Nukleinske kiseline

Literatura:
ppt,
Praktikum,
Bilo koji od predloženih udžbenika iz biohemije

Sadržaj predavanja

- Hemijska struktura nukleinskih kiselina
- Watson-Crickov model B DNK
- Centralna dogma molekularne biologije
- Zašto je DNK (a ne RNK) izabrana za čuvanje genetske informacije?
- Zašto dvostruki heliks?
  - Osobine baza (tautomerija, interakcije u nepolarnoj sredini i vodi)
- Dimenzije DNK i organizacija u ćeliji
- Polimorfizam DNK i konzervatizam RNK
- Ponašanje DNK
  - uvijanje-razvijanje
  - interakcije sa ligandima
- Genom
  Proteom

From the beginning, the study of nucleic acids has drawn together, as though by a powerful unseen force, a galaxy of scientists of the highest ability. Striving to tease apart its secrets, these talented individuals have brought with them a broad range of skills from other disciplines while many of the problems they have encountered have proved to be soluble only by new inventions. Looking at their work, one is constantly made aware that scientists in this field appear to have enjoyed a greater sense of excitement in their work than is given to most. Why?

For over 60 years, such men and women have been fascinated and stimulated by their awareness that the study of nucleic acids is central to the knowledge of life.

Michael Blackburn et al., **Nucleic Acids in Chemistry and Biology,** RSC Publishing, 2006

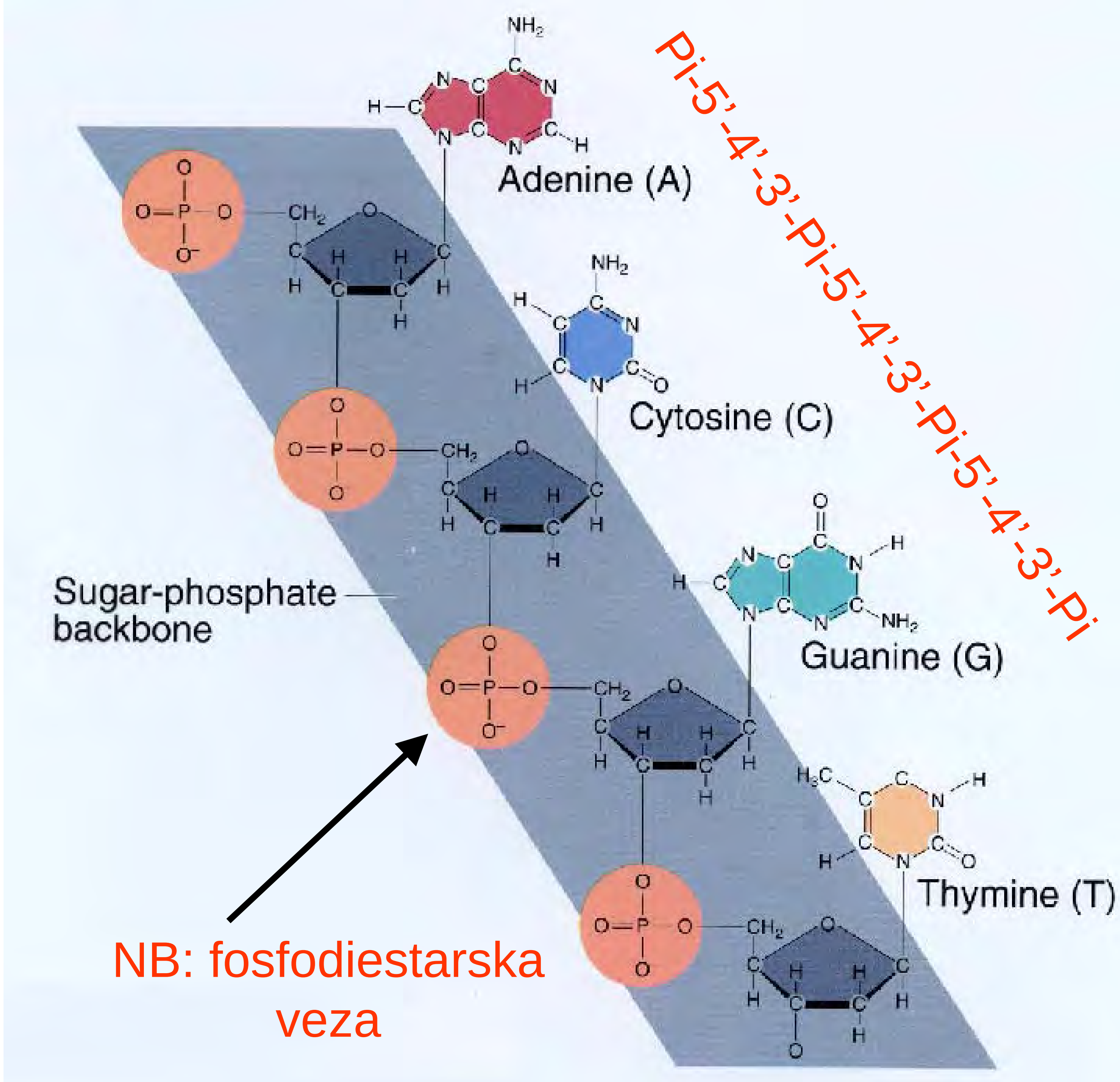
Lekar, Friedrich Miescher (1869) izolovao supstancu (nukleoprotein) koju je nazvao nuclein (potiče iz jedra).

Čist preparat (bez proteina) izolovao Richard Altman 1889 i nazvao ga nukleinska kiselina

Figure 1.5 The laboratory at Tübingen where Miescher isolated nuclein (courtesy of the University of Tübingen Library, Tübingen, Federal Republic of Germany).

