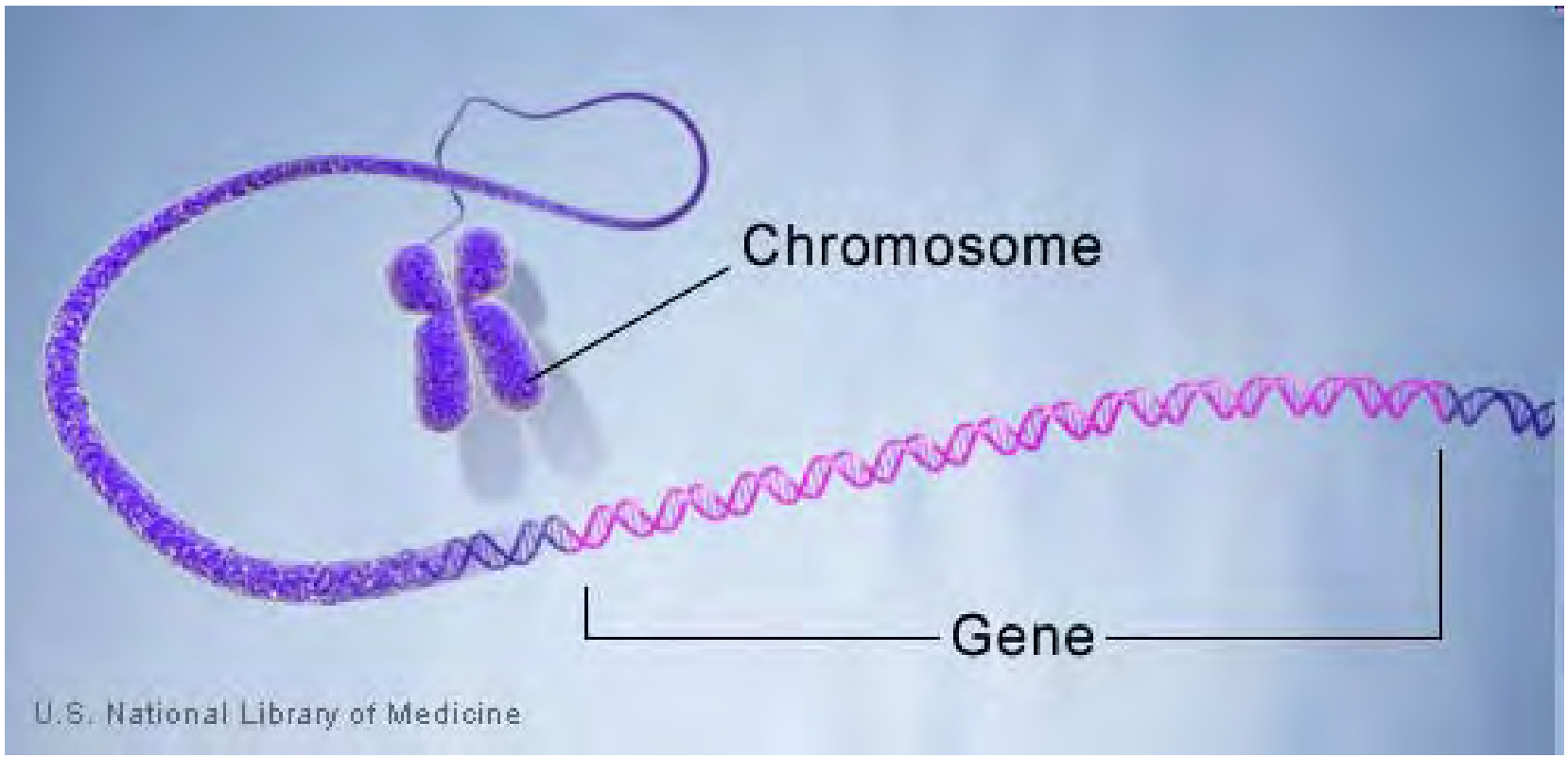
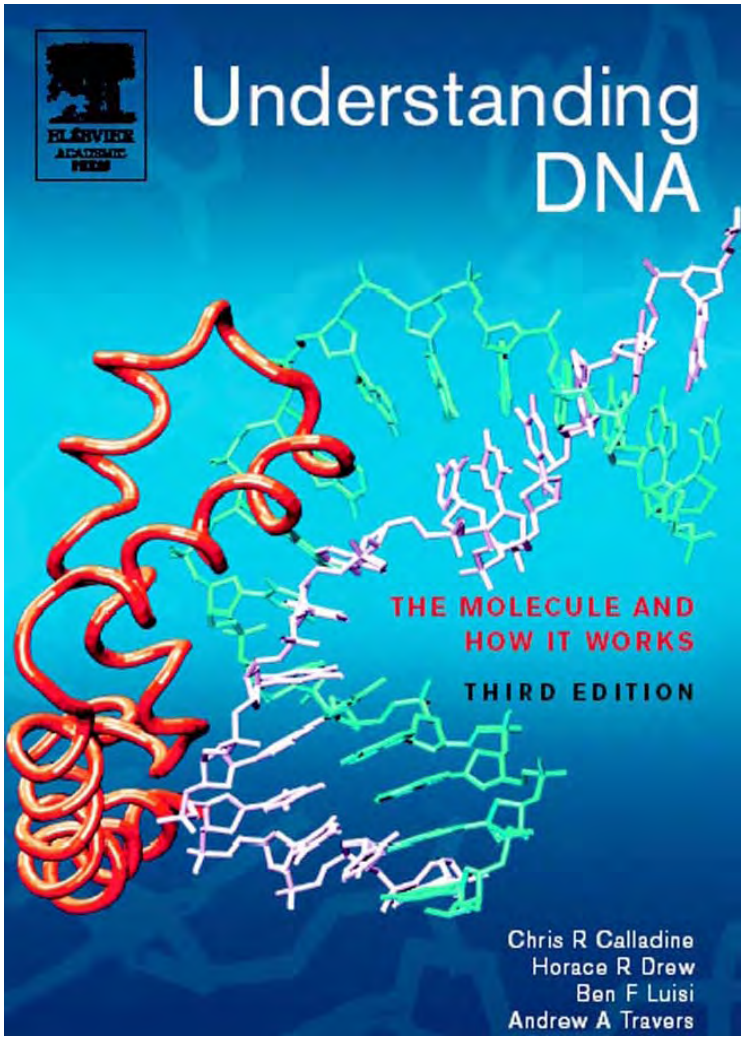
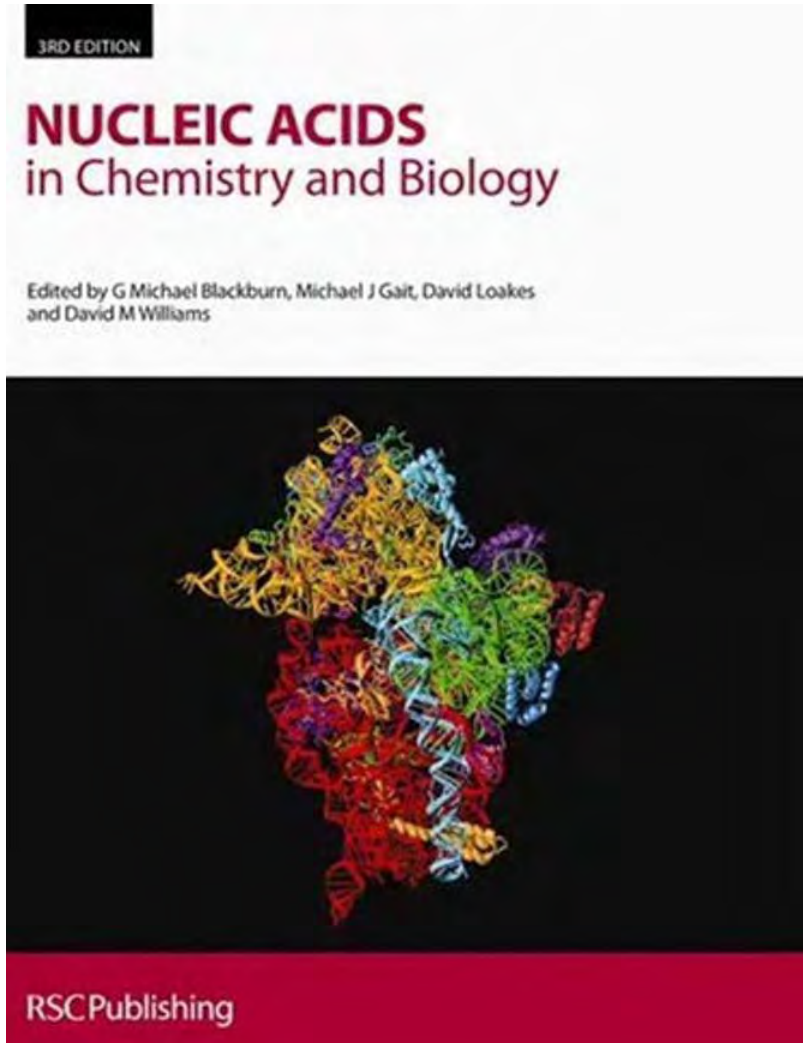
Stvarna struktura DNK

- Neobične (ne-kanonske) strukture DNK
- Modulacija strukture DNK u zavisnosti od sekvence
- Cirkularna DNK i superuvijanje



