



HEMIJA PRIRODNIH ORGANSKIH JEDINJENJA

**AMINOKISELINE, PEPTIDI, PROTEINI**

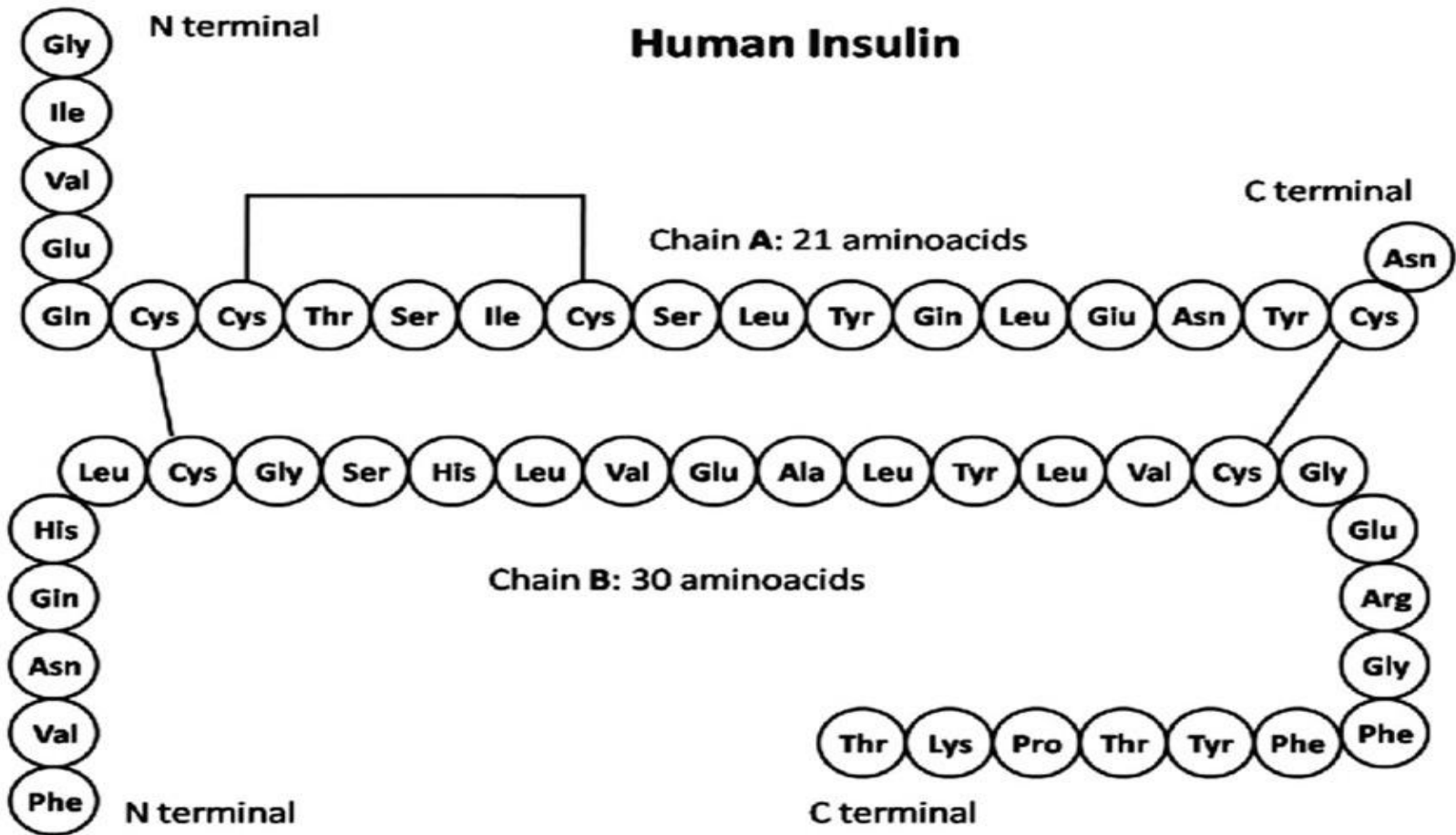
**Paul Langerhans**

**Insulin**

**Frederick Sanger, Nobelovac**

**1922, Toronto**





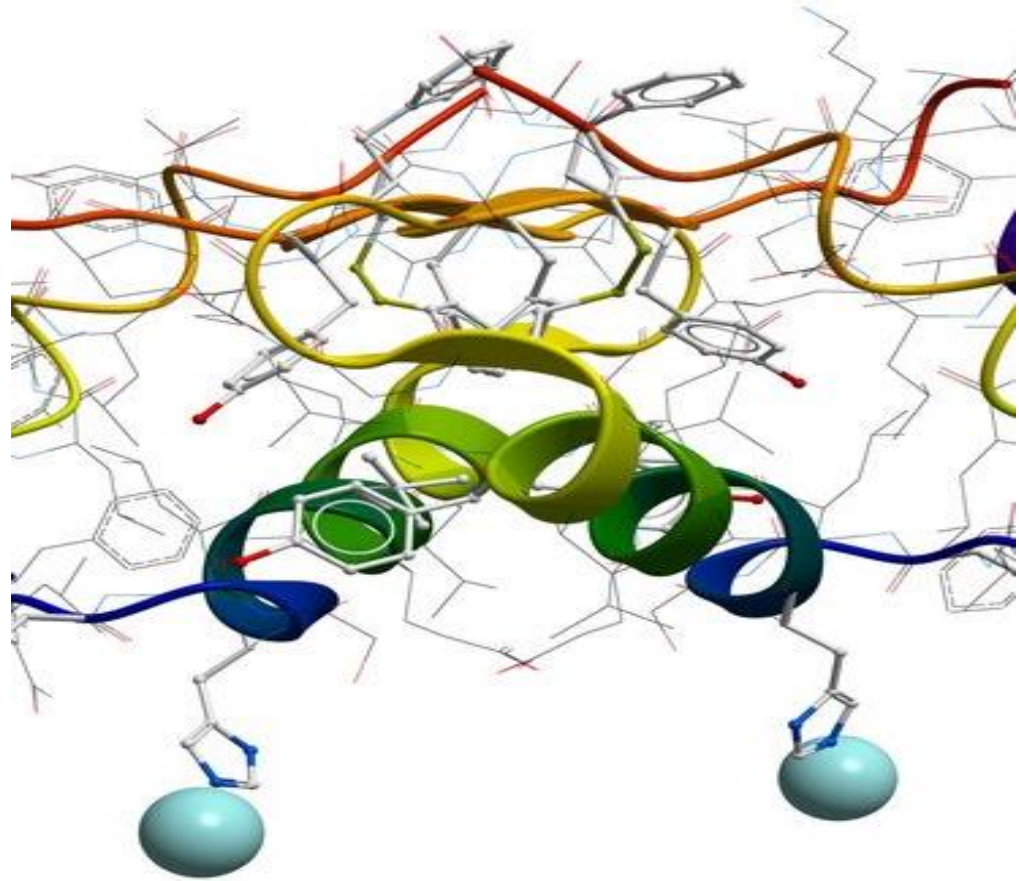
**Frederick Sanger, Nobelovac**

**Paul Langerhans**

**Insulin**

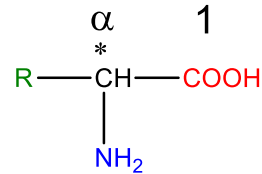
**Frederick Sanger, Nobelovac**

**1922, Toronto**

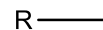


## POJAM AMINOKISELINE

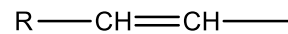
- Aminokiseline su organske kiseline
- Kao organski molekul to je  $\alpha$ -aminokarbonska kiseline
- Kao biomolekul to je  $\alpha$ -aminokiselina



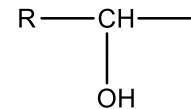
alifatične



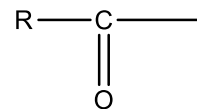
polienske



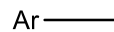
hidroksi



keto



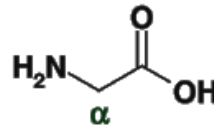
aromatične



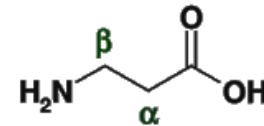
## POJAM AMINOKISELINE

- Aminokiseline su organske kiseline
- Kao organski molekul to je  $\alpha$ -aminokarbonska kiseline
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### Alpha-Amino Acid

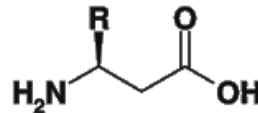


### Beta-Amino Acid

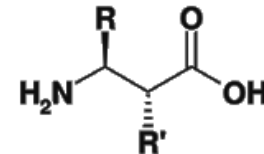


Po položaju bazne amino grupe mogu biti  $\alpha$ ,  $\beta$ ...

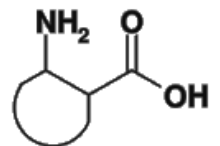
### Substituted Beta-Amino Acids:



3-substituted  
("beta<sup>3</sup>-amino acids")



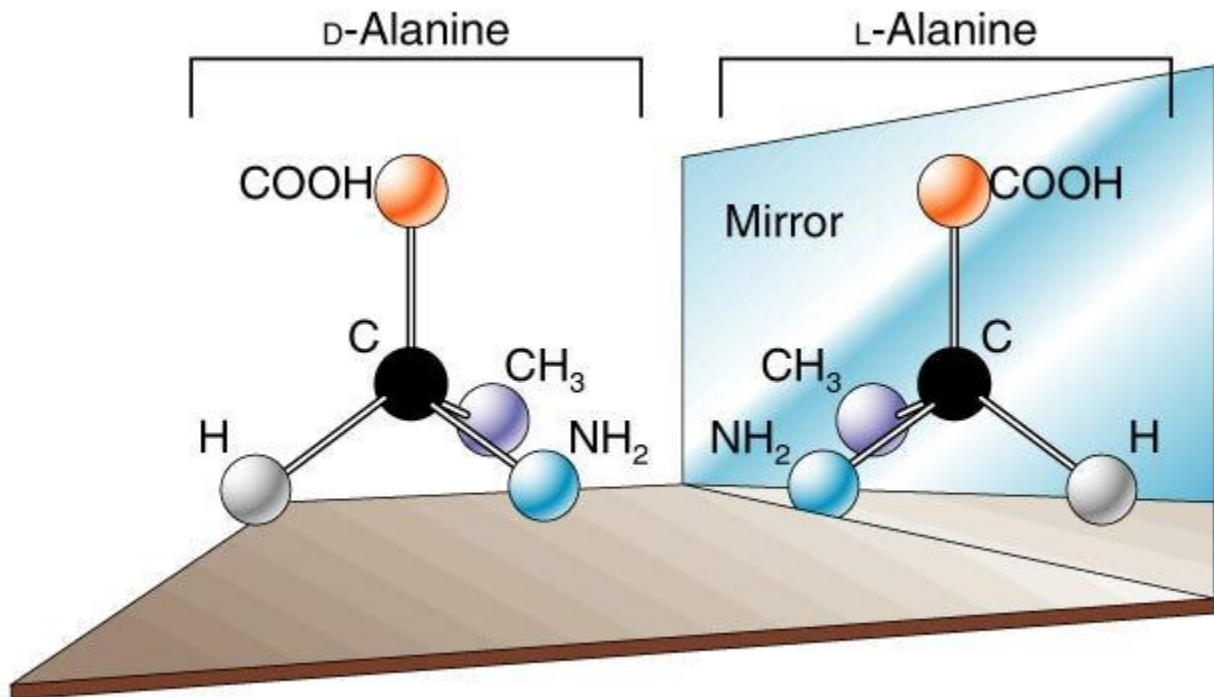
2,3-bis substituted



cyclic

## POJAM AMINOKISELINE

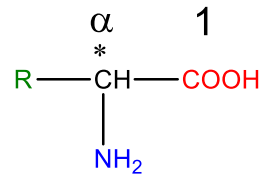
- U prirodi postoji i manji broj AK D-konfiguracije, npr u mikroorganizmima. U sastavu nekih antibiotika ih ima takođe.



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## POJAM AMINOKISELINE

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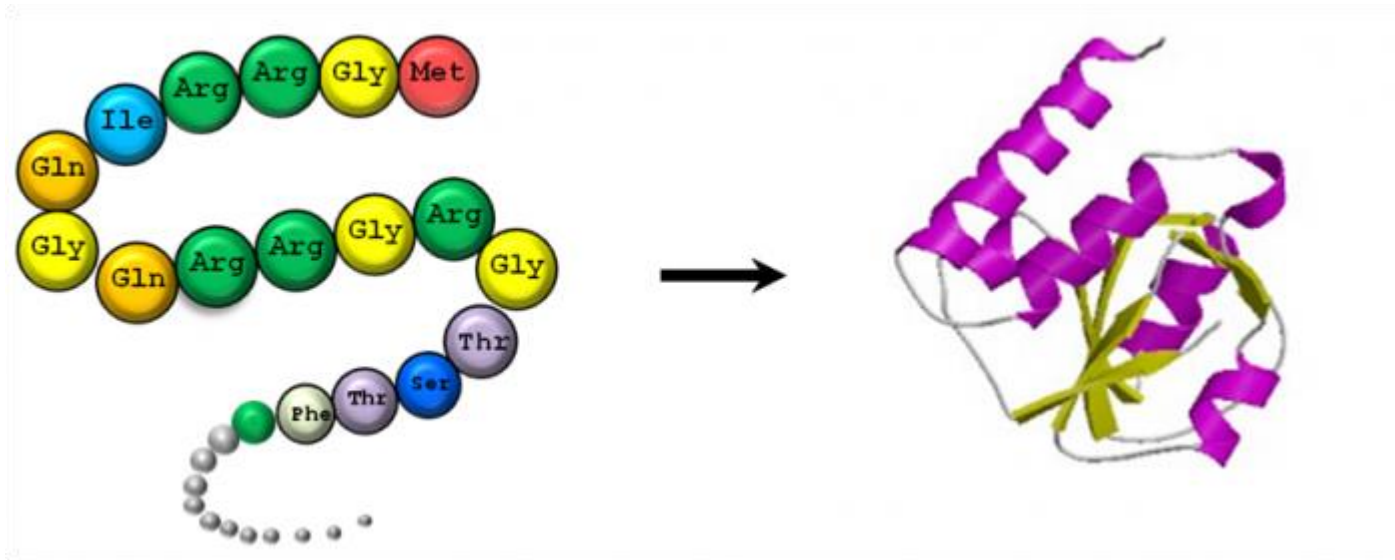


Po rastvorljivosti se dijele na lipofilne i lipofobne.



## POJAM AMINOKISELINE

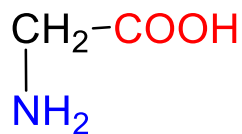
- Postoji preko 200 aminokiselina
- Samo njih 20 su proteinske
- $\alpha$ -aminokiseline na  $\alpha$ -C atomu uvijek imaju dvije funkcionalne grupe: karboksilnu i amino



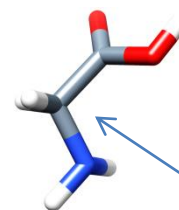
20 proteinskih aminokiselina gradi proteine

## NEPOLARNE AMINOKISELINE

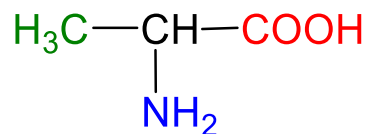
### Alifatske zasićene nesupstituisane monoamino-monokarboksilne kiseline



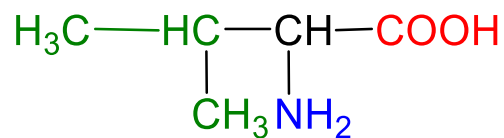
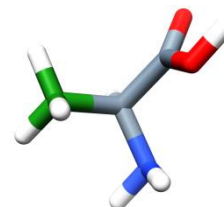
glicin, Gly, G



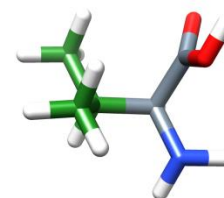
nema hiralan  $\alpha$ -C



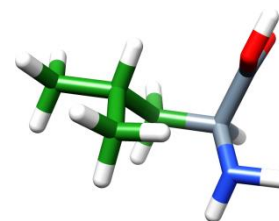
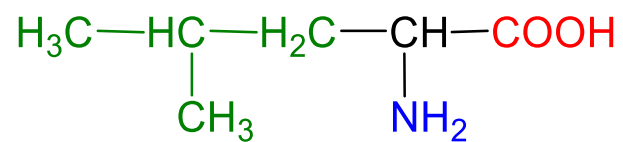
alanin, Ala, A



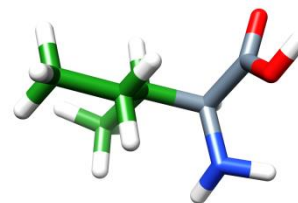
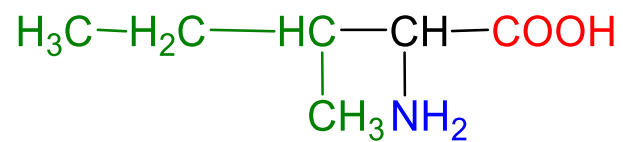
valin, Val, V



## NEPOLARNE AMINOKISELINE

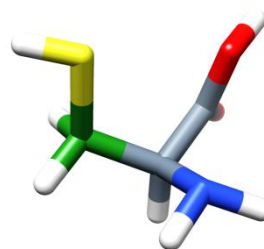
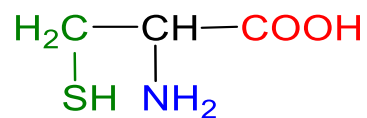


leucin, Leu, L

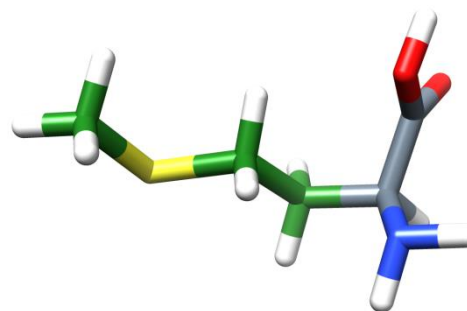
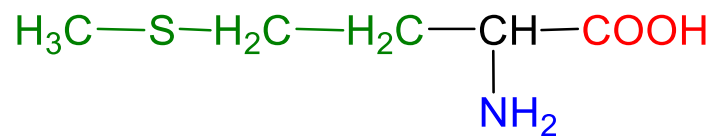


izoleucin, Ile, I

## AMINOKISELINE SA SUMPOROM (tio-aminokiseline)

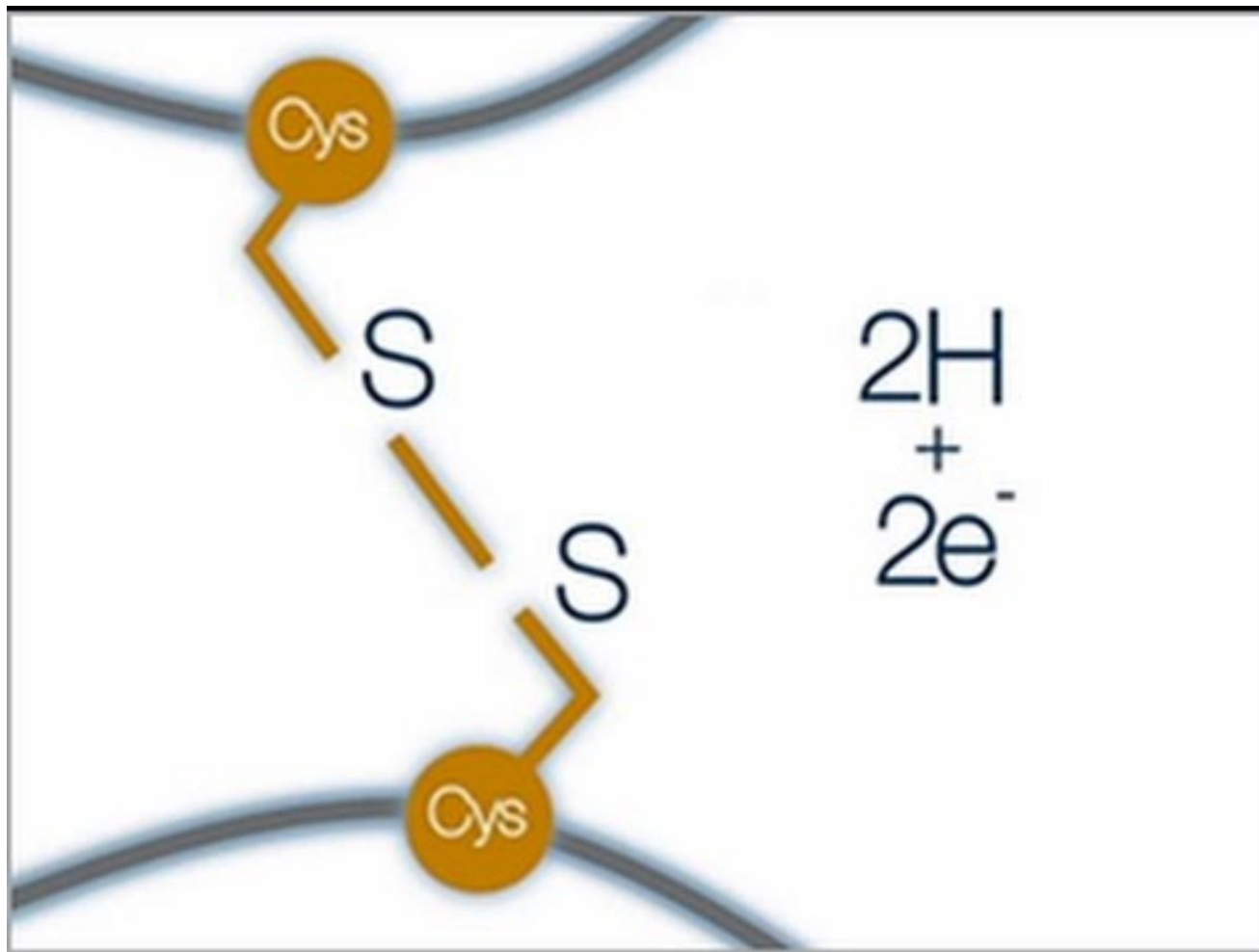


cistein, Cys, C



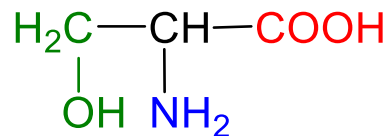
metionin, Met, M

## AMINOKISELINE SA SUMPOROM (tio-aminokiseline)

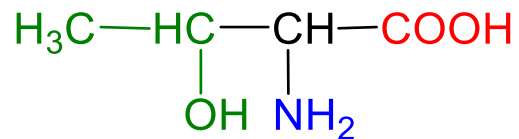
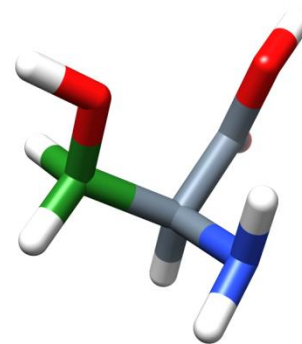