Laboratorija u Tibingenu u kojoj je Mischer izolovao nuclein

Šećer-fosfatna kičma

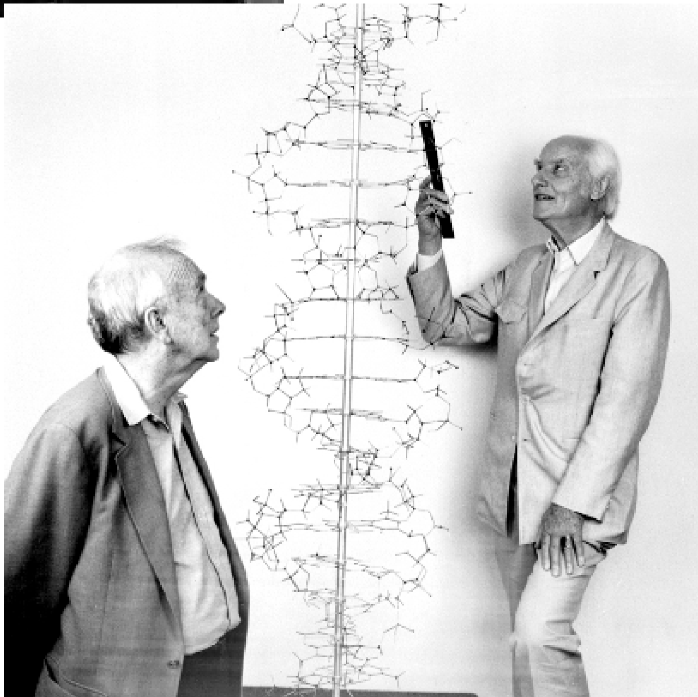


NB: fosfodiestarska veza

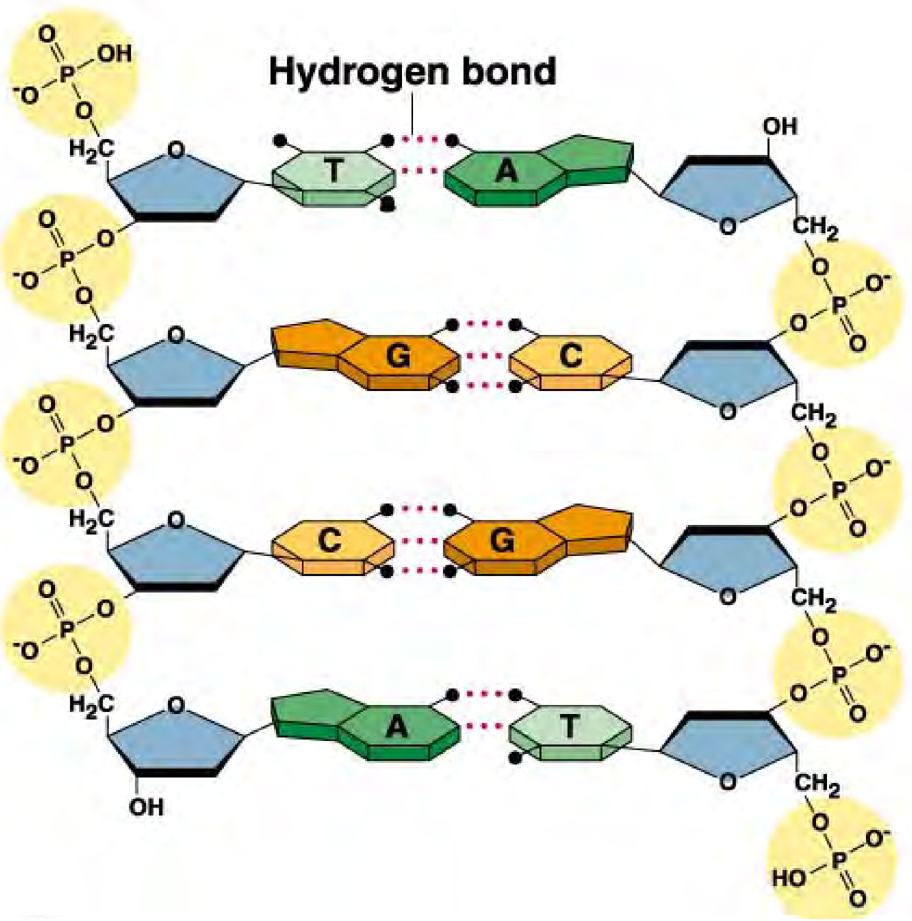
Rosalind Franklin

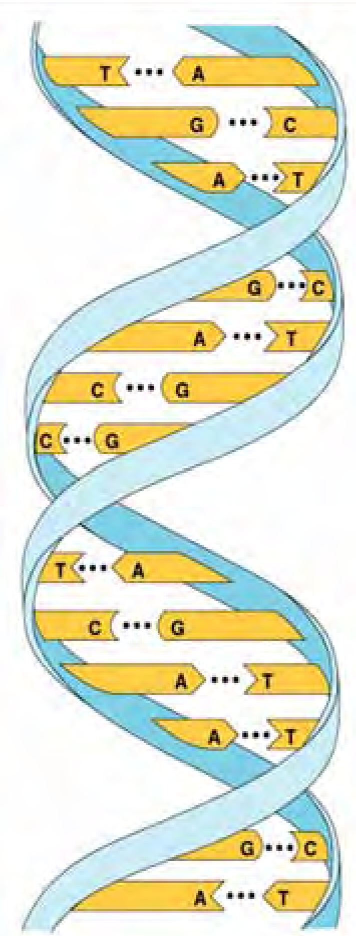
Maurice Wilkins

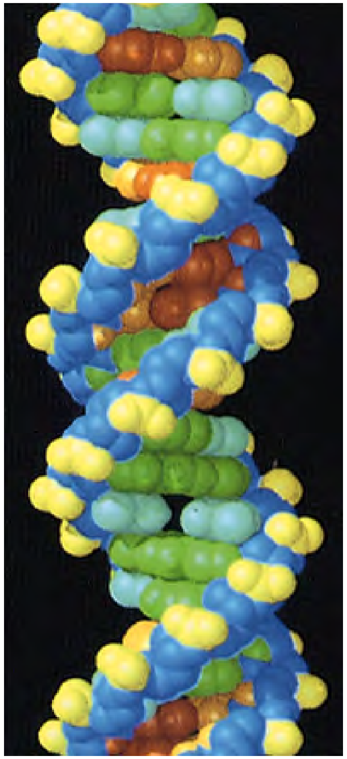
Jim Watson &
Francis Crick
1952-2002



W-C model B-DNK

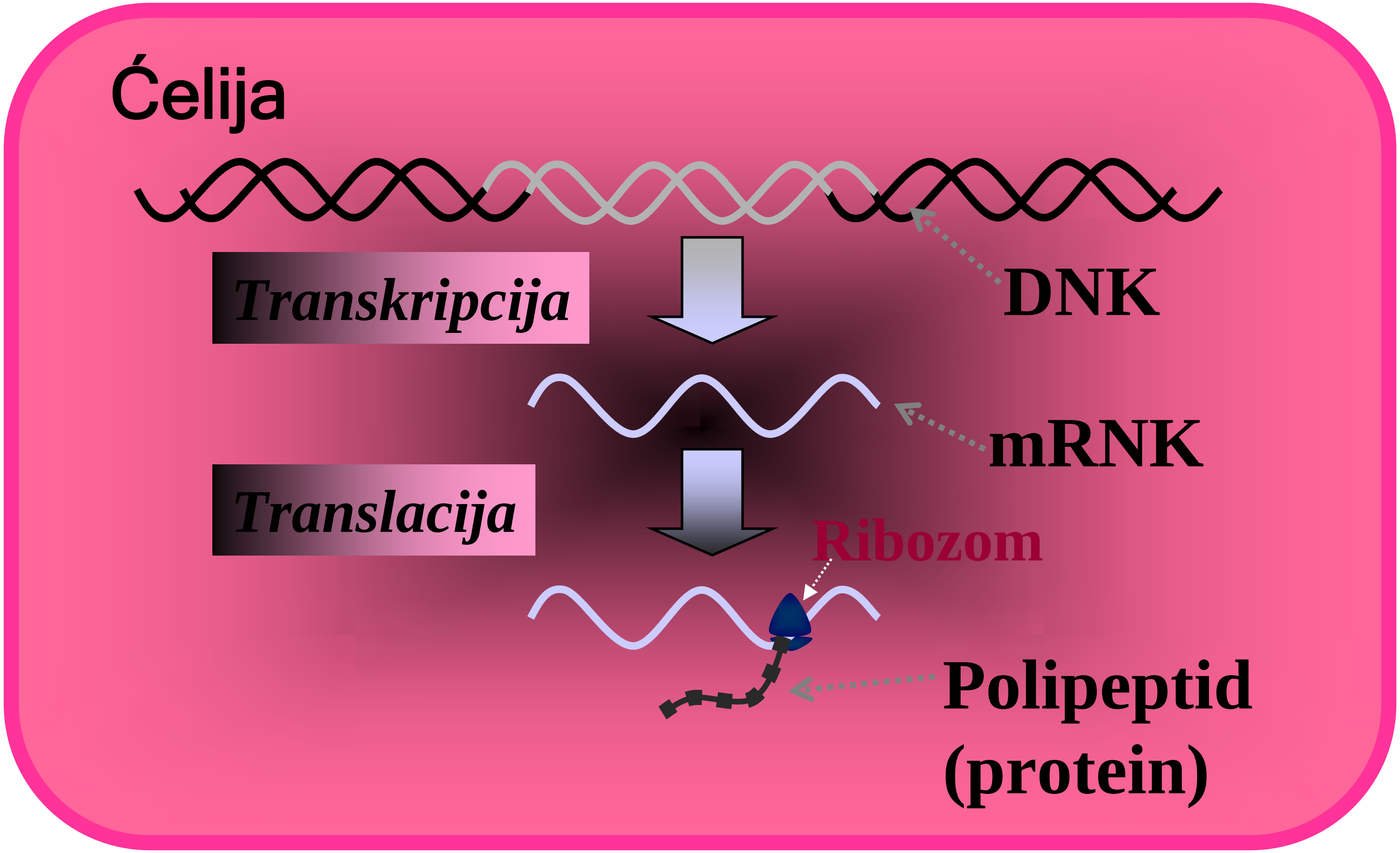




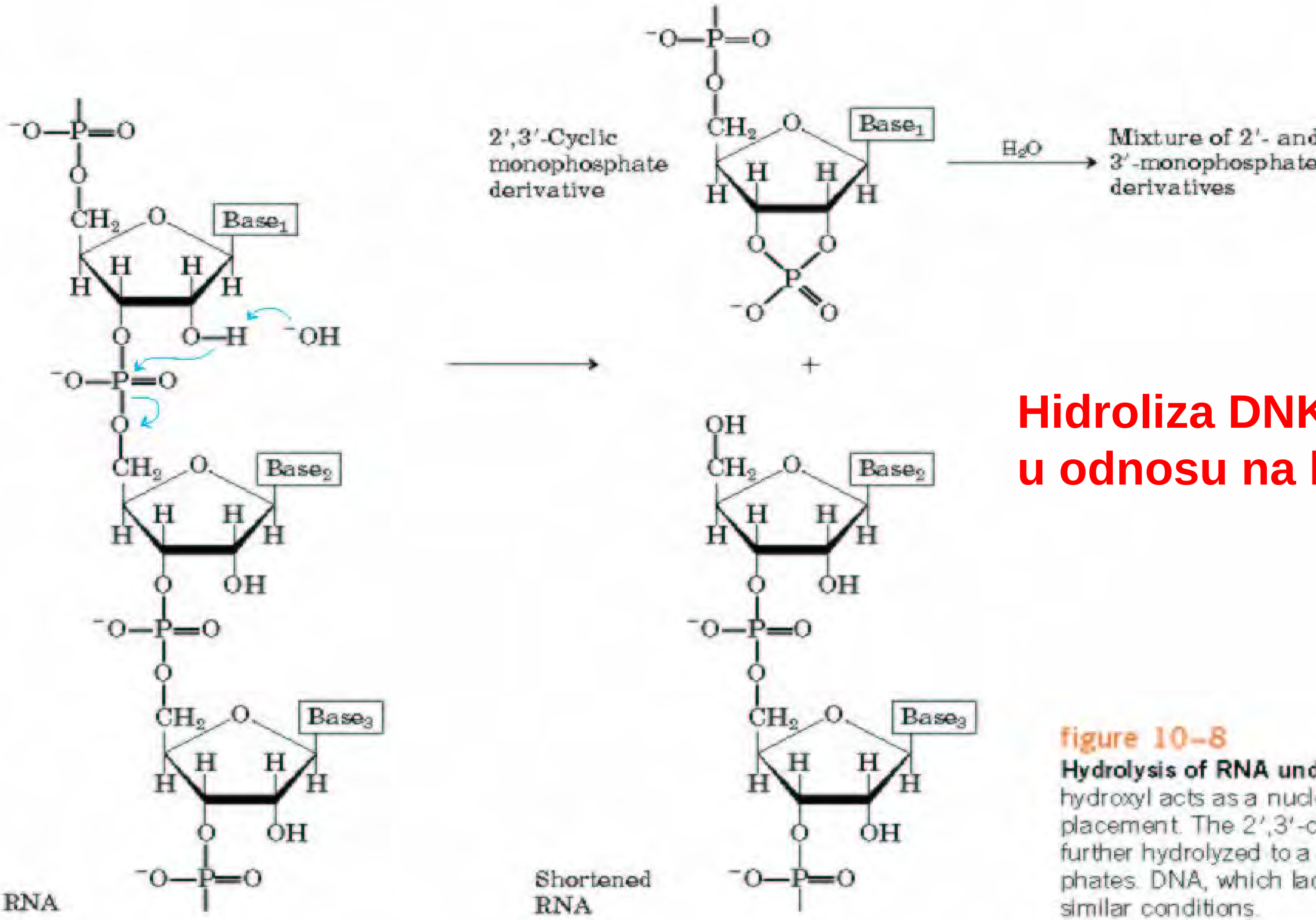


Biološke implikacije modela DNK su bile očigledne: hemijska priroda gena (genetske informacije)!!!!

Centralna dogma molekularne biologije (F. Crick 1957)



Zašto je DNK izabrana za čuvenje genetske informacije?



Hidroliza DNK je 100x sporija u odnosu na hidrolizu RNK !

figure 10–8

Hydrolysis of RNA under alkaline conditions. The 2′ hydroxyl acts as a nucleophile in an intramolecular displacement. The 2′,3′-cyclic monophosphate derivative i further hydrolyzed to a mixture of 2′- and 3′-monophosphates. DNA, which lacks 2′ hydroxyls, is stable under similar conditions.

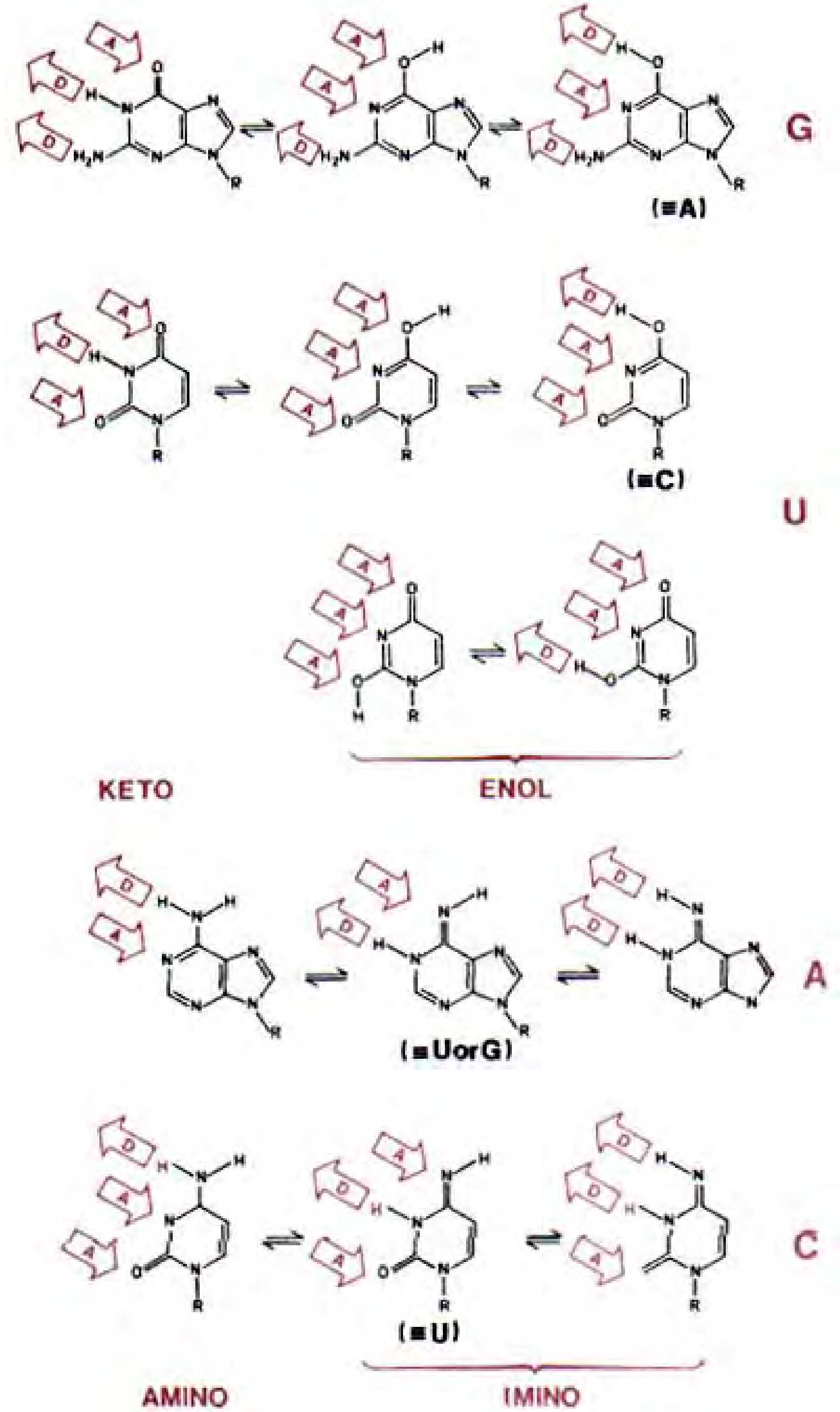
Zašto dvostruki heliks?

- Osobine baza:
  - Tautomerija
  - Horizontalne interakcije
    - Komplementarne vodonične veze u NEPOLARNOJ SREDINI
  - Vertikalne interakcije
    - “STEKING INTERAKCIJE” U VODI

Tautomerne strukture baza

- Keto vs. enol
- Amino vs imino

- Do ~1950 smatralo se da su baze u enolnom obliku. Zašto?

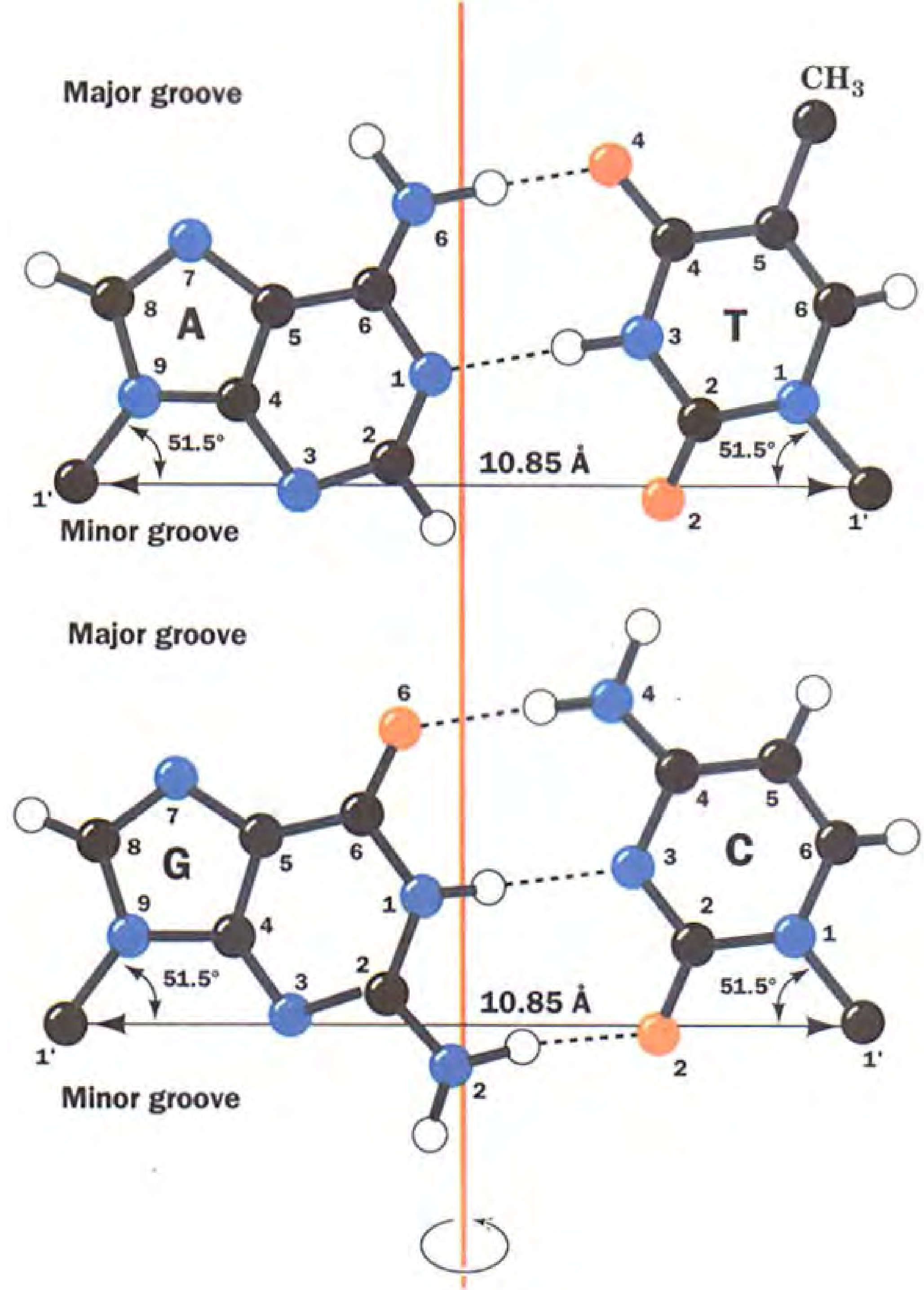


Horizontalne interakcije:

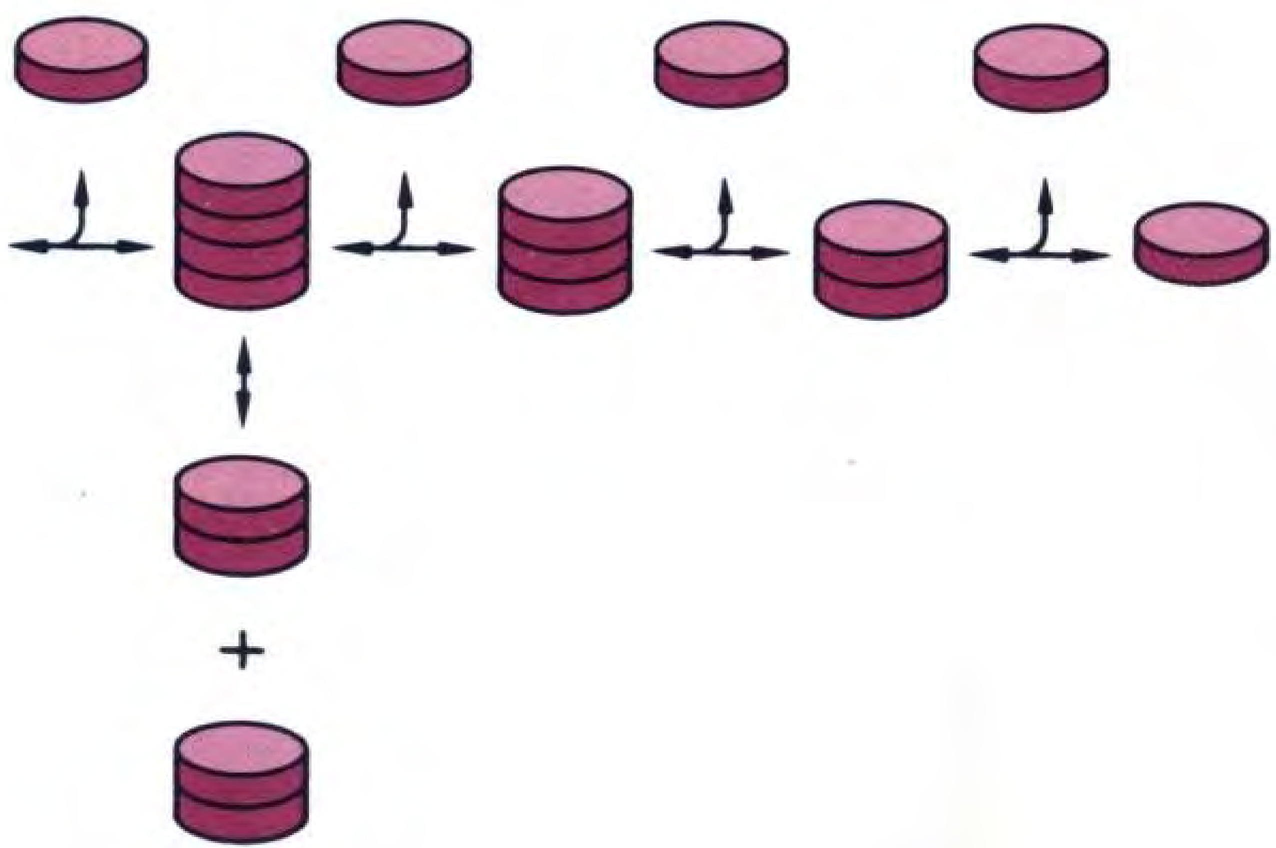
vodonično vezivanje
 među bazama.