Stvarna struktura DNK





Neobične (ne-kanonske) strukture DNK

Palindromi
”Mirror repeat”?
Trostruki heliks
Četvorostruki heliks

Palindrome

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

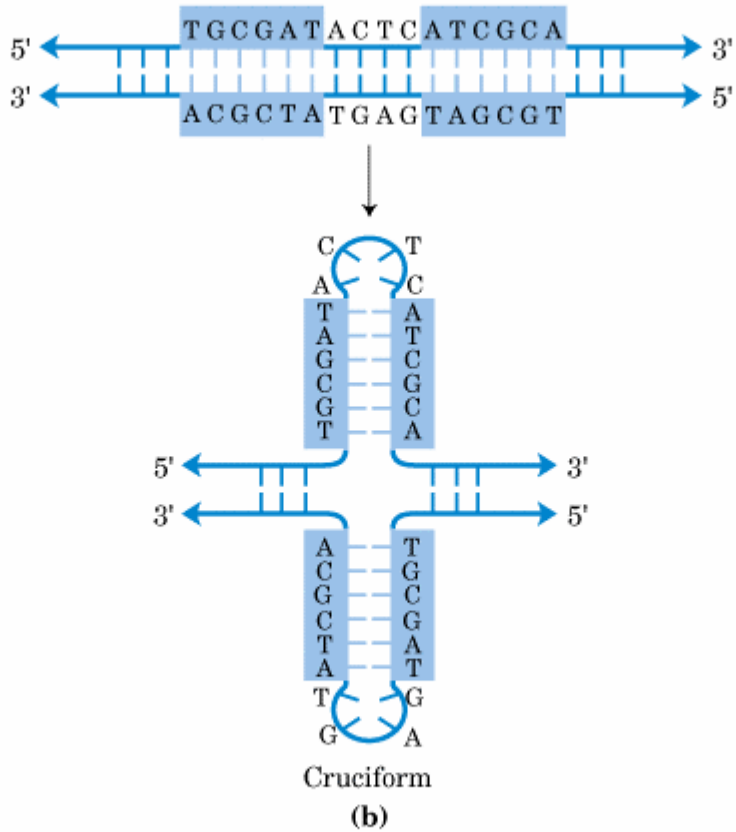
A A T C G T G C A C G A T T

Mirror repeat

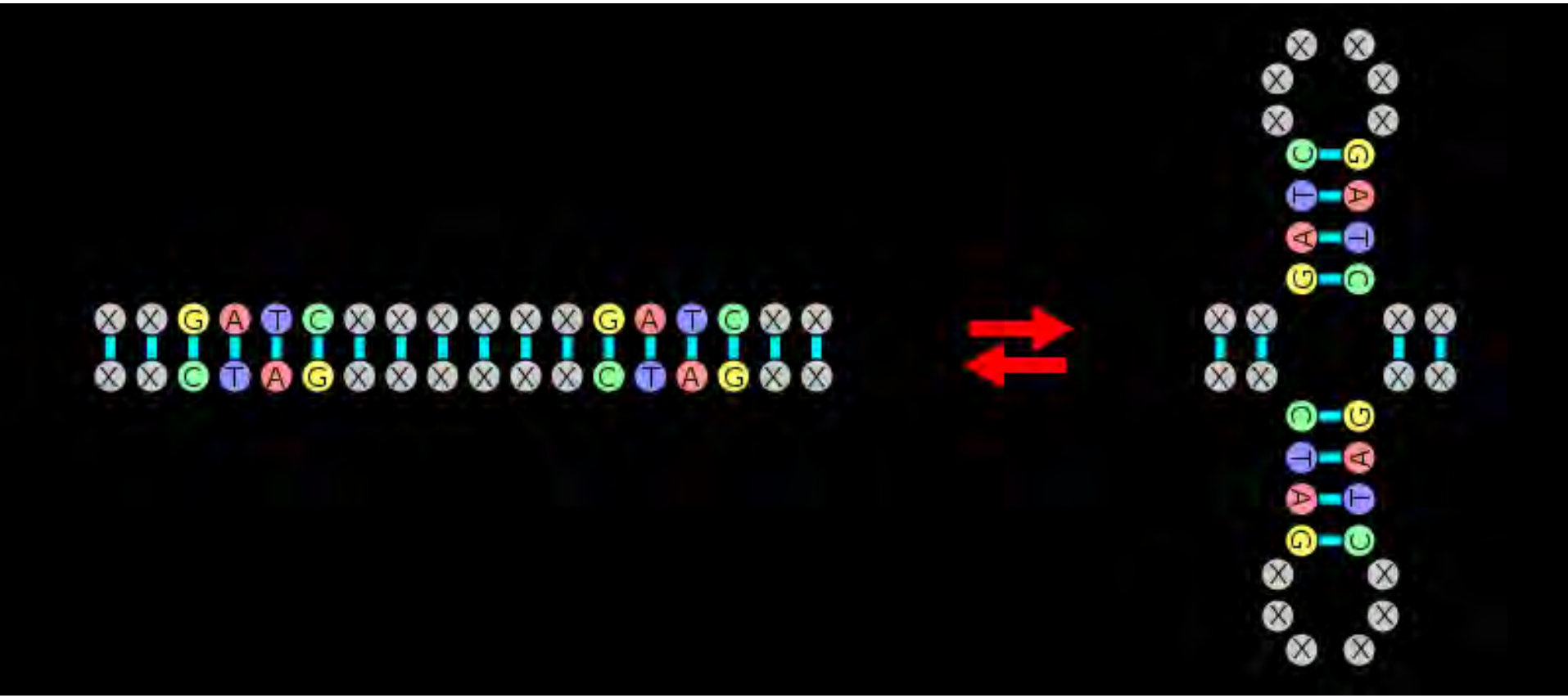
T T A G C A C C A C G A T T

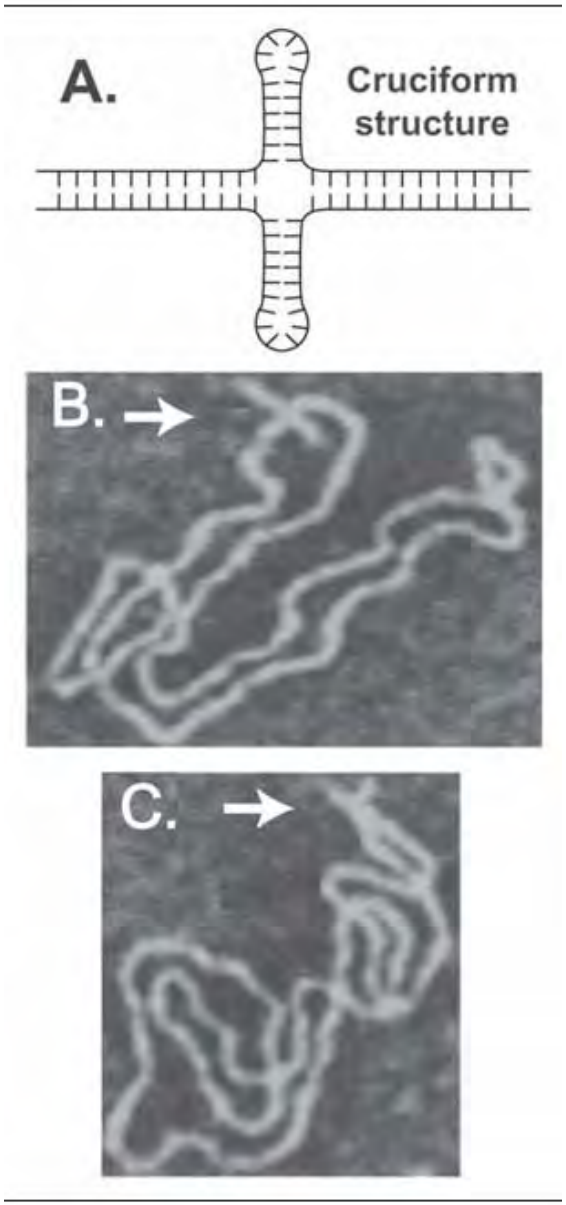
A A T C G T G G T G C T A A

Palindrom u ravnoteži sa “krstom”



Oblik krsta obrazuje 2 petlje, nastaje na mestima aktivnog metabolizma

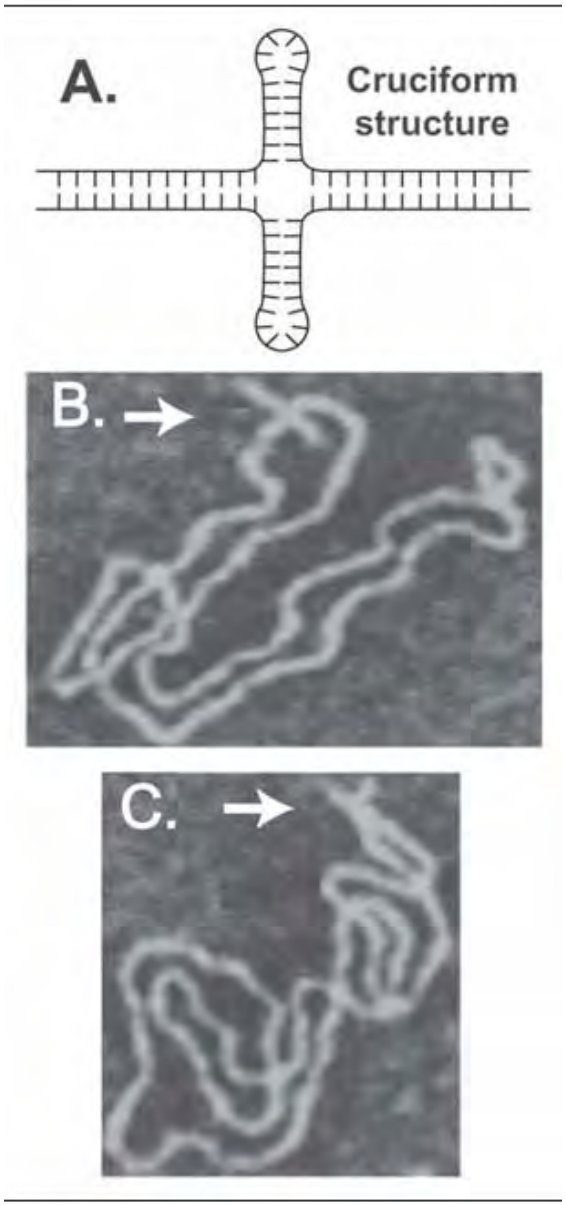

G A T C
C T A G
G A T C
C T A G



Pri kojim uslovima nastaje konformacija krsta?

Konformacija krsta nastaje iz palindromskih sekvenci pri niskoj jonskoj sili kada dolazi do odbijanja među fosfatima u fosfo-diestarskoj kičmi.....

.....pri fiziološki relevantnim uslovima krst prelazi u konformaciju tipa **X** !!!

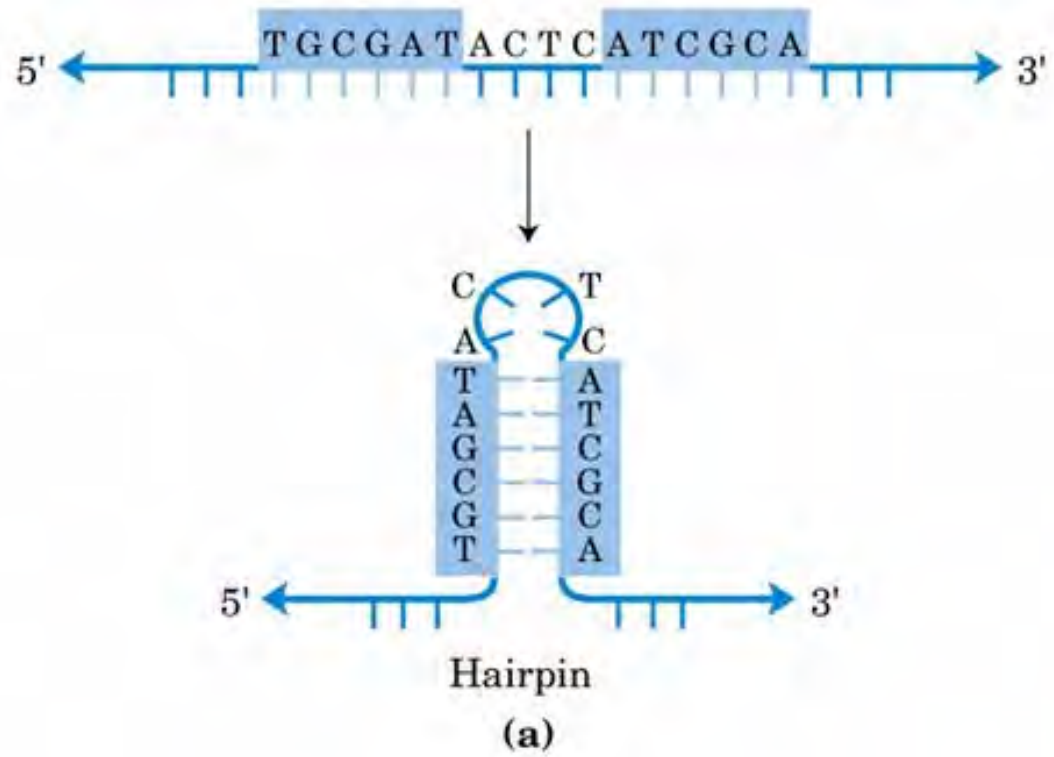


Pri kojim uslovima nastaje konformacija krsta?

Konformacija krsta nastaje iz palindromskih sekvenci pri niskoj jonskoj sili kada dolazi do odbijanja među fosfatima u fosfo-diestarskoj kičmi.....

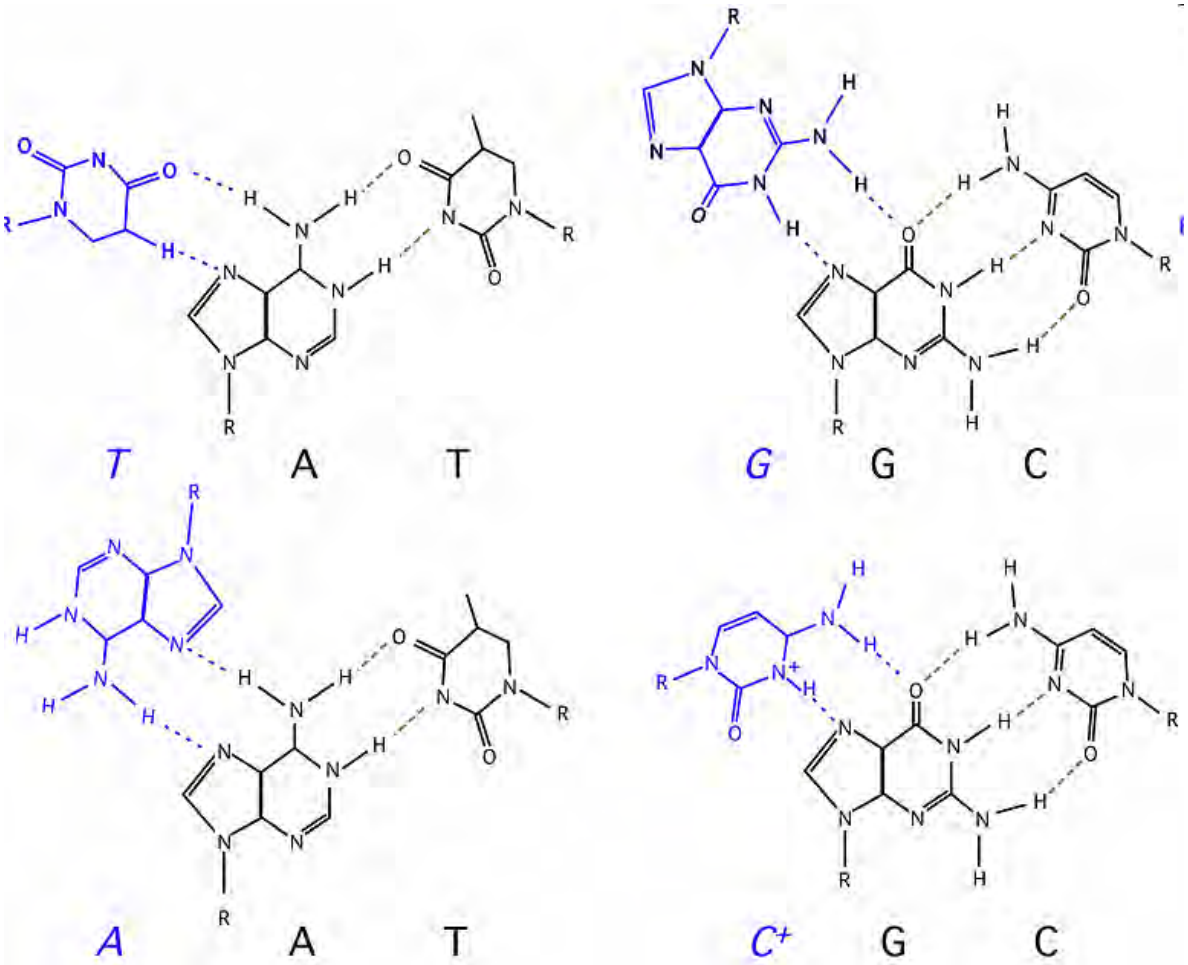
.....pri fiziološki relevantnim uslovima krst prelazi u konformaciju tipa **X** !!!

