Nukleinske kiseline

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

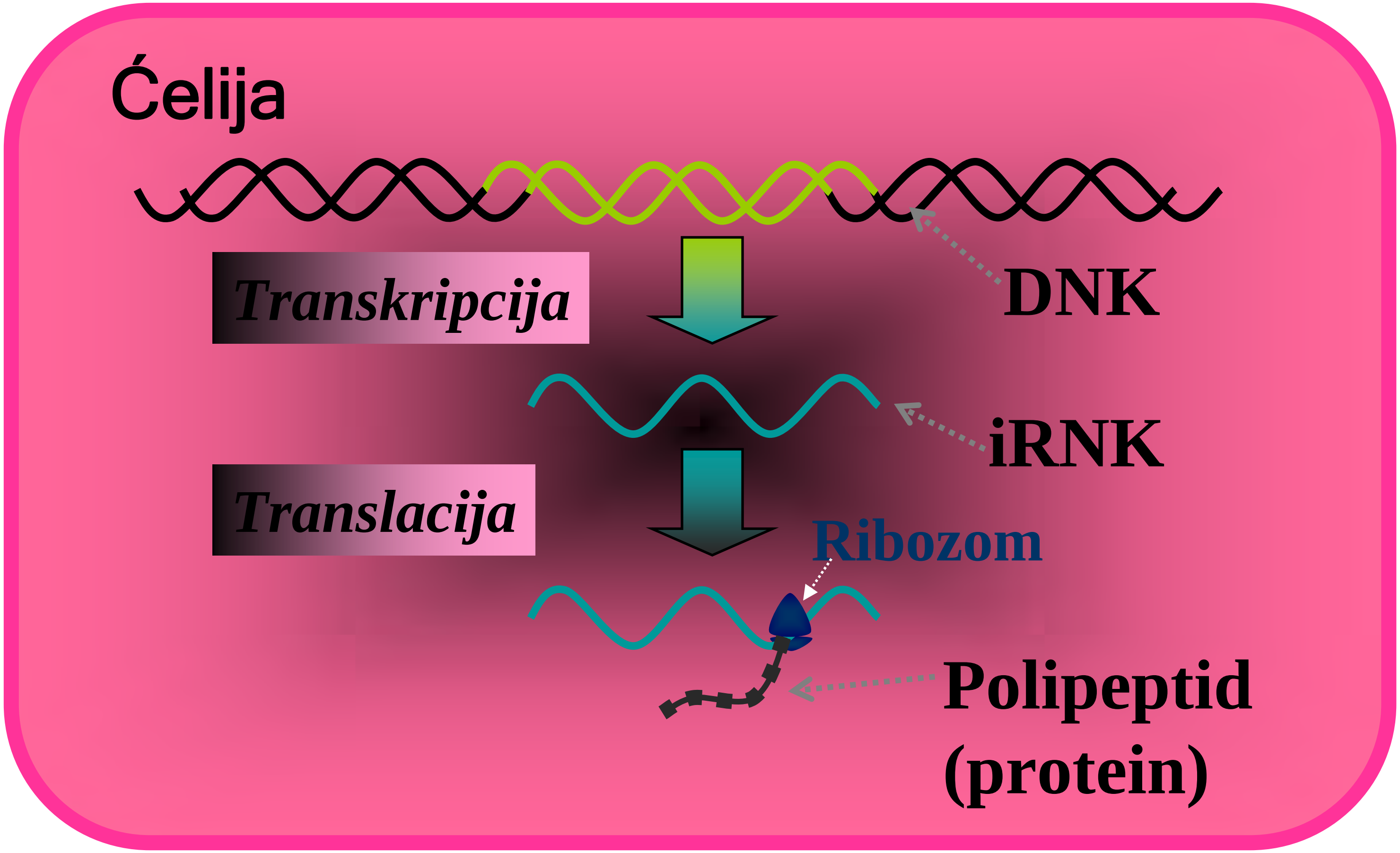
Sadržaj predavanja

Odnos proteina i nukleinskih kiselina

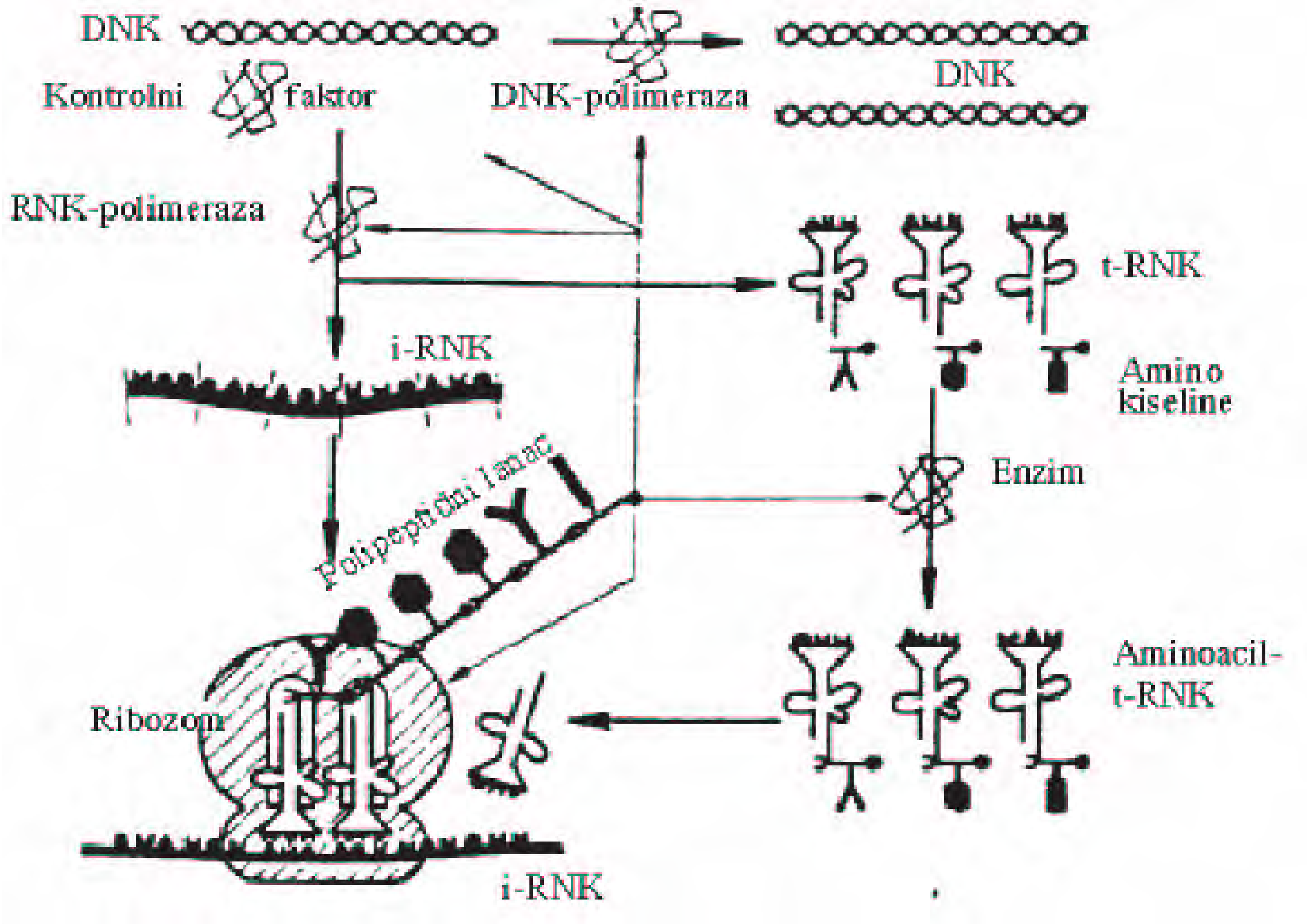
– Otkriće DNK
– Osnovna hemijska struktura DNK
– Otkriće DNK kao nosioca genetske informacije

- Dimenzije DNK i organizacija u ćeliji
- Kako izgleda (preparat) DNK?
- Zašto su nukelinske baze izabrane za čuvanje i prenos genetske informacije?

“Centralna dogma molekularne biologije”



Biosintetički ciklus samoreprodukcije ćelije



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

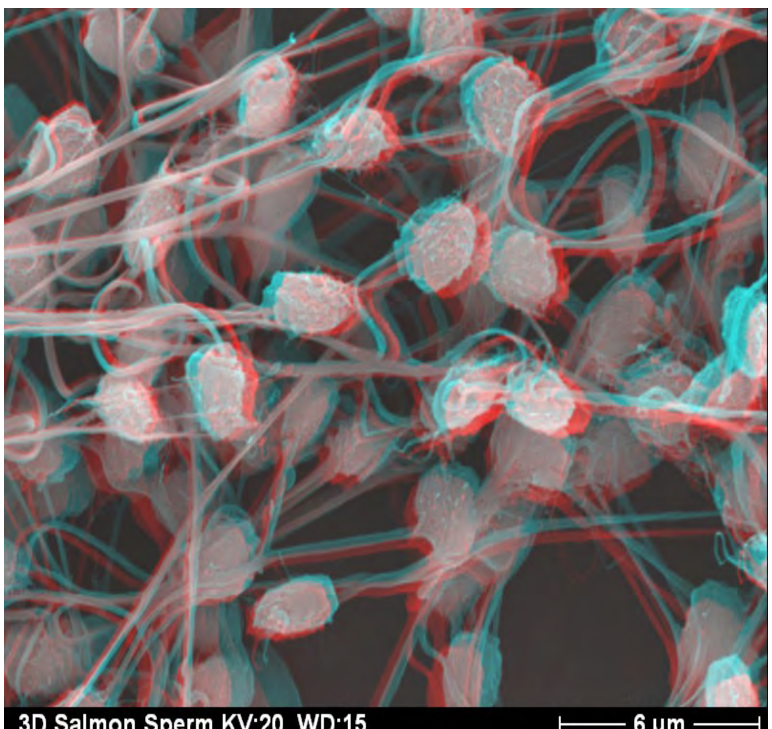
Zašto se time bavio?

Očekivao je da će tu naći nešto interesantno!

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

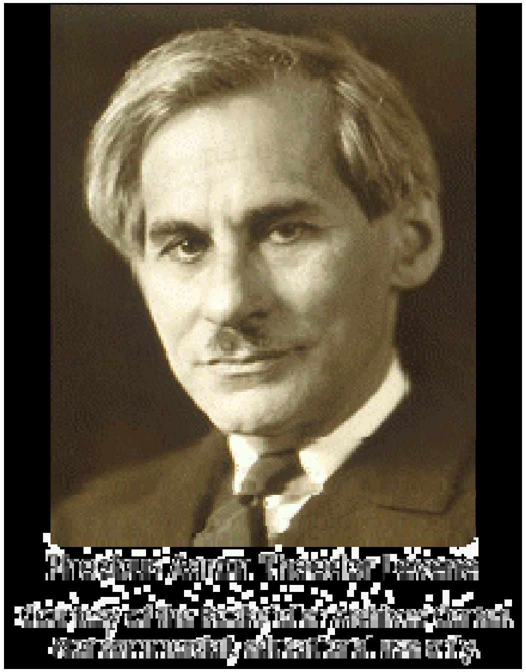
- Supstanca je bila toliko neobična da je Hoppe-Seyler (Miescherov profesor i “otac biohemije”) ponovio postupak izolovanjem DNK iz sperme lososa! pre nego što je dozvolio da se rad Miescher-a publikuje.



Određivanje hemijske strukture nukleinskih kiselina

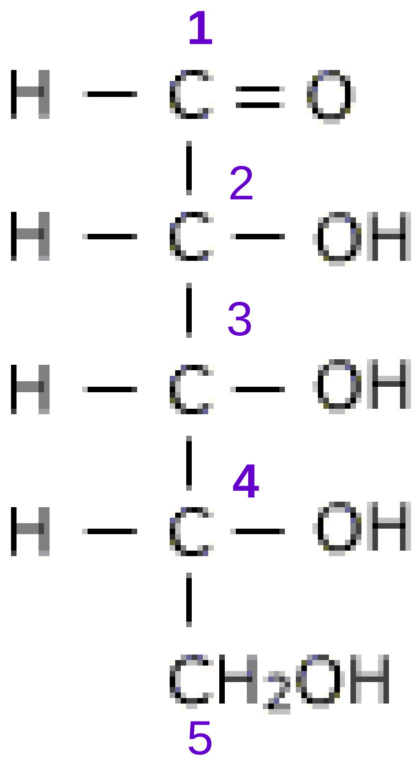
- Istraživanja je započeo Levene (1909) određivanjem strukture nukleotida u:

- timusnoj NK (otporna na alkalnu hidrolizu): DNK
- i biljnoj NK (podložna alkalnoj hidrolizi): RNK

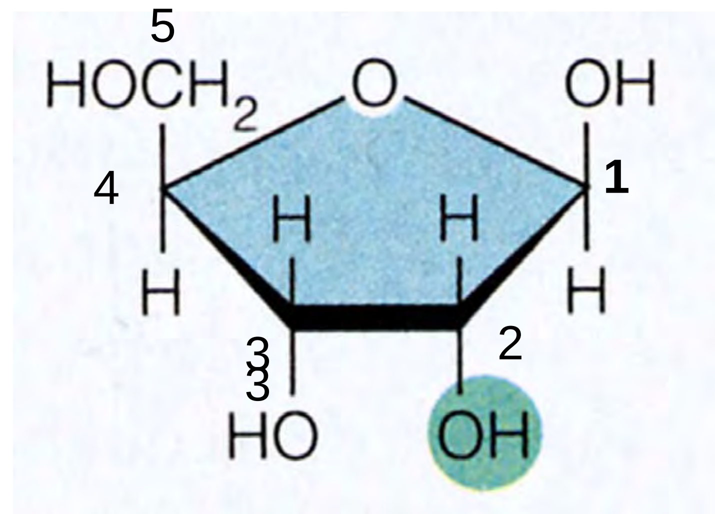


Phoebus Levene

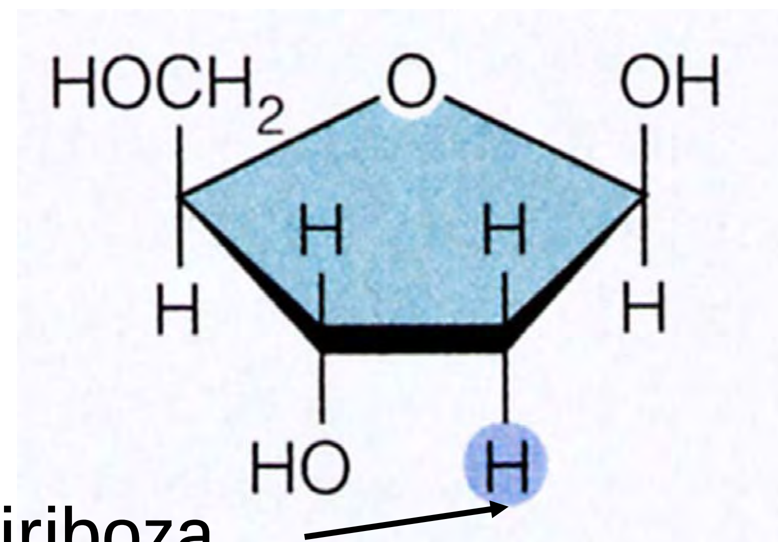
Radio sa Albrecht Kosselom i Emil Fischerom, ekspertima za biomakromolekule s kraja 19/početka 20 veka