Može se videti samo u
nepolarnom rastvaraču.

Funkcija: komplementarno
prepoznavanje baza.

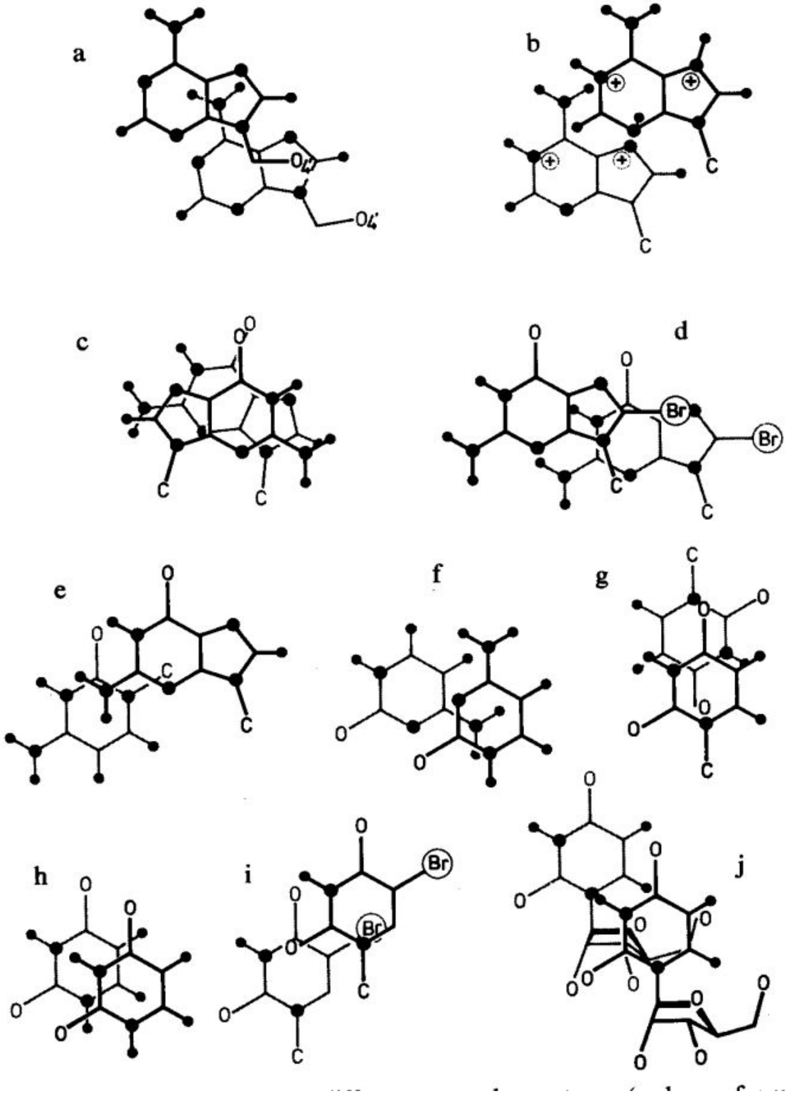


Interakcije baza u vodi: “slepljivanje” “stacking” baza



“Slepljivanje” baza u kristalnom stanju

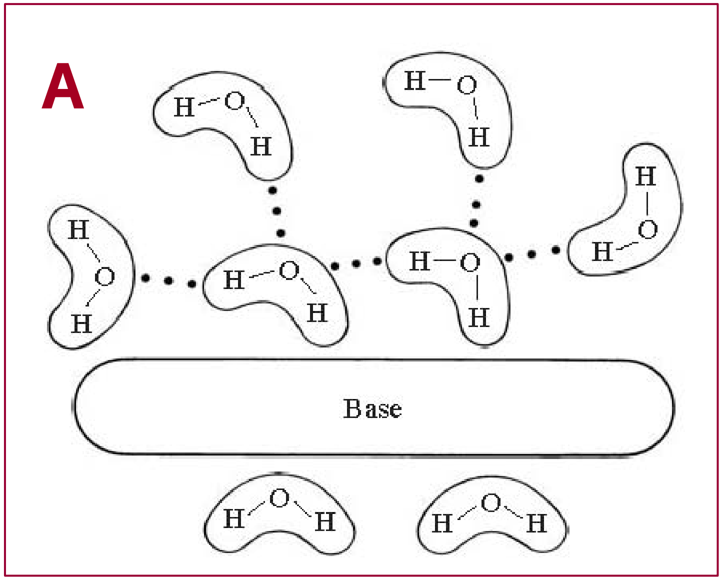
Baze su kristalisane iz vode!

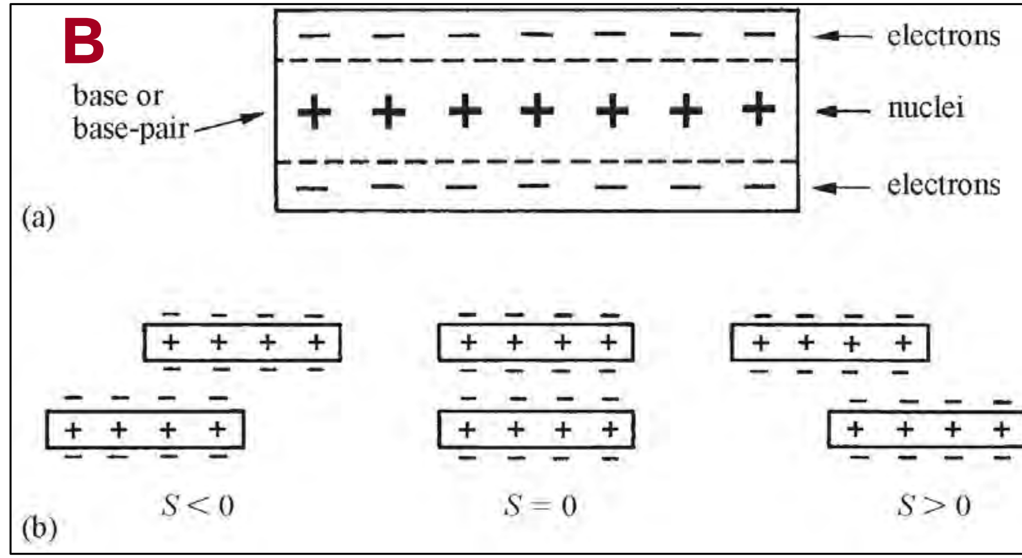


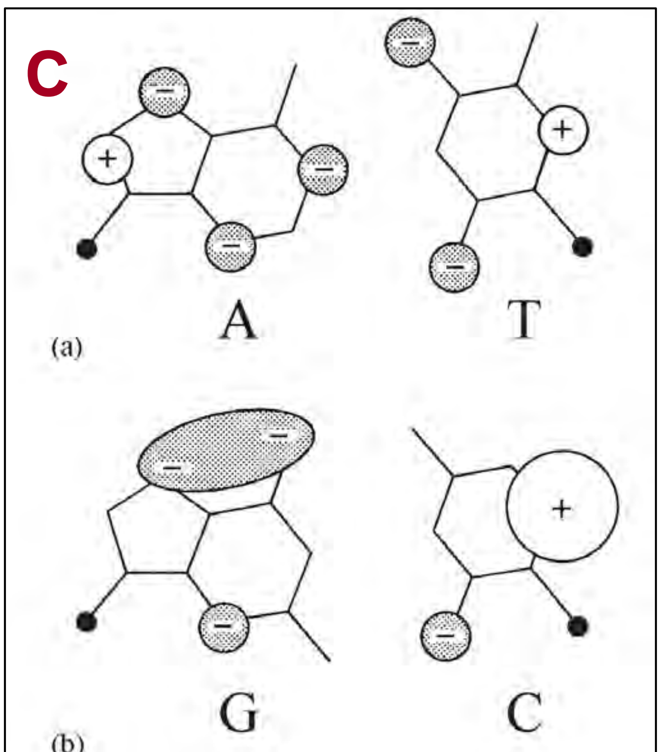
Energija (ΔG) “stacking” interakcija

Stacked dimers		Stacking energies [kcal/mole dimer]
↑C·G G·C↓		−14.59
↑C·G A·T↓	↑T·A G·C↓	−10.51
↑C·G T·A↓	↑A·T G·C↓	− 9.81
↑G·C C·G↓		− 9.69
↑G·C G·C↓	↑C·G C·G↓	− 8.26
↑T·A A·T↓		− 6.57
↑G·C T·A↓	↑A·T C·G↓	− 6.57
↑G·C T·A↓	↑T·A C·G↓	− 6.78
↑A·T A·T↓	↑T·A T·A↓	− 5.37
↑A·T T·A↓		− 3.82

Zaključak:







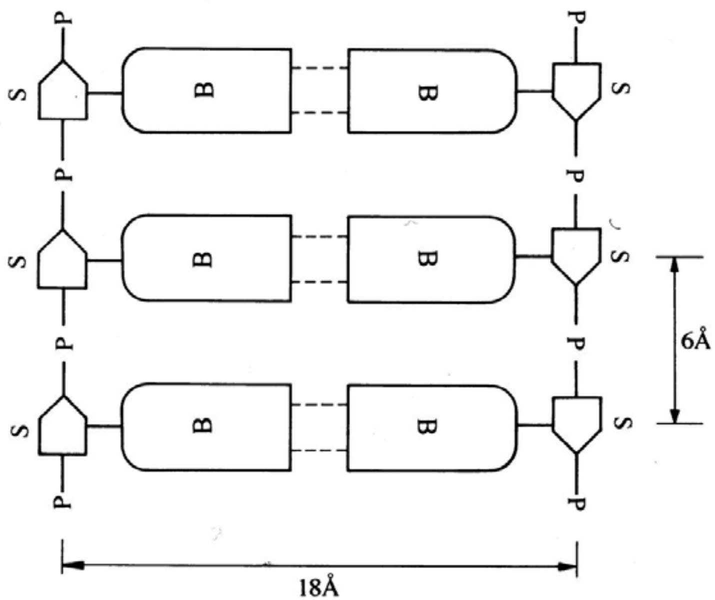
Objašnjenje “stacking” interakcija među bazama (Chris Hunter):

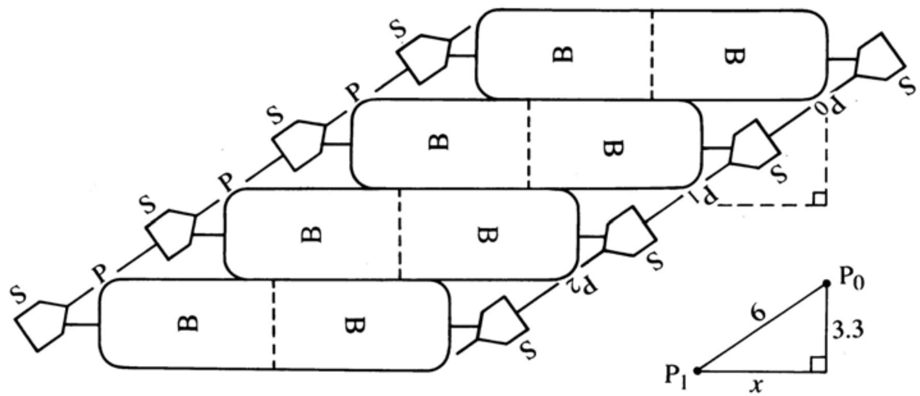
A: hidrofobni efekat;

B: baze na donjoj i gornjoj površini imaju (mali) višak negativnog naelektrisanja;

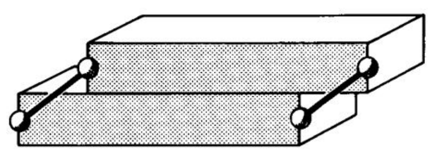
C: parcijalne šarže na bazama.

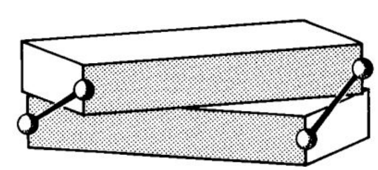
Zašto dvostruki heliks?



