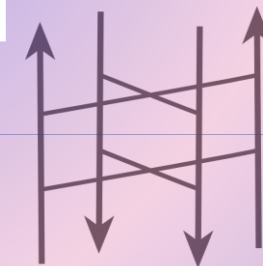
X - form



Triplex

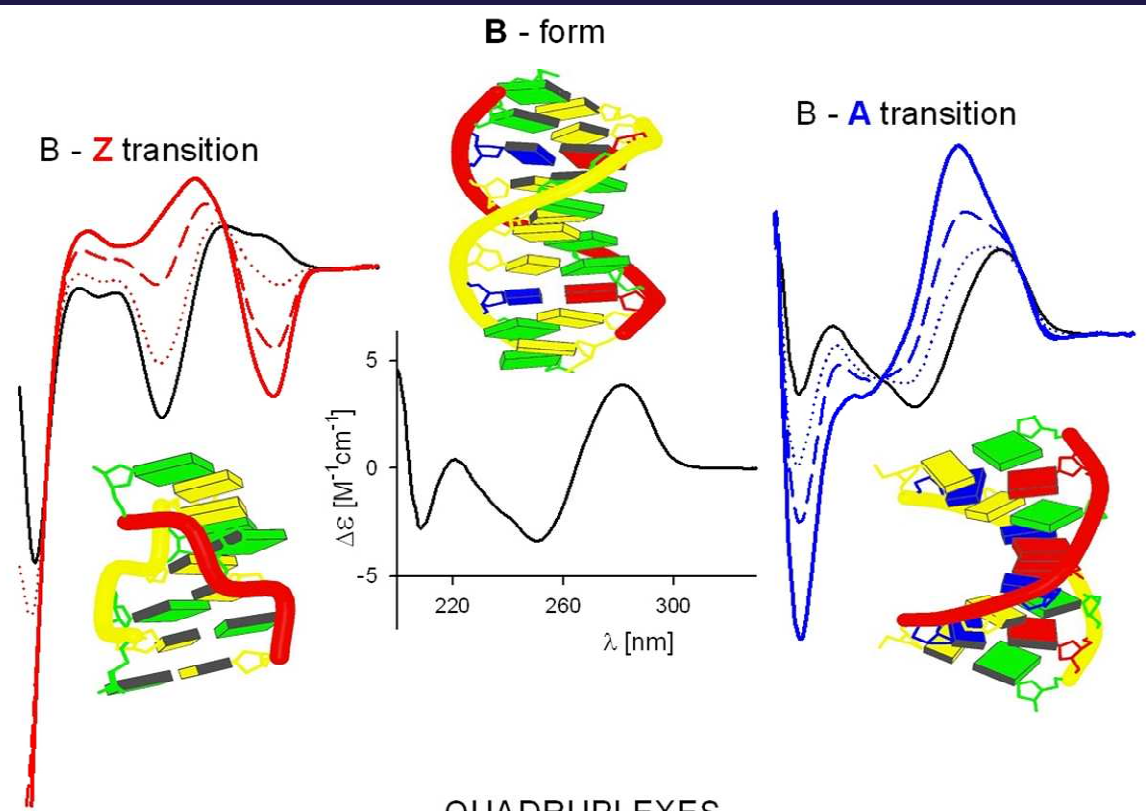


G - tetraplex

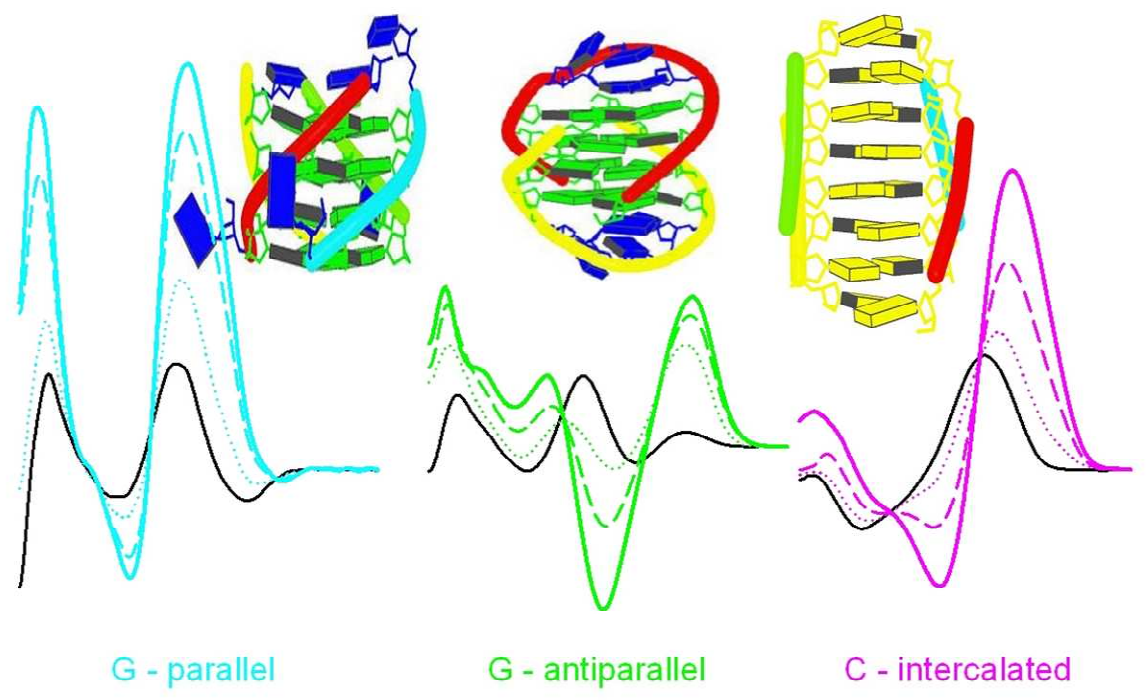


C - tetraplex





QUADRUPLEXES



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

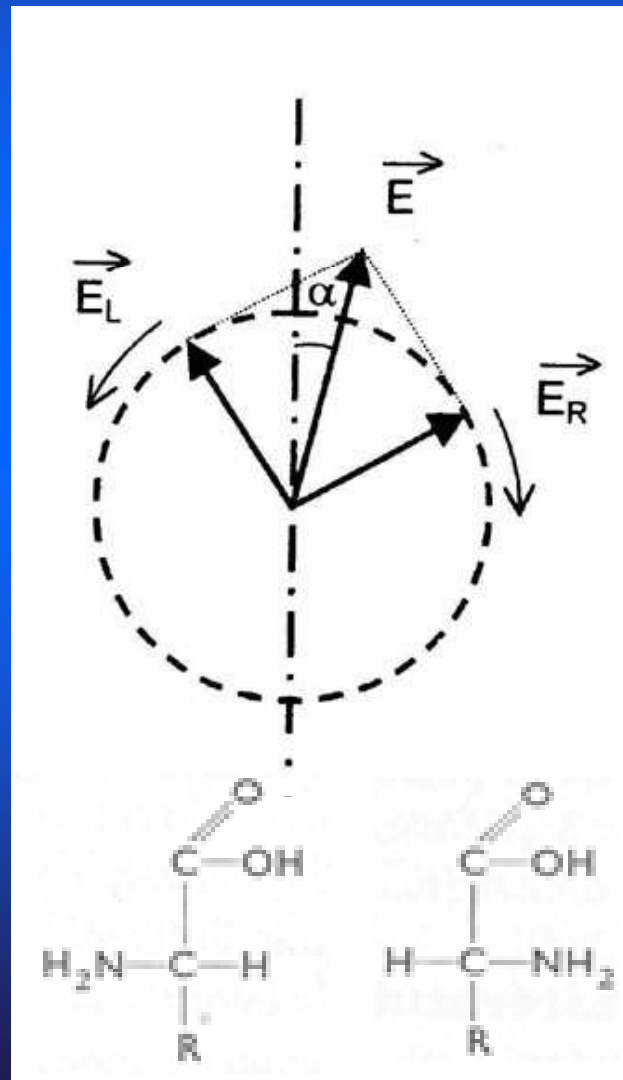


Cirkulární dichroismus a optická aktivita biopolymerů

) optická aktivita – chirální látky (aminokyseliny, cukry) úhel stočení roviny polarizovaného světla, ORD

Paprsek, levo-, pravotočivý, paprsek prochází prostředím...

Phenomenon, veličina



glycin

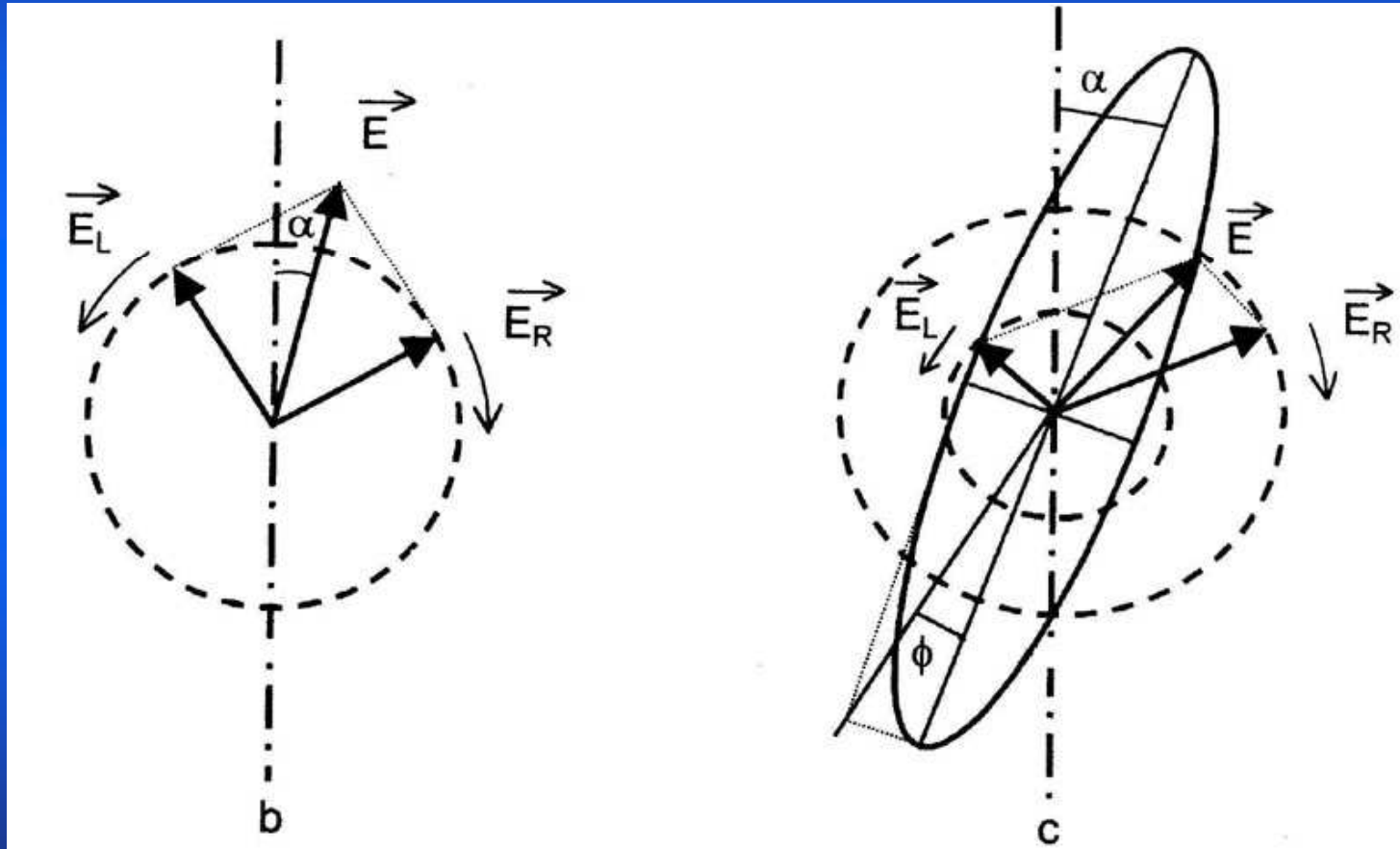
Specific rotation
 $[\alpha]_{\lambda}^T = \alpha/c$



Cirkulární dichroismus a optická aktivita biopolymerů

) optická aktivita – chirální látky (aminokyseliny, cukry) úhel stočení roviny polarizovaného světla, ORD

) CD – princip, veličiny, elipticita, ΔA , $\Delta \epsilon$, vztah mezi ORD a CD



Elipticita

φ [°]

$$\operatorname{tg} \theta = b/a = \frac{\epsilon_L - \epsilon_R}{\epsilon_L + \epsilon_R}$$

Cirkulární dichroismus $\Delta \epsilon$

$$\Delta \epsilon = \epsilon_L - \epsilon_R = \frac{\Delta A}{cl}, \quad \theta = 3300 \cdot \Delta \epsilon$$

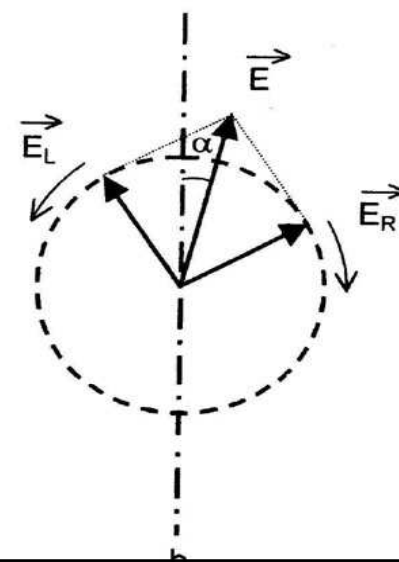
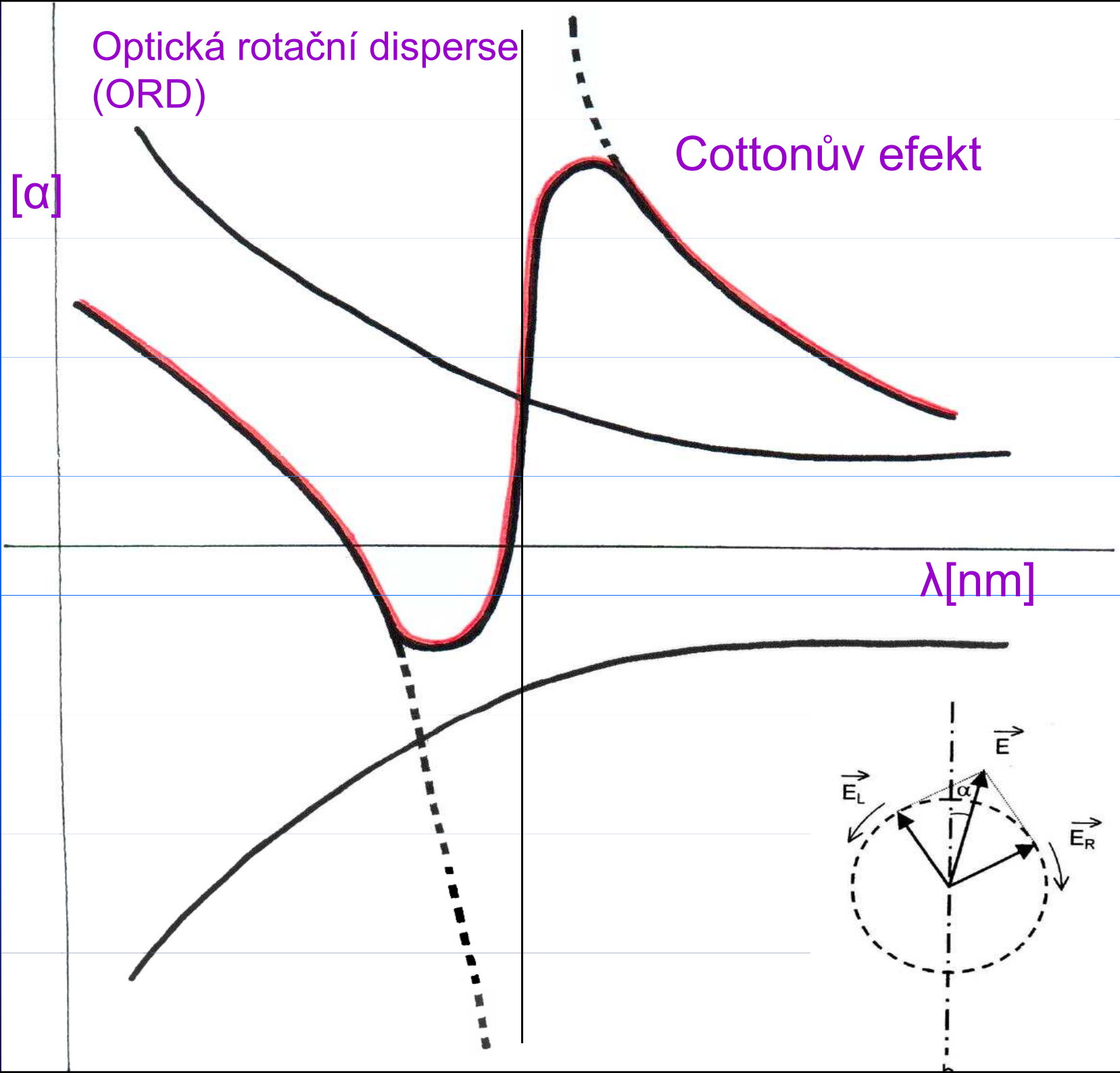


Optická rotační disperse
(ORD)

Cottonův efekt

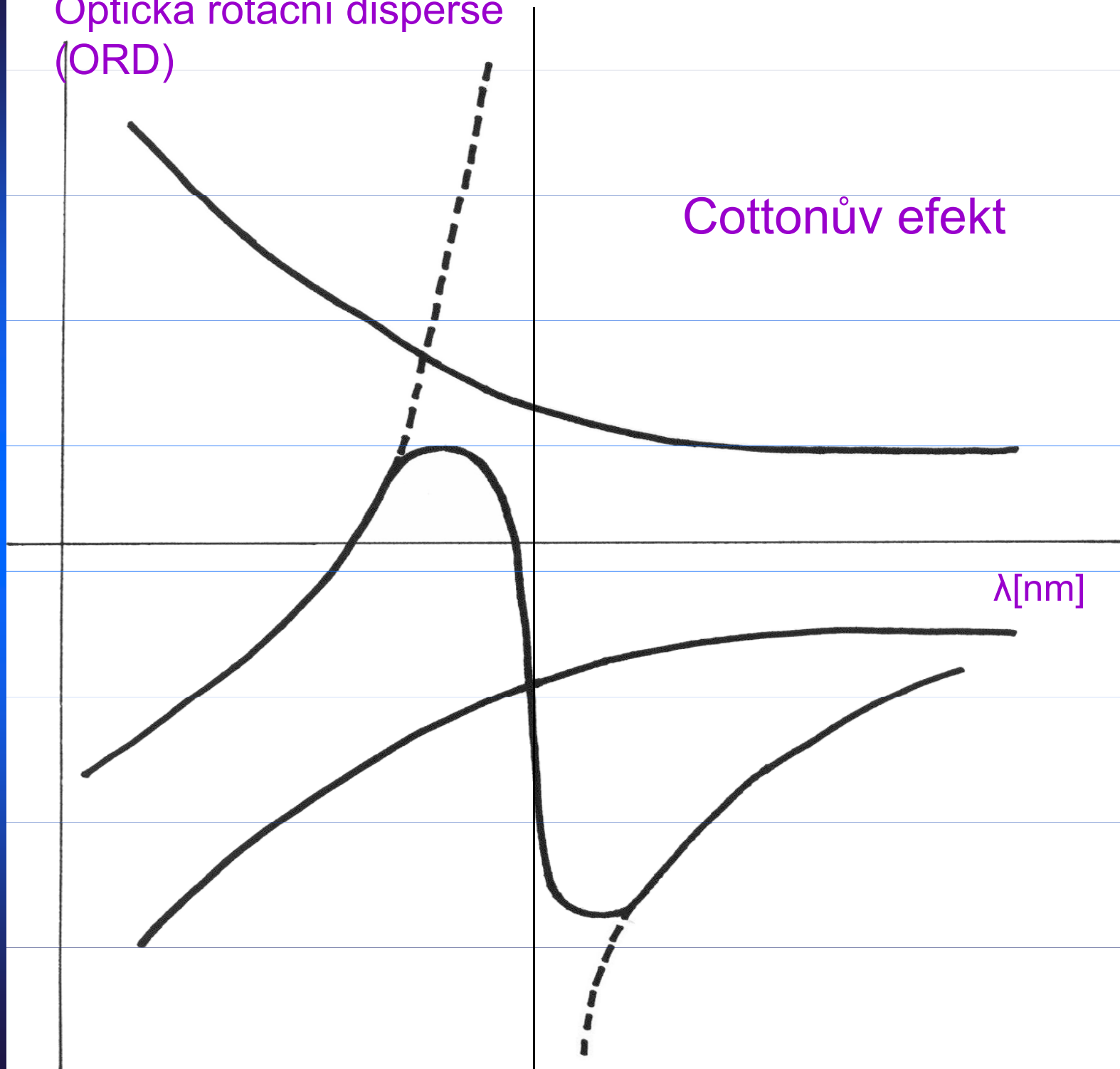
$[\alpha]$

λ [nm]



Optická rotační disperse
(ORD)