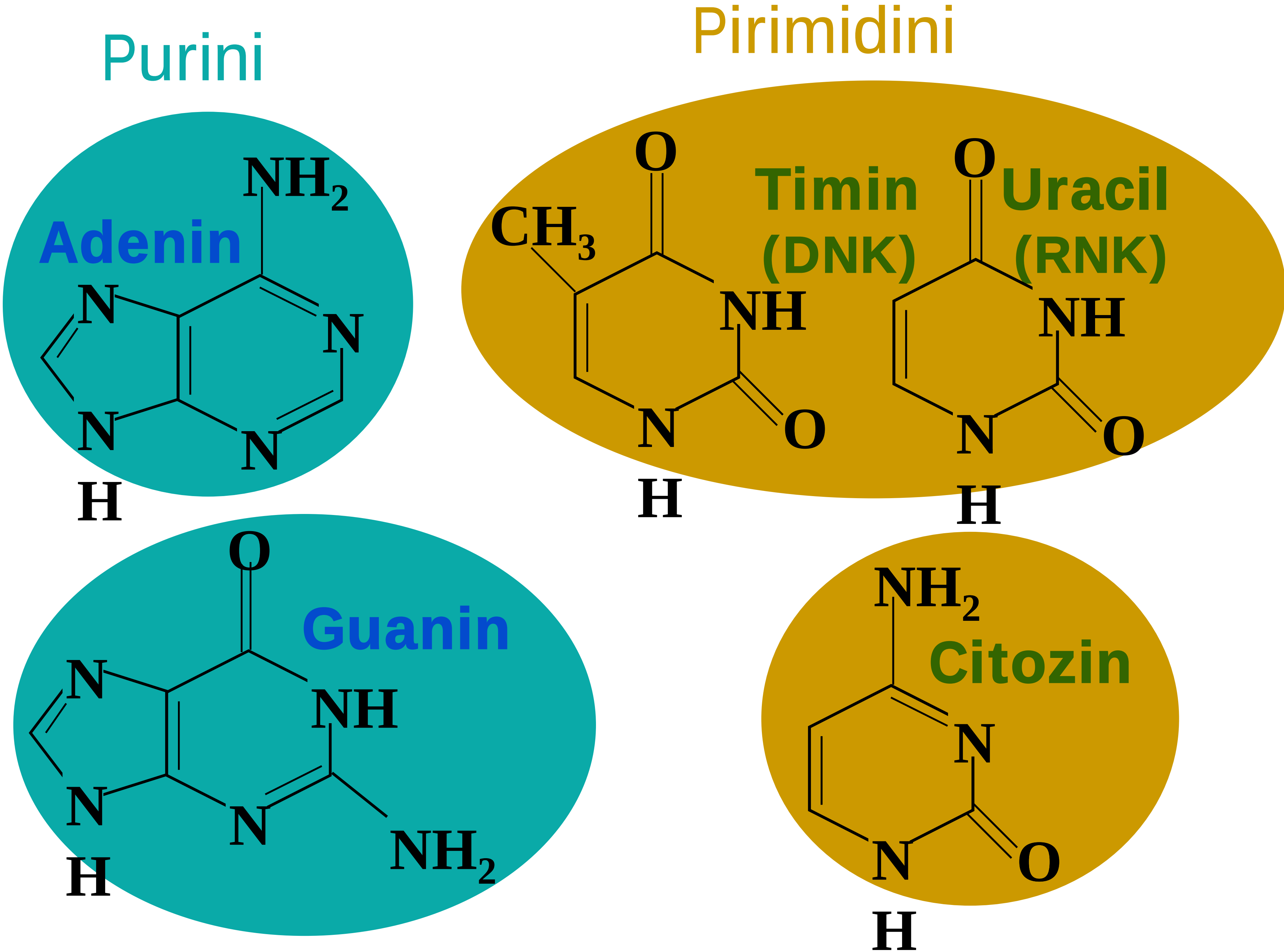
D - Ribose



Riboza



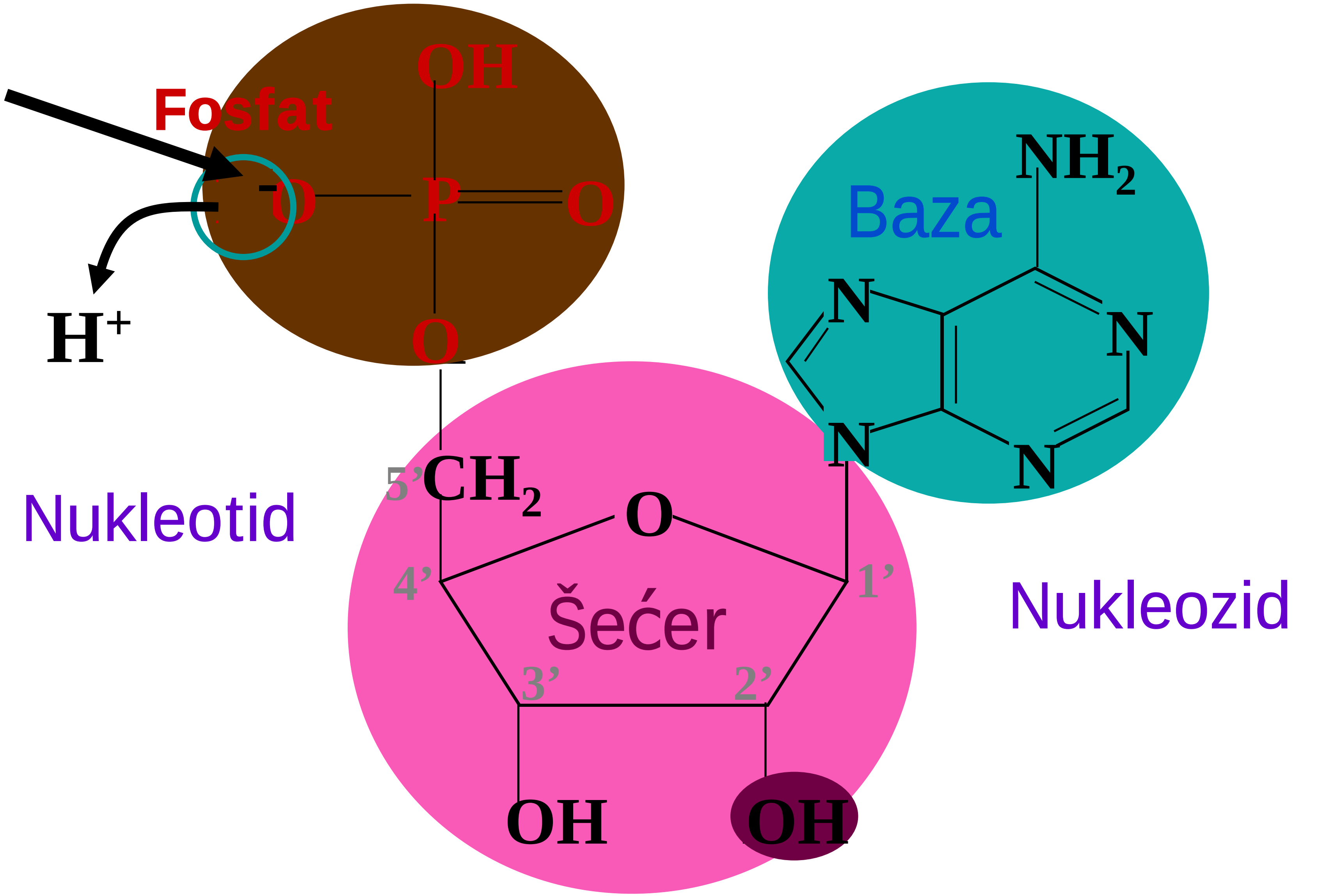
Dezoksiriboza



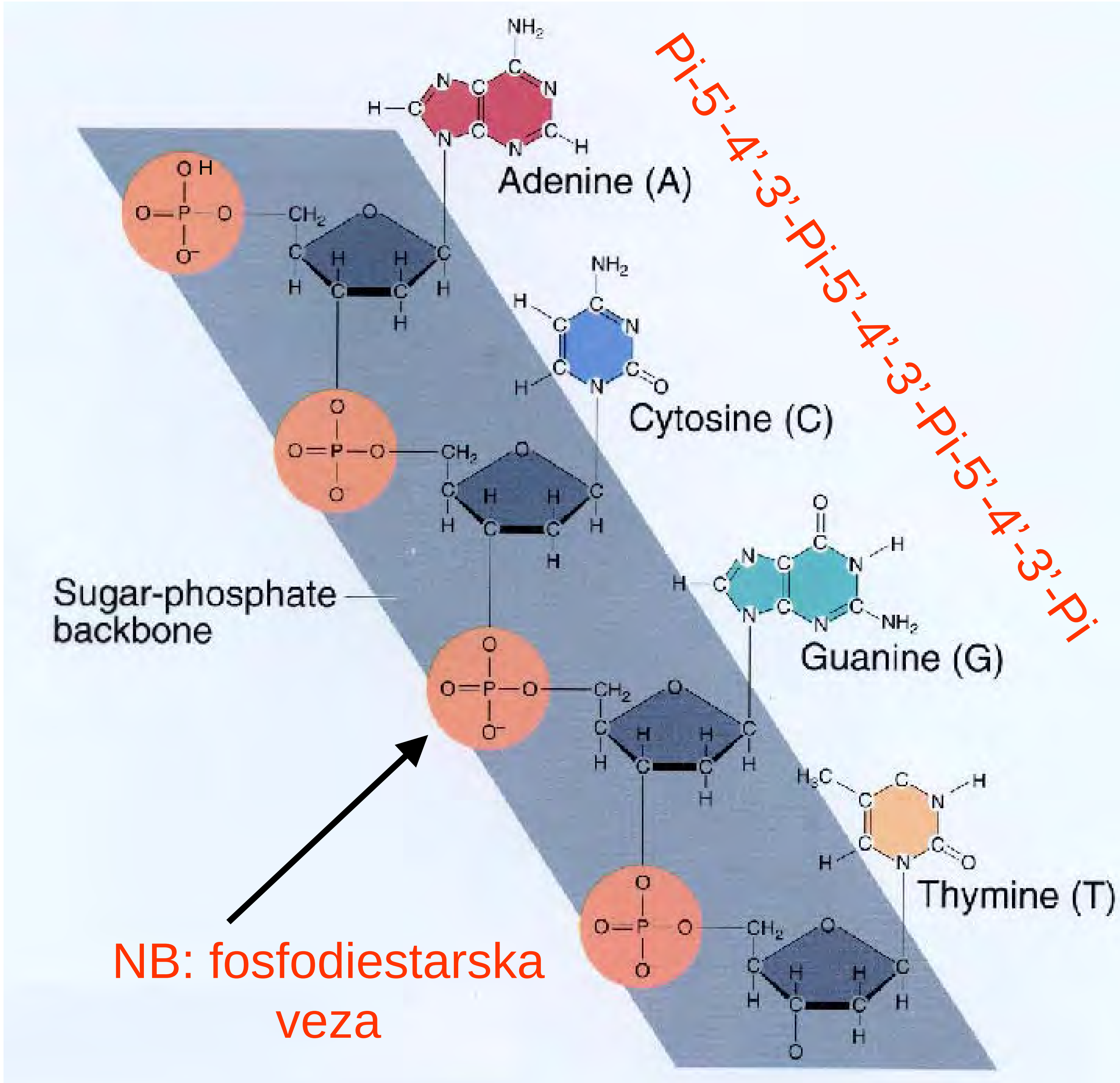
Purini
Pirimidini
Adenin
Guanin
Timin
(DNK)
Uracil
(RNK)
Citozin

Nukleotid

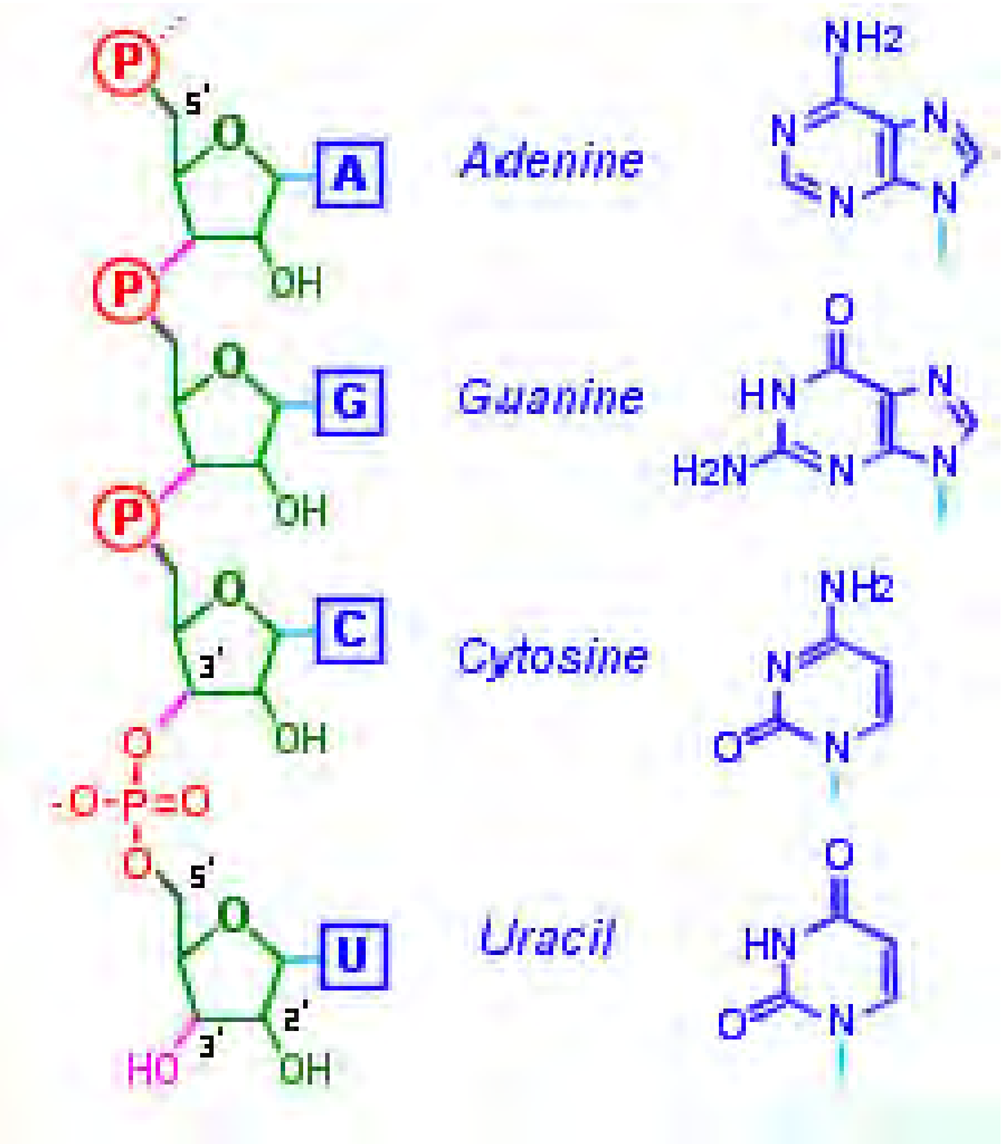
Adenozin monofosfat (AMP)



Šećer-fosfatna kičma

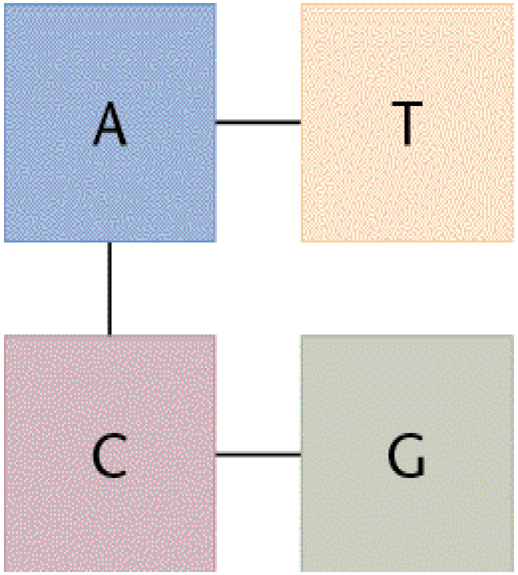


RNK vs. DNK



P
5'
O
A
Adenine
NH2
N
N
N
N
OH
P
O
G
Guanine
O
HN
N
H2N
N
N
OH
P
O
C
3'
Cytosine
NH2
N
O
N
OH
O
-O-P=O
O
5'
O
U
Uracil
O
HN
O
N
2'
3'
HO
OH

Primenom metoda koje su bile na raspolaganju početkom 20. veka zaključeno je da se četiri komponente DNK nalaze u približno istom odnosu što je rezultovalo u hipotezi o TETRANUKLEOTIDNOJ strukturi DNK.....