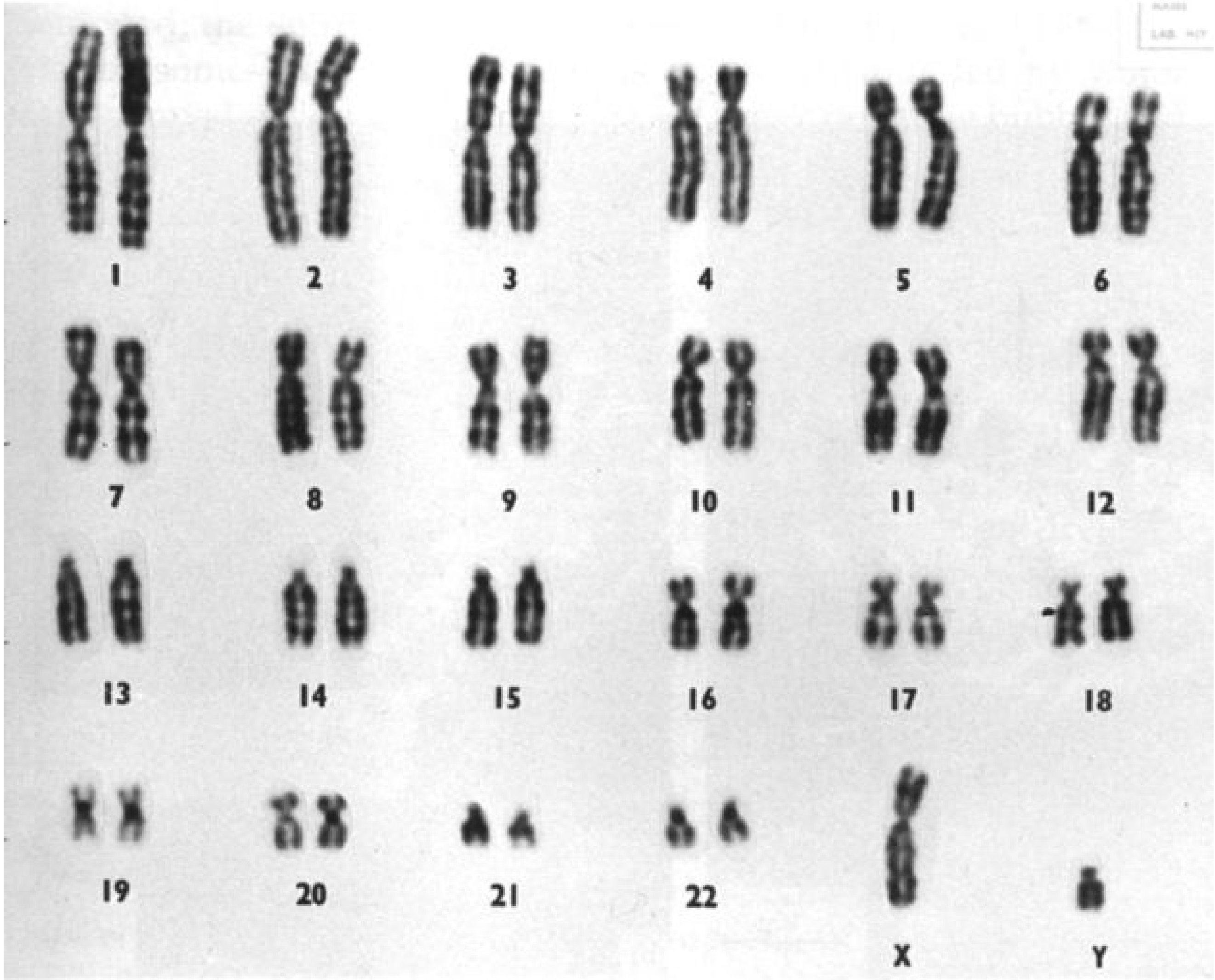


(a) (b)


(a)


(b)

Organizacija DNK u ćelijama eukariota!

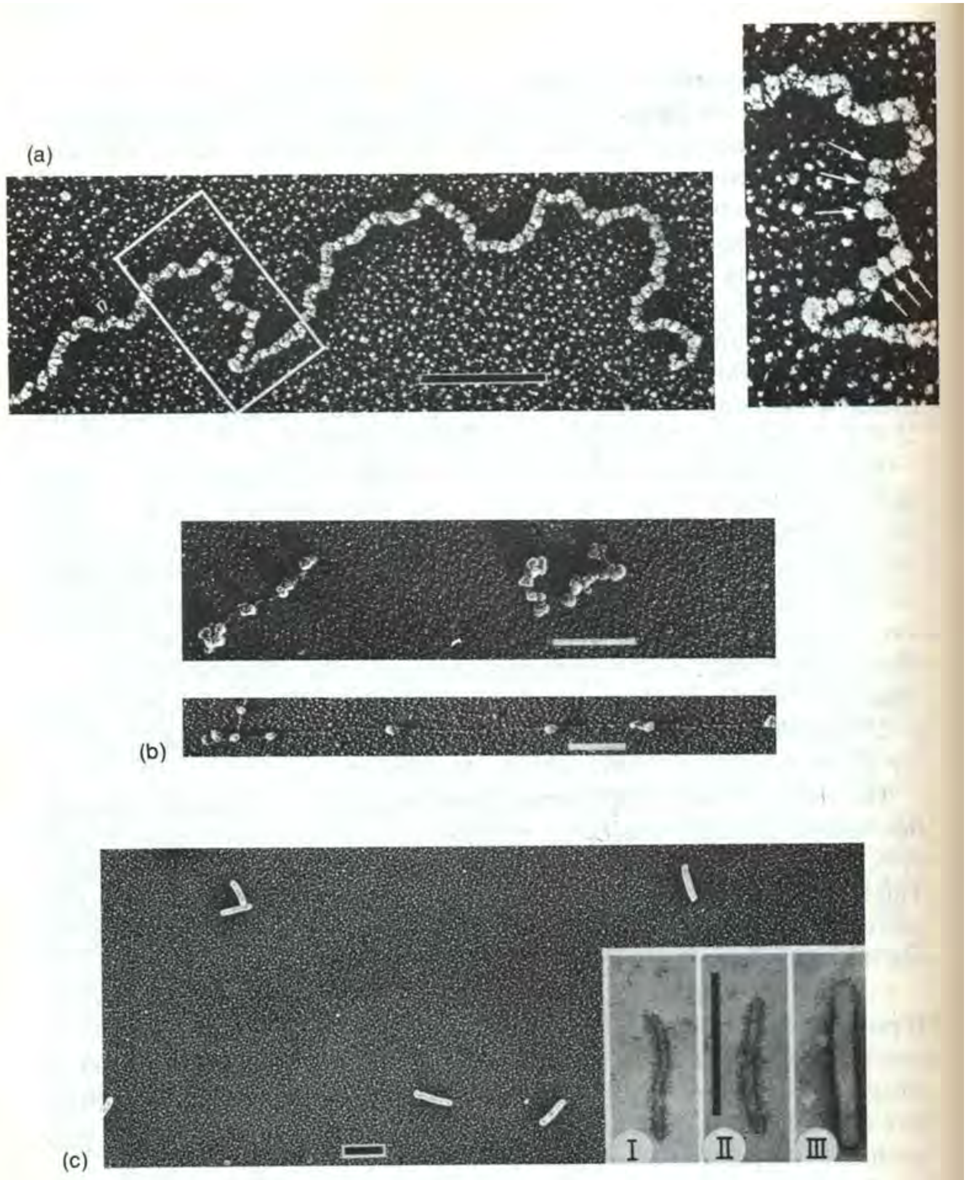


Dimenzije DNK: relativne dužine

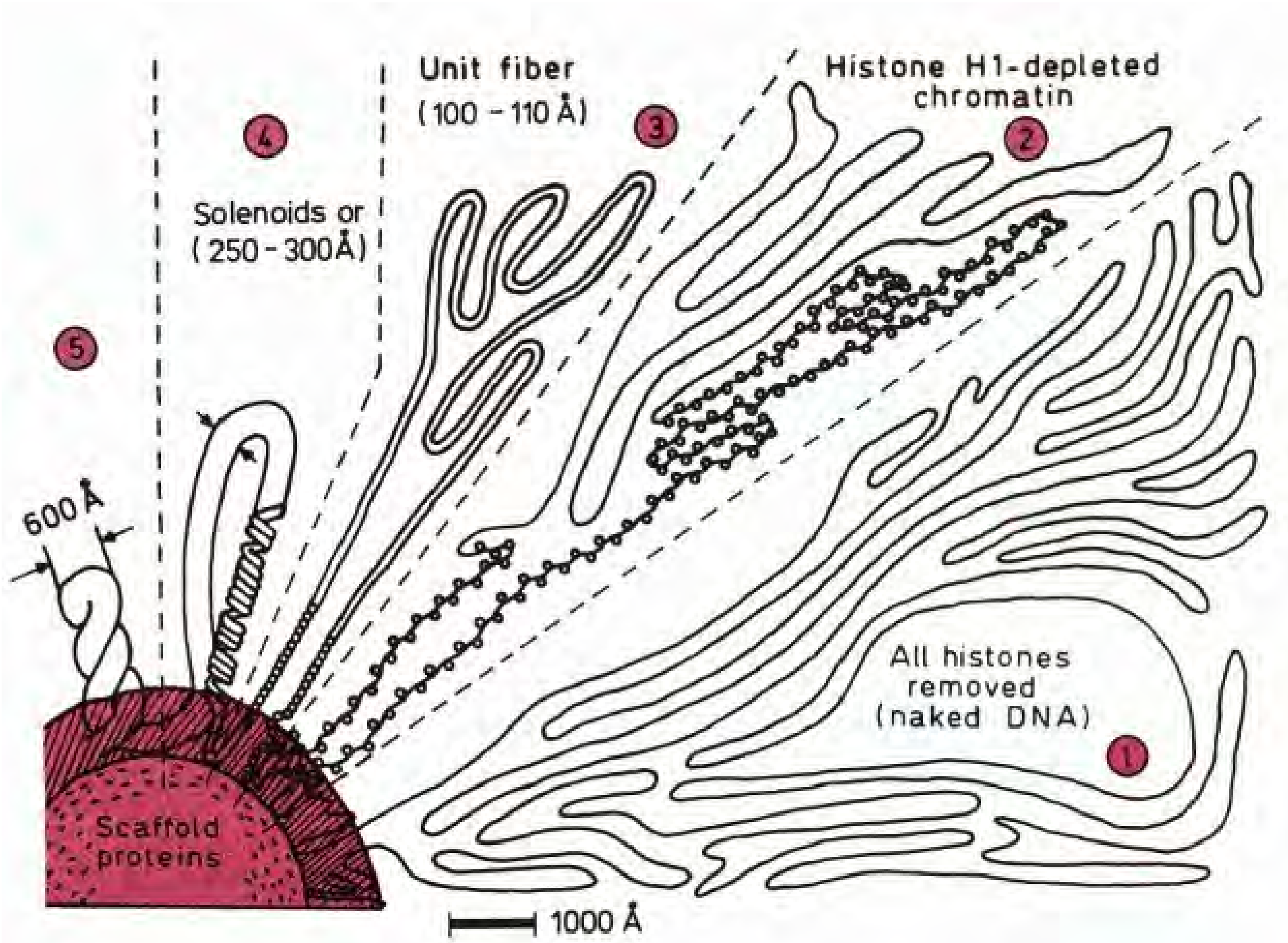
- **Dužina (m)**
- **Ukupna DNK u humanim ćelijama 1m**
- **DNK u humanom hromozomu 10^{-2} m**
- **Prečnik lopte humane DNK 10^{-8} m**
- **Prečnik molekula DNK 10^{-9} m (~2 nm)**
- **Prečnik atoma 10^{-10} m (1 Å)**

- **Dužina x 10^{6}**
- **1000 km**
- **10 km**
- **1 cm**
- **2.4 mm**

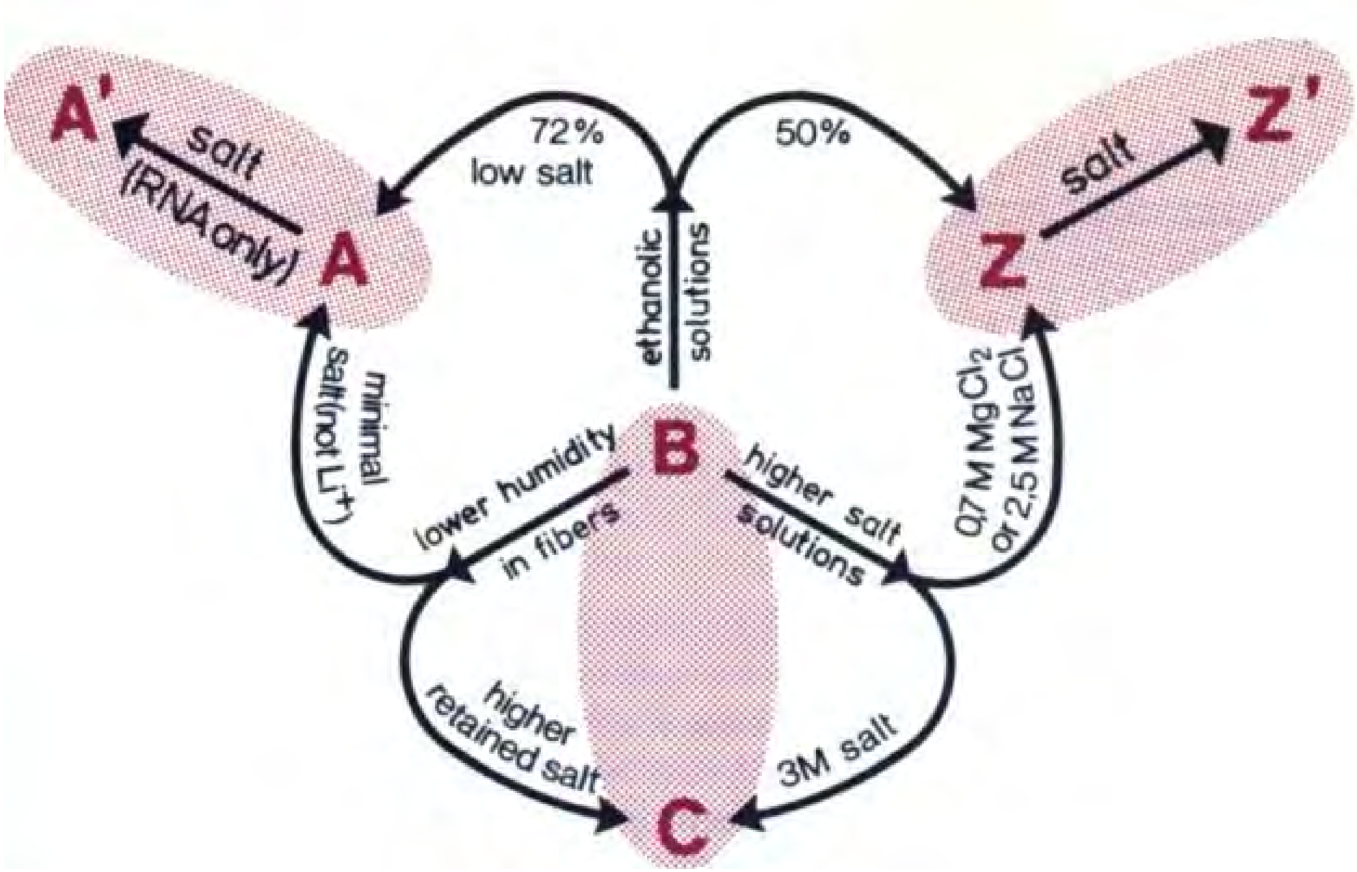
Kako izgleda preparat DNK: efekat jonske sile



