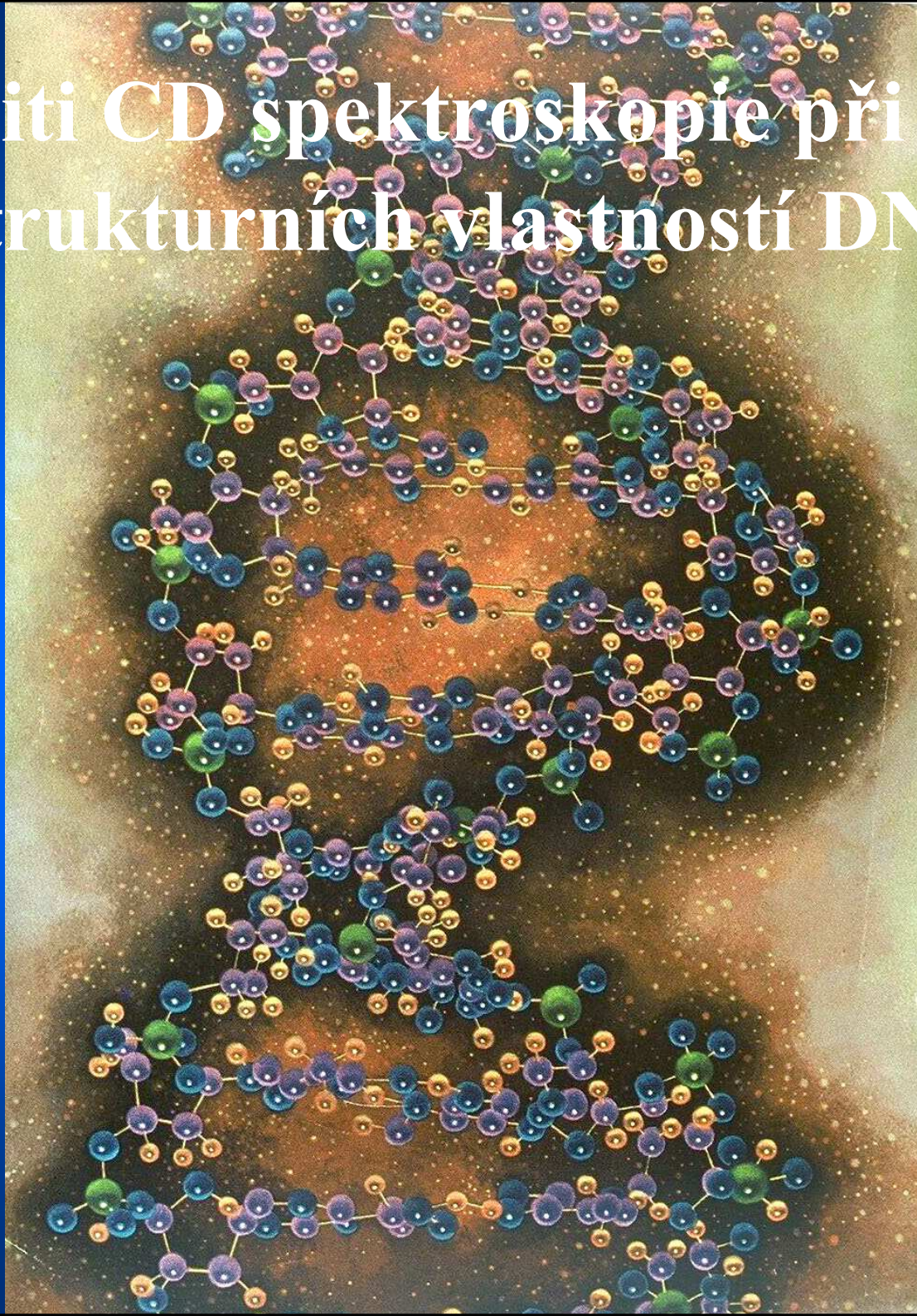


Použití CD spektroskopie při studiu strukturních vlastností DNA

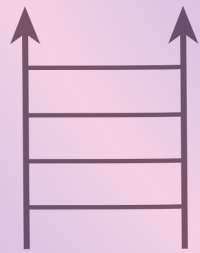




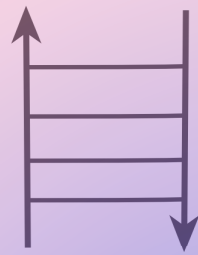
Hairpin



Coiled - coil



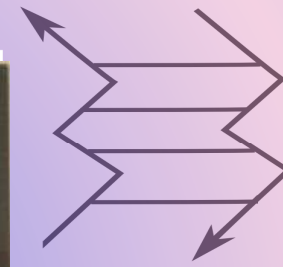
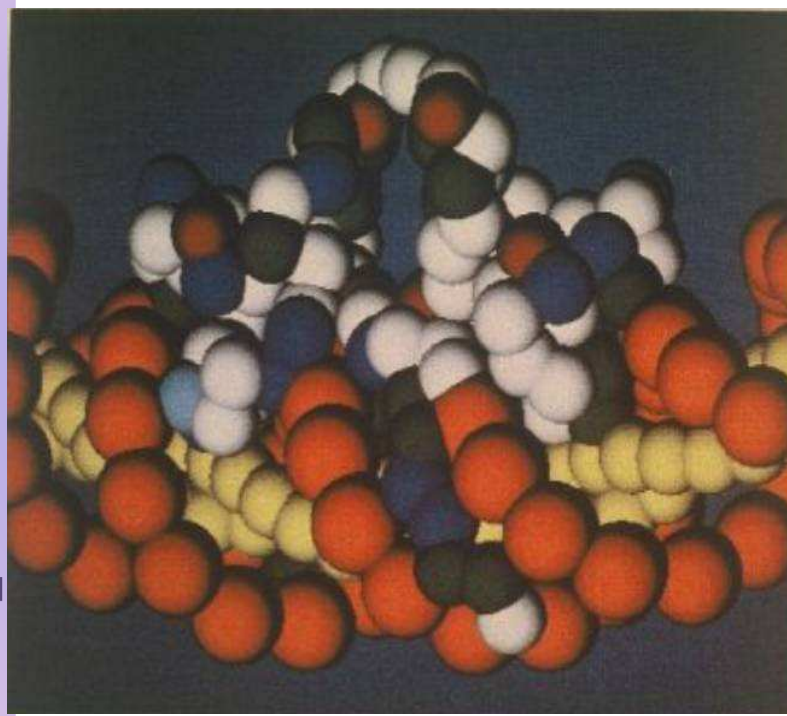
Parallel Homoduplex



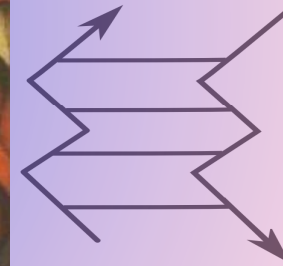
B - form



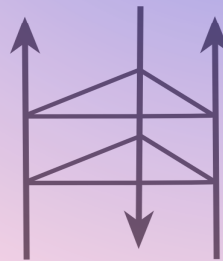
A - form



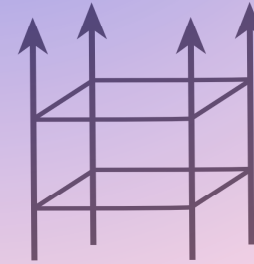
Z - form



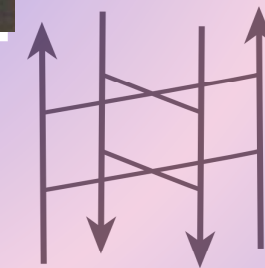
X - form



Triplex



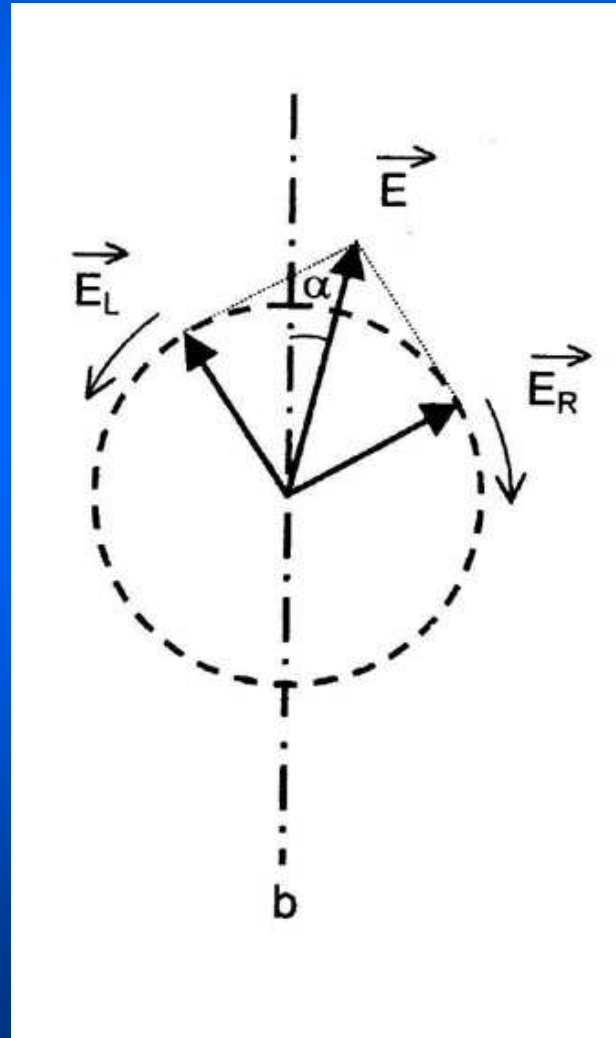
G - tetraplex



C - tetraplex

Cirkulární dichroismus a optická aktivita biopolymerů

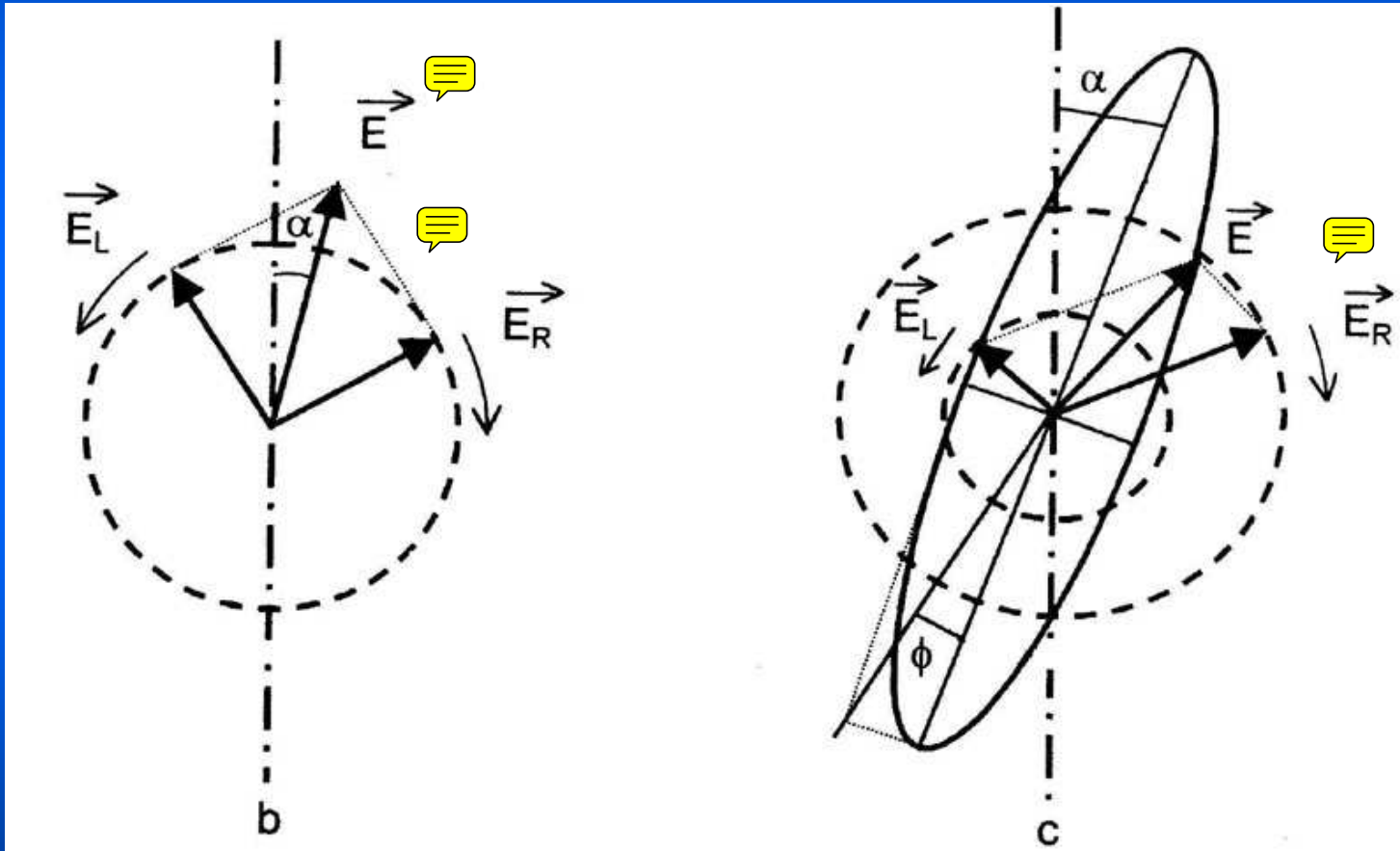
) optická aktivita – chirální látky (aminokyseliny, cukry) úhel stočení roviny polarizovaného světla, ORD



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 [ψ]

$$\text{tg } \varphi = b/a = \frac{\epsilon_L - \epsilon_R}{\epsilon_L + \epsilon_R}$$

Cirkulární dichroismus $\Delta \epsilon$

$$\Delta \epsilon = \epsilon_L - \epsilon_R = \Delta A / l c$$

Podmínky vzniku CD



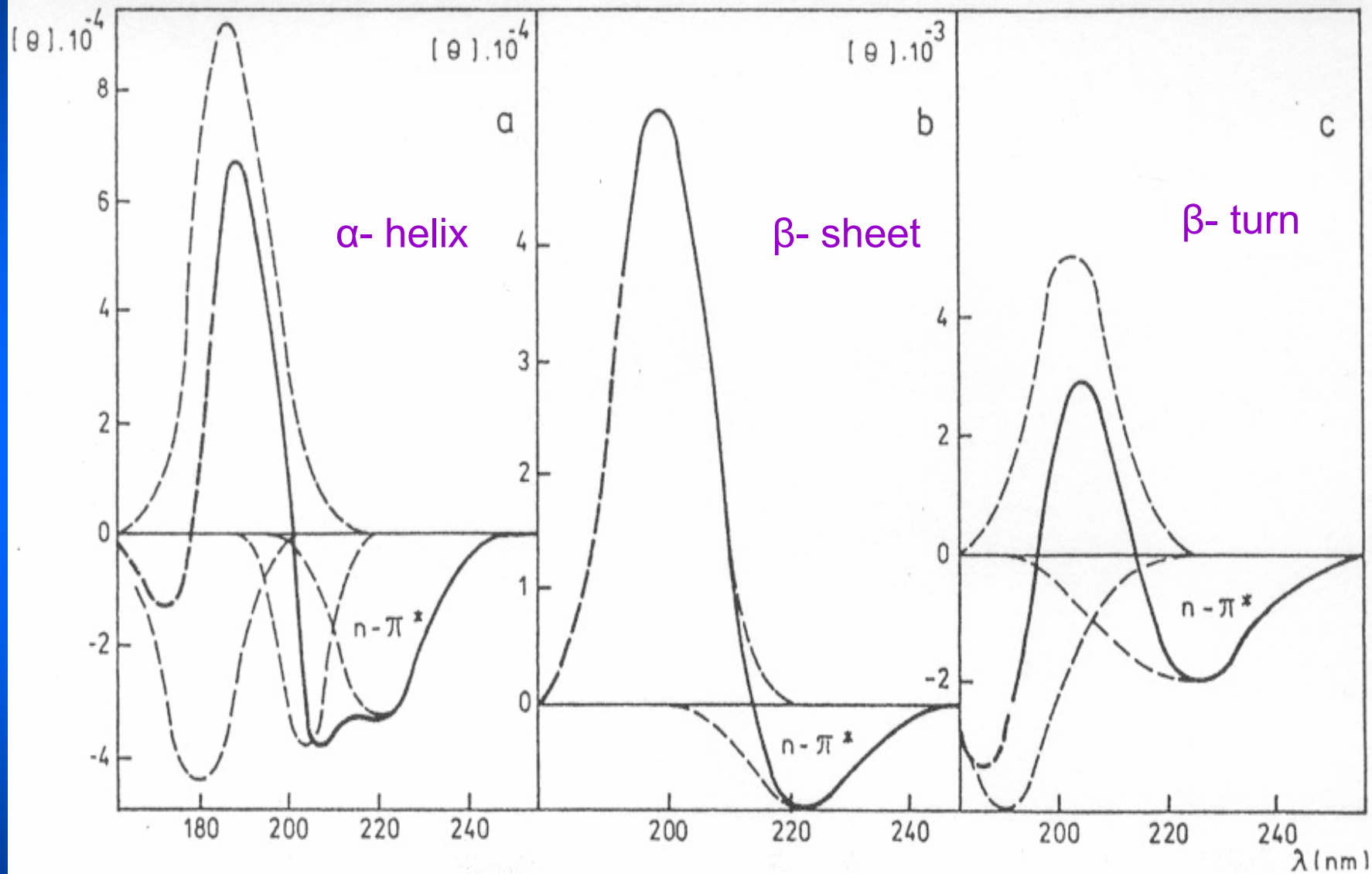
BÁZE

*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

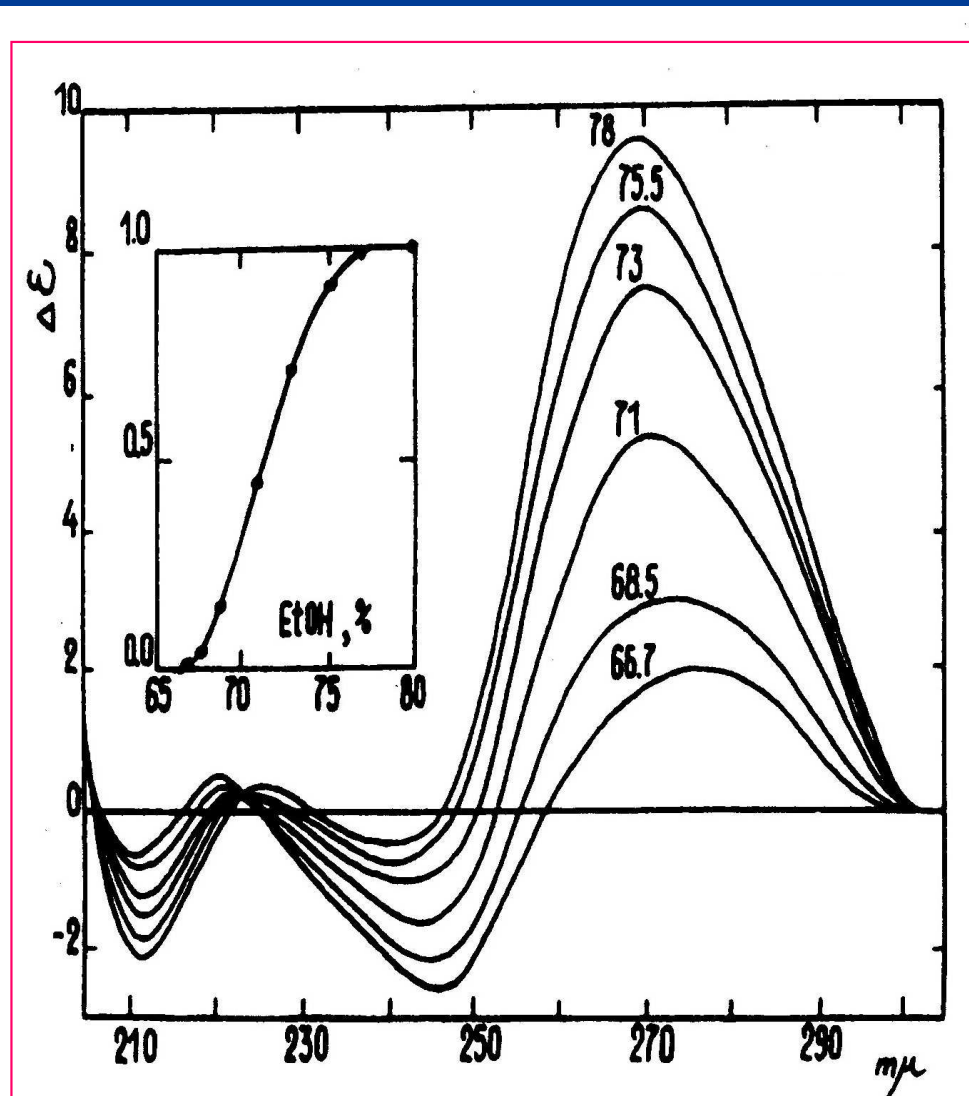
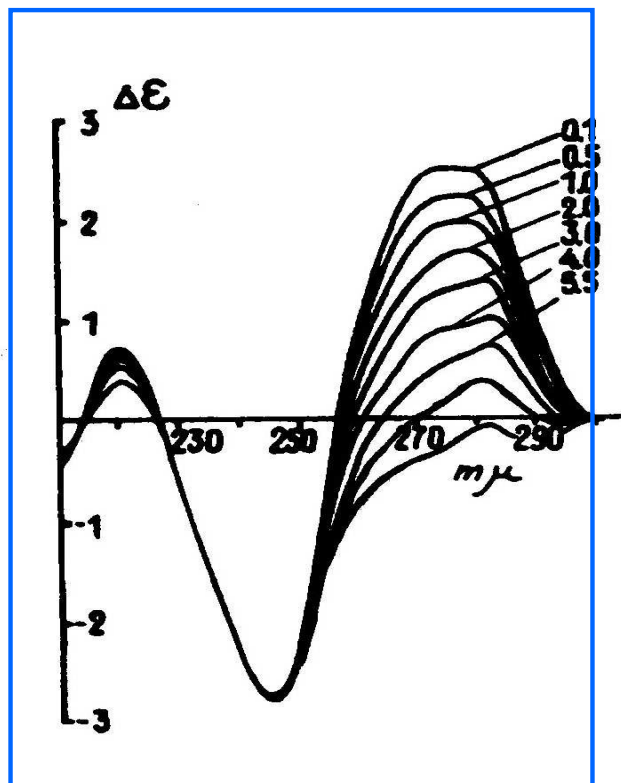
Rozlišení mezi kooperativními a nekooperativními změnami

Nevýhody

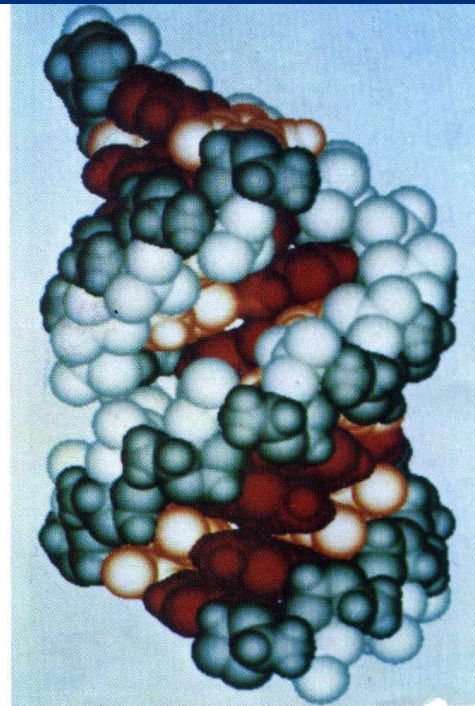
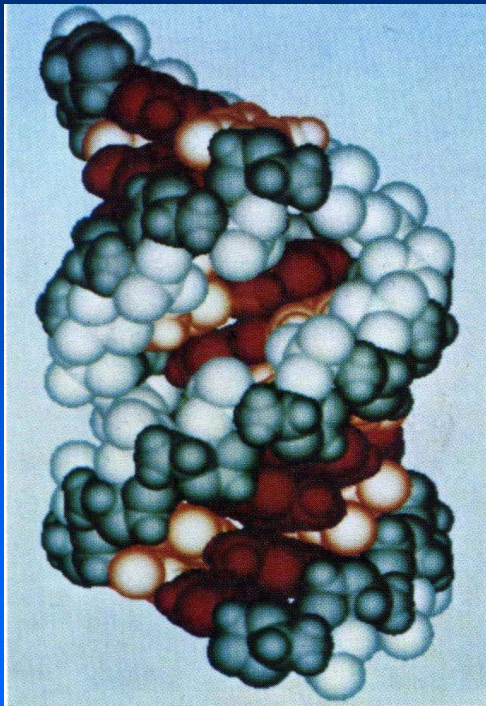
Chybí explicitní vztah mezi spektrem CD a strukturou