(Klug & Cummings 1997)

Tetranukleotidna struktura DNK je bila u suprotnosti sa određivanjem molekulske mase DNK ~ 10^6 Da!!!!

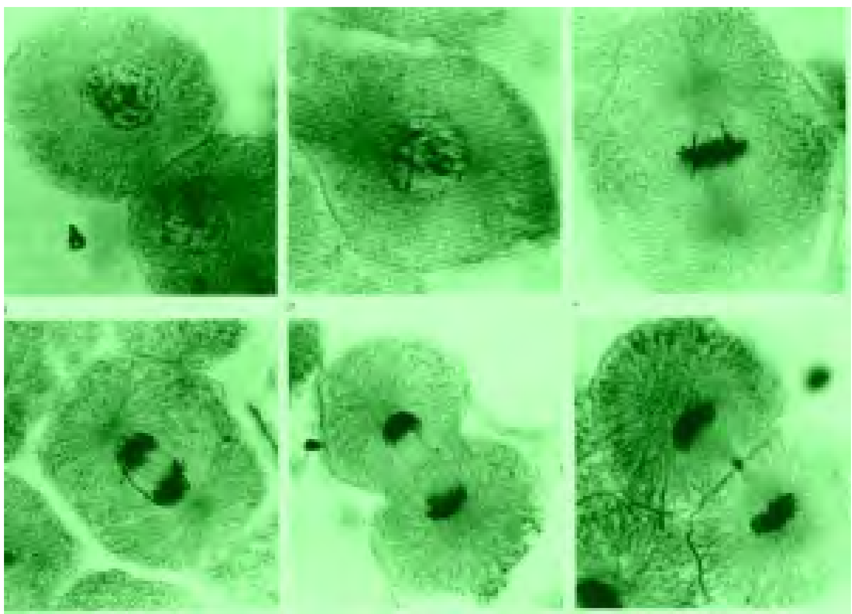
Eksperimentalni dokazi da je DNK (a ne proteini!) nosilac genetske informacije:

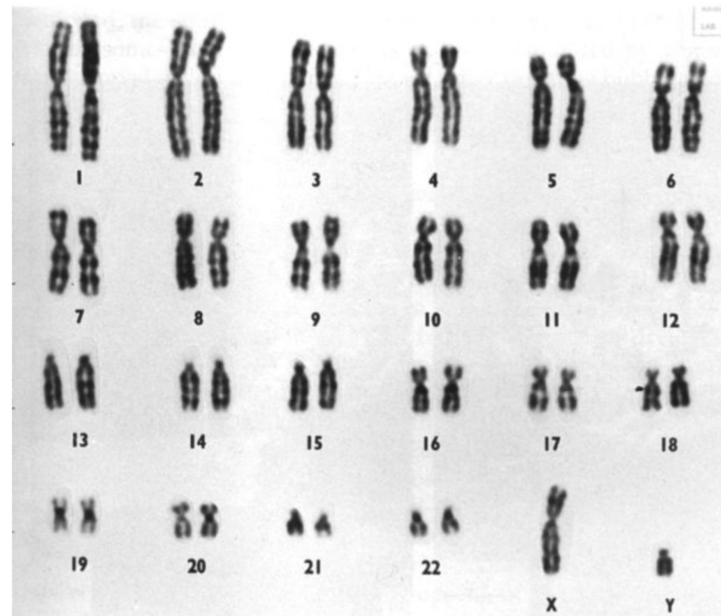
- Otkriće “genetske transformacije” (Frederick Griffith, 1928. g.)

- DNK je “transformišući princip” (Oswald T. Avery i Maclyn McCarty,rane 1940.g.)

- Definitivan dokaz da je DNK nosilac genetske informacije (Alfred Hershey i Martha Chase, 1952. g)

- Otkriće anilinskih boja izazvalo veliki interes kod biologa za bojenje ćelijskih struktura!!!!
- Walter Flemming otkrio (1870 g), na osnovu specifičnog bojenja, hromozome koji se mogu videti samo tokom deobe ćelije; povezao otkriće sa zakonima genetike (Mendel)!

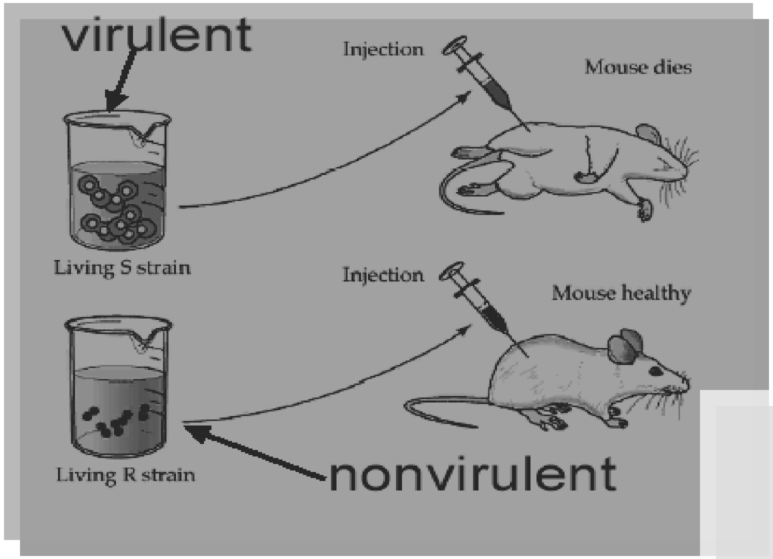


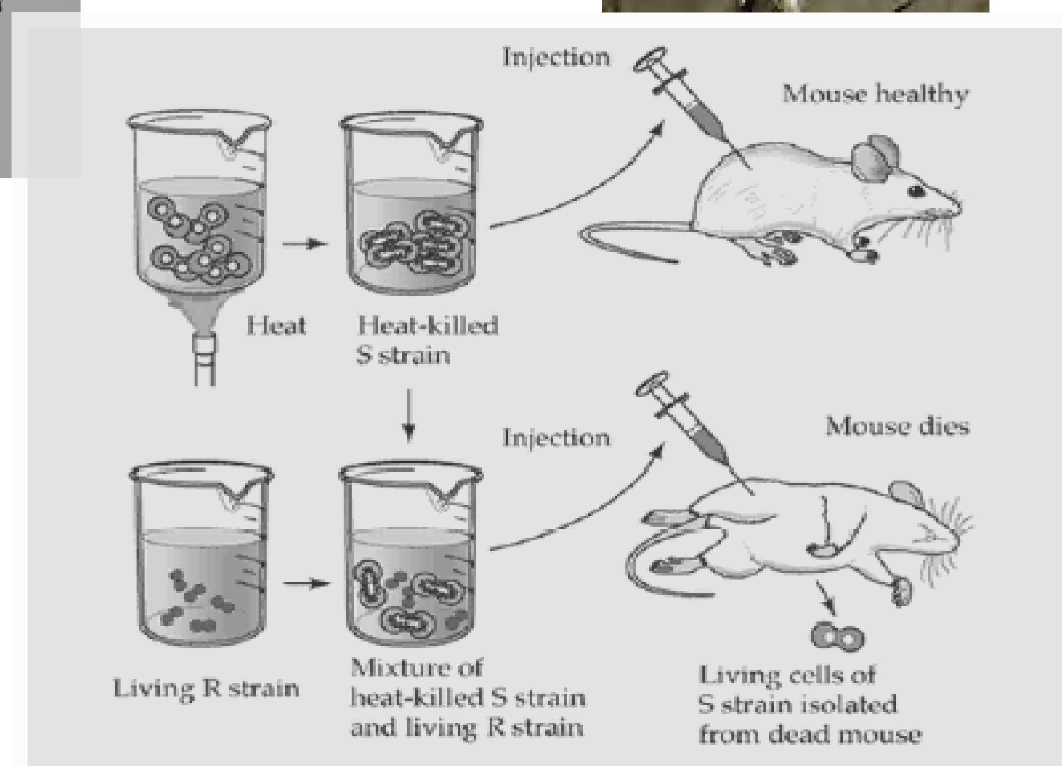


Fleming je spekulisao da je hromozom = nuclein
E.B. Wilson dokazao (1900 g.) hromatin = nuclein
1920: **Nukleinske kiseline su glavna komponenta hromozoma!!!!**

Otkrića ispred svog vremena!!!

Otkriće “genetske transformacije” (Frederick Griffith, 1928. g.)

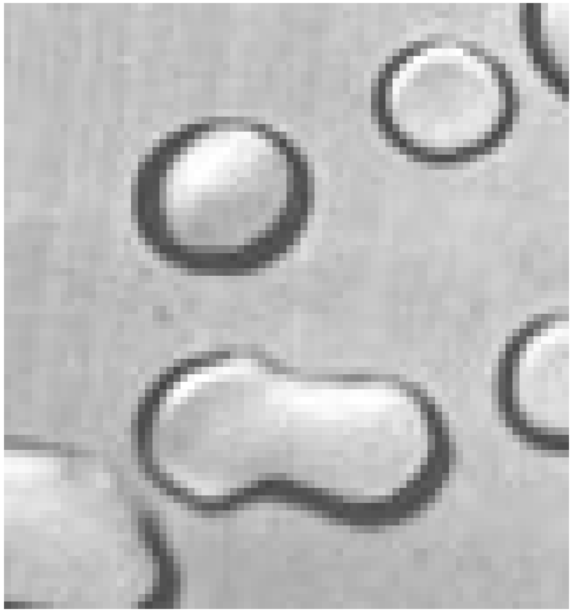




Pneumococcus

-**virulentni S** soj izaziva upalu pluća;
-**nevirulentni R** soj ne izaziva upalu pluća

“Transformišući princip” je DNK !!! (Oswald T. Avery i Maclyn McCarty,rane 1940.g.)



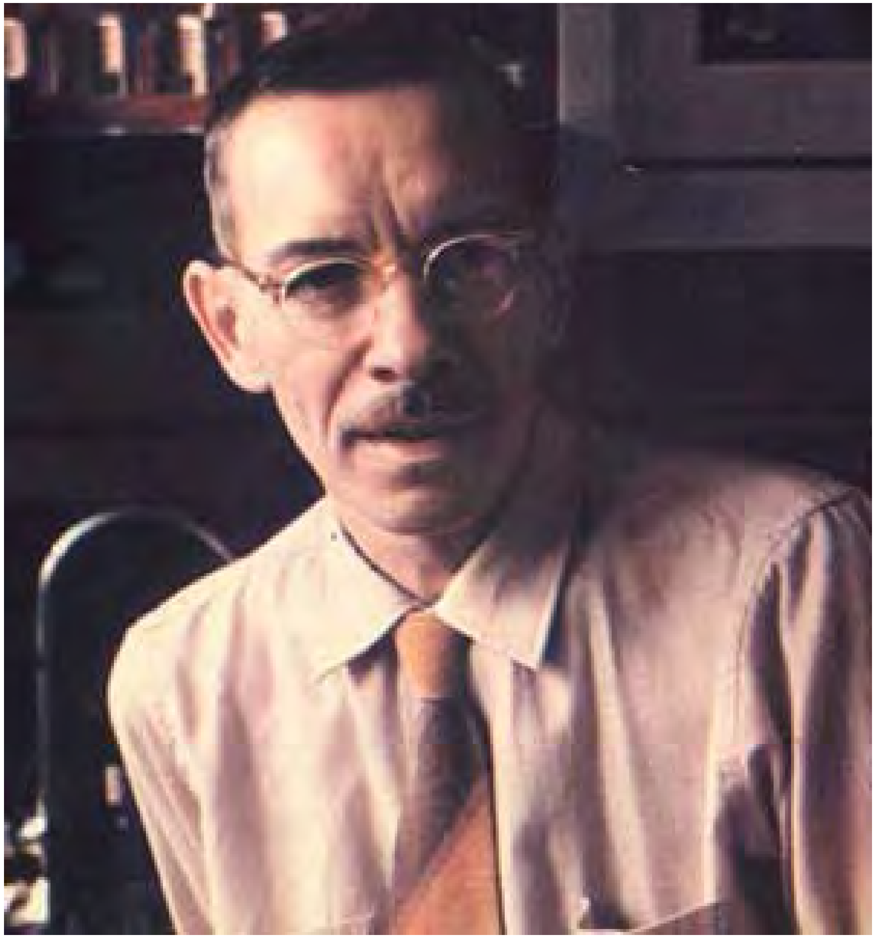
Ekstrakti S bakterija ubijenih toplotom sadržavali su proteine, RNK i DNK.

Koja od ovih supstanci izaziva transformaciju?