## AMINOKISELINE SA HIDROKSILNOM GRUPOM

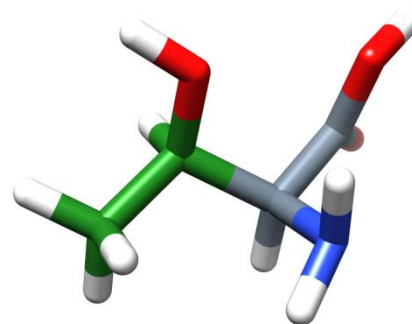
### Alifatske oksiamino-monokarboksilne kiseline



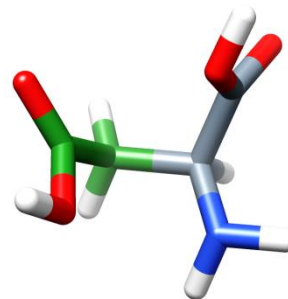
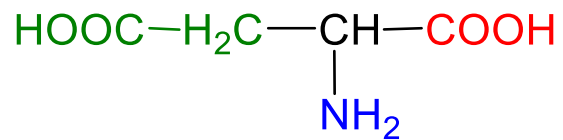
serin, Ser, S



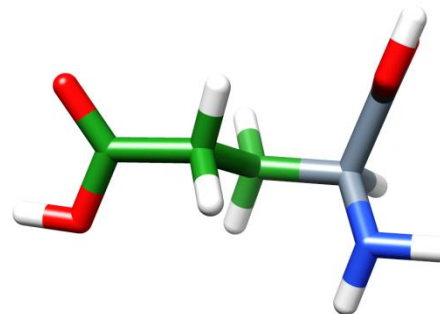
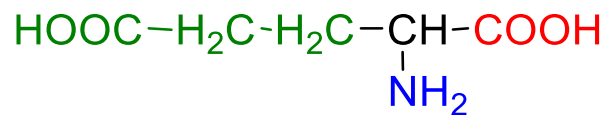
treonin, Thr, S



## DIKARBOKSILNE AMINOKISELINE

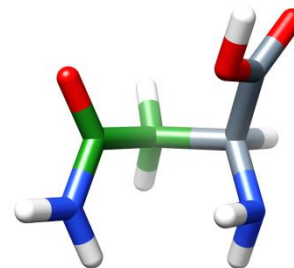
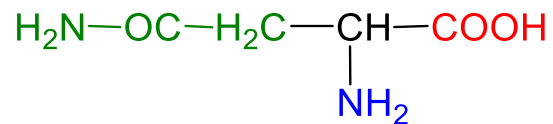


Asparaginska kiselina, Asp, D

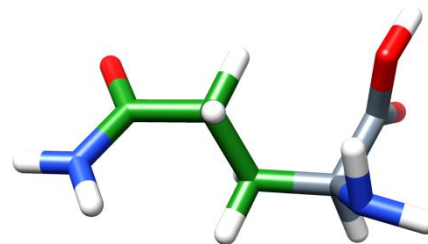
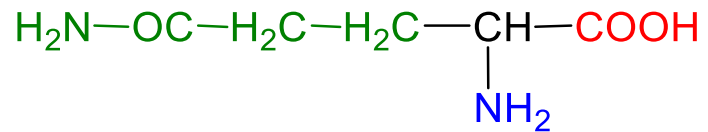


Glutaminska kiselina, Glu, E

## AMIDI DIKARBOKSILNIH AMINOKISELINA



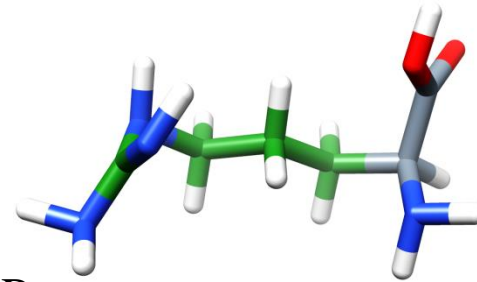
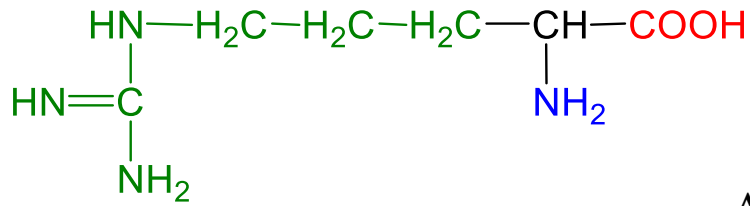
Asparagin, Asn, N



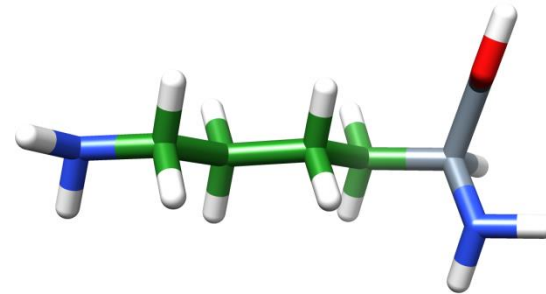
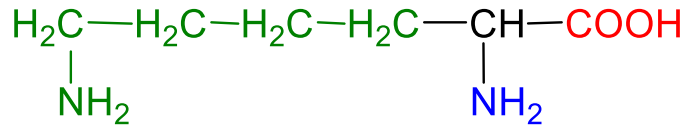
Glutamin, Gln, Q



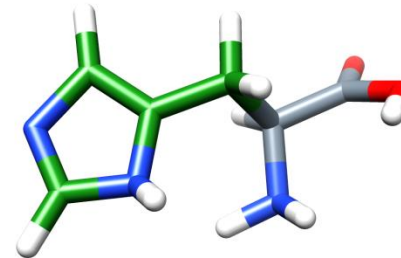
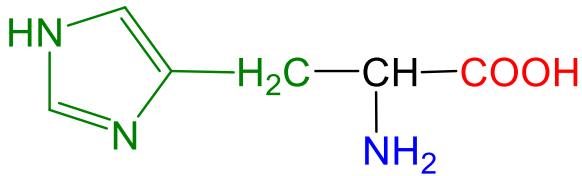
## BAZNE AMINOKISELINE



Arginin, Arg, R

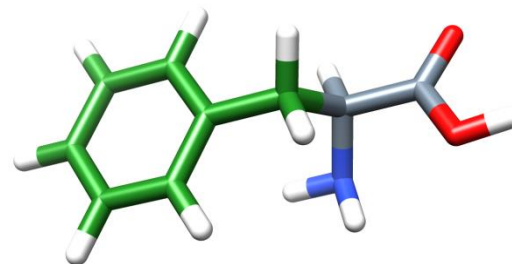
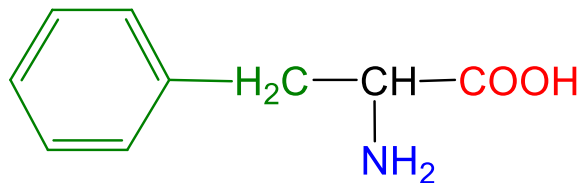


Lizin, Lys, K

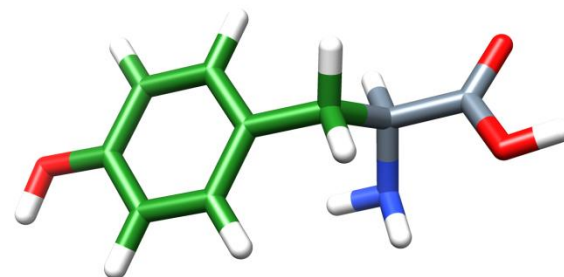
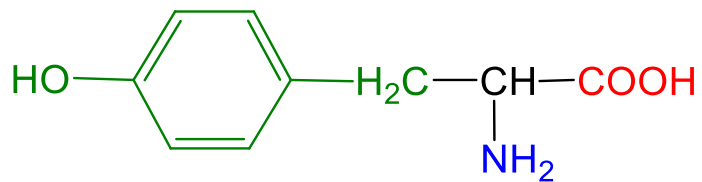


Histidin, His, H

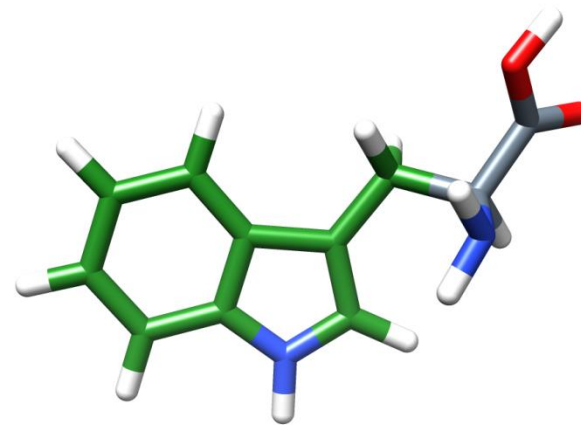
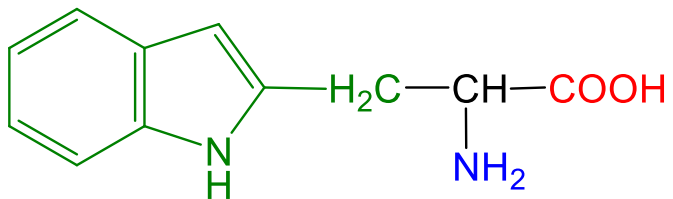
## AROMATIČNE AMINOKISELINE



Fenilalanin, Phe, P

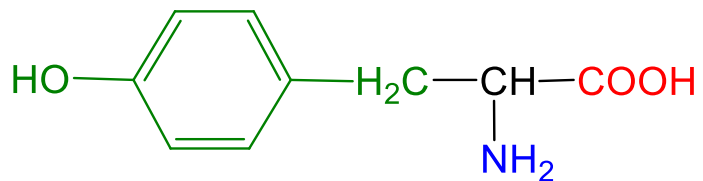


Tirozin, Tyr, Y

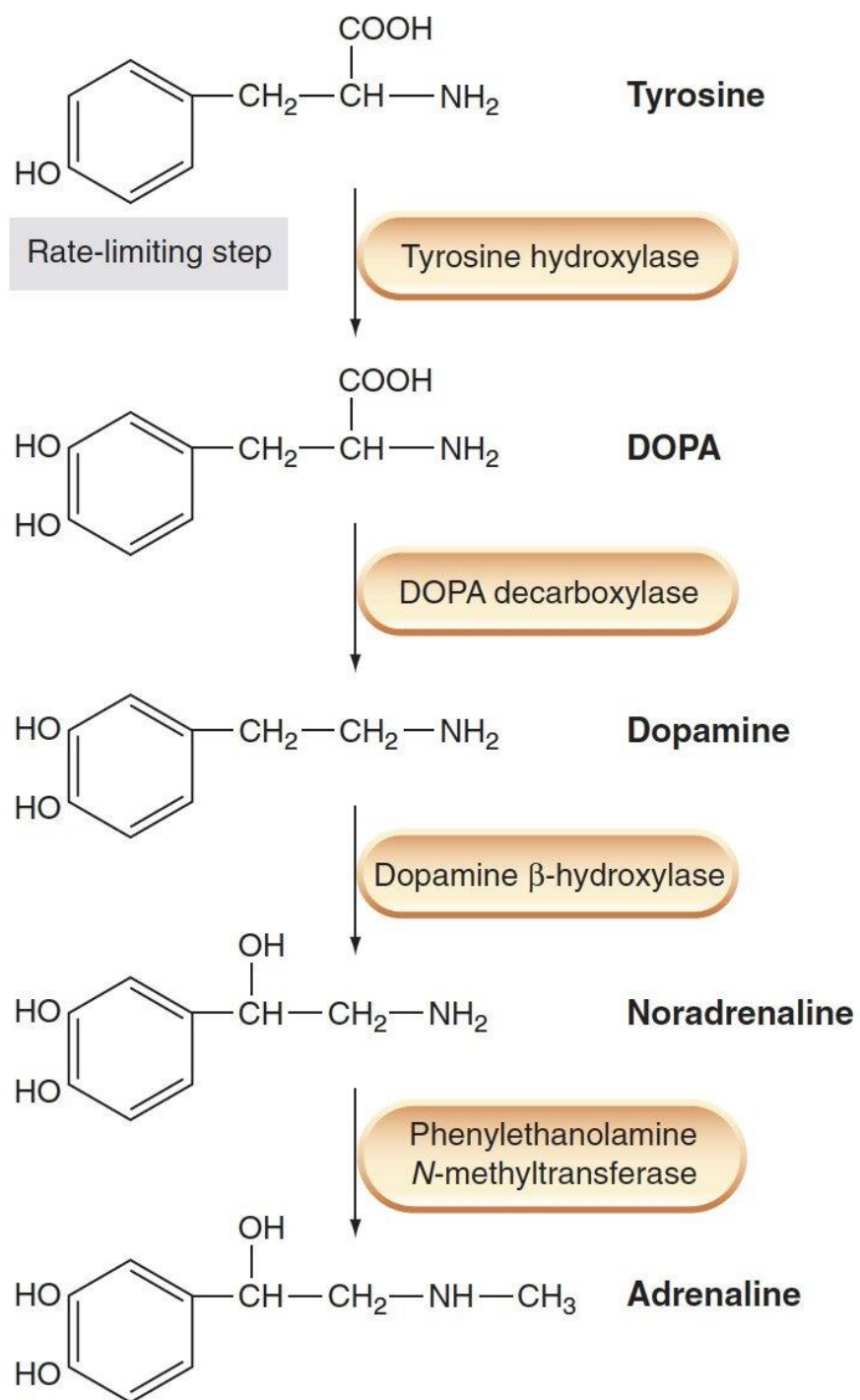


Triptofan, Trp, W

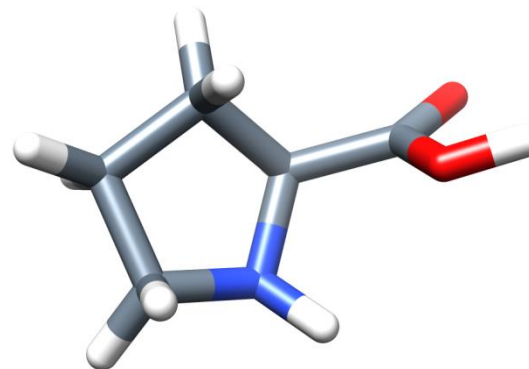
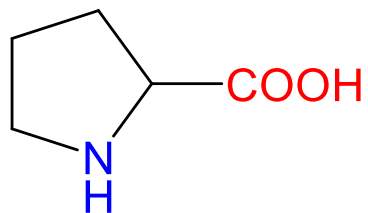
## AROMATIČNE AMINOKISELINE



Tirozin, Tyr, Y

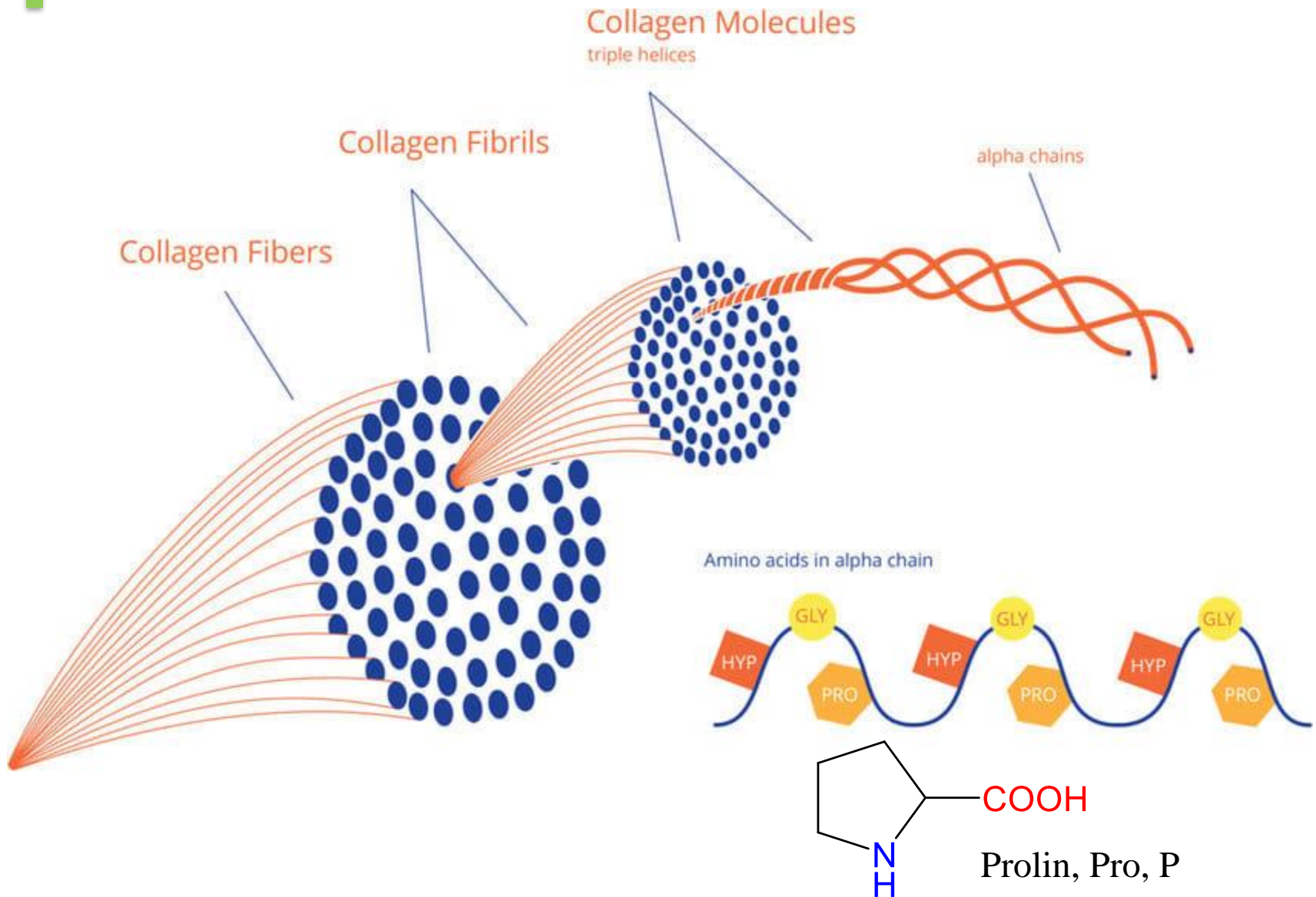


## AMINOKISELINE SA PRSTENOM



Prolin, Pro, P

# AMINOKISELINE SA PRSTENOM

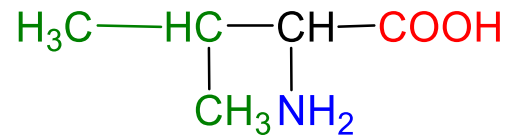
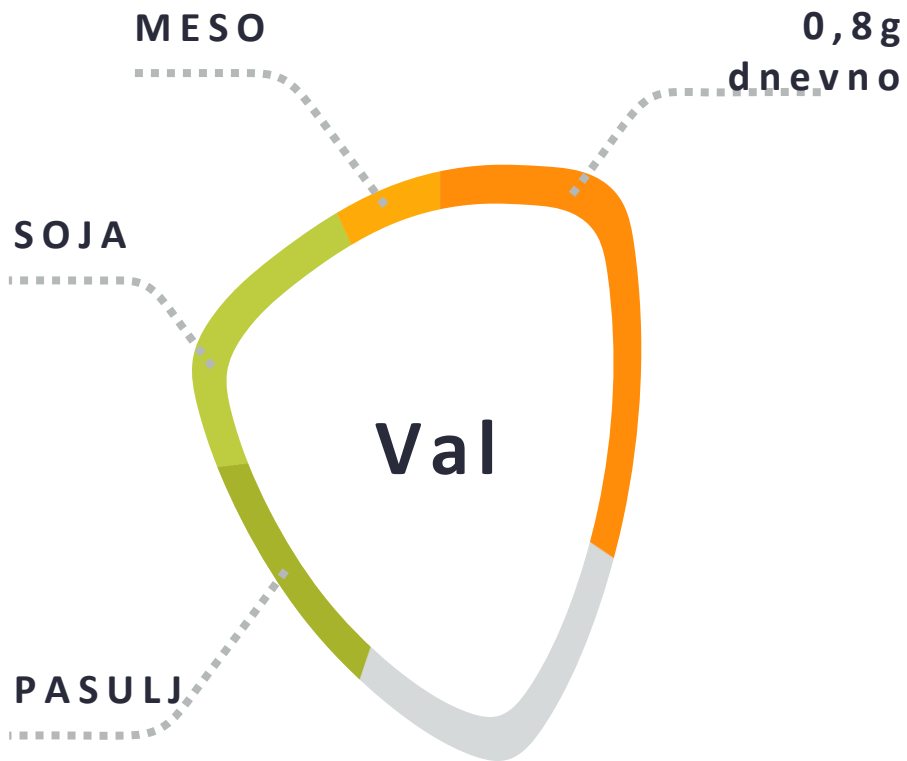