Příklady nekooperativních a kooperativních změn

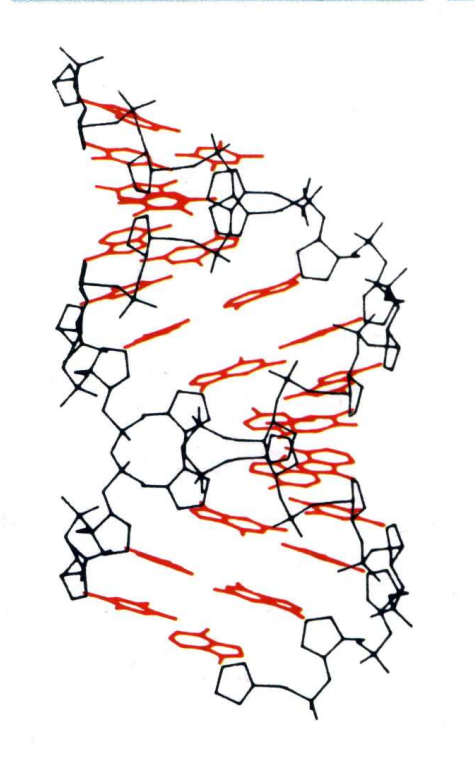
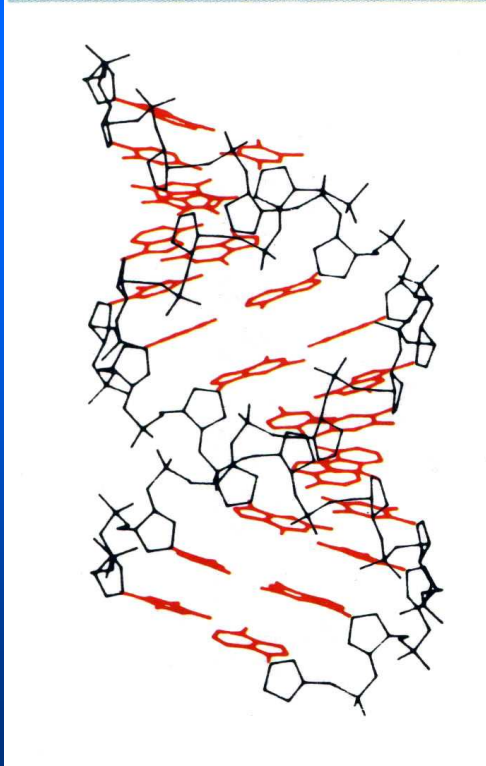
Nekooperativní změny
v rámci téže struktury

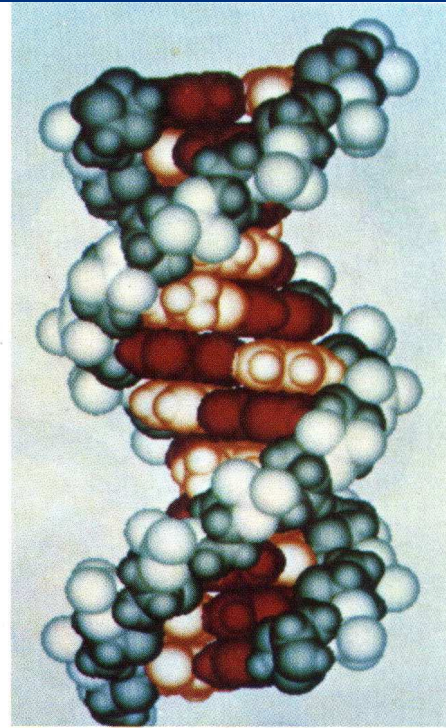
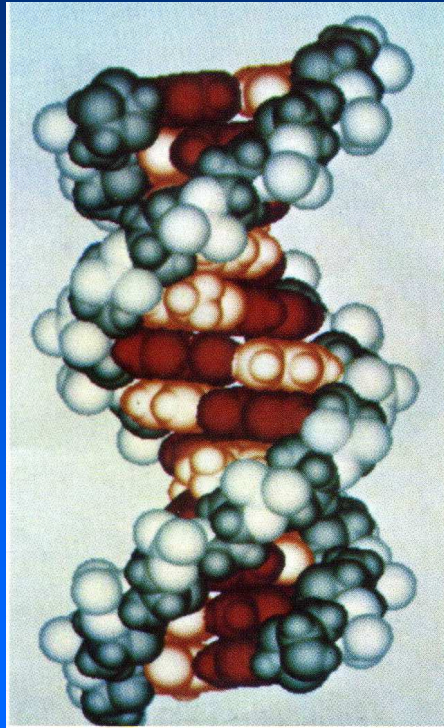


Kooperativní změny mezi diskretními strukturami

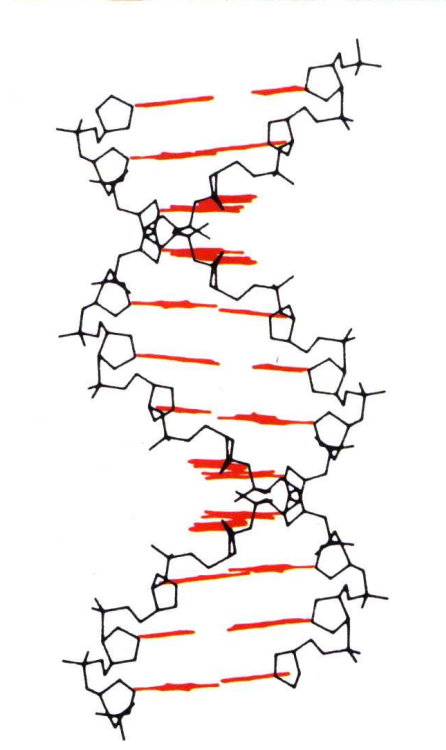
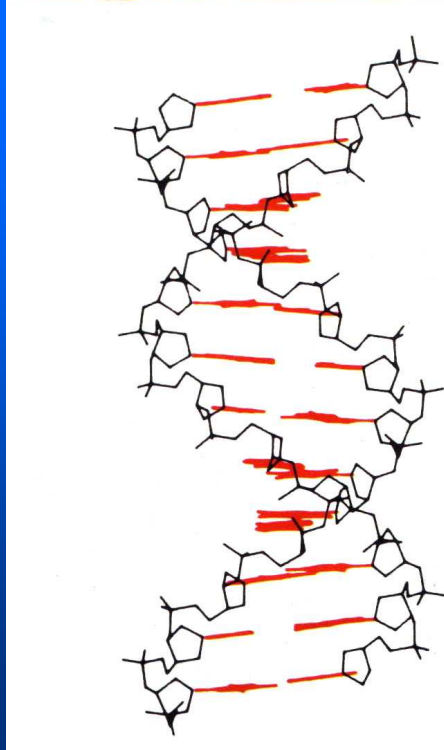


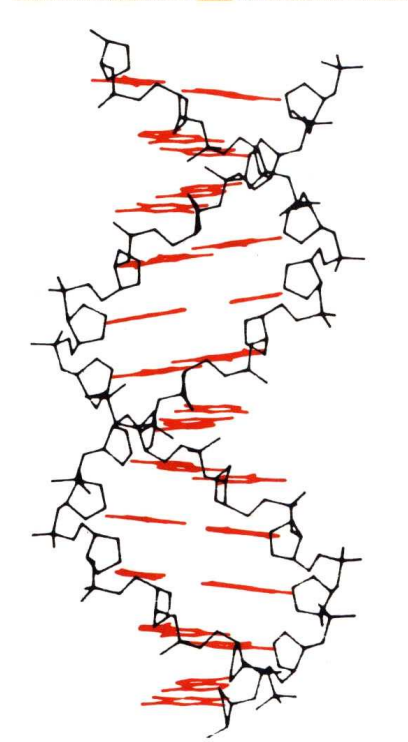
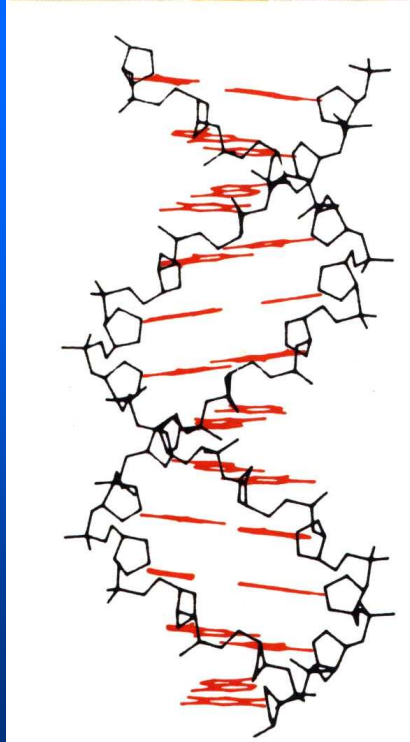
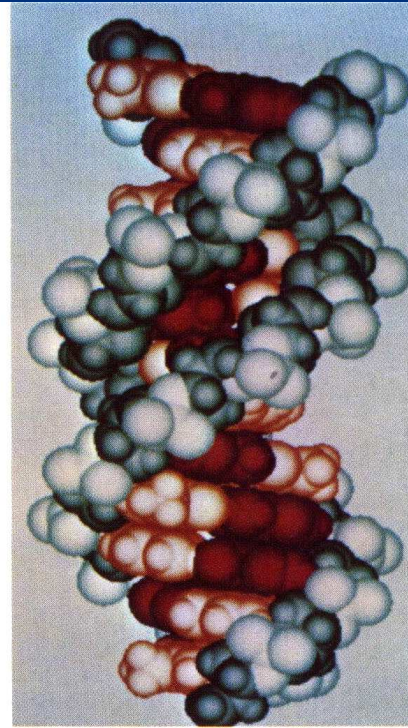
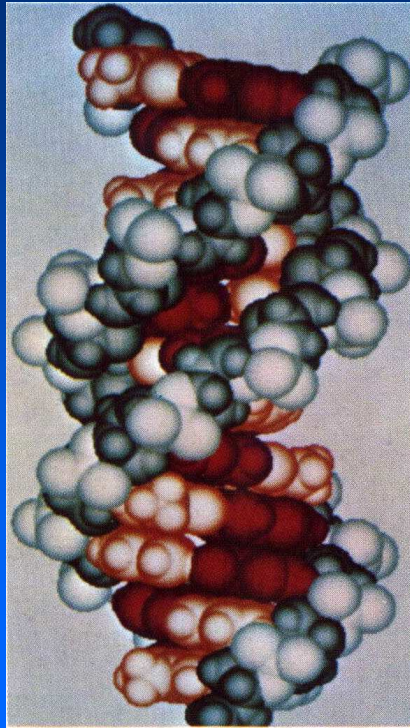
A-DNA





B-DNA





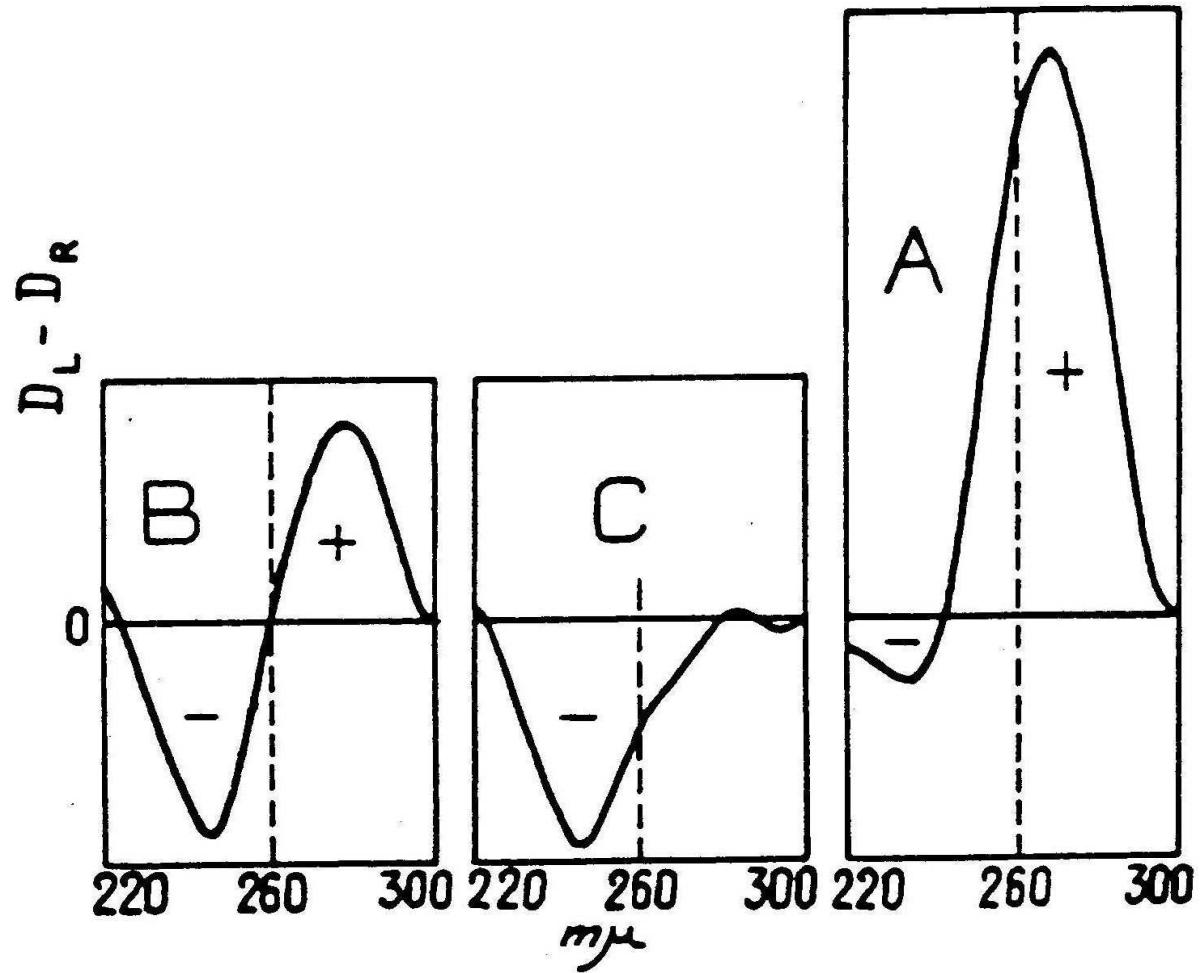
C-DNA

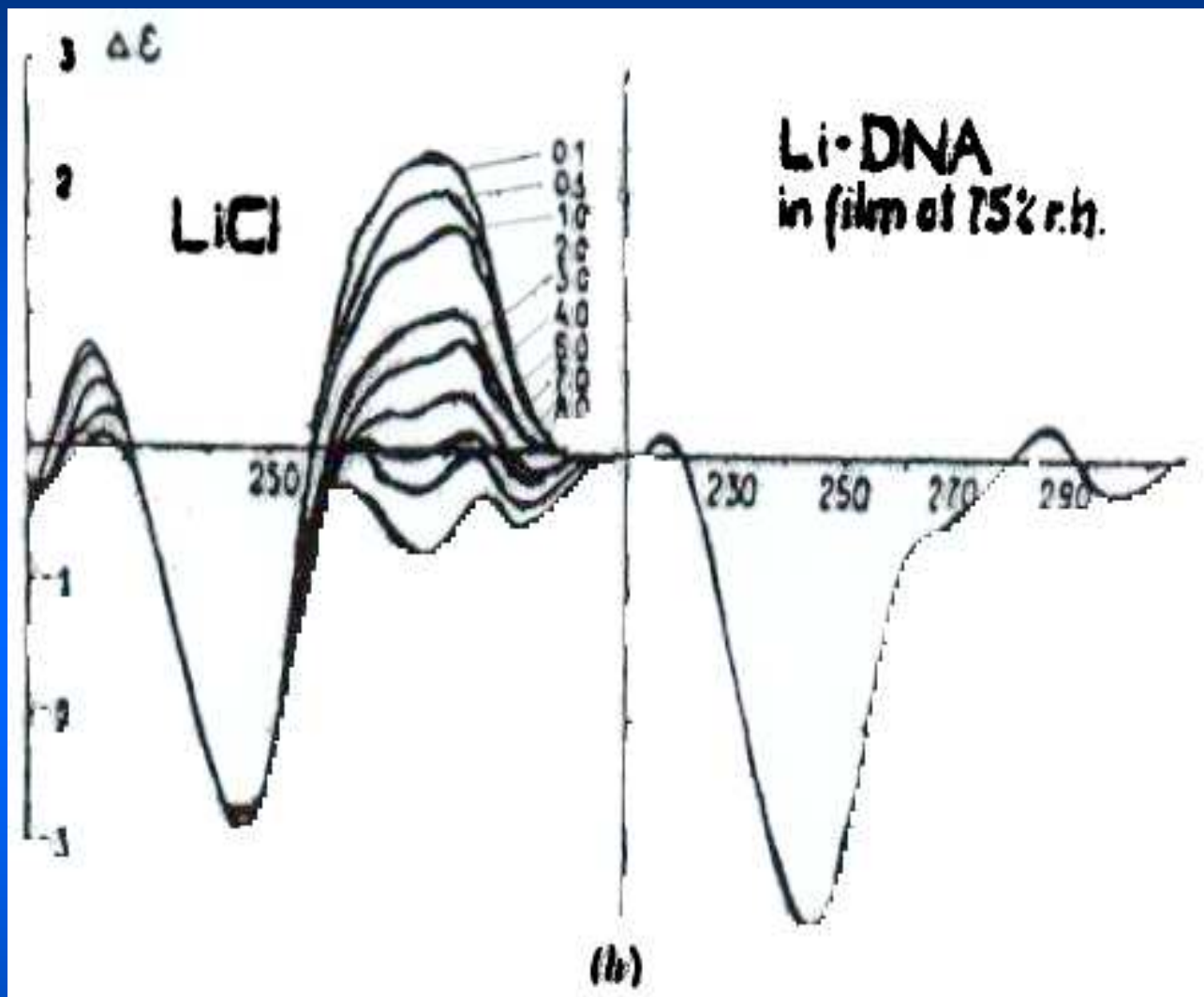
D-DNA

E-DNA

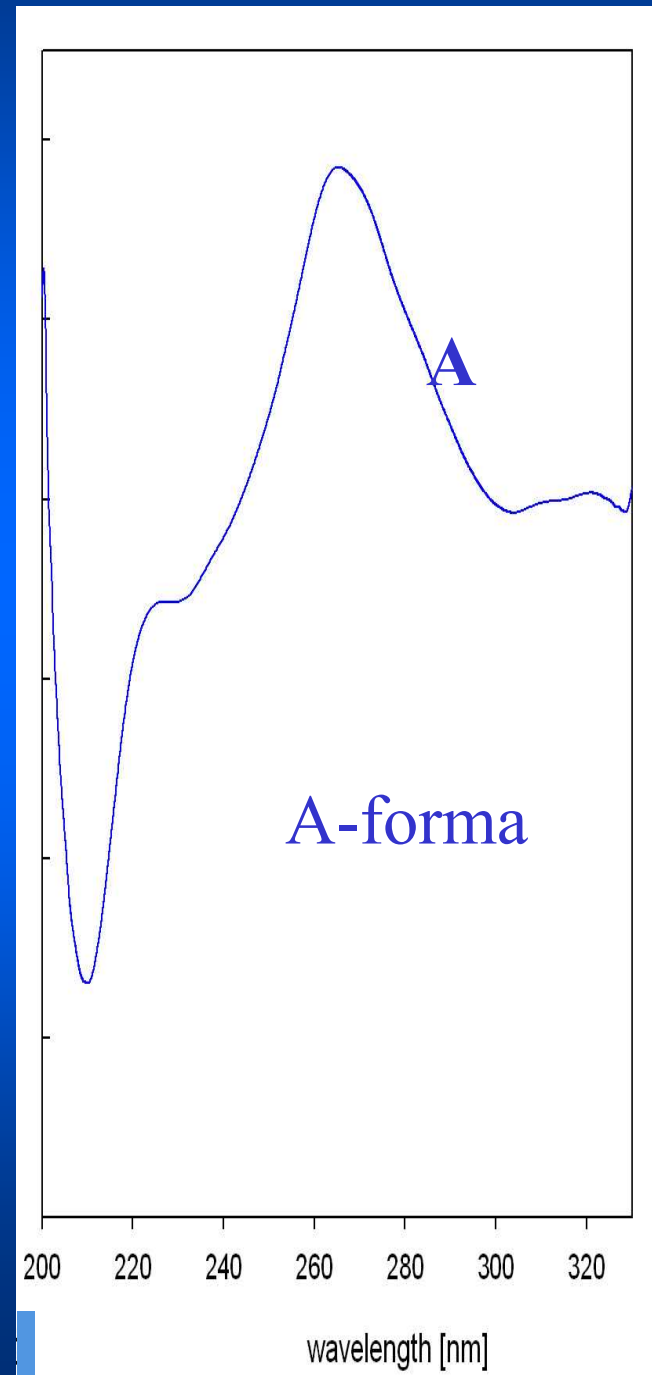
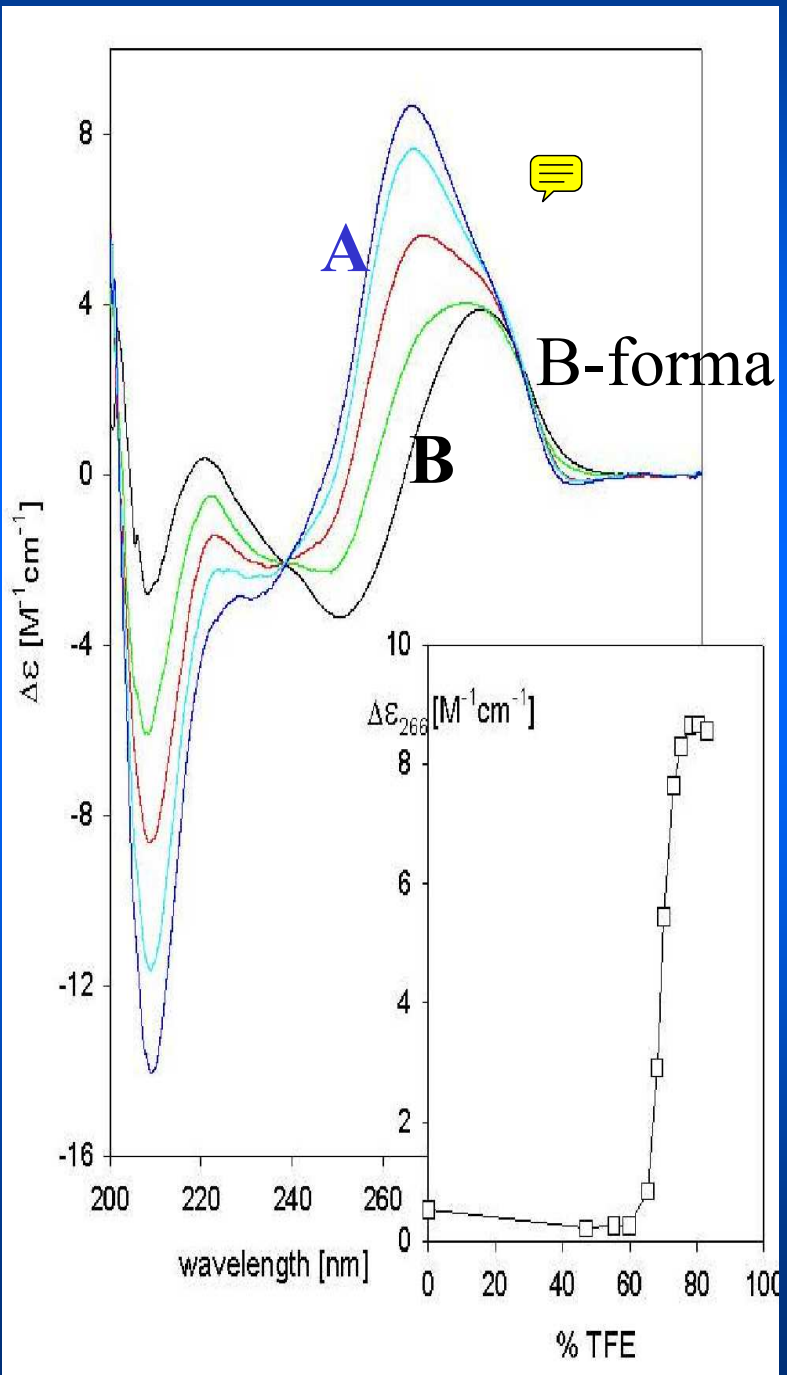
T-DNA

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

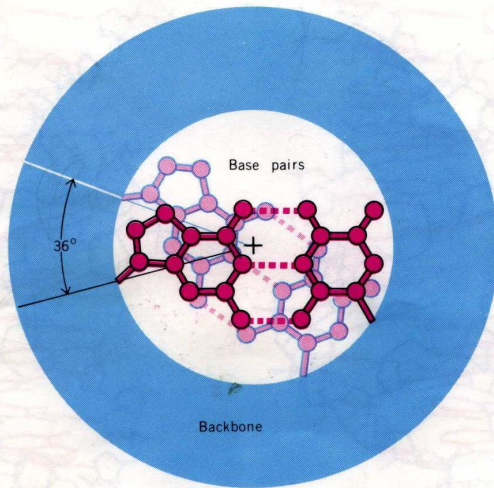
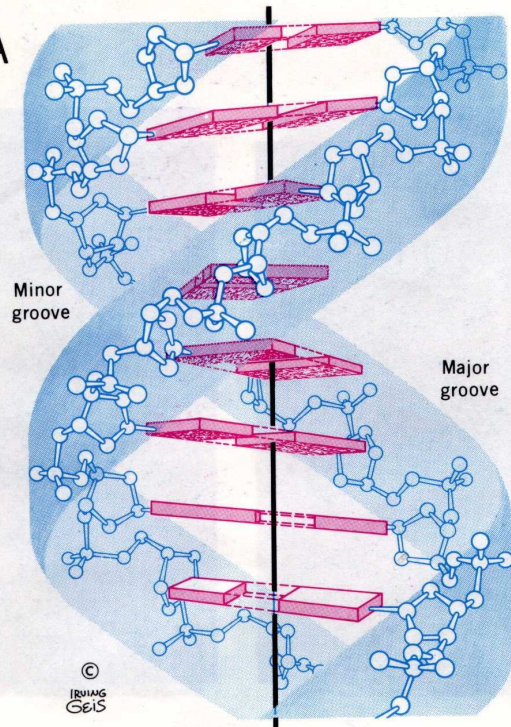