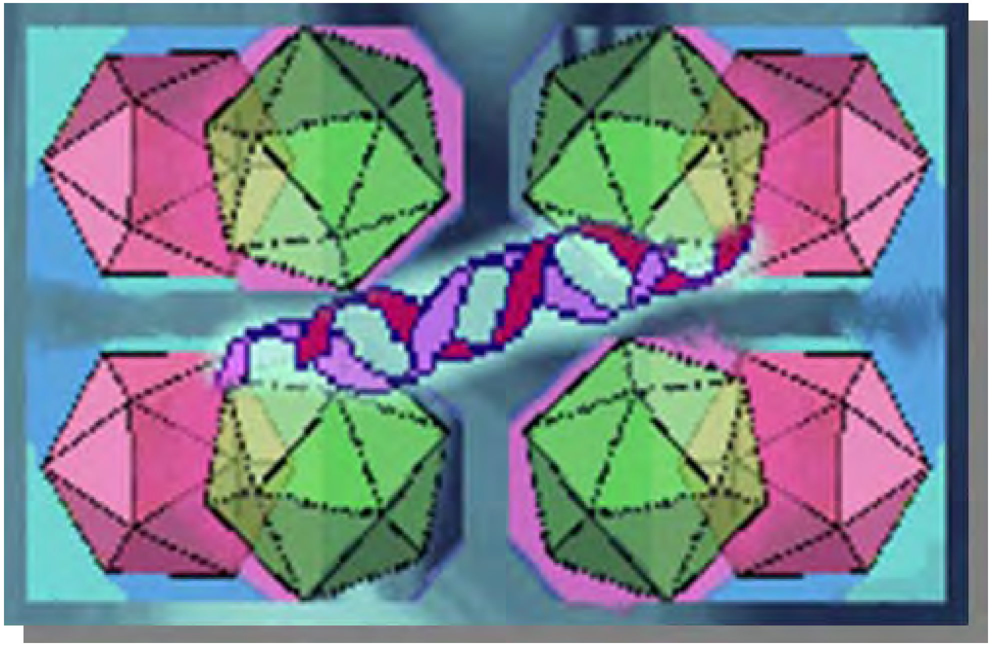
Odlučili su da primene proces eliminacije:...

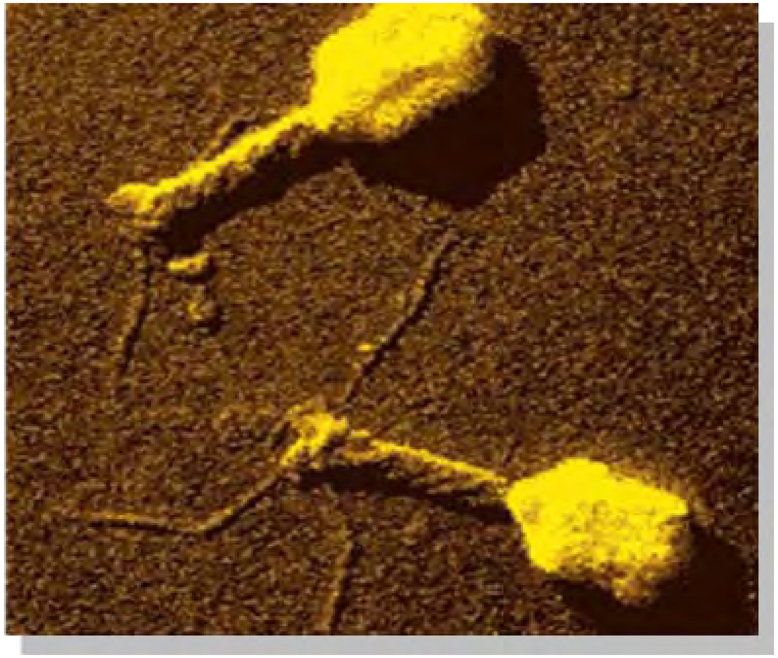
- Odstranjivanje proteina i lipida (ekstrakcijom) ne utiče na aktivnost;
- U ekstrakt su dodali:
  - Proteazu (da se razore proteini)
  - RNAzu (da se razori RNK)
  - DNAzu (da se razori DNK)
- Elementarni sastav prečišćenog “transformišućeg principa” odgovara DNK;
- “Transformišući princip” po osobinama (spektri, ultracentrifuga, viskoznost, elektroforeza) odgovara DNK.
- Za transformaciju je isključivo odgovorna DNK!!!.....
- ....otkriće prošlo nezapaženo!!!!!

Amerikanci, Alfred Hershey i Martha Chase (1952) su definitivno dokazali da je DNK nosilac genetske informacije

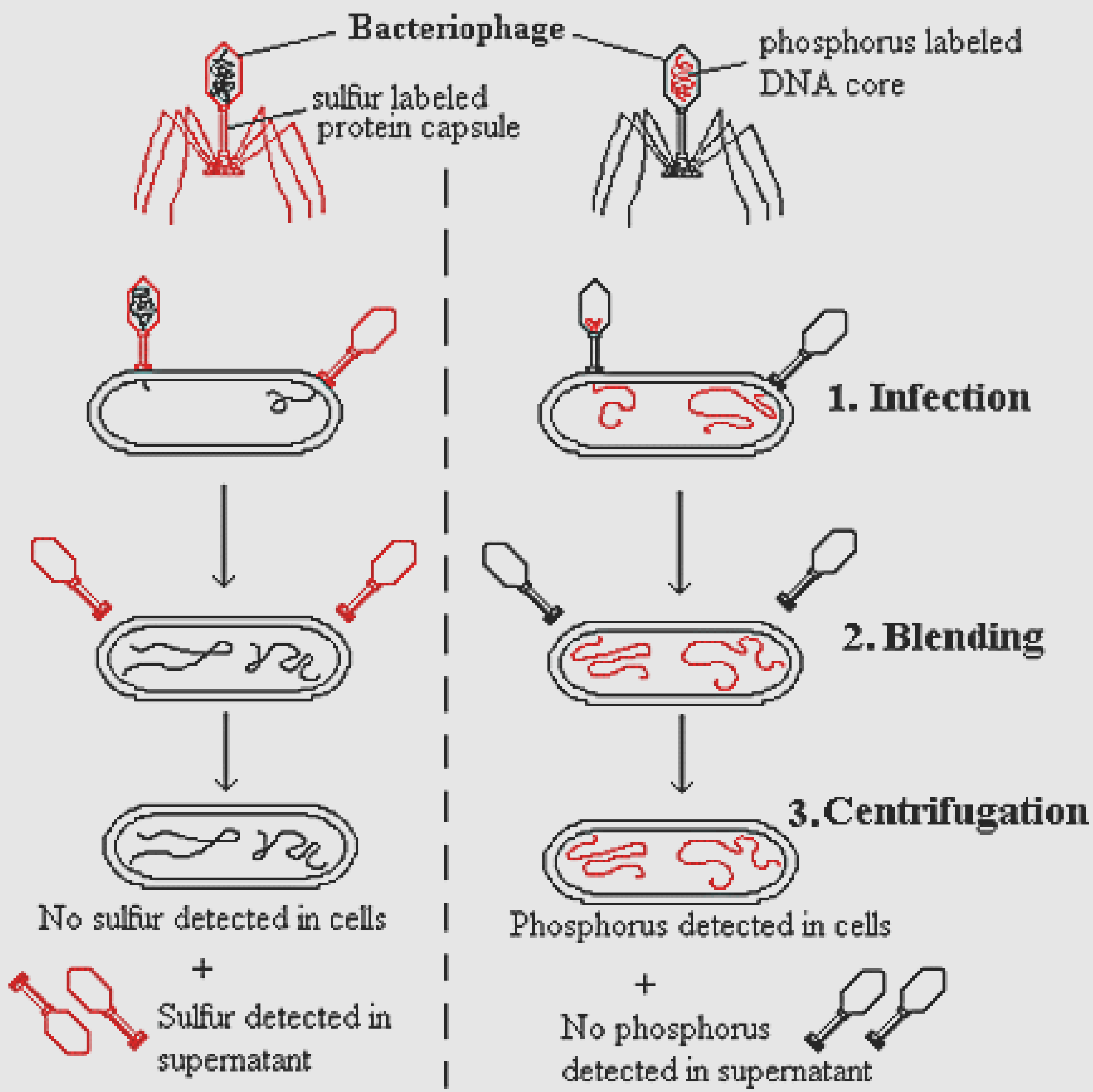


Oni su koristili bakteriofag (virus koji napada bakterije)





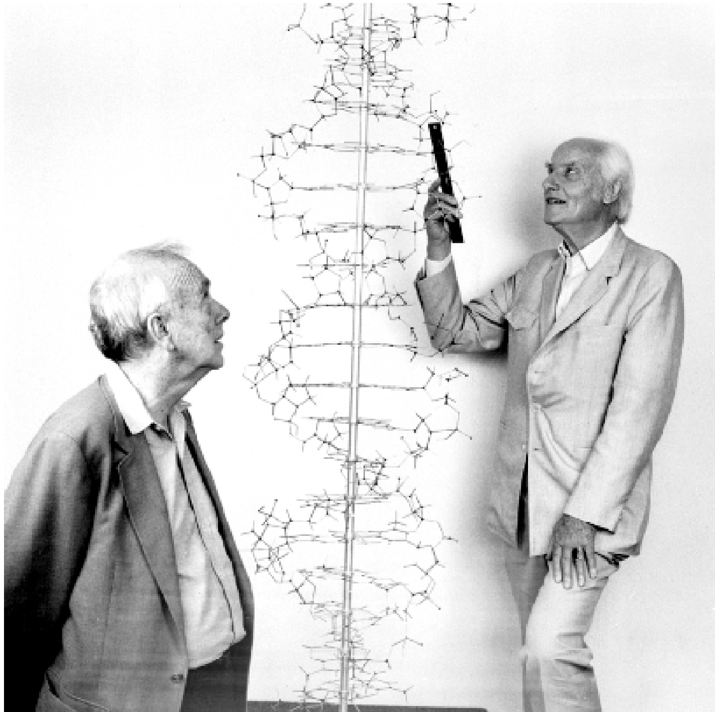
Virusi su organizovane asocijacije makromolekula: nukleinske kiseline se nalaze unutar protektivne proteinske ljuske.



The Hershey-Chase Experiment

- Kako izgleda 3-D struktura molekula DNK?
  - rendgenska strukturna analiza!!!

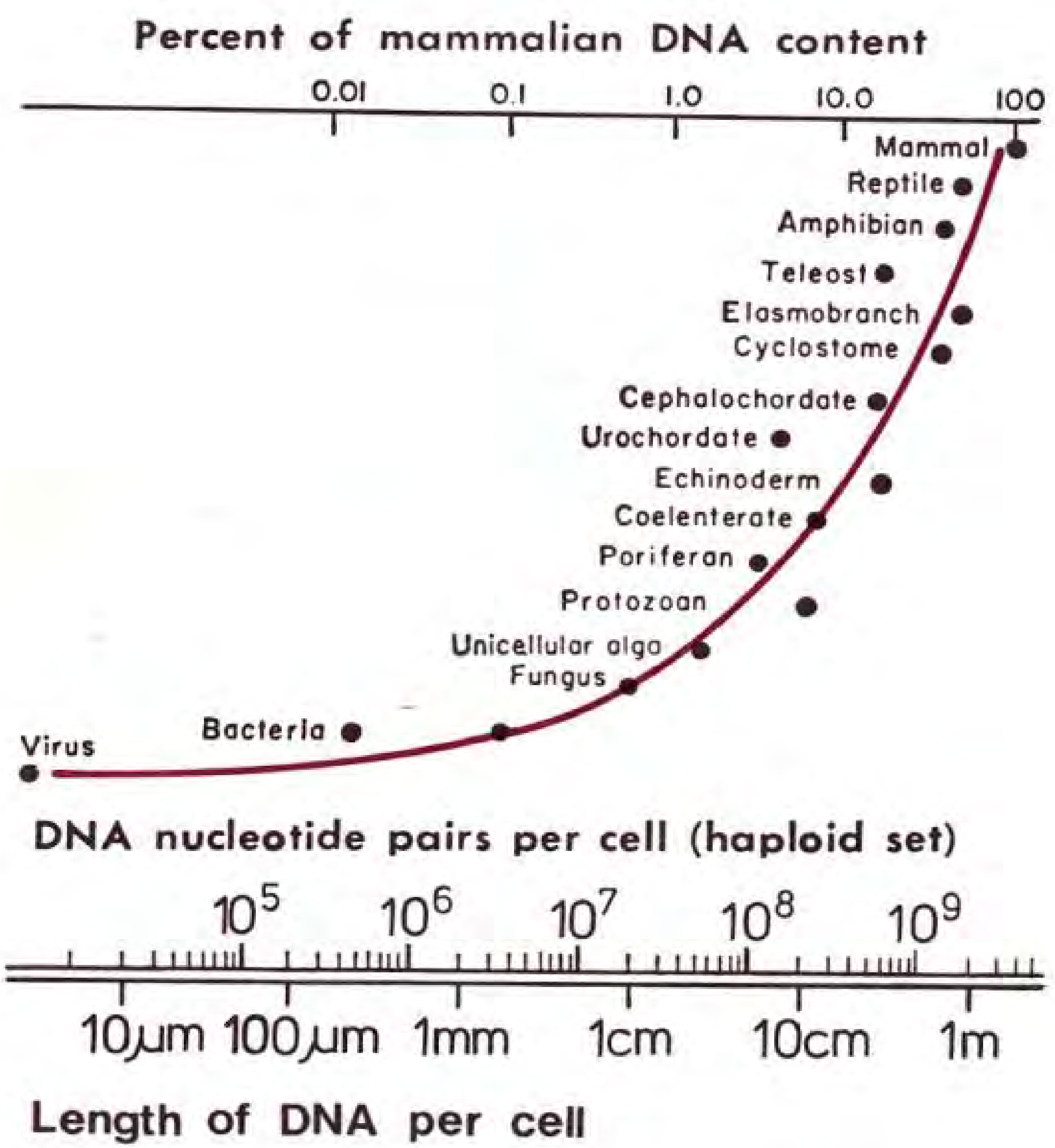
- Kako molekul DNK čuva i prenosi genetsku informaciju????