Konformacija ukosnice formira petlju – često na mestu aktivnog metabolizma



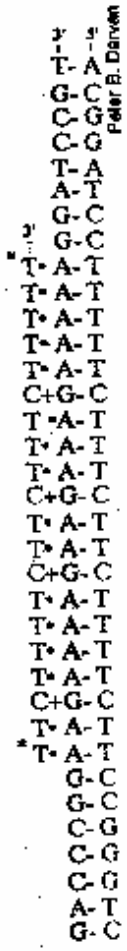
Više (hiper) lančana DNK: trostruki i četvorostuki heliksi

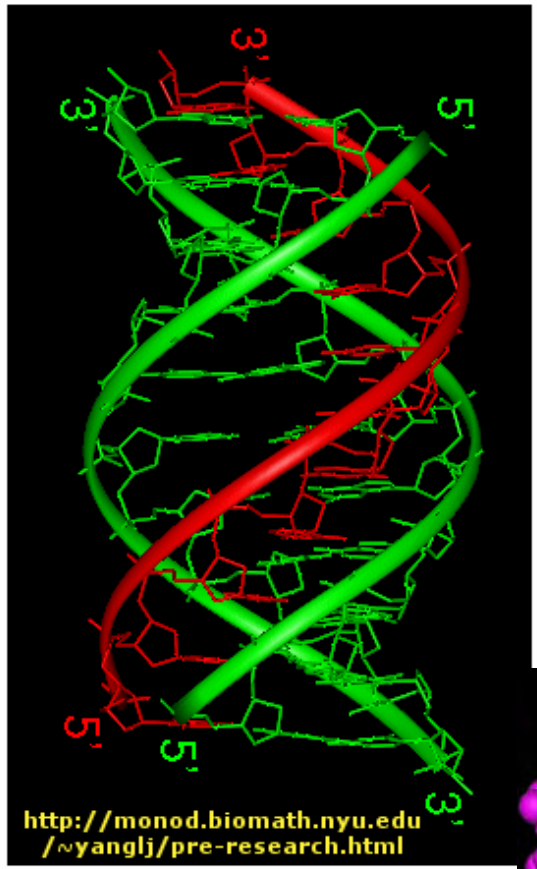
Trostruki heliks

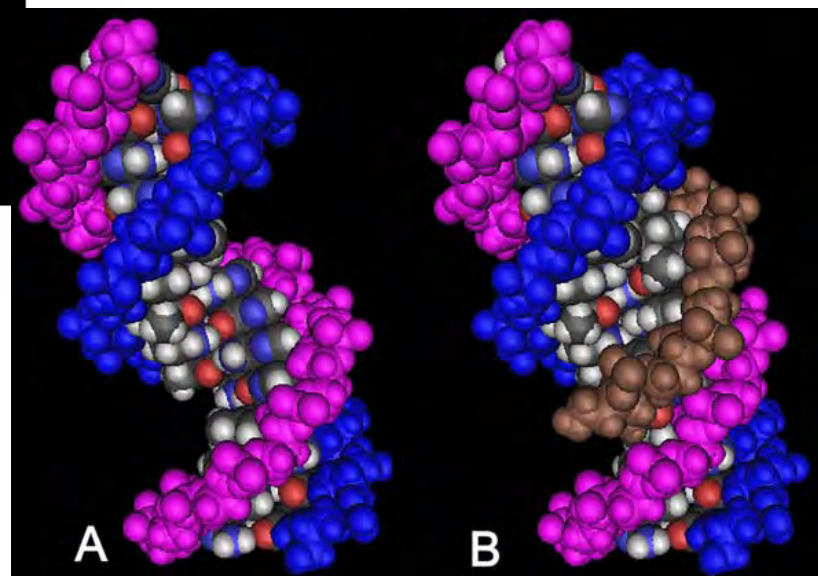


Purini u velikoj brazdi B-DNK mogu da grade dodatne H-veze Hoogsteen tipa!!! Sparivanje obuhvata 2 pirimidina i 1 purin. U slučaju 2 purina treća baza se prevrće.

Trostruki heliks

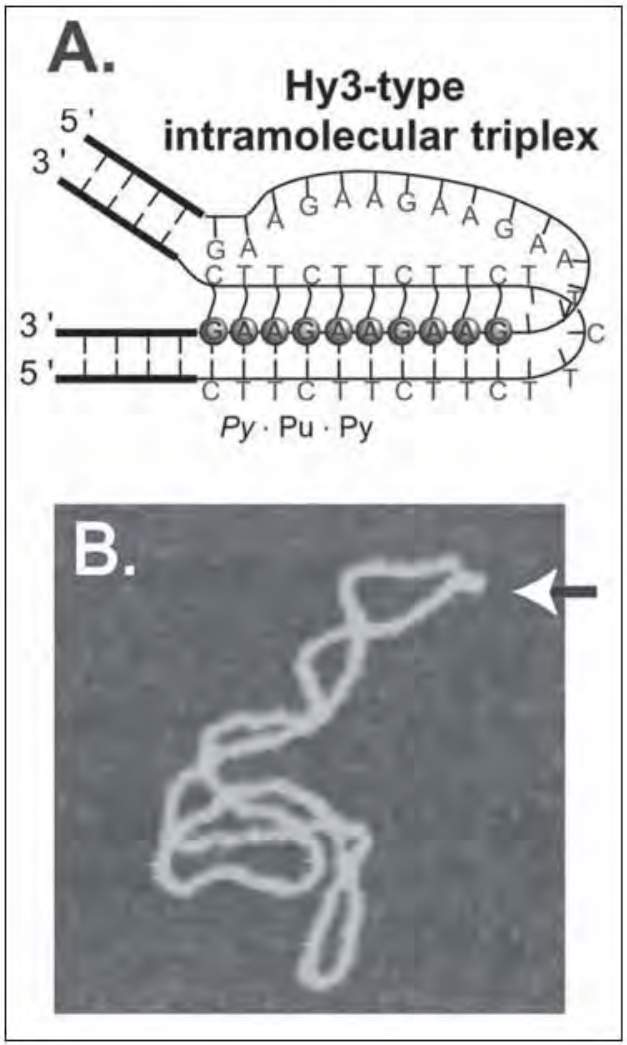


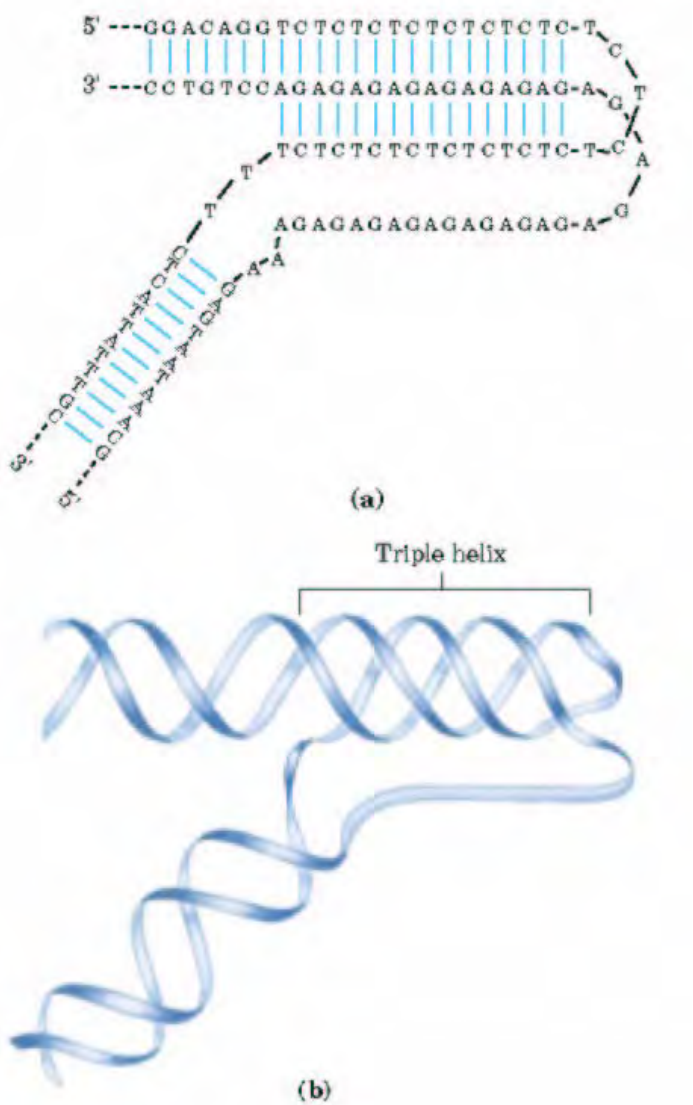




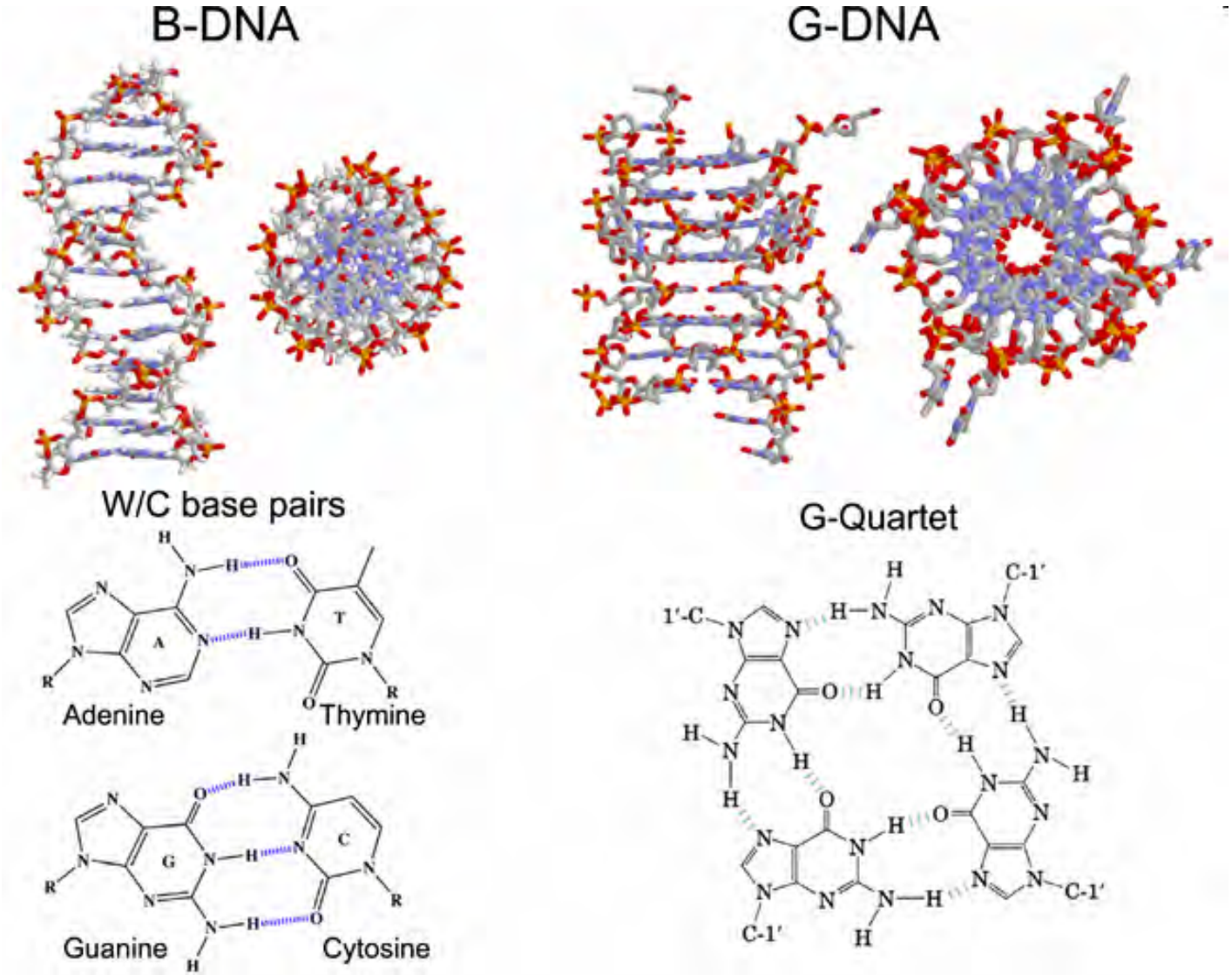
H-DNK: treći niz gradi vodonične veze Hoogsteenovog tipa sa purinima iz B-heliksa.