Cottonův efekt



CD

ORD

+

λ

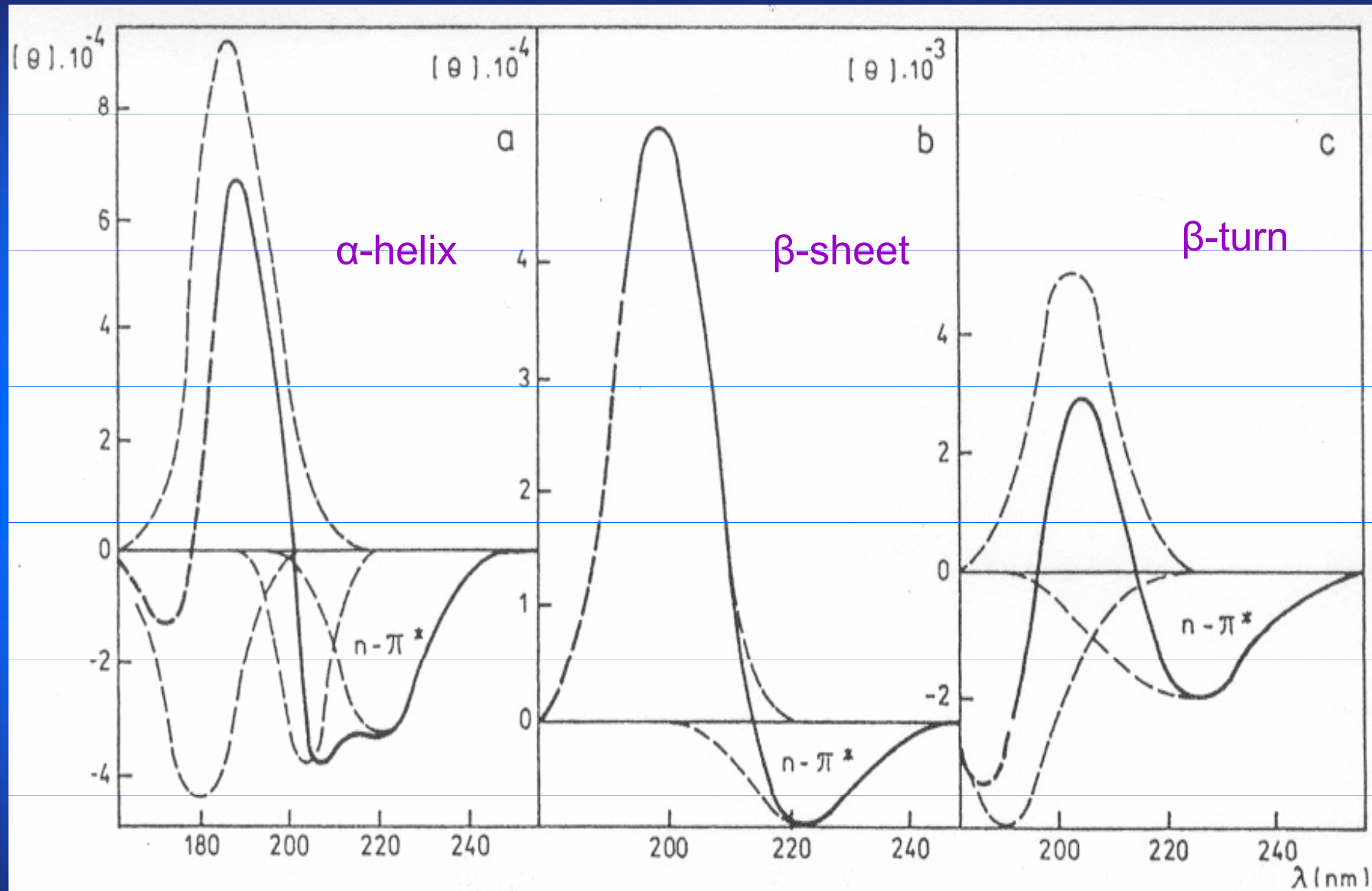
ORD

-

CD



CD of proteins

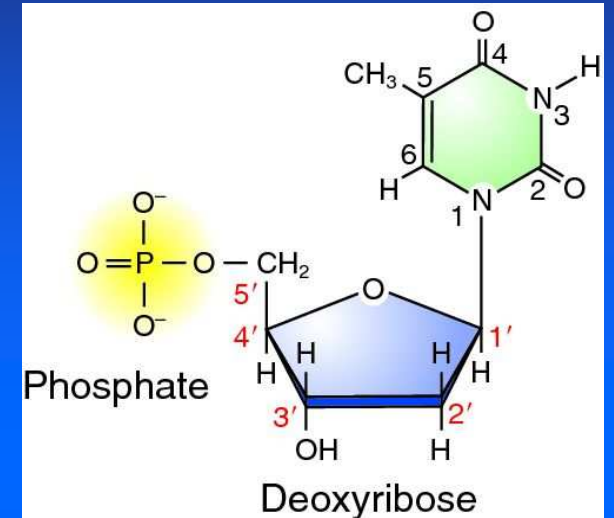


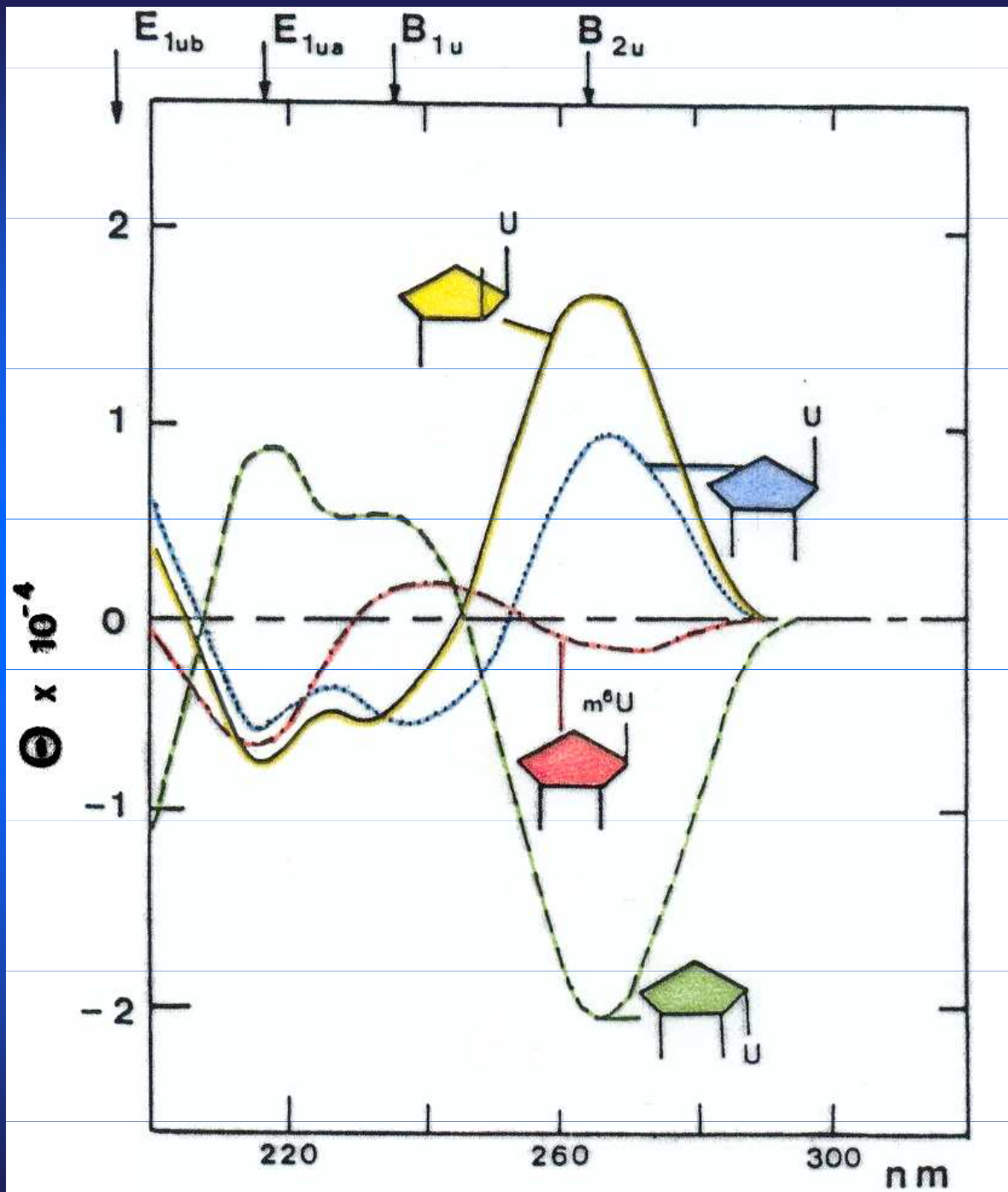
Podmínky vzniku CD DNA



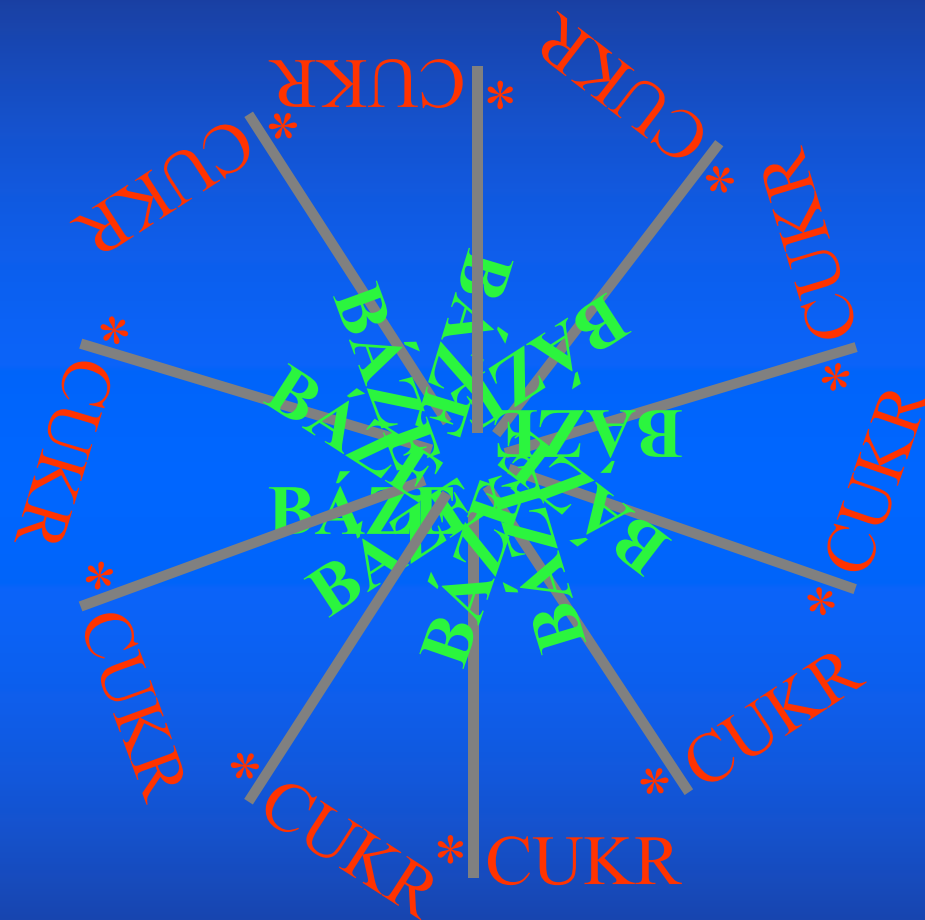
BÁZE

*CUKR





Podmínky vzniku CD



Cirkulární dichroismus a optická aktivita biopolymerů

-) optická aktivita – chirální látky (aminokyseliny, cukry) úhel stočení roviny polarizovaného světla, ORD
-) CD – princip, veličiny, elipticita, ΔA , $\Delta \epsilon$, vztah mezi ORD a CD
-) **Výhody a nevýhody CD spektroskopie ve srovnání s jinými metodami studia biopolymerů**

Výhody

Citlivost - nízká koncentrace studované látky
snadná rozpustnost
i v extrémních podmínkách

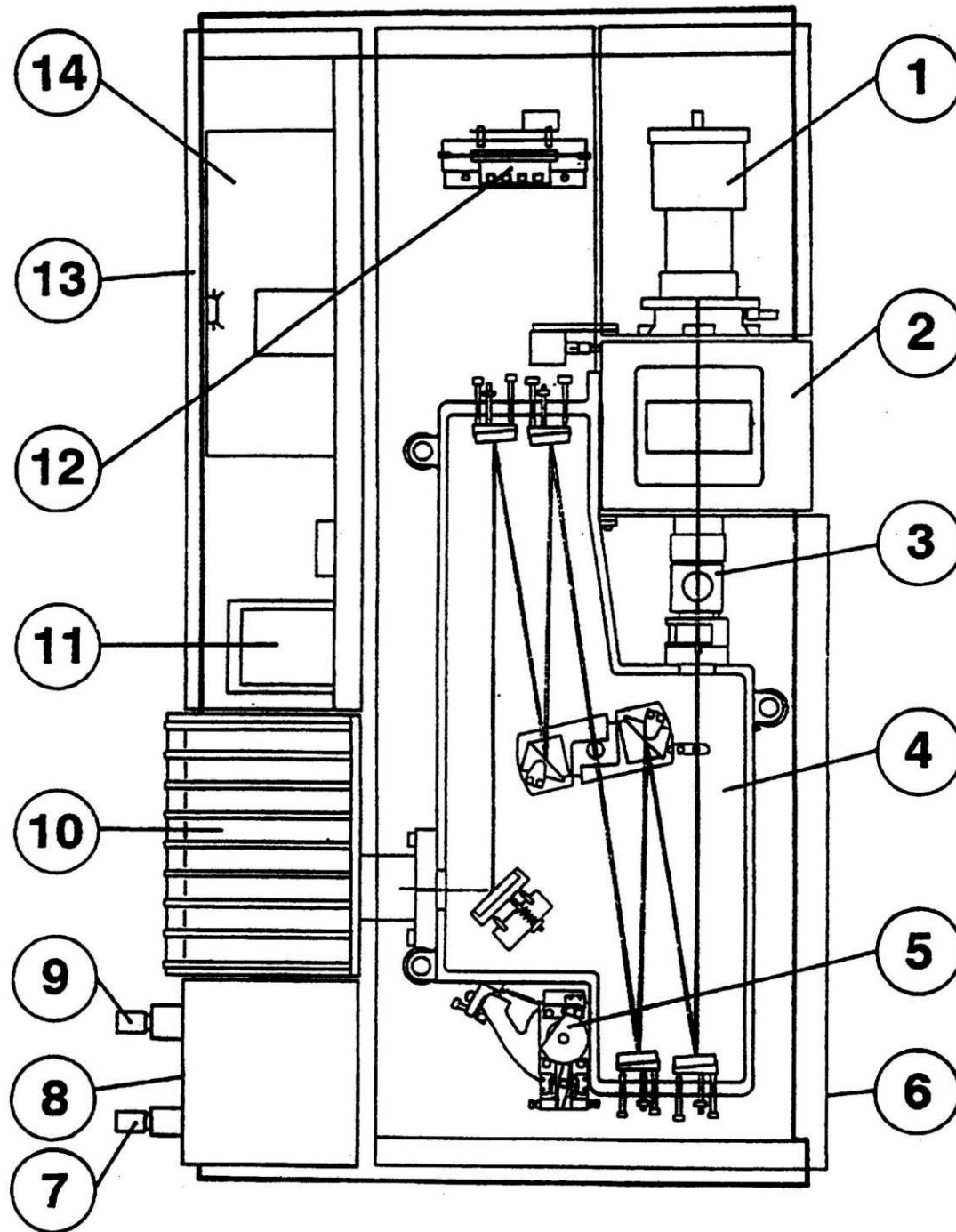
Snadná manipulace - titrace
přechody mezi různými strukturami
celý konformační prostor

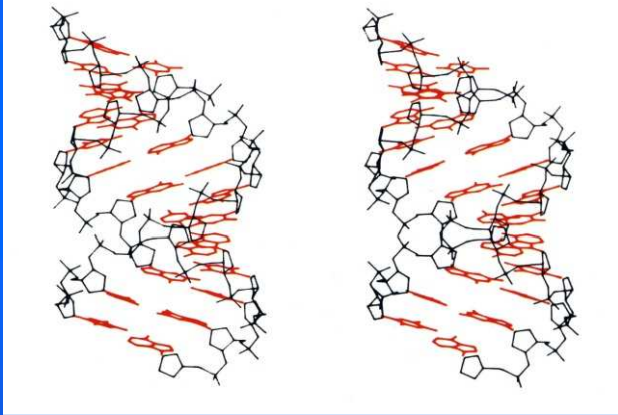
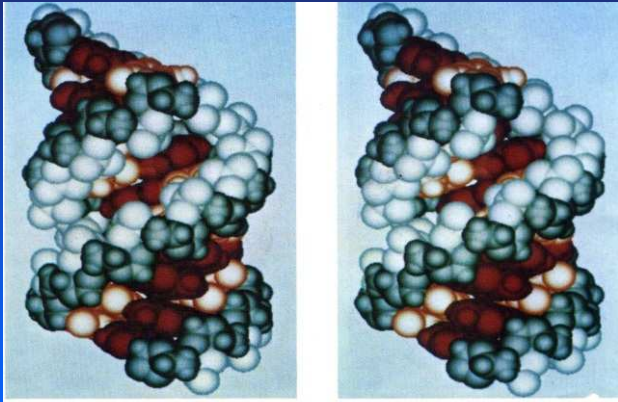
Rozlišení mezi kooperativními a nekooperativními změnami

Nevýhody

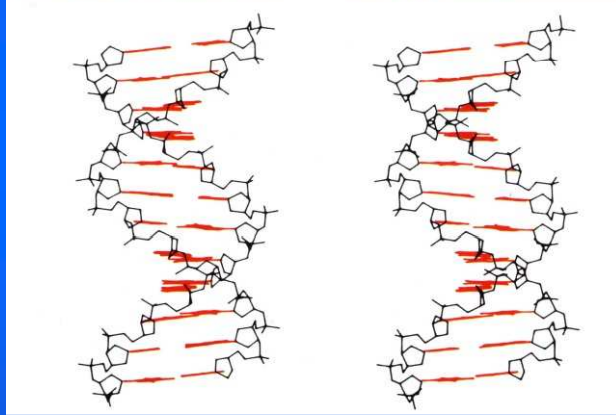
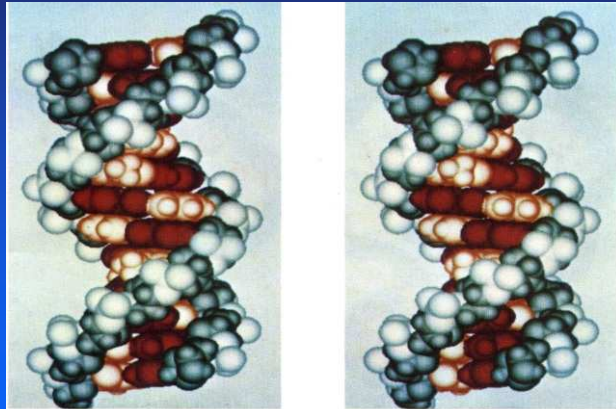
Pro složité molekuly jakými je DNA chybí explicitní vztah mezi spektrem CD a strukturou



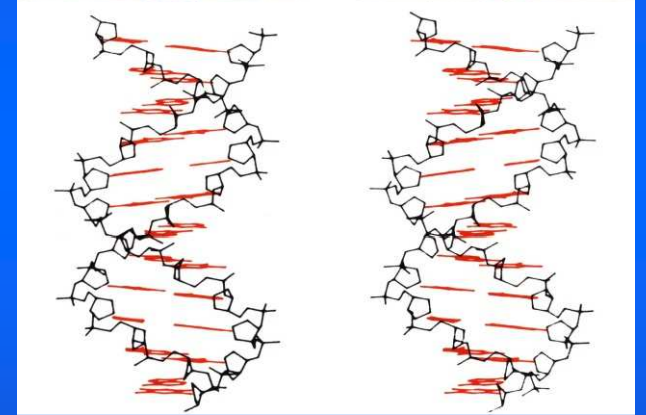
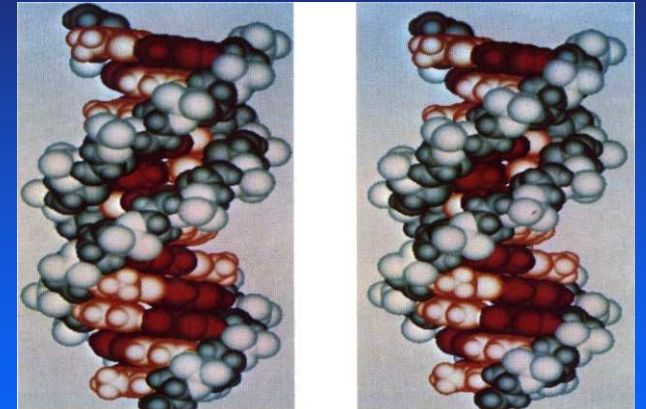