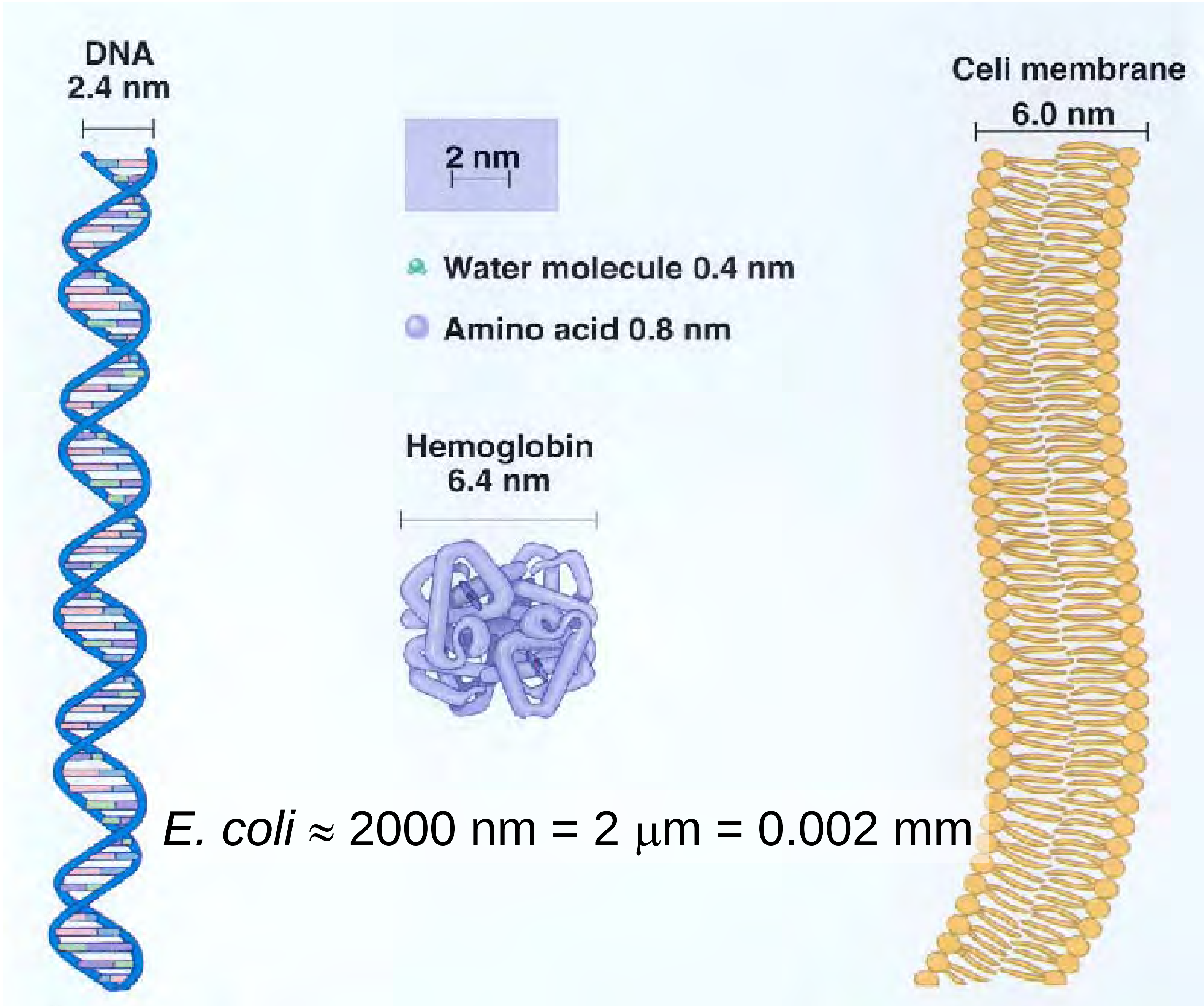
Jim Watson &
Francis Crick

1952-2002

Dužina molekula DNK po ćeliji



Relativne veličine biomolekula



E. coli ≈ 2000 nm = 2 μm = 0.002 mm

Dimenzije DNK: relativne dužine

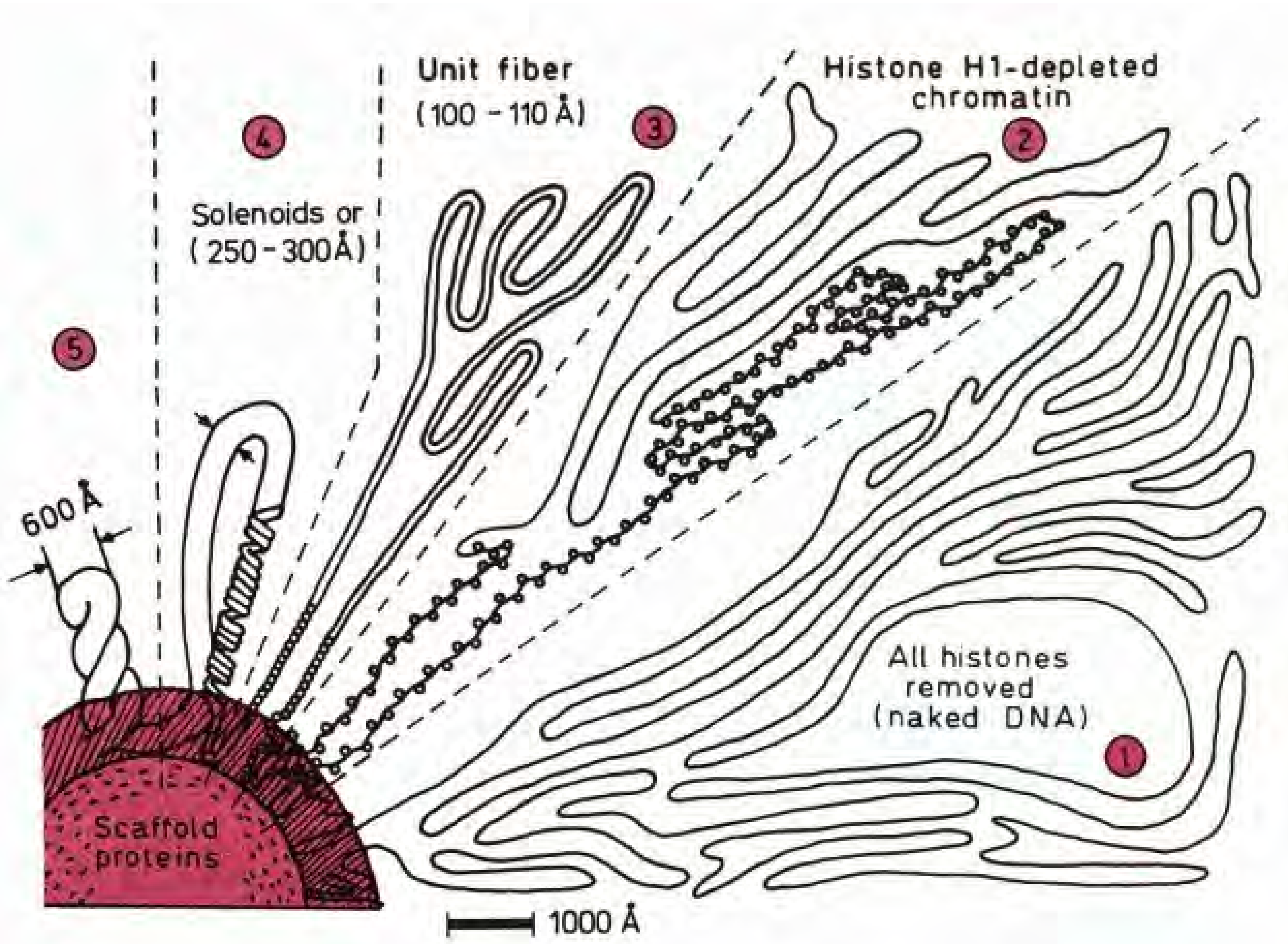
- Dužina (m)

- 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} (1 nm)
- Prečnik atoma 10^{-10} (1 Å)

- Dužina x 10^{6}

- 1000 km
- 10 km
- 1 cm
- 1 mm

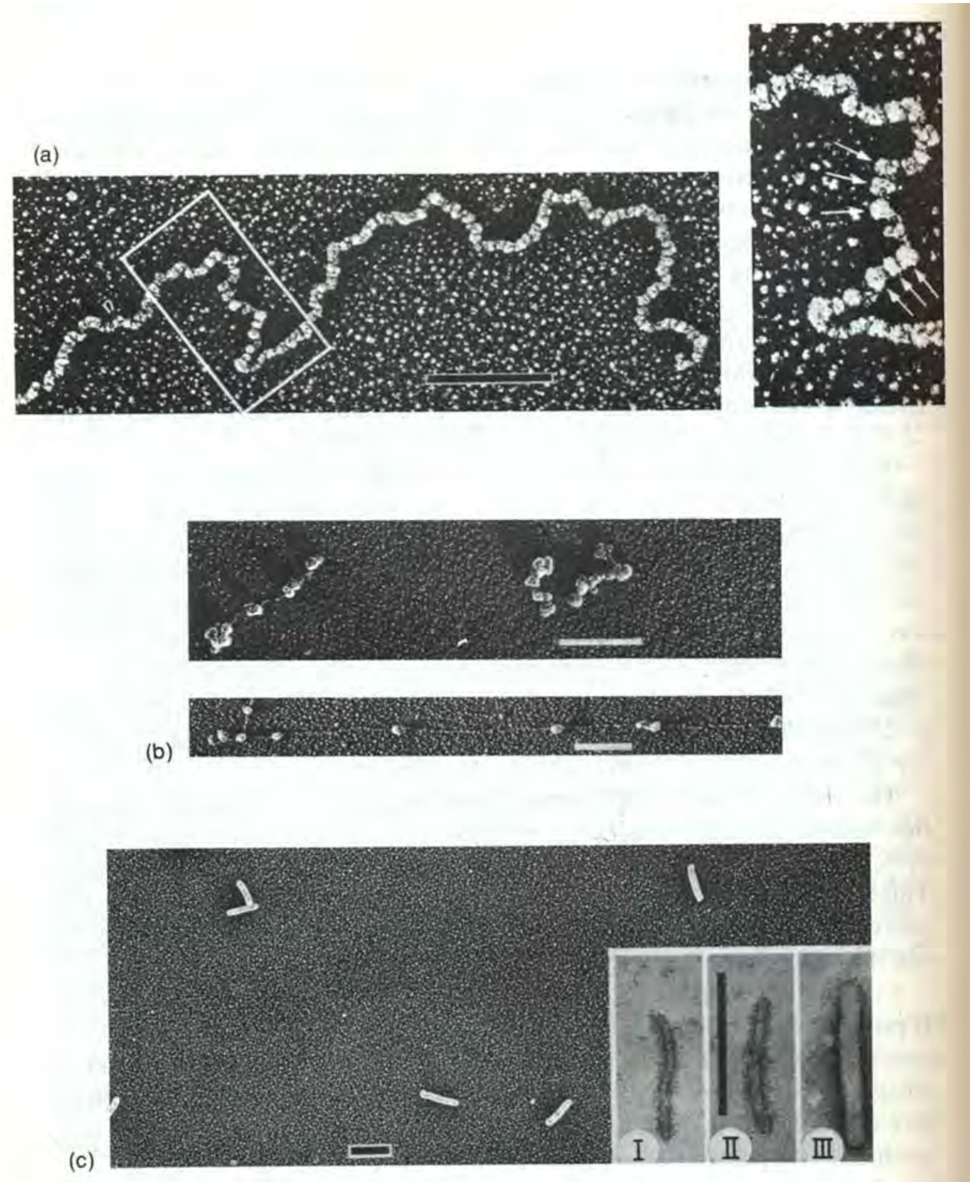
Organizacija DNK u ćeliji



Izolovanje DNK

- Kako se izoluje DNK iz biološkog materijala?
- Sličnosti i razlike u odnosu na izolovanje proteina!
- Osnovni problem:
  - veličina molekula DNK: ako se pažljivo radi dobijaju se fragmenti molekulske mase 10^7 D

Kako izgleda preparat DNK: efekat jonske sile



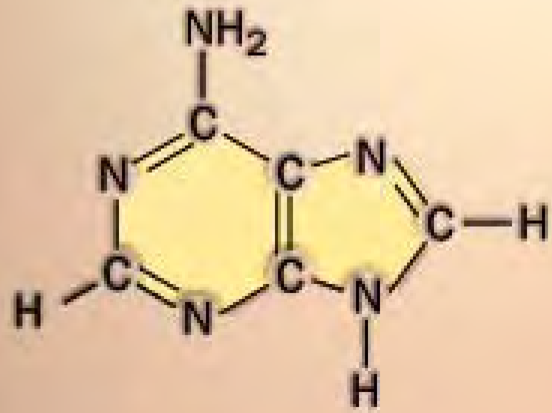
Zašto su baze izabrane za čuvanje i prenos genetske informacije?

- Tautomerija baza

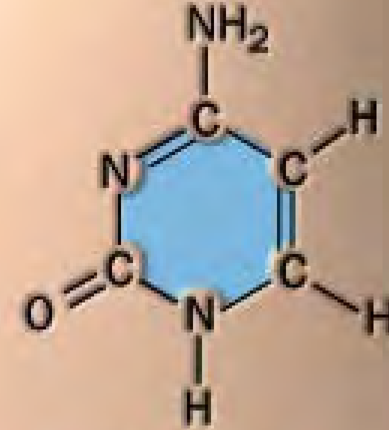
- Horizontalne i vertikalne interakcije među bazama:

  - Vodonične veze u nepolarnoj sredini/rastvaraču: komplementarnost/prepoznavanje baza

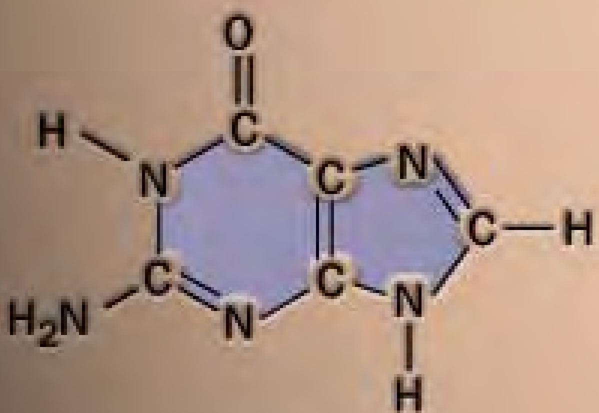
  - Slepljivanje ("stacking") baza
  (uključuje i hidrofobni efekat) u vodi: stabilnost



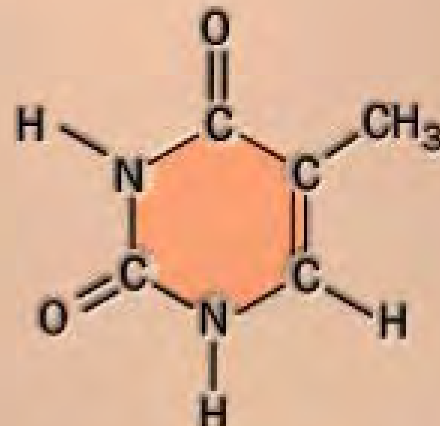
Adenine (A)



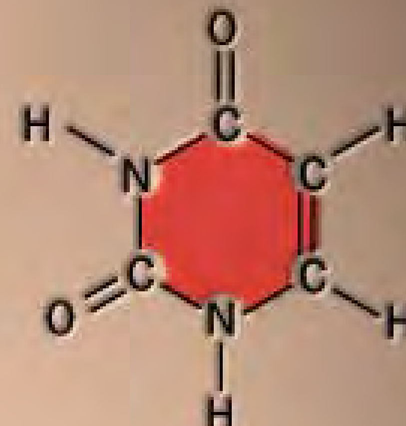
Cytosine (C)



Guanine (G)