Javlja se kod dugih polipurinskih ili polipirimidinskih sekvenci koje sadrže “mirror repeat”
Nađen je regionima DNK odgovornim za ekspresiju nekih gena kod eukariota!

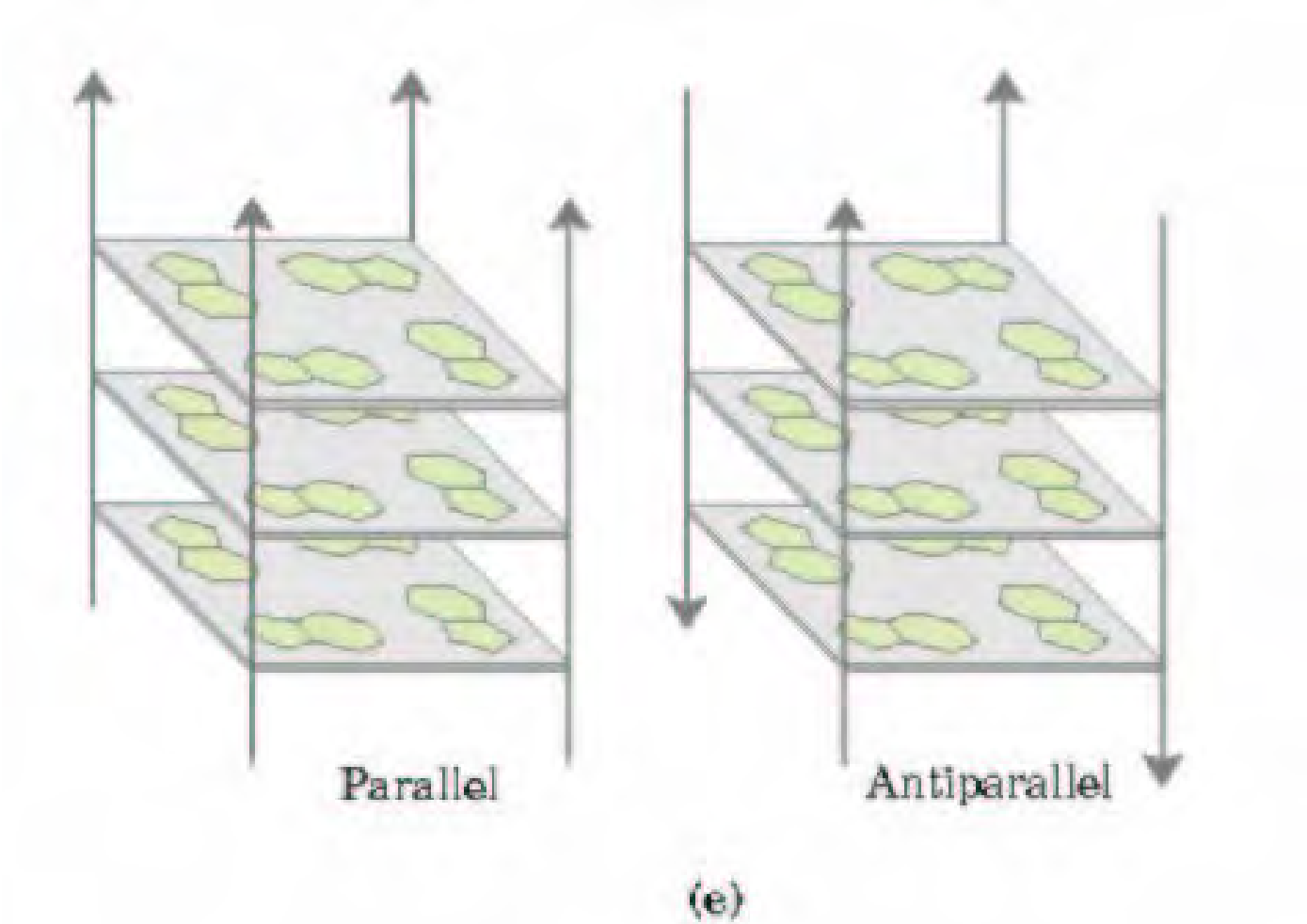




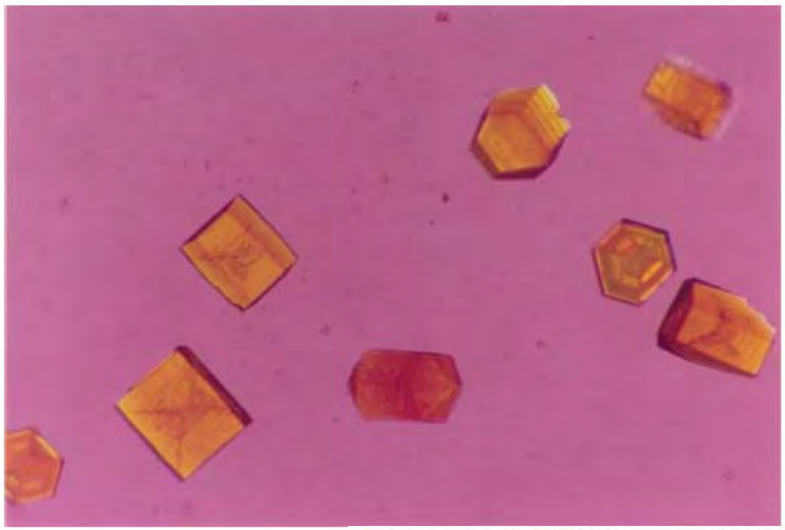
Sekvence DNK sa visokim sadržajem guanina mogu da nagrade stabilne četvorostruke helikse (tetraplekse)

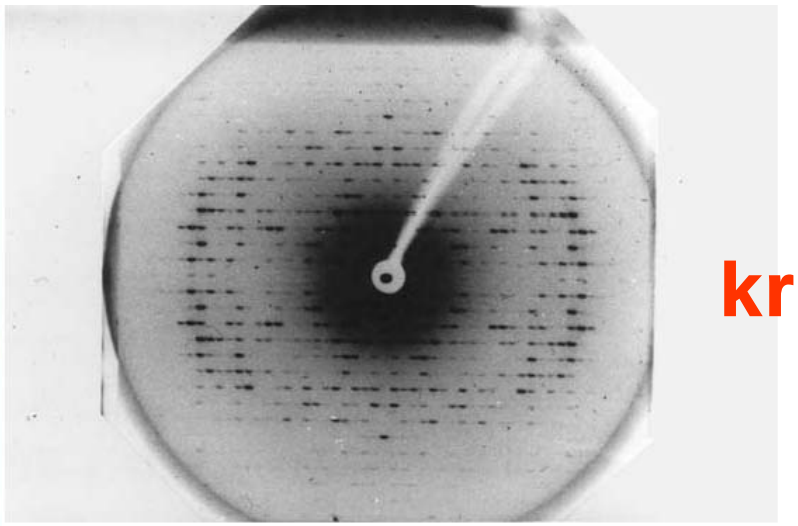


Sekvence DNK sa visokim sadržajem guanina mogu da nagrade stabilne četvorostruke helikse (tetraplekse)