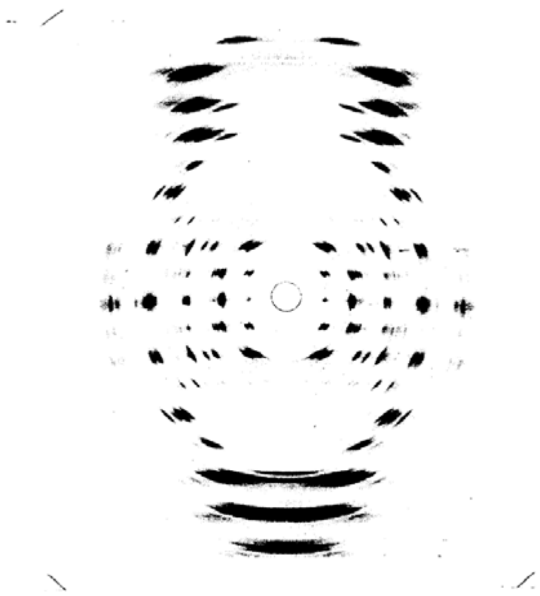
Organizacija DNK u jedru

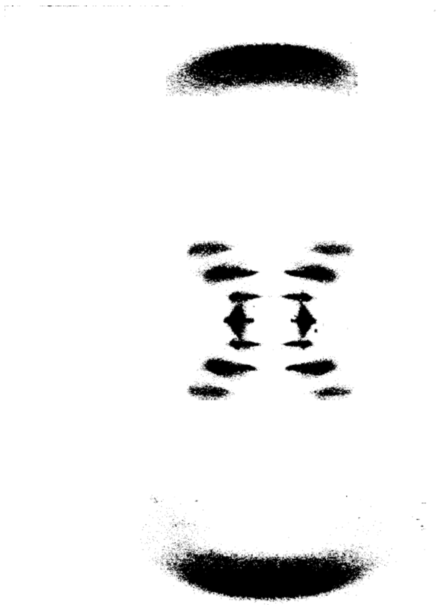


Polimorfizam DNK vs. konzervatizam RNK



Preparat DNK se u zavisnosti od uslova izolovanja može dobiti u više konformacija (polimorfizam), a RNK (kao i nativni protein) uvek samo u jednoj (konzervatizam).

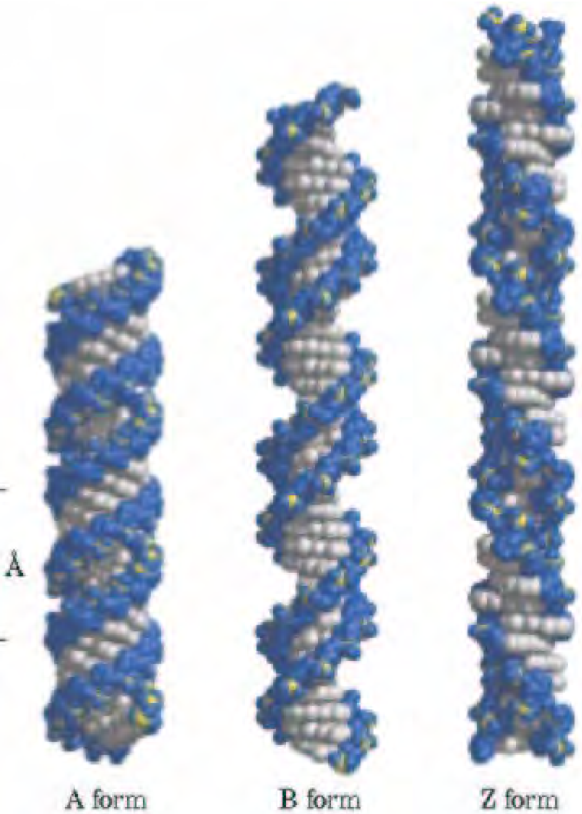




Difrakcione slike A-DNK (levo) i B-DNK (desno)

NB: preparati se nalaze u rastvoru u kojem su taloženi/kristalisani

Polimorfizam DNK: standardne (prosečne) konformacije DNK



	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrmidines; syn for purines

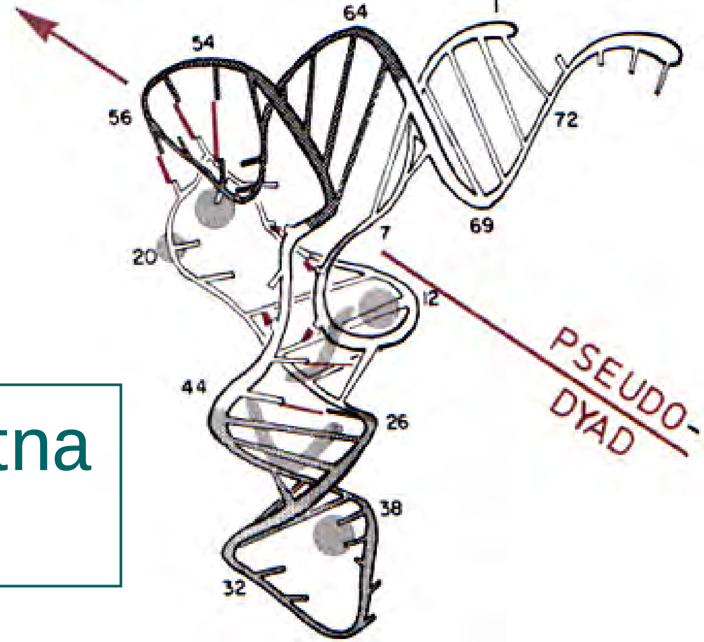
figure 10–19
Comparison of A, B, and Z forms of DNA. Each struc-

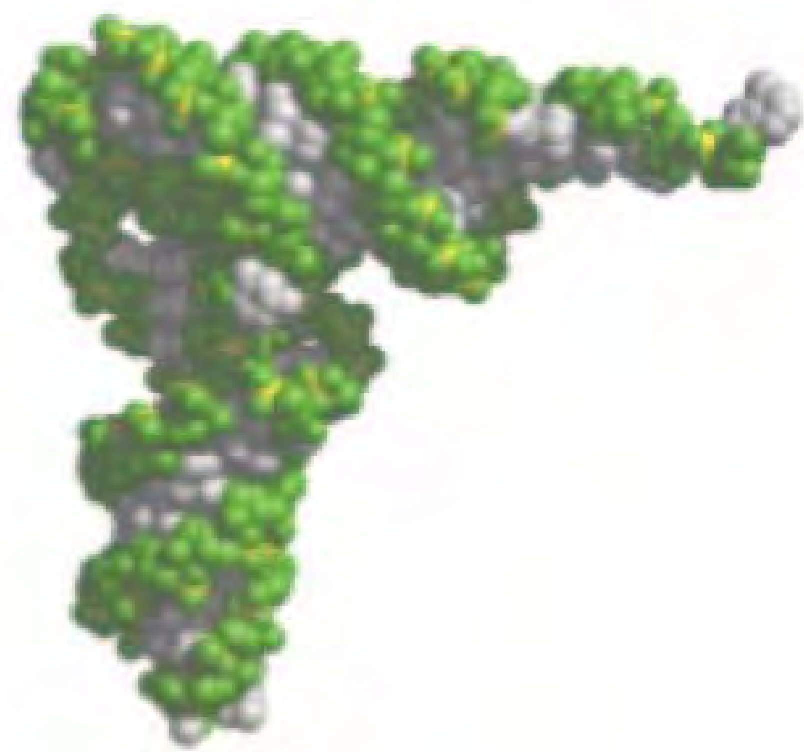
Informaciona RNK

figure 10–25

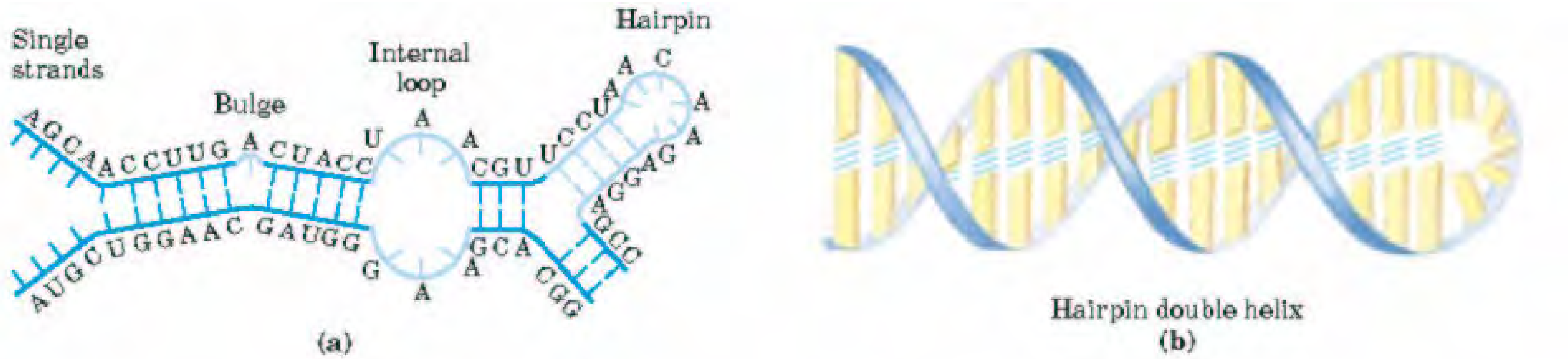
Typical right-handed stacking pattern of single-stranded RNA. The bases are shown in white, the phosphate atoms in yellow, and the riboses and phosphate oxygens in green. Green is used to represent RNA strands in succeeding chapters, just as blue is used for DNA.

Transportna RNK





Sekundarna struktura tRNK, rRNK

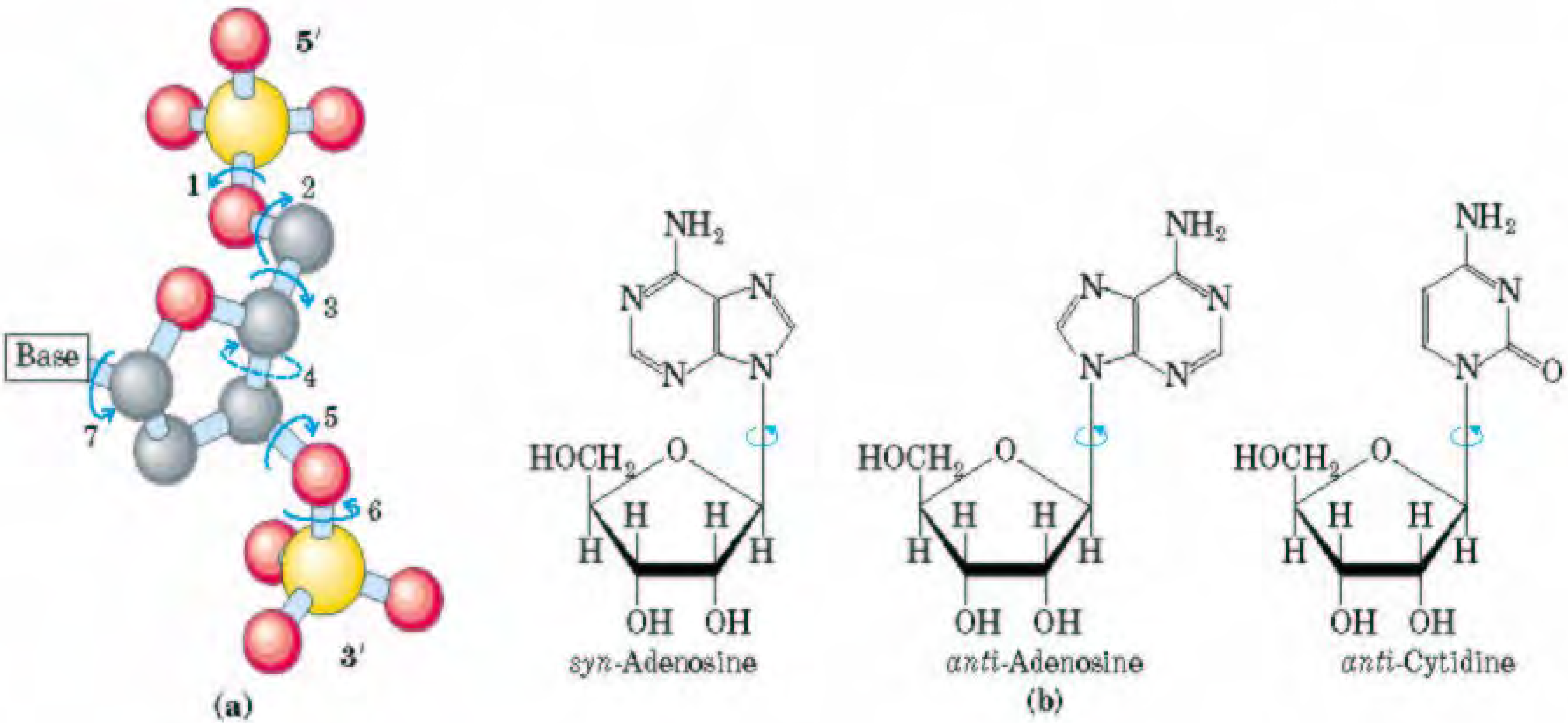


Konzervatizam RNK:

RNK kristališe slično proteinima samo u jednoj konformaciji.