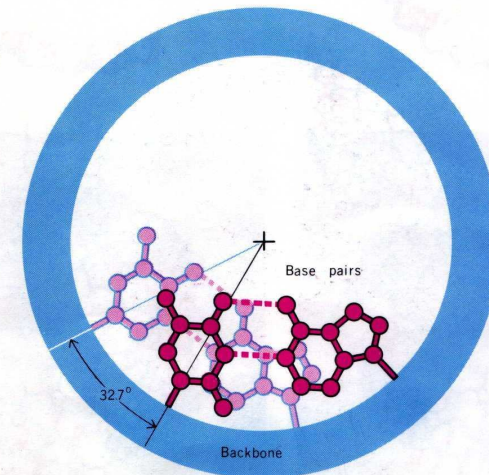
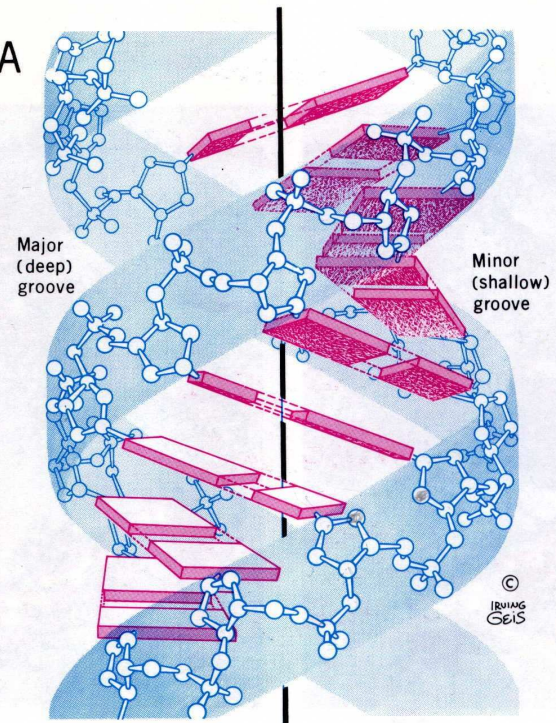
Jed **B-A** přechod DNA NA poskytují charakteristická CD spektra **A** -forma RNA

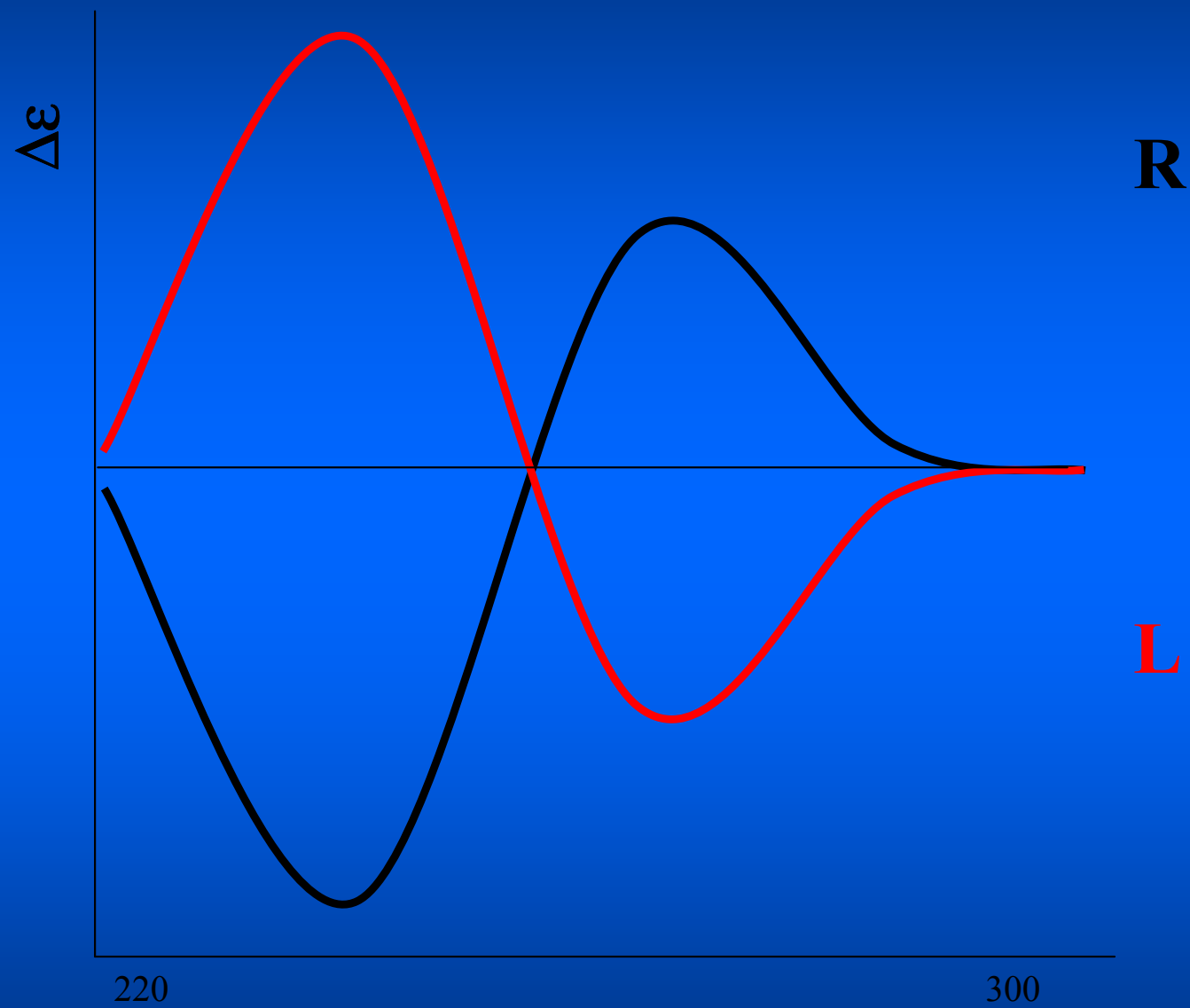


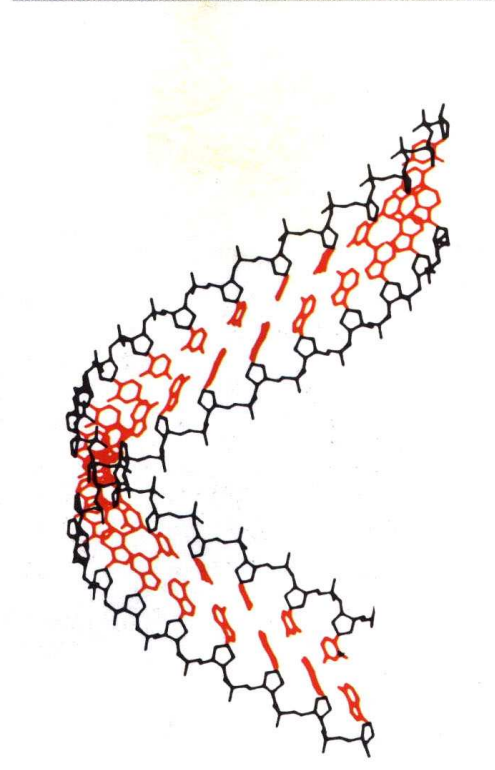
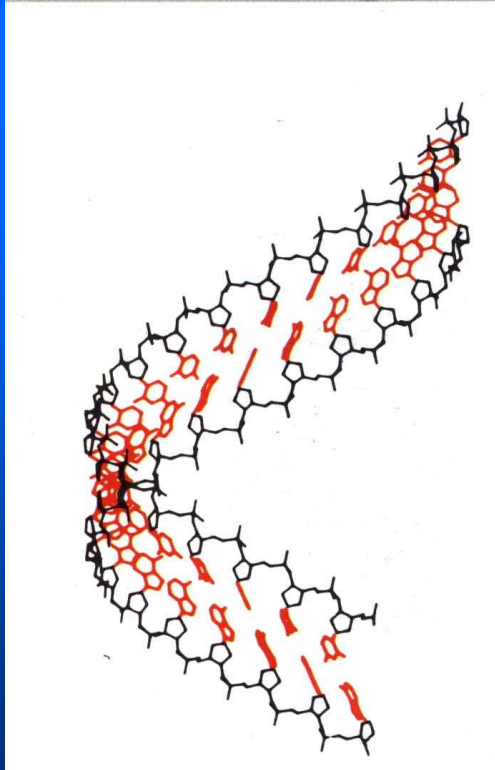
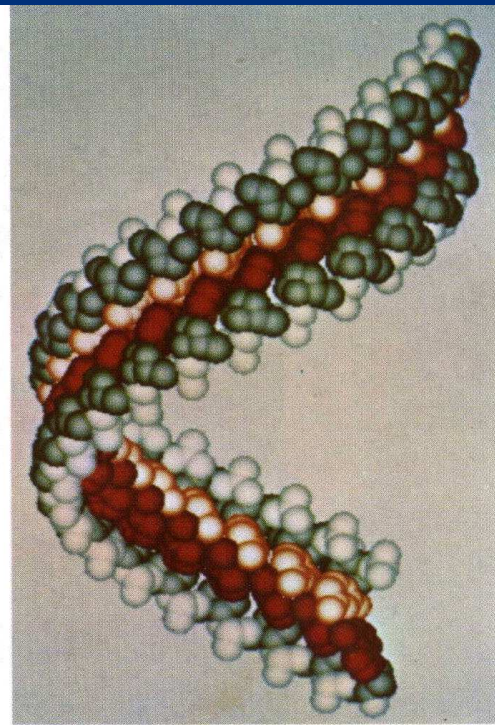
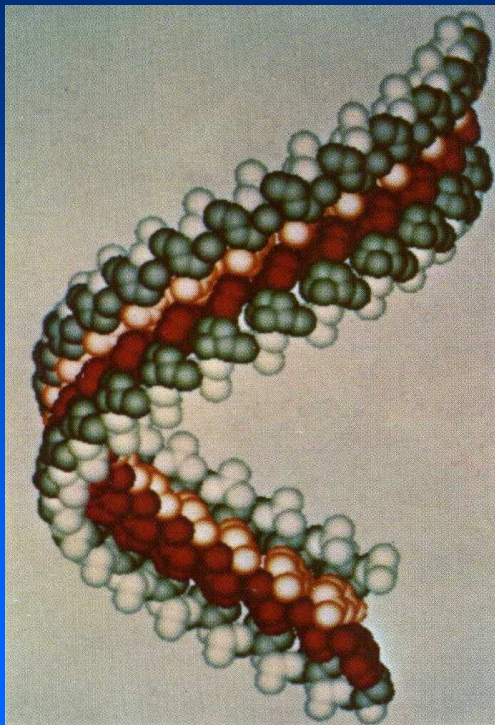
B DNA

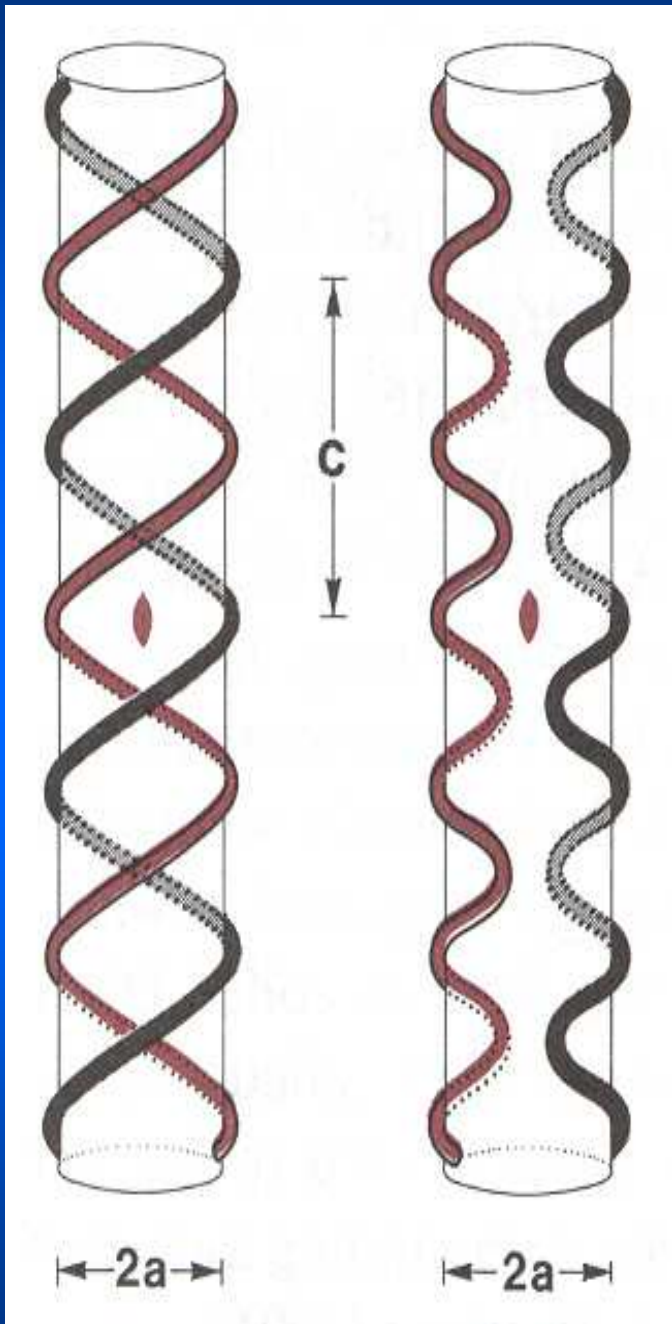


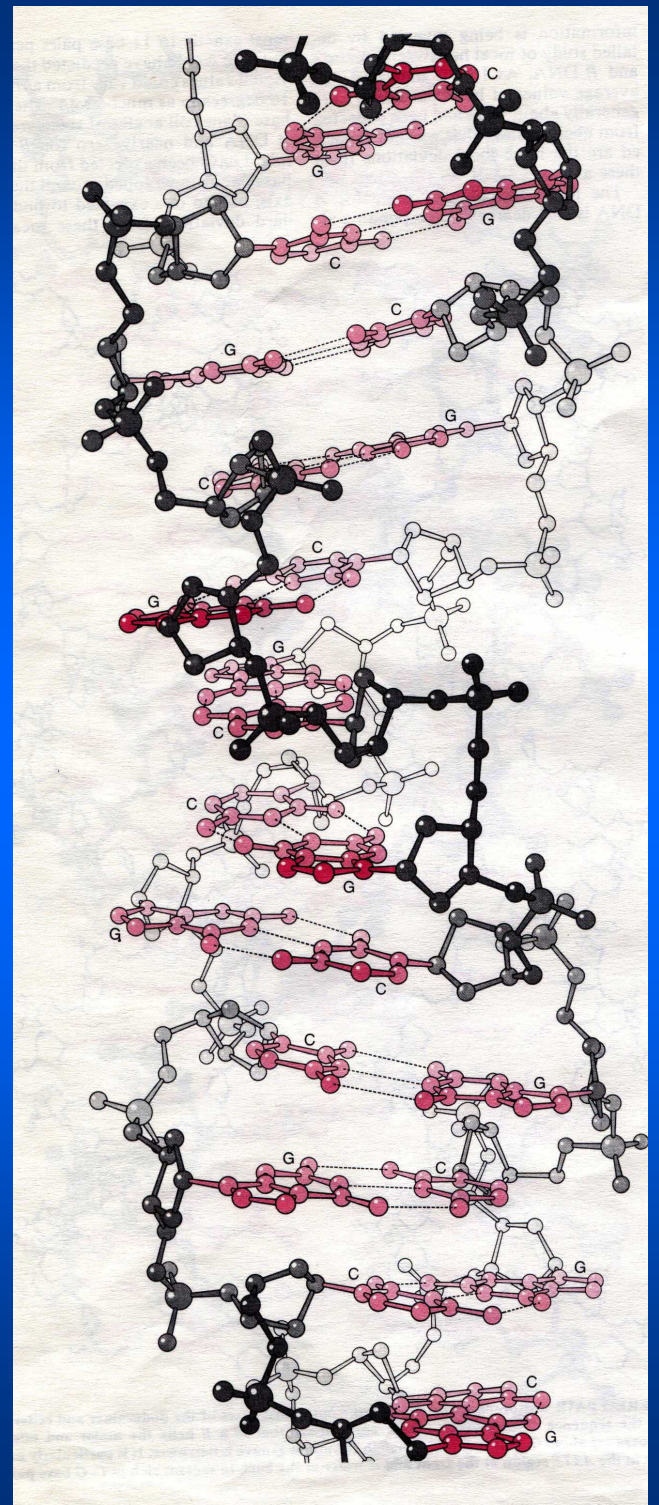
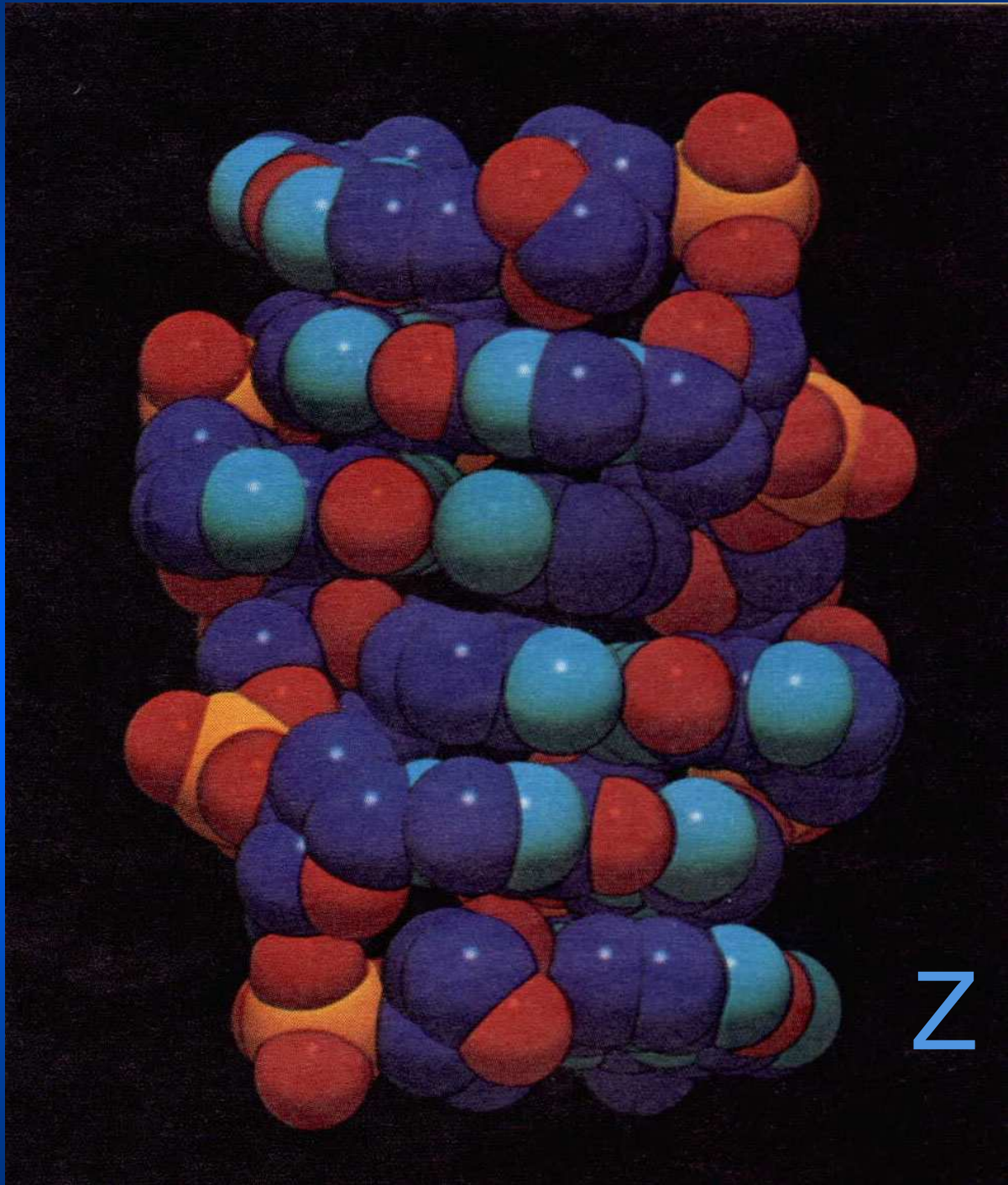
A DNA