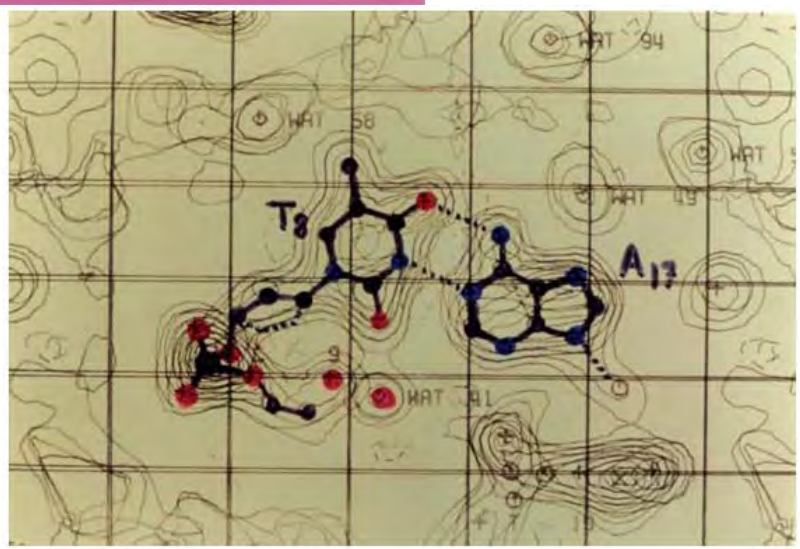


Određivanje strukture kristala sintetičkih oligonukleotida različitih sekvenci





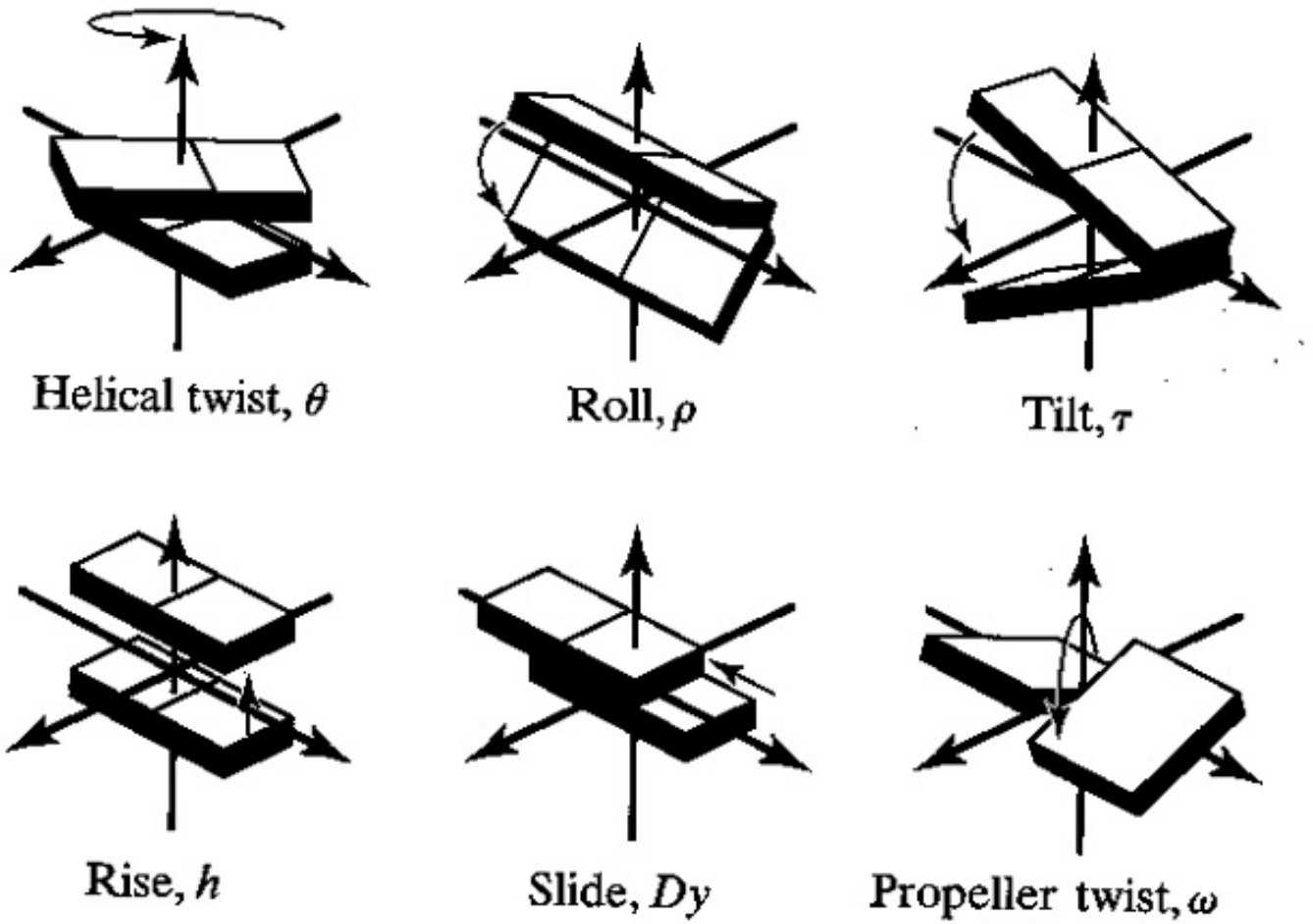
kristal



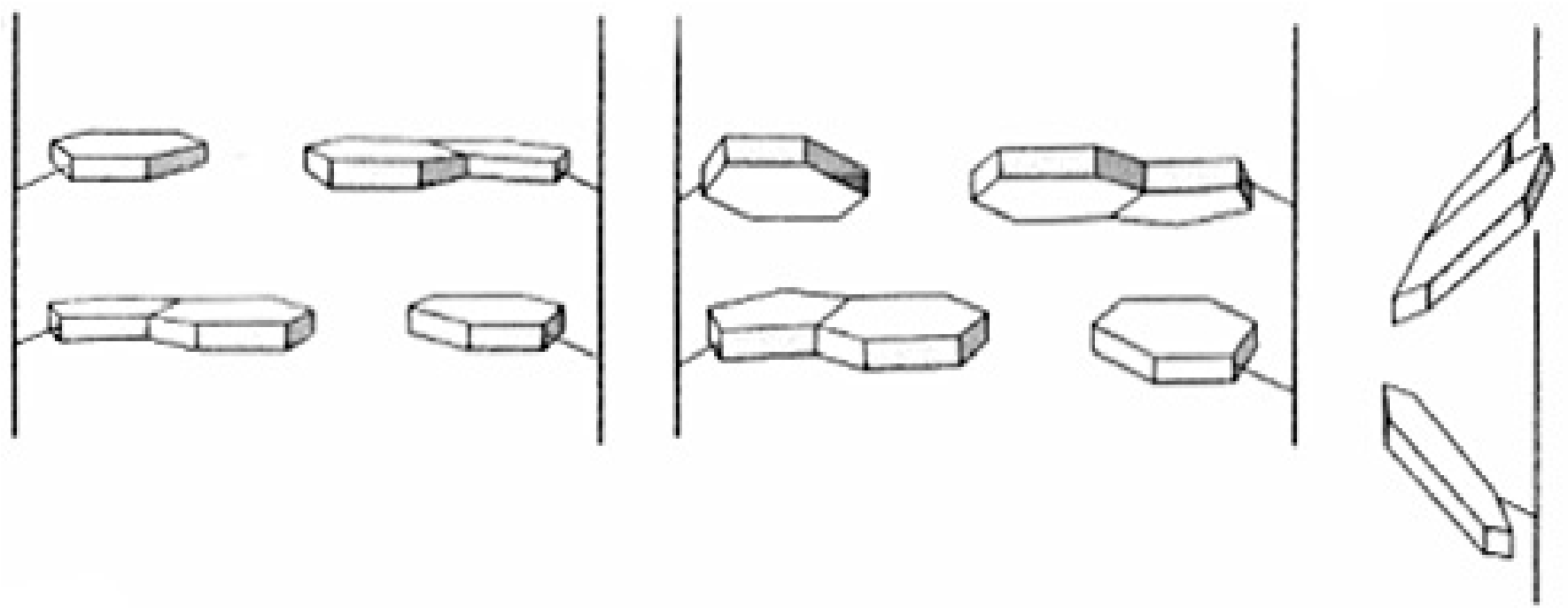
Difrakcione slike

vlakno

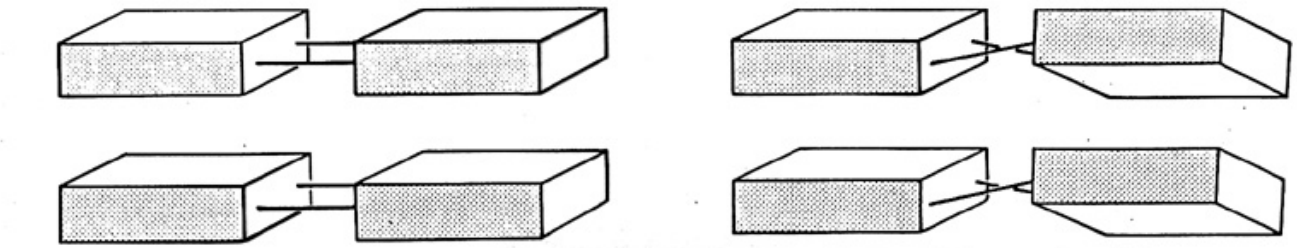
Pomeranje baza u odnosu na x, y i z osu (Euler-ovi stepeni slobode u kretanju čvrstih tela)



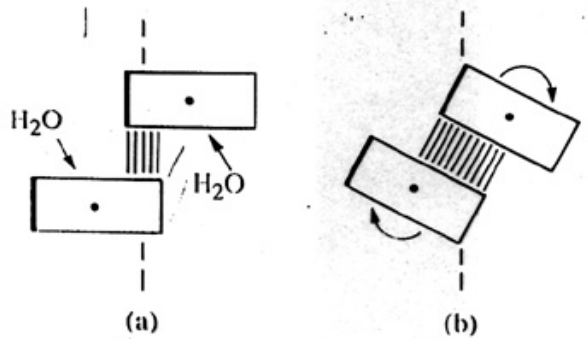
“Roll”



“Efekti drugog reda”: propelersko izvijanje



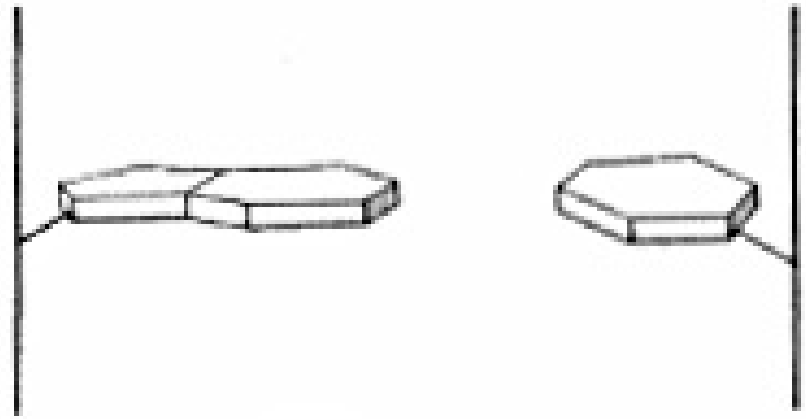
Propeller twist need not alter roll. The roll angle remains zero in part (b), because the mean planes of the base-pairs remain parallel.

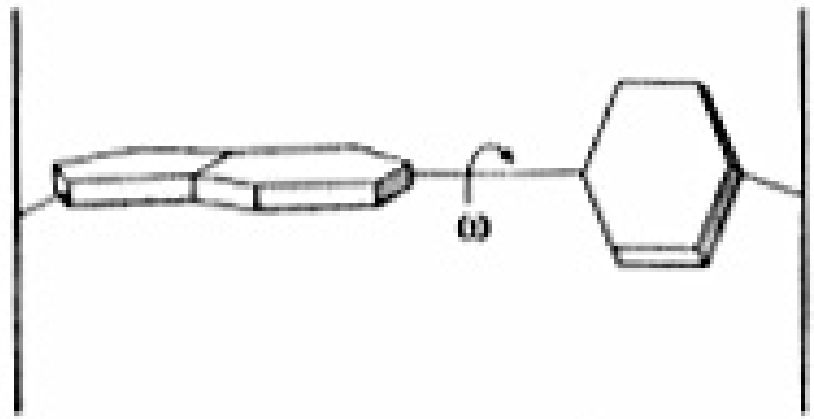


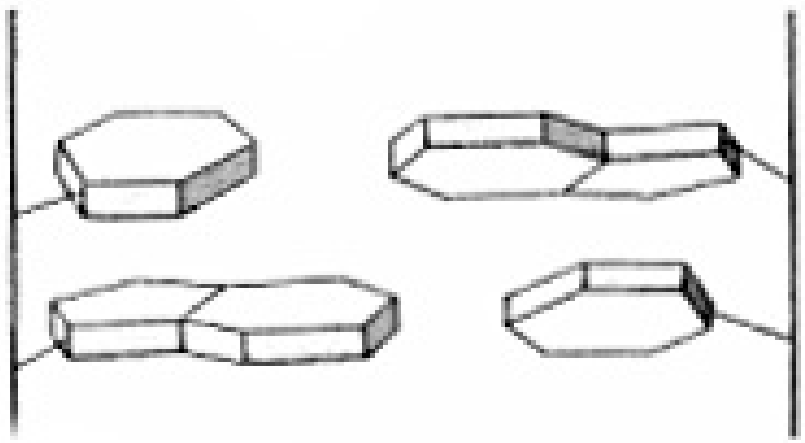
Propeller twist, as in (b), allows greater overlap of bases within the same strand and reduces the area of contact between the bases and water.

Propelersko izvijanje je veće (15-25^o) u regionima sa AT parovima nego u regionima sa GC parovima (5 - 15^o)

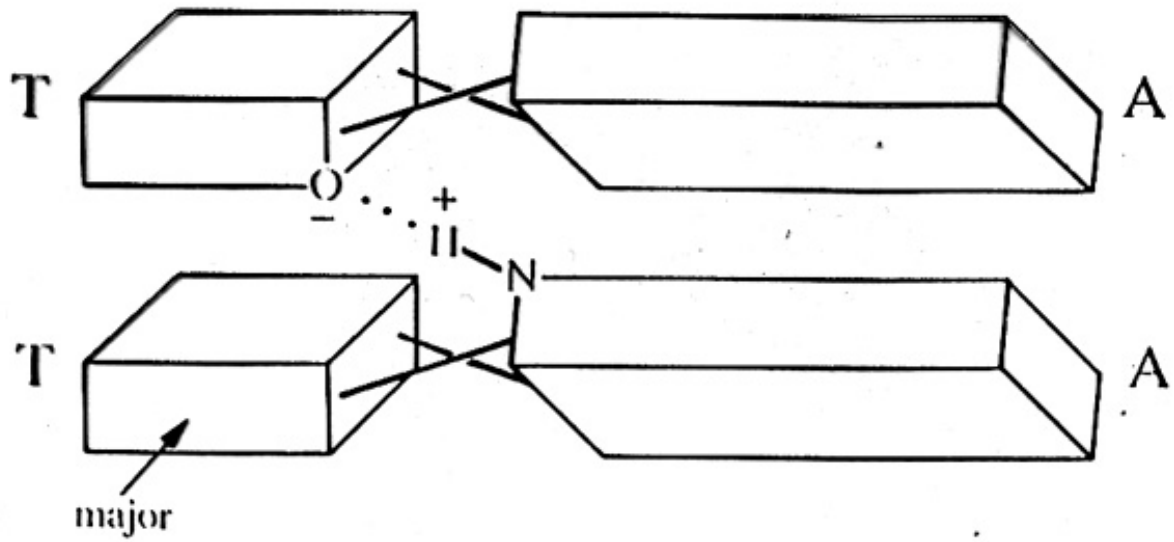
Propelersko izvijanje (“twist”)