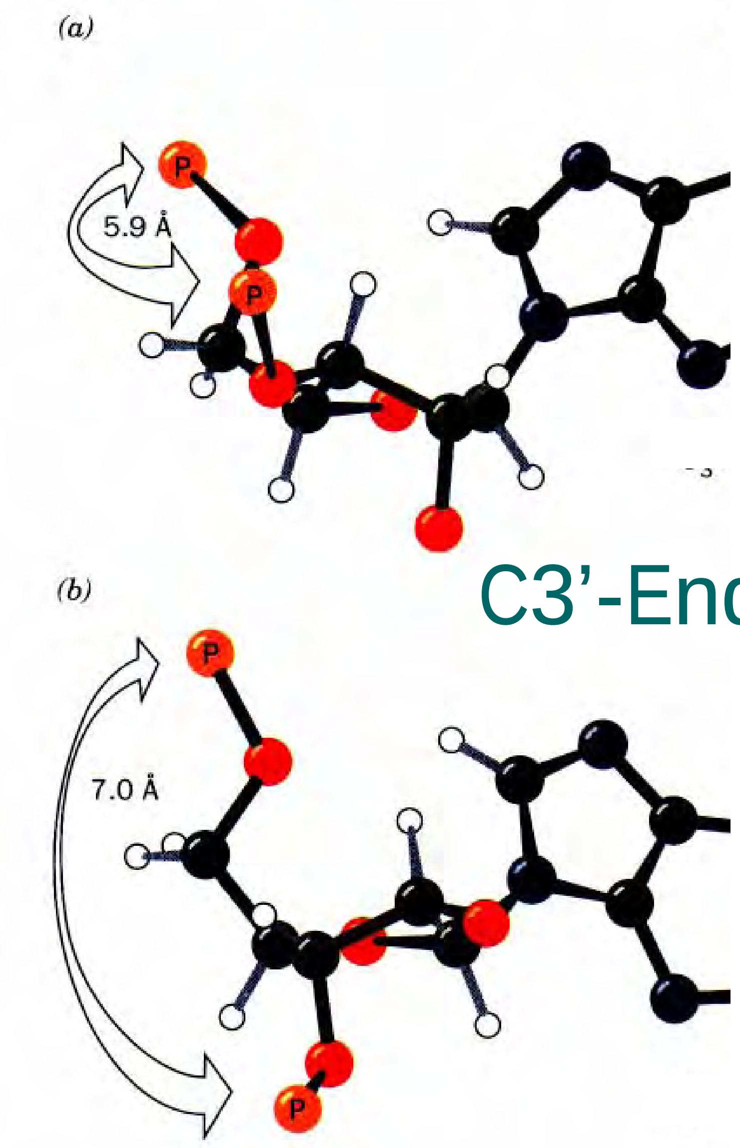
Helikoidne oblasti: A-heliks

Zašto polimorfizam DNK i konzervatizam RNK?



Konformaciona analiza nukleotida

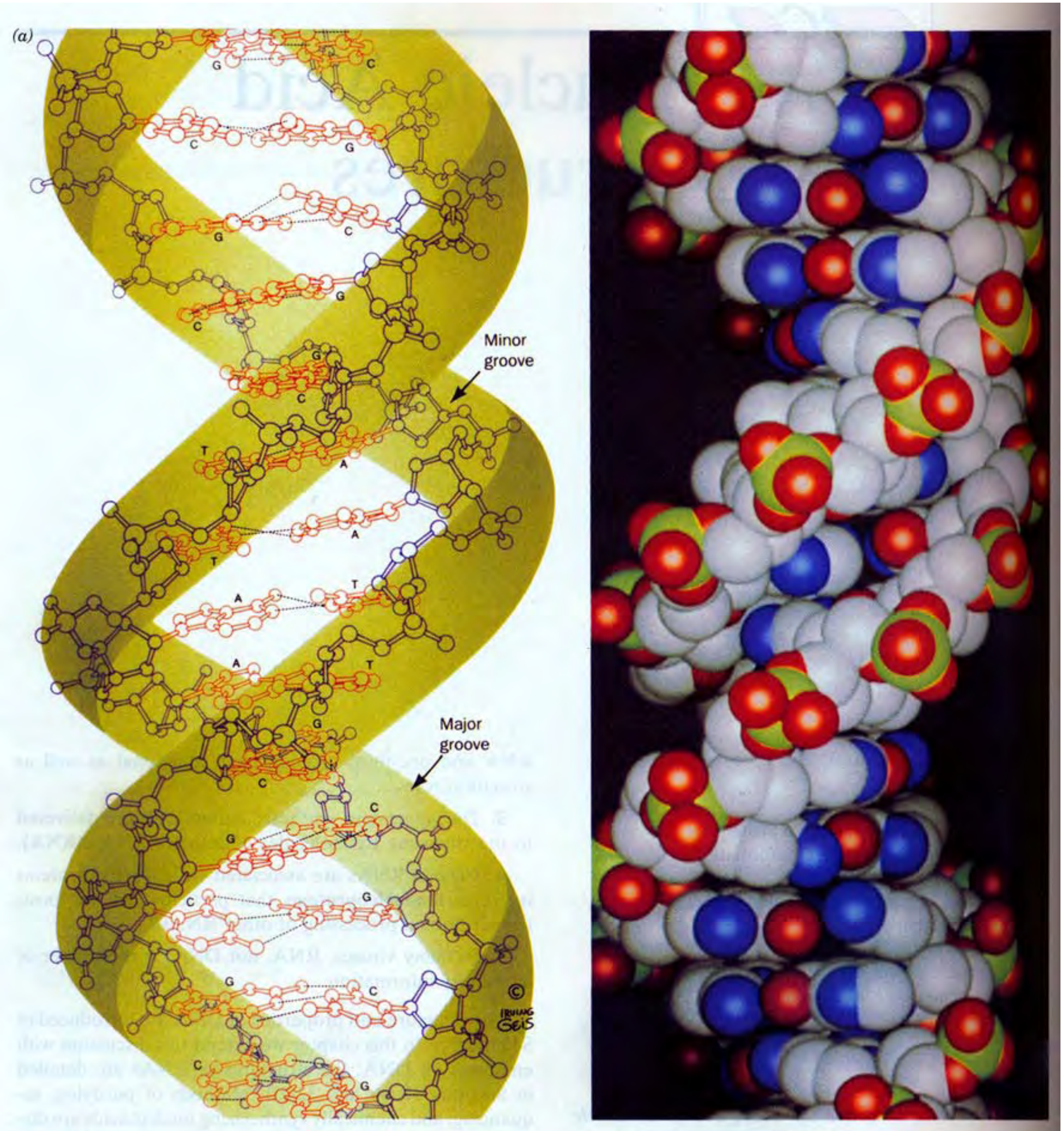
Efekat konformacije šećera na fosfodiestarsku kičmu!



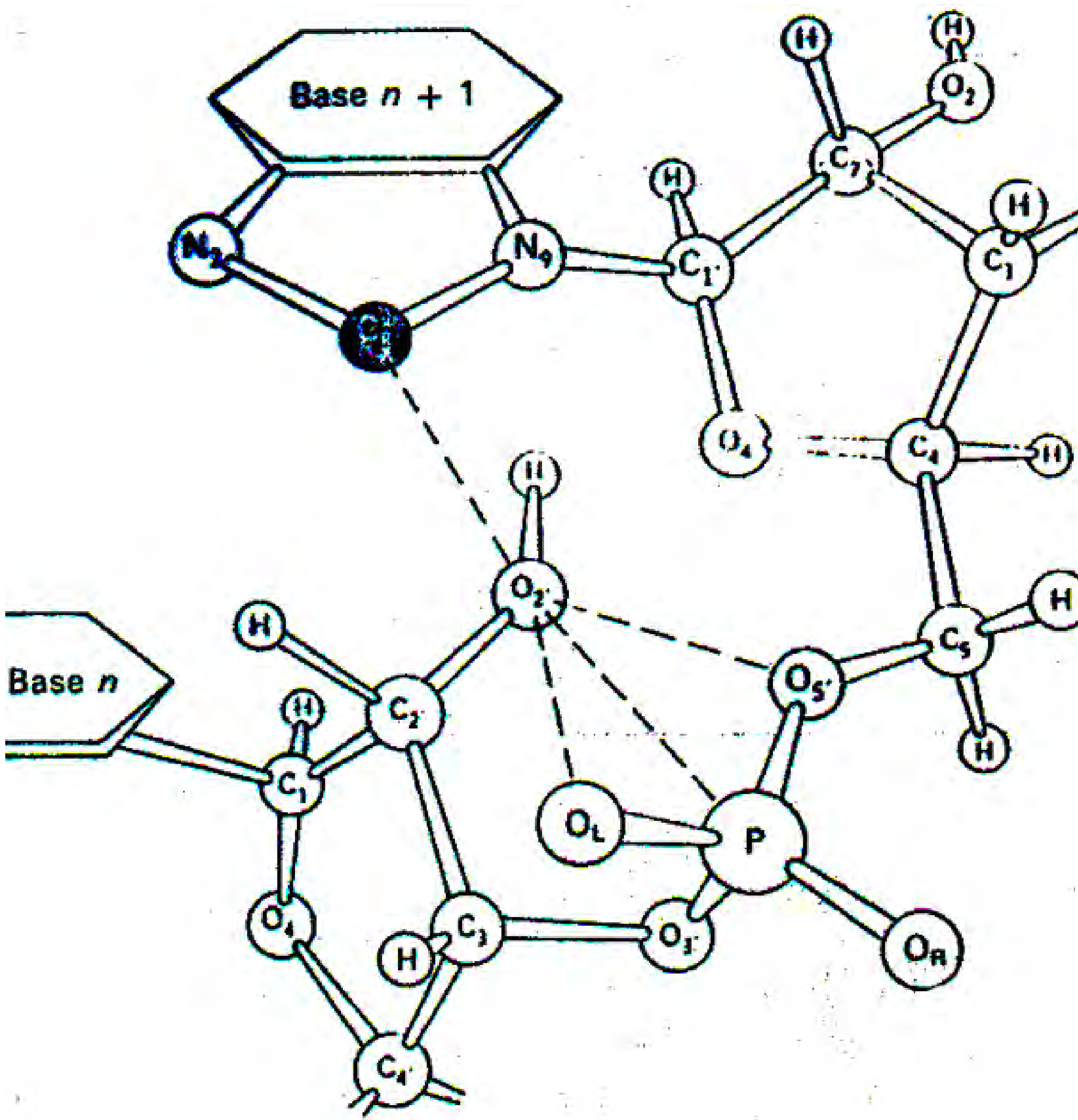
C3’-Endo

C2’-Endo

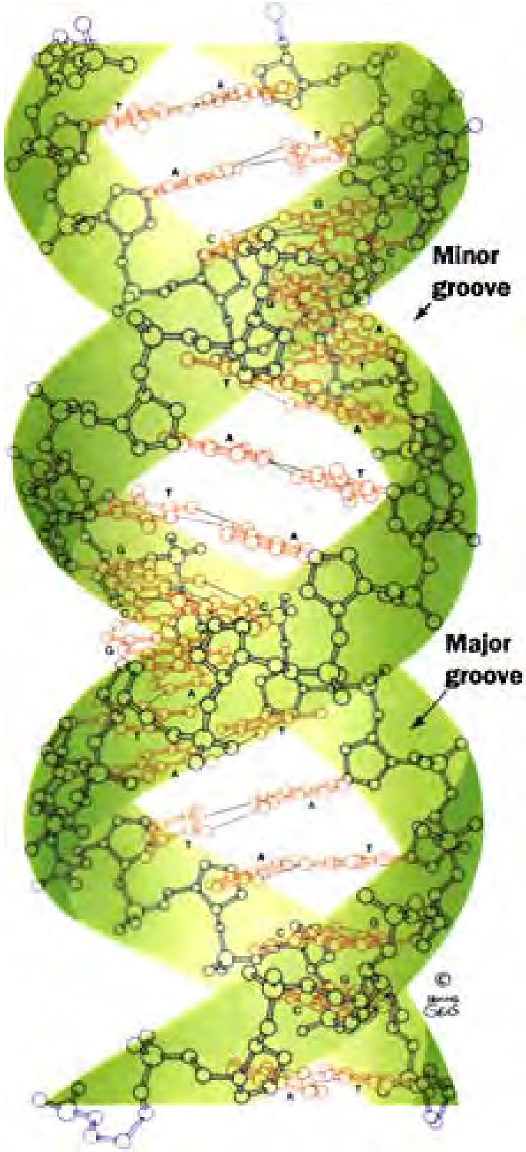
Zašto riboza ne može u B-DNK….?

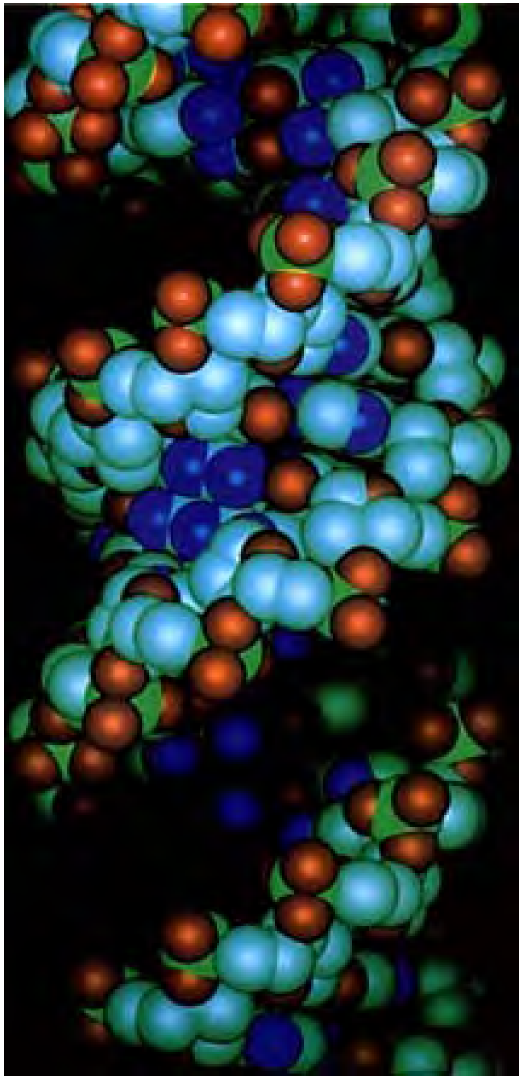


Zašto riboza ne može u B-DNK?