A

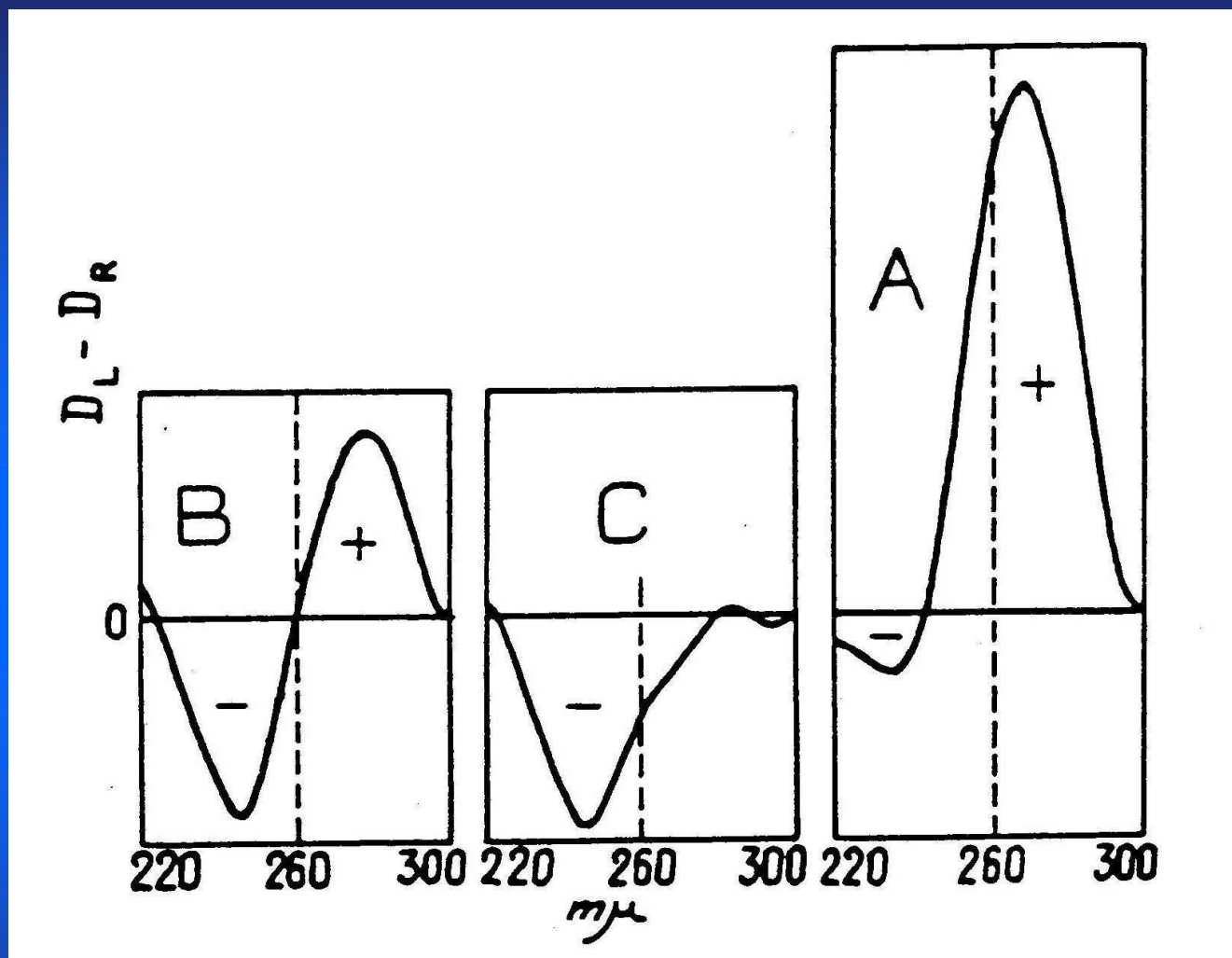


B



C,D,E,T



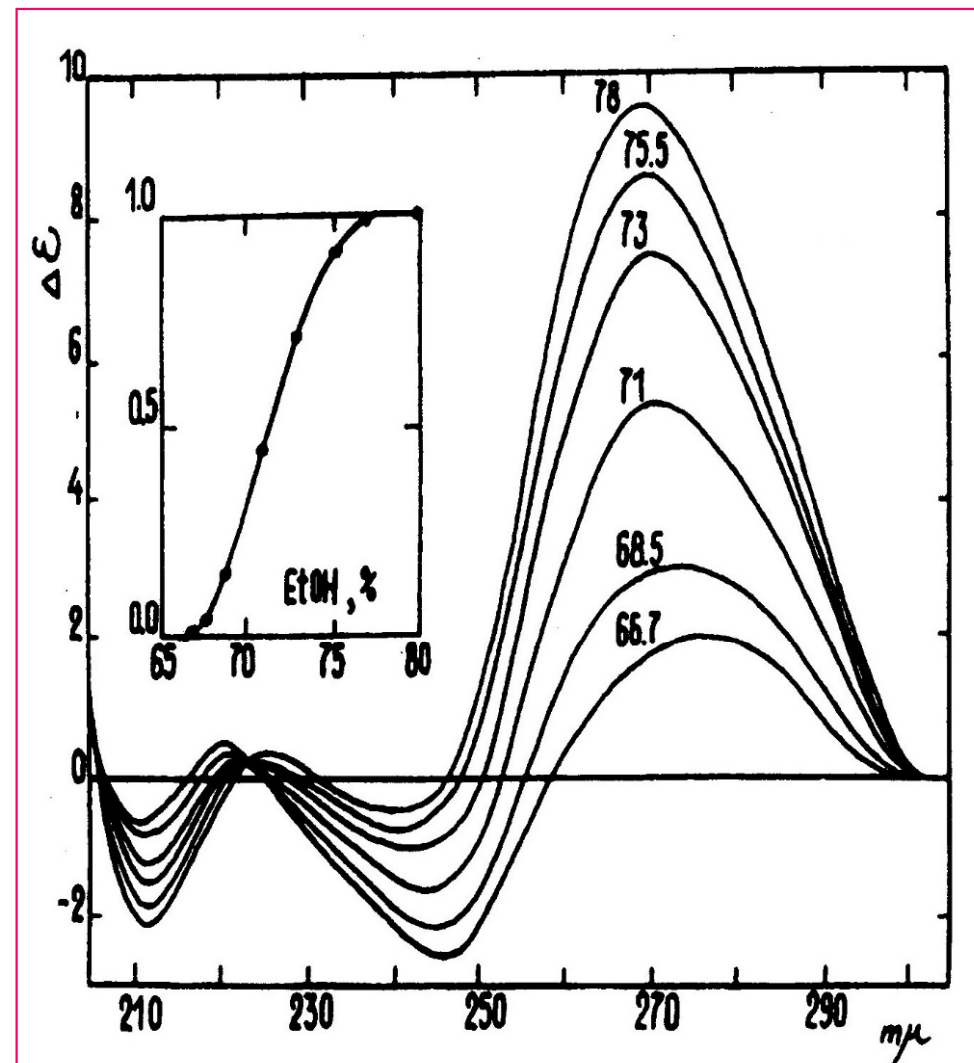
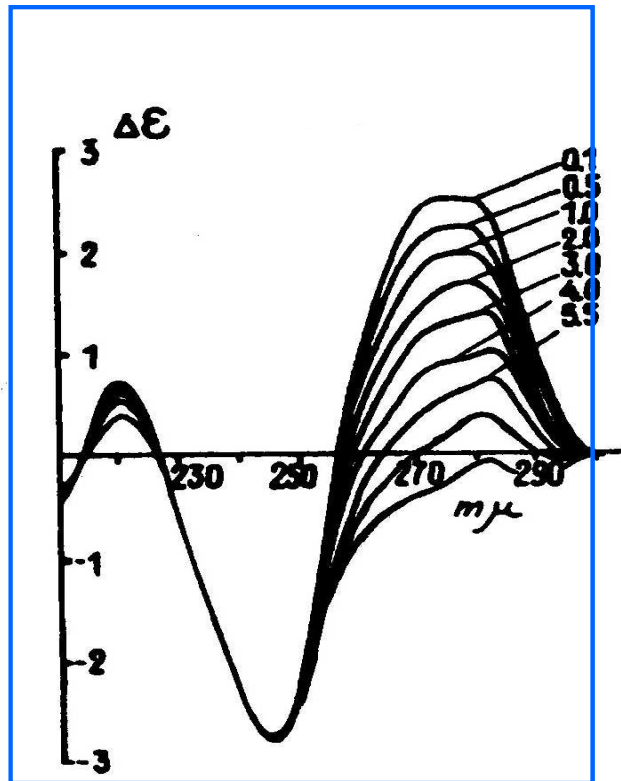


Tunis-Schneider, M.J.B. + Maestre, M.F.



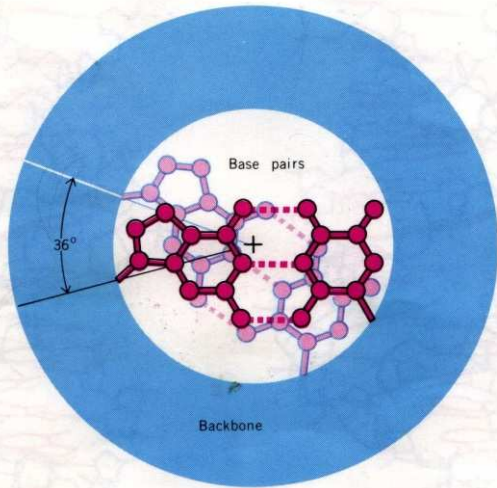
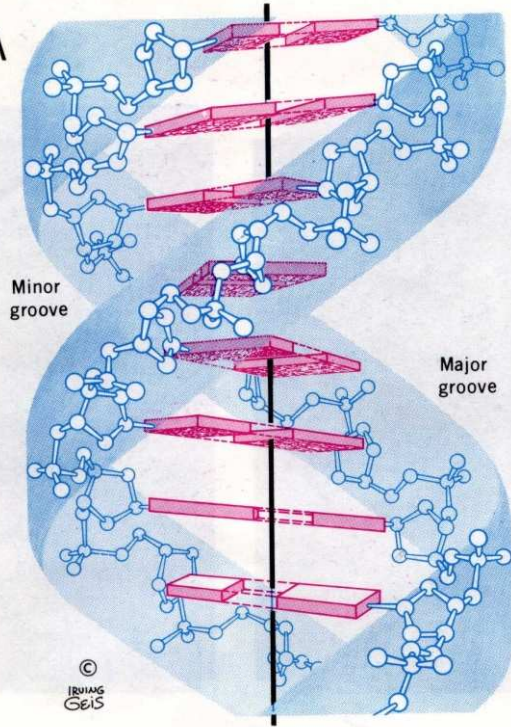
Příklady nekooperativních a kooperativních změn

Nekooperativní změny
v rámci téže struktury

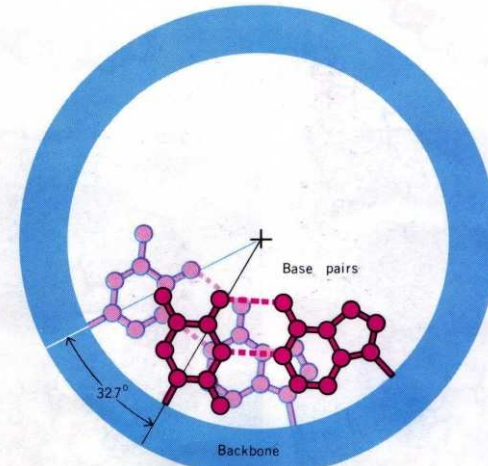
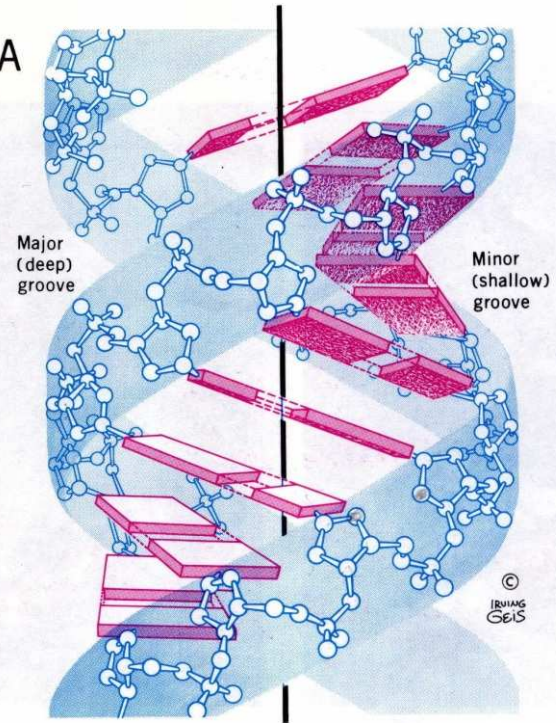


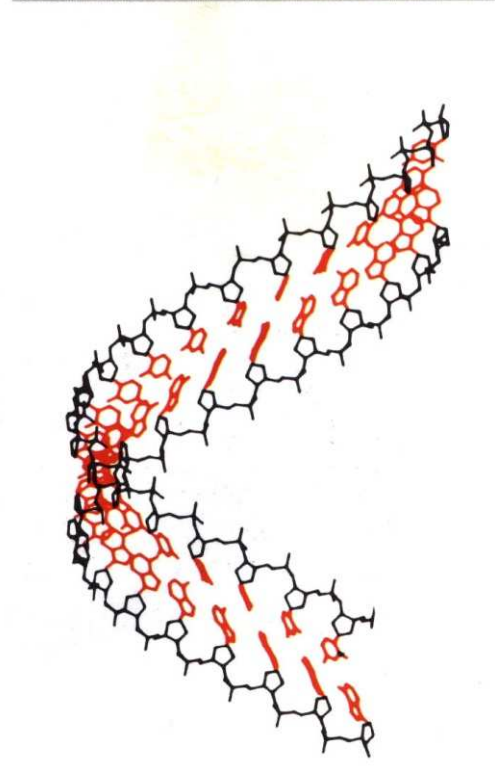
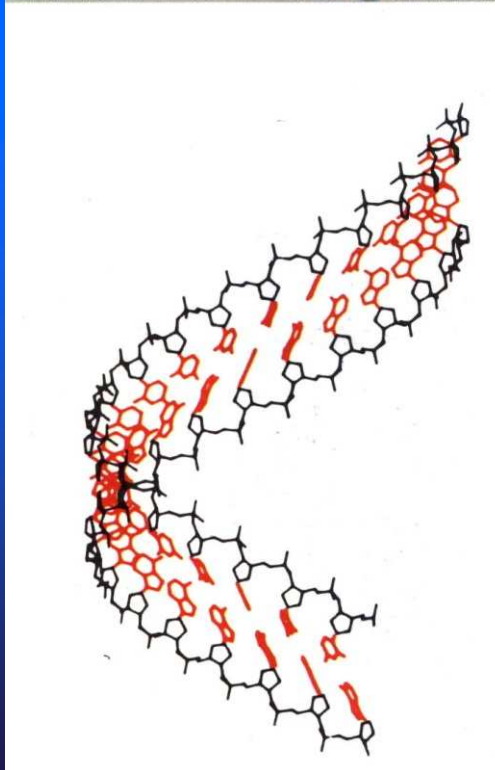
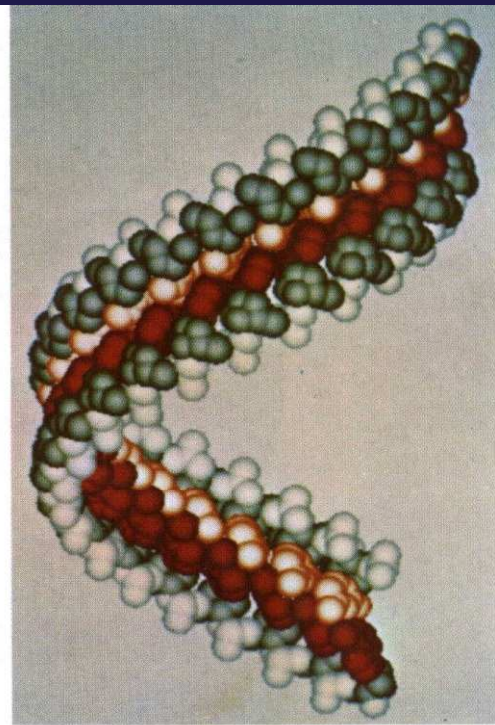
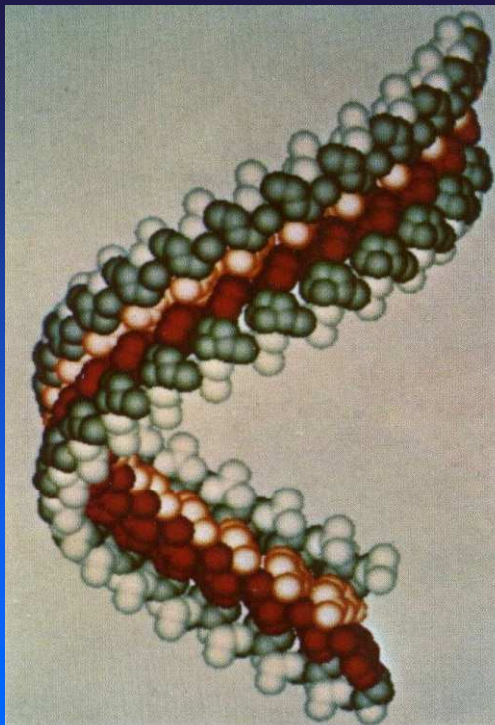
Kooperativní změny mezi diskrétními strukturami