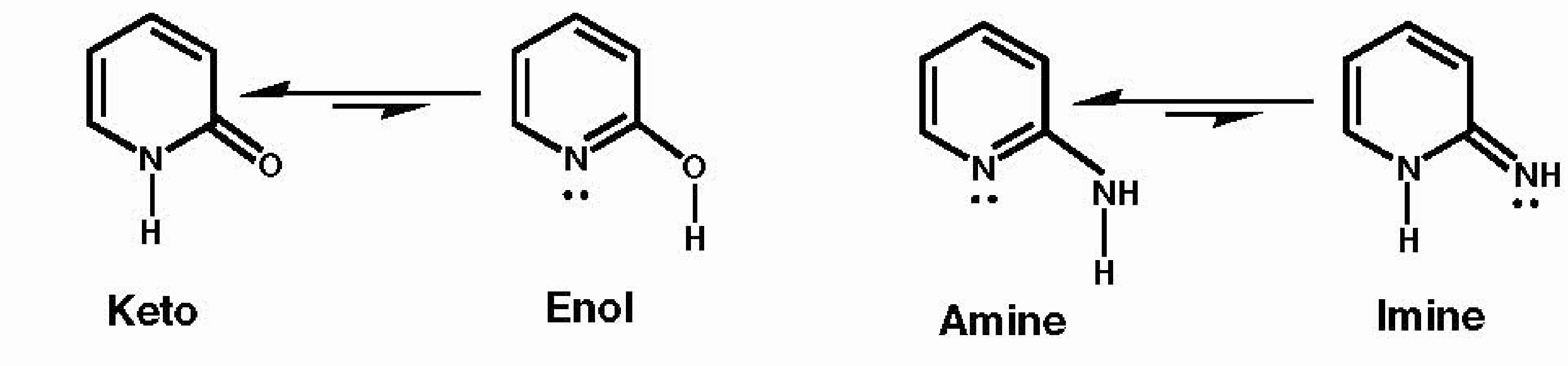


Thymine (T)

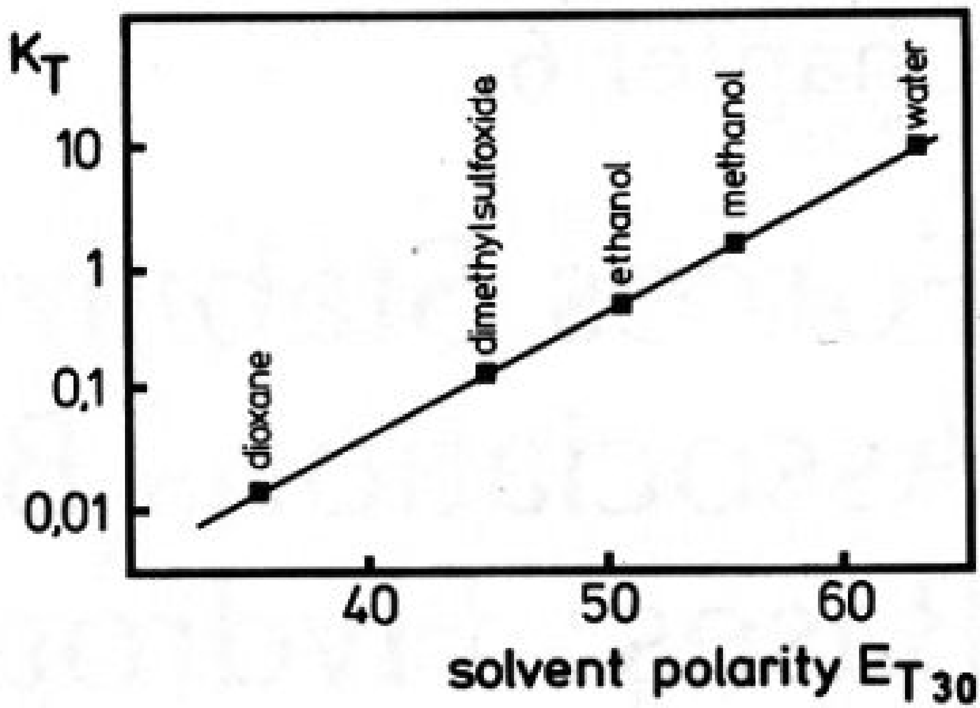


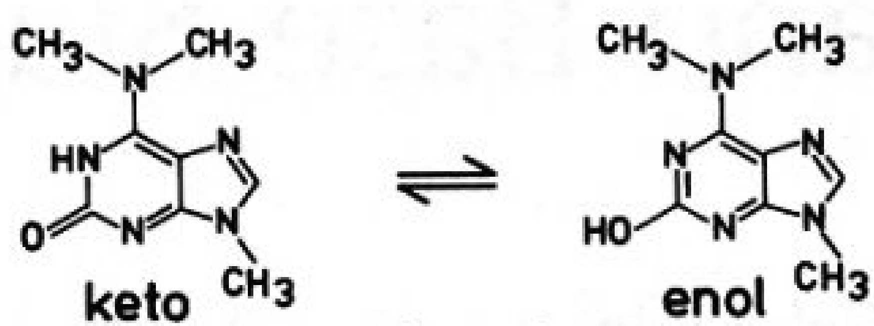
Uracil (U)

Keto – enolna izomerija (tautomerija)



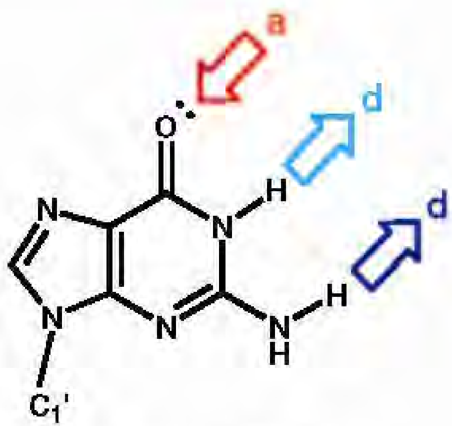
Keto ⇆ enolna ravnoteža i polarnost rastvarača



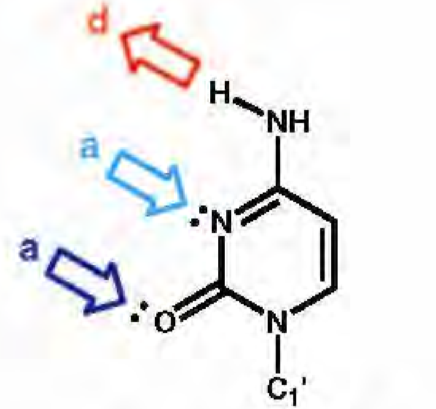


$$K_T = \frac{[\text{keto}]}{[\text{enol}]}$$

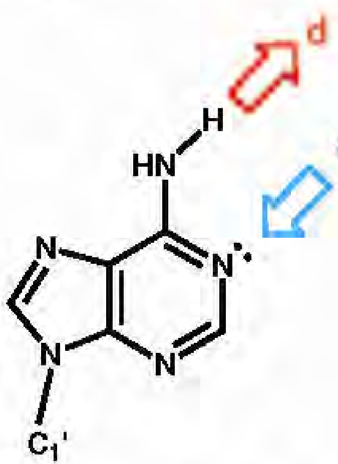
Vodonično vezivanje među bazama



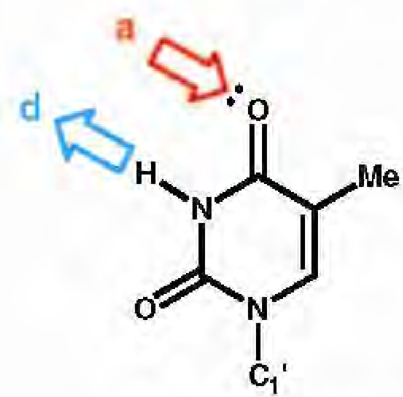
Deoxyguanosine dG



Deoxycytidine dC

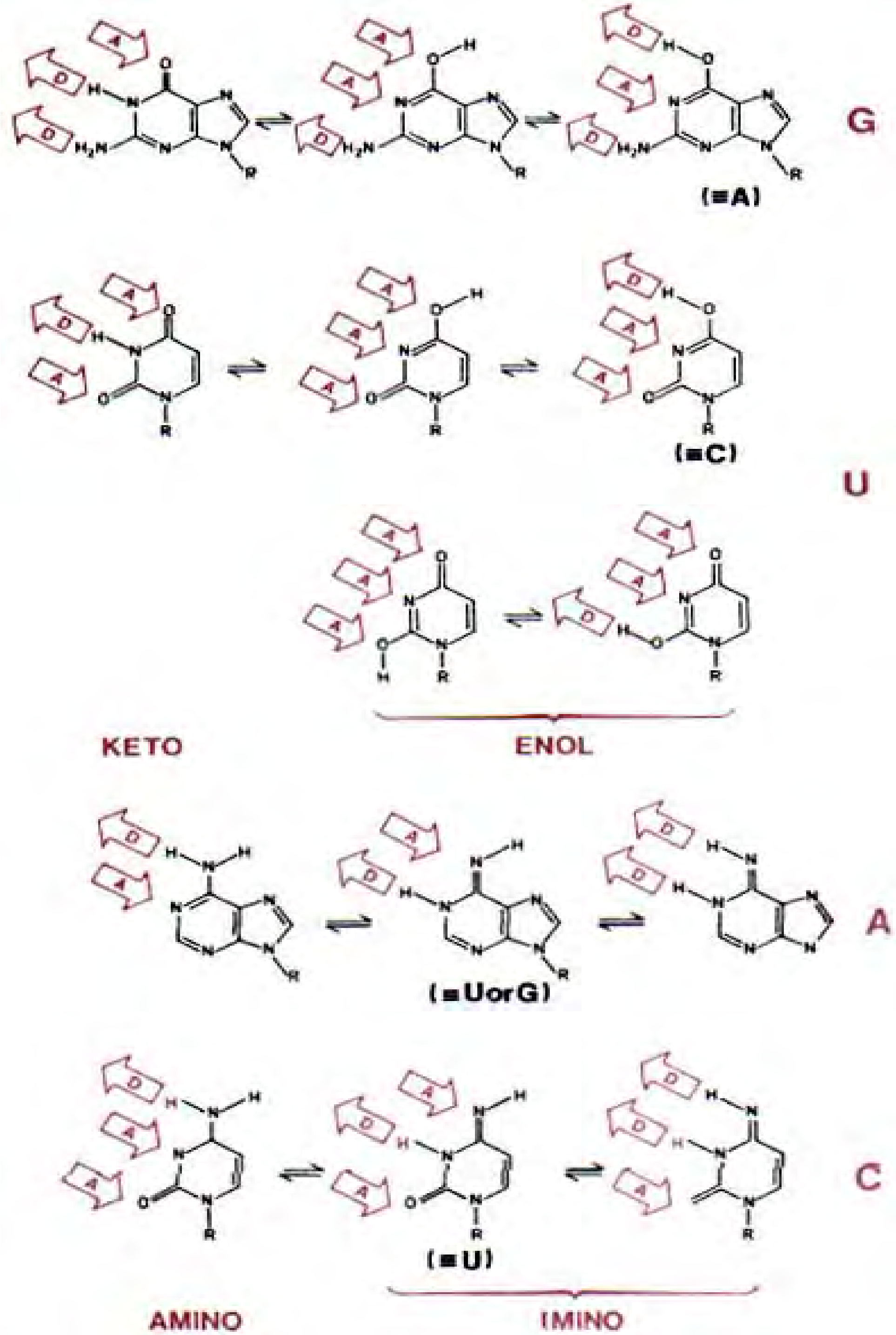


Deoxyadenosine dA



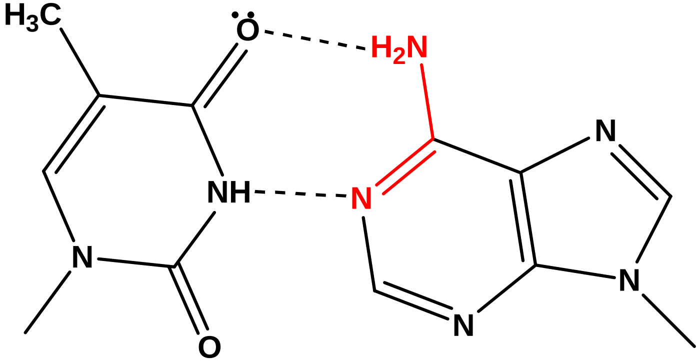
Deoxythymidine dT

Tautomerne strukture baza i vodonično vezivanje

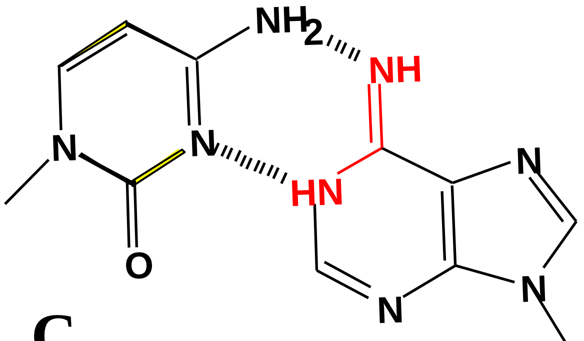


Spontane mutacije zbog prisustva male količine tautomernih oblika baza

(1 na 10^8 - 1 na 10^{10} parova baza)