# Esencijalnost

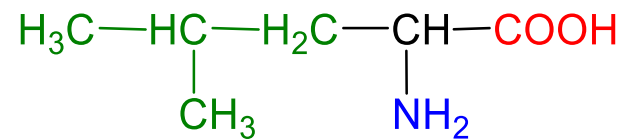
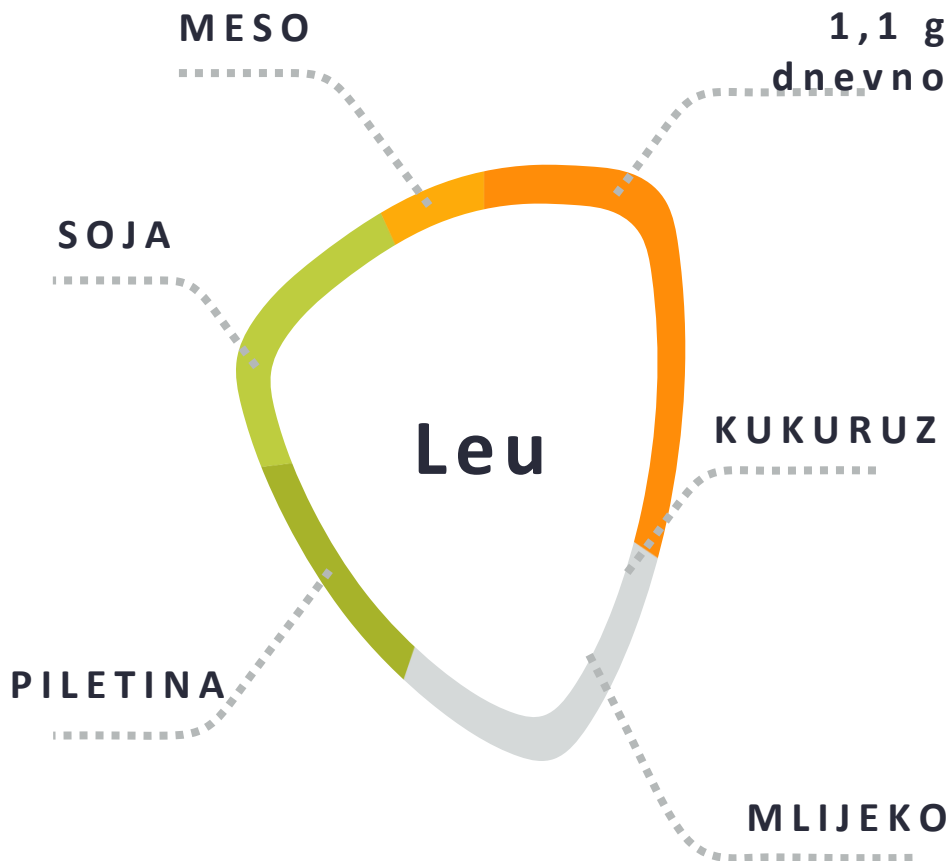
- odnosi se na biološku funkciju aminokiselina
- esencijalne AK ćelija NE MOŽE da biosintetiše
- njihova potreba se mora regulisati eksternim putem (isхранom)



# ESENCIJALNE AMINOKISELINE

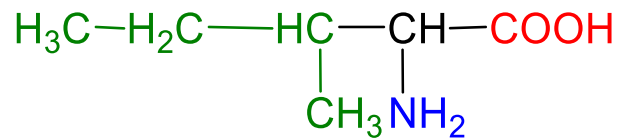
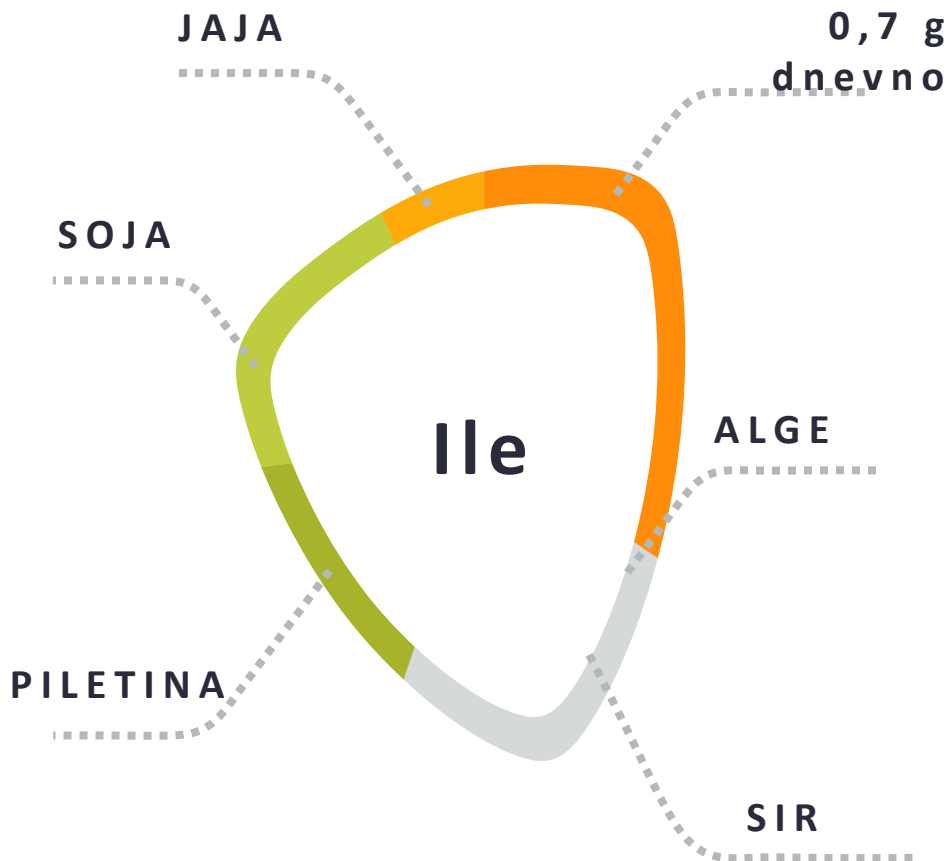


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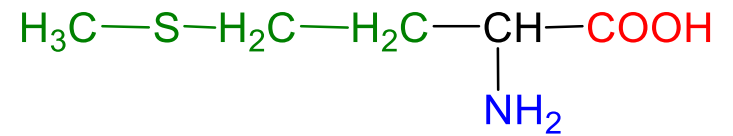
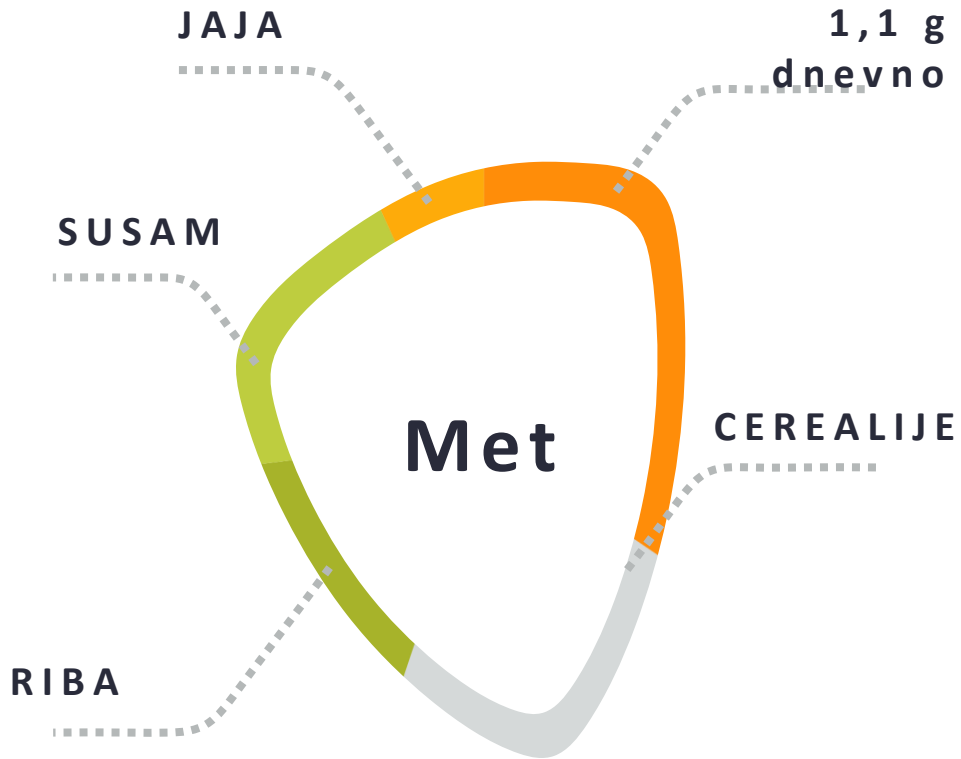




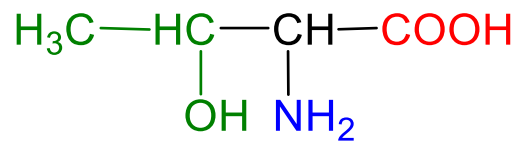
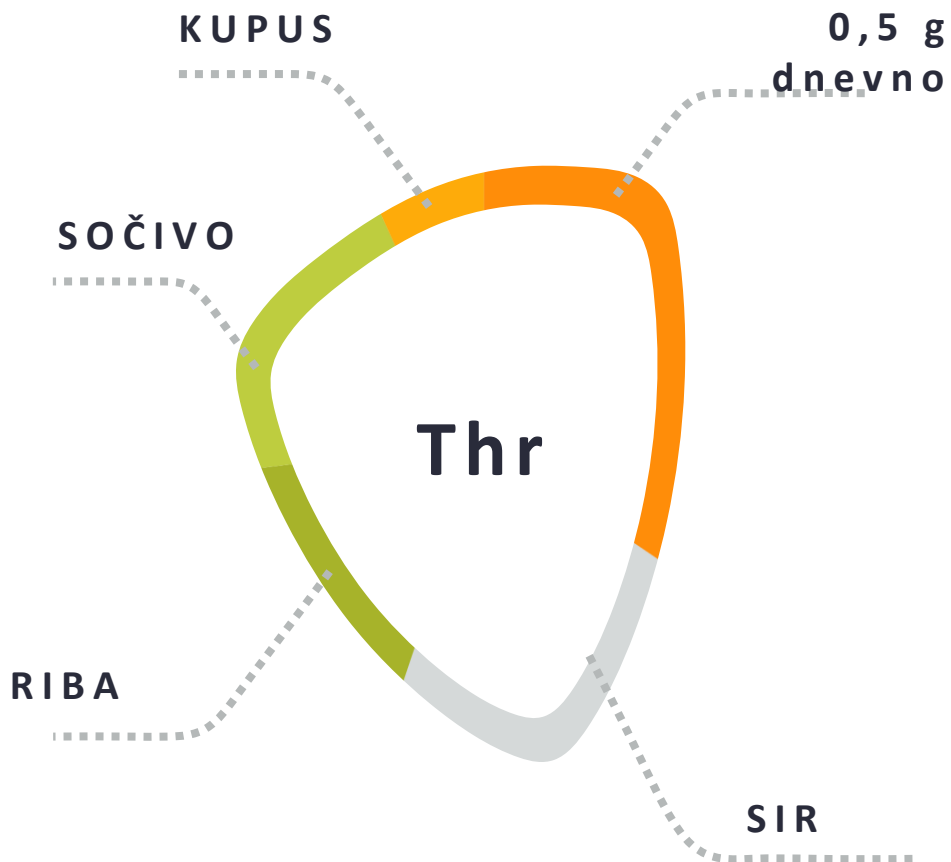
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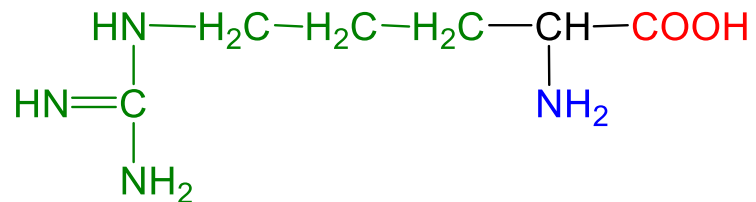
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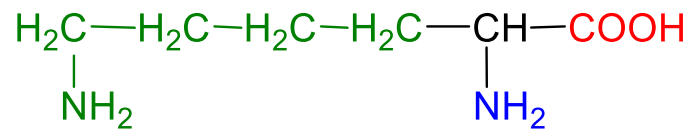
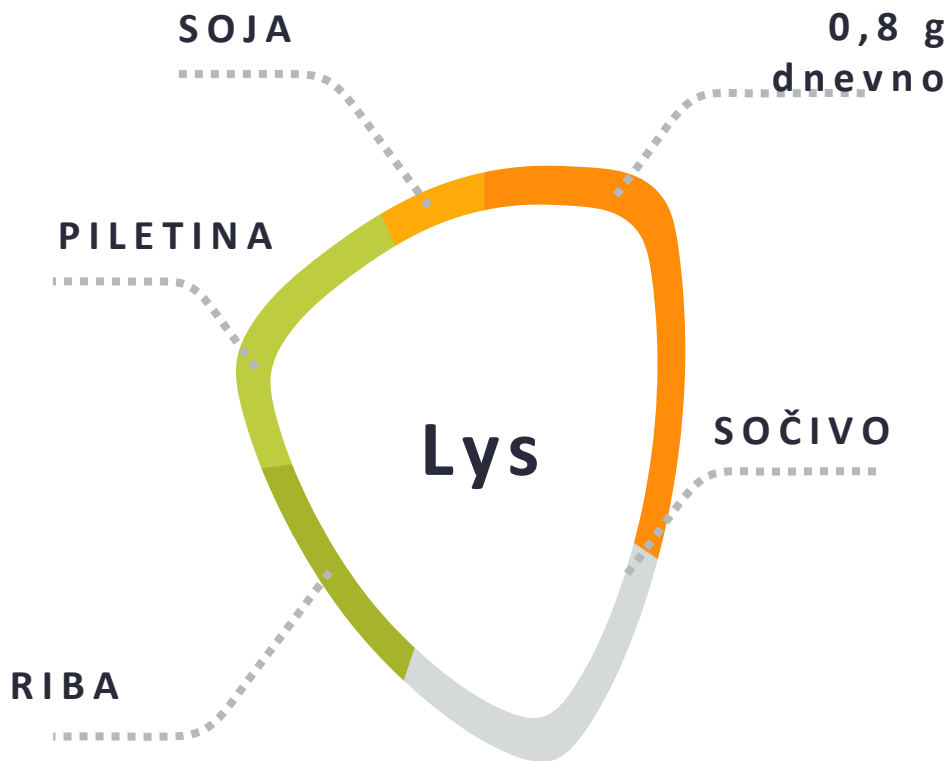
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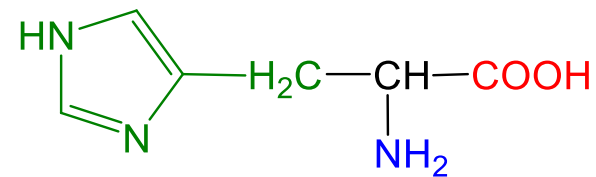
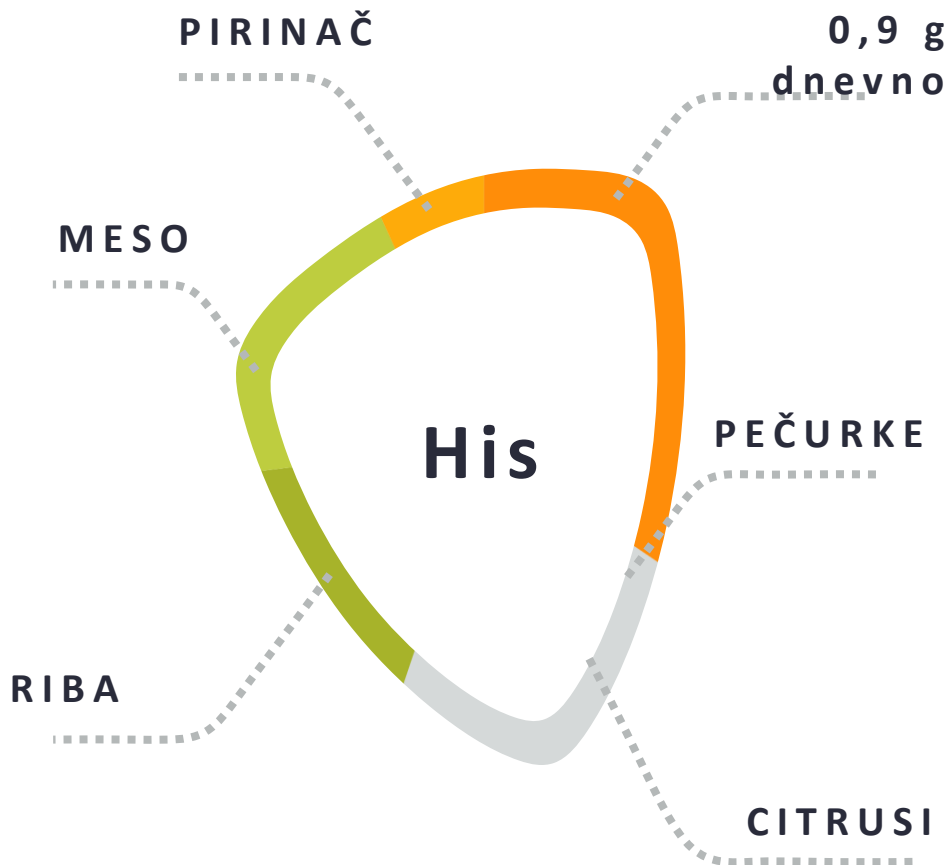
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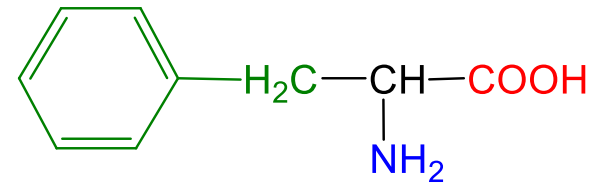
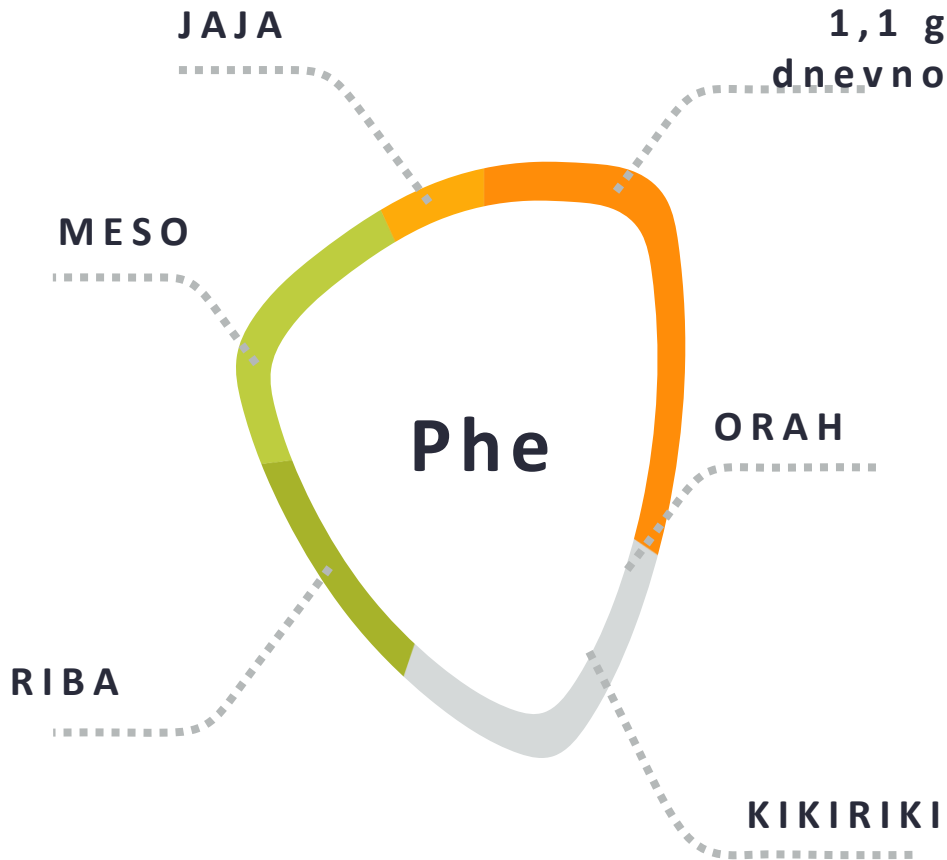
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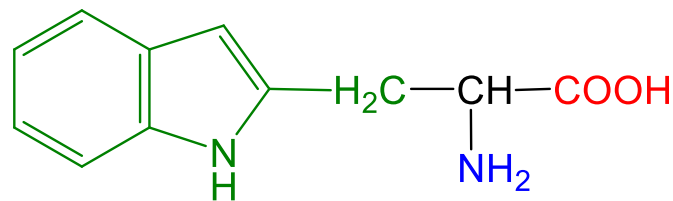
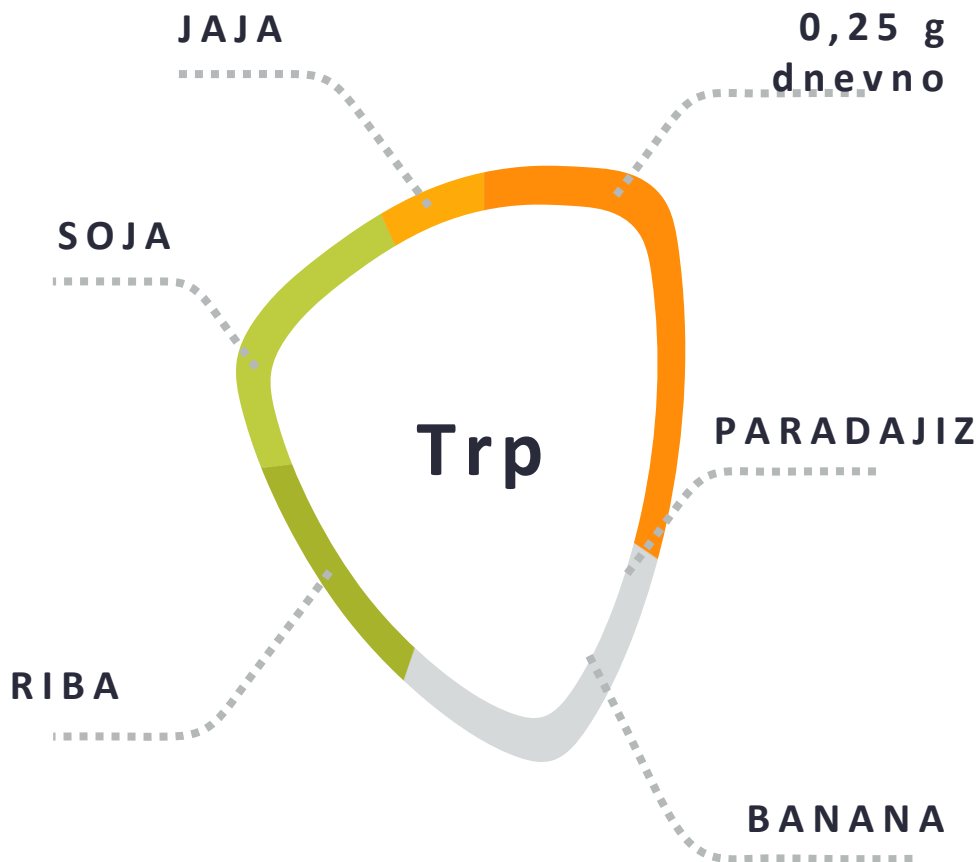
# ESENCIJALNE AMINOKISELINE