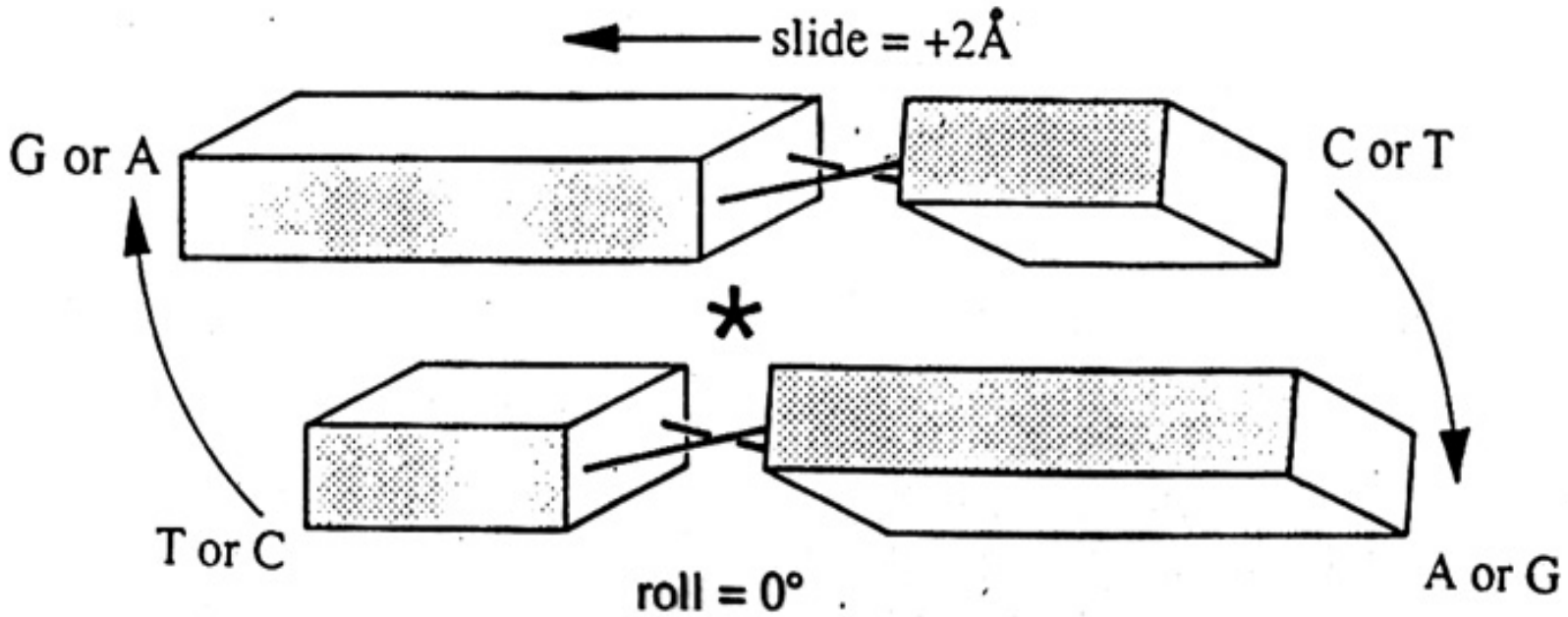


Sekvenca A-T



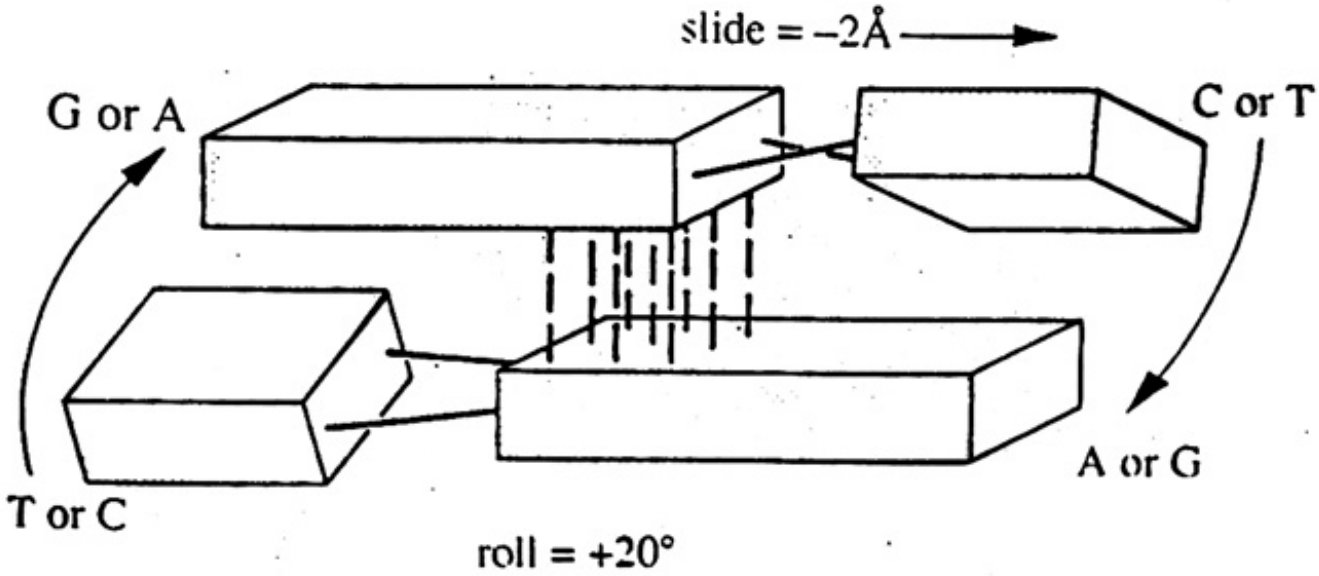
Propeller-twisted A–T pairs, showing a possible additional hydrogen bond between the base-pairs in the major groove (as proposed originally by Hillary Nelson).

Sekvenca pirimidin-purin



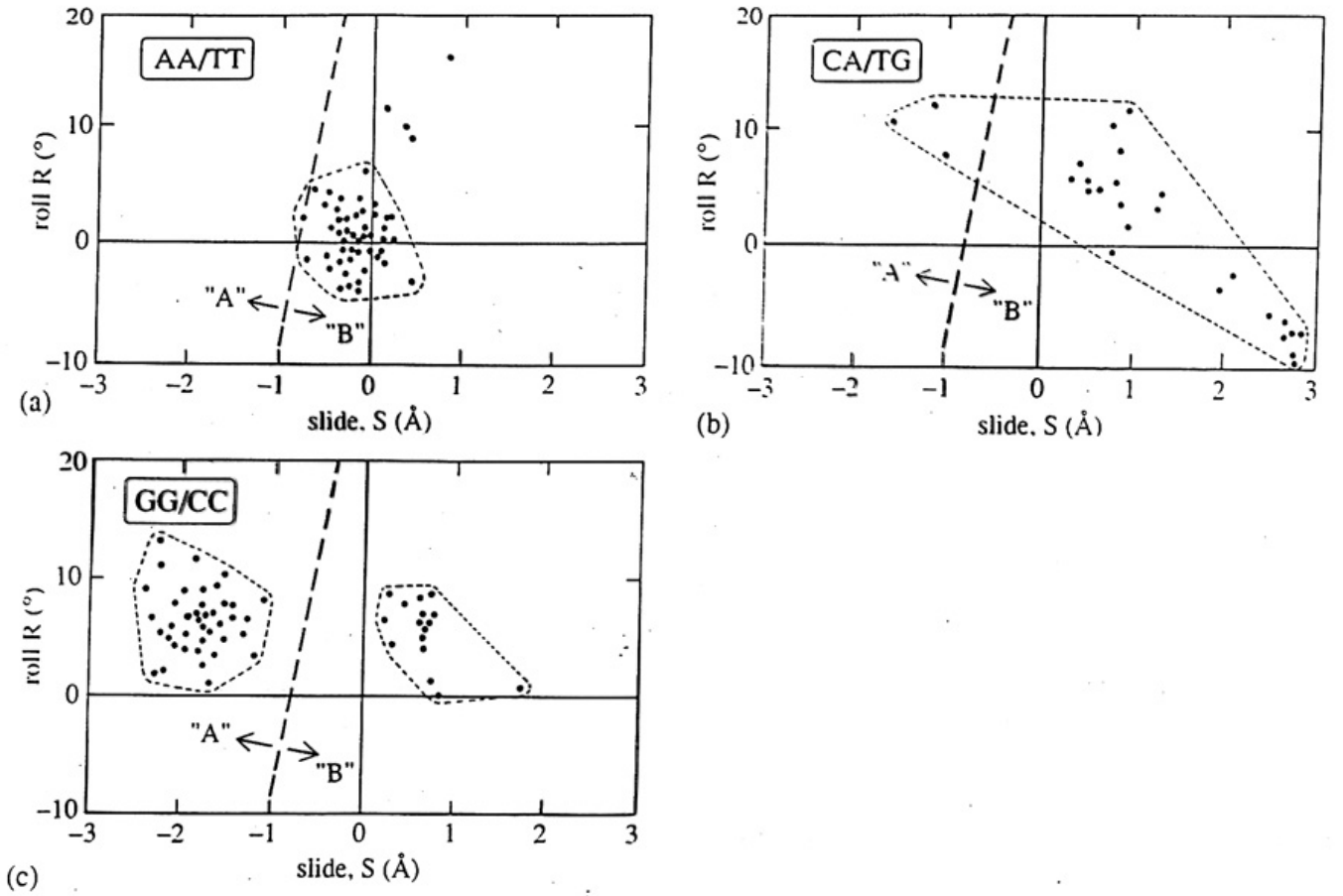
A pyrimidine–purine step with zero roll: positive slide is needed to avoid a steric clash at *, if the base-pairs have propeller twist.

Sekvenca purin-pirimidin



A pyrimidine–purine step in an alternative configuration, having negative slide and positive roll, due to the cross-chain stacking of purines.

Eksperimentalni rezultati



Plots of roll versus slide for many base-pair steps of oligomeric DNA as studied by X-ray diffraction. Separate plots are given for three of the ten distinct steps by sequence : AA/TT is a 'rigid' step, CA/TG is a 'flexible' step and GG/CC is a 'bistable' step. From El Hassan and Calladine (1997). *Philosophical Transactions of the Royal Society*, **A 355** 43–100.

Konformacija B i A heliksa molekula DNK

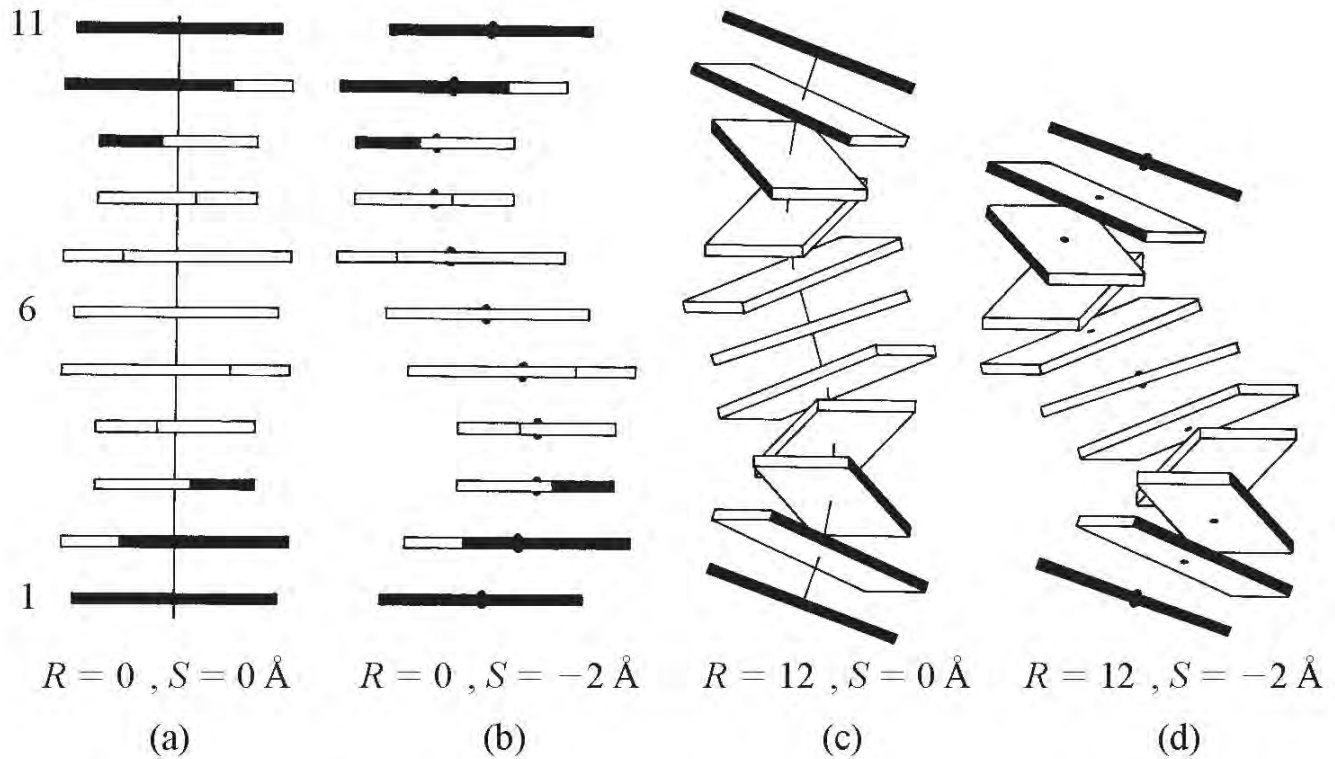


Figure 3.14 One complete helical turn of DNA having $T = 36°$, showing the effects of introducing uniform roll R or slide S at each step. Broadly, (a) corresponds to the 'B' form of DNA, while (d) corresponds to the 'A' form as shown in Fig. 2.7. Parts (b) and (c) correspond to structures intermediate between 'B' and 'A' which have, in fact, been seen recently in DNA crystals by X-ray diffraction.

Efekat sekvence na konformaciju DNK

Različite sekvence DNK preferiraju ili B ili A heliks:

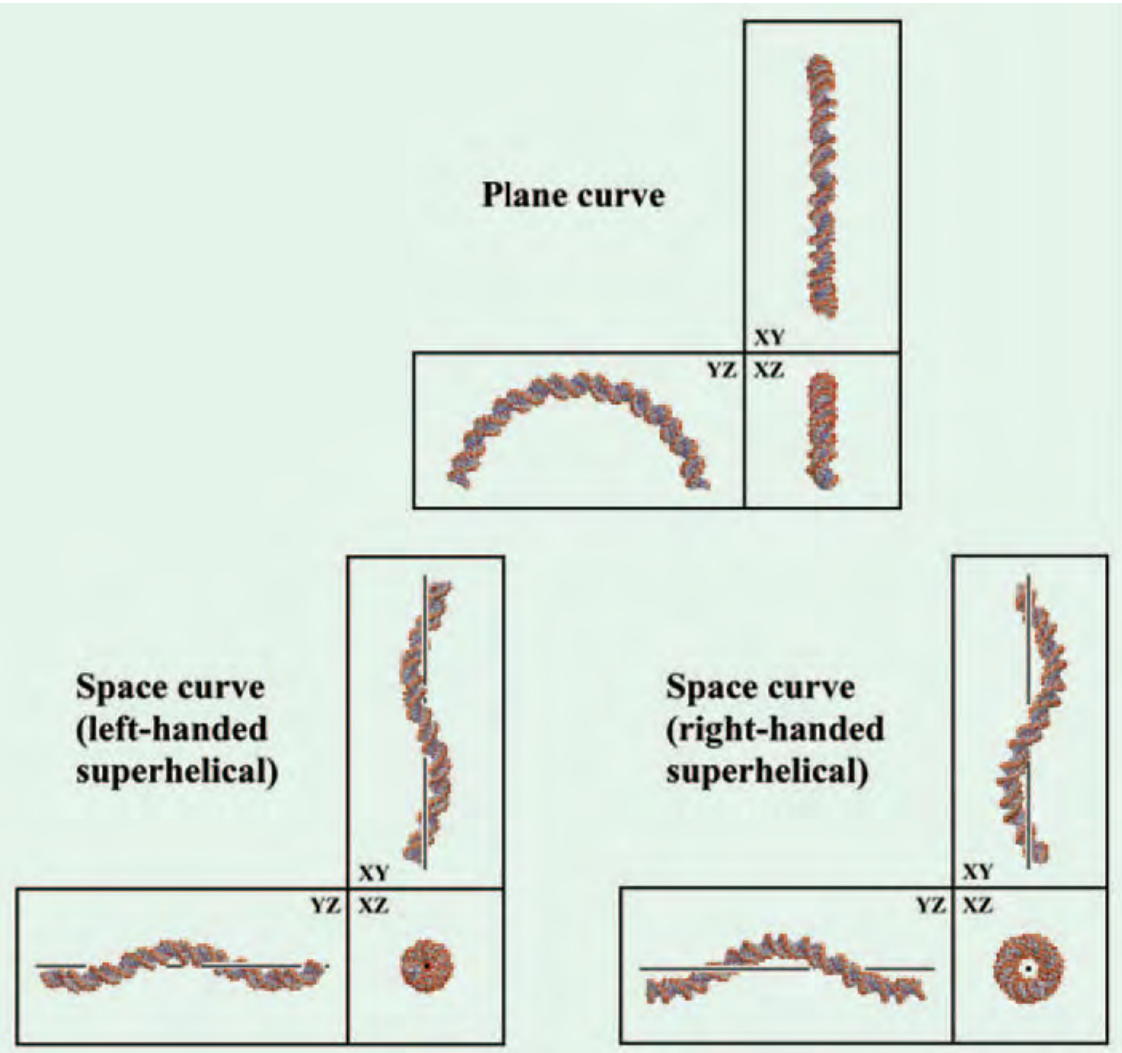
- AA/TT se neće naći u A heliksu
- CA/TG ili GG/CC mogu da se nađu u B i A heliksu