T A



C A

Amino

Retki imino tautomer

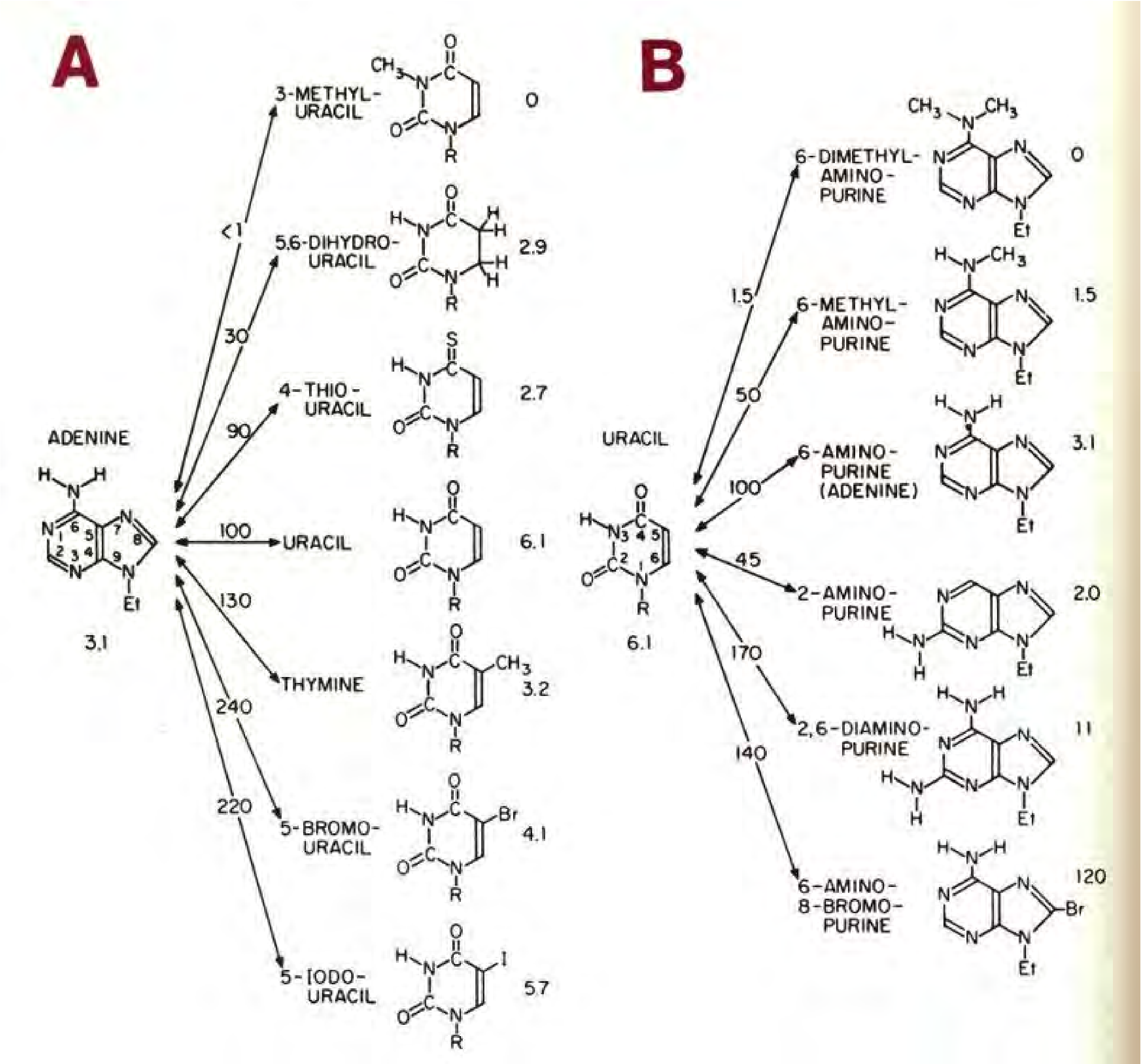
Normalno sparivanje baza

Pogrešno sparivanje

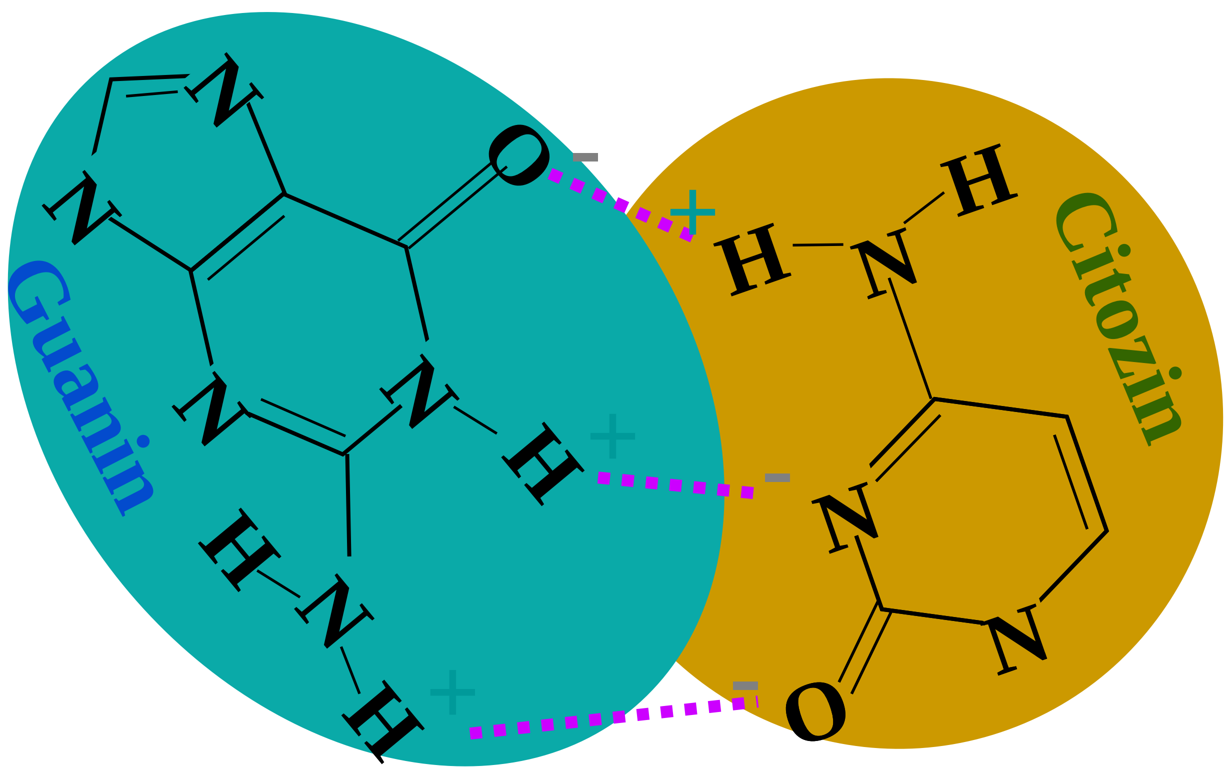
Vodonično vezivanje među bazama

- Horizontalne interakcije među bazama u nepolarnim rastvaračima
  - prepoznavanje baza
  - tipovi vodoničnog vezivanja među bazama

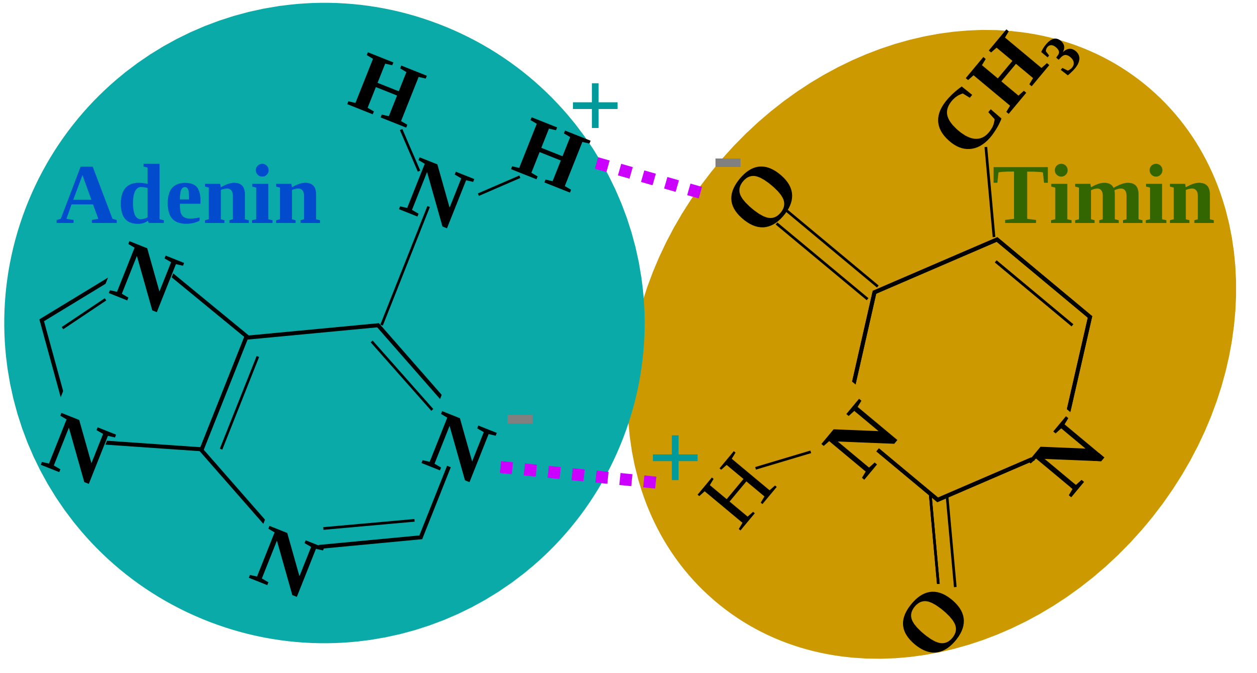
Konstante vezivanja baza u nepolarnom rastvaraču



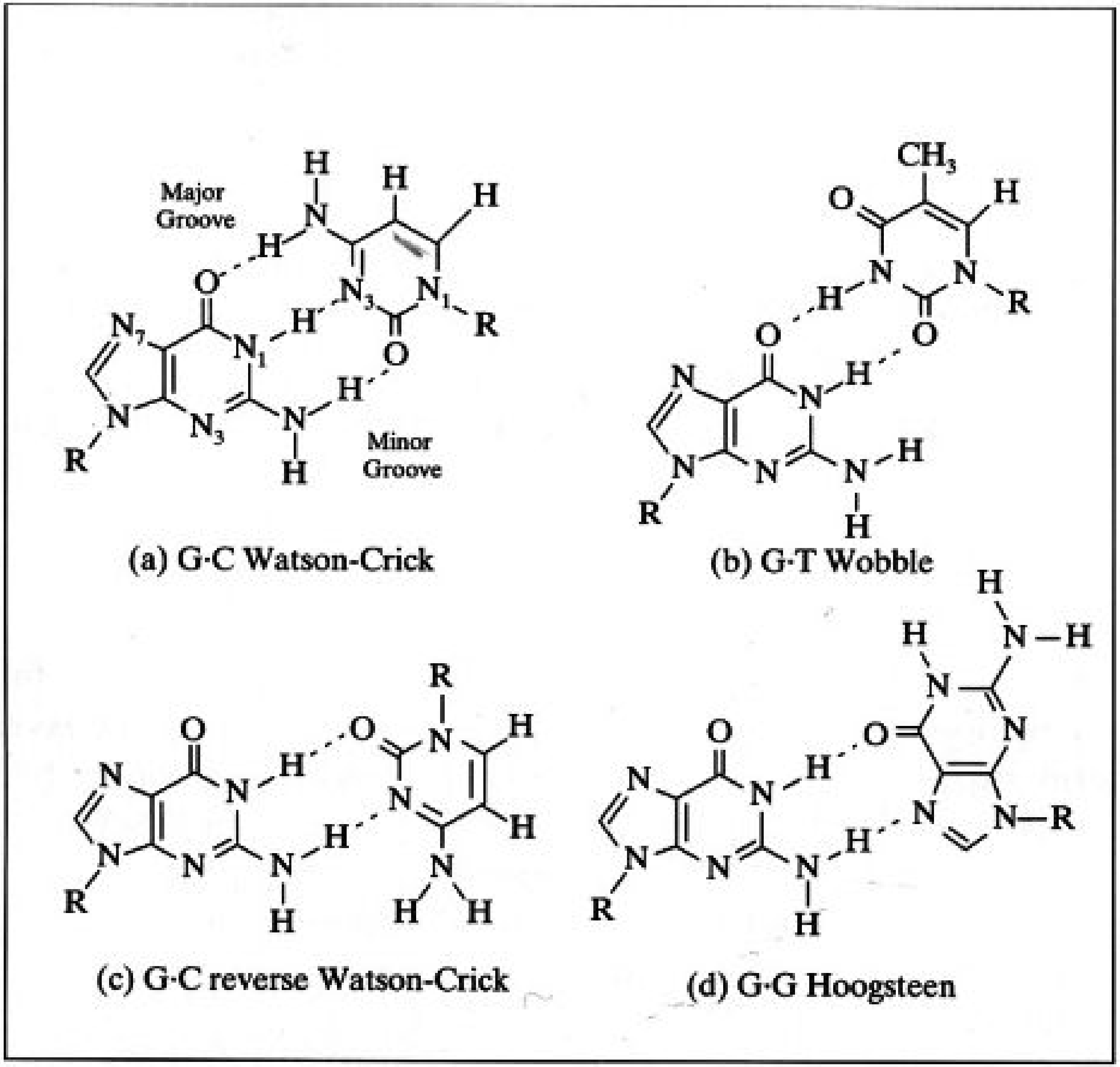
W-C sparivanje baza: guanin i citozin



W-C sparivanje baza:
adenin i timin



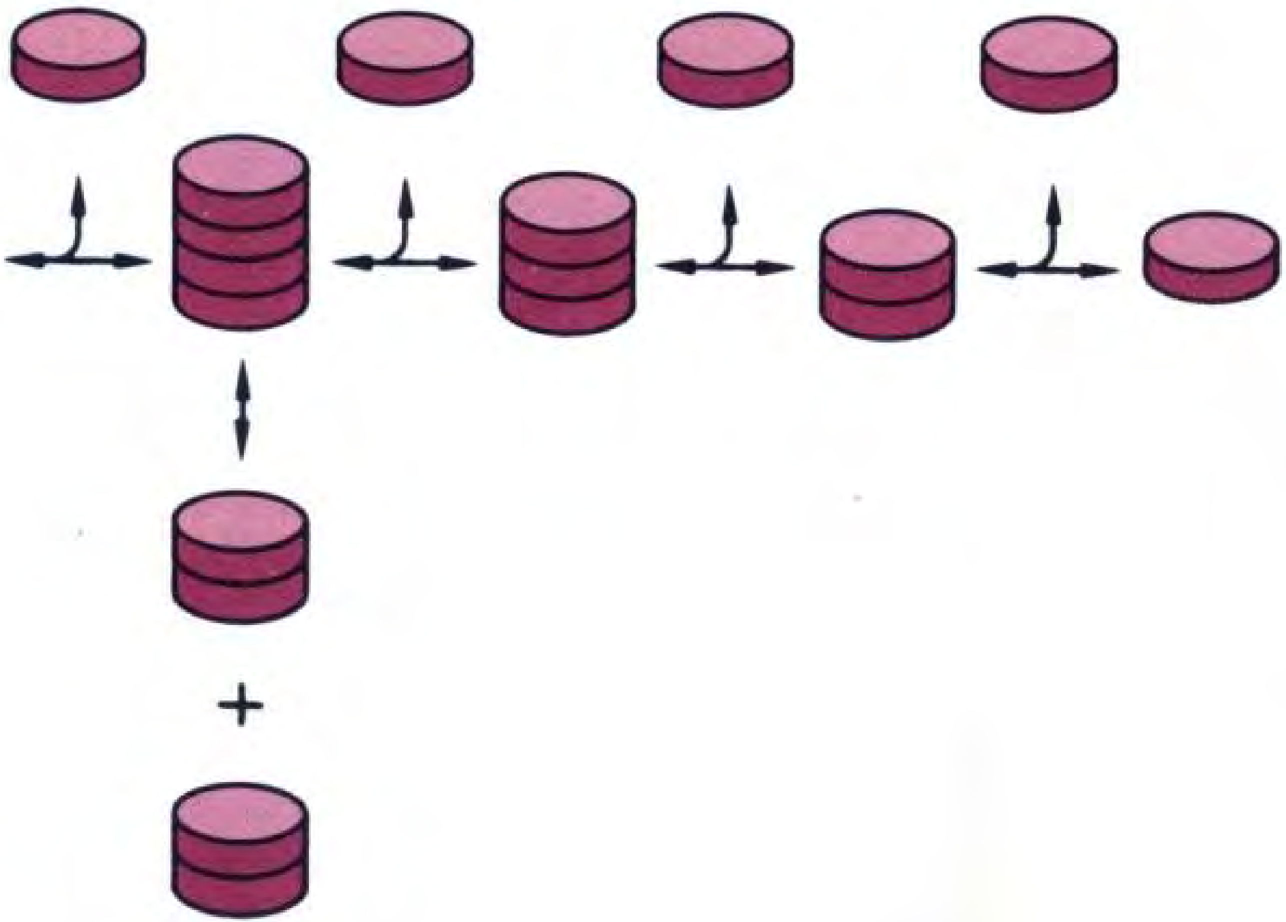
Primeri vodoničnog vezivanja među bazama



Slepljivanje (”stacking”) baza

- Vertikalne interakcije među bazama u vodi
- Dokazi za “stacking” interakcije
- Termodinamika