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# ESENCIJALNE AMINOKISELINE

Essential amino acids	Non-essential amino acids synthesised from essential amino acids	Non-essential amino acids
Phenylalanine	Tyrosine (from Phe)	Glycine
Methionine	Cysteine (from Met)	Alanine
Lysine		Serine
Threonine		Proline
Leucine		Glutamate
Isoleucine		Glutamine
Valine		Aspartate
Tryptophan		Asparagine
Histidine*		
Arginine*		

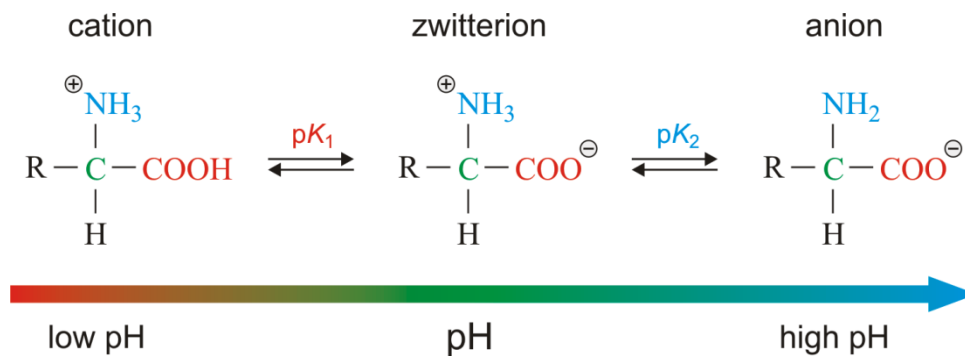
# Zdravo ili ne?



# AMINOKISELINE

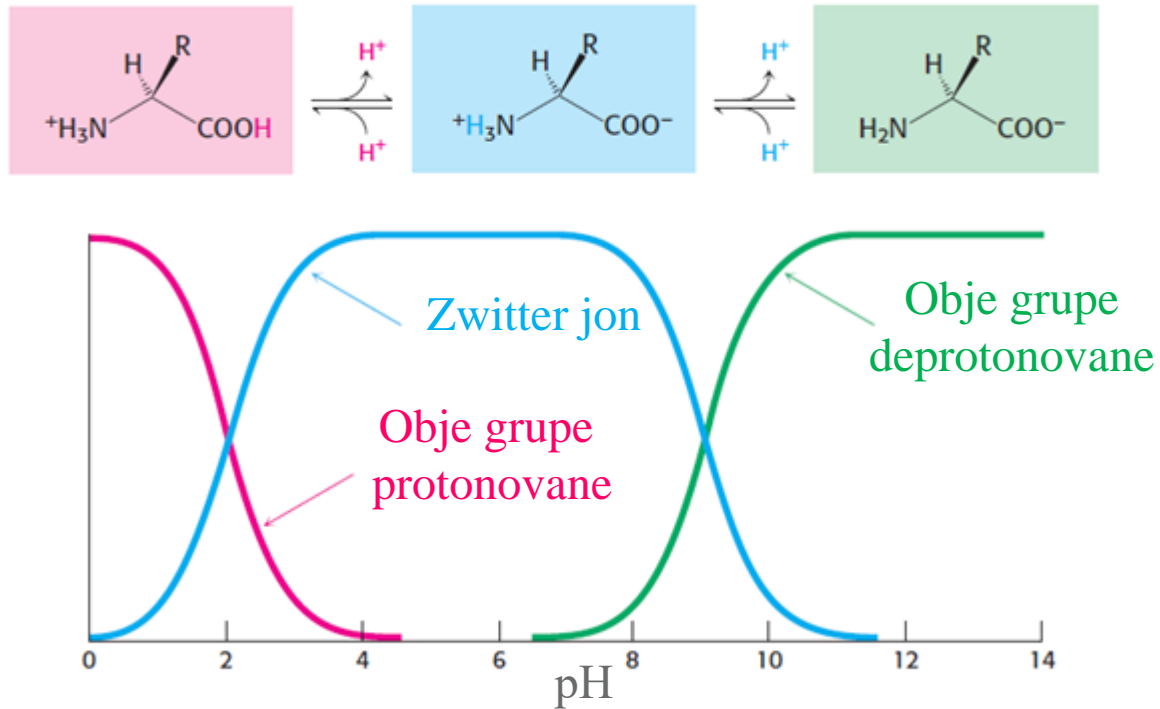
## Hemijsko ponašanje

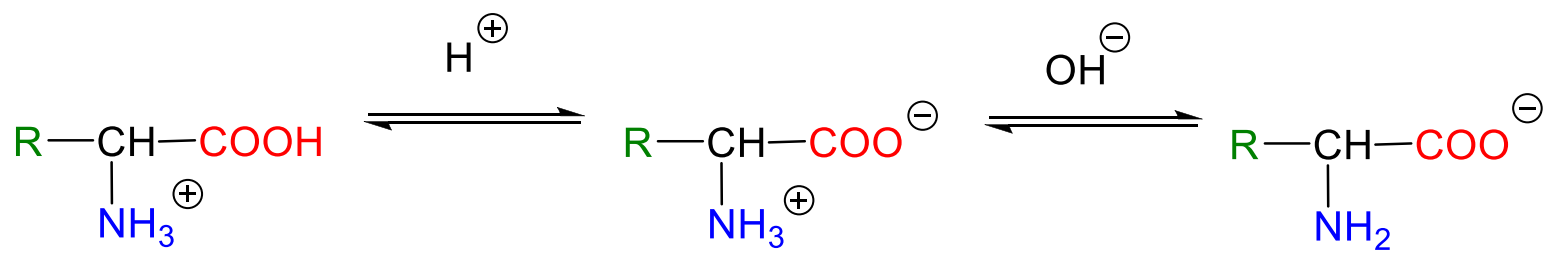
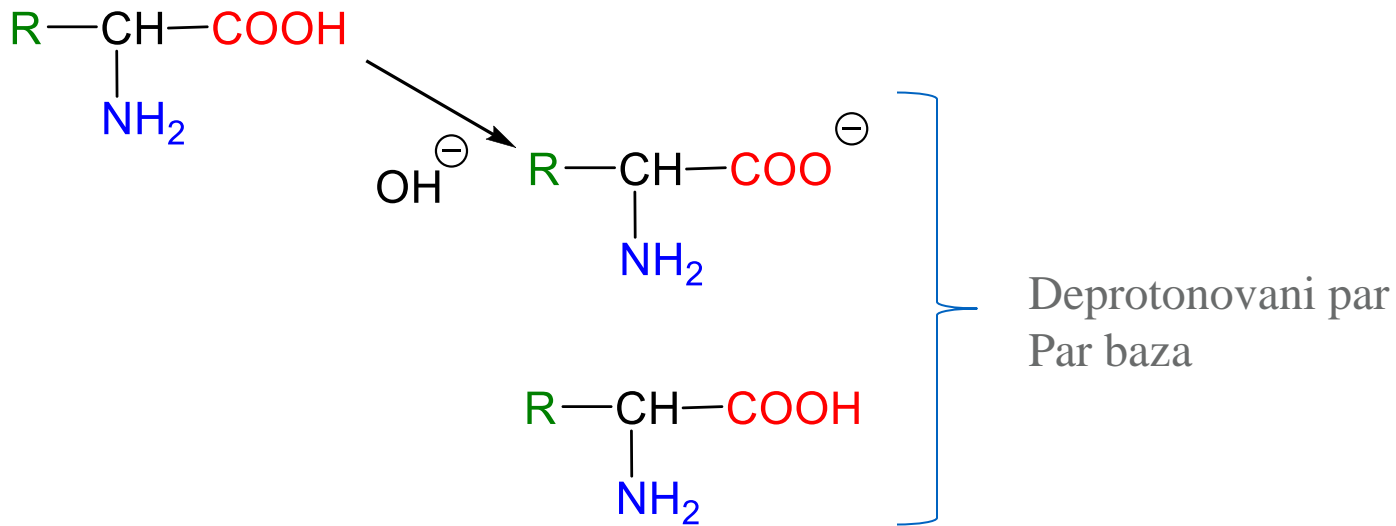
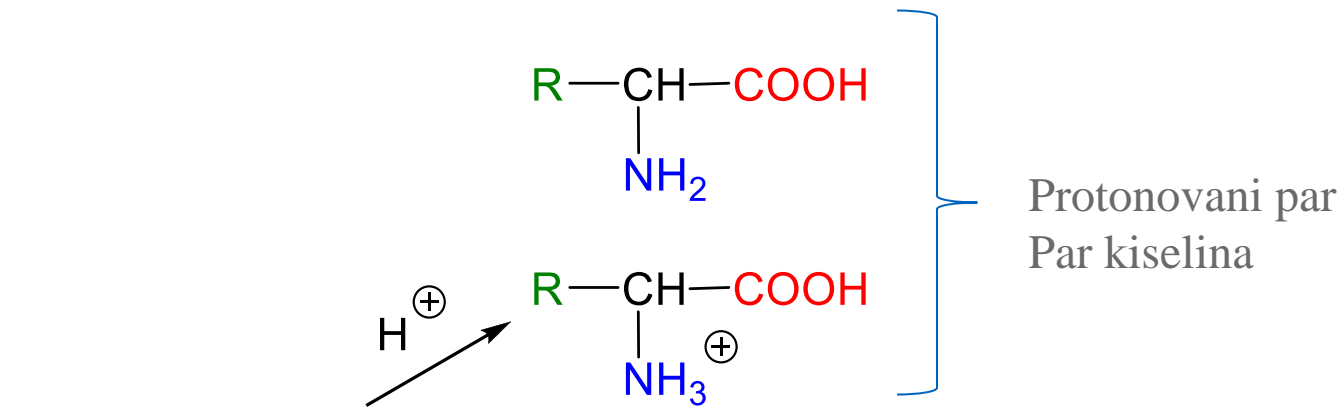
-zbog svoje specifične strukture u kojoj su na jednom C atomu vezane i -COOH i -NH<sub>2</sub> grupa, AK se različito ponašaju u odnosu na pH u kojoj egzistiraju



## Hemijske osobine aminokiselina

- Struktura aminokiseline jeste dinamička ravnoteža svih oblika u kojima ona egzistira u rastvoru

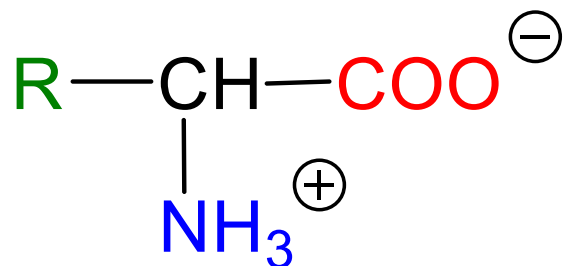




## Hemijske osobine aminokiselina

### Egzistiranje u obliku Zwitter jona uslovljava da:

- Rastvorne u jako polarnim rastvaračima
- Visoka tačka topljenja, na temperaturama višim od 80 °C se raspadaju
- Karakteristični ponašanje u kiseloj i baznoj sredini

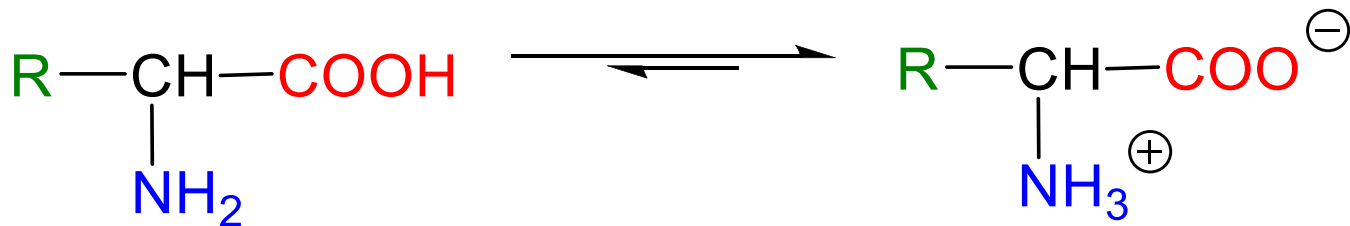


## Hemijske osobine aminokiselina

**Sve aminokiseline izuzev Asp, Glu, His, Tyr su dobro rastvorne u :**

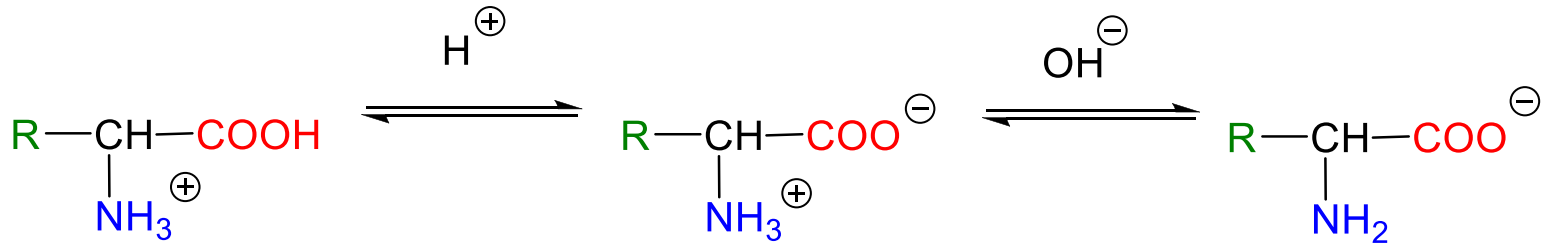
- $\text{H}_2\text{O}$
- $\text{NH}_3$
- Polarnim rastvaračima (EtAc, DMSO, THF..)

**U slabo polarnim (MeOH, EtOH..) i nepolarnim ( $\text{CCl}_4$ ,  $\text{CHCl}_3$ ) su nerastvorne**

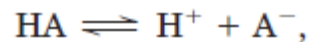


Ravnoteža pomjerena ka energetski stabilnijoj strukturi

## Hemijske osobine aminokiselina



$$K_{a1} = \frac{\left[ \begin{array}{c} \text{R}-\text{CH}-\text{COO}^{\ominus} \\ | \\ \text{NH}_3^{\oplus} \end{array} \right] \left[ \text{H}^{\oplus} \right]}{\left[ \begin{array}{c} \text{R}-\text{CH}-\text{COOH} \\ | \\ \text{NH}_3^{\oplus} \end{array} \right]} \quad K_{a2} = \frac{\left[ \begin{array}{c} \text{R}-\text{CH}-\text{COO}^{\ominus} \\ | \\ \text{NH}_3^{\oplus} \end{array} \right] \left[ \text{OH}^{\ominus} \right]}{\left[ \begin{array}{c} \text{R}-\text{CH}-\text{COO}^{\ominus} \\ | \\ \text{NH}_2 \end{array} \right] \left[ \text{H}_2\text{O} \right]}$$



$$K_{\text{eq}} = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} = K_a$$

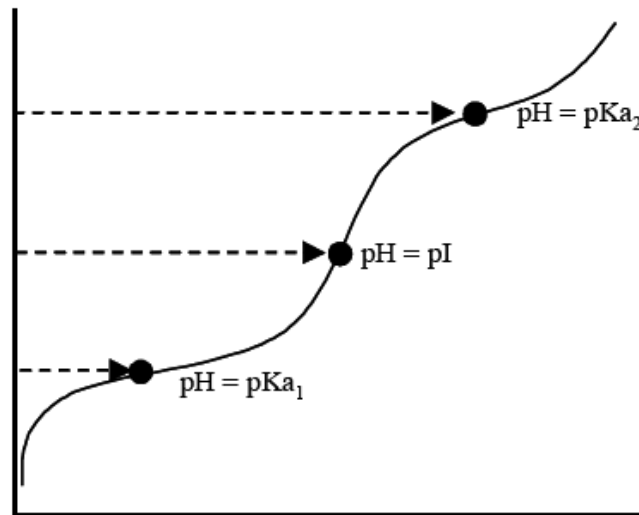


## Hemijske osobine aminokiselina

$$pK_a = \log \frac{1}{K_a} = -\log K_a$$

$$pH = pK_a - \log \frac{[HA]}{[A^-]}$$

- Što je mogućnost disocijacije protona veća, kiselina je jača i njena pKa vrijednost je manja
- Za tačno određivanje kiselosti aminokiselina potrebno je konstruisati titracionu krivu