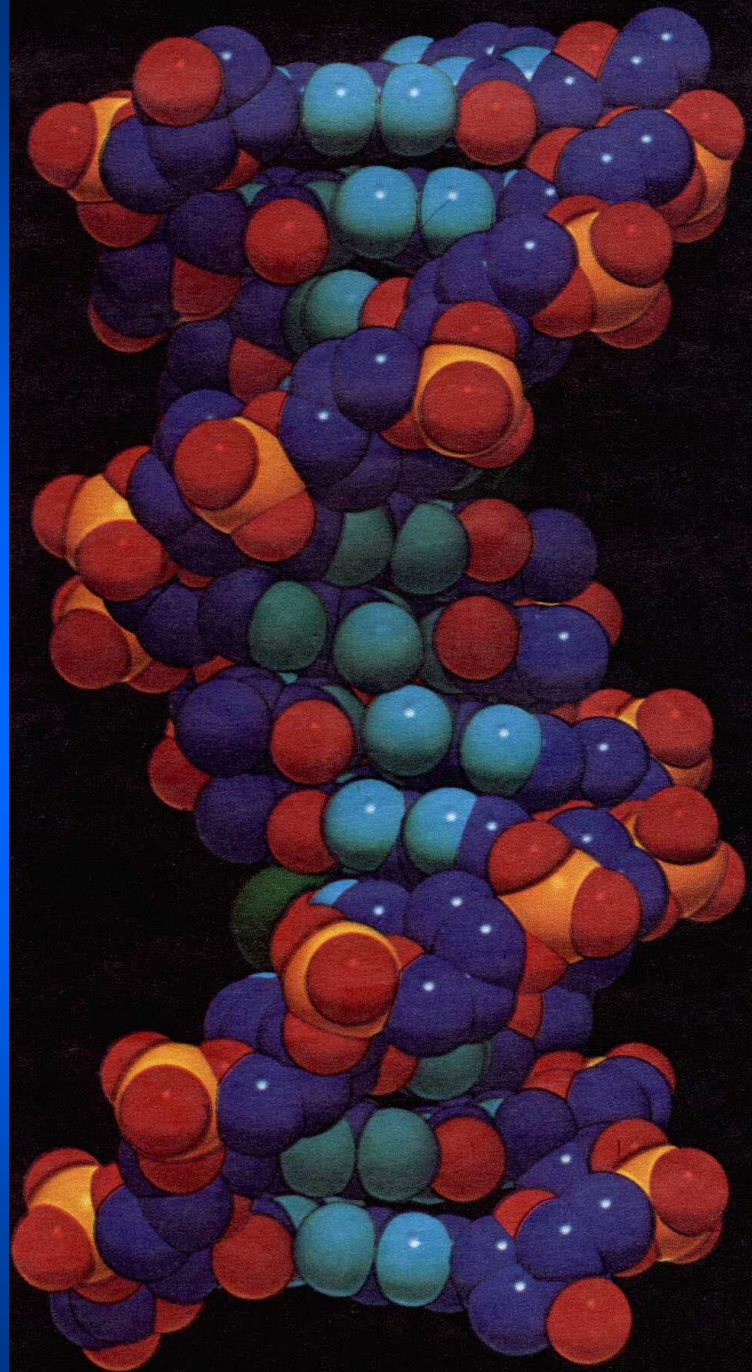




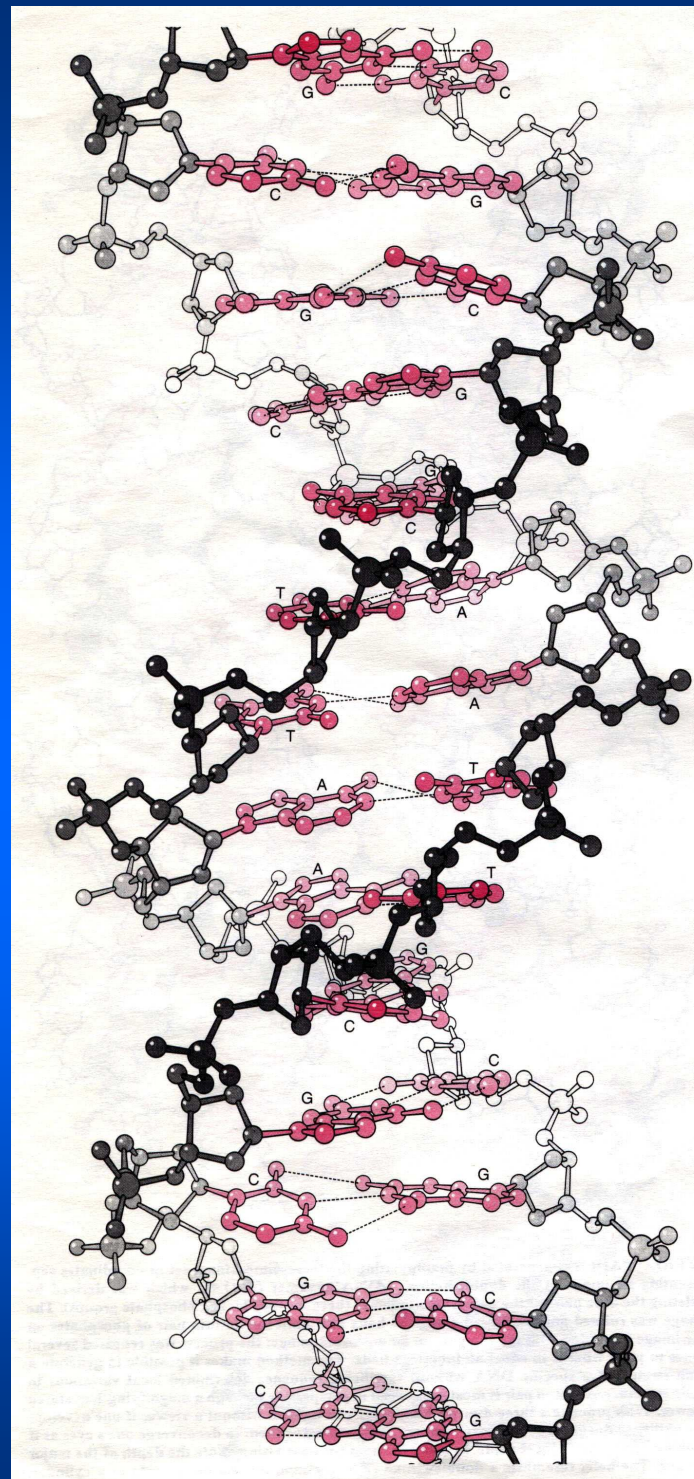


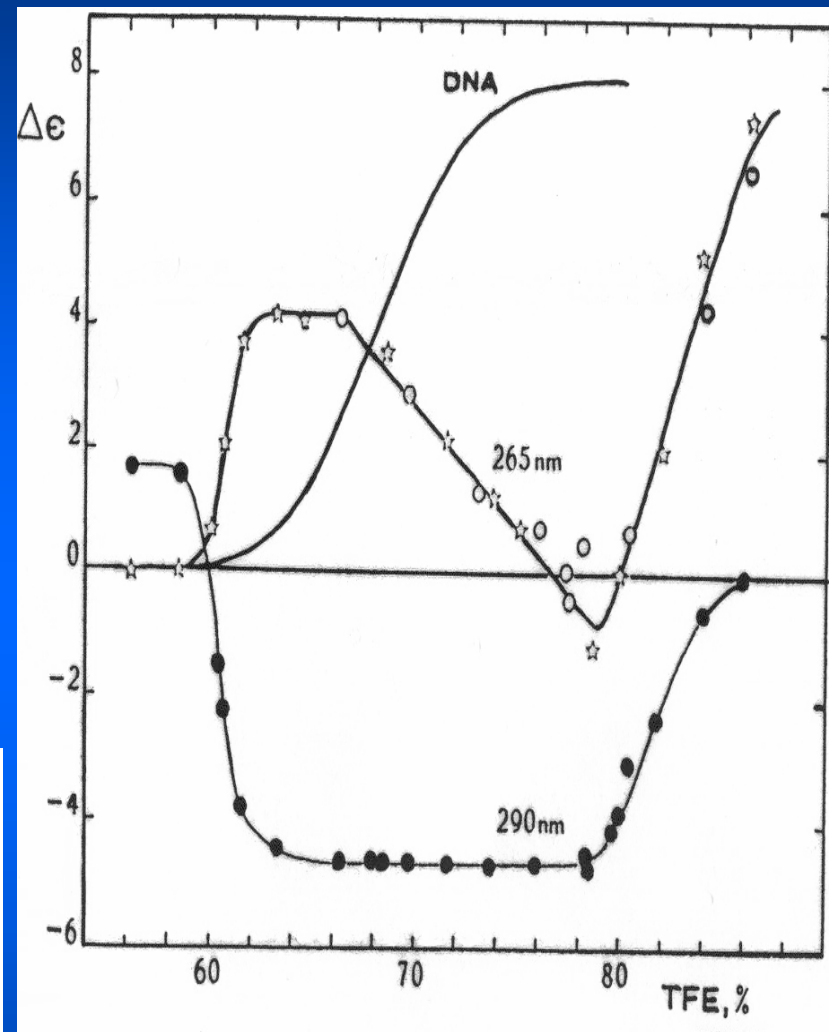
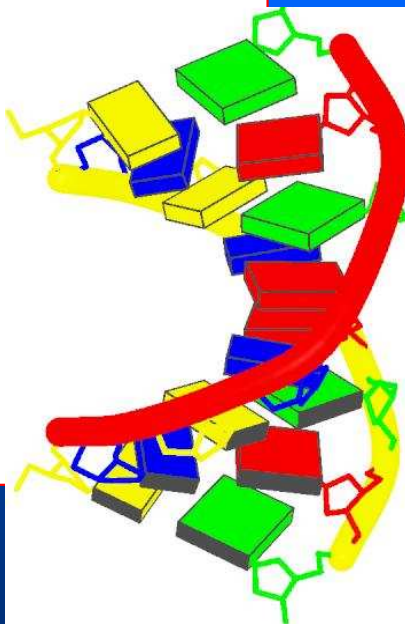
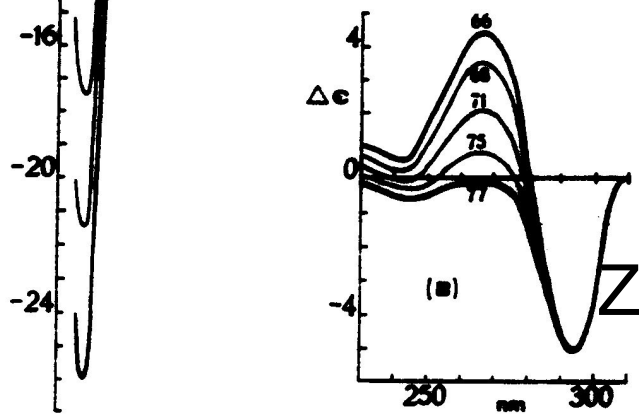
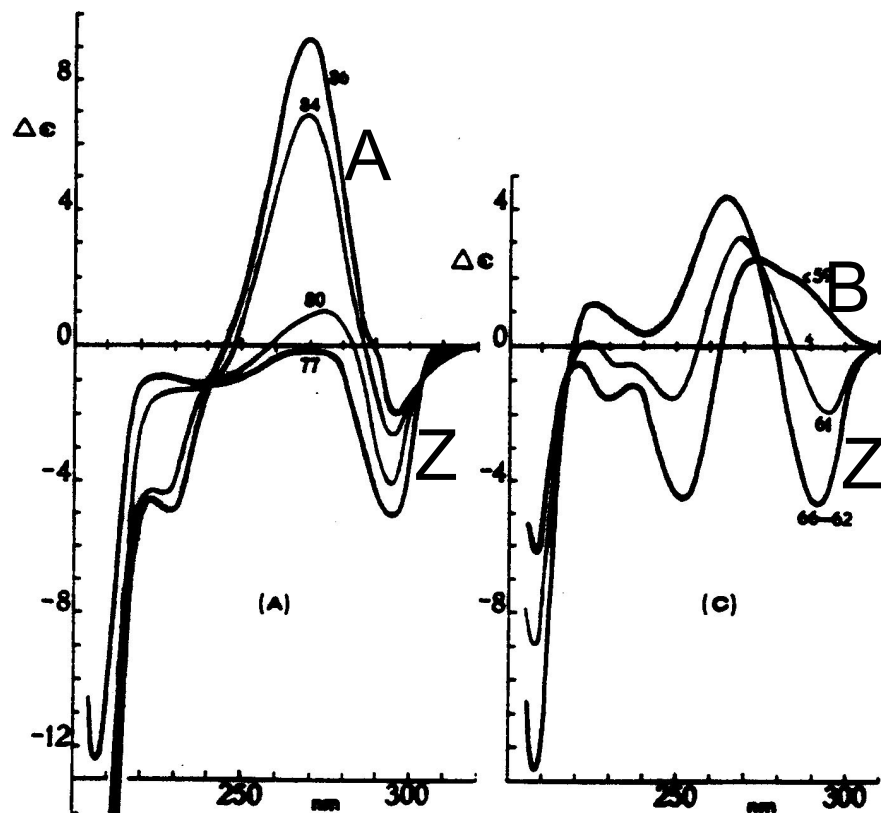


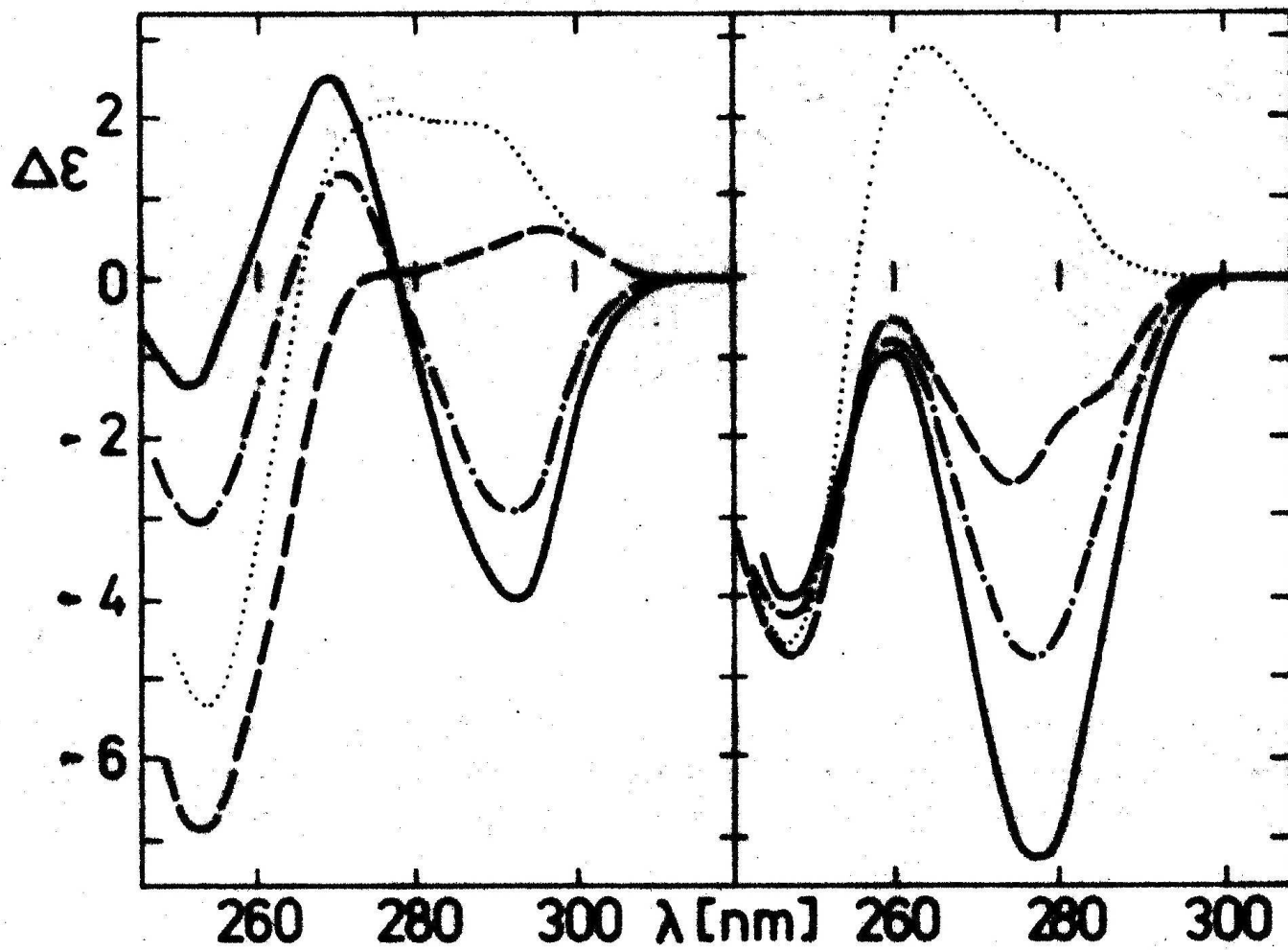


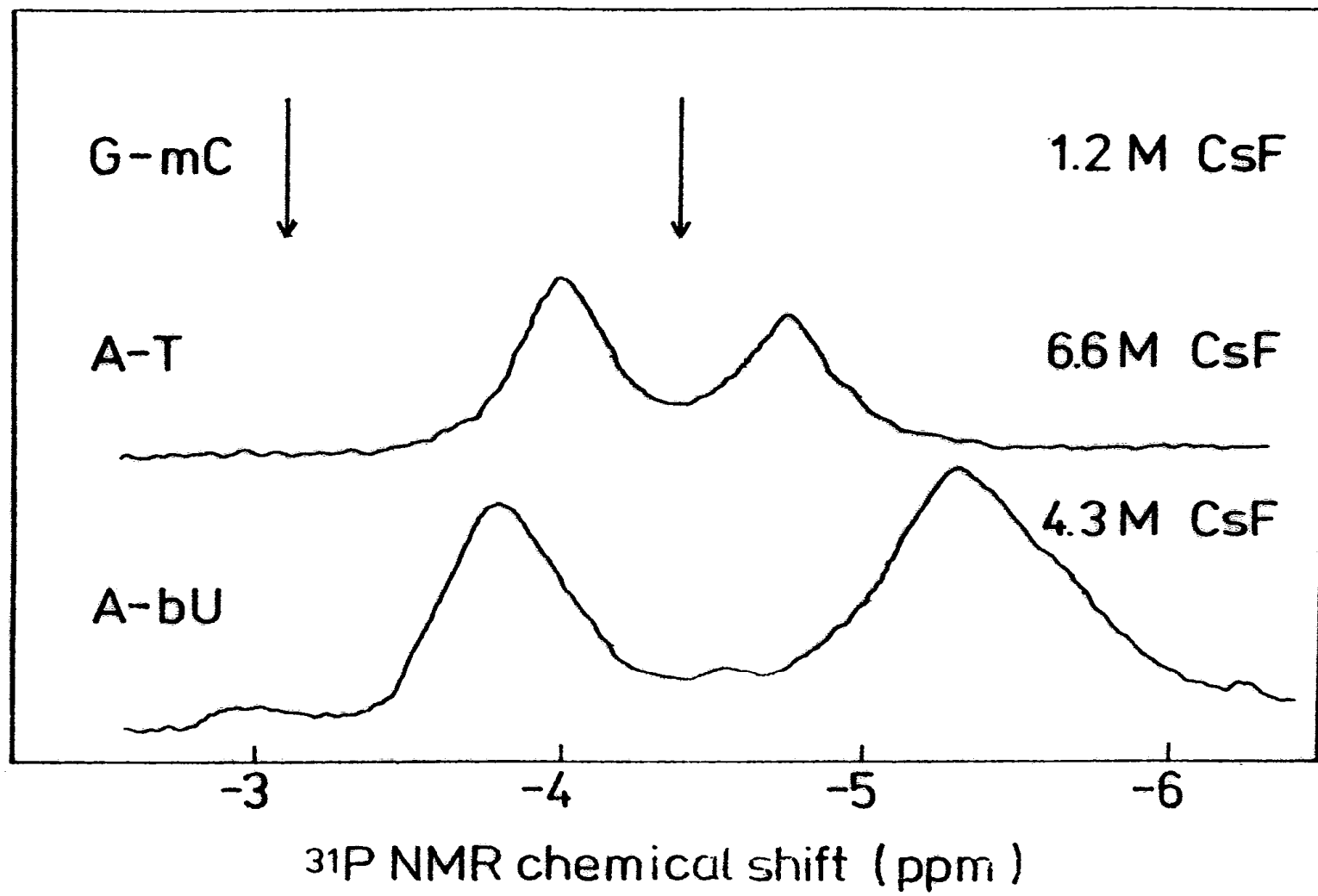


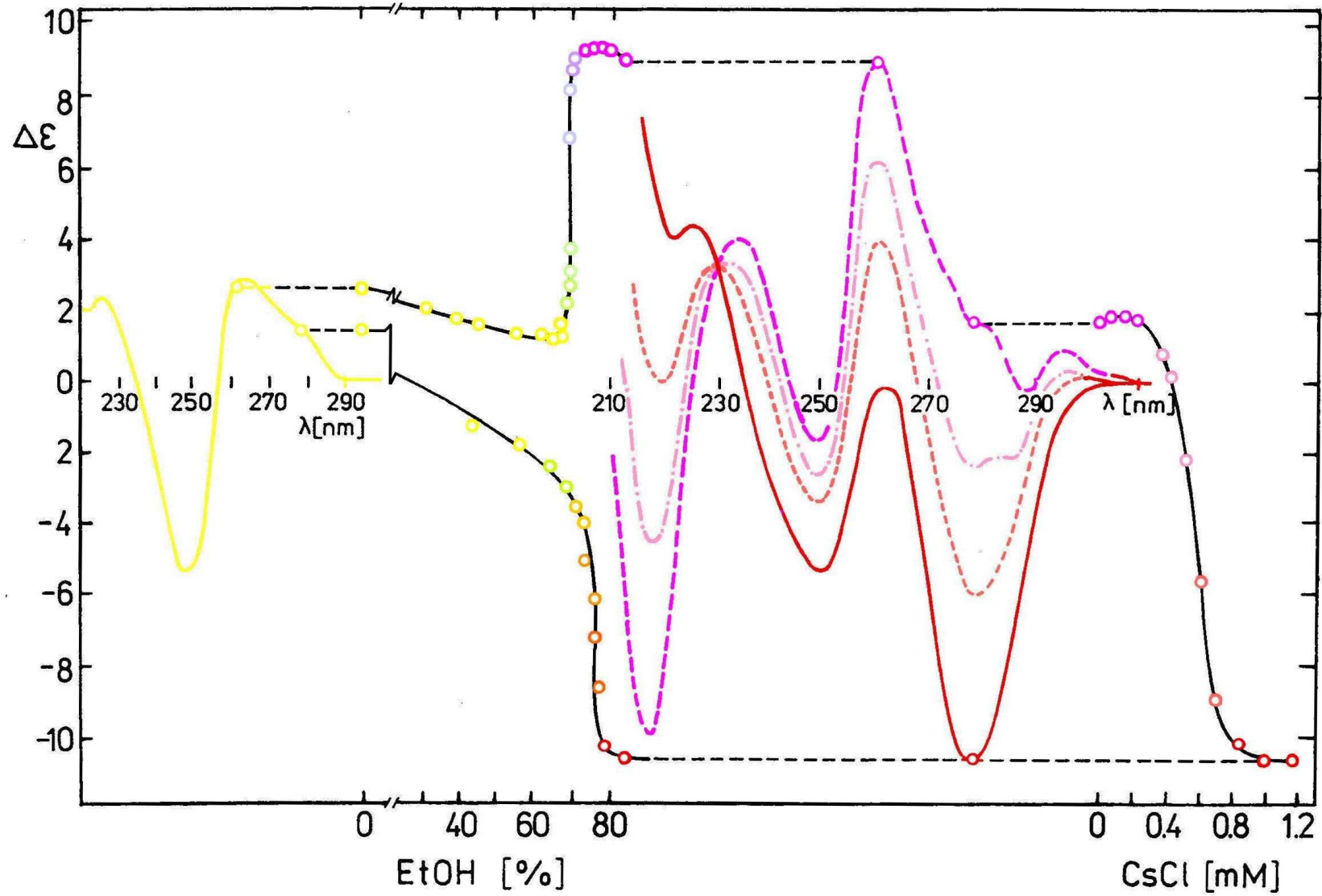
B

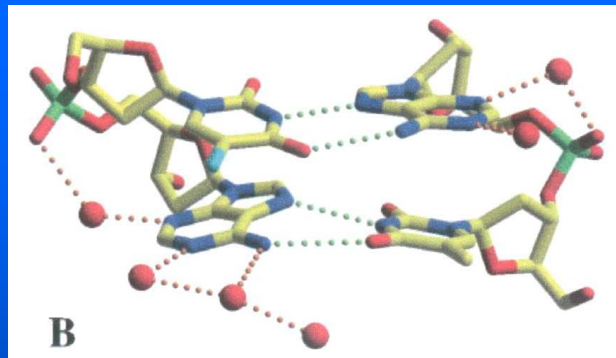
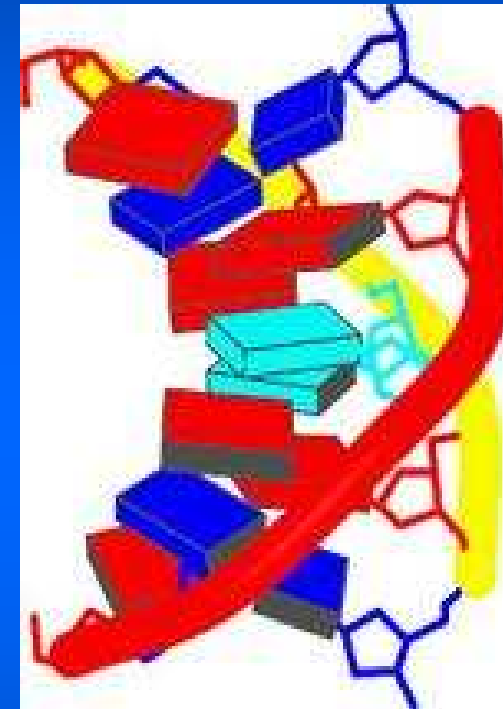
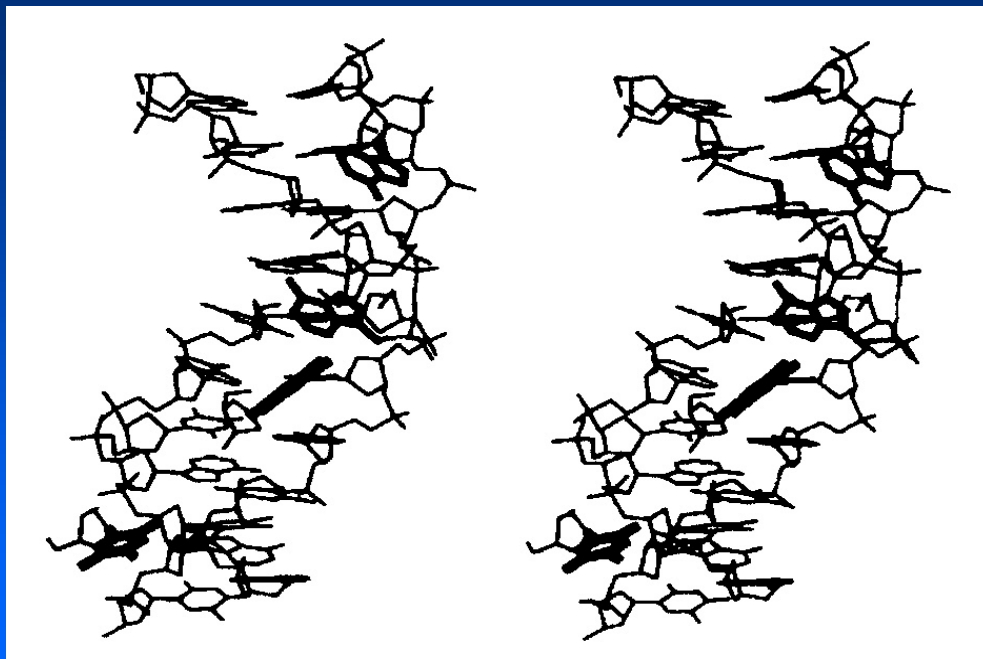










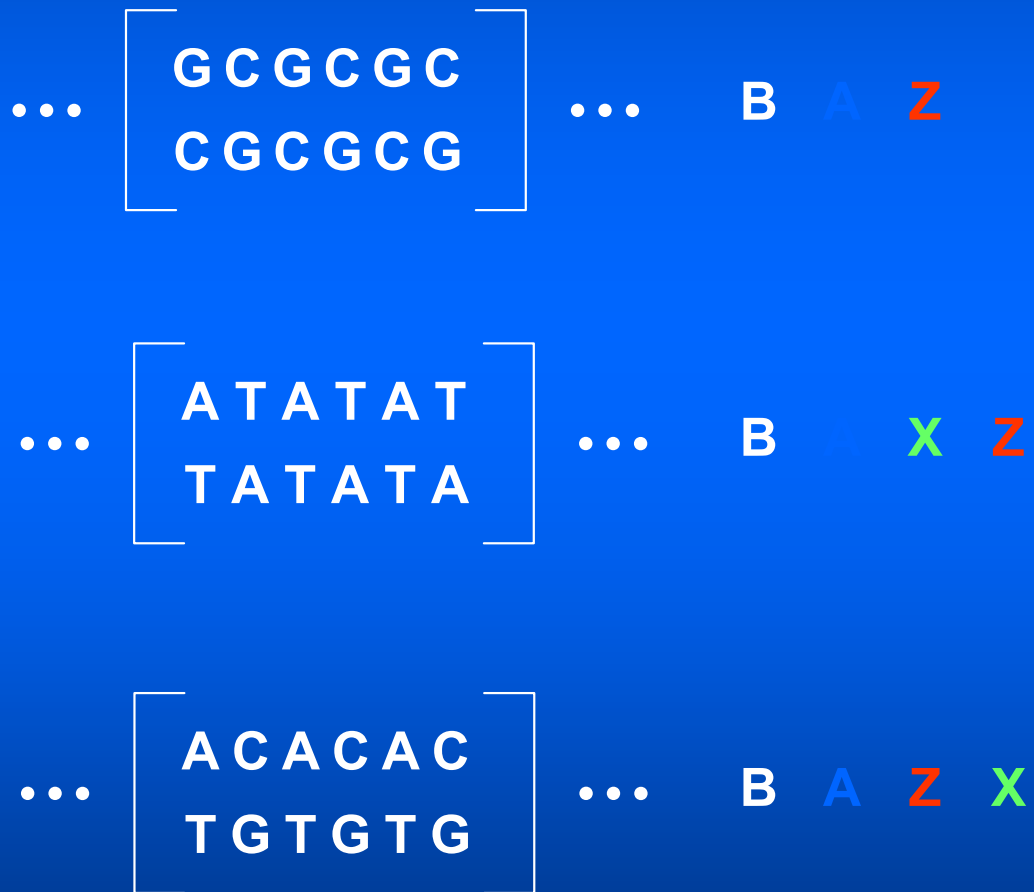


ALTERNATING A-T FRAGMENT WITH HOOGSTEEEN BASE PAIRING

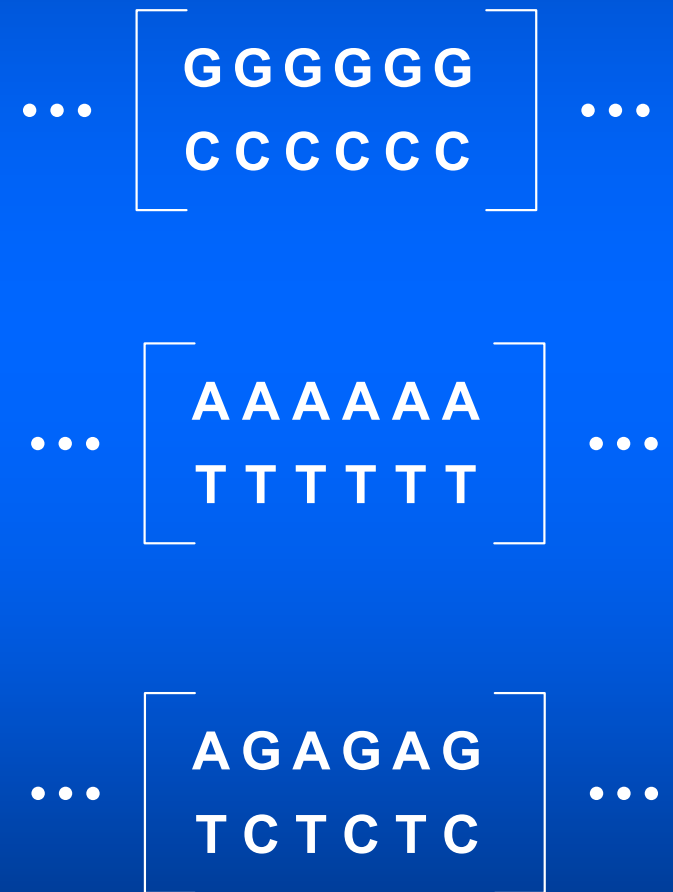
Subirana, J. *Proc.Nat.Acad.Sci.USA* , **99**, pp. 2806, 2002.