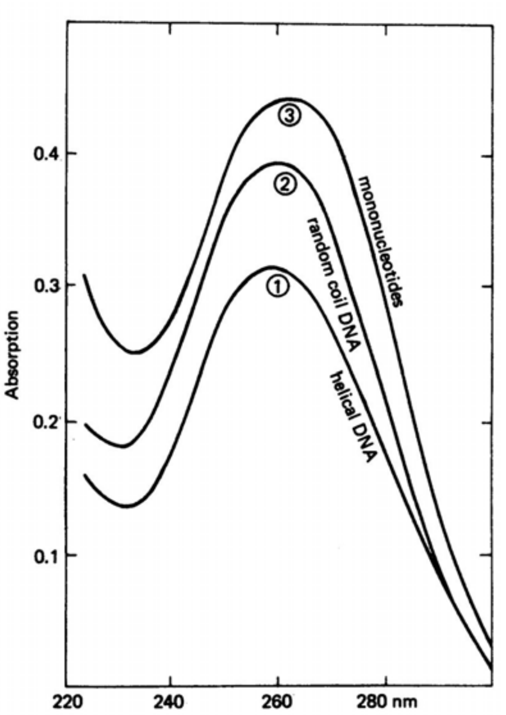


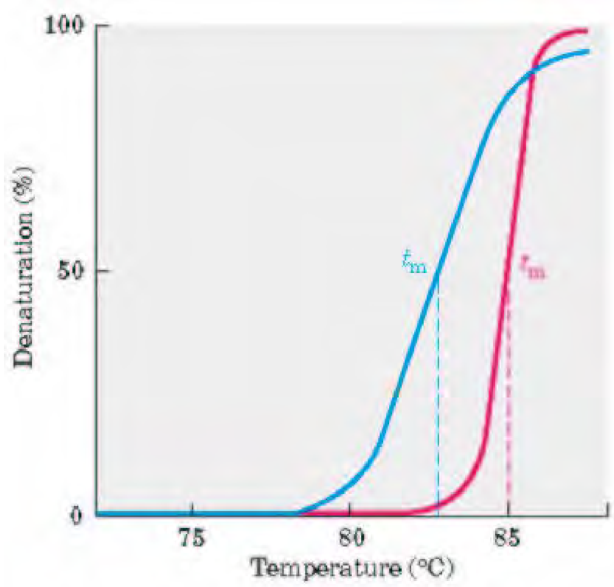
.....a može u A-DNK

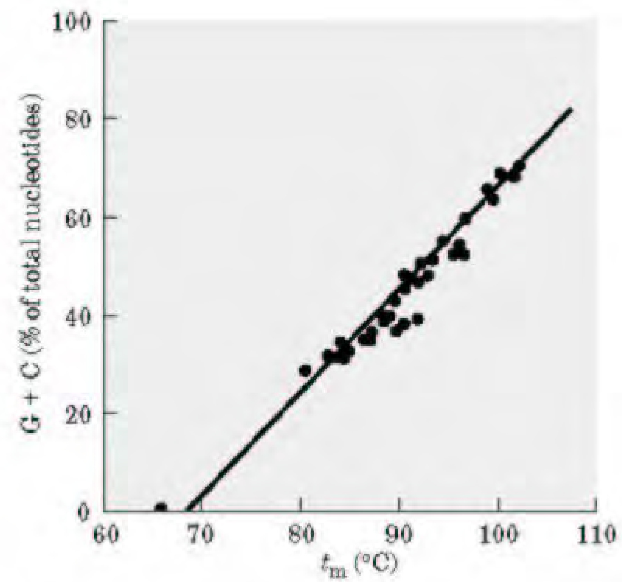




Konformacioni prelazi (denaturacija/renaturacija) DNK



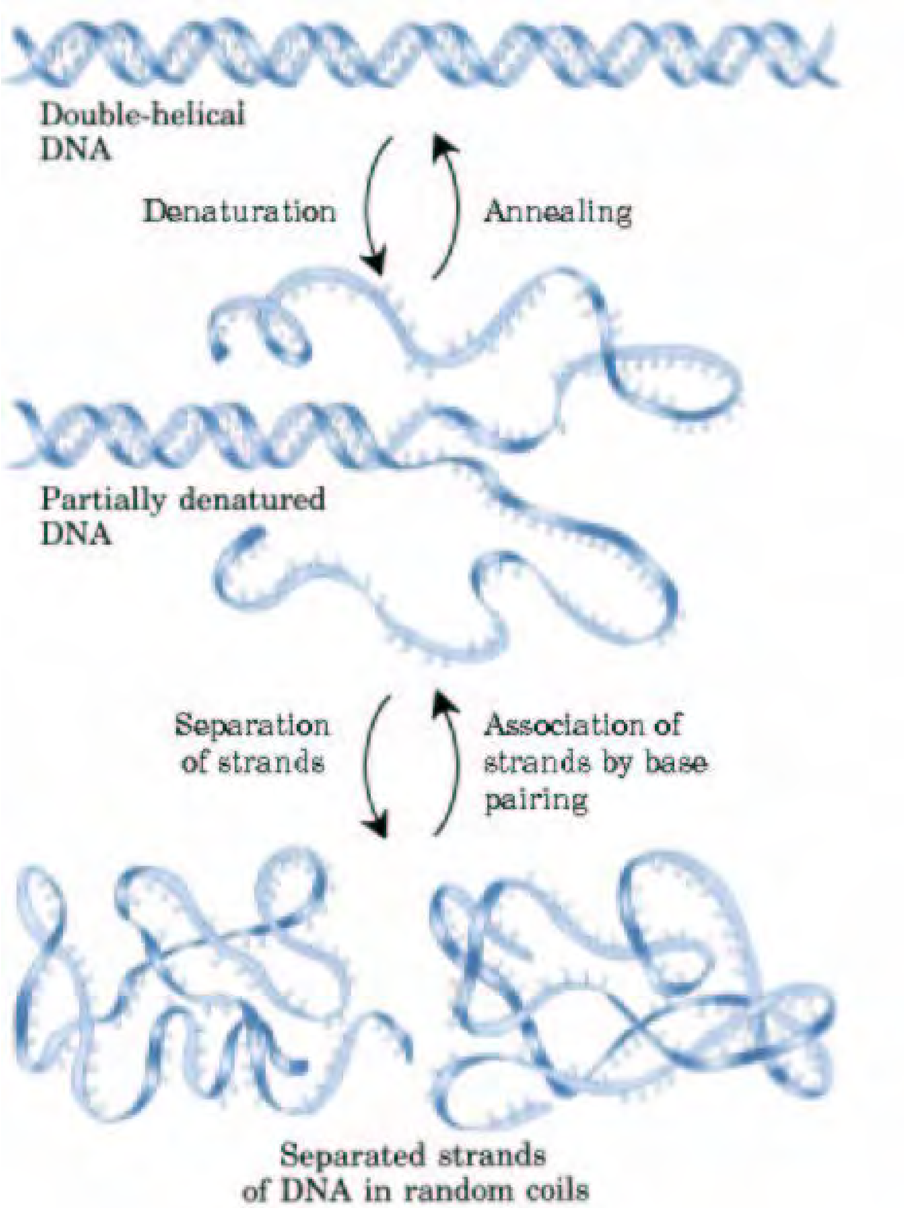




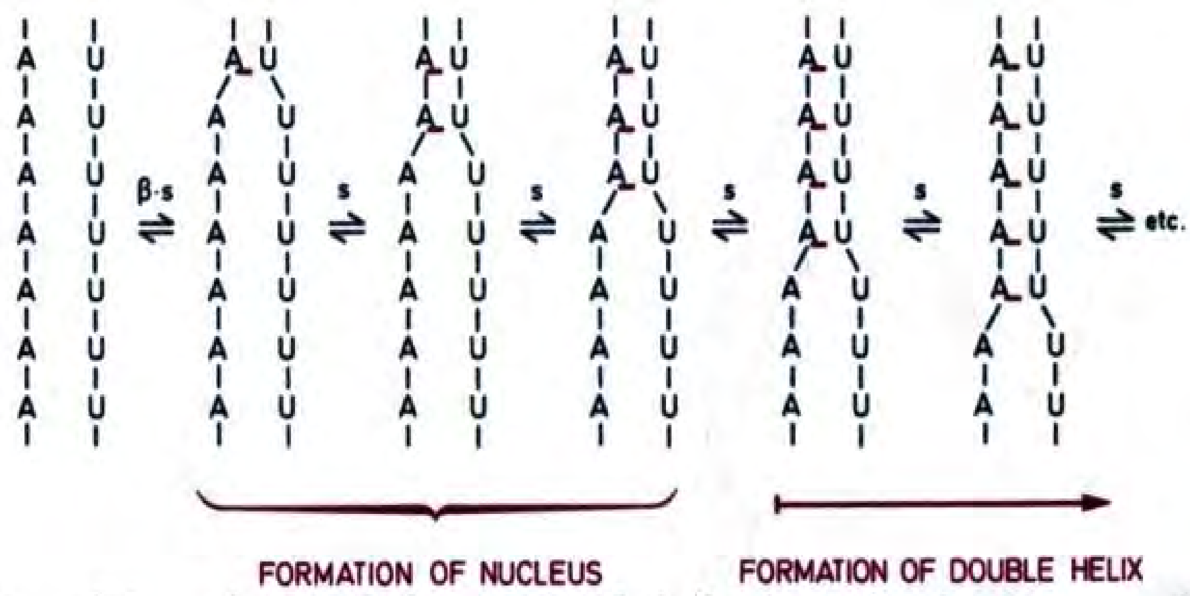
Tm se uzima kao mera stabilnosti date DNK

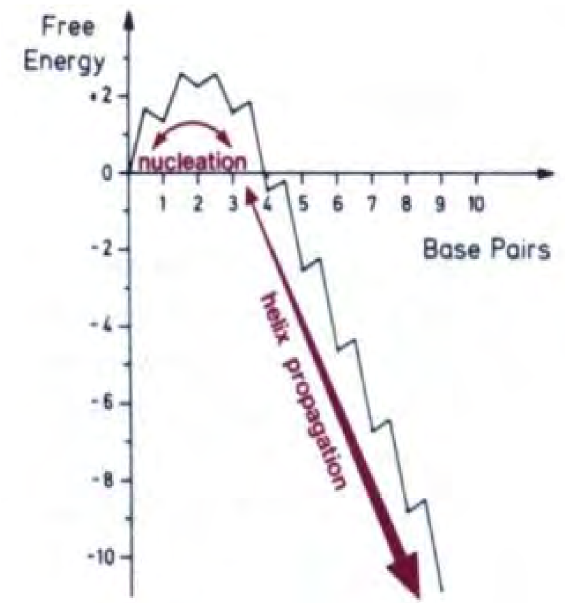
Komentar o mehanizmu prelaza!

Denaturacija DNK



Kooperativni mehanizam denaturacije/renaturacije DNK

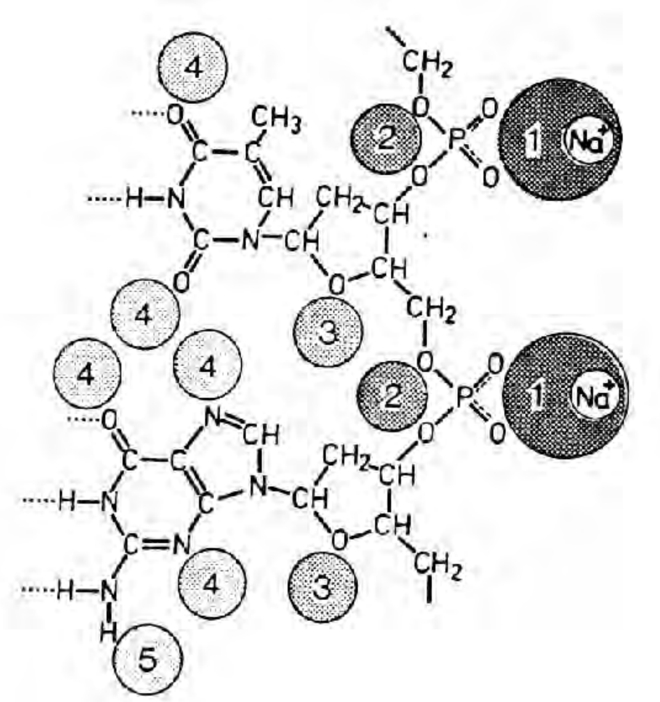




Interakcije DNK sa vodom: voda četvrta komponenta strukture DNK!

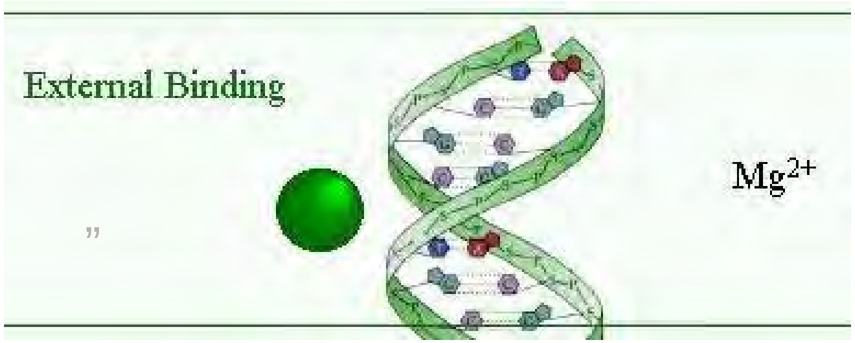
Primarna hidrataciona ljuska:
-nije propustljiva za katjone
-20 molekula H_2O/nukleotid u dvostrukom heliksu DNA:
- (5-6 H_2O hidratiše fosfatne kiseonike,
- 5-6 H_2O hidratiše fosfodiesterske O i furanozne O´ ,
- 8-9 H_2O hidratiše funkcionalne grupe u bazama (amino, imino, keto)

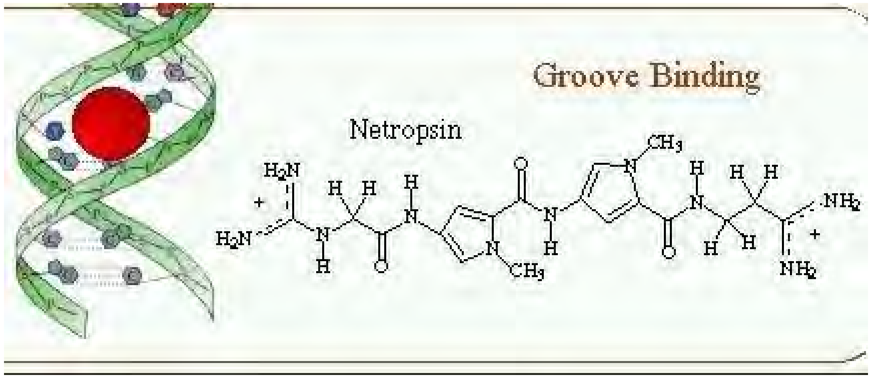
Sekundarna hidrataciona ljuska:
skoro se ne razlikuje od okolne vode, propustljiva je za jone