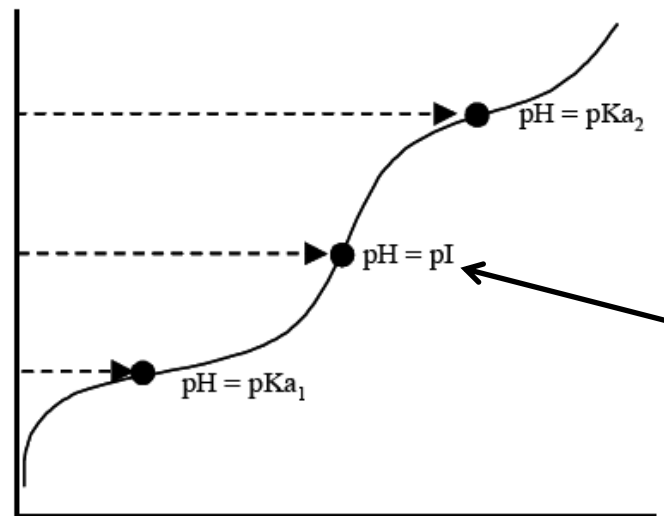


## Hemijske osobine aminokiselina

$$pK_a = \log \frac{1}{K_a} = -\log K_a$$

$$pH = pK_a - \log \frac{[HA]}{[A^-]}$$

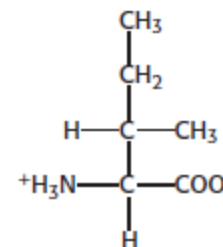
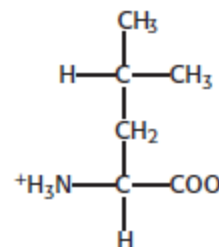
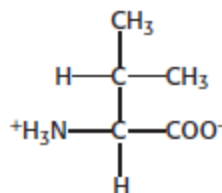
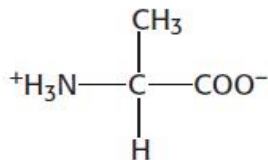
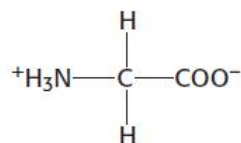
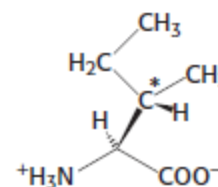
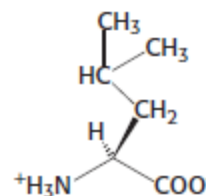
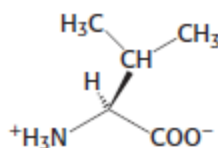
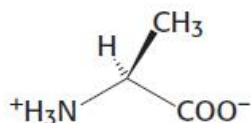
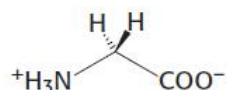
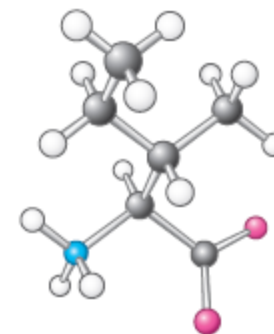
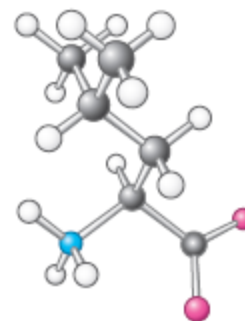
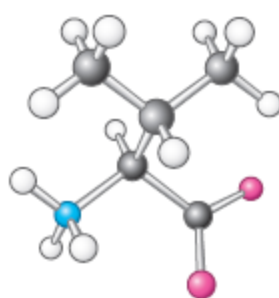
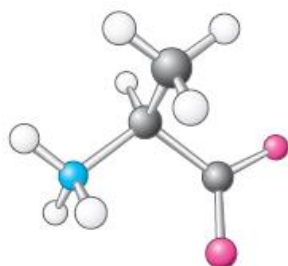
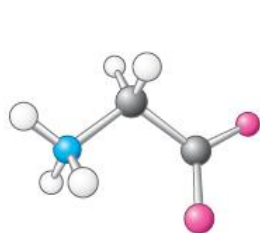
- Što je mogućnost disocijacije protona veća, kiselina je jača i njena pKa vrijednost je manja
- Za tačno određivanje kiselosti aminokiselina potrebno je konstruisati titracionu krivu



Najmanju rastvorljivost u vodi aminokiseline pokazuju na pI

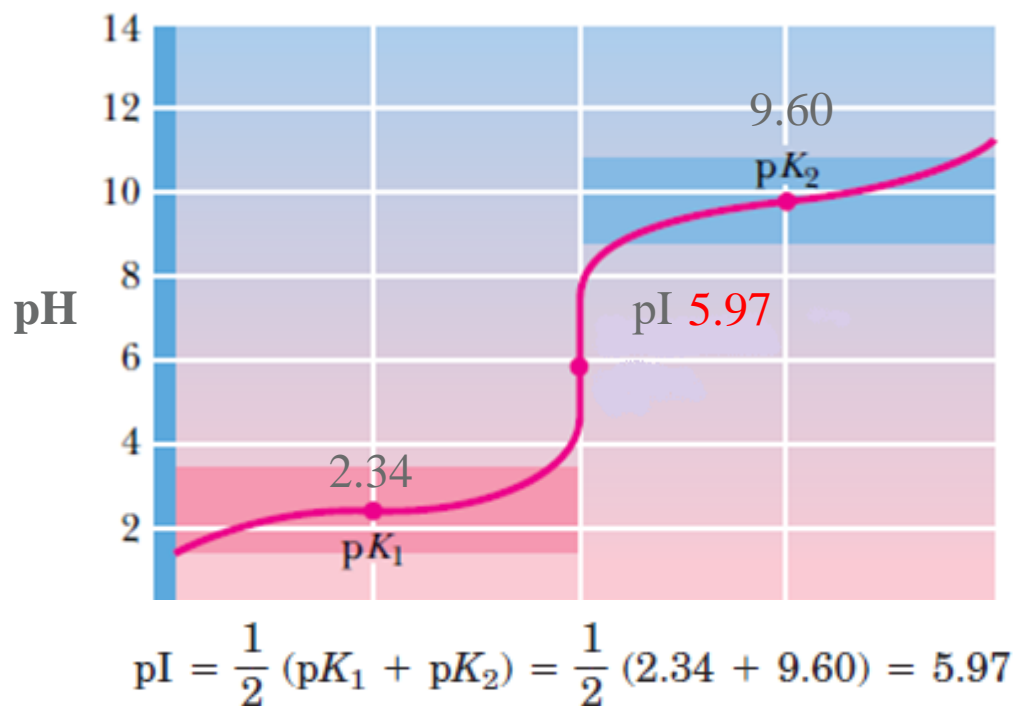
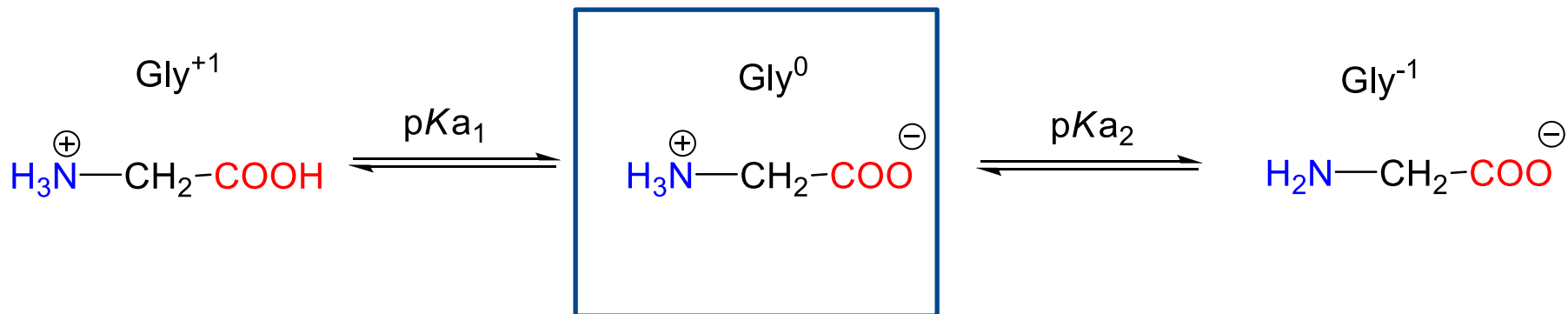
# Hemijske osobine aminokiselina

## Hemijsko ponašanje neutralnih aminokiselina



## Hemijske osobine aminokiselina

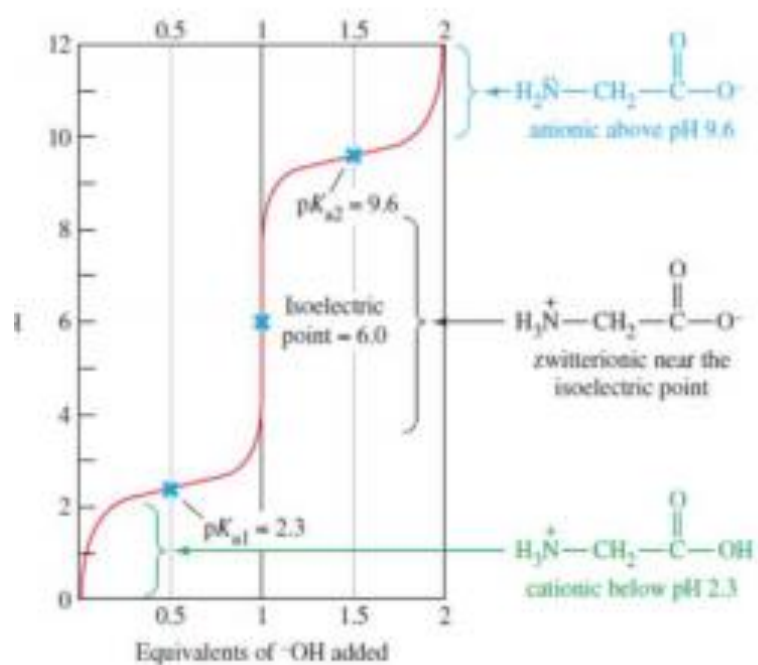
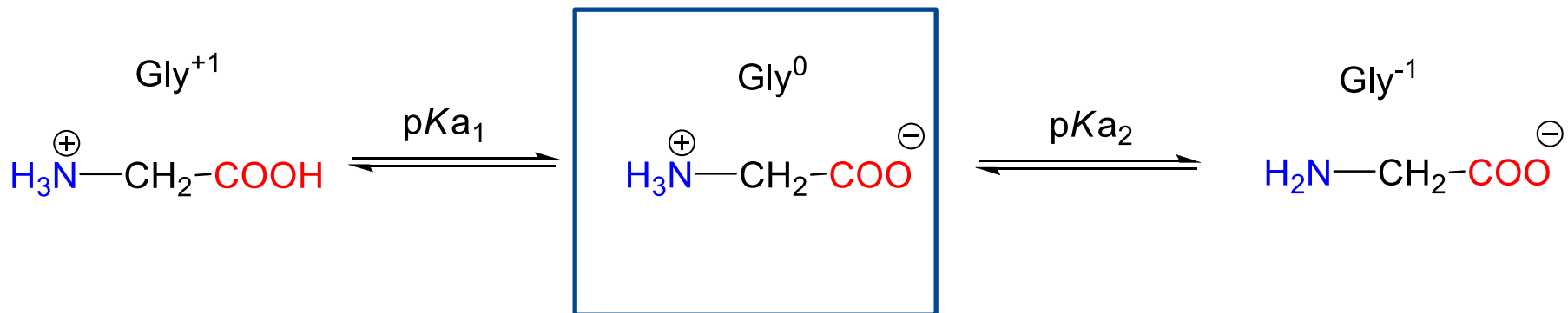
### Hemijsko ponašanje neutralnih aminokiselina



$$K_1 = \frac{[\text{Gly}^0][\text{H}_3\text{O}^+]}{[\text{Gly}^+]}$$
$$K_2 = \frac{[\text{Gly}^-][\text{H}_3\text{O}^+]}{[\text{Gly}^0]}$$

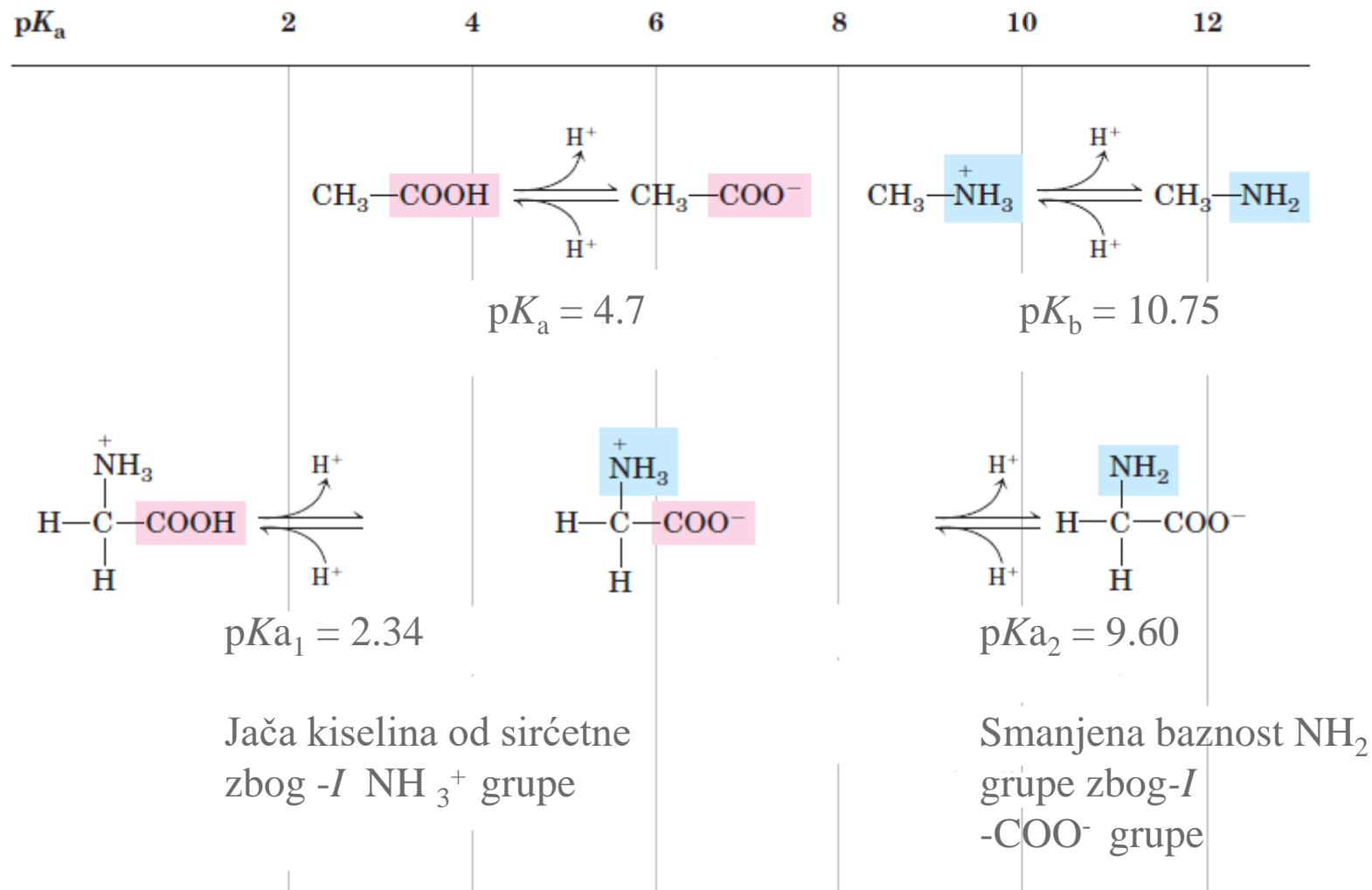
## Hemijske osobine aminokiselina

### Hemijsko ponašanje neutralnih aminokiselina



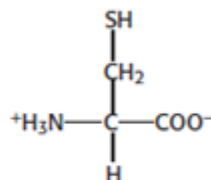
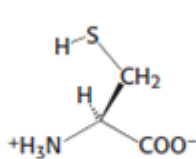
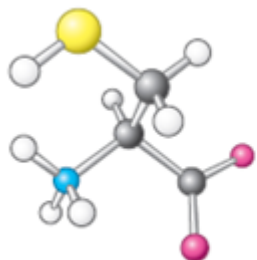
# Hemijske osobine aminokiselina

## Hemijsko ponašanje neutralnih aminokiselina

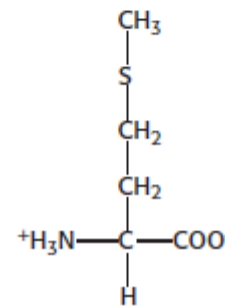
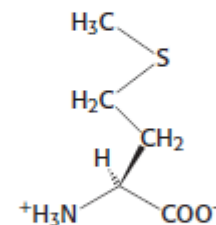
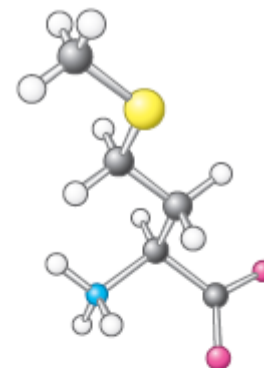


## Hemijske osobine aminokiselina

### Hemijsko ponašanje aminokiselina sa sumporom



Cys

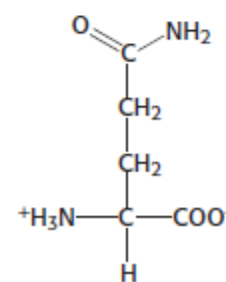
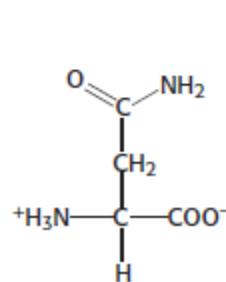
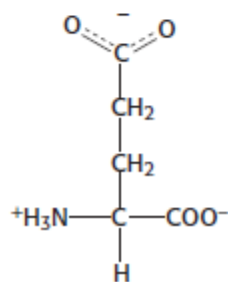
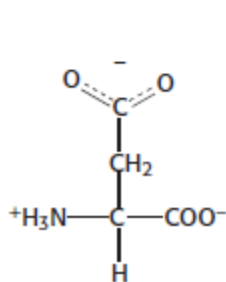
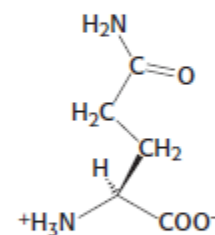
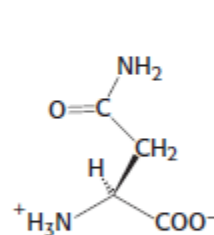
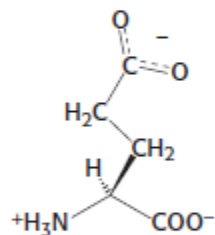
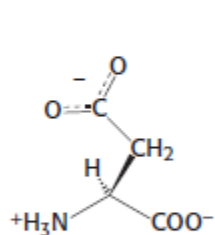
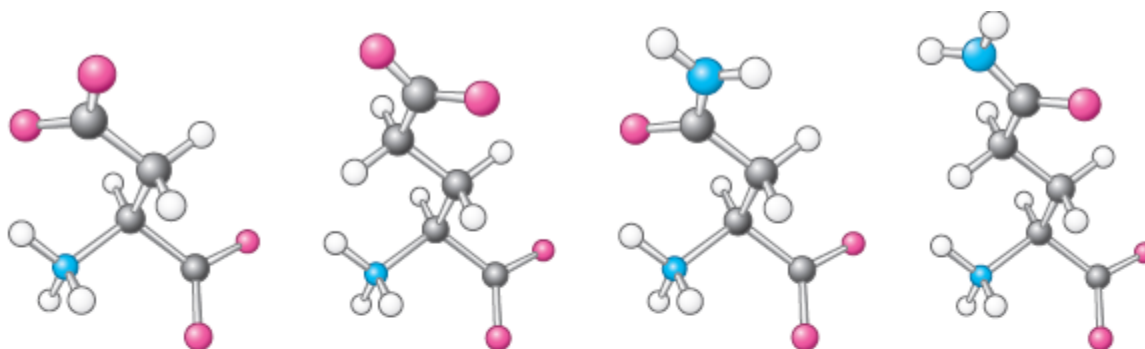


Met

Postoji mogućnost disocijacije tiolne grupe u jako baznoj sredini. Na titracionoj krivoj postoji  $pK_{a3} = pK_{aR}$

## Hemijske osobine aminokiselina

Hemijsko ponašanje dikarbonskih aminokiselina i amida dikarbonskih kiselina



Asp

Glu

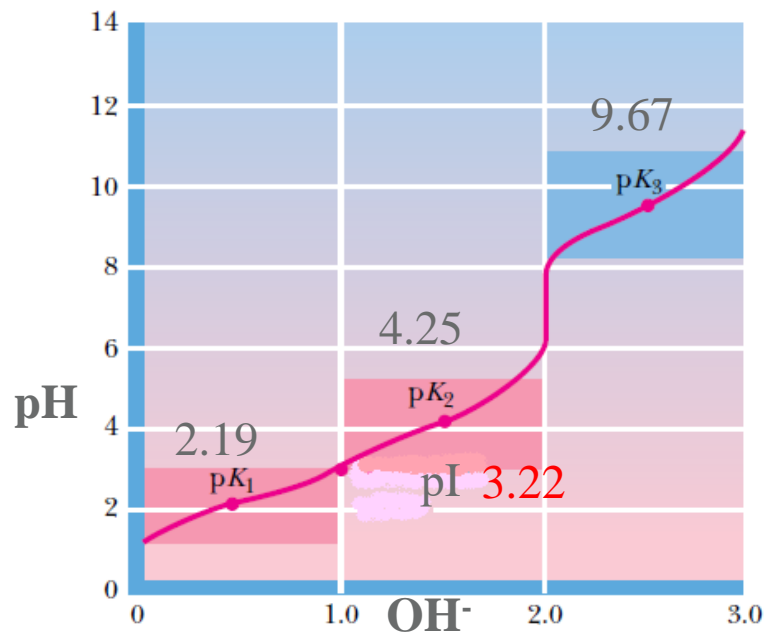
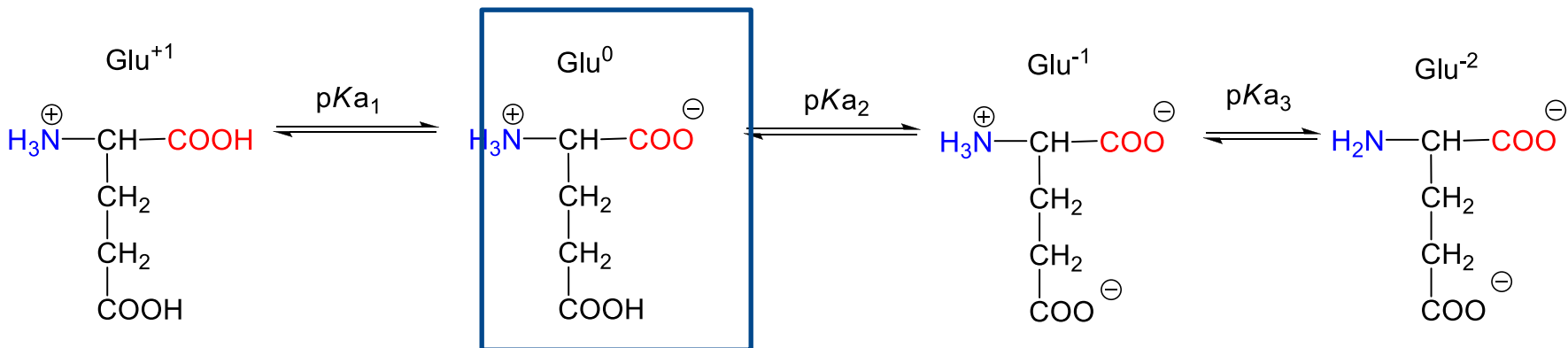
Asp