(što delimično objašnjava zašto DNK preferira ova dva tipa heliksa!!!)

Funkcionalni značaj:

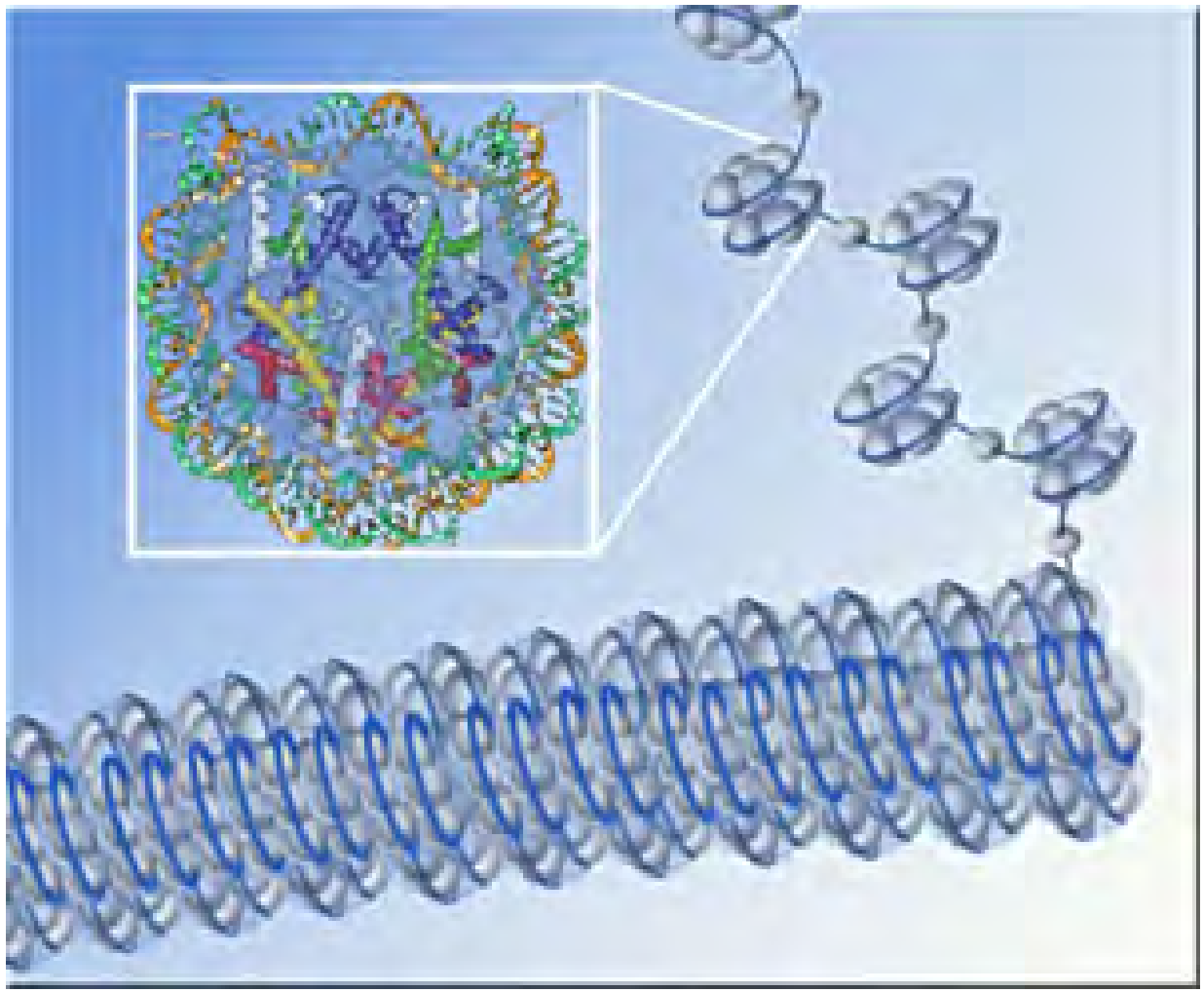
- Uvijanje DNK
- Vezivanje (određenih) proteina
- Ekspresija gena

Zakrivljenost DNK



- Slobodnoj DNK u rastvoru svojstvena je mala zakrivljenost (10-15^{o} po zavoju heliksa i to samo za određene sekvence).
- DNK vezana za bazne proteine može mnogo više da se zakrivi, često 40-50^{o} po zavoju heliksa (TATATA)!

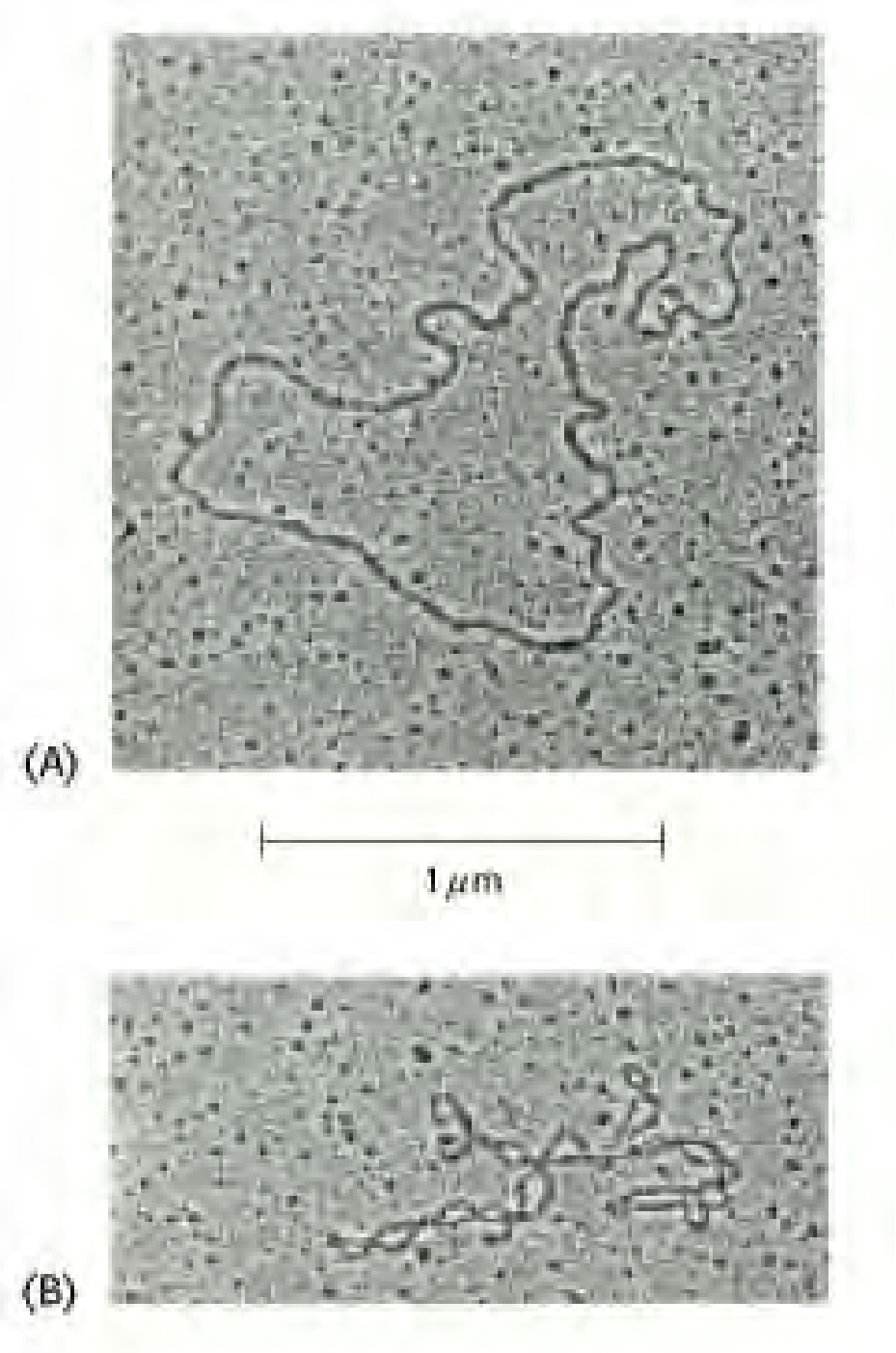
Nukleozom

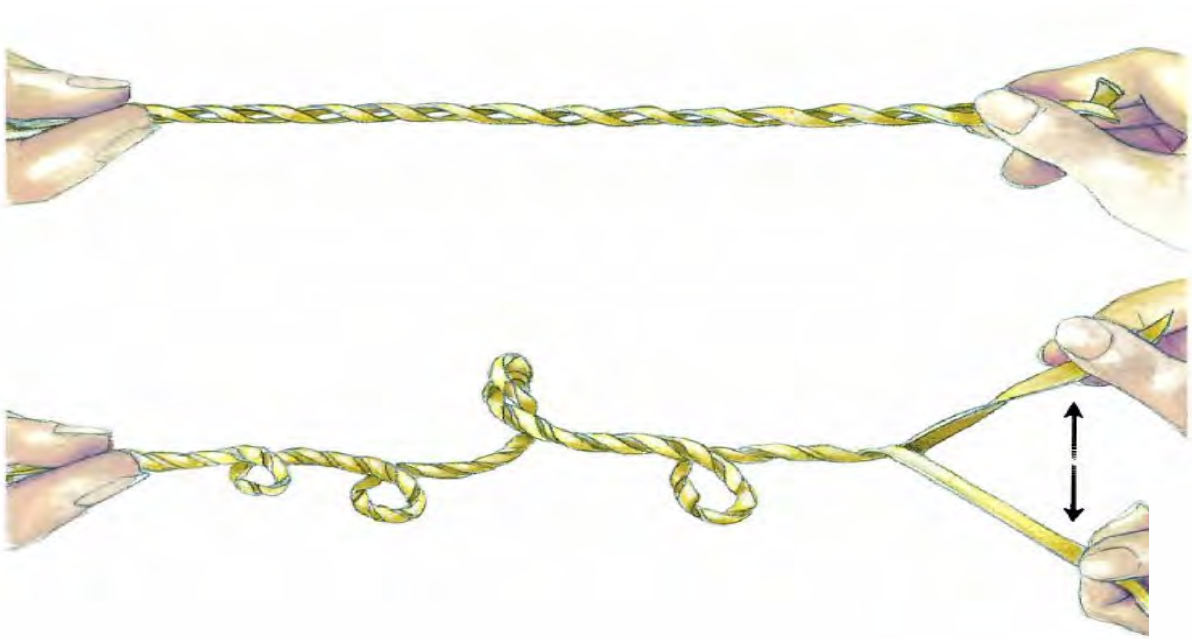


Uvijanje DNK (roll!!) oko histona

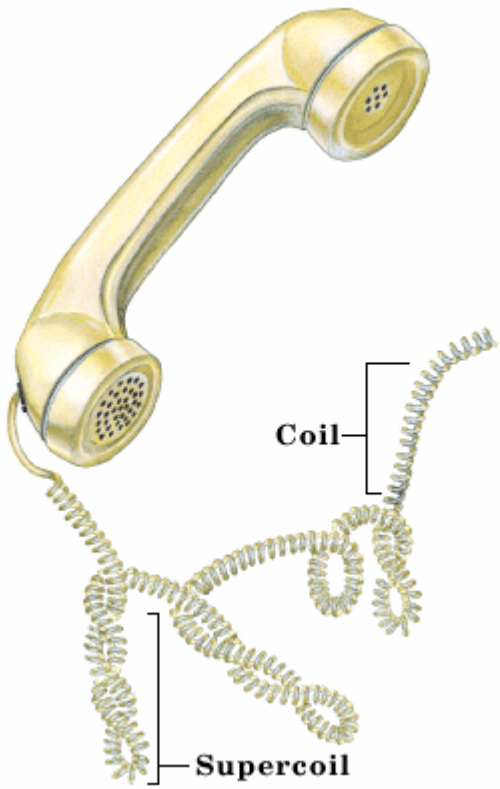
Cirkularna DNK i superuvijanje

Elektronski mikrograf relaksirane (A) i **superuvijene** (B) cirkularne DNK iz mitohondrija.