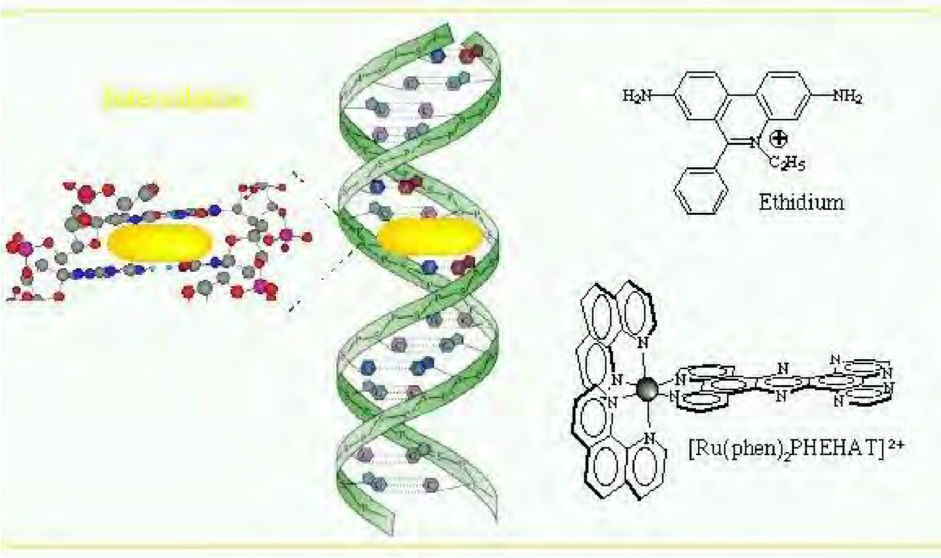
Preferentna mesta za vezivanje vode

Vezivanja liganada za DNK



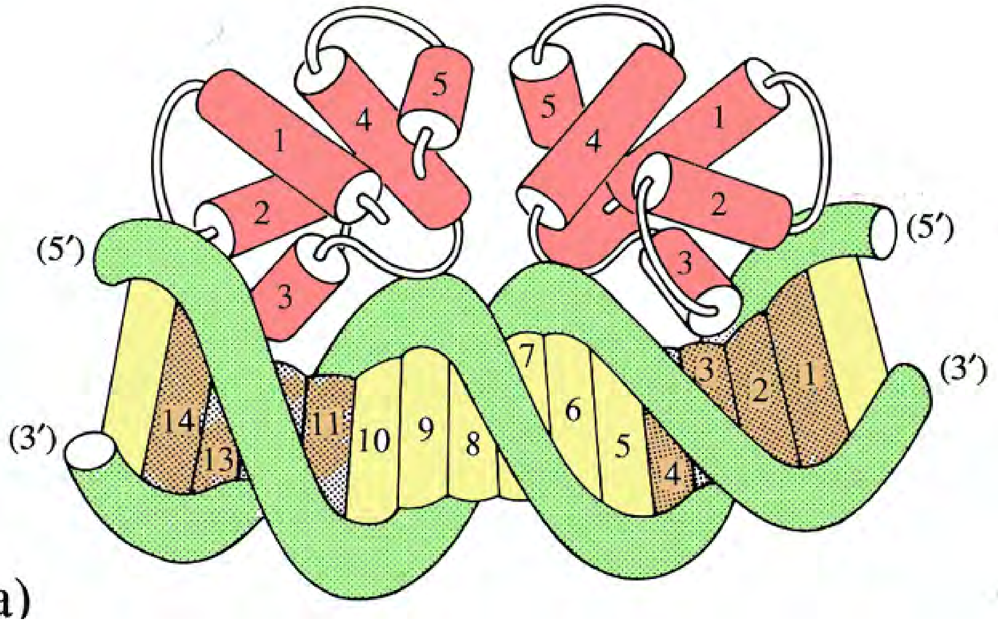


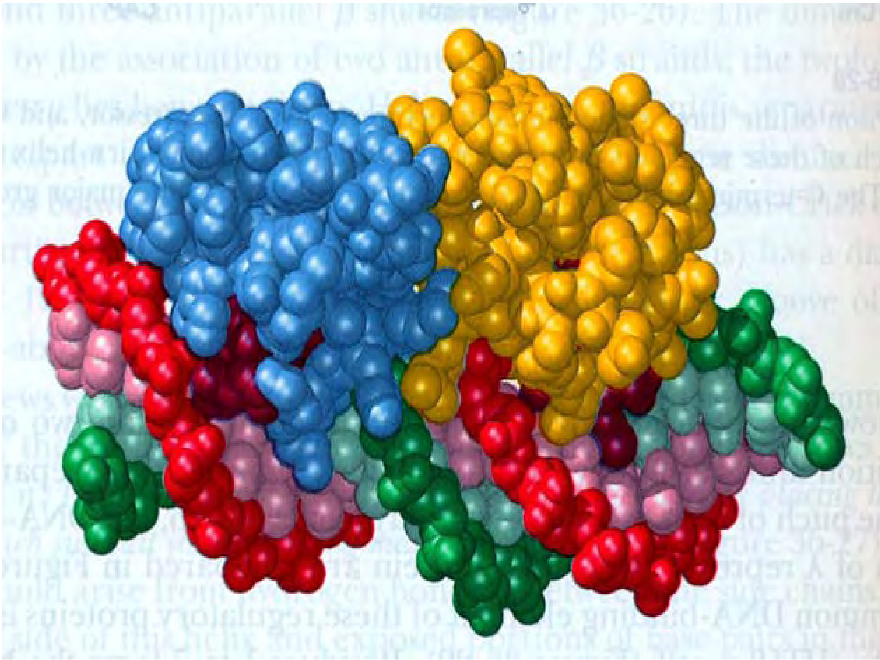
Vezivanje u brazdi



„Interkalacija“ – umetanje planarnih aromatičnih sistema medju parove baza

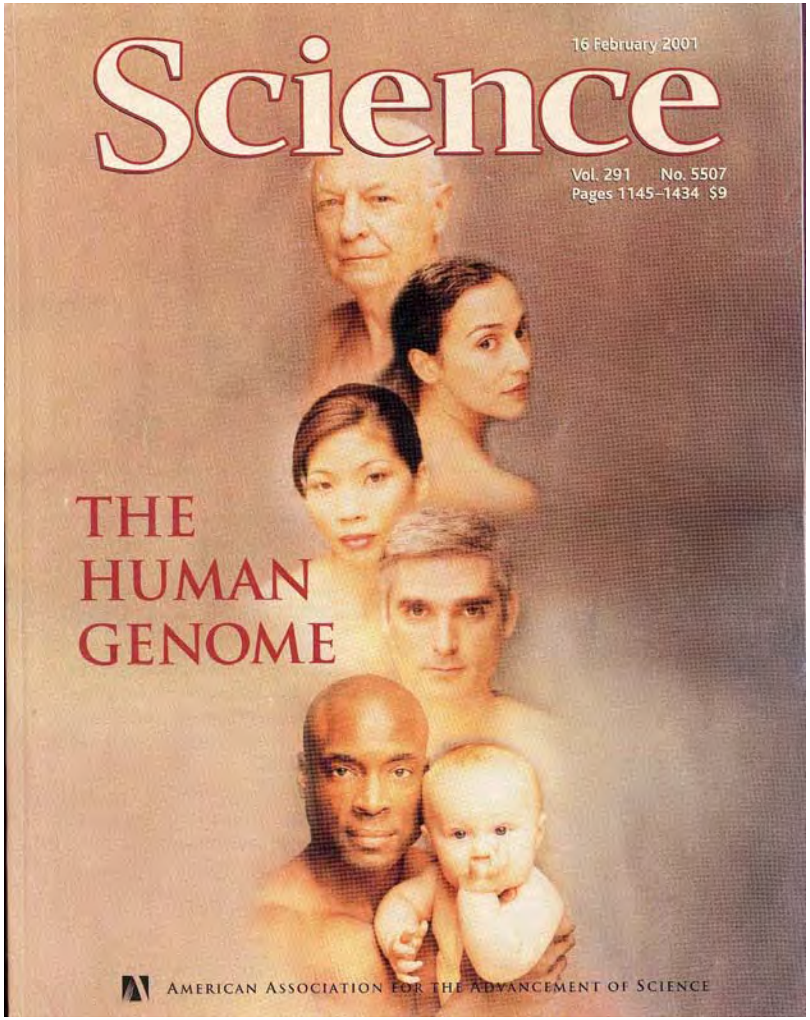
Vezivanje proteina za DNK





434 represorni protein

Projekat humanog genoma (HGP)

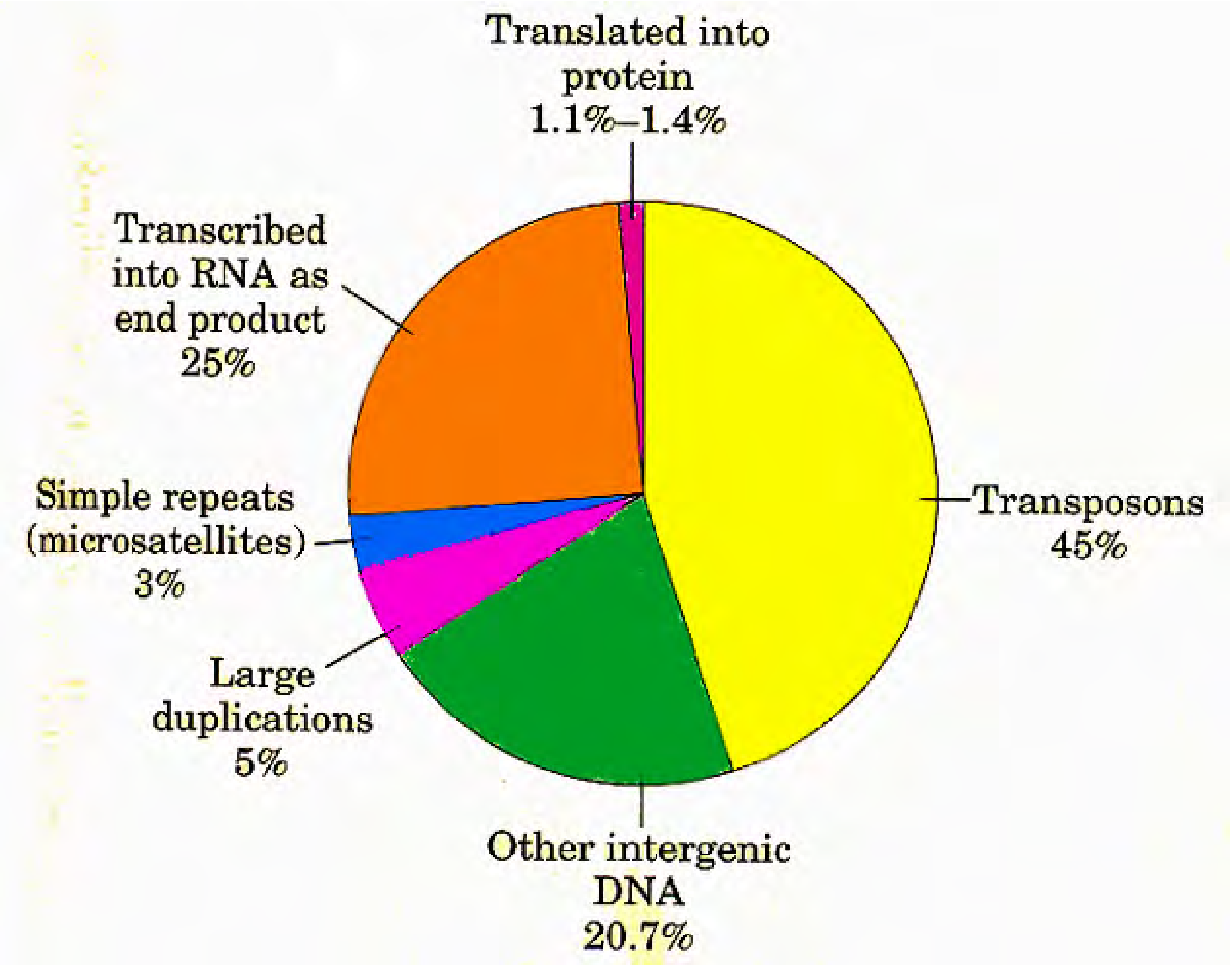


- Šta su uradili?
- Zašto su to uradili?
- Šta to znači za čovečanstvo?

Ciljevi HGP

- Napraviti fizičku mapu 24 humana hromozoma
- Identifikovati celokupan set gena & mapirati ih na njihovim hromozomima
- Odrediti sekvencu procenjenih 3 milijarde parova baza (genom)
- Analizirati genetske varijacije među ljudima
- Mapirati i sekvencirati genome model-organizama

Humani genom



Variation between individuals

- your DNA is 99.9% the same as any other human on Earth
- humans & chimpanzees share about 98.5% of their genes
- banana DNA & human DNA are about 50% the same

Primena u medicini i bilogiji

- Genetske bolesti
- Mete (“targets”) za lekove
- Fundamentalna biologija

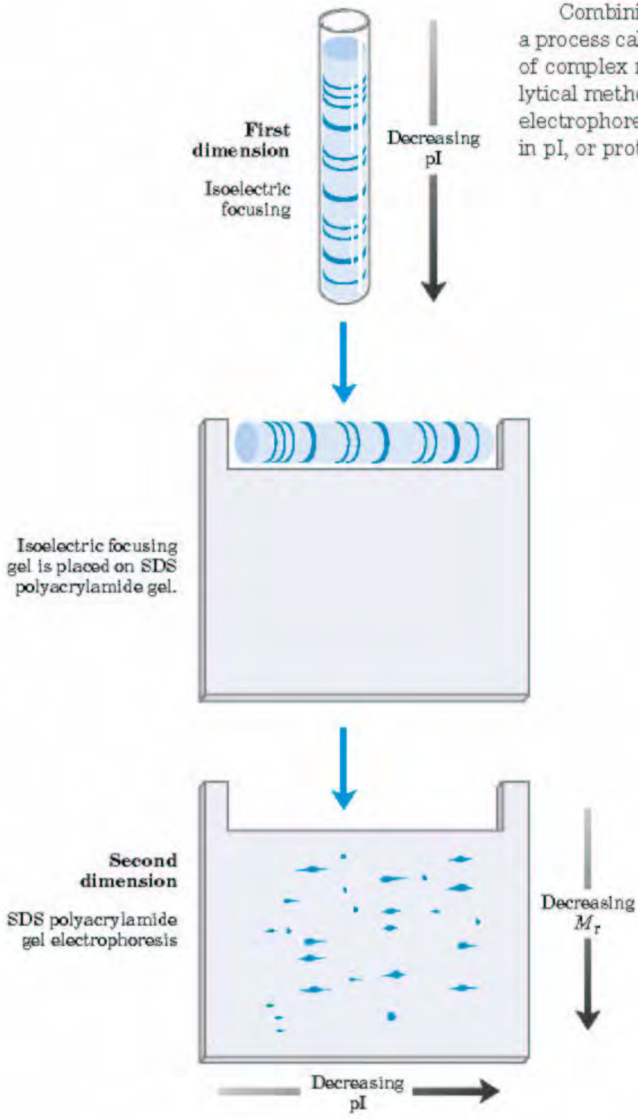
Humani proteom

- Analogija sa genomom:

- Svi eksprimirani proteini u ćeliji (organizmu):
  - identifikacija
  - modifikacije
  - količina
  - lokalizacija
  - međusobne interakcije

2D elektroforeza može da razdvoji više od 1000 proteina

Combining isoelectric focusing and SDS electrophoresis sequentially in a process called **two-dimensional electrophoresis** permits the resolution of complex mixtures of proteins (Fig. 5–22). This is a more sensitive analytical method than either electrophoretic method alone. Two-dimensional electrophoresis separates proteins of identical molecular weight that differ in pI, or proteins with similar pI values but different molecular weights.



(a)

(b)

figure 5–22
Two-dimensional electrophoresis. (a) Proteins are first separated by isoelectric focusing in a cylindrical gel. The gel is then laid horizontally on a second, slab-shaped gel, and the proteins are separated by SDS polyacrylamide gel electrophoresis. Horizontal separation reflects differences in pI; vertical separation reflects differences in molecular weight. **(b)** More than 1,000 different proteins from *E. coli* can be resolved using this technique.