Gln



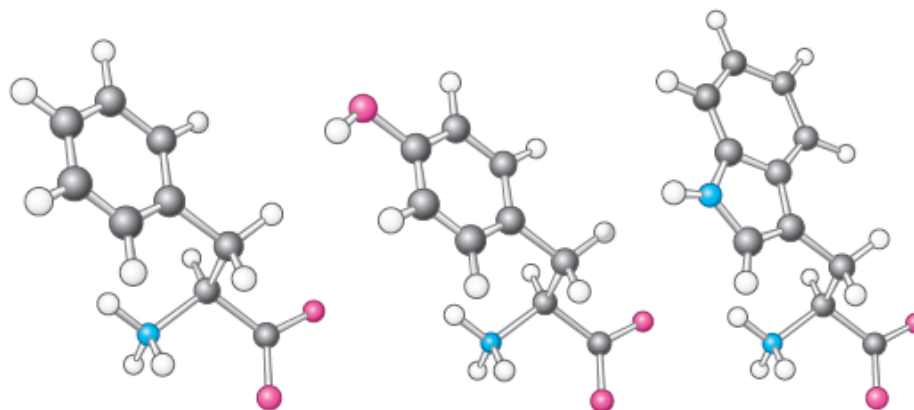
## Hemijske osobine aminokiselina

Hemijsko ponašanje dikarbonskih aminokiselina i amida dikarbonskih kiselina

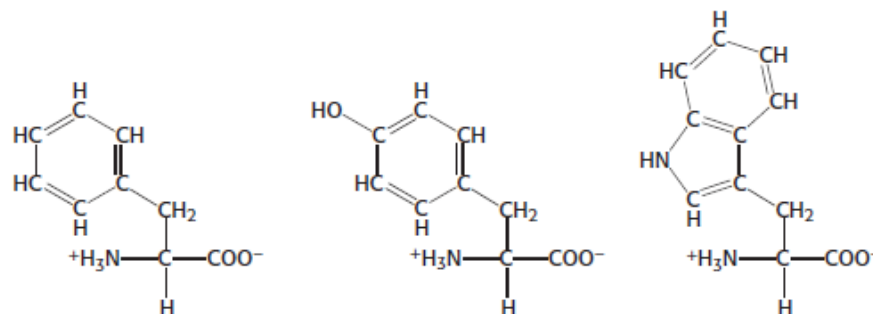
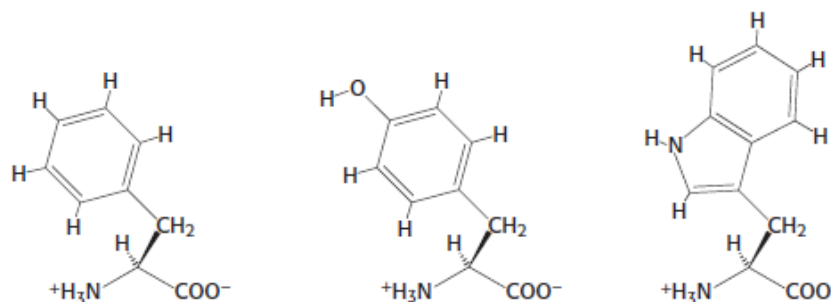


# Hemijske osobine aminokiselina

## Hemijsko ponašanje aromatičnih aminokiselina



Moguća disocijacija u ekstremnim pH uslovima



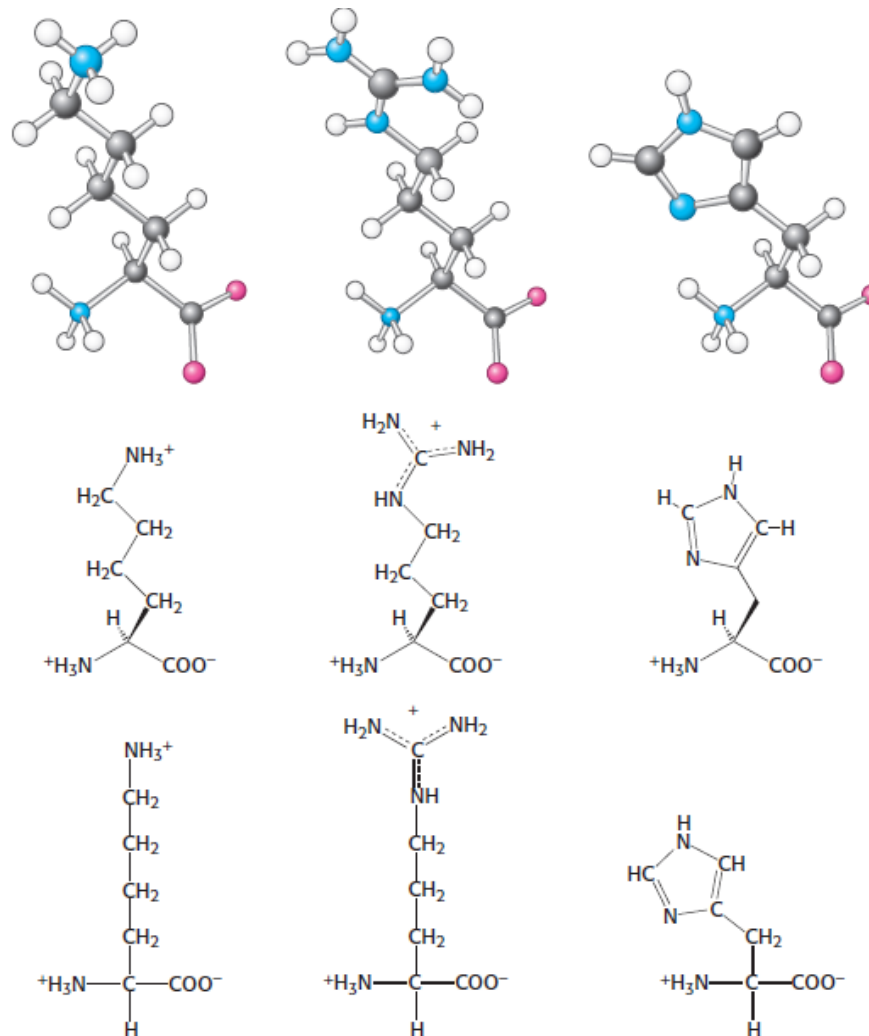
Phe

Tyr

Trp

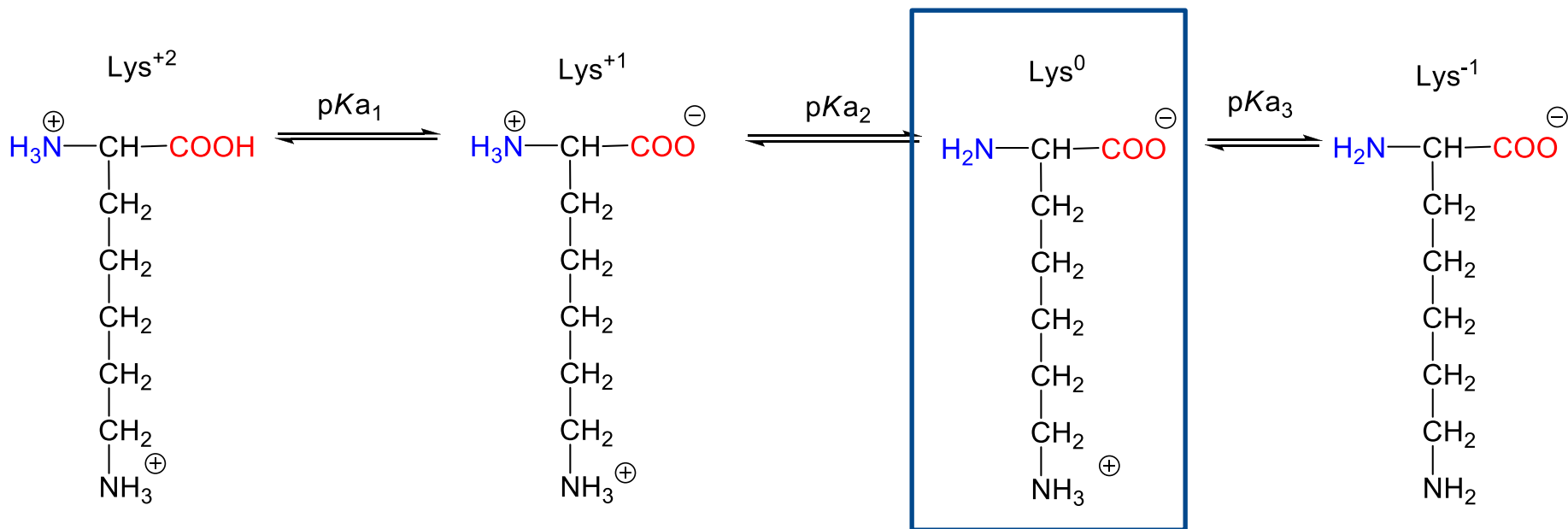
# Hemijske osobine aminokiselina

## Hemijsko ponašanje baznih aminokiselina



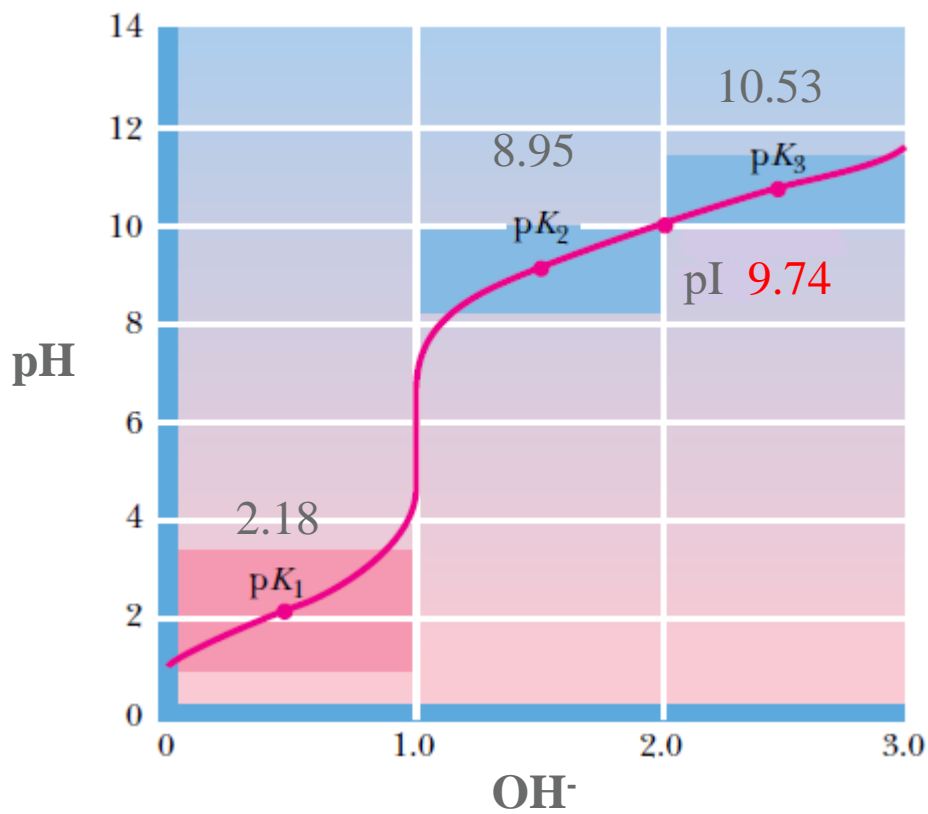
# Hemijske osobine aminokiselina

## Hemijsko ponašanje baznih aminokiselina



## Hemijske osobine aminokiselina

### Hemijsko ponašanje baznih aminokiselina



# AMINOKISELINE

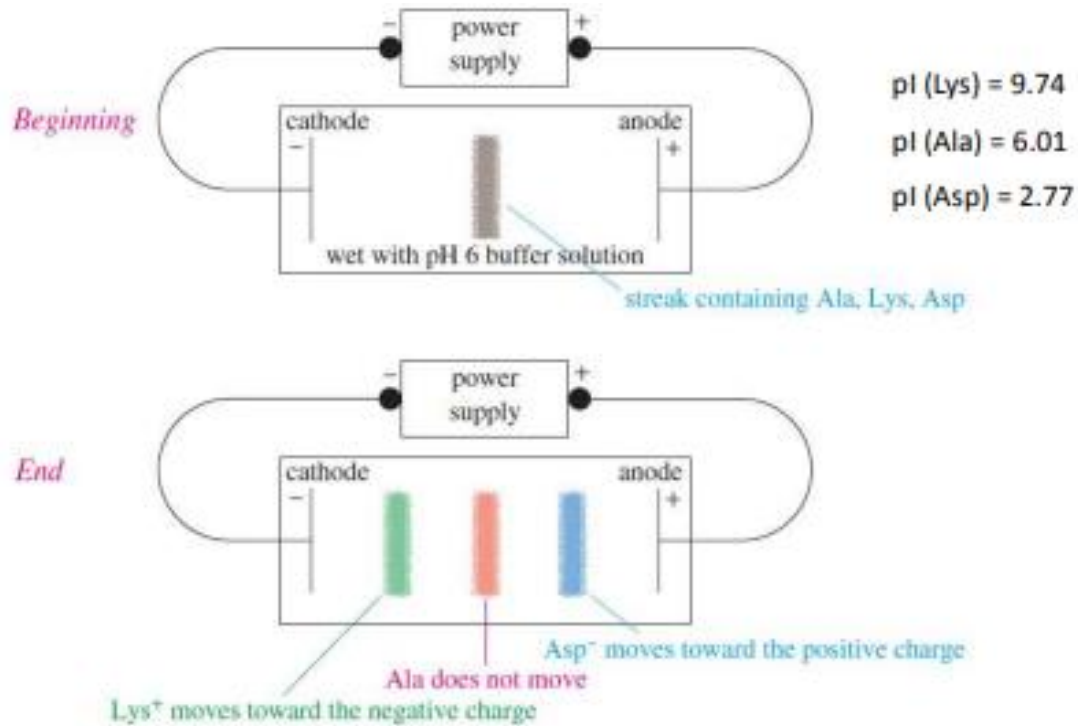
## Odvajanje

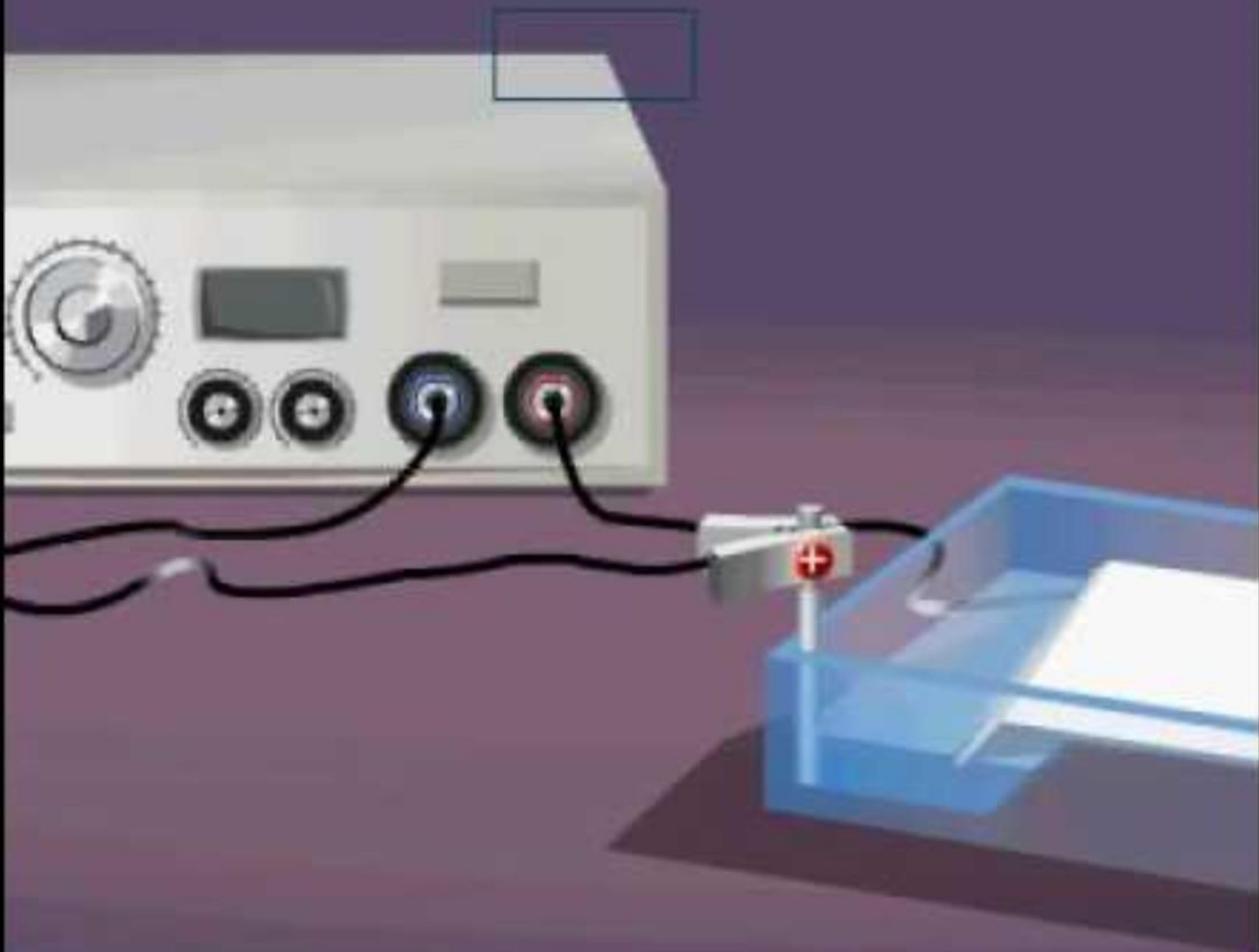
- Različite AK imaju različito  $pI$  na različitim  $pH$
- Podešavanjem  $pH$  mogu se odvojiti AK u rastvoru