Modeli za superuvijanje



Načini superuvijanja DNK:”toroidno” ili solenoidno vs isprepletano ili plektonomično

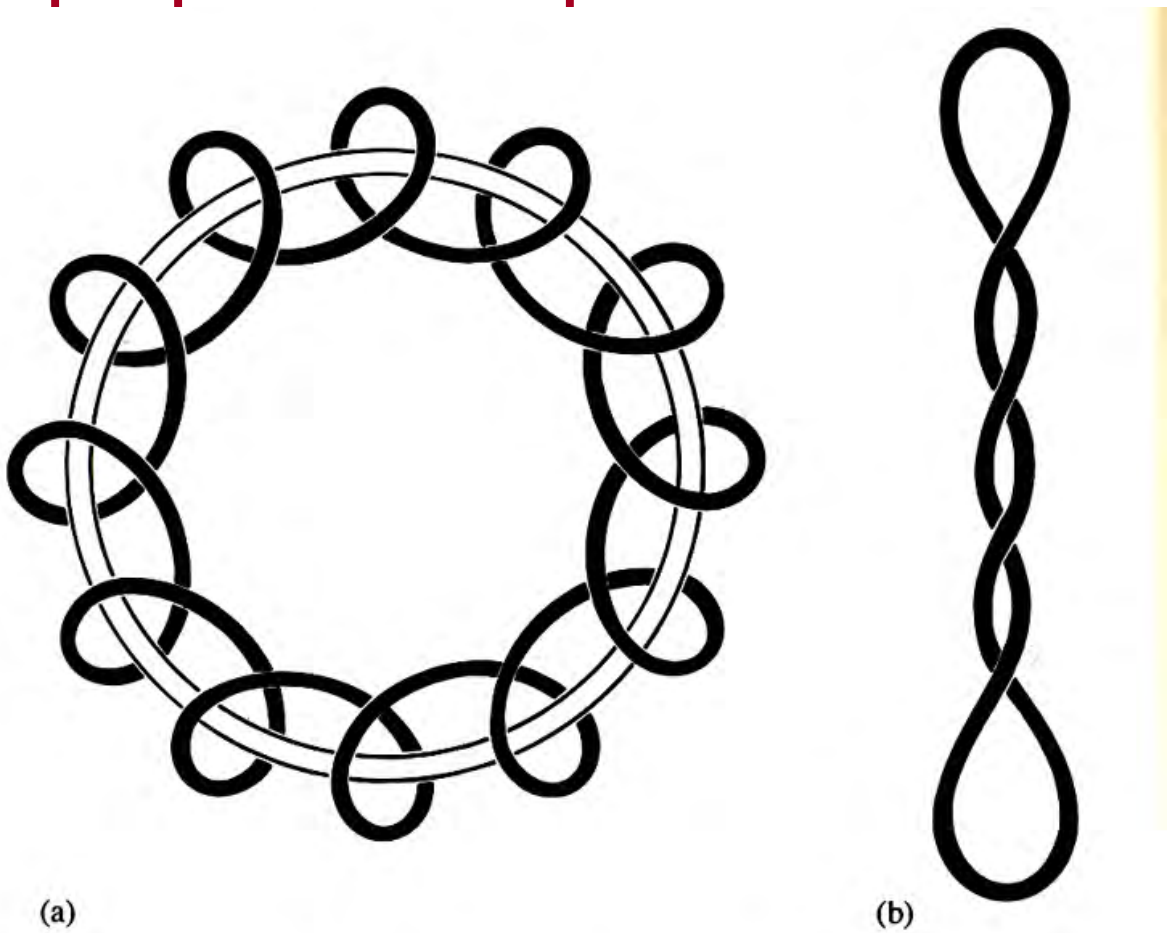


Figure 6.2 Two general varieties of DNA supercoil. In (a), the DNA coils into a series of spirals about an imaginary toroid or ring (shown here by open lines); and so this kind of wrapping is known as ‘toroidal.’ In (b), the DNA crosses over and under itself repeatedly; and so this kind of wrapping is known as ‘interwound.’

Super-uvijanje dugačke DNK u hromozomima

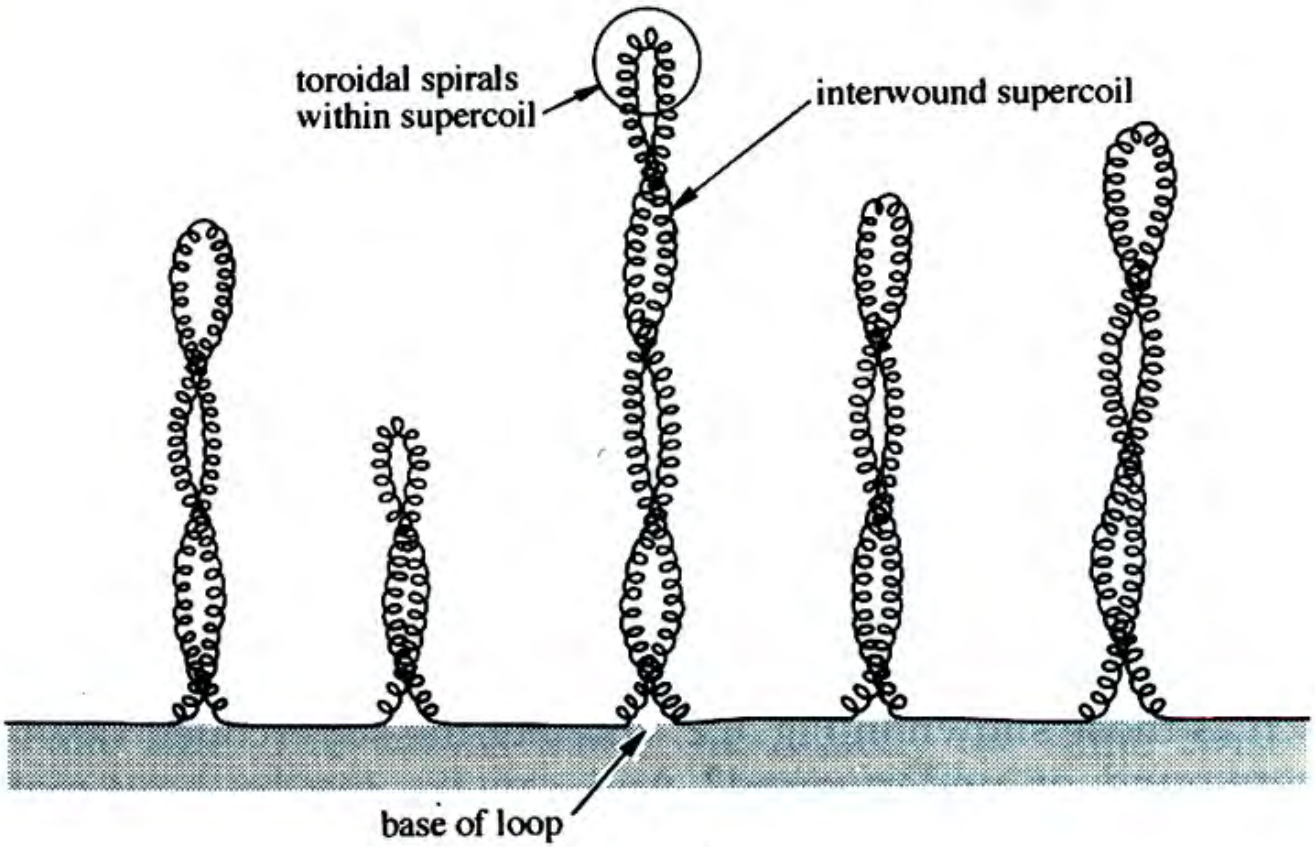
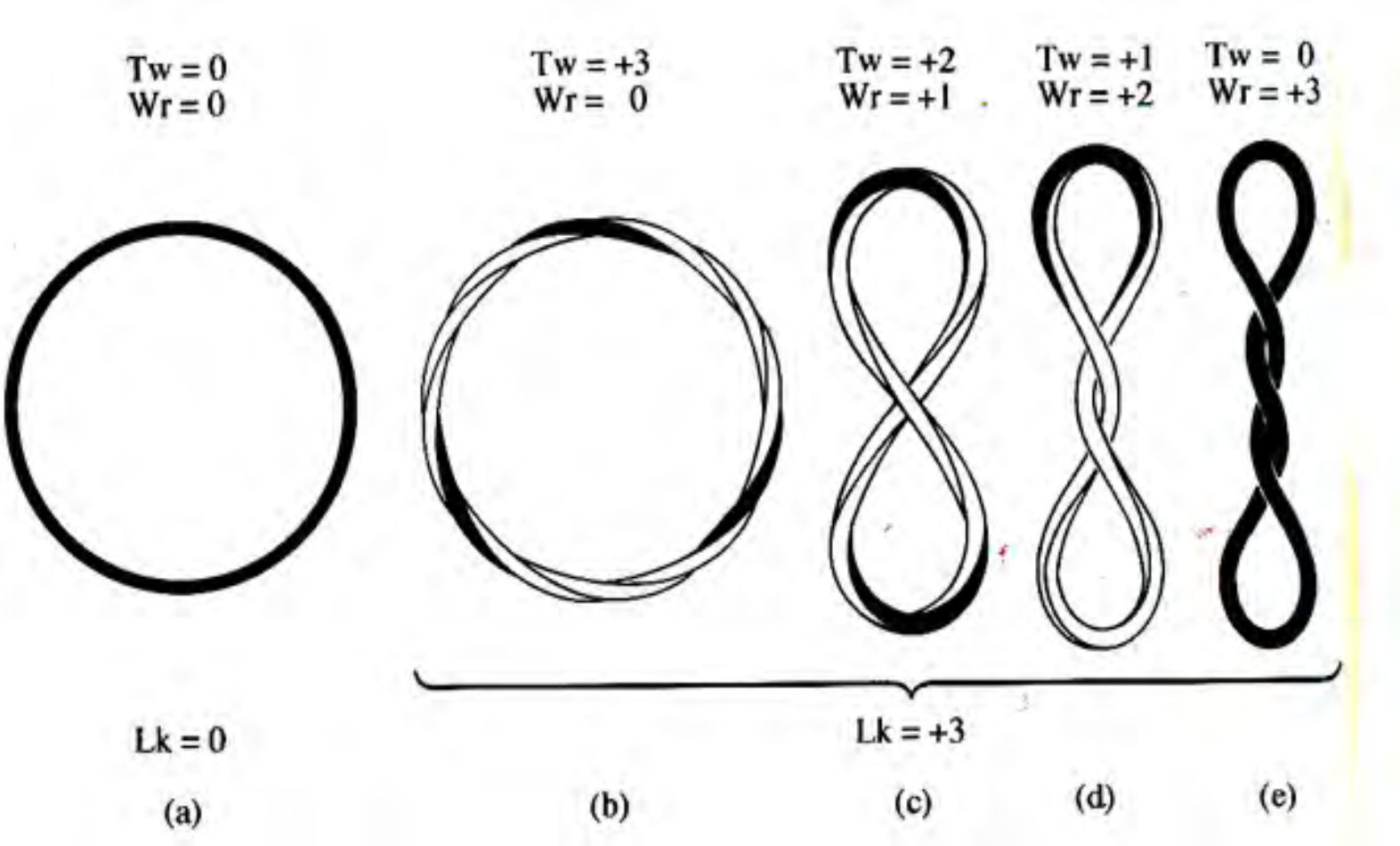


Figure 6.3 The division of a long, linear DNA molecule into loops generates end-restraint at the base of every loop, if the two ends are attached to some support or 'scaffold.' This kind of looped-linear arrangement is thought to be typical of the chromosomal DNA found in higher organisms.

Topologija superuvijanja DNK



Tw: “twist”(uvrtanje) Wr: ”writhe” (previjanje)

Lk: “linking number”

Lk = Tw + Wr (Lk pokazuje koliko se puta 2 polinukleotidna niza uvijaju jedan oko drugog!)

- Molekuli DNK koji se međusobno razlikuju po “linking” broju nazivaju se topoizomeri.

- Nastajanje topoizomera katalizuju enzimi: topoizomeraze!!!