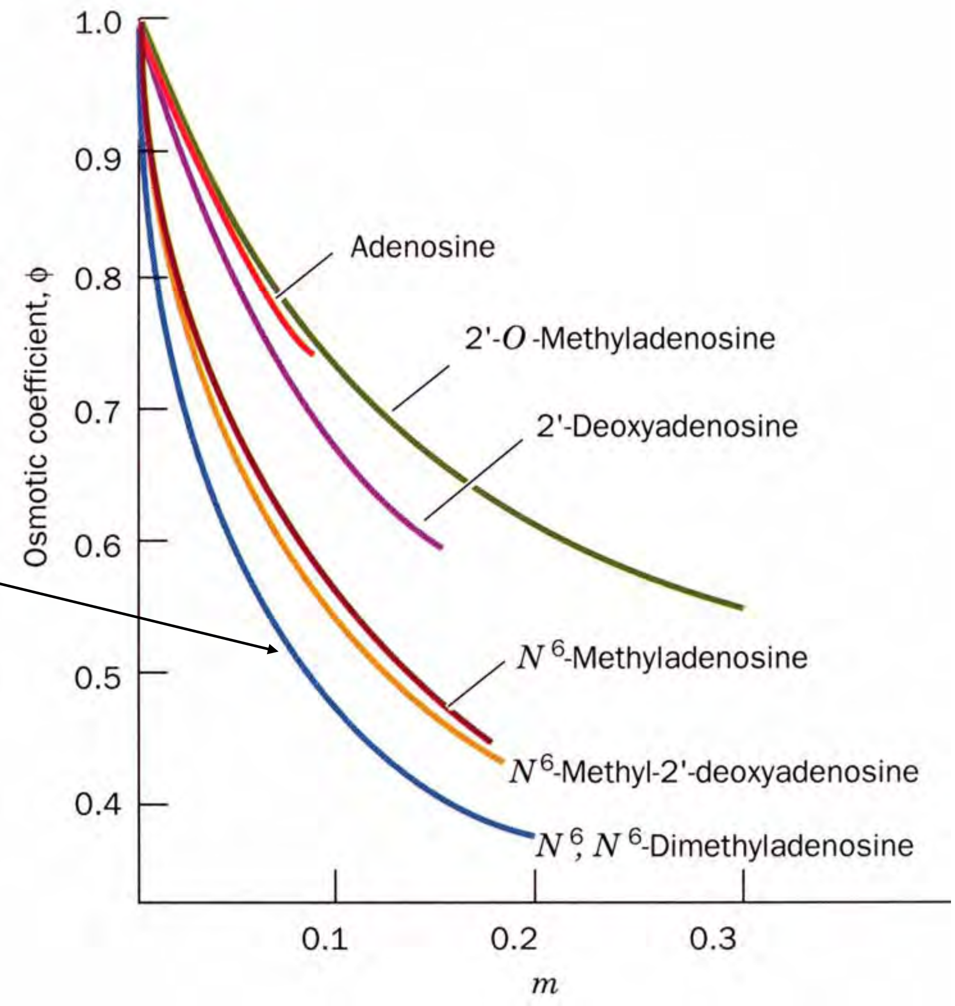
Interakcije baza u vodi:
“slepljivanje” “stacking” baza



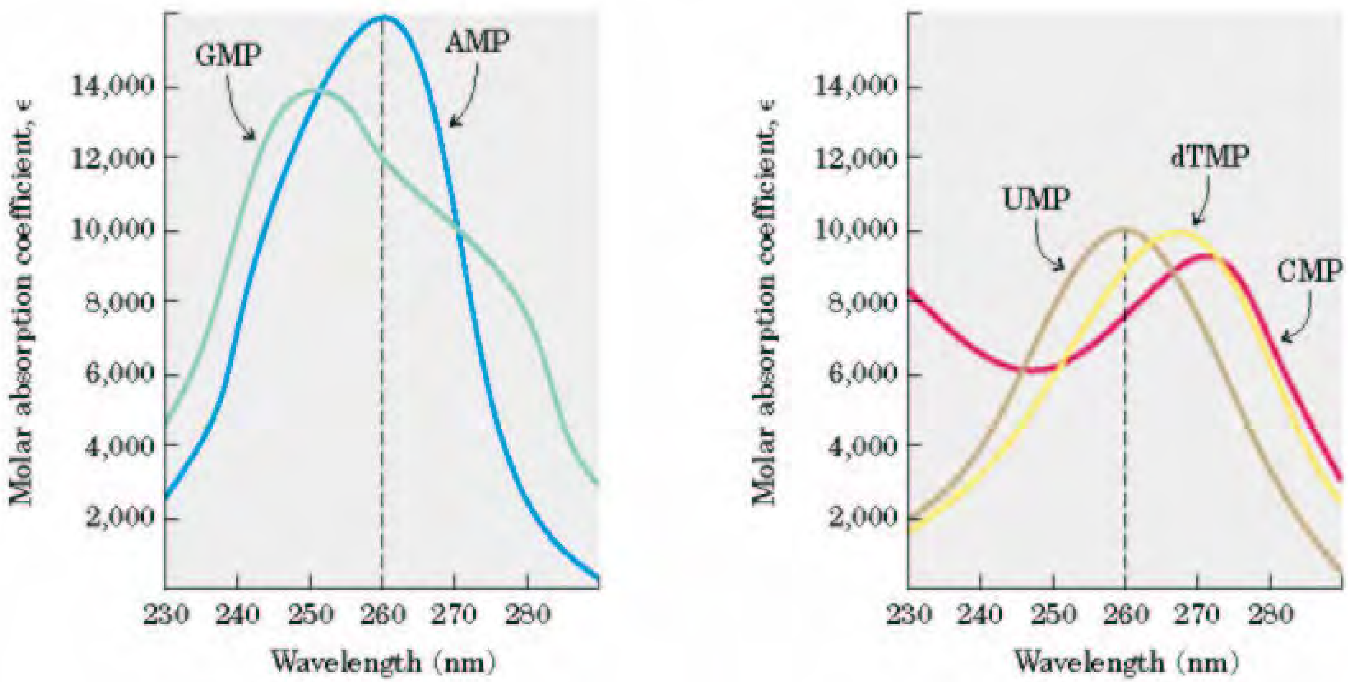
Dokazi za "stacking" baza u vodenom rastvoru

Smanjenje osmotskog pritiska nije zbog vodoničnog vezivanja!

Zašto?



UV spektri baza



Molar absorption coefficient at 260 nm, ϵ_{260} ($M^{-1}cm^{-1}$)	
AMP	15,400
GMP	11,700
CMP	7,500
UMP	9,900
dTMP	9,200

Da li bi se na osnovu UV spektara moglo videti slepljivanje baza?

Termodinamički parametri za slepljivanje baza

TABLE 3.9 ENERGIES FOR BASE-STACKING BETWEEN AND WITHIN NUCLEOBASES

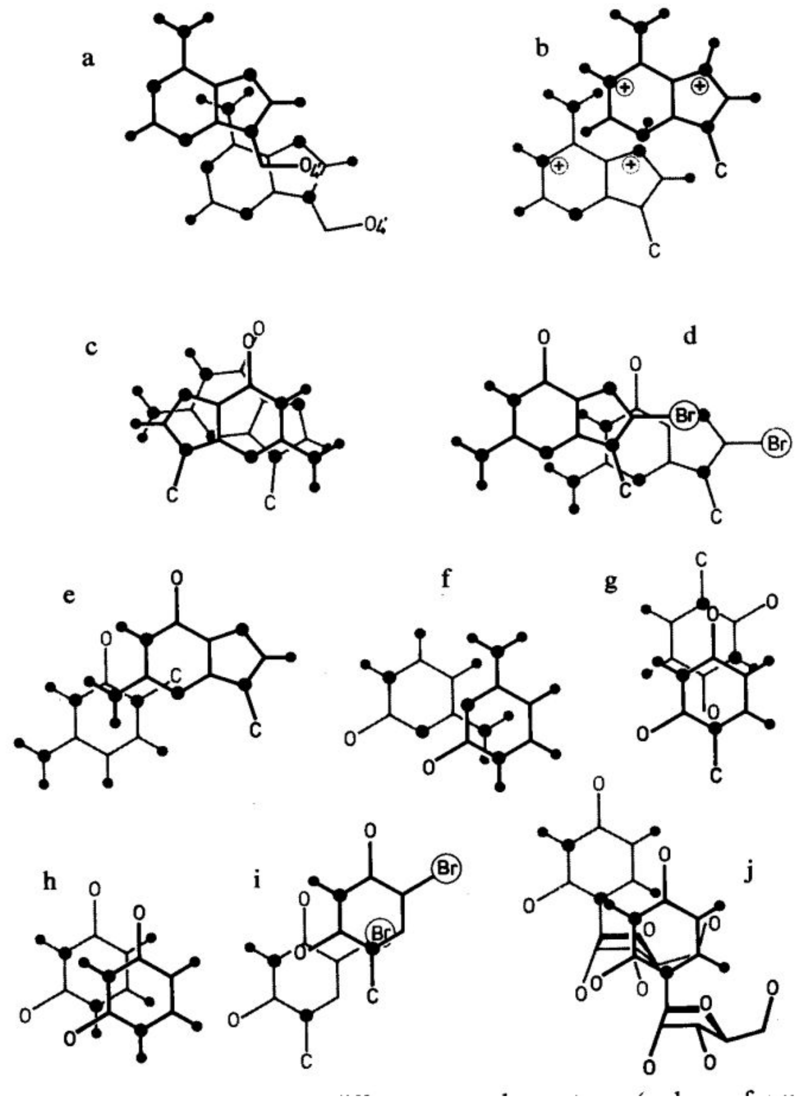
Compound	$\Delta H°$ (kJ/mol)	$\Delta S°$ (J/mol K)	$\Delta G°$ (kJ/mol)
Nucleobases and Nucleosides			
Purine	−17.3 ± 0.8	−54	−1.8
Ribosylpurine	−10.3 ± 0.4	−29	−1.6
2′-Deoxyadenosine	−27 ± 4	−74	−4.1
Cytidine	−11.5 ± 0.4	−41	0.3
Uridine	−11.1 ± 0.4	−41	1.2
Thymidine	−10 ± 1	−37	0.24
Dinucleotides			
ApA	−35 ± 2	−118 ± 6	0
CpC	−35 ± 2	−124 ± 6	1.8
ApU	−30 ± 2	−102 ± 7	0.0

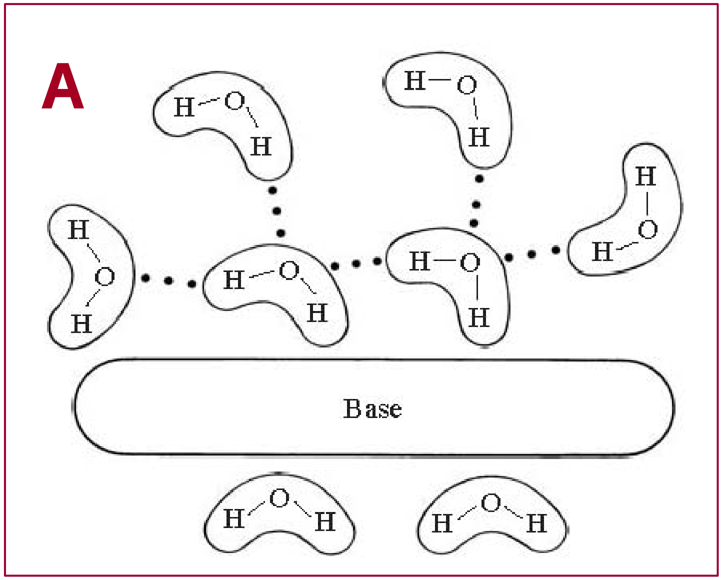
Zaključak:

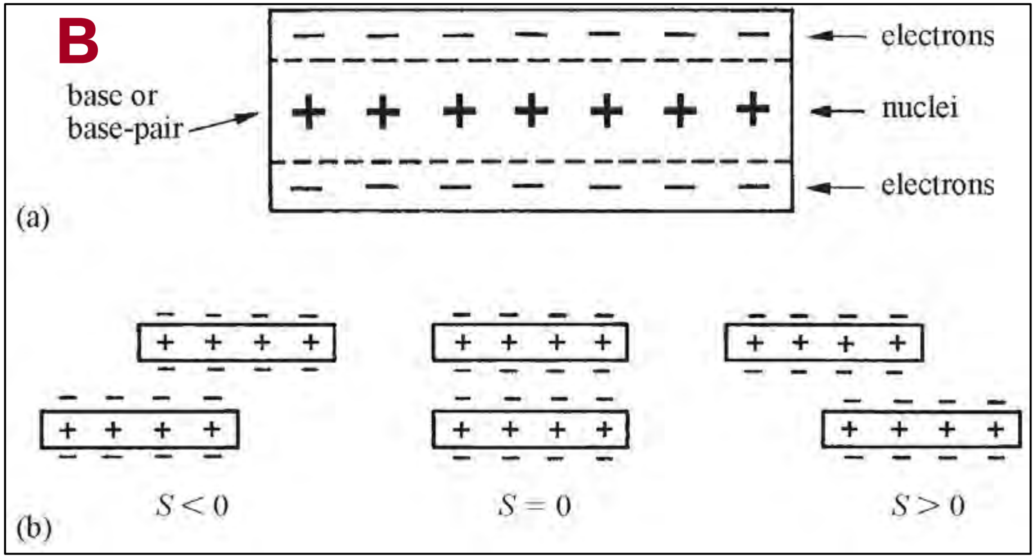
“Slepljivanje” baza u kristalnom stanju

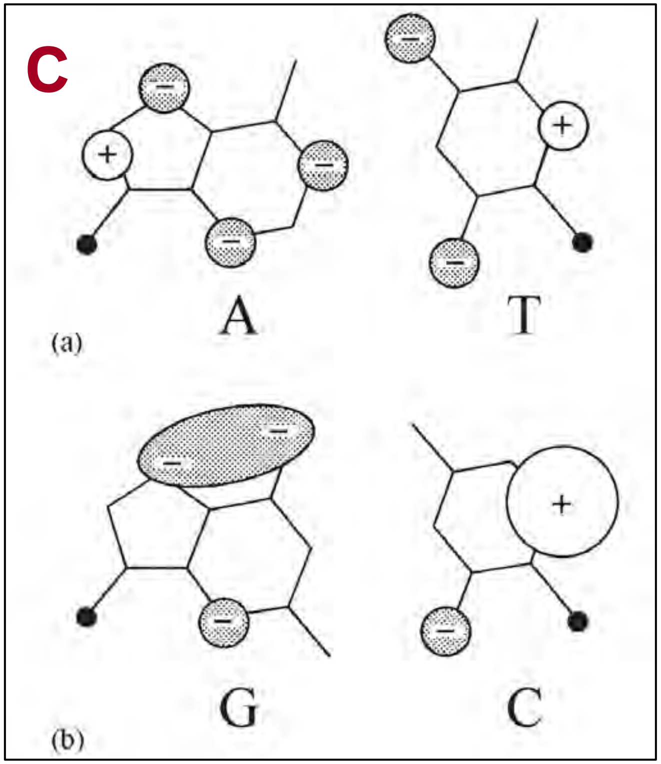
Baze su kristalisane iz vode!

Šta vidimo sa ovih slika?









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.

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

Šta zaključujemo na osnovu podataka iz tabele?