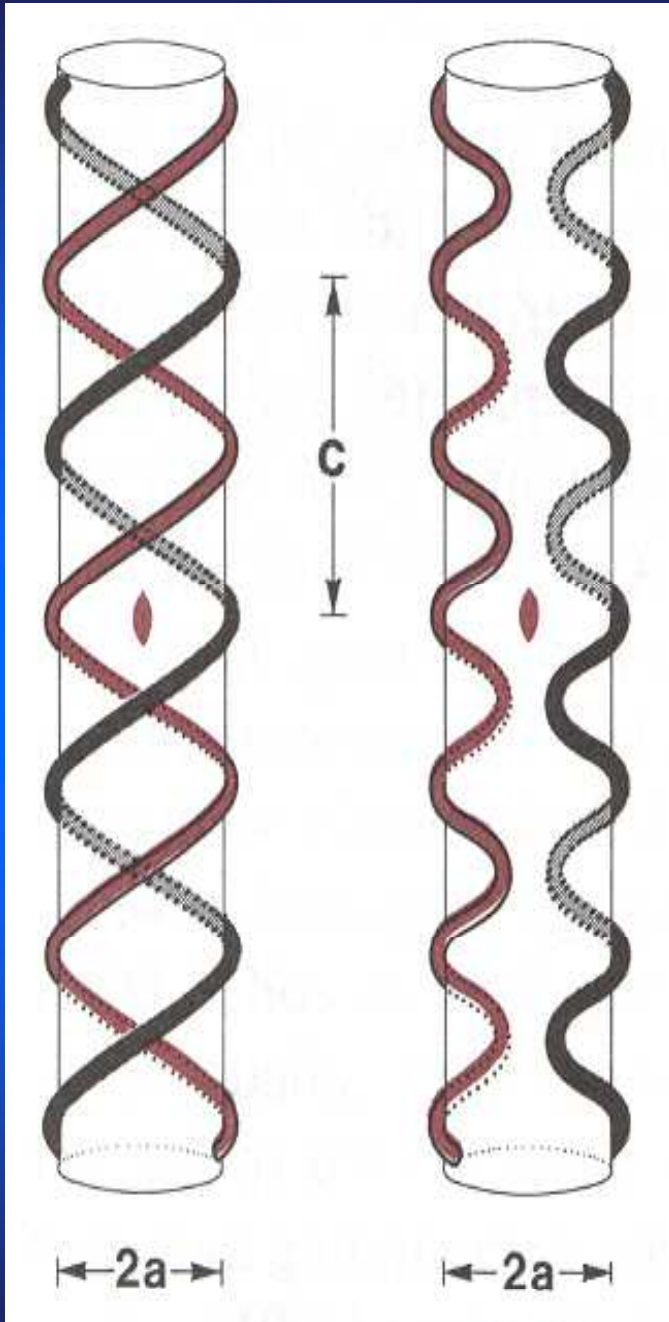
B DNA

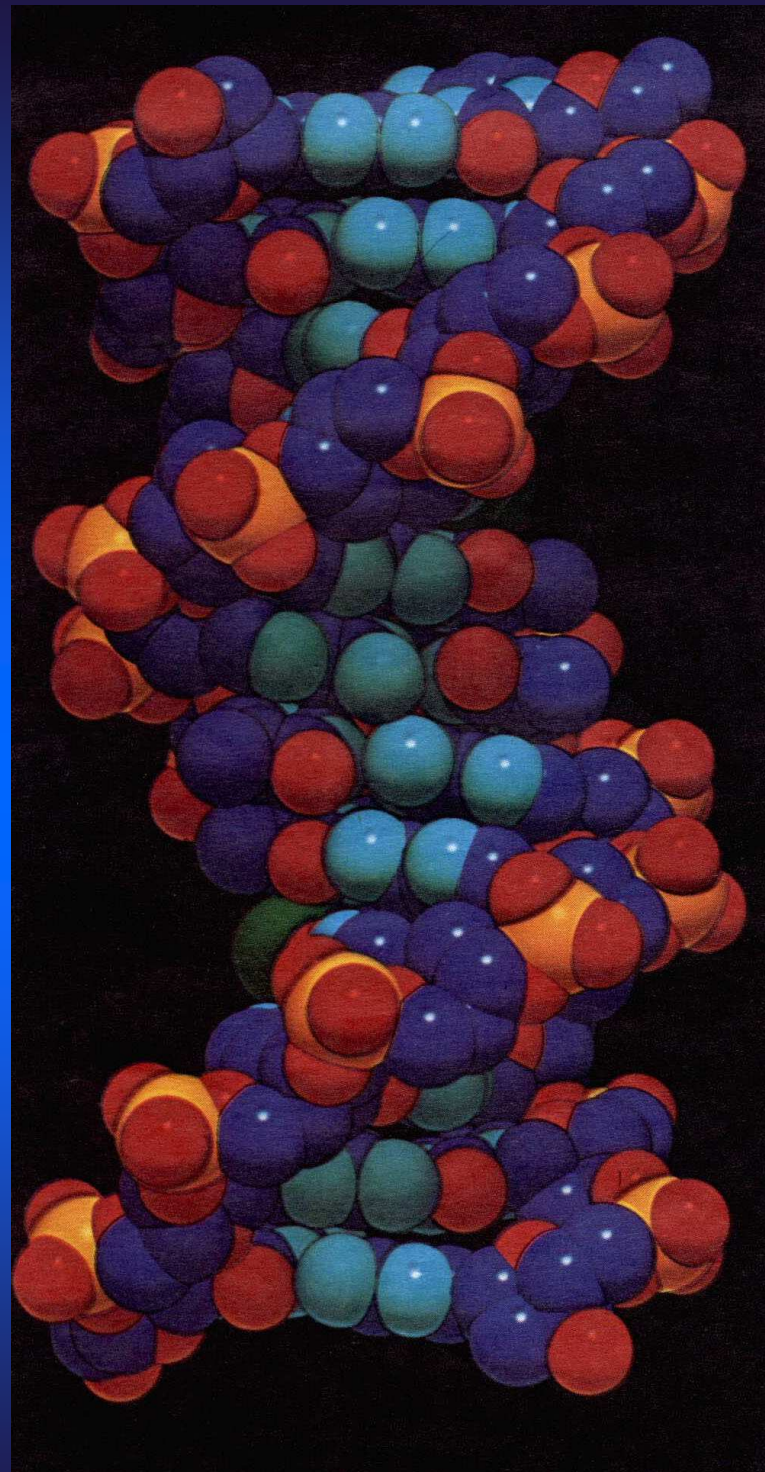


A DNA



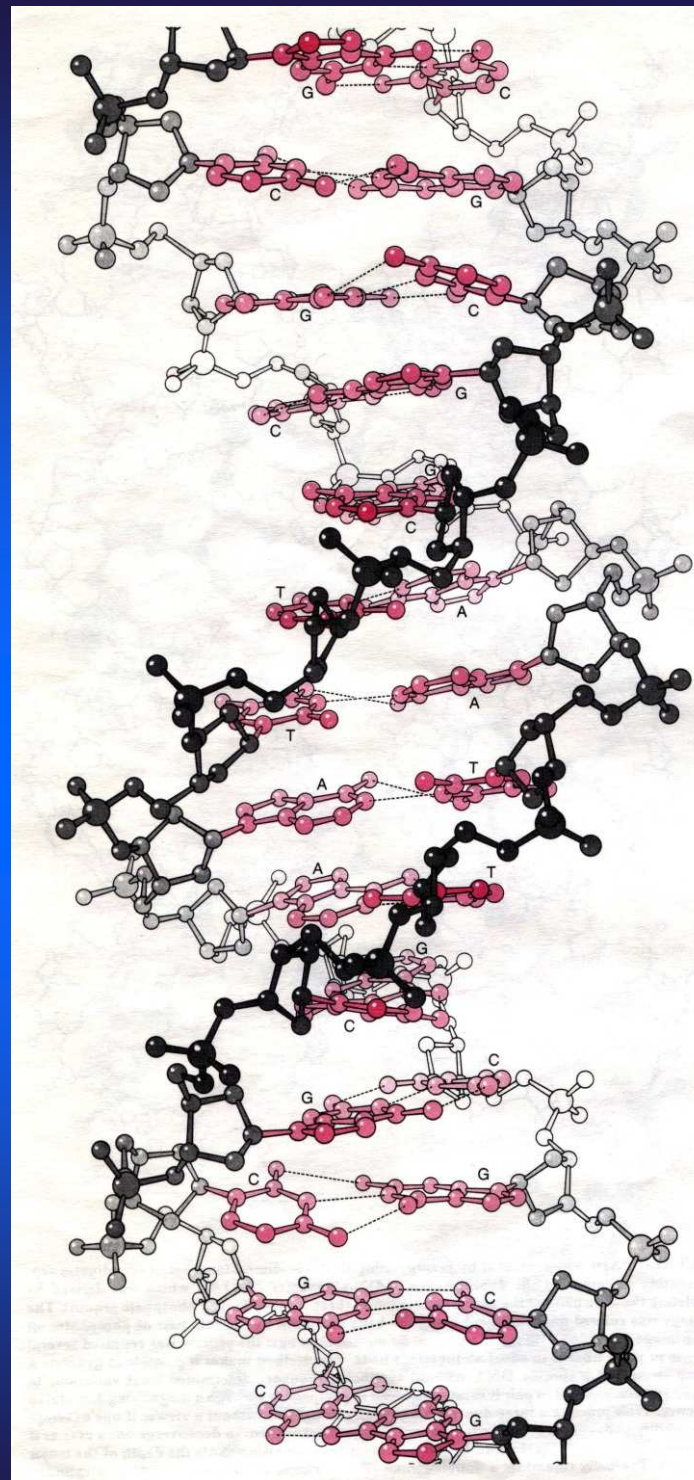


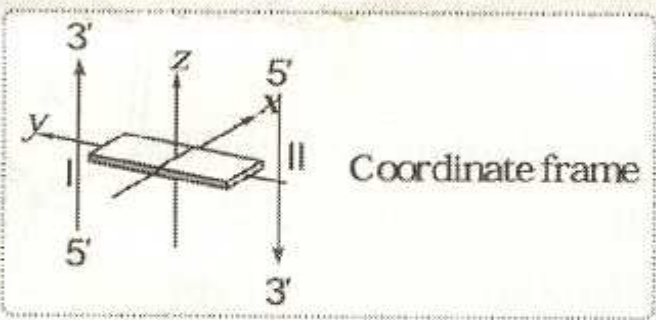
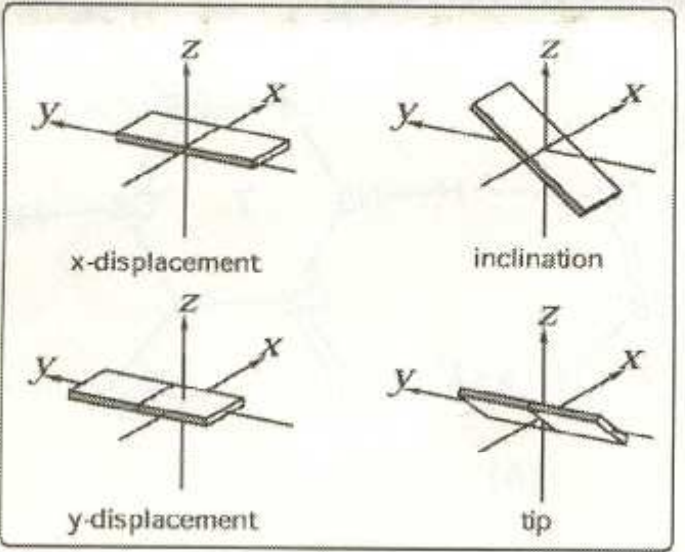
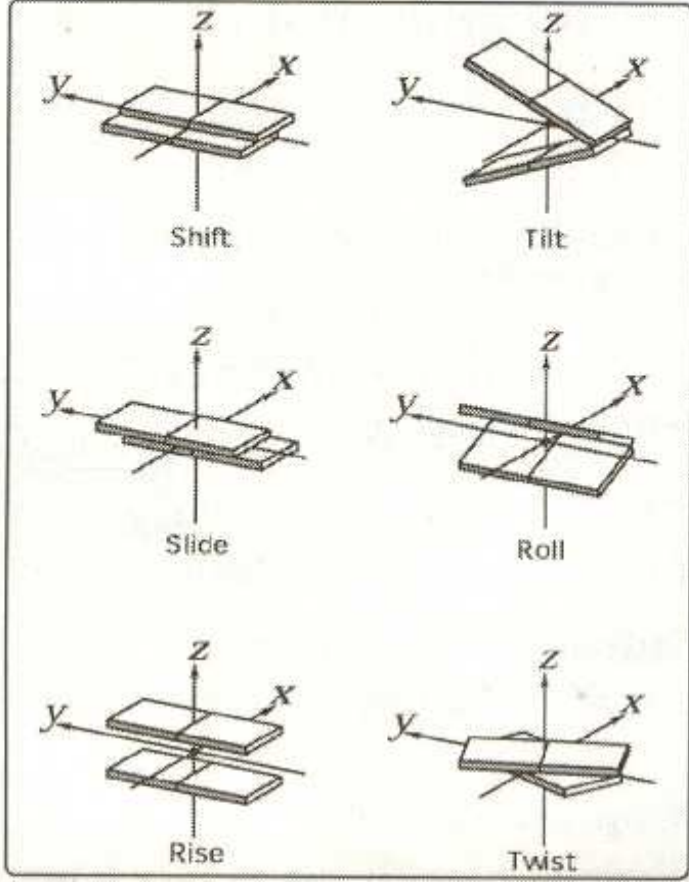
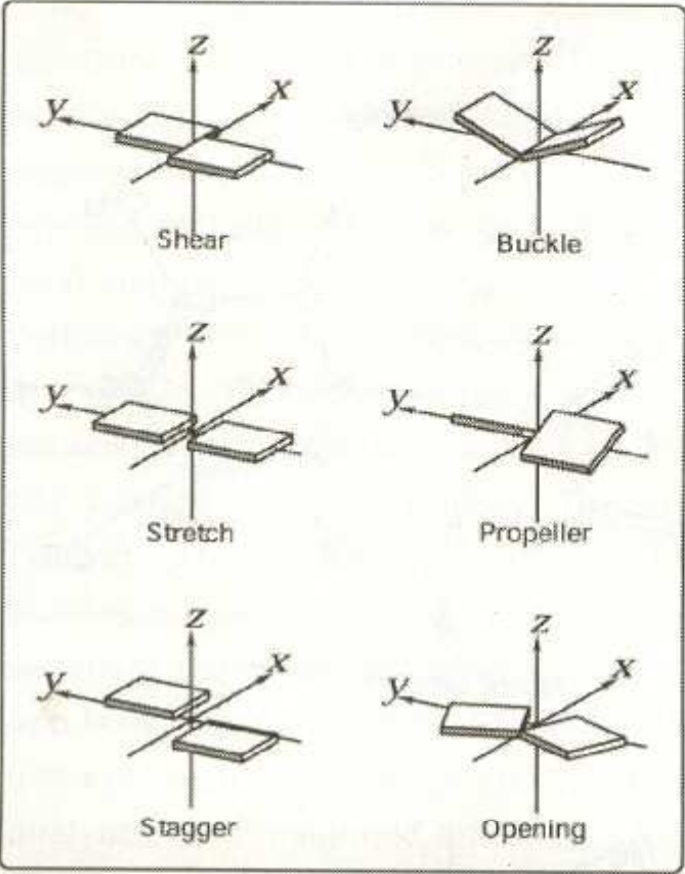




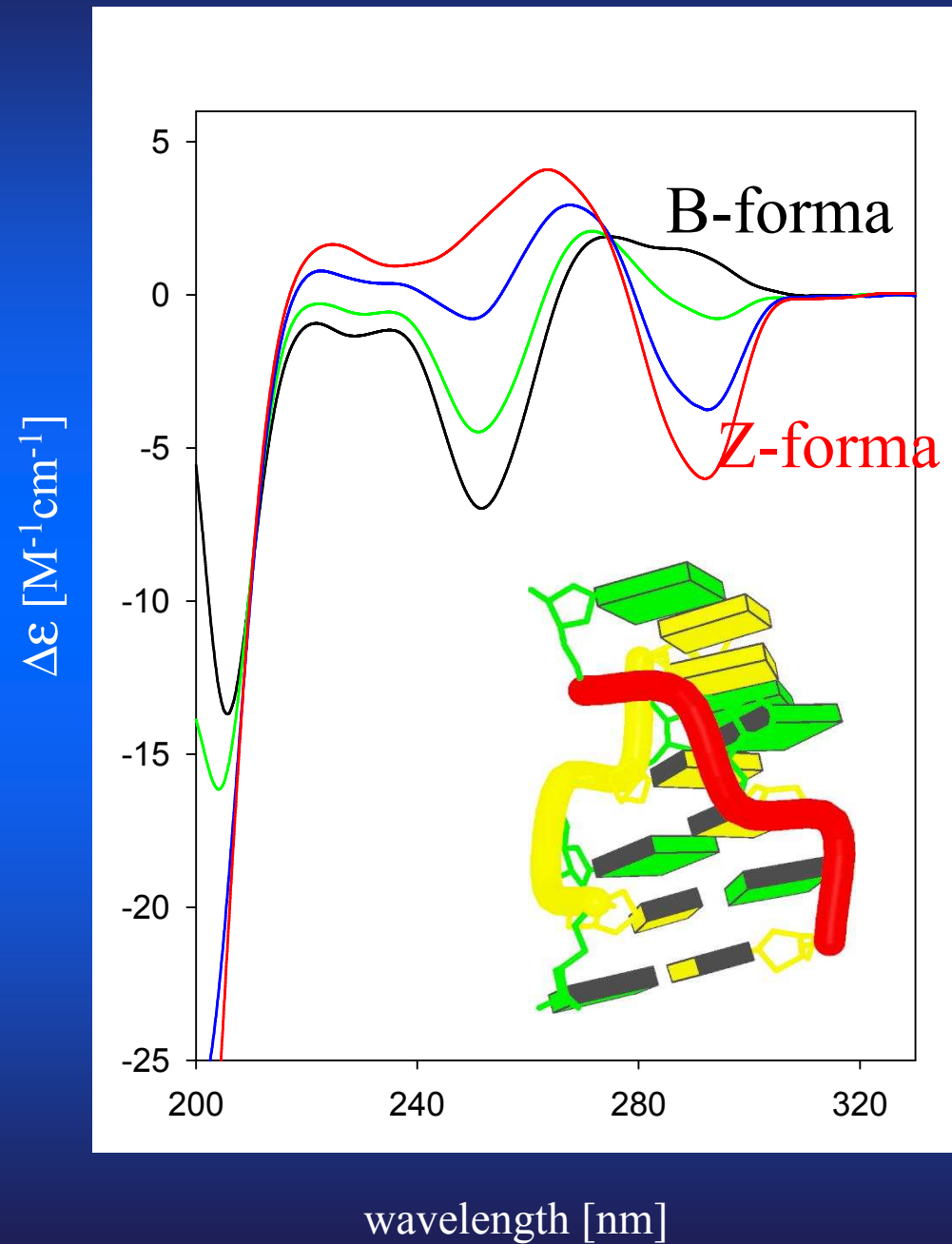
Dickerson

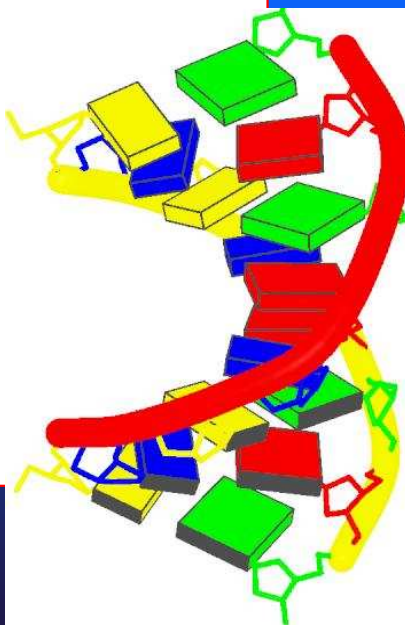
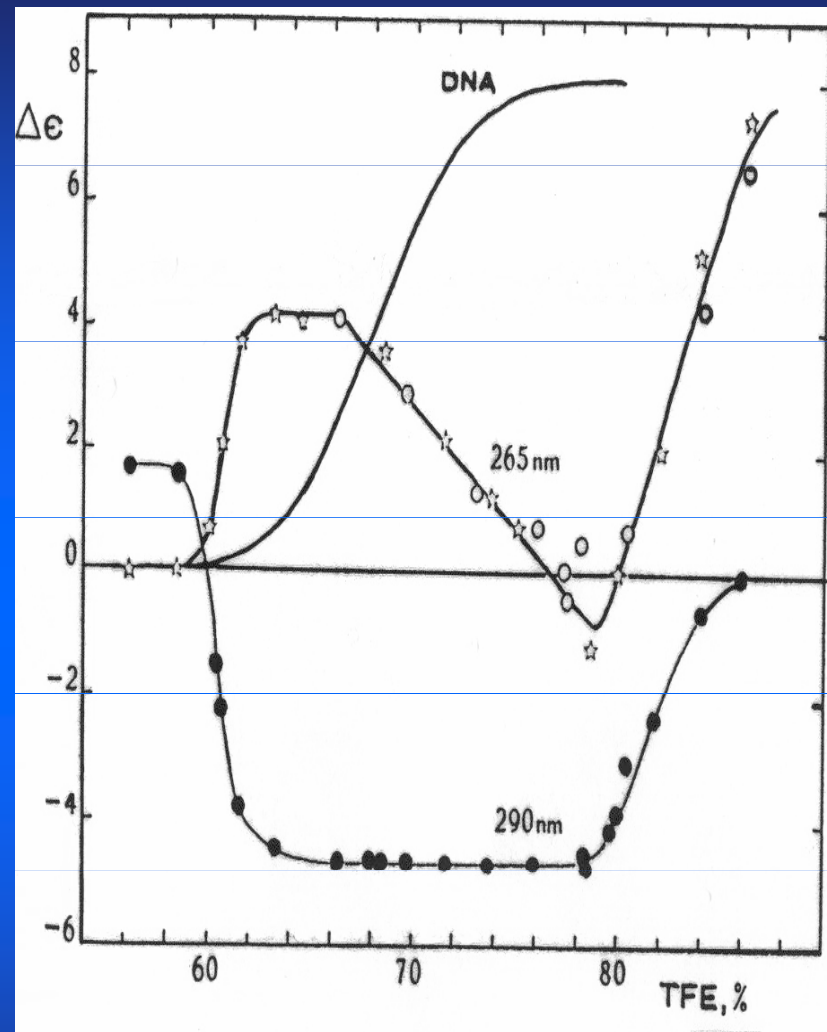
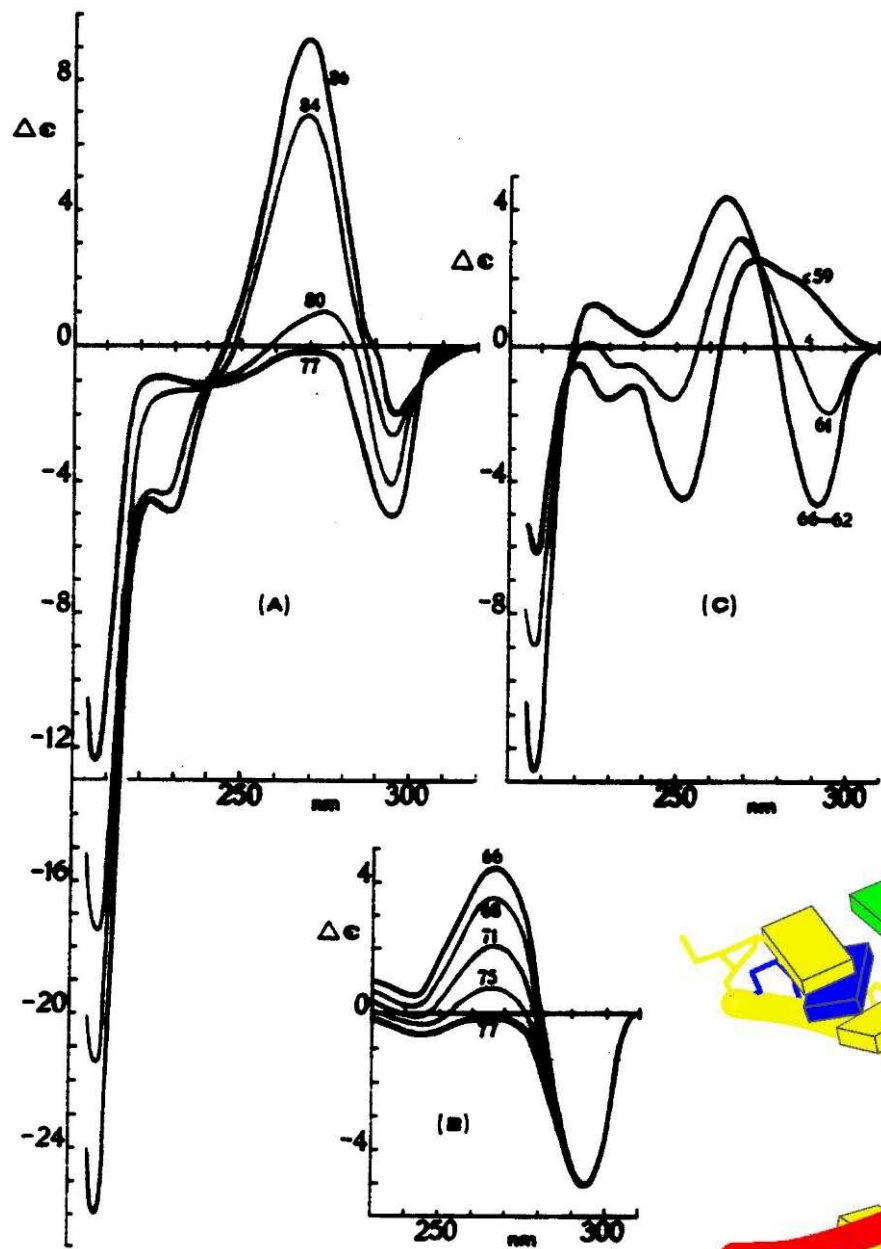
B



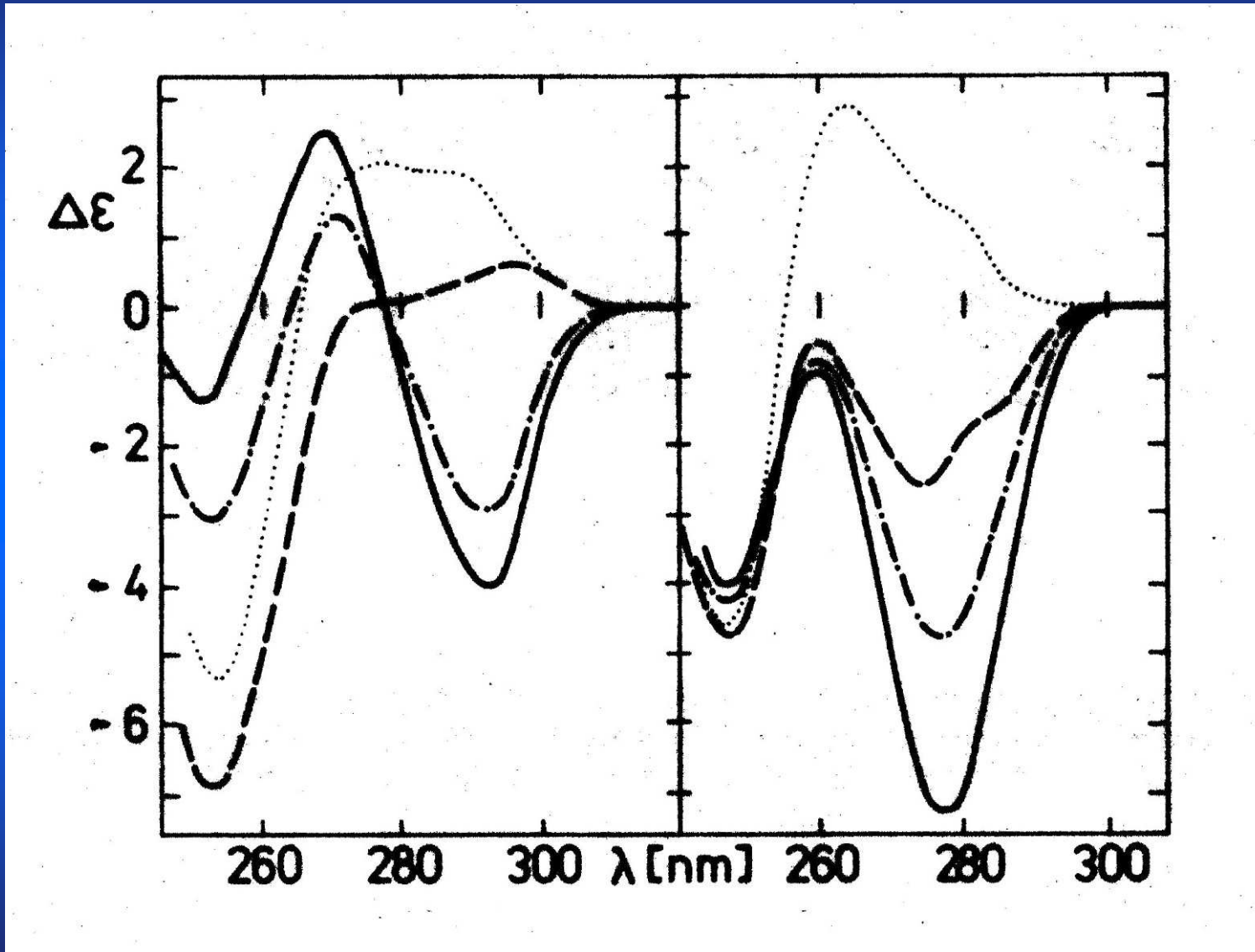


CD spectral changes accompanying B-Z transition of poly(dG-dC)

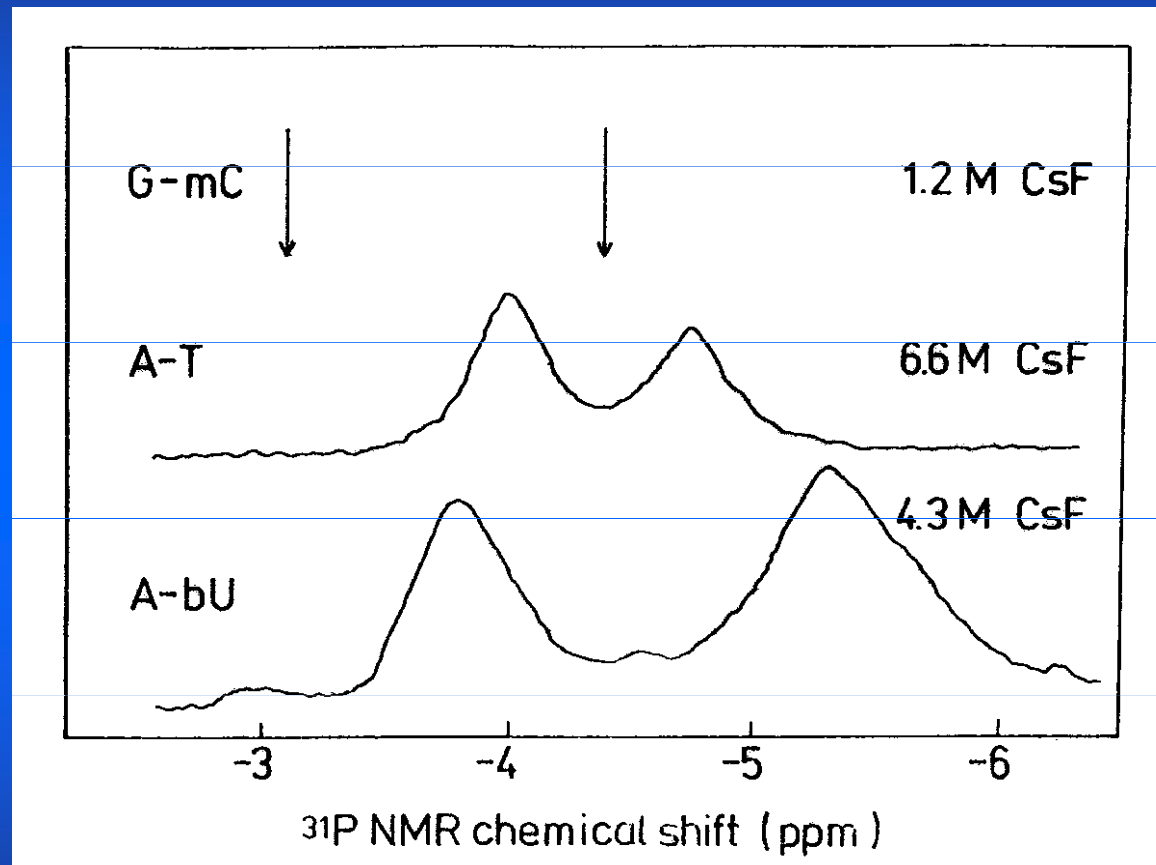




ATATATATATATATATATATATATATATATATATATA

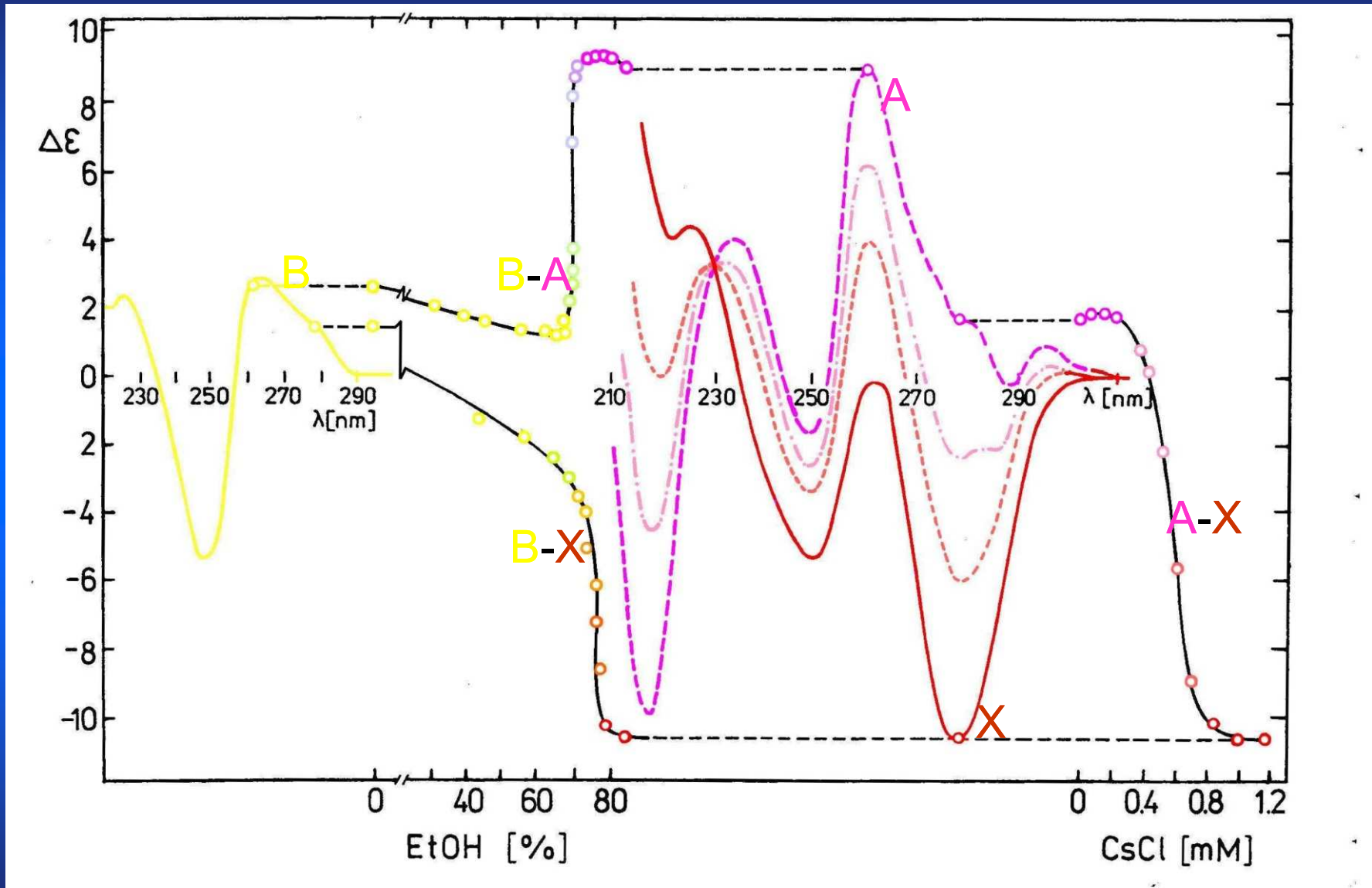


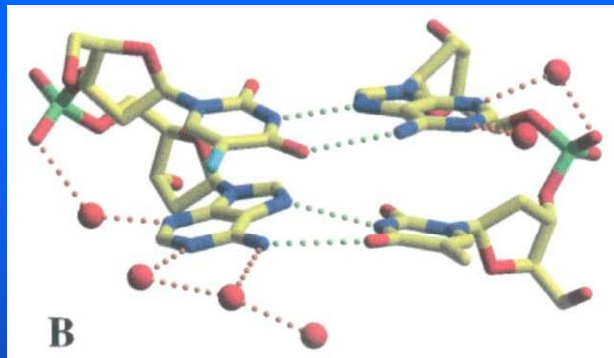
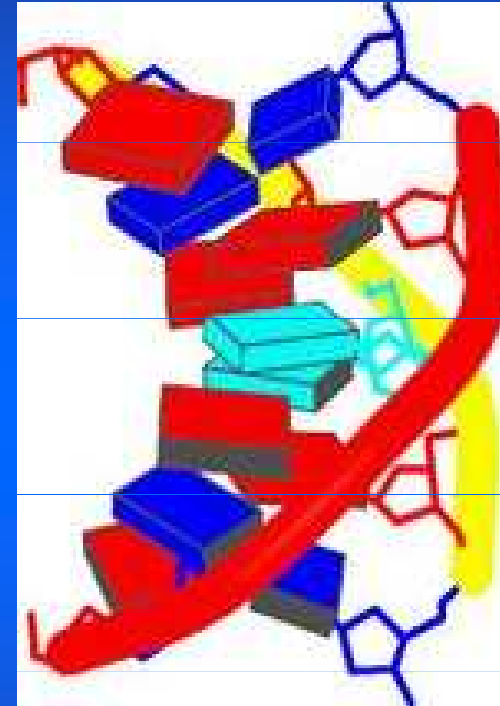
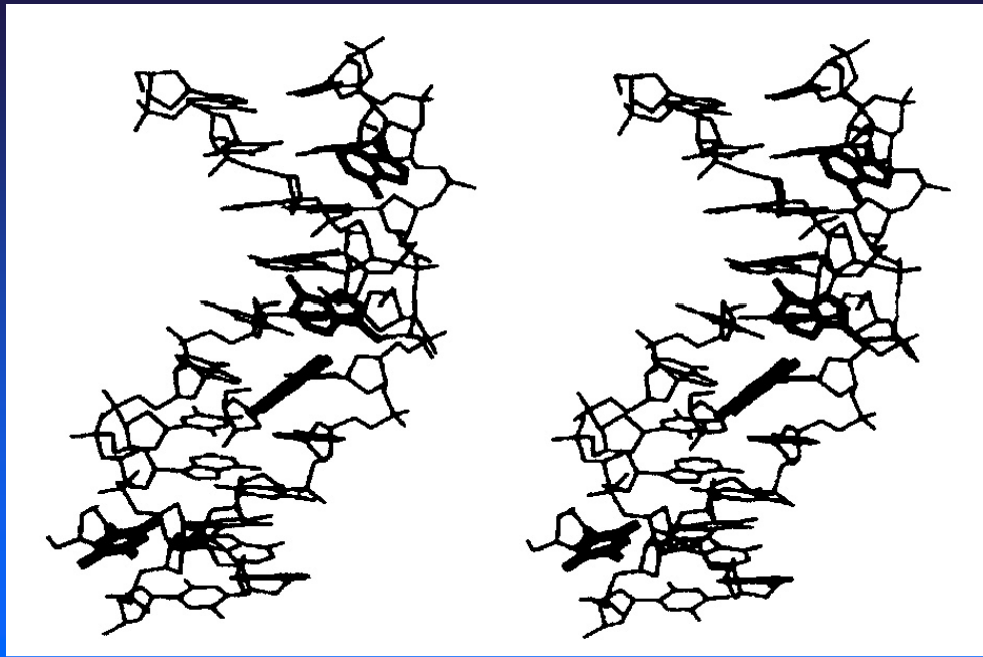
ATATATATATATATATATATATATATATATATATATA



X-DNA







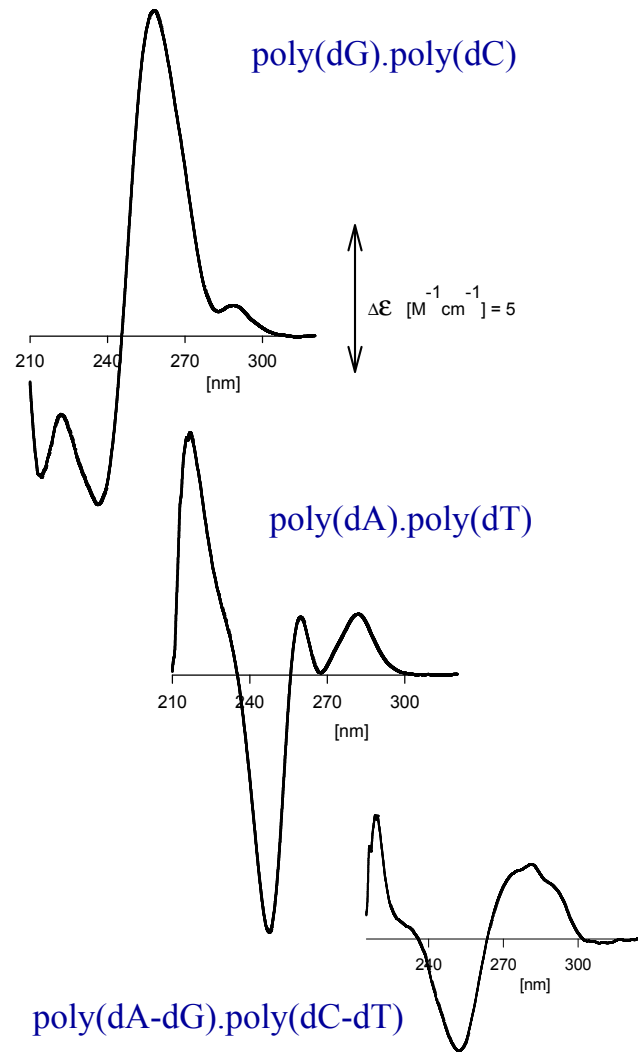
ALTERNATING A-T FRAGMENT WITH HOOGSTEN BASE PAIRING

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



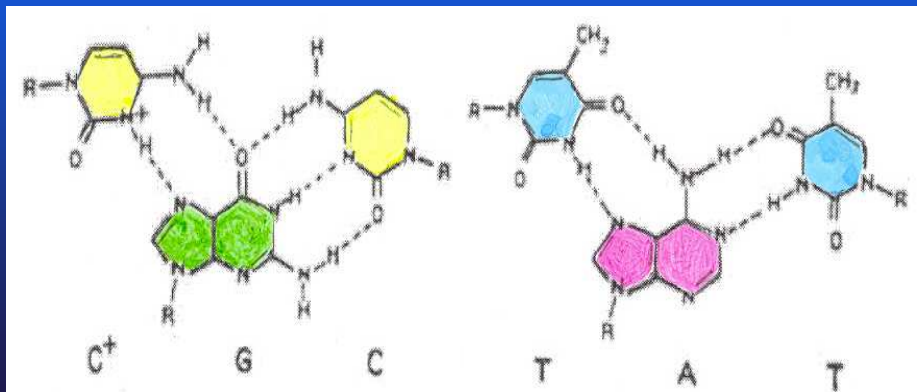
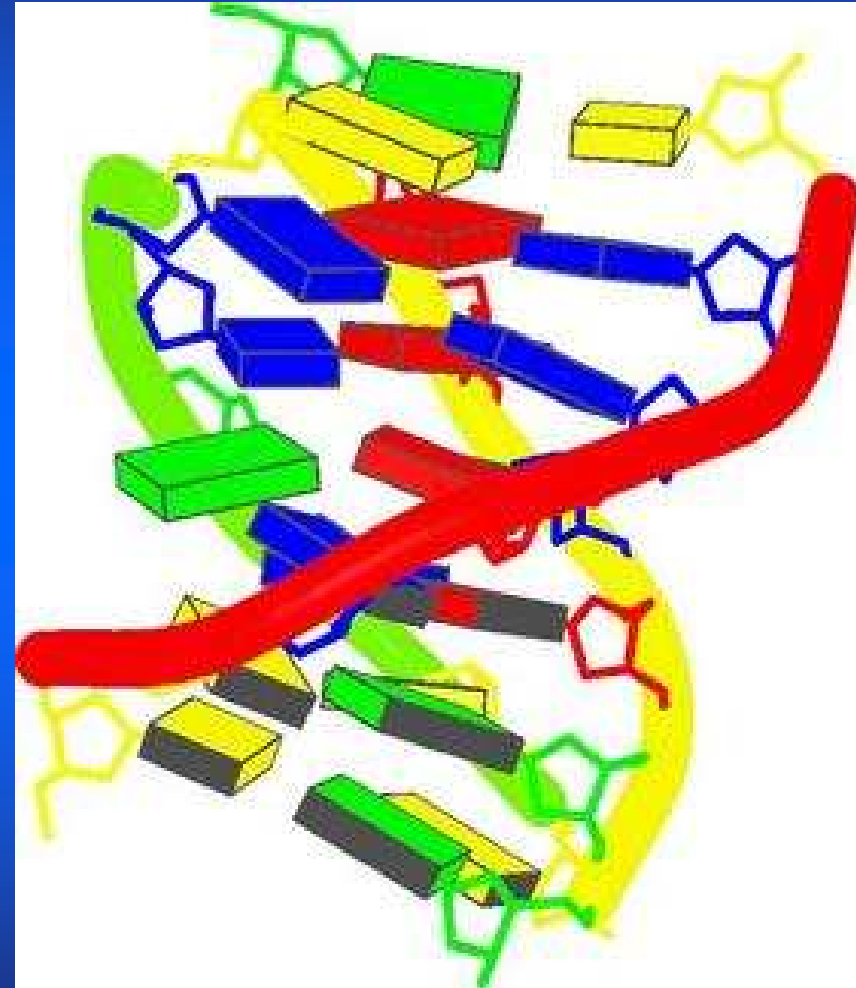
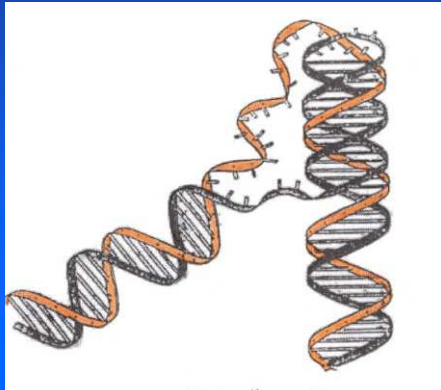
Alternating (Pu-Py)_n

(Pu)_n · (Py)_n complexes



DNA Triplex

Pyrimidine. Purine. Pyrimidine

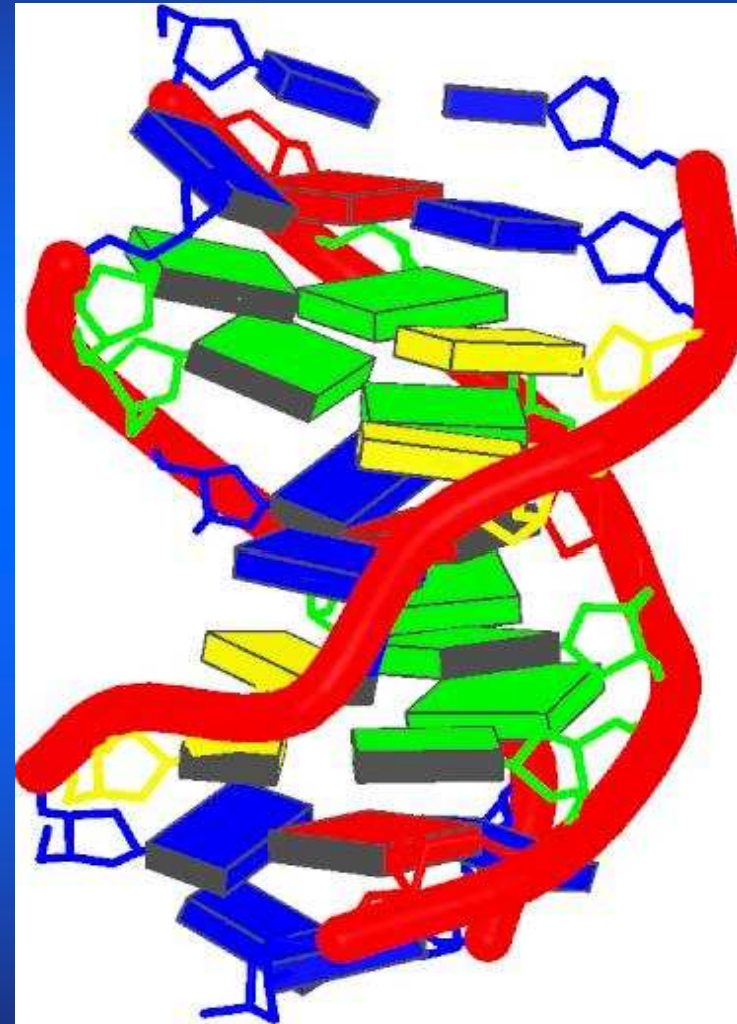
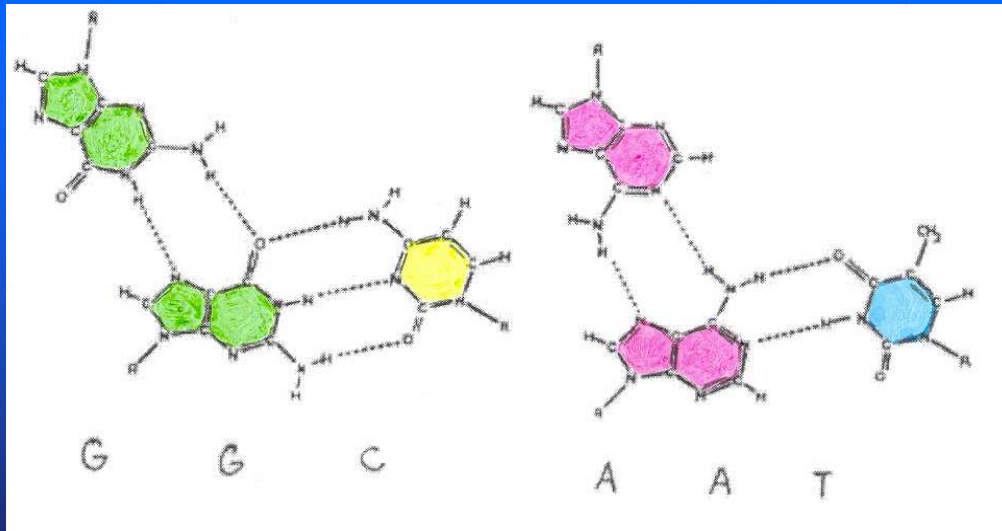


Radhakrishnan, I., Patel, D.J. (1994)



DNA TRIPLEX

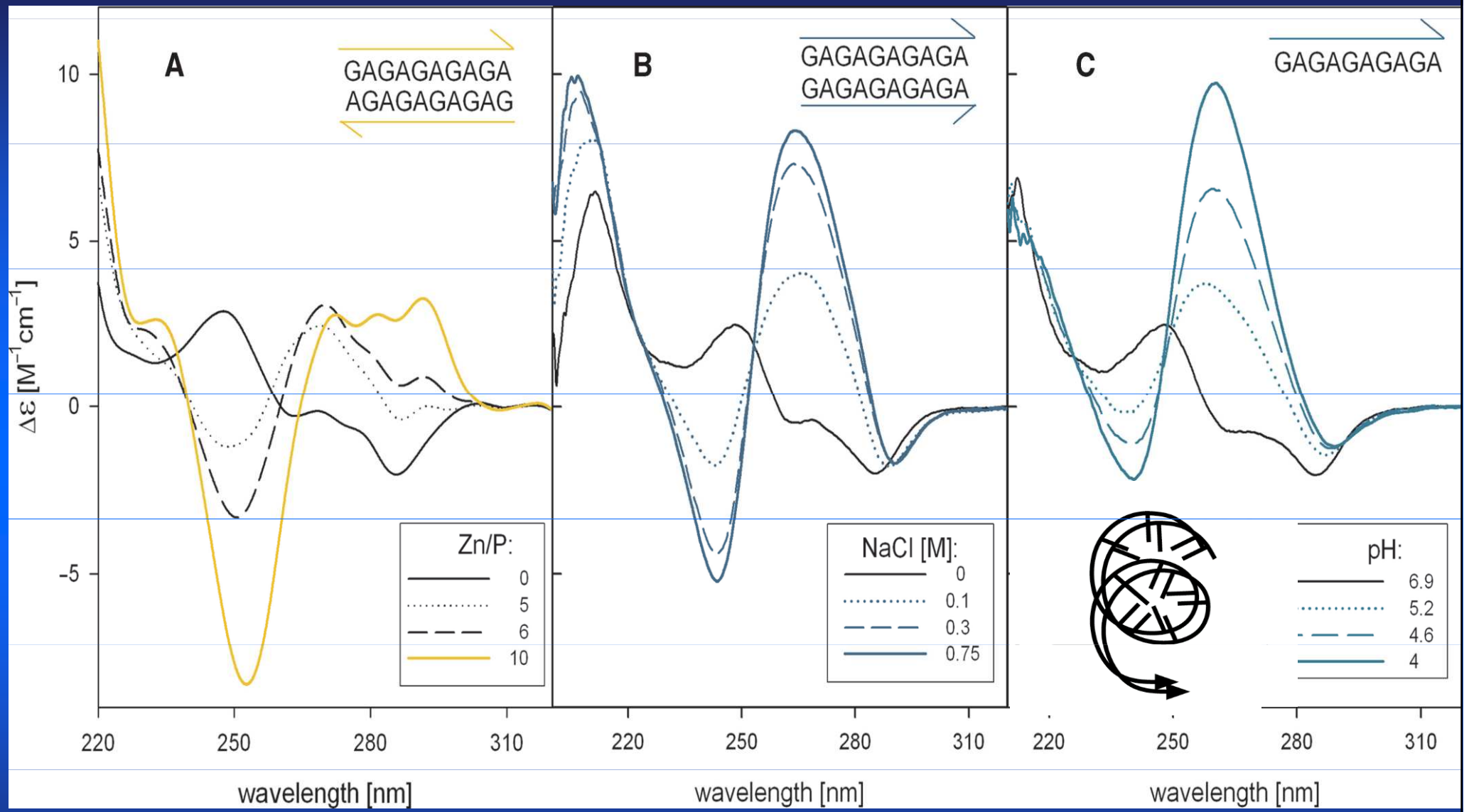
TCCTCCTTTT TAGGAGGATTTT TGGTGGT



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

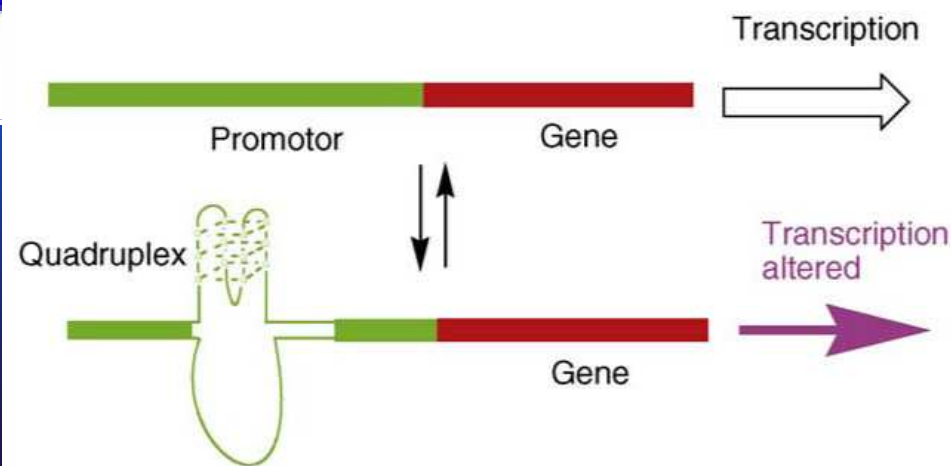
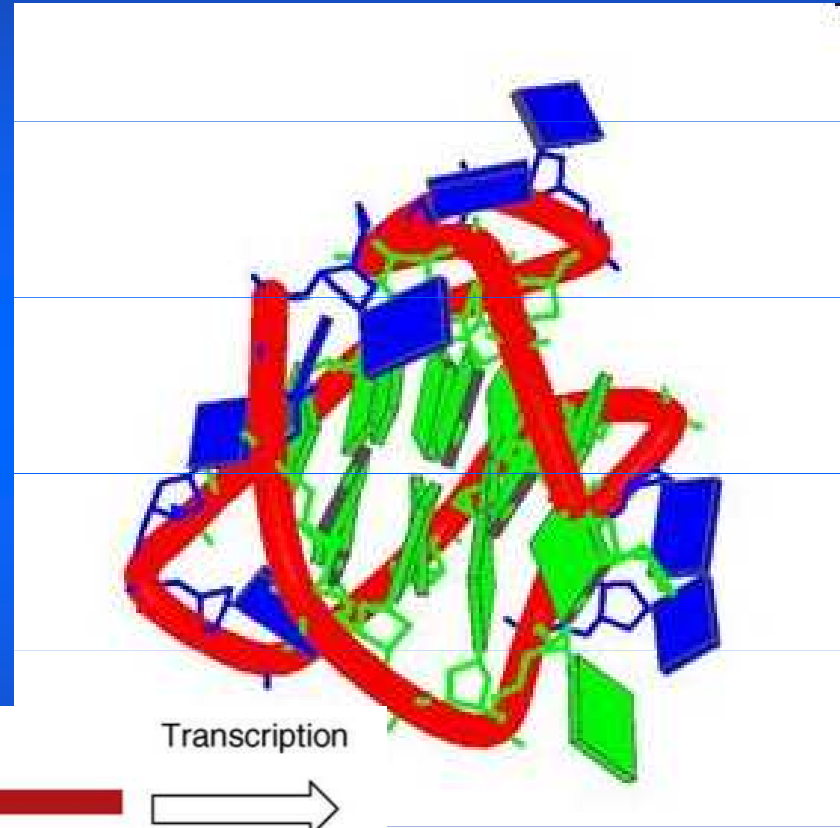
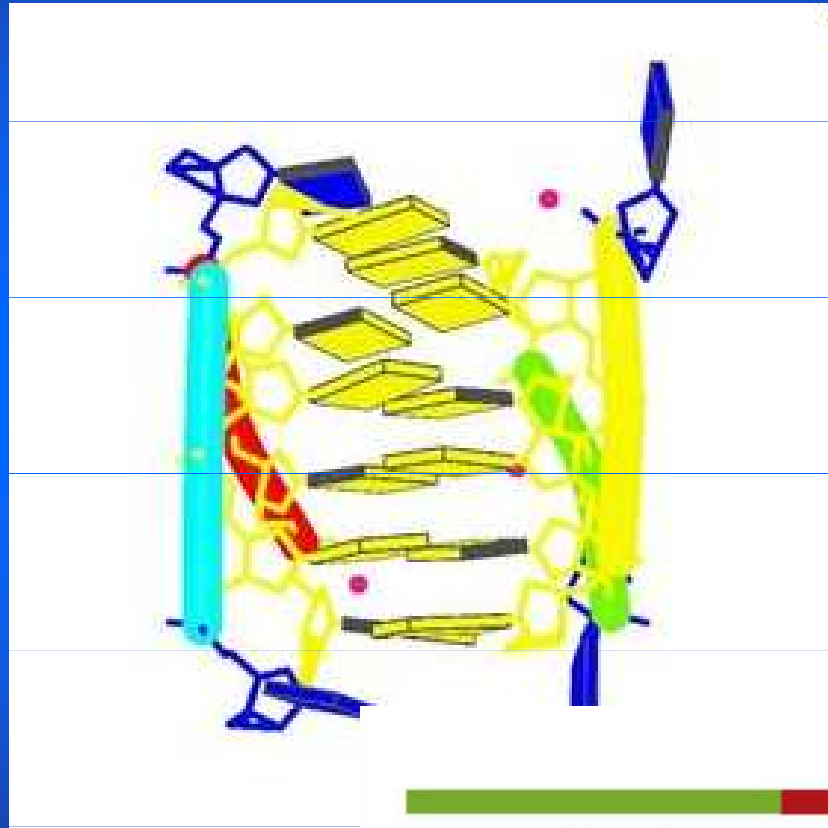
Pyrimidine. Purine. Purine





Quadruplexes

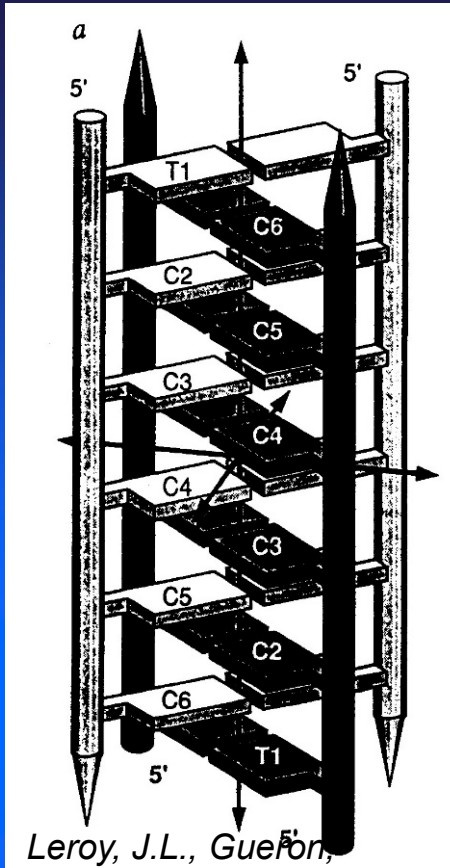
frequently occur in promoters of genes and were shown to control their expression.



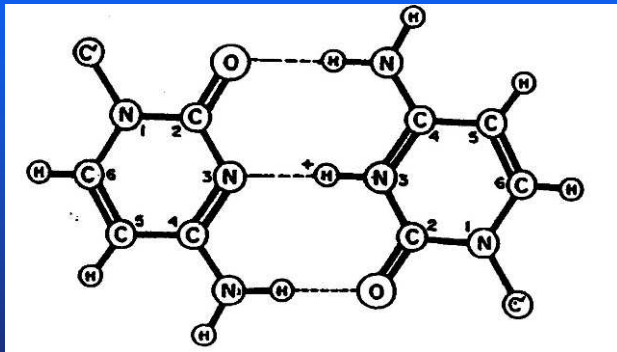
Wang, Y., Patel, D.J. (1994)



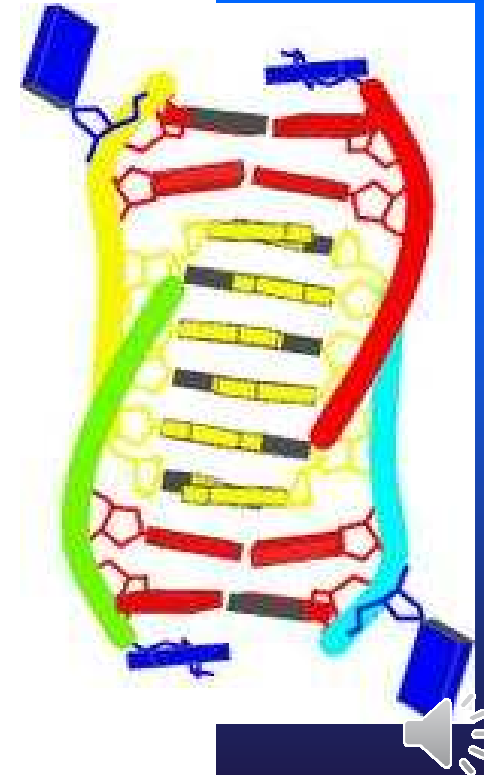
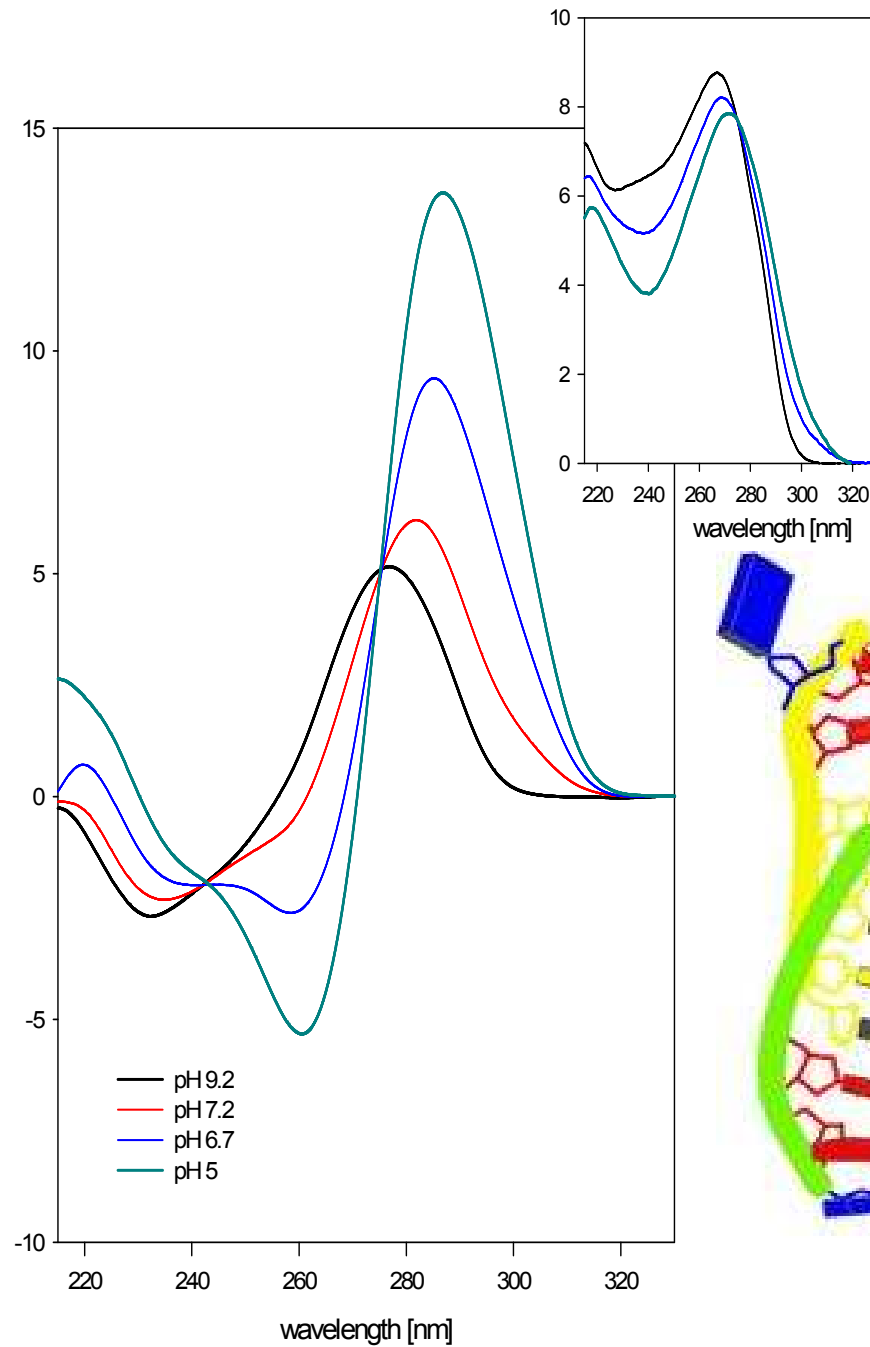
i - motif

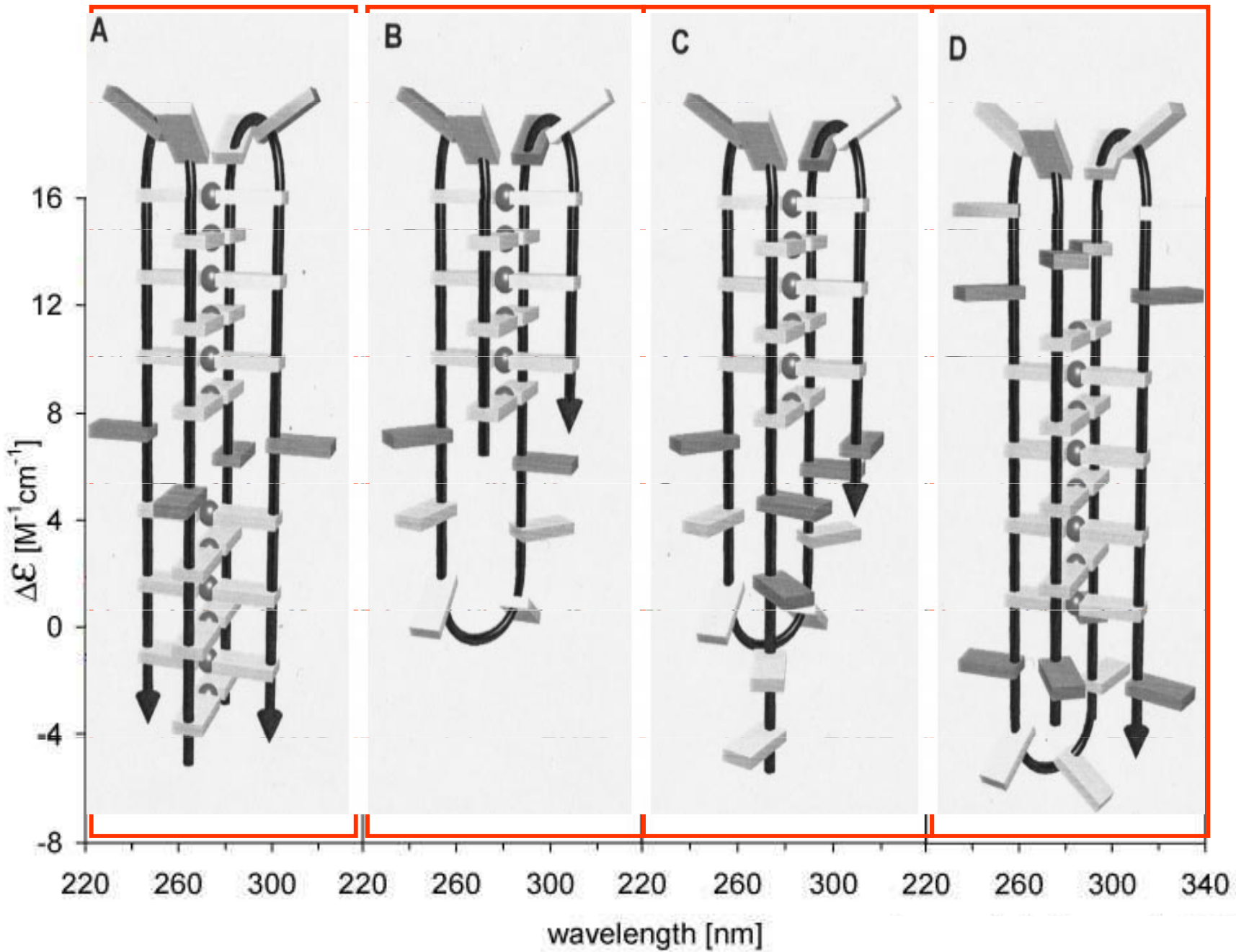


Leroy, J.L., Guéron, M., 1995



Two parallel-bonded duplexes are intercalated in the antiparallel fashion

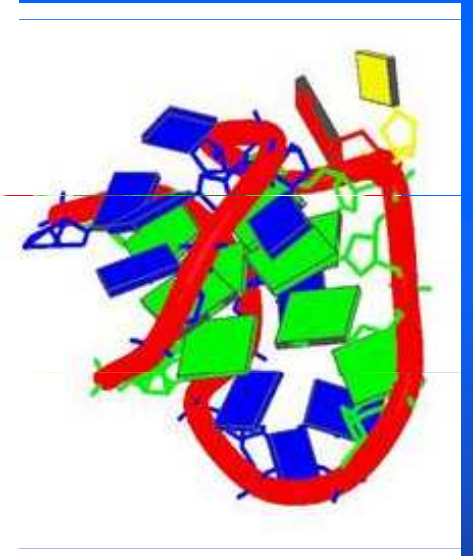
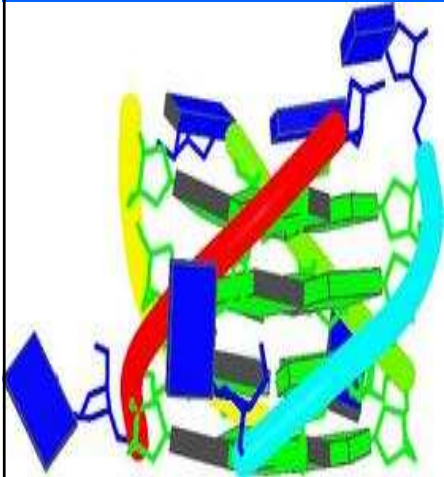
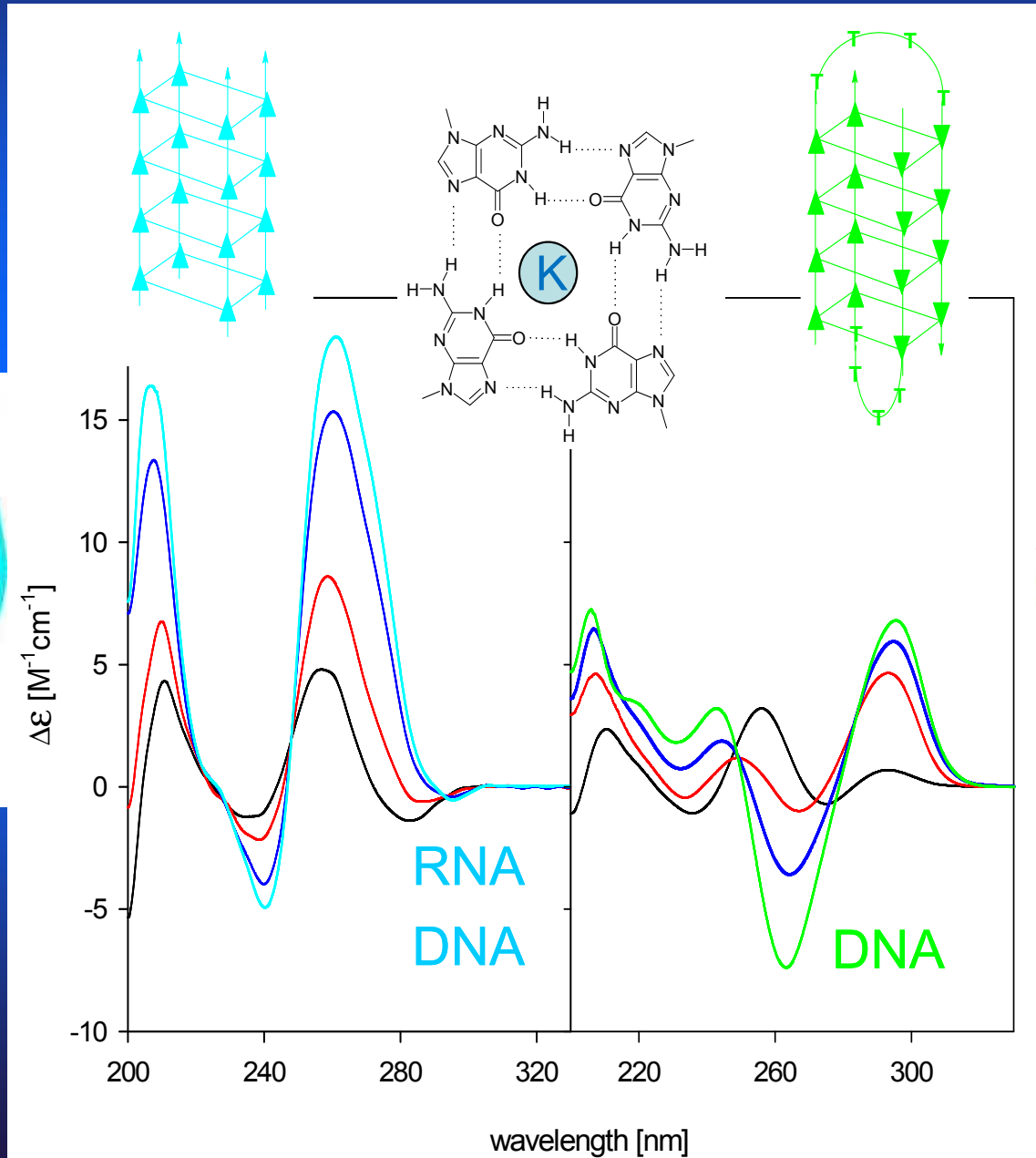




TCCCA CCTTCCCA CCCTCCCC ACCCTCCCA

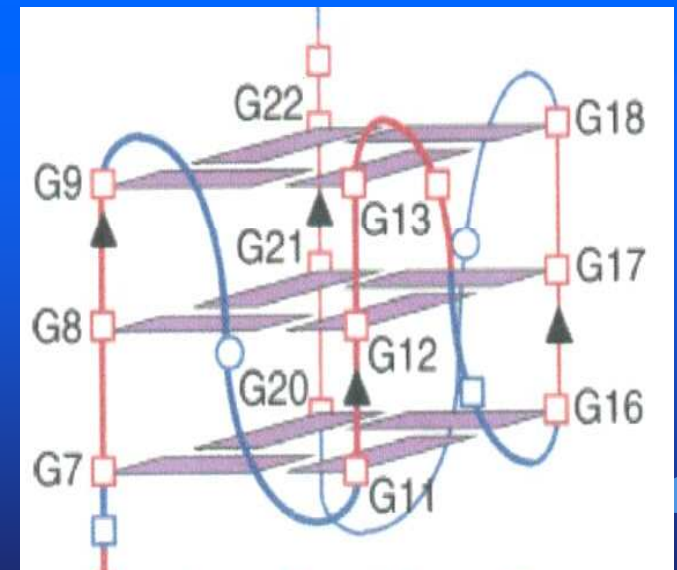
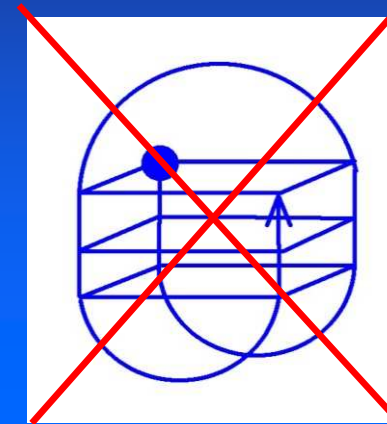
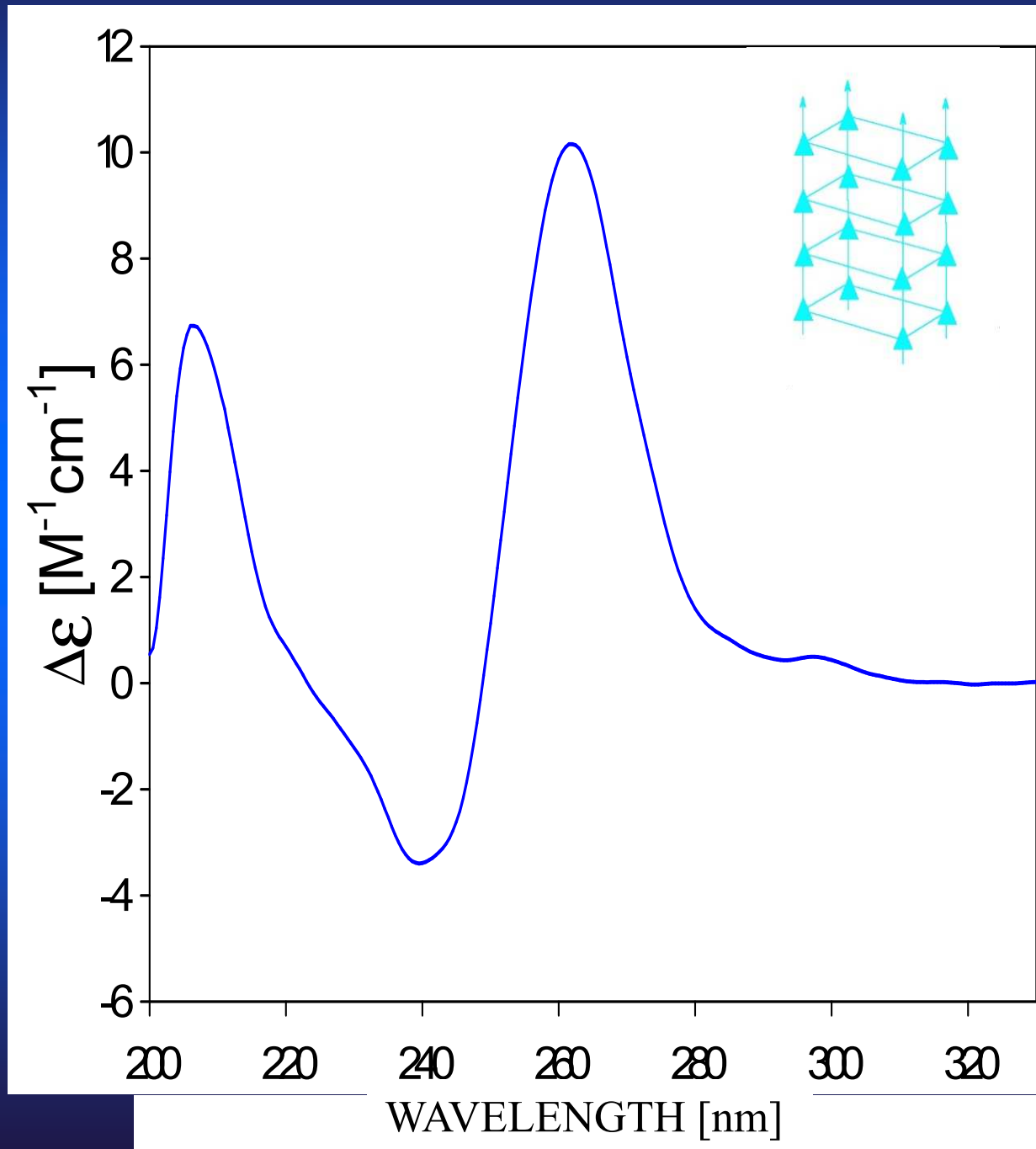


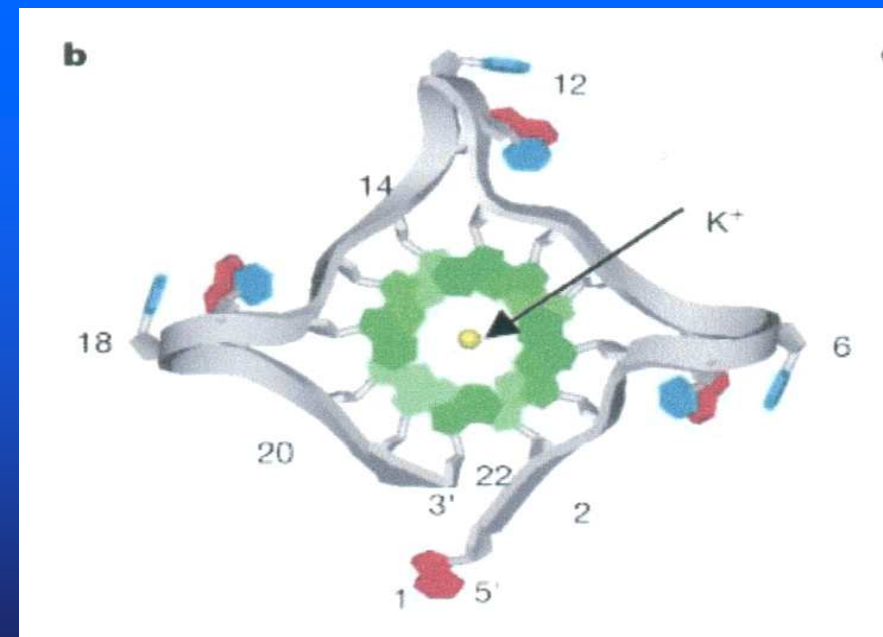
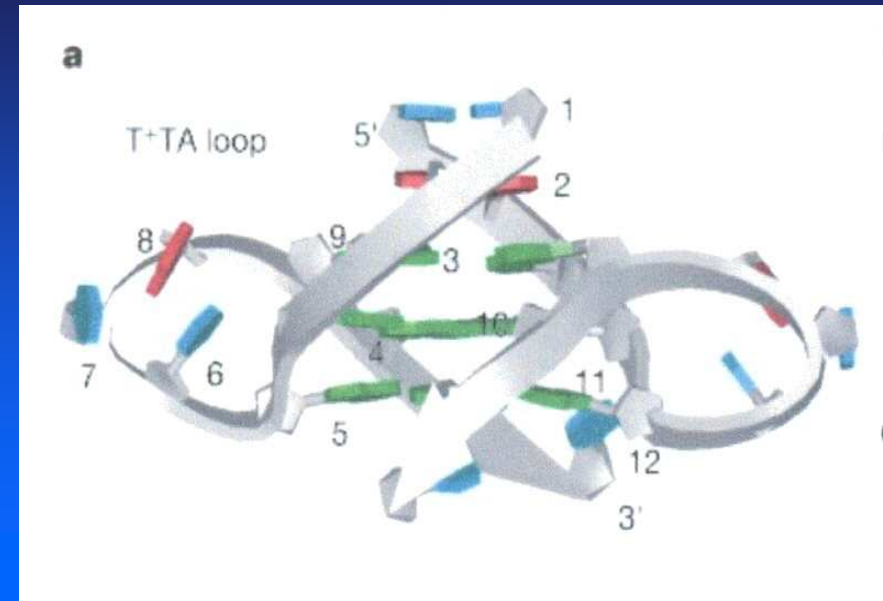
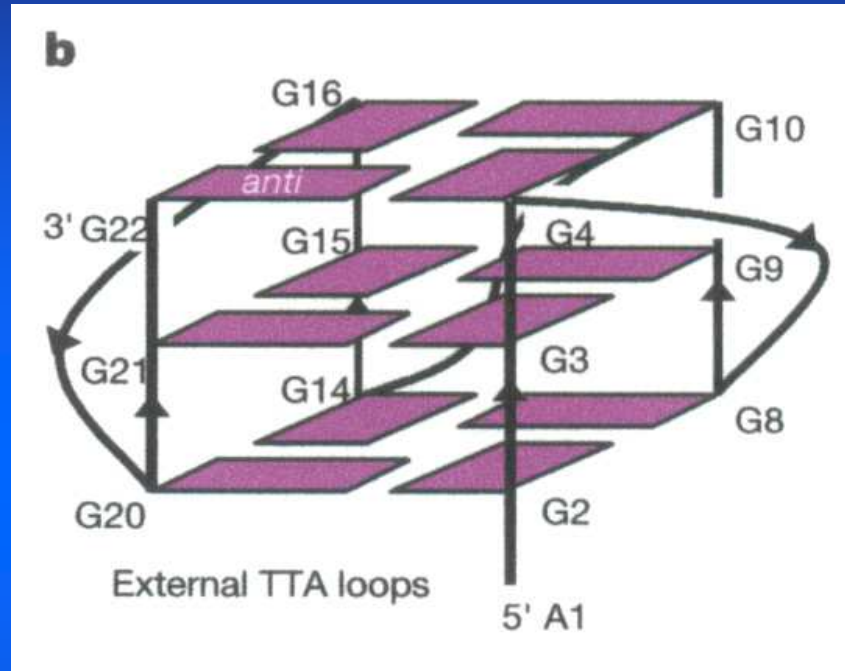
CD spectra reflecting formation of a **parallel** and **antiparallel** guanine quadruplex



Fragment Pu-27 promotoru c-myc:

TGGGGAGGGGTGGGGAGGGGTGGGGGAAGG



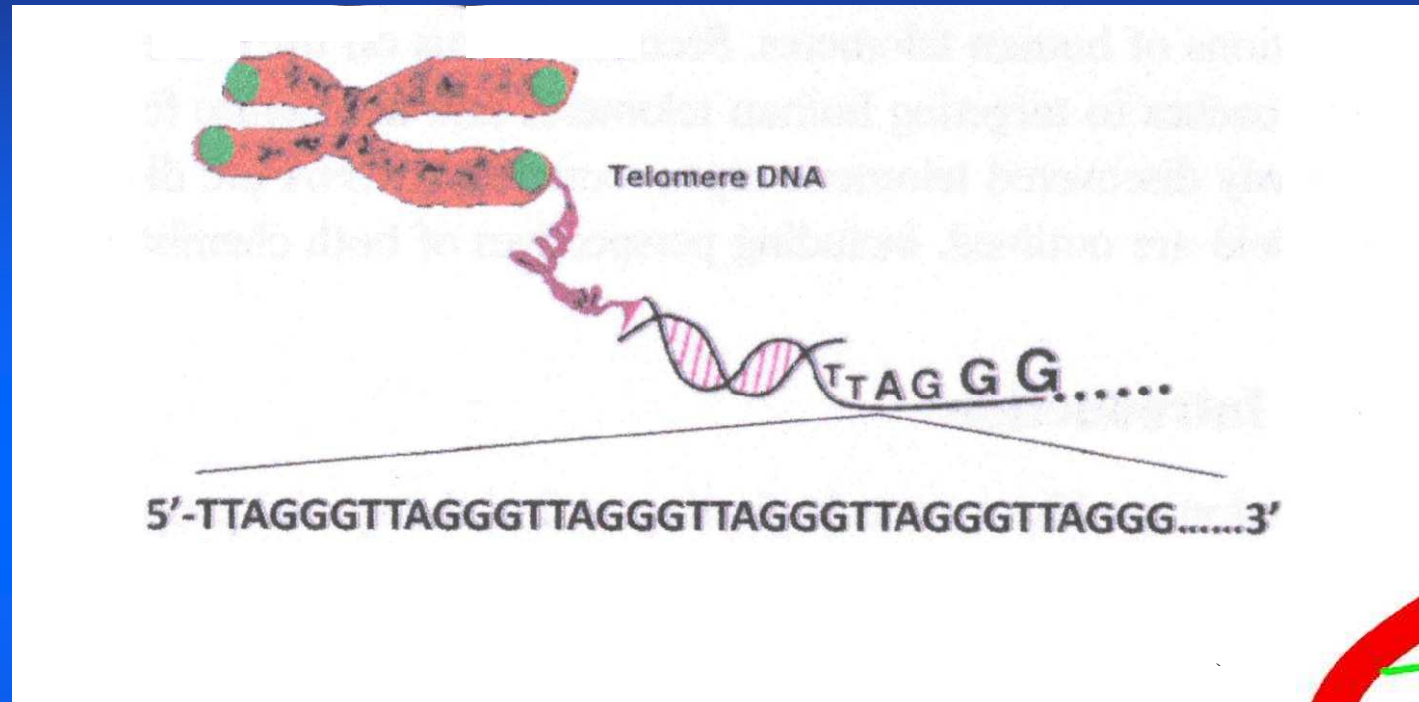


Parkinson, G.N., Lee, M.P.H, Neidle, S.
Nature **417** (2002) 876-880.

d[AGGG(TTAGGG)3]