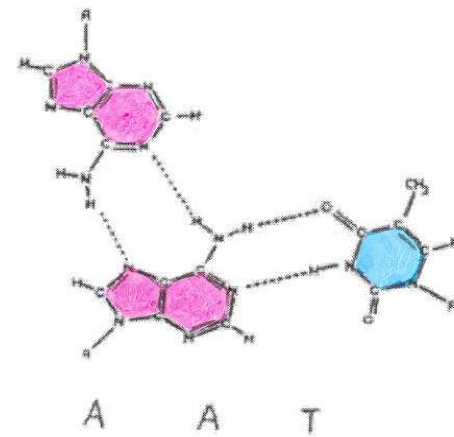
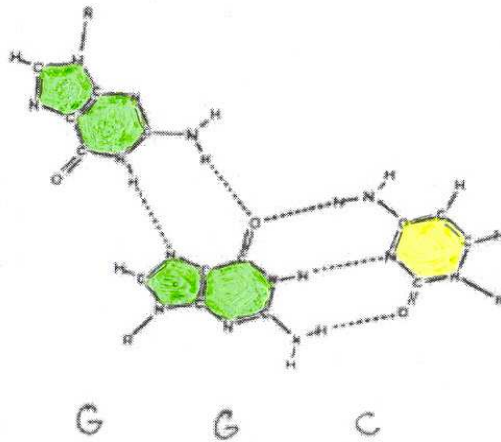
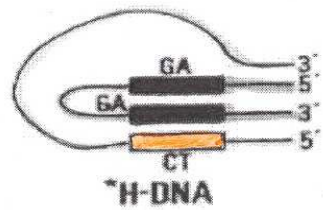
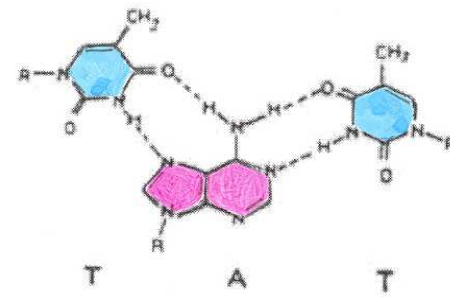
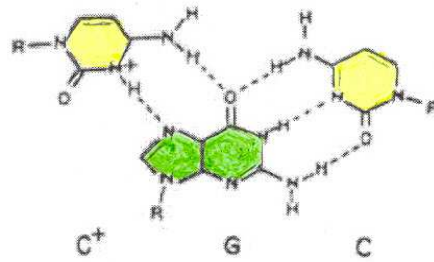
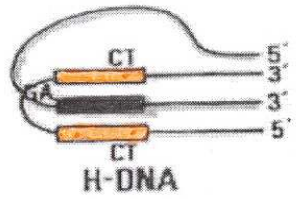
Biochemistry , **43**, pp. 4092 - 4100, 2004.

Dinukleotidová opakování (Pu-Py)_n



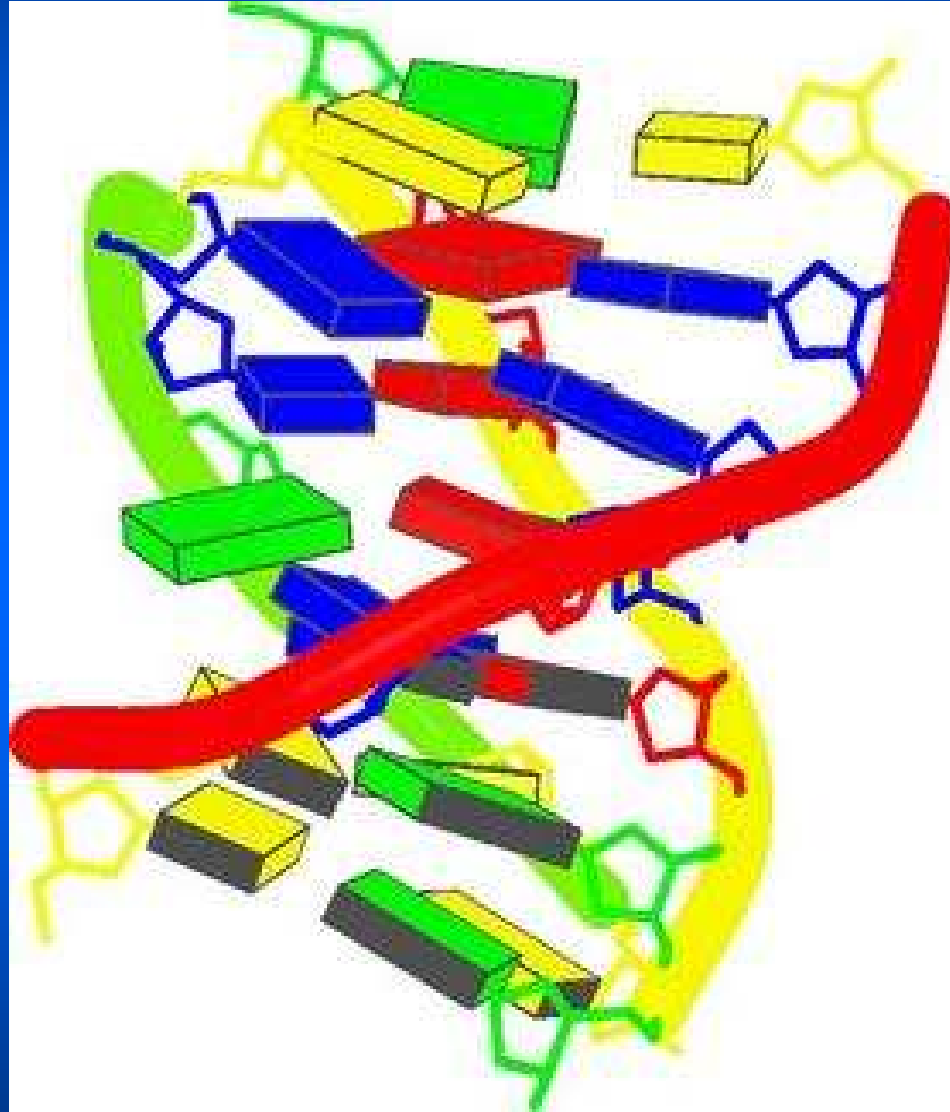
Mononukleotidová opakování (Pu)_n · (Py)_n





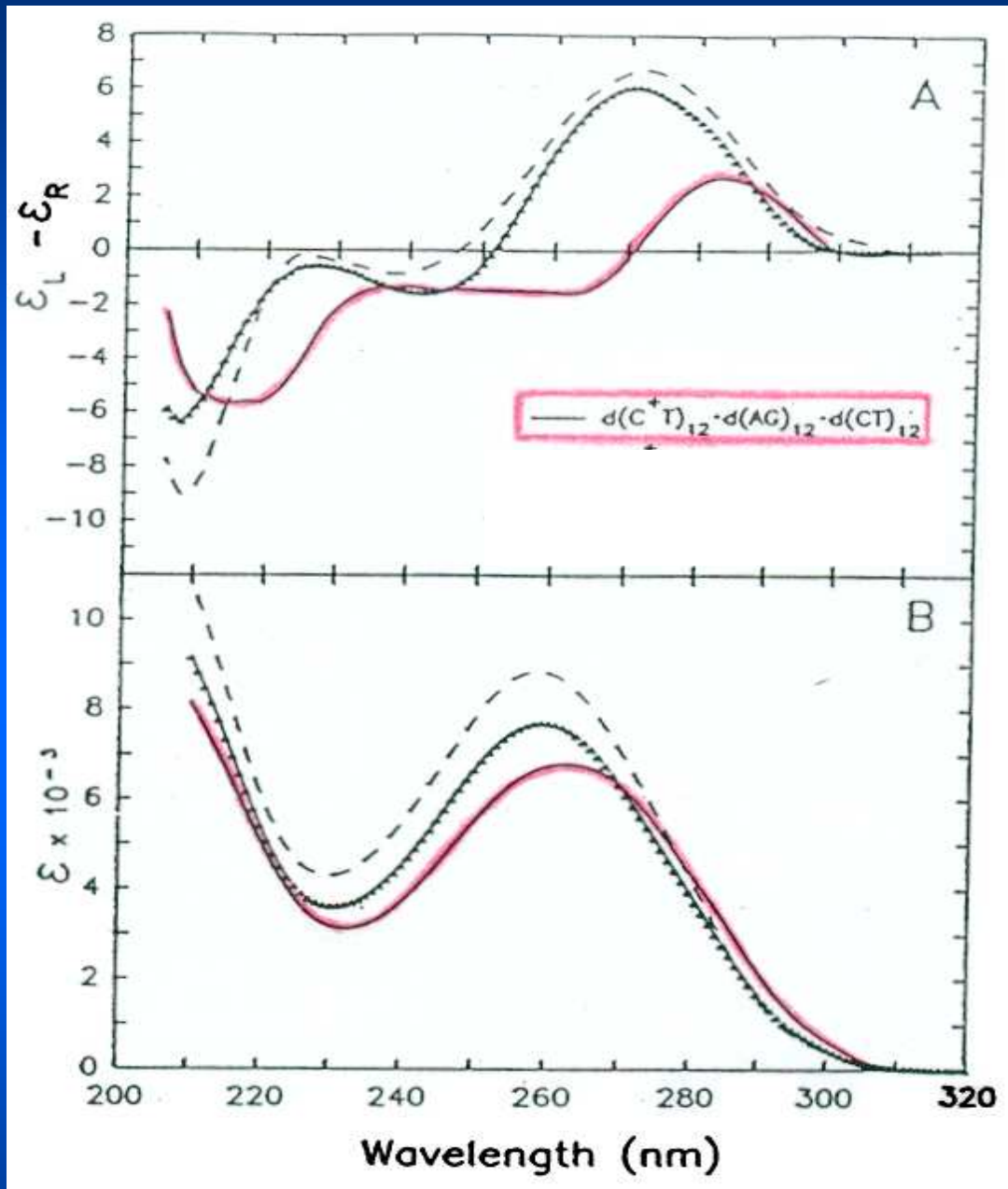
Pyrimidine. Purine. Pyrimidine DNA Triplex

CCTATTC
GAATAGG
CTTGTCC



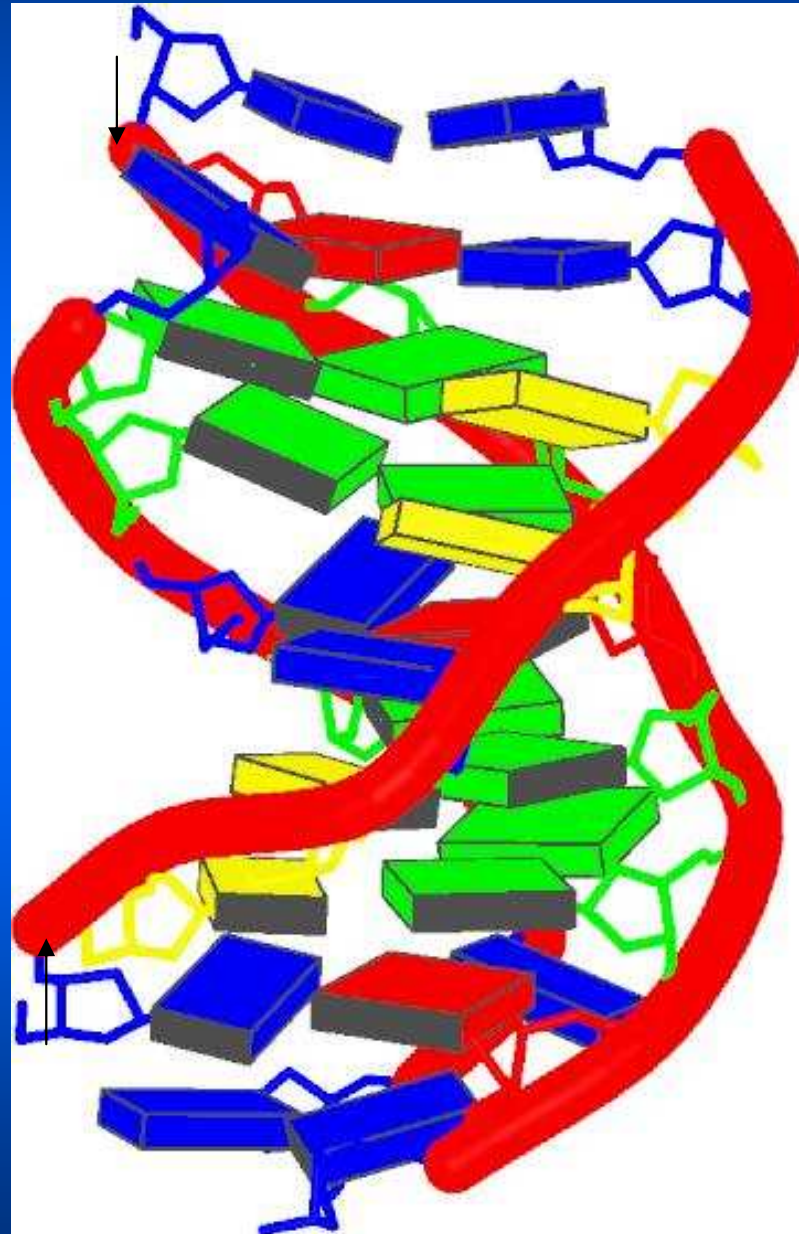
[Radhakrishnan, I., Patel, D.J.](#)

Pyrimidine.Purine.Pyrimidine DNA Triplex *Structure* , 2, pp. 17, 1994.

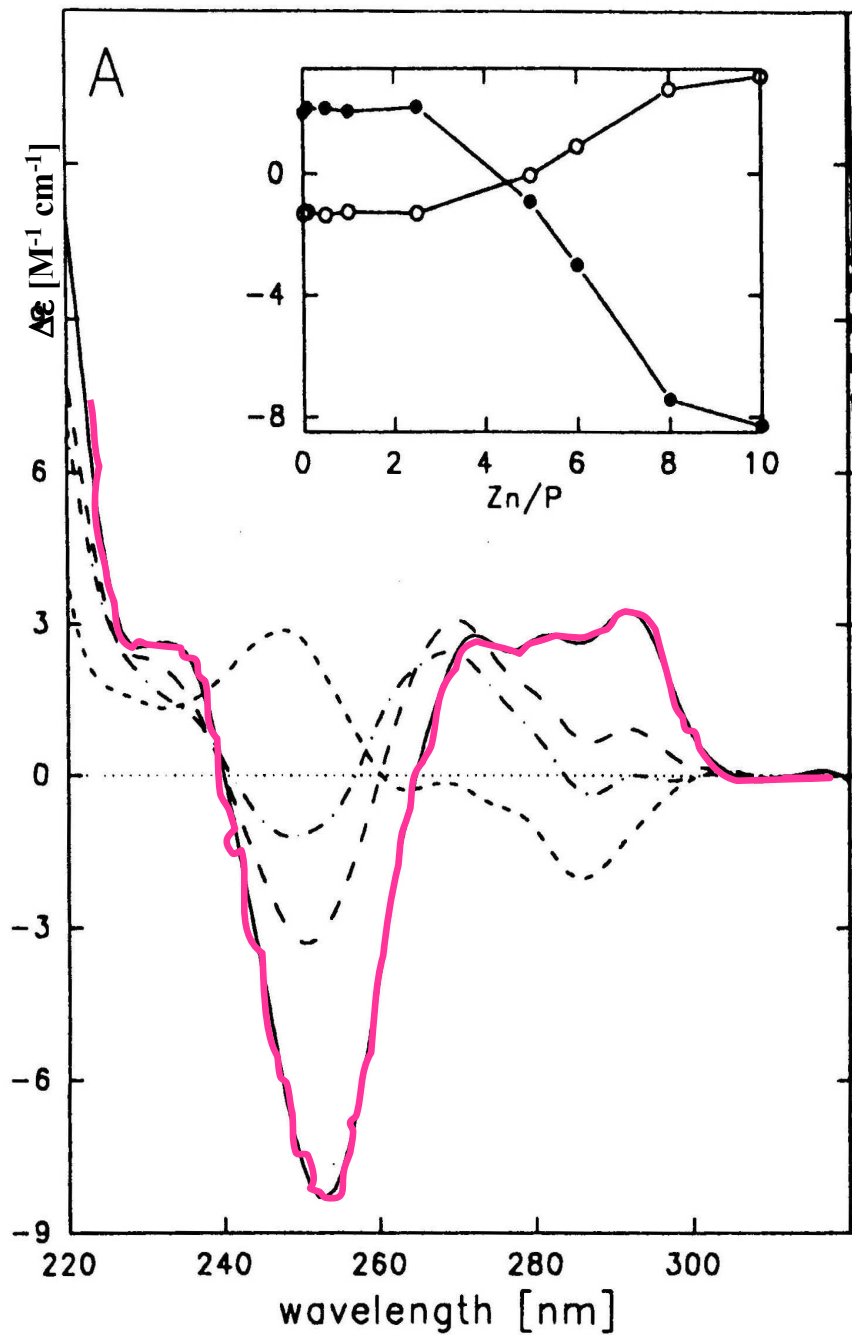


Purine.Purine.Pyrimidine DNA Triplex

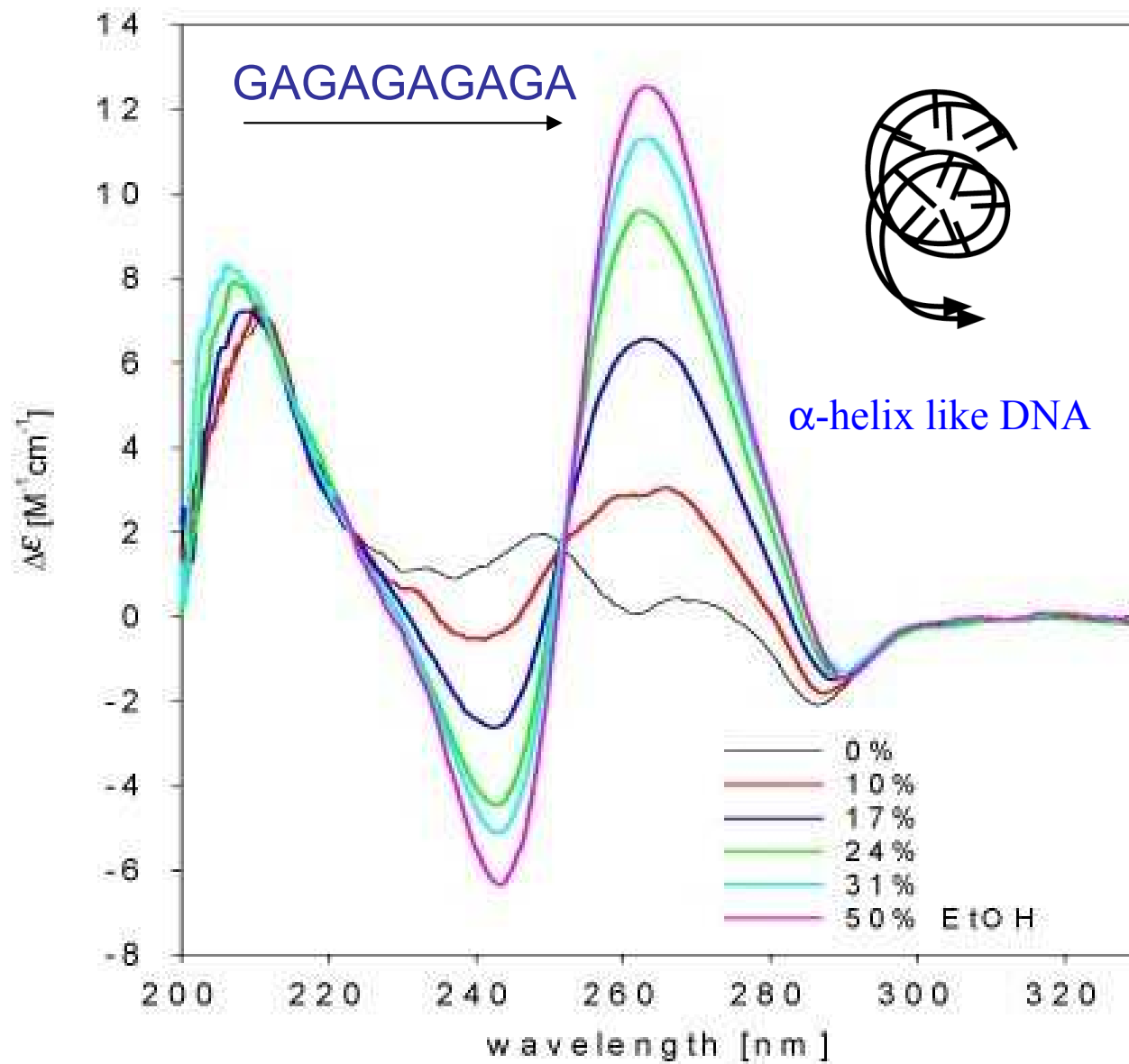
TAGGAGGATTTTTTGGTGGT TCCTCCTT



$\Delta\epsilon$ [$M^{-1} cm^{-1}$]