# Aminokiseline odvajanje

## Odvajanje elektroforezom

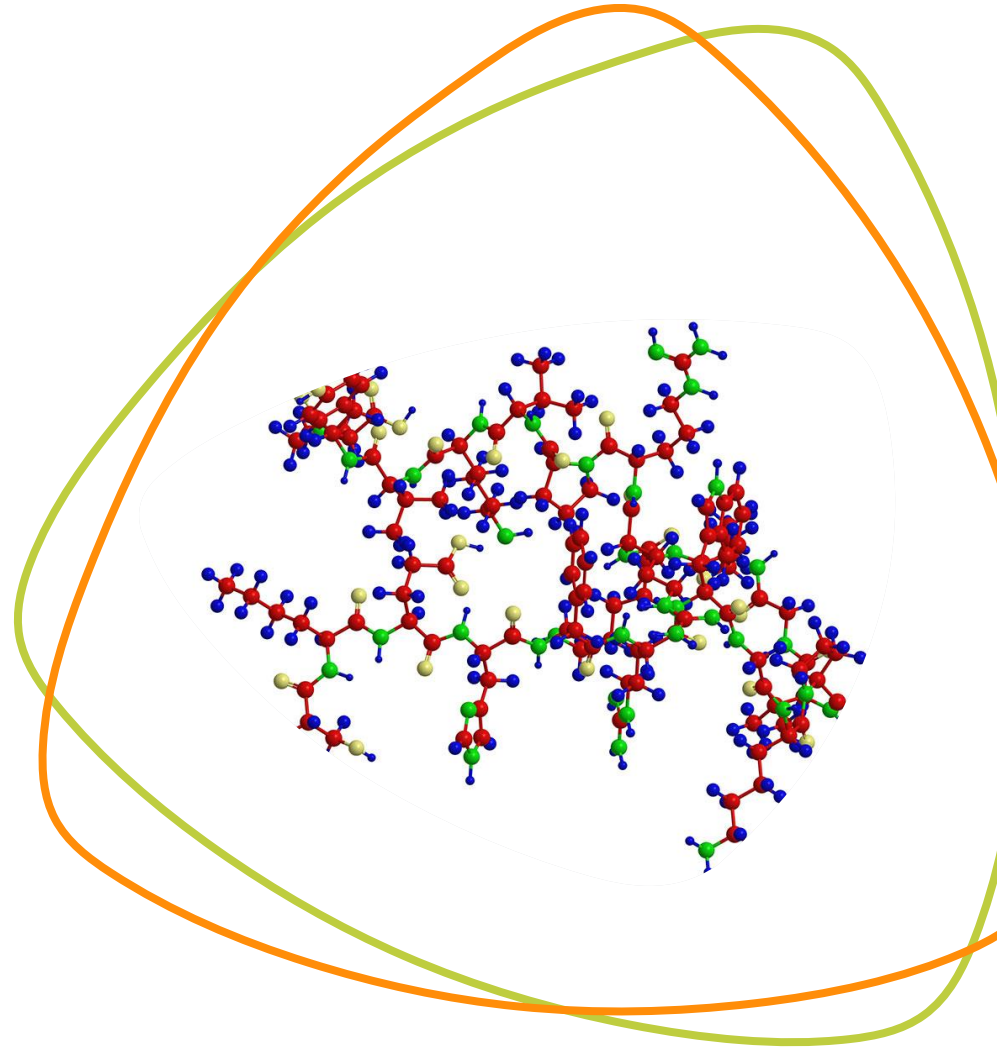






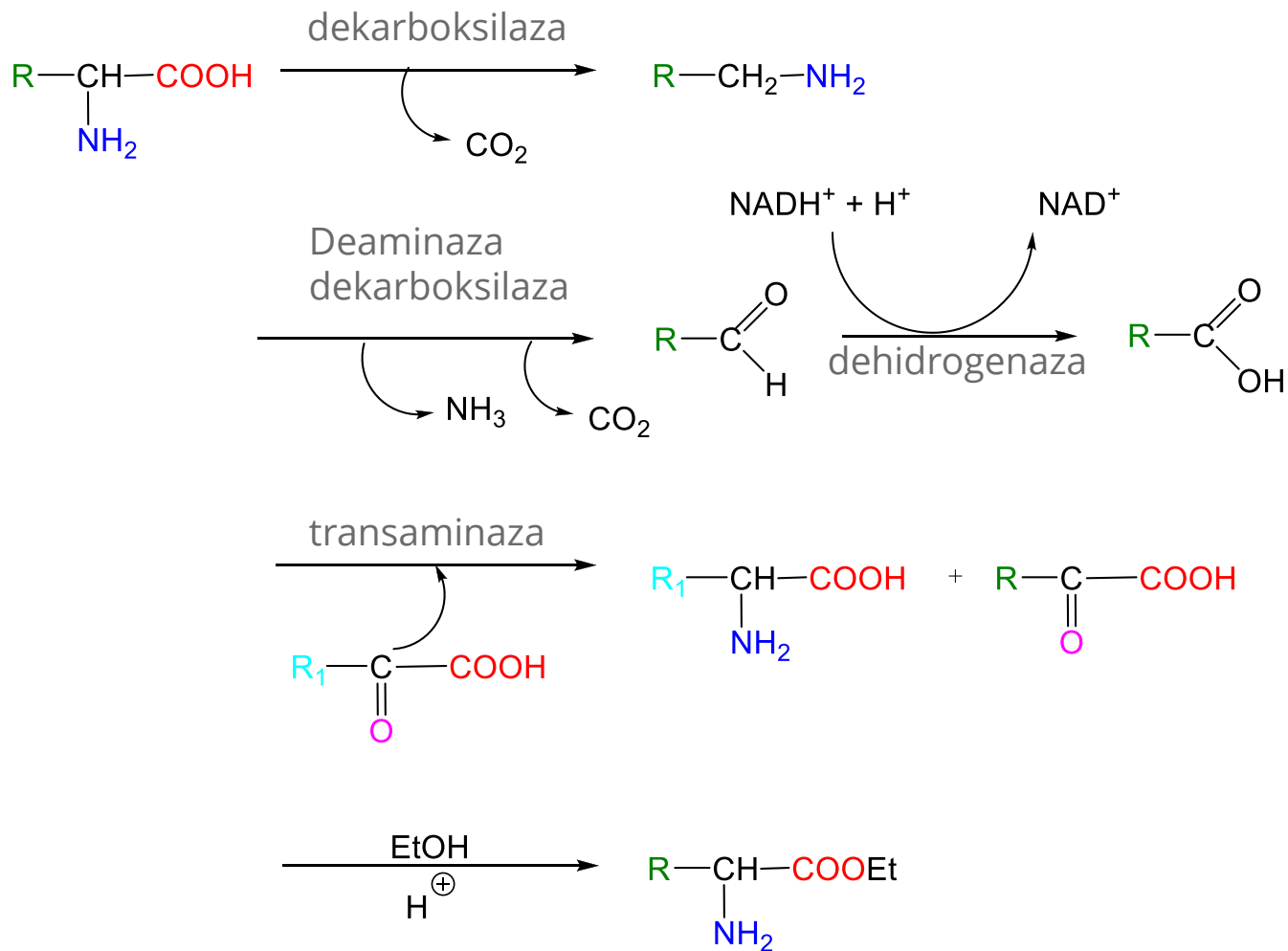
# AMINOKISELINE

## Hemijske reakcije



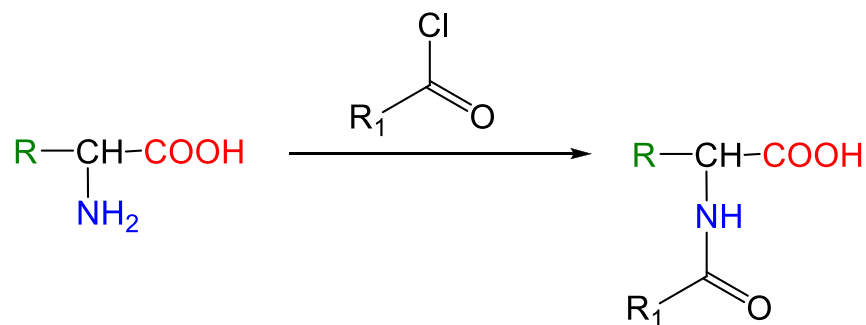
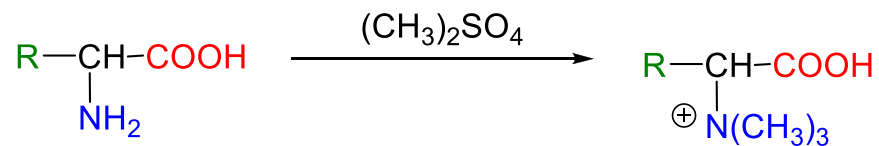
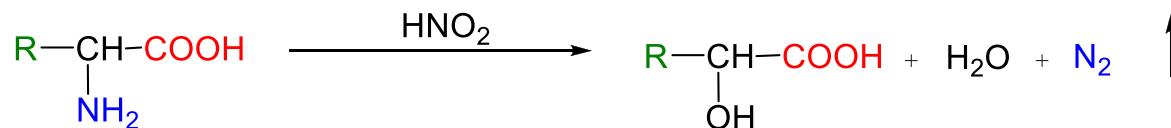
# Aminokiseline

## Hemijske reakcije



# Aminokiseline

## Hemijske reakcije



# AMINOKISELINE

## DOBIJANJE AMINOKISELINA

HEMIJSKA SINTEZA -D, L-AK

IZOLOVANJE IZ BIOLOŠKOG  
MATERIJALA -L-AK

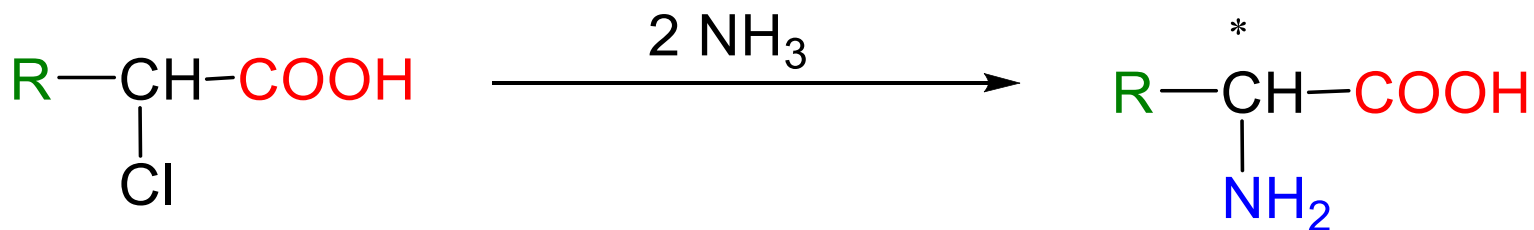
MIKROBIOLOŠKI POSTUPCI  
DOBIJANJA -D,L-AK

## Aminokiseline

### Dobijanje

#### Hemijska sinteza

- **Uvijek se dobija racemska smješa L- i D-izomera**
- ***Izomere je potrebno odvojiti***
- ***Najjednostavniji postupak dobijanja je amonoliza halogenkarbonskih kiselina***

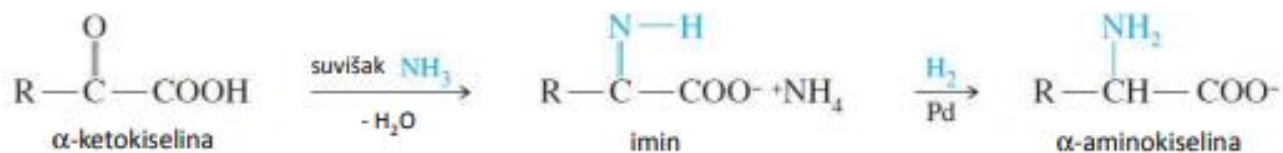


# Aminokiseline

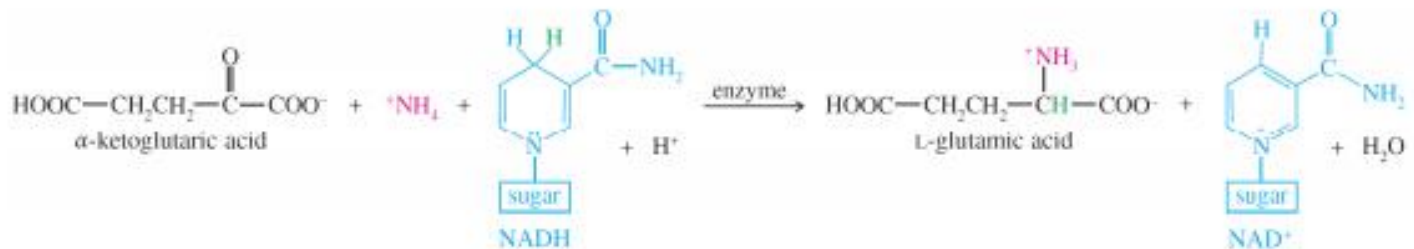
## Dobijanje

### Hemijska sinteza

- Reduktivna aminacija**



• reduktivna aminacija je **biomimetička** metoda, jako nalikuje biosintezi aminokiselina:

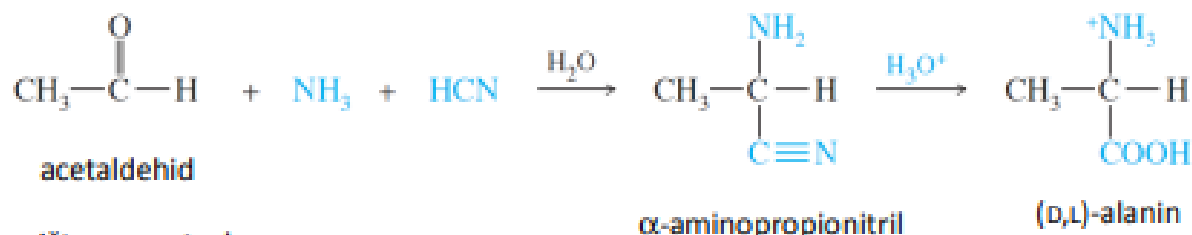


# Aminokiseline

## Dobijanje

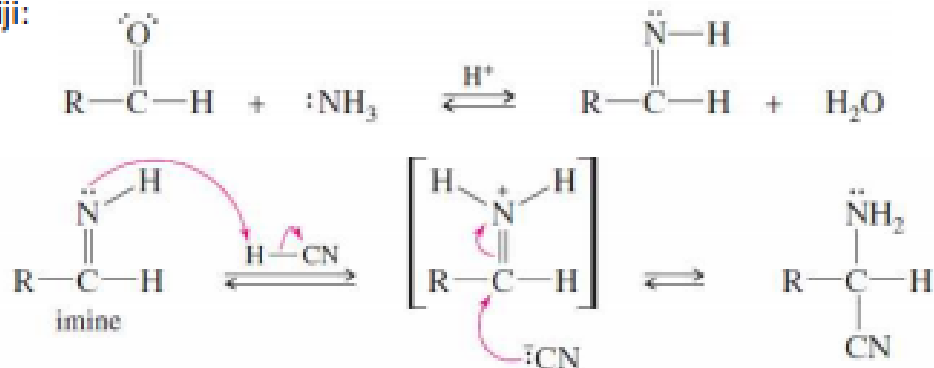
### Hemijska sinteza

- Streckerova sinteza**



- puno korištena metoda

- mehanizam reakcije uključuje nastanak imina (iz aldehida i amonijaka), koji podliježe cijanhidrinskoj reakciji:

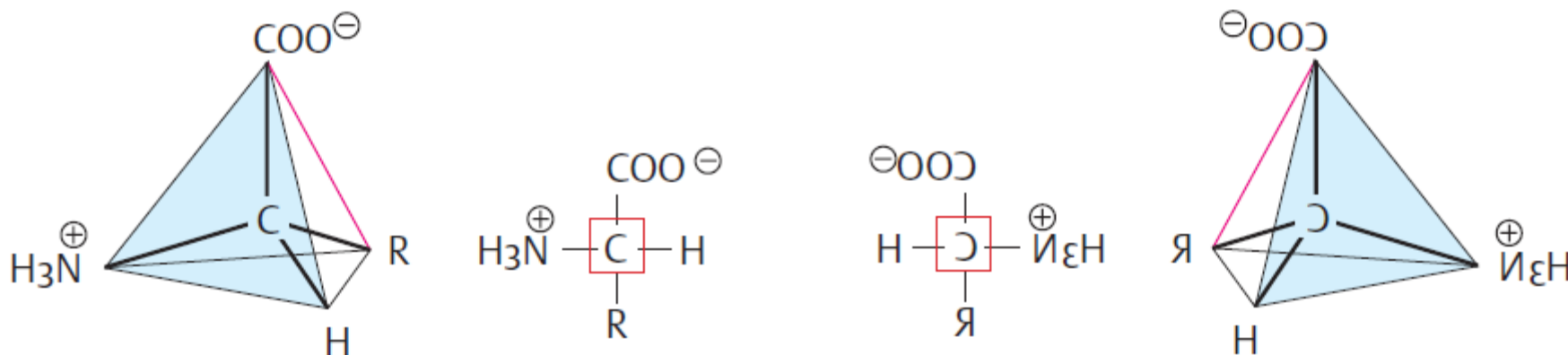


# Aminokiseline

## Dobijanje

### Hemijska sinteza

- Optičko odabiranje
- *L-i D- oblici daju različite poliedarske kristale*
- *Kristali se mogu odvojiti prostim odabiranjem pod mikroskopom*





### Hemijska sinteza

- Hemijsko razlaganje
- *Biohemijsko razlaganje*

***Metoda 1: Oksidaze i dekarboksilaze nekih mikroorganizama djeluju samo na L-oblike a D-oblici ostaju u podlozi. Upravo je to nedostatak što se dobijaju D-aminokiseline***

***Metoda 2: Asimetrična sinteza se zasniva na osobini nekih enzima da katalizuju građenje amidne veze samo L-oblika AK***

***Metoda 3: Asimetrična hidroliza je zasnovana na osobini acilaza iz bubrega i karboksipeptidaza iz pankresasa da brže hidrlizuje N-acil-derivate L-AK id D-izomera. Oslobođena L-aminokiselina se obično taloži sa etil-alkoholom***

# Aminokiseline

## Dobijanje

### Izolovanje iz biološkog materijala \_L

- Sitnjenje biološkog materijala
- *Ekstrakcija*
- *Hidroliza*



## Izolovanje iz biološkog materijala \_L

- ***Hidroliza***
  1. ***Kiseli postupak***
  2. ***Bazni postupak***
  3. ***Hidroliza pod uticajem proteolitičkih enzima***

## Izolovanje iz biološkog materijala \_L

- **Hidroliza**

1. **Kiseli postupak**

- **Vrši se u prisustvu 5-6 M HCl ili 5-6 M H<sub>2</sub>SO<sub>4</sub>**
- **Inertna atmosfera**
- **12-72 h u zavisnosti od stabilnosti peptidne veze**
- **Nedostatak: raspadanje pojedinih AK**

**Thr, Ser se razlažu 10-20 %**

**Asn, Gln prevode se u Asp, Glu**

**Cys do H<sub>2</sub>S**

**Trp, Thr se potpuno razlažu**

**Phe, Tyr-neenzimsko tamnjenje**

## Aminokiseline

### Dobijanje

## Izolovanje iz biološkog materijala \_L

- *Hidroliza*

### 2. Bazni postupak

- *Vrši se u prisustvu 5-6 M Ba(OH)<sub>2</sub> ili 5-6 M Ca(OH)<sub>2</sub>*
- *Inertna atmosfera*
- *Pod pritiskom od 5-7 x 10<sup>7</sup> Pa*
- *6-7 h u zavisnosti od stabilnosti peptidne veze*
- *Nedostatak: raspadanje pojedinih AK*

*Ser do Ala i Gly*

*Thr do Ala, Gly*

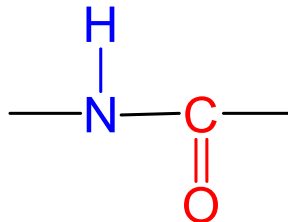
*Cys do H<sub>2</sub>S*

### Izolovanje iz biološkog materijala **\_L**

- **Hidroliza**

#### **3. Pomoću proteolitičkih enzima (po tipu C-N hidrolaze)**

- **Sukcesivna hidroliza uz upotrebu većeg broja enzima**
- **Raaskidanje peptidne veze**
- **Dobijaju se visoko prečišćeni proizvodi i to isključivo L-aminokiseline**
- **Postupak je skup ali se koristi za potrebe farmaceutske, kozmetičke, industrije hrane**



## Aminokiseline

### Dobijanje

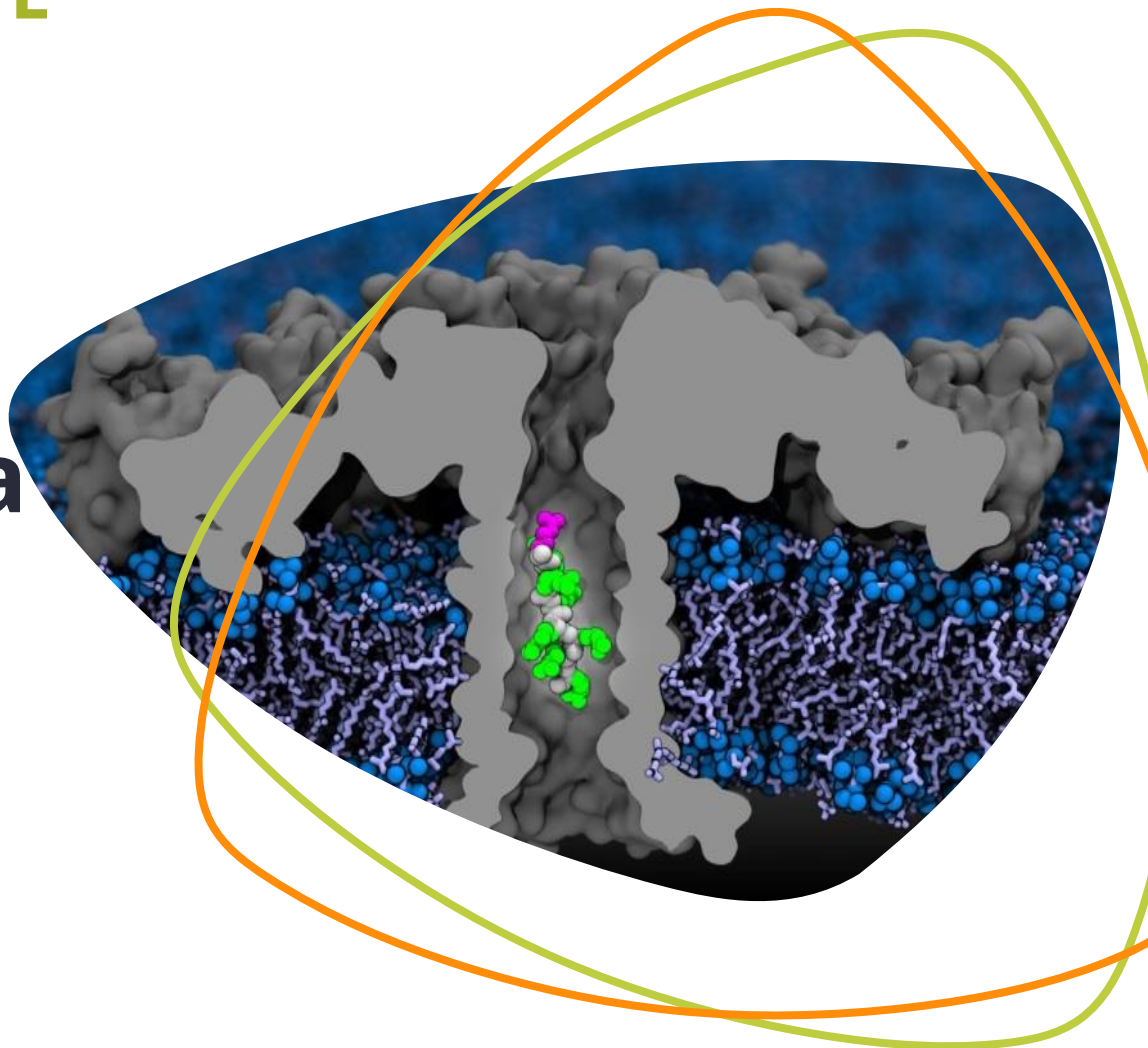
#### **Mikrobiološki postupci dobijanja**