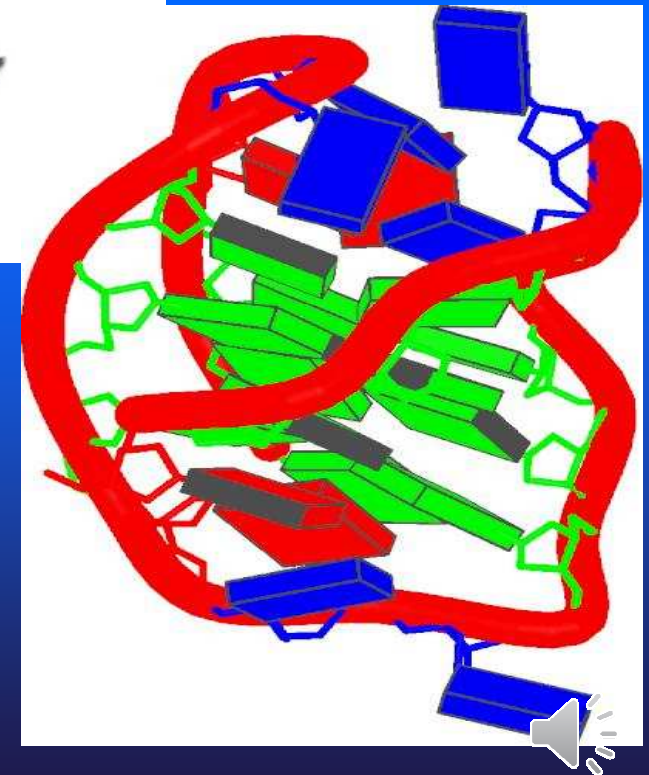


Human telomeric DNA forms quadruplex



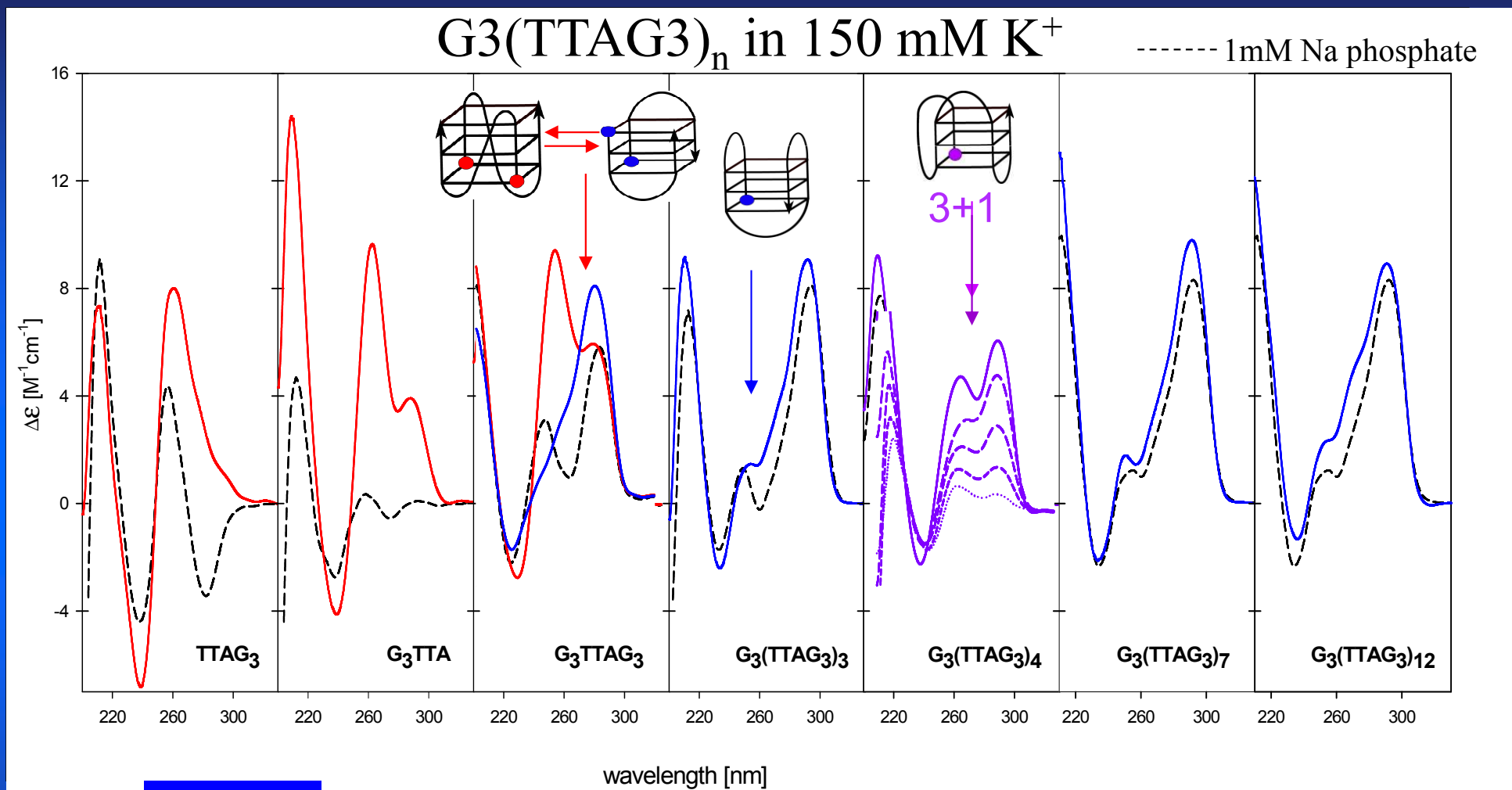
Telomeric DNA is associated with aging

The telomere quadruplex became a target for developing anticancer drugs

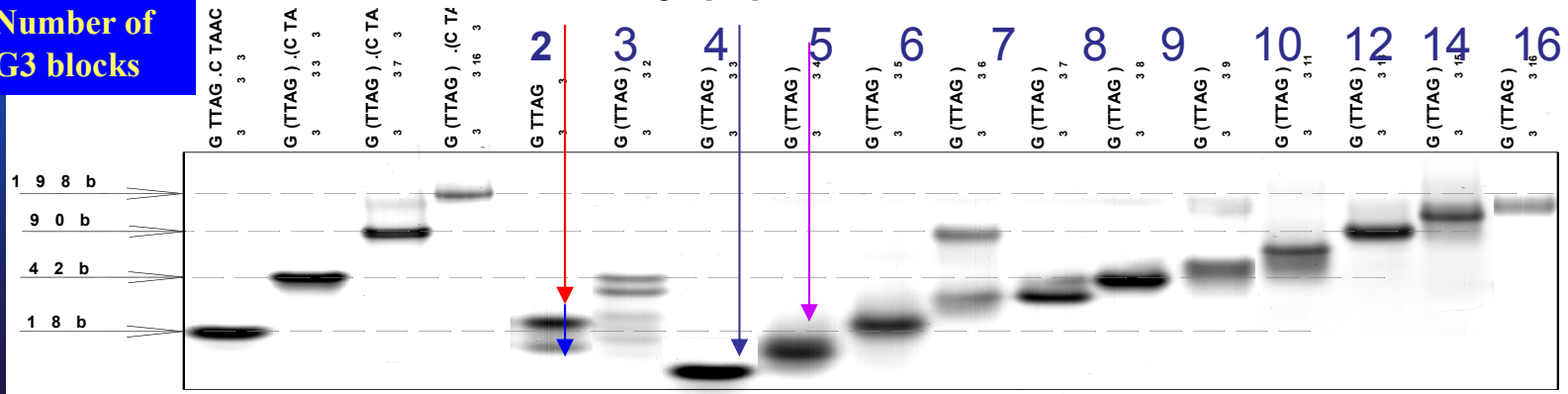


Guanine quadruplex topology of human telomere DNA is governed by the number of (TTAGGG) repeats.

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



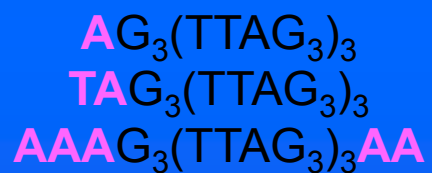
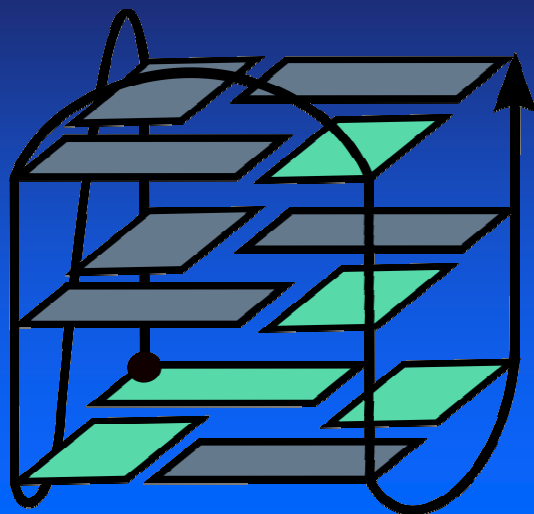
Number of G3 blocks



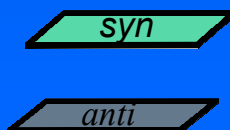
17



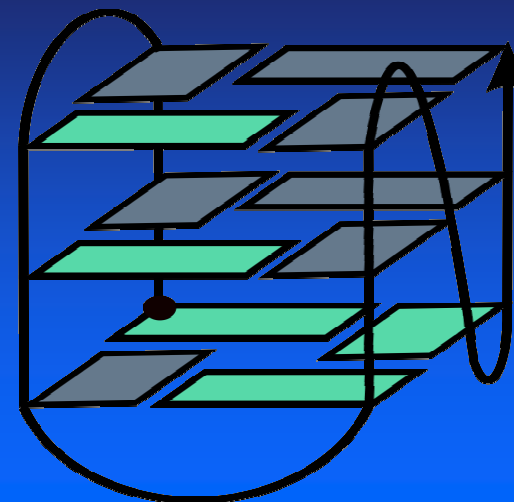
3 + 1



K⁺



3 + 1

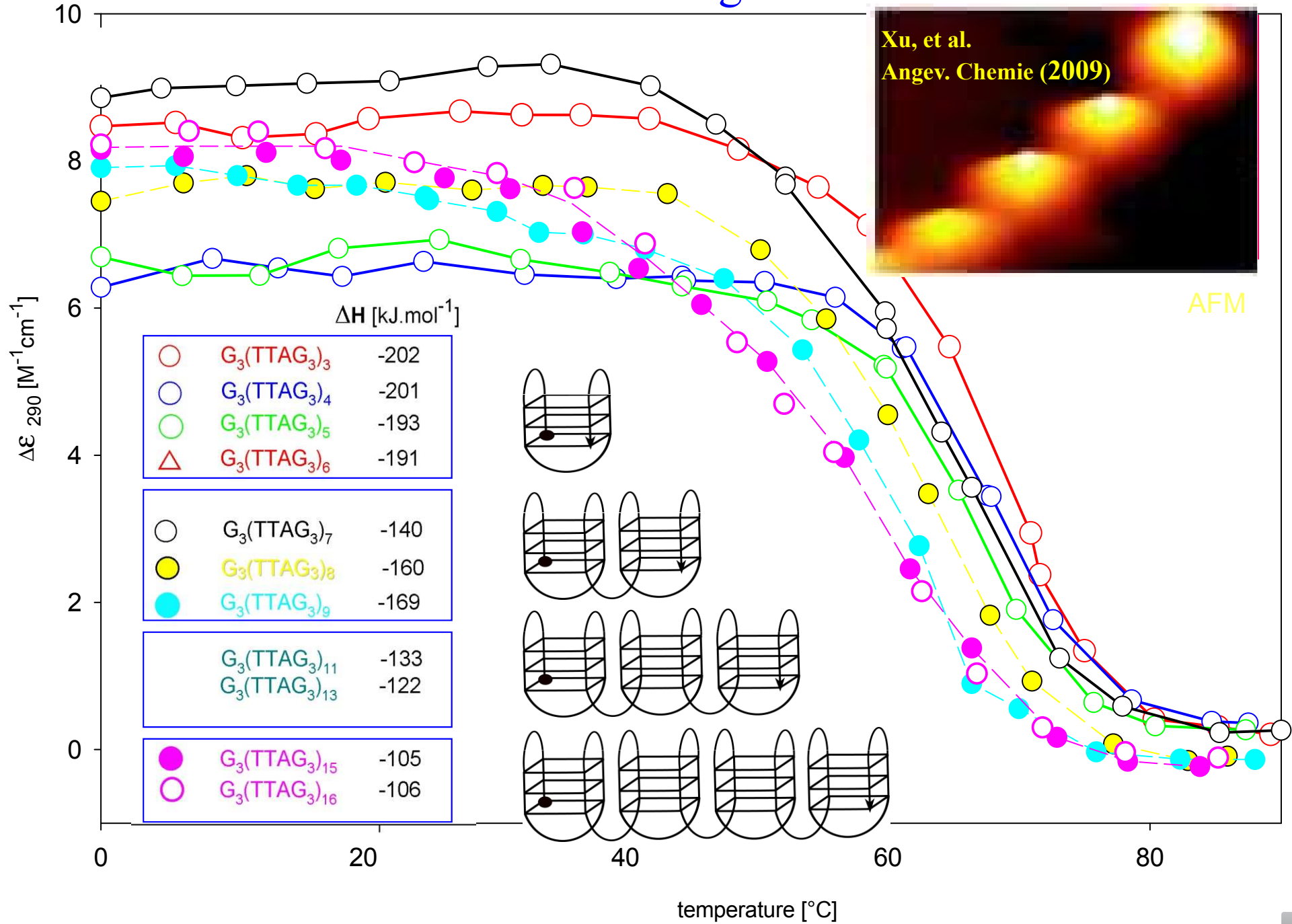


- Luu, K.N., Phan, A.T., Kuryavyi, V., Lacroix, L., Patel, D.J. (2006) J.Am.Chem.Soc., 128, 9963-9970.
- Ambrus, A., Chen, D., Dai, J., Bialis, T., Jones, R.A., Yang, D. (2006) Nucleic Acids Res. 34, 2723-2735.

- 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



$AG_3(TTAG_3)_3$

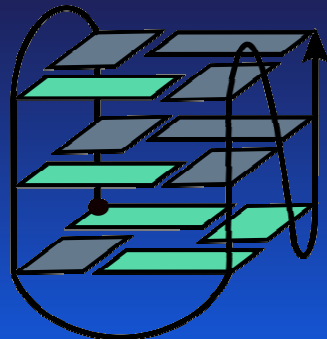
$TAG_3(TTAG_3)_3$

$AAAG_3(TTAG_3)_3AA$

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

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

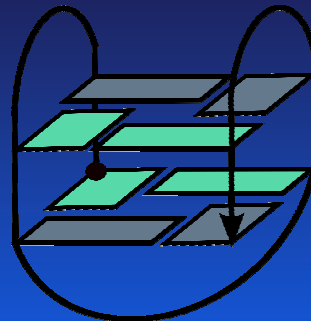
3 + 1



$TAG_3(TTAG_3)_3TT$

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

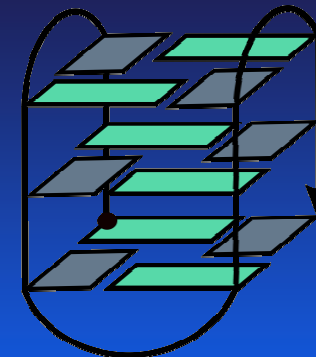
BASKET
two tetrads



$G_3(TTAG_3)_3T$

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

CHAIR



$AG_3(TTAG_3)_3$

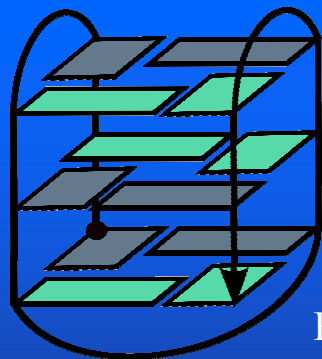
He et al.: Nucleic Acids Res. 32 (2004)
5359-5367.

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 μ M strand concentration in CD



$G_3(TTAG_3)_3$

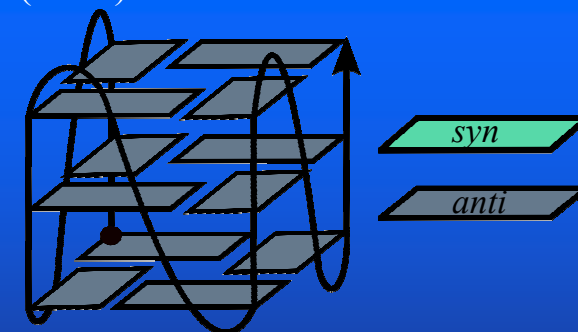
$AG_3(TTAG_3)_3$

$TTAG_3(TTAG_3)_3$

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

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

Parkinson, Lee, Neidle: $AG_3(TTAG_3)$
Nature 417 (2002) 876-880.



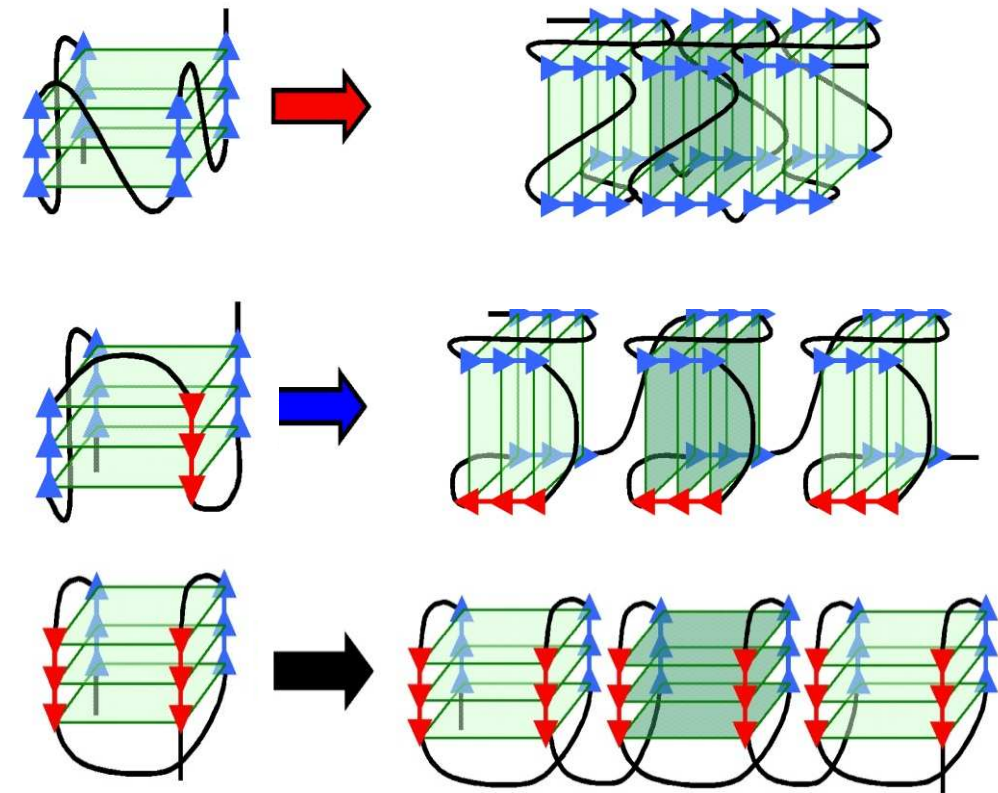
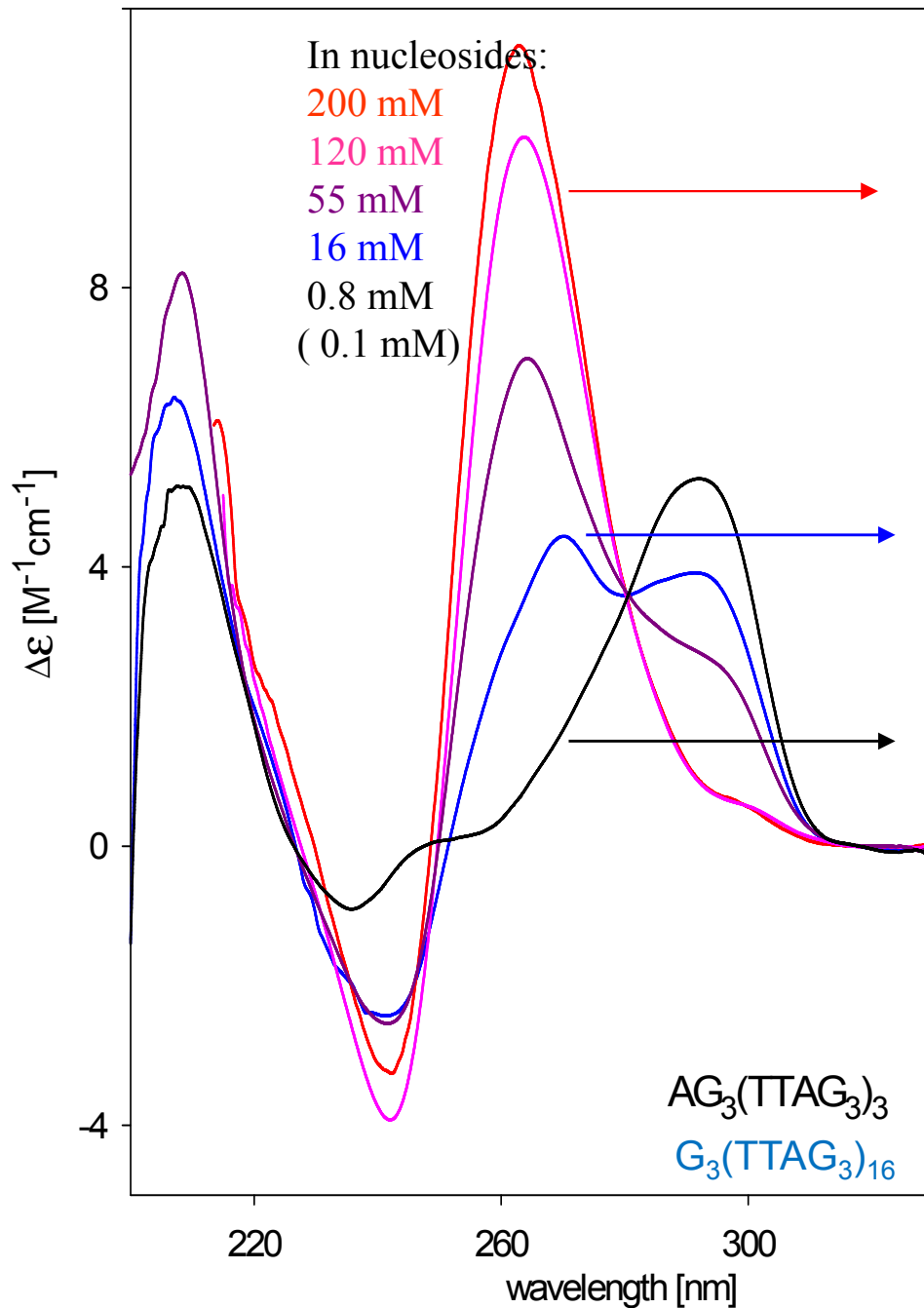
BASKET

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.



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 μm)

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



CD spektroskopie a konformační vlastnosti nukleových kyselin

Otázky

-) Co je optická aktivita, chirální látky, optická rotace, cirkulární dichroismus
-) Jaké jsou (dvě) podmínky vzniku cirkulárního dichroismu (příslušná látka musí být chirální a absorbovat světlo)
-) které komponenty odpovídají za vznik CD nukleových kyselin a proteinů
-) Jaké jsou výhody a nevýhody metody CD ve srovnání s dalšími metodami struktury biopolymerů
-) Co je podstatou unikátní citlivosti metody CD ke strukturním změnám v NA?
-) Co je optická rotační disperse a Cottonův efekt?
-) Podstata nekooperativních a kooperativních změn
-) Globální charakteristika forem B, A a Z DNA (zejména žlábků, obrácená topologie párů bází v případě Z formy)
-) nekanonické sekundární struktury DNA
-) typy čtyřřetězcových uspořádání NA