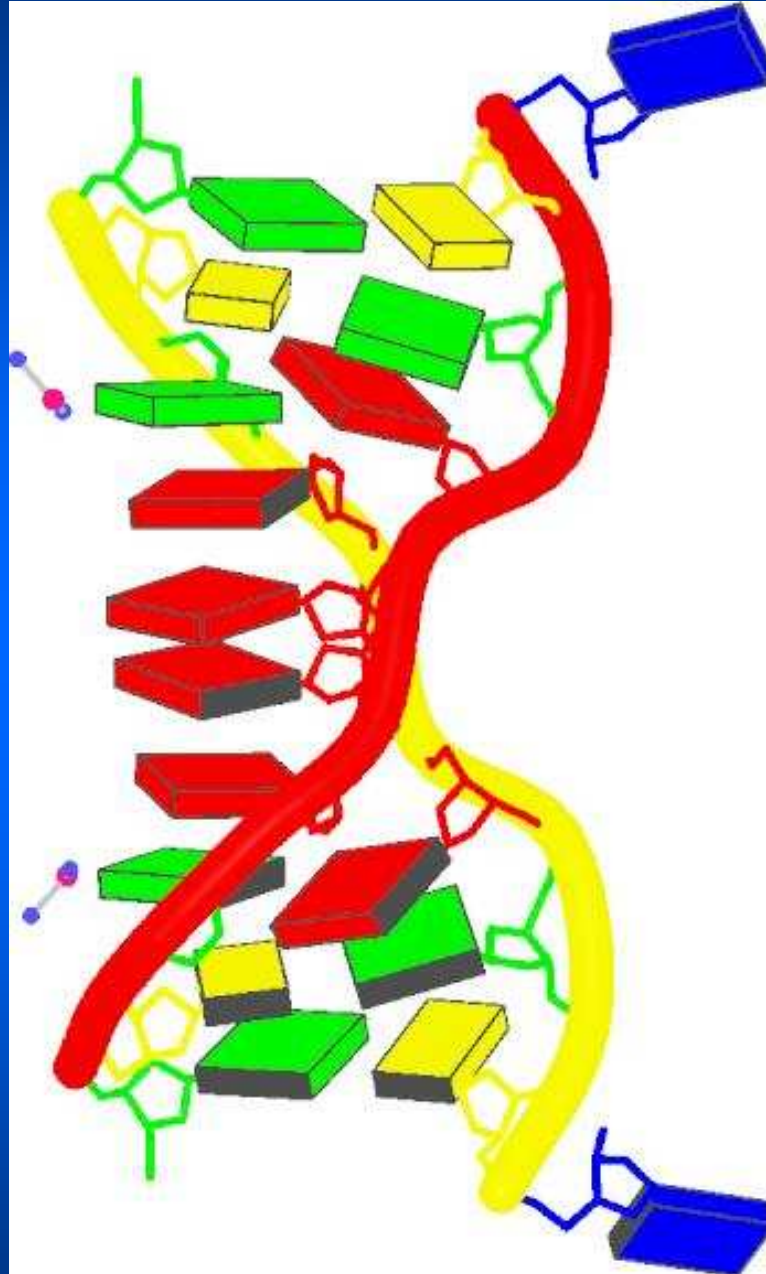


CTCTCTCTC
GAGAGAGAG
AGAGAGAGA

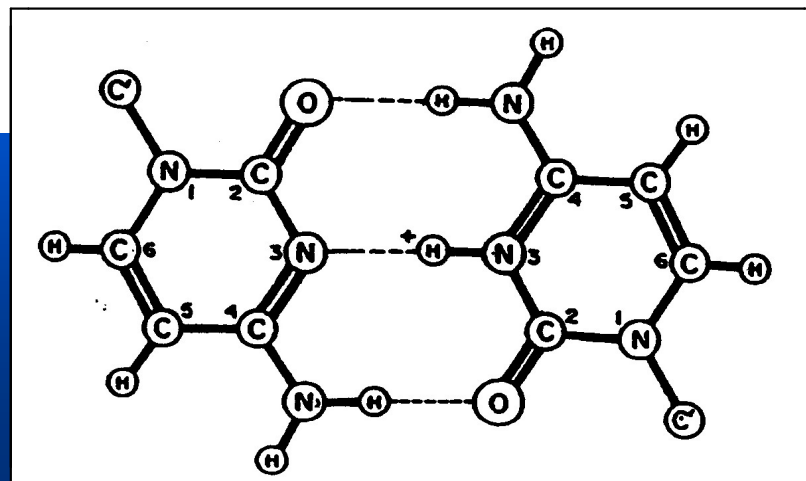
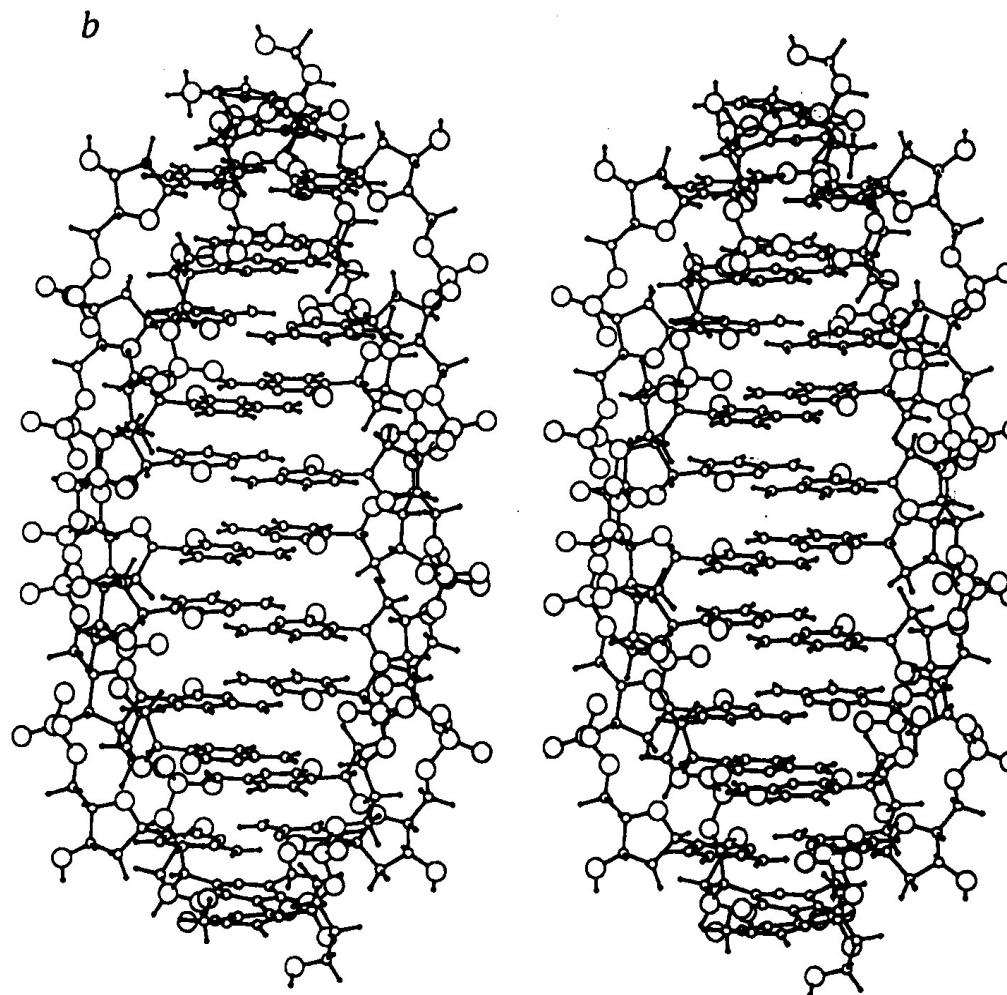
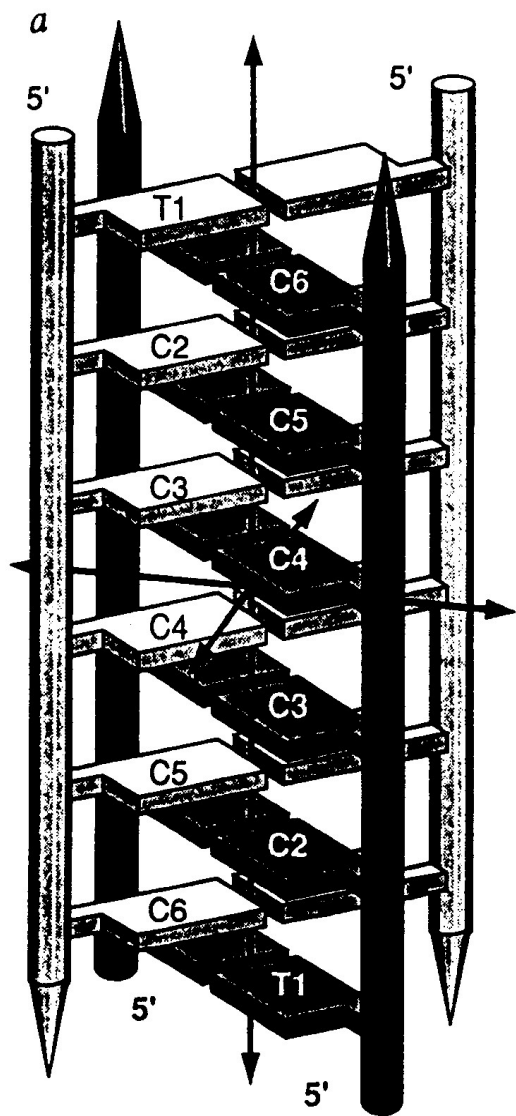


A ZIPPER-LIKE DNA DUPLEX

d(GCGAAAGCT)



Shepard, W. et al.,
Structure 6, pp. 849 - 861, 1998.

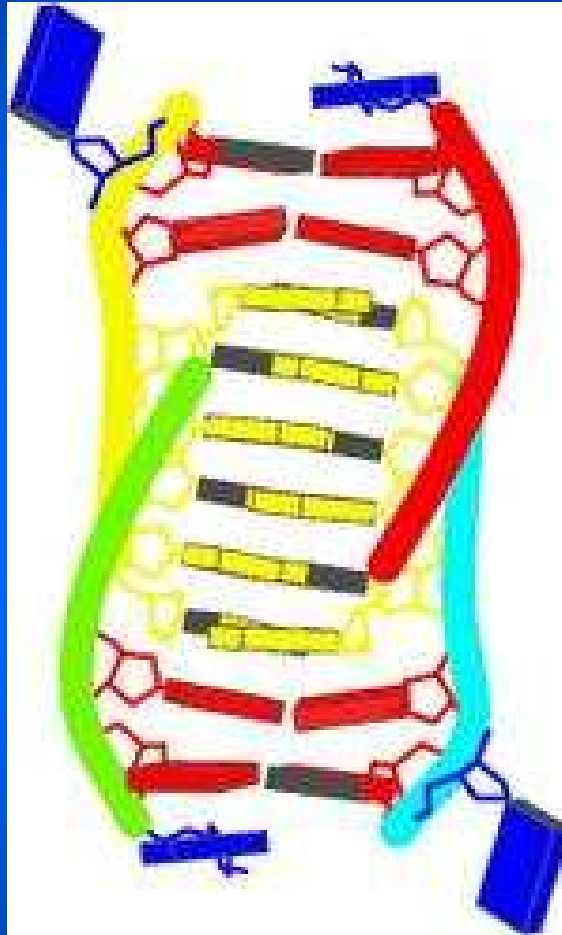


i - tetraplex

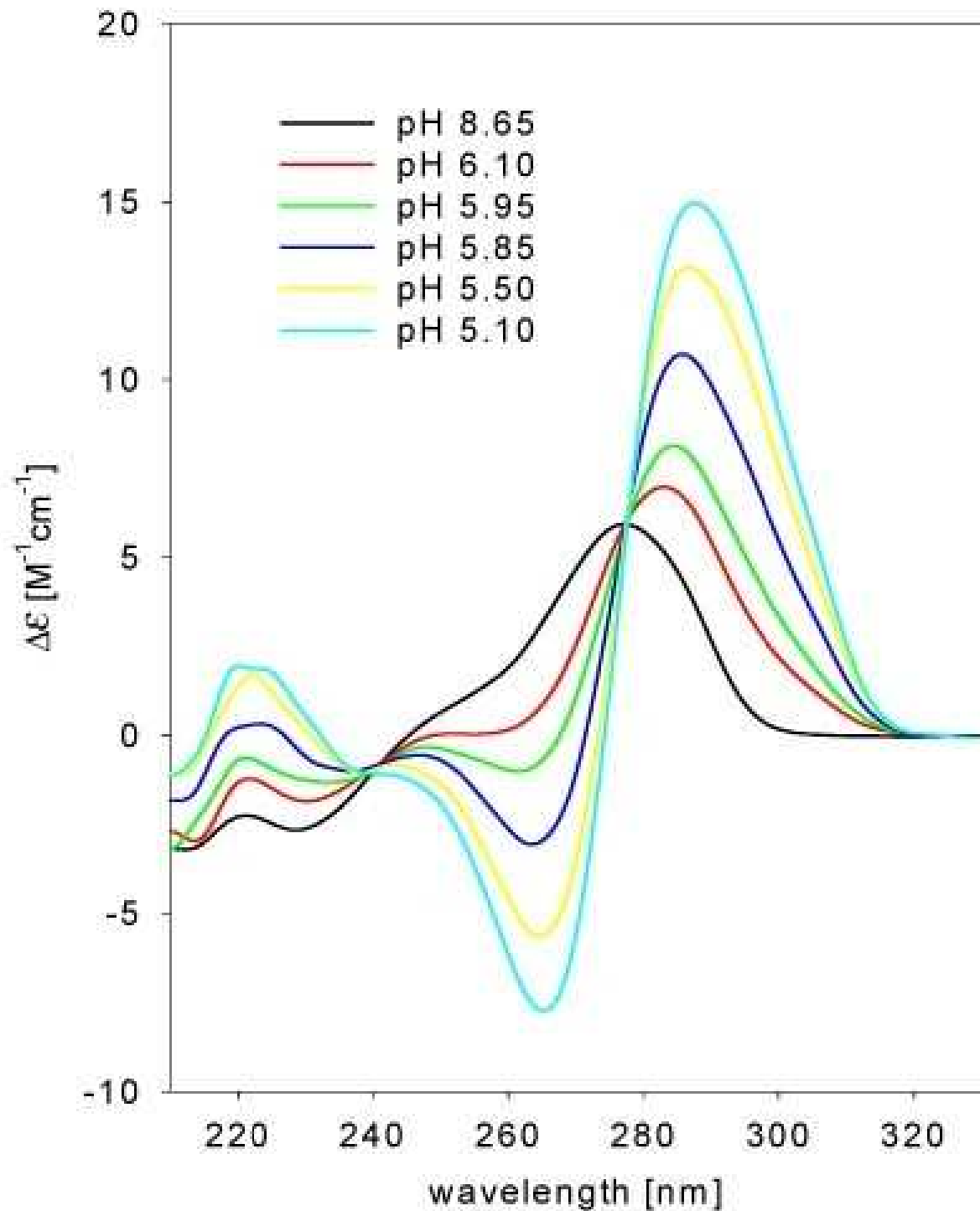
Guéron, Leroy

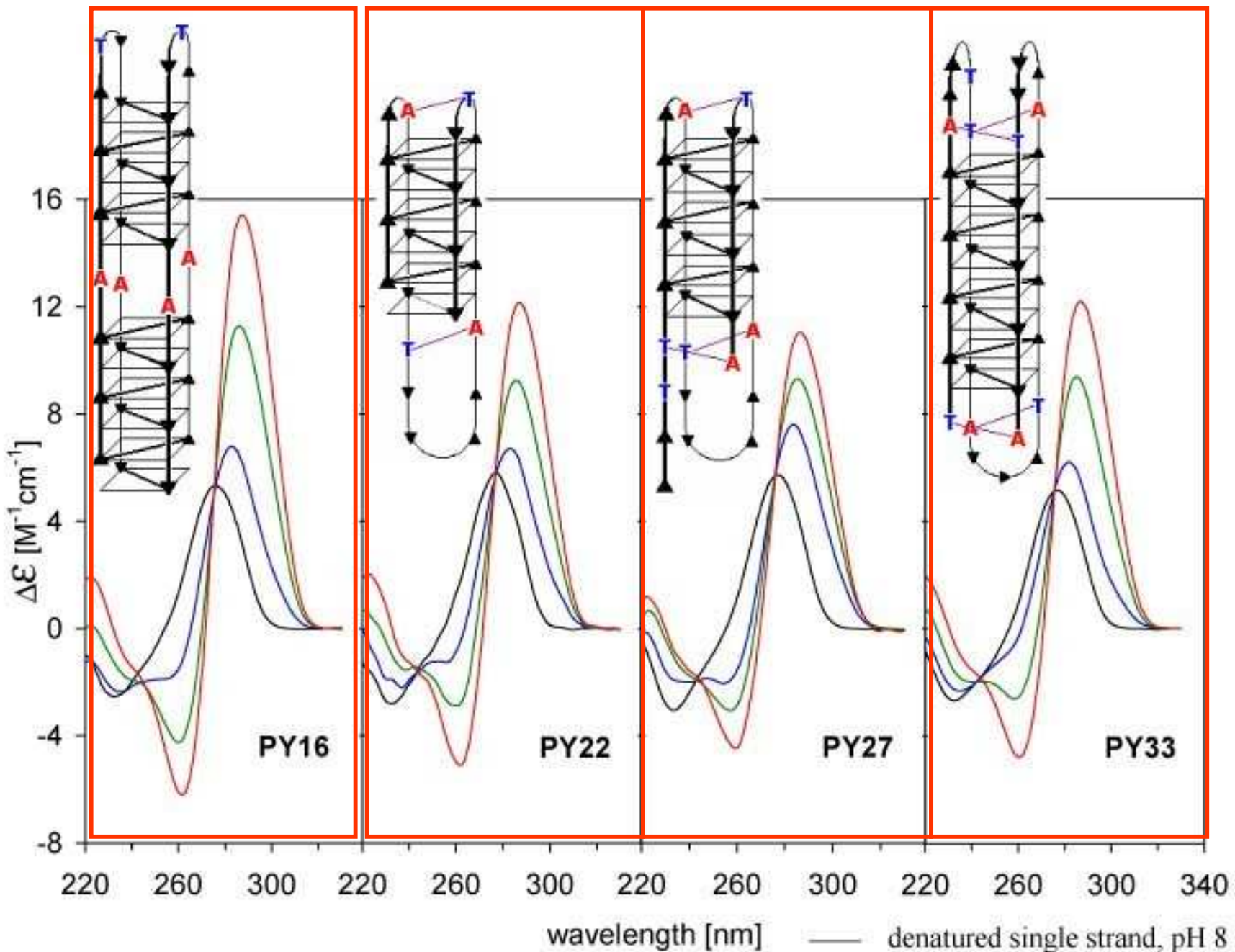
Intercalated tetraplex of human telomeric DNA

D(C C C A A T)



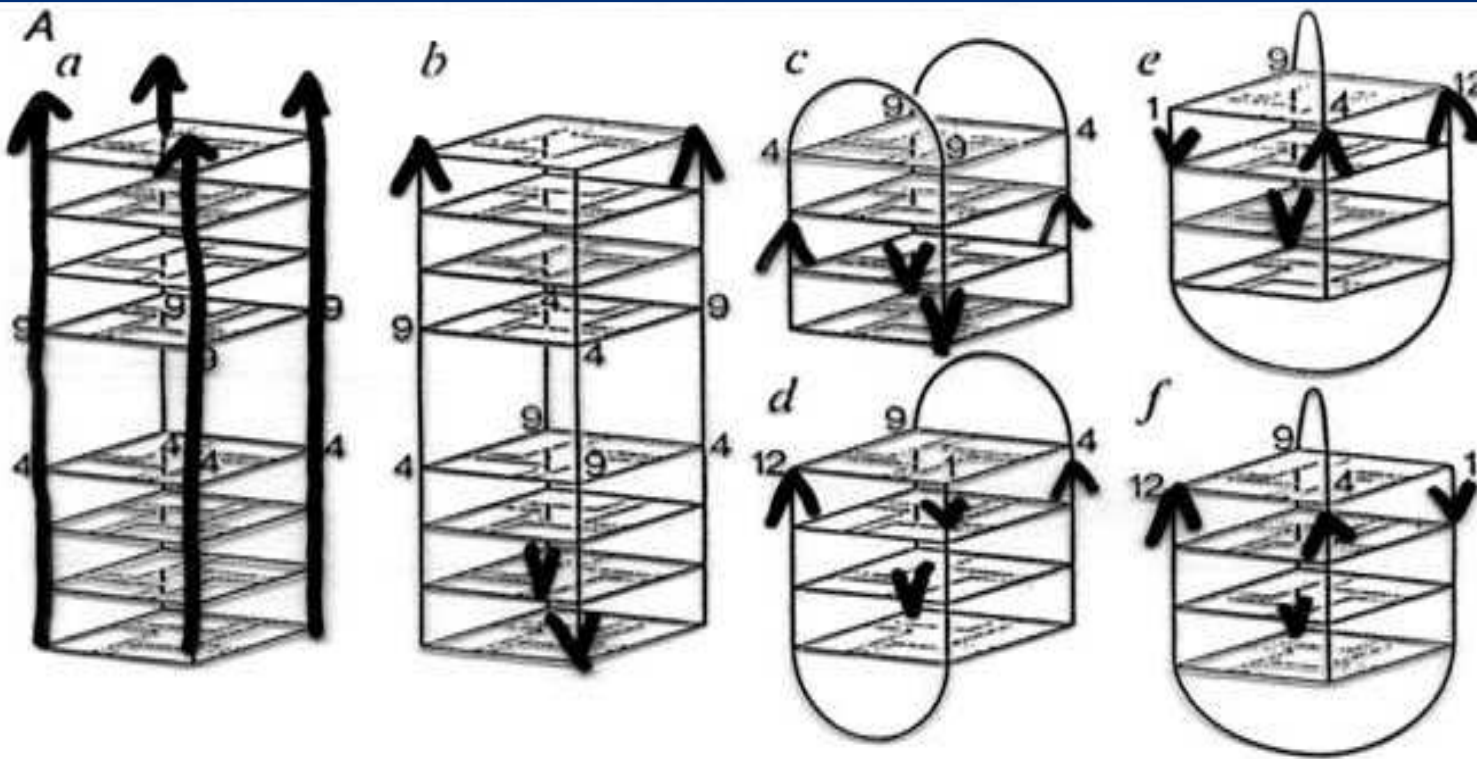
Berger, I., Kang, C., Fredian, A., Ratliff, R., Moyzis, R., Rich, A.
Nat.Struct.Biol., **2**, pp. 416 - 425, 1995



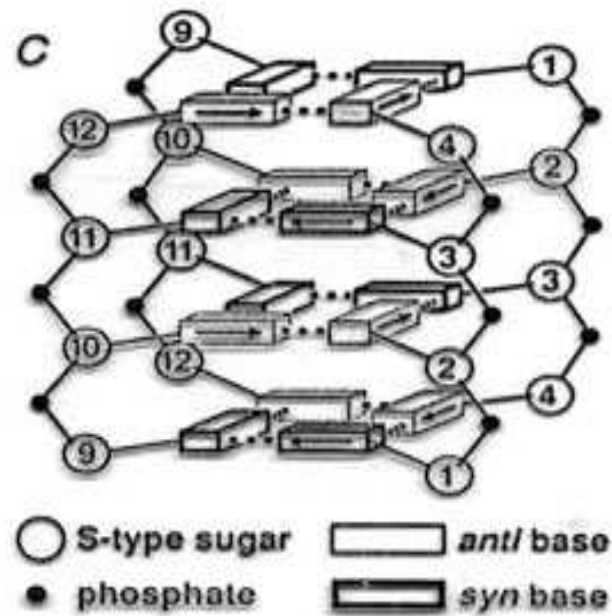
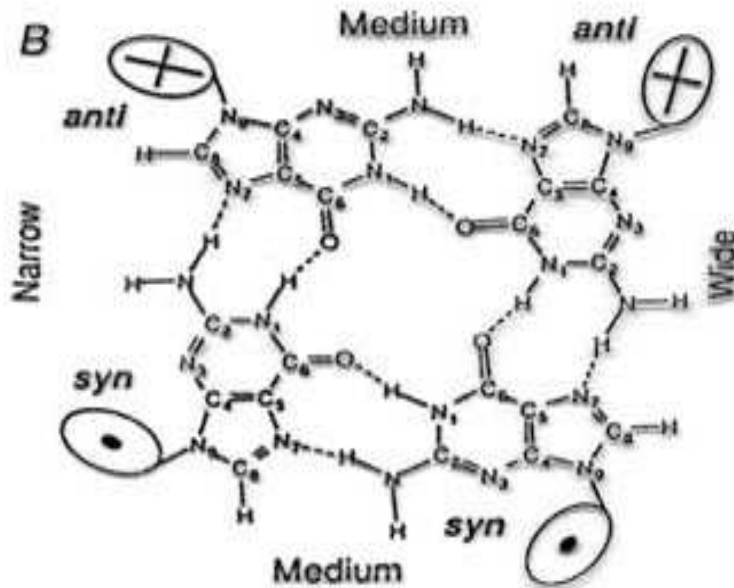