- **Korišćenje visokospecifičnih oblika mikroorganizama koji se razvijaju na hranljivoj podlozi**
- ***Izvor ugljenika je urea***
- ***Neorganske soli kao izvor azota,  $NH_4^+$ ,  $NO_3^-$***
- ***Faktori rasta: biotin, vitamin B1, B2 i B6***

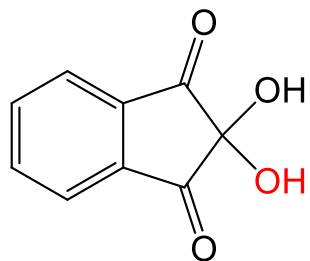
# AMINOKISELINE

## Identifikacija

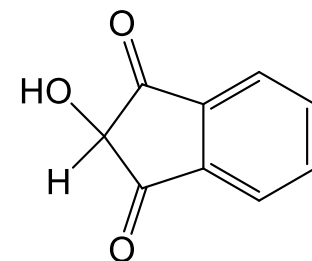
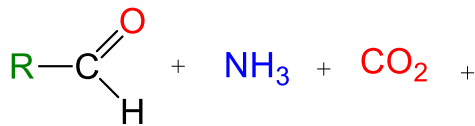
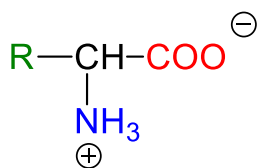
- Hromatografija na tankom sloju silika-gela(TLC)
- Spektrofotometrijska identifikacija (UV-Vis)
- Infracrvena spektroskopija (IR)
- Nuklearna magnetna rezonanca (NMR)
- Masena spektrometrija (MS)
- Enzimsko određivanje



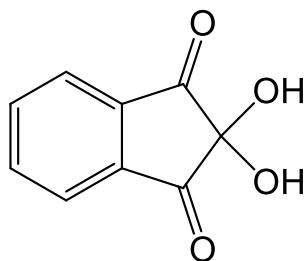




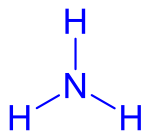
ninhydrin



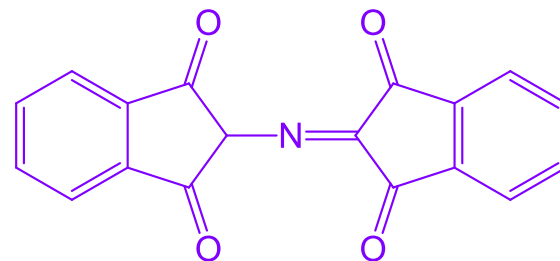
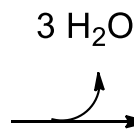
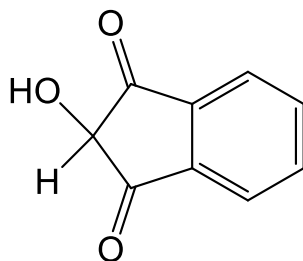
hidridantin



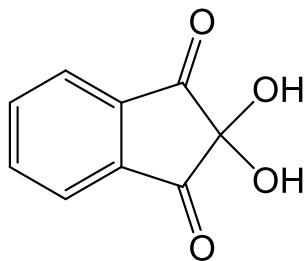
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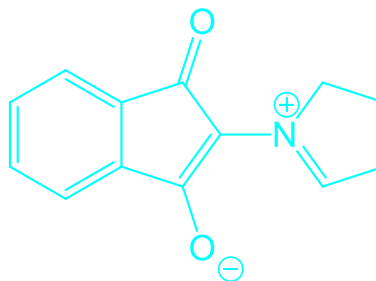
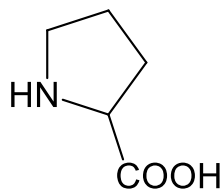
+



$\lambda_{\text{max}} = 570 \text{ nm}$



+

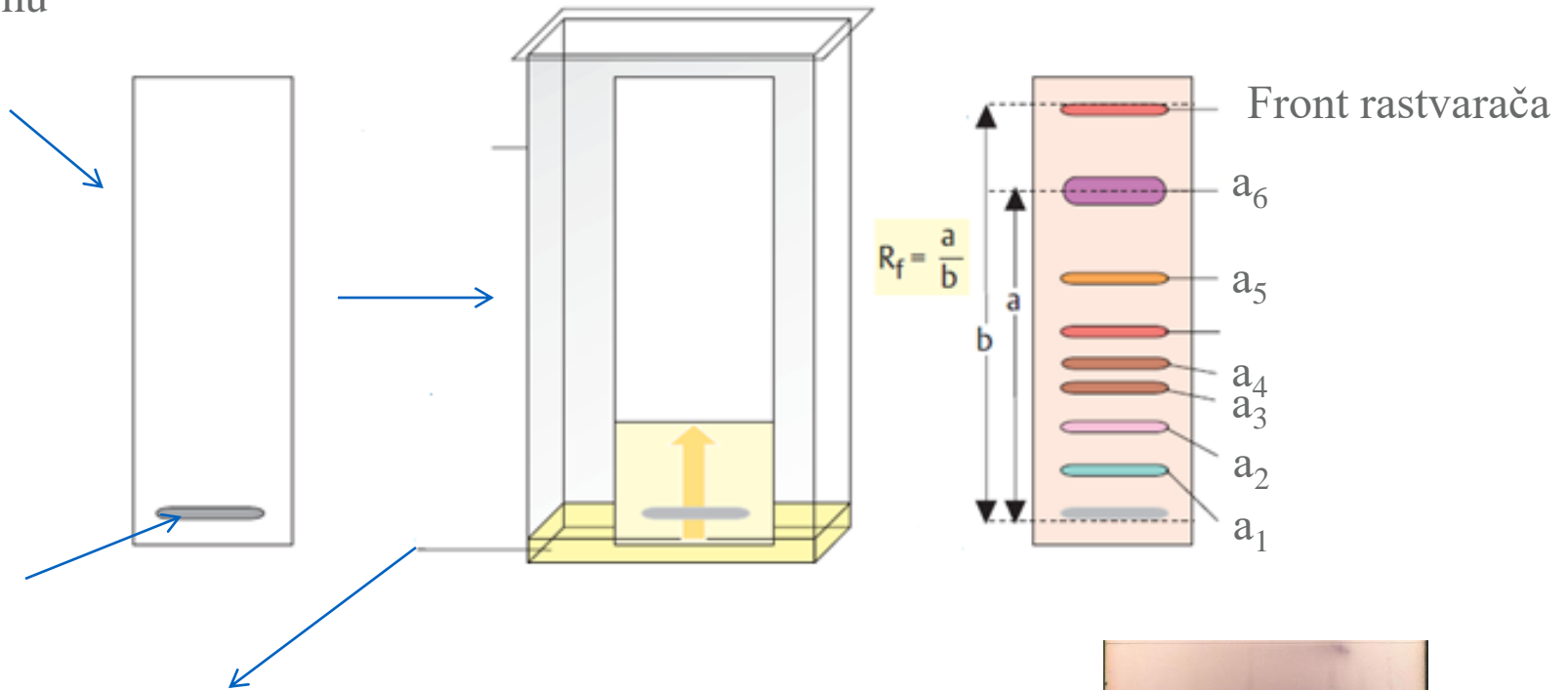


$\lambda_{\text{max}} = 440 \text{ nm}$

# TLC-tankoslojna hromatografija

Tanki sloj  
 $\text{SiO}_2$  na  
aluminijumu  
ili staklu

Kada za  
hromatografiju



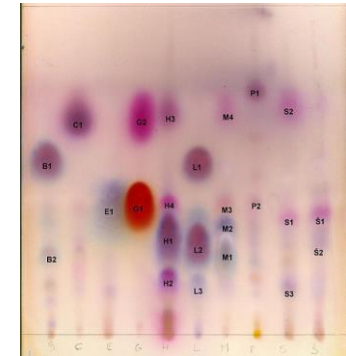
startna  
mrlja

Front rastvarača

$$R_f = \frac{a}{b}$$

eluent

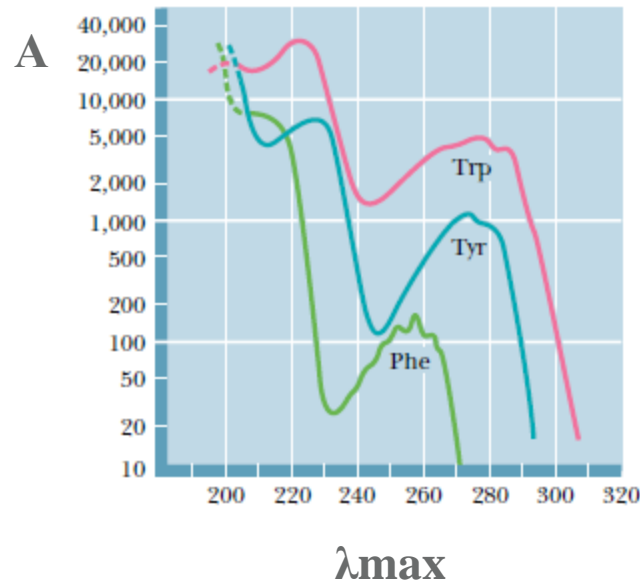
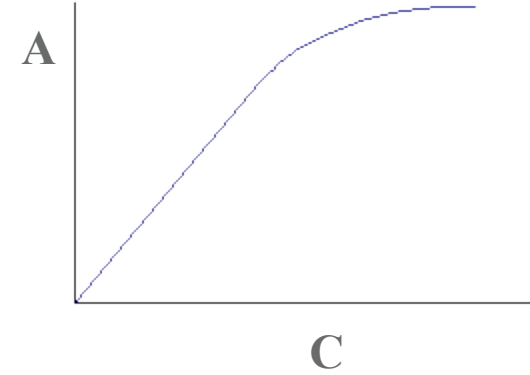
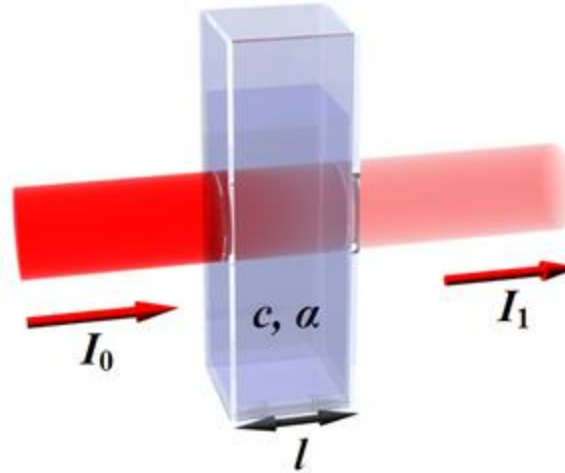
i-ButOH:Acc(glac):  $\text{H}_2\text{O}$  = 4:1:1



# UV-Vis spektrofotometrija

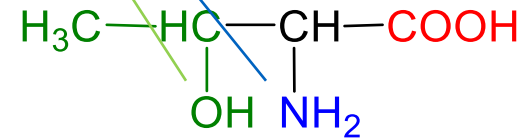
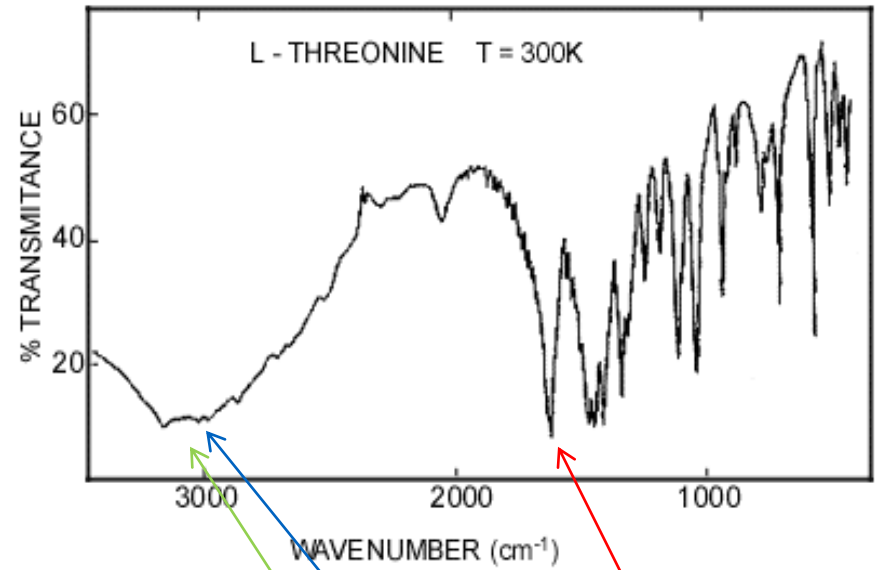
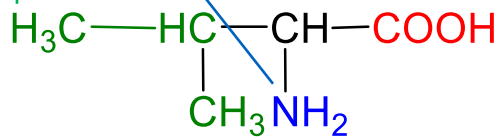
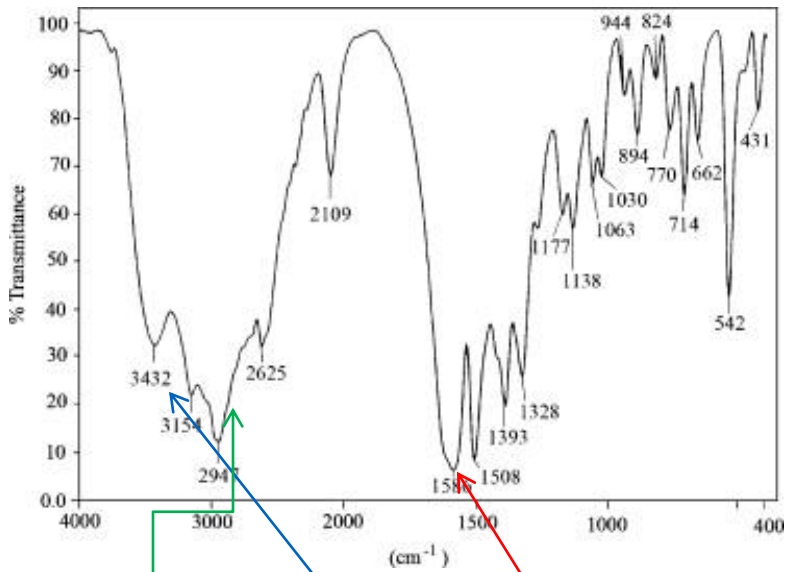
$$A = \log_{10} \left( \frac{I_0}{I} \right) = \log_{10} (\%T^{-1}) = \epsilon l c$$

Lambert-Beer-ov zakon

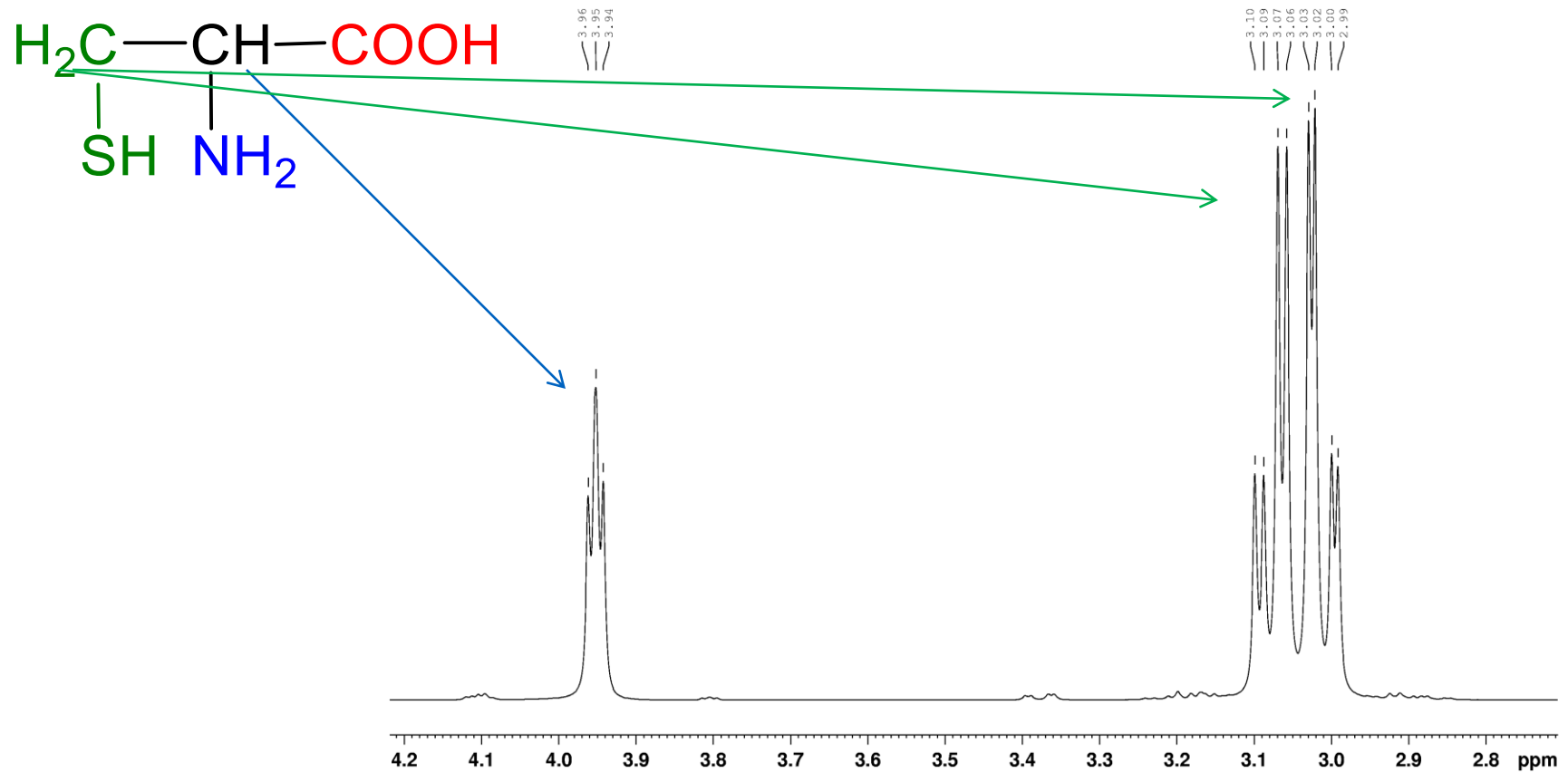


Dobijaju se karakteristični apsorbicioni spektri koji se koriste za identifikaciju naročito aromatičnih aminokiselina

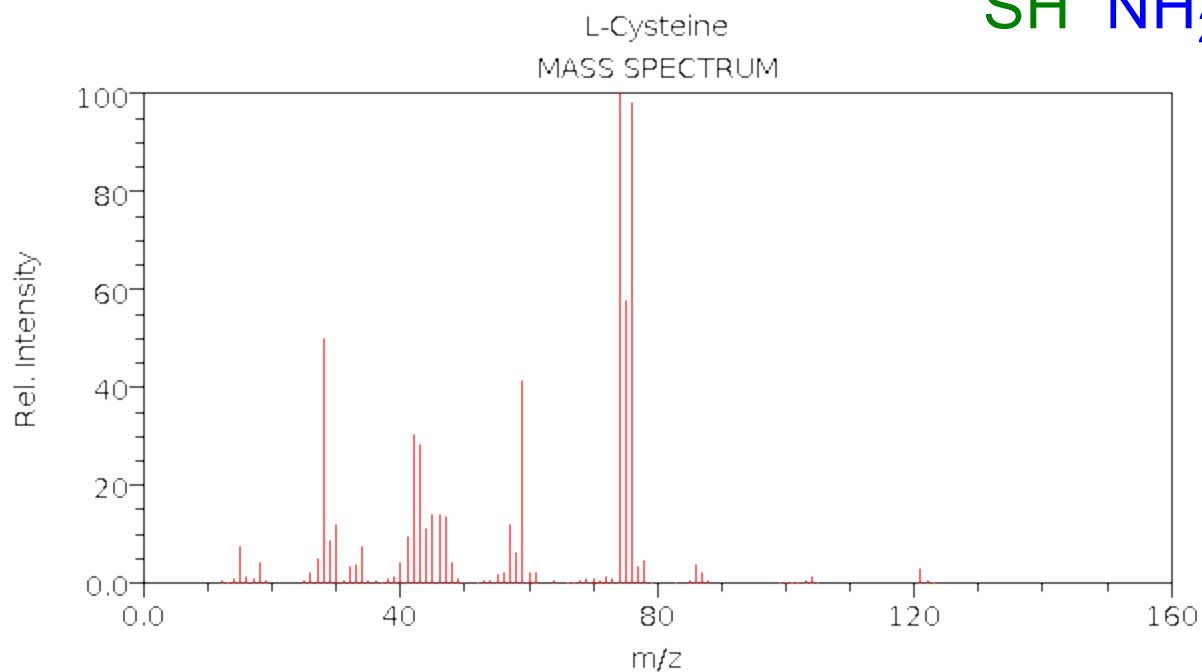