Fragmenty promotoru c-myc

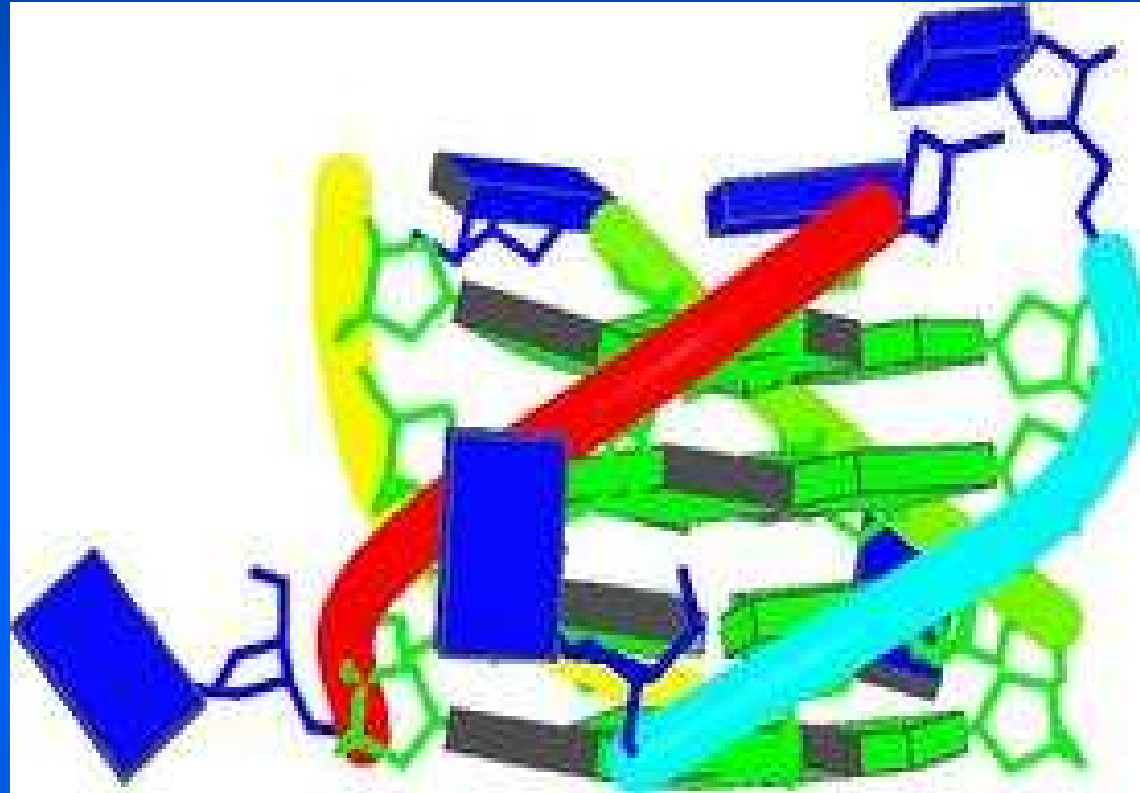
TCCCA CCTTCCCA CCCTCCCC ACCCTCCCA



G-tetraplex



PARALLEL-STRANDED GUANINE TETRAPLEX

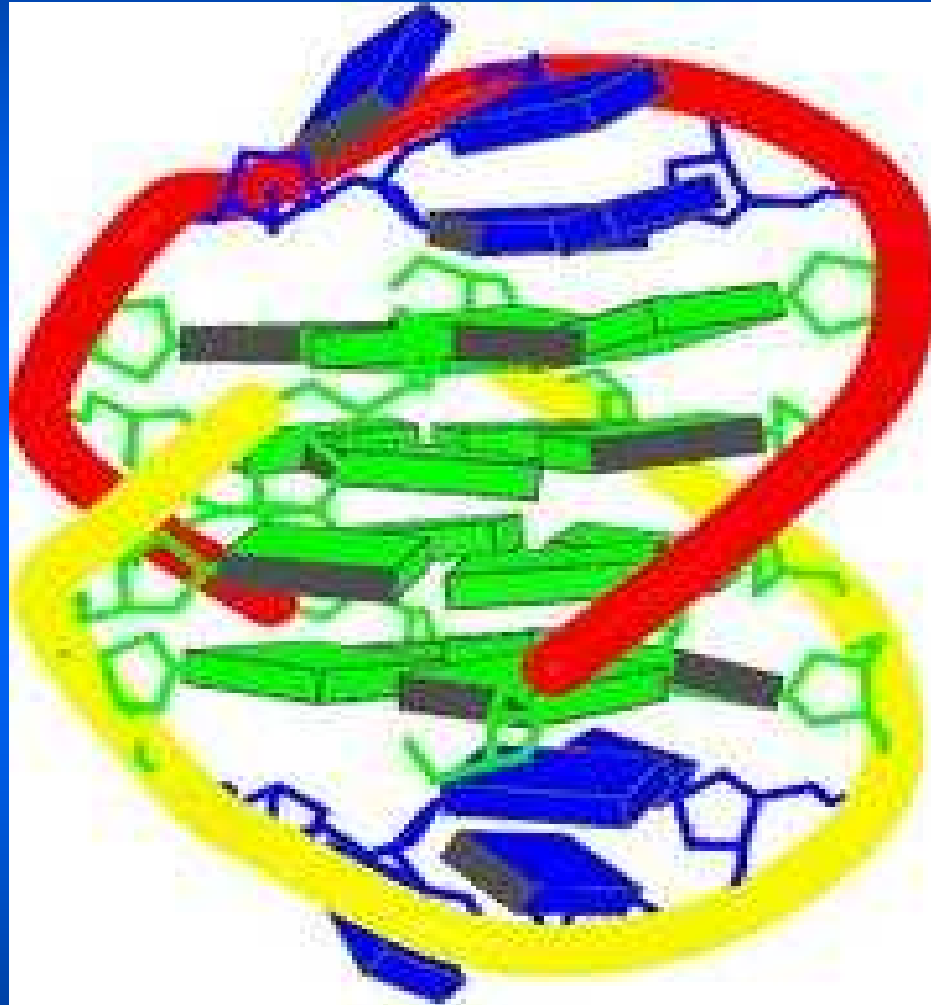


d(T G G G G T)

Phillips, K., Dauter, Z., Murchie, A.I.H., Lilley, D.M.J., Luisi, B.
J.Mol.Biol., **273**, pp. 171 - 182, 1997.

OXYTRICHA TELOMERIC DNA

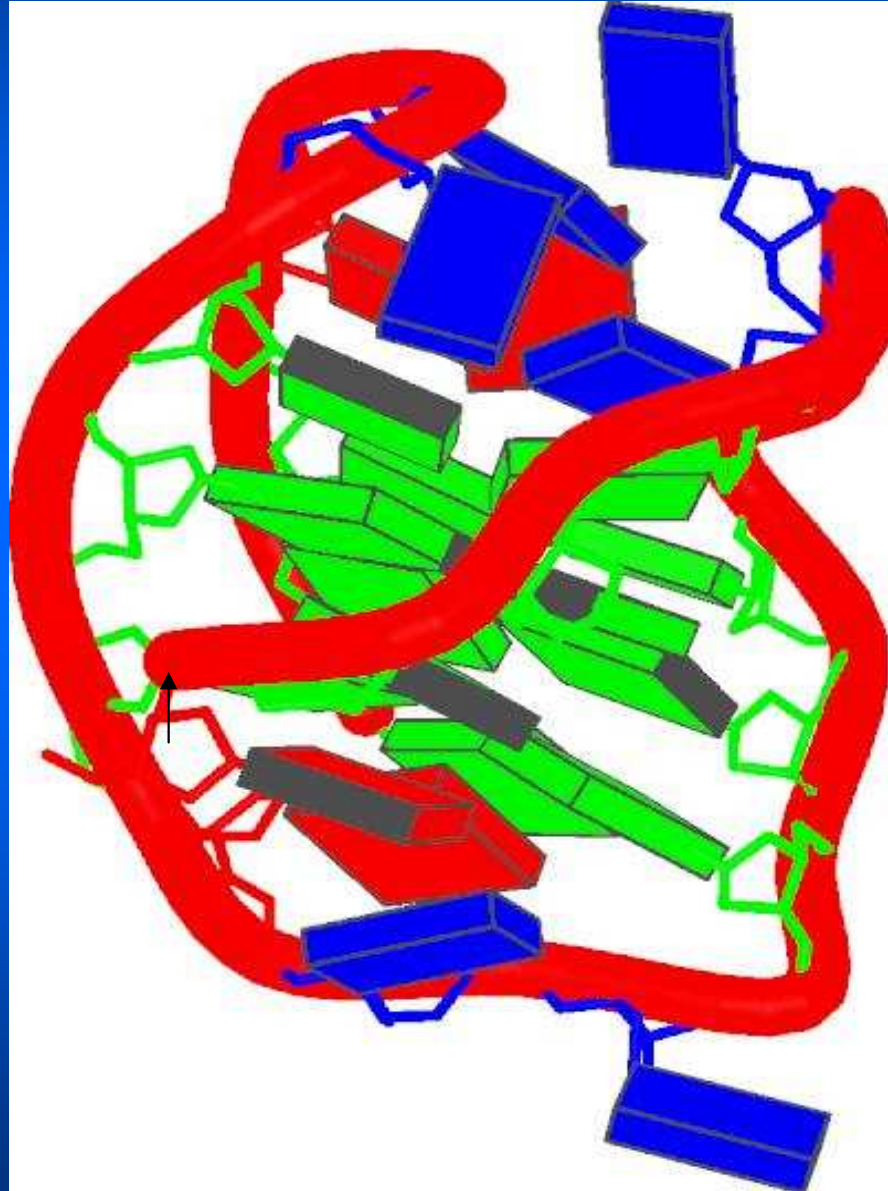
G4T4G4



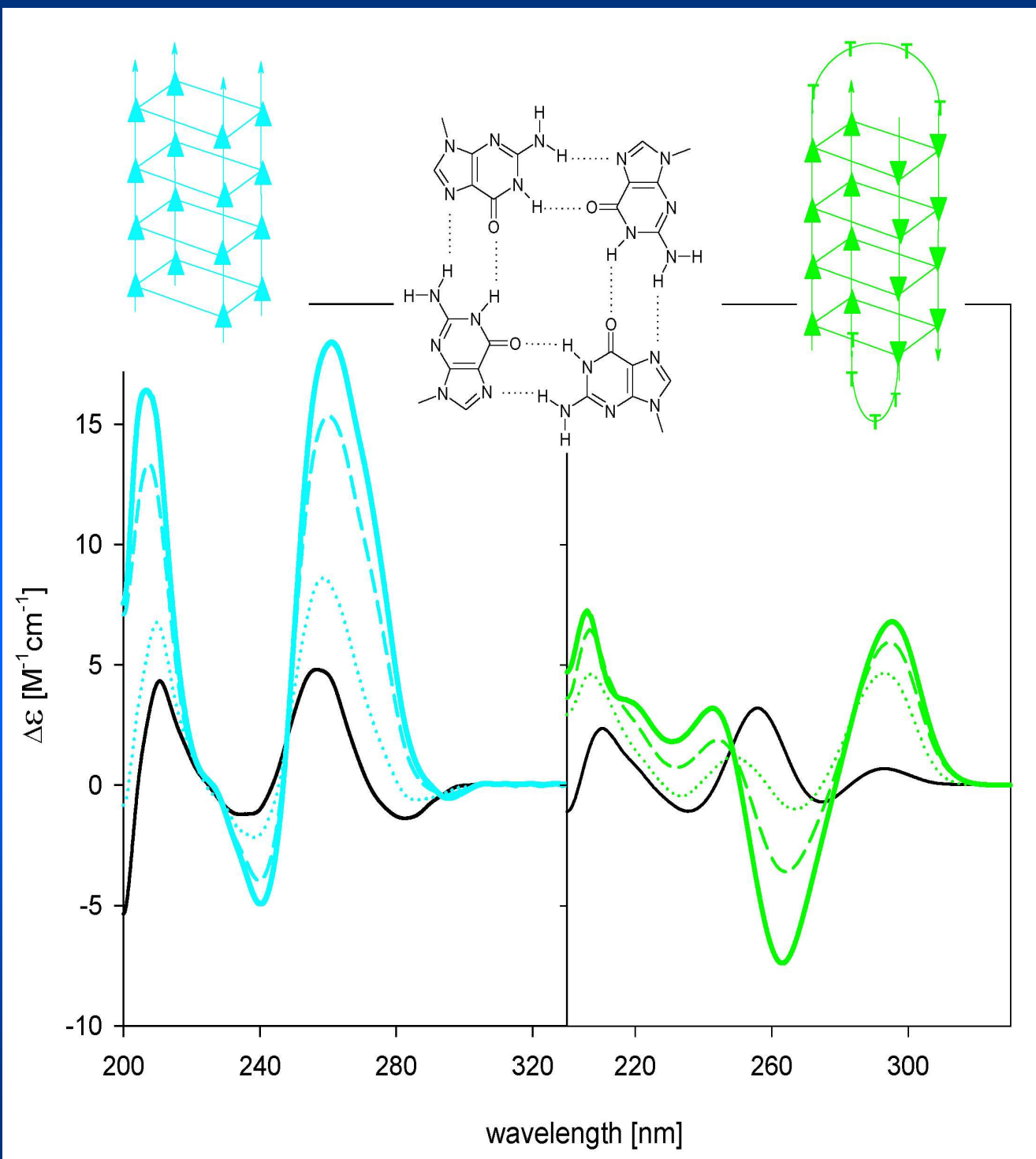
Haider, S.M., Parkinson, G., Neidle, S.
J.Mol.Biol., **320**, pp. 189 - 200, 2002

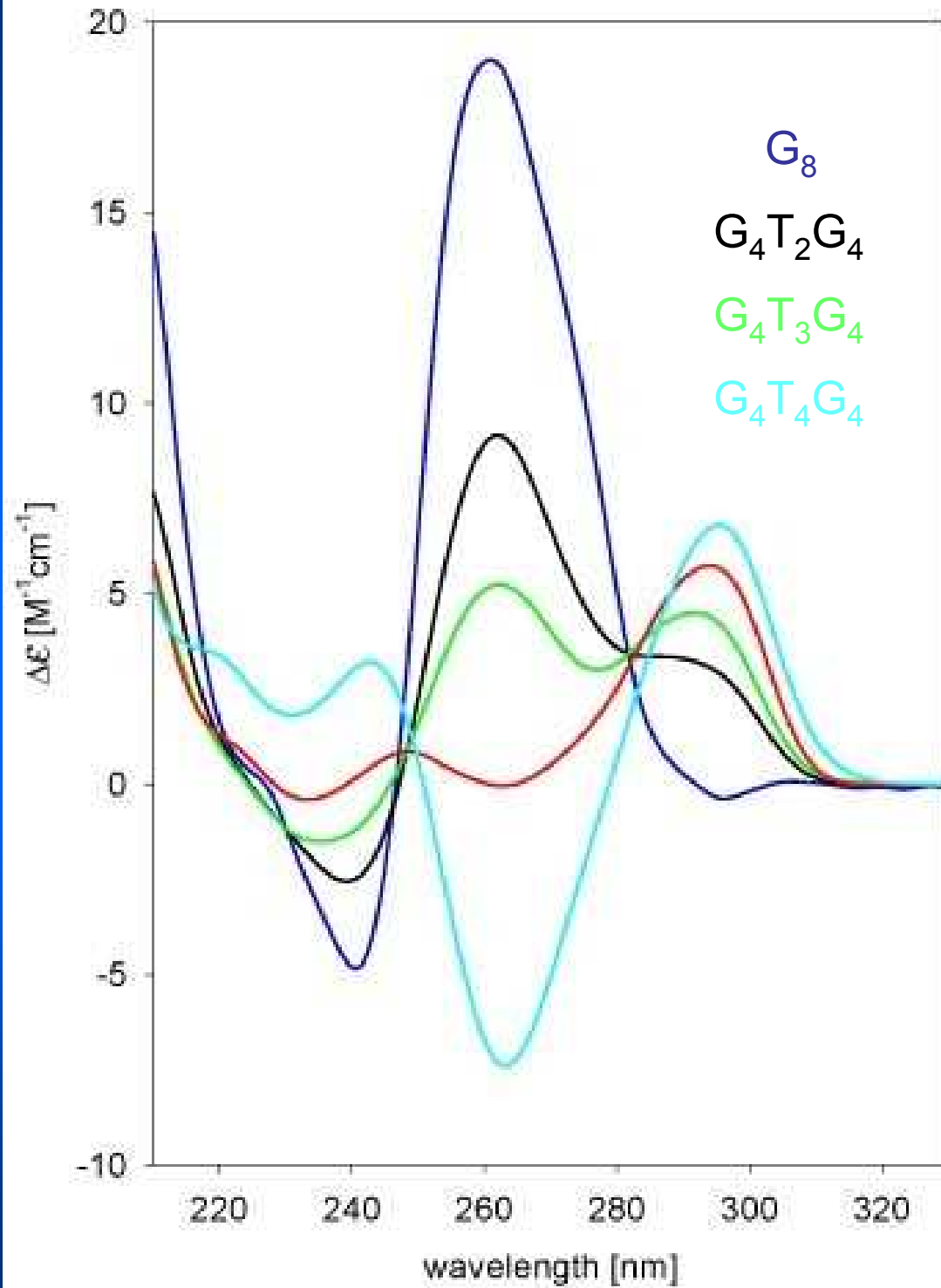
HUMAN TELOMERIC REPEAT

5'-d(AGGGTTAGGGTTAGGGTTAGGG)-3'



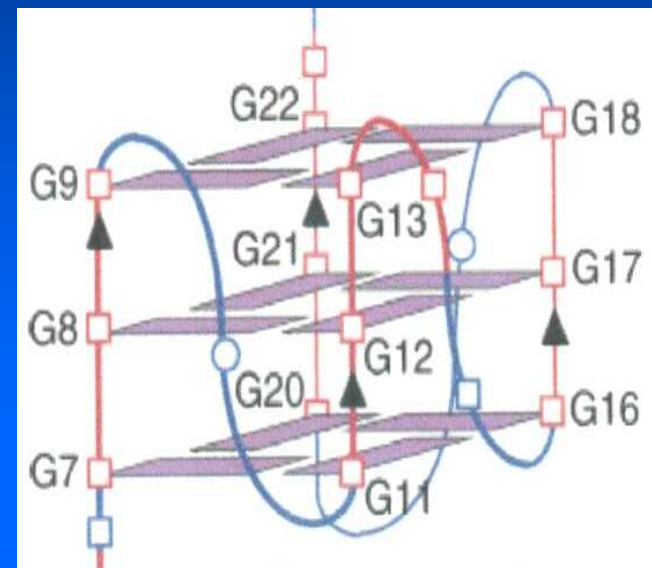
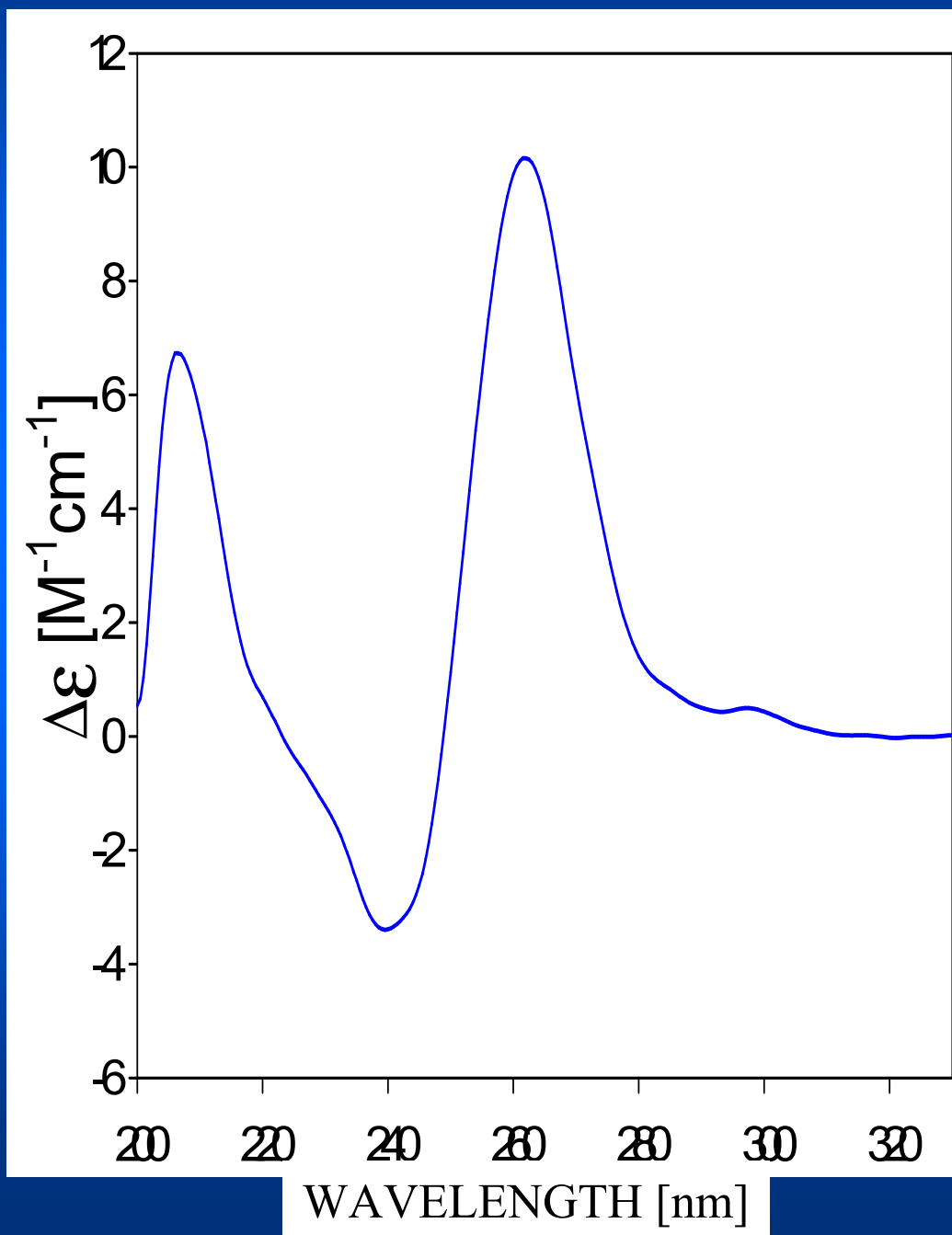
Wang, Y., Patel, D.J.
Structure, 1, pp. 263, 1993





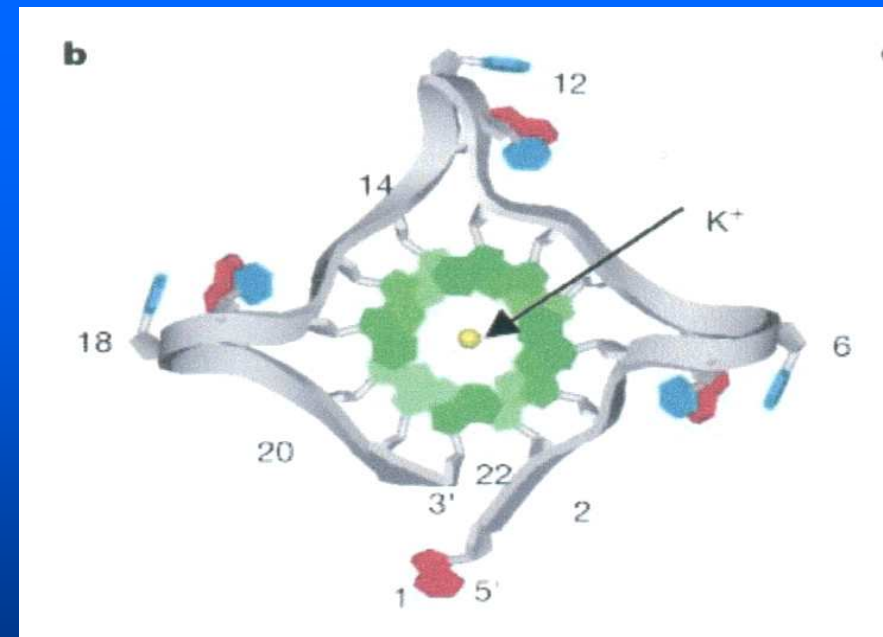
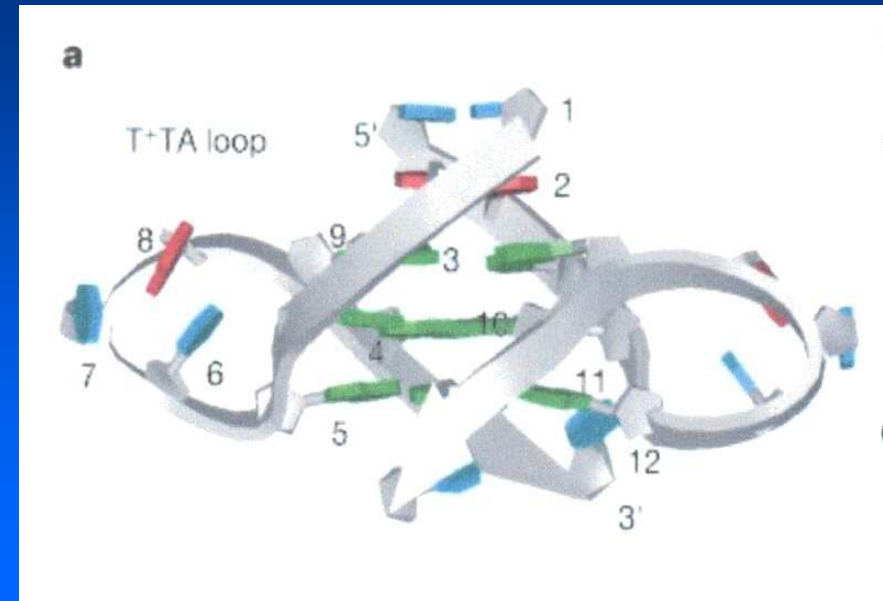
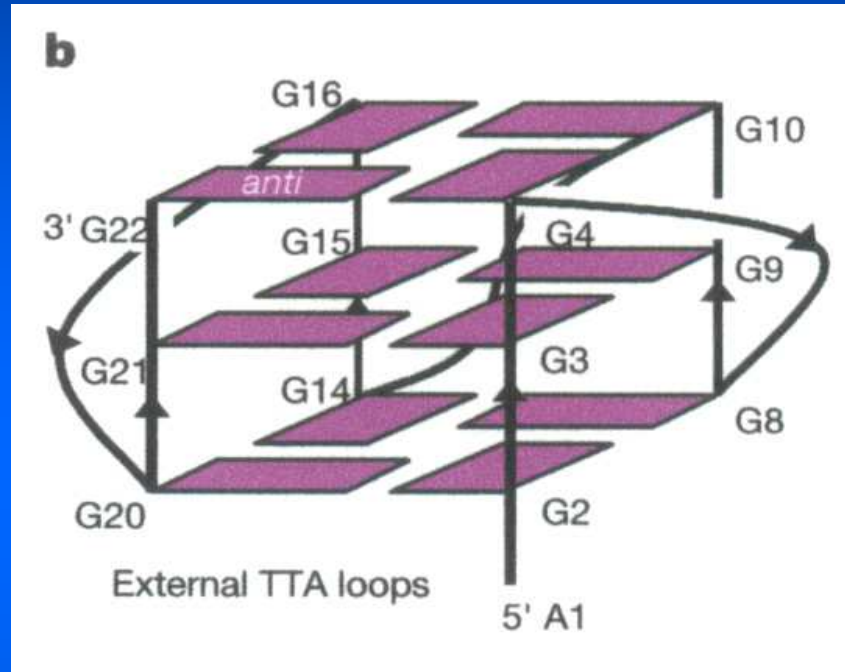
Fragment Pu-27 promoter c-myc:

TGGGGAGGGGTGGGGAGGGGTGGGGGAAGG

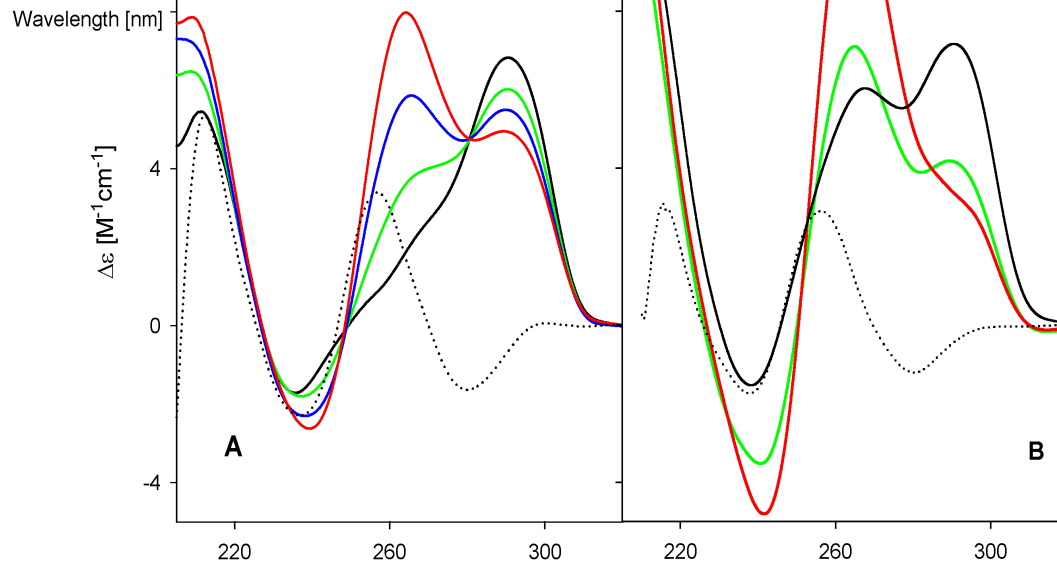
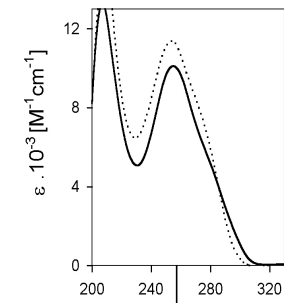
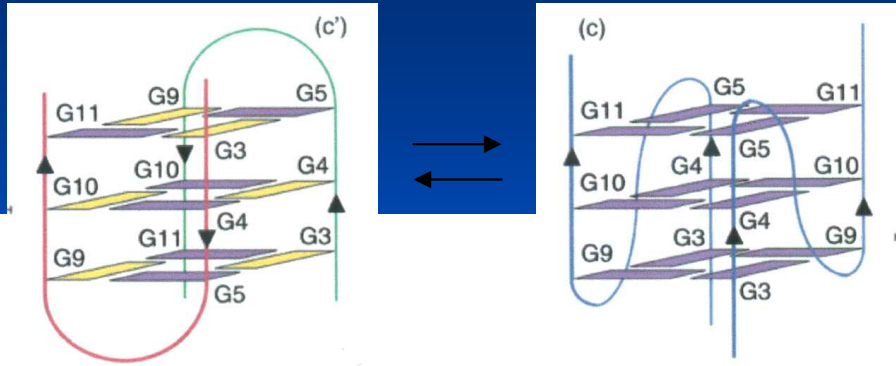


Pan, A.T. et al.:

J. Am. Chem. Soc. **126**(2004)8710



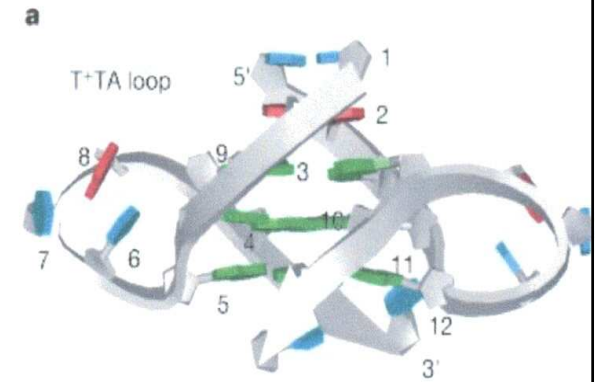
Parkinson, G.N., Lee, M.P.H., Neidle, S.
Nature **417** (2002) 876-880.

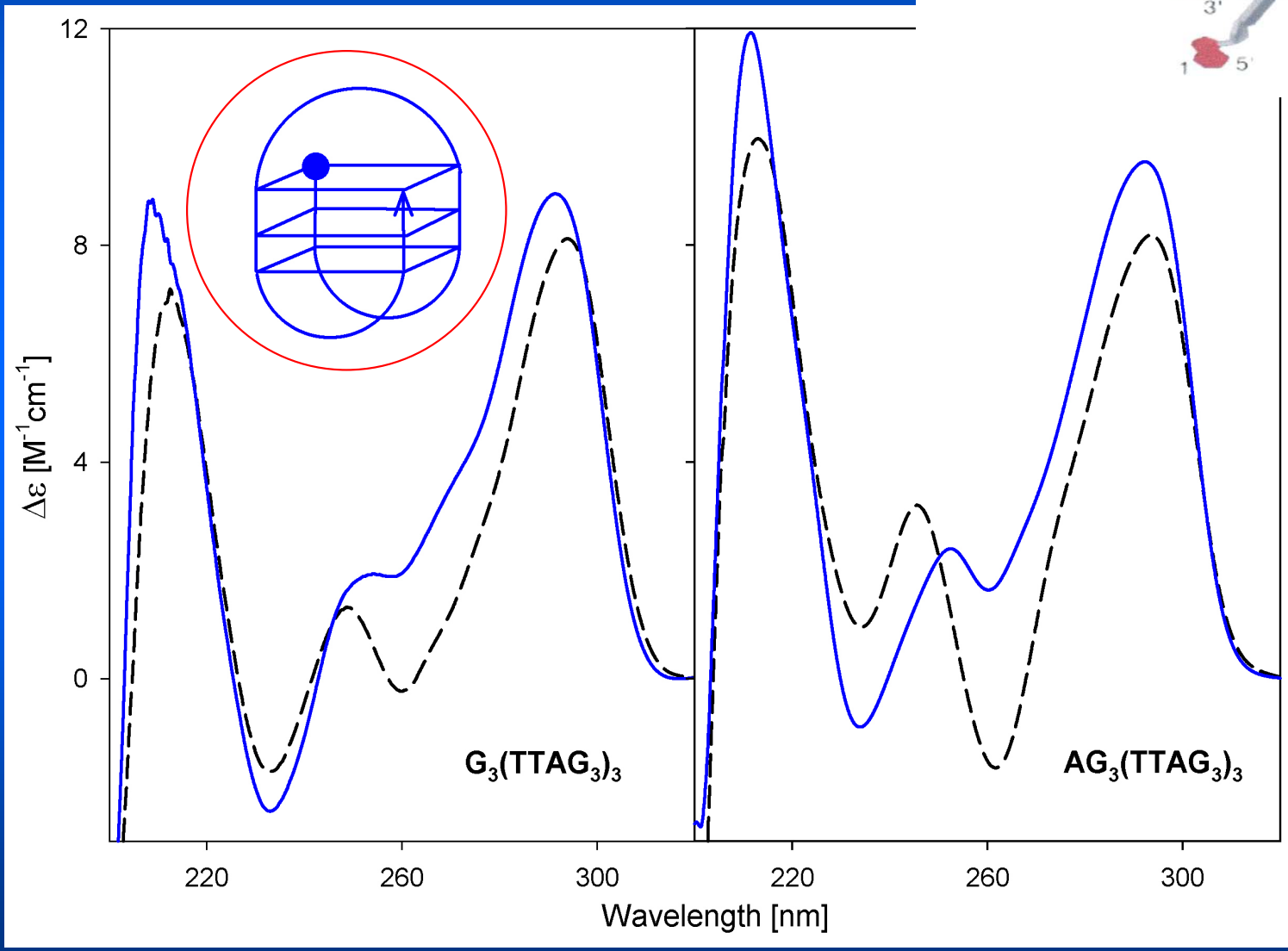
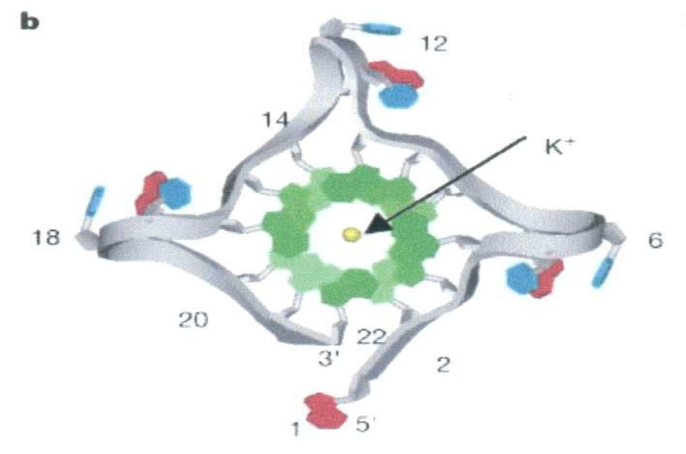


d(GGGTTAGGG)

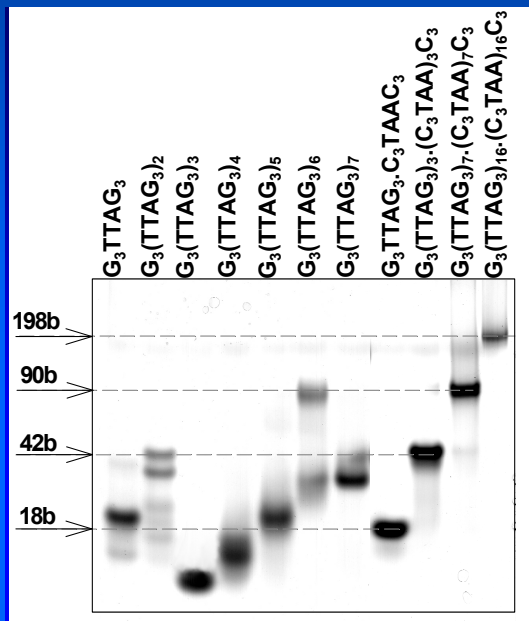
Wavelength [nm]

d(TAGGGTTAGGGT)

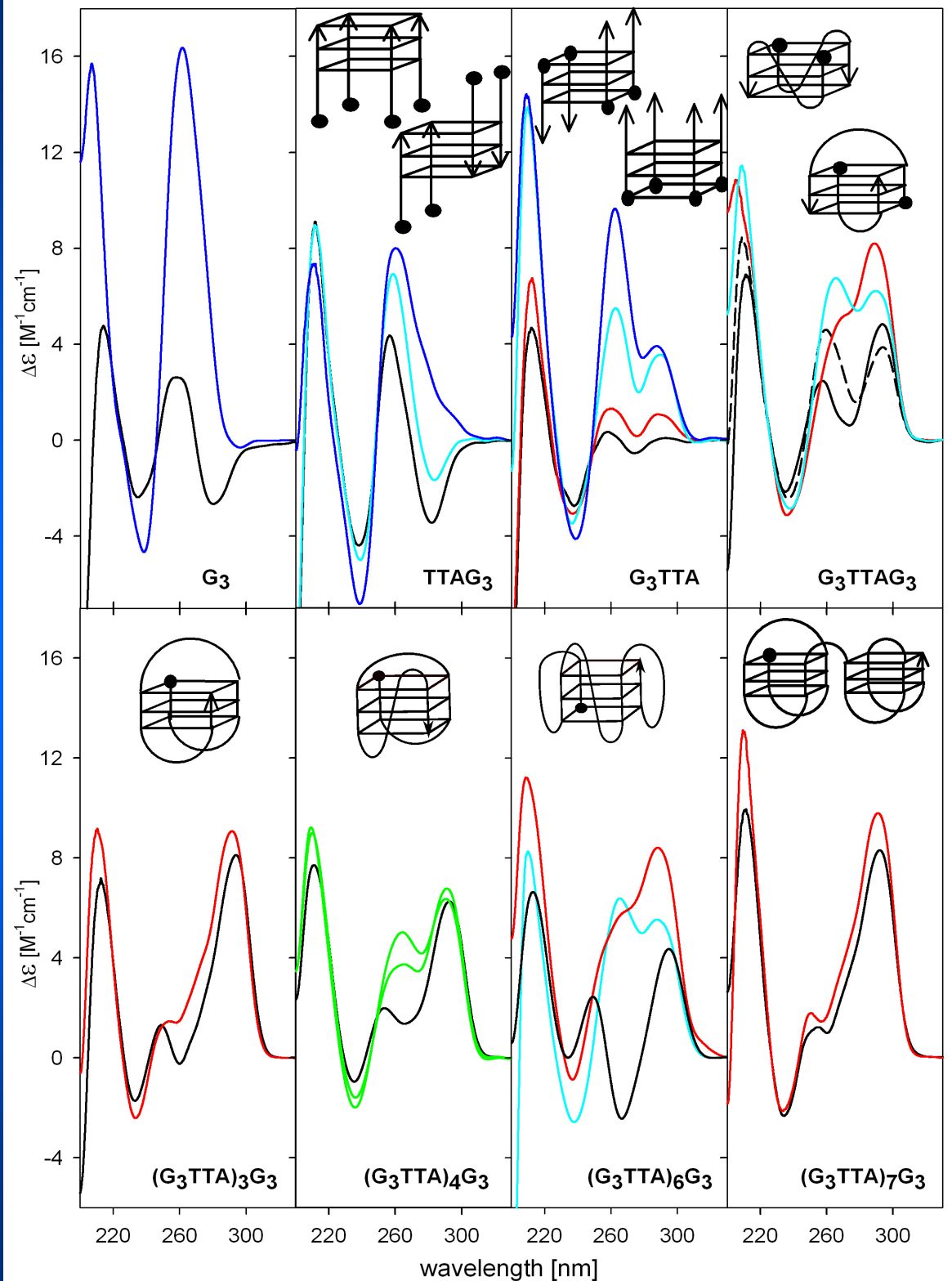


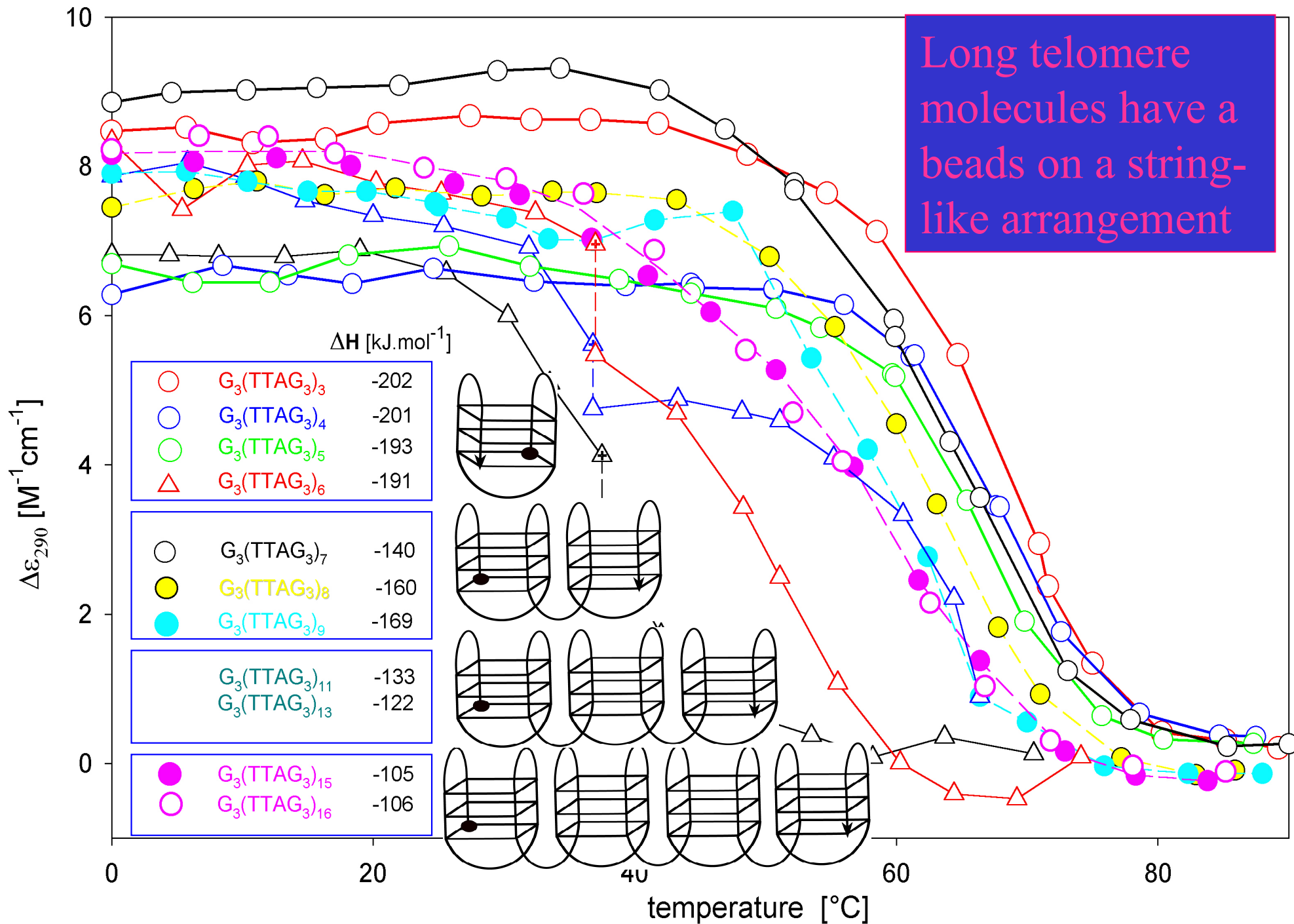


Quadruplexes of the human telomere sequence



The topology of quadruplex arrangements of the human telomere DNA sequence depends on the number of $(TTAG_3)$ repeats.





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

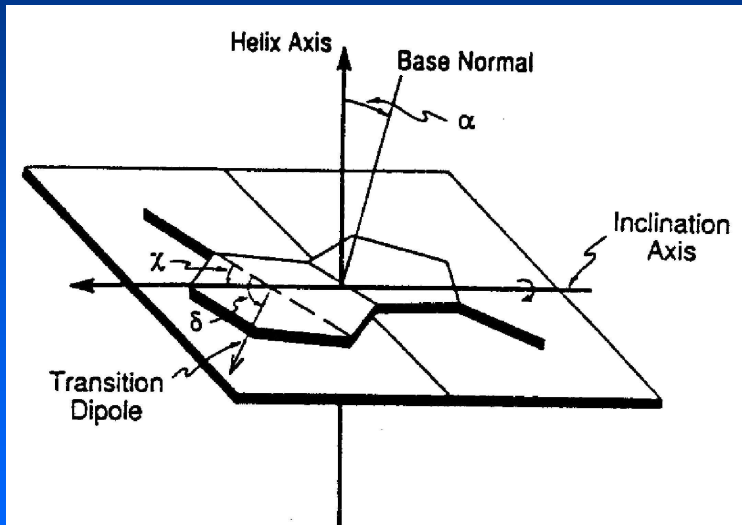
Závislost rozdílu intenzity fluorescence, excitované vlevo a vpravo kruhově polarizovaným světlem na vlnové délce excitačního záření. (~ 200 nm až vlnová délka emise)

Cirkulárně polarizovaná luminiscence (emise)-CPL (CPE)

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

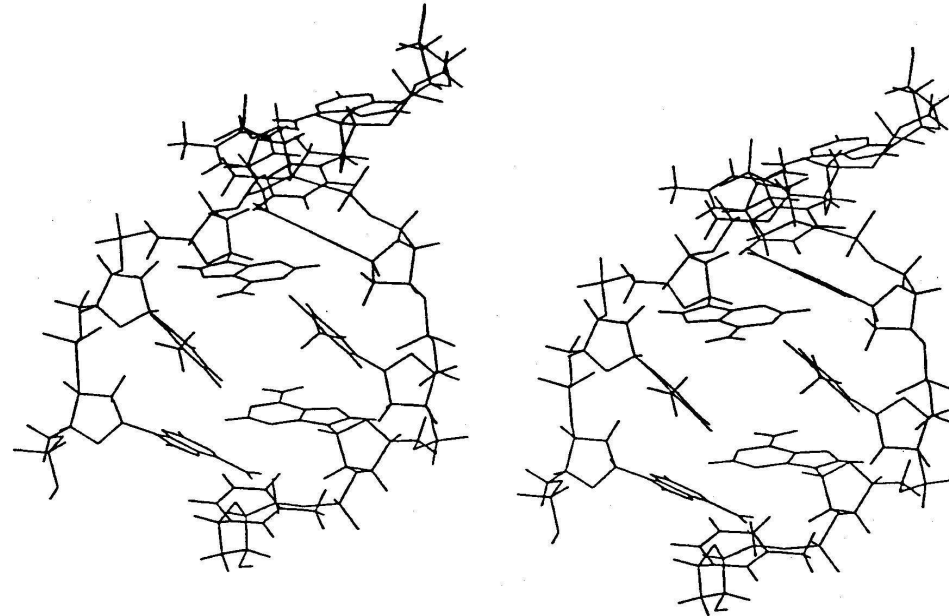
Cirkulární diferenciální Ramanův rozptyl-Raman CID

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



$$LD = A_{||} - A_{\perp}$$

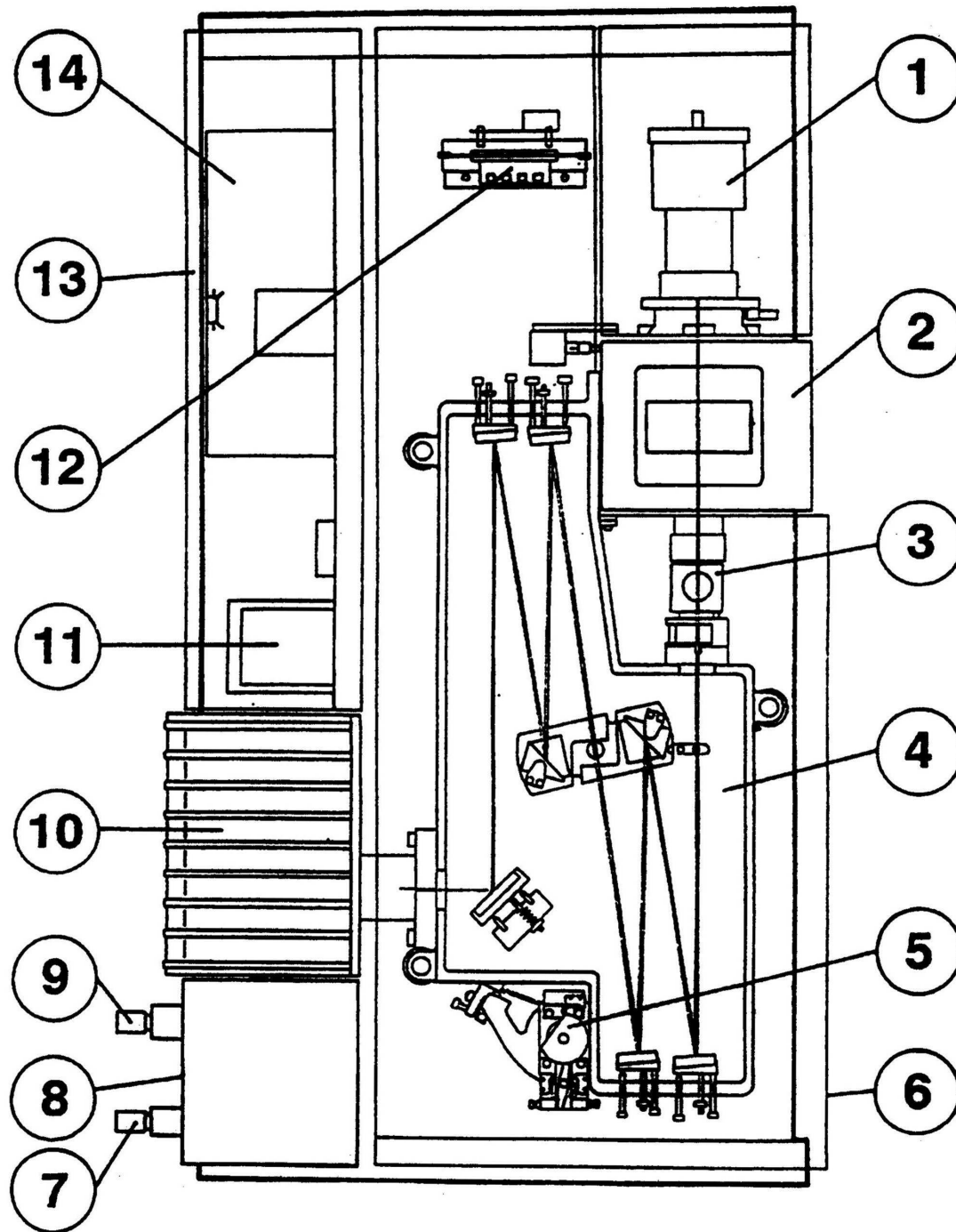
	α [deg]	χ [deg]
dA	20.9	95.2
dT	39.7	60.8



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		} 7 bp / turn
20°	31°	40°	36°	ApT	
			66°	TpA	





NOTE: 2010/10/10
2010/10/10
2010/10/10
2010/10/10
2010/10/10
2010/10/10



polystat cc1

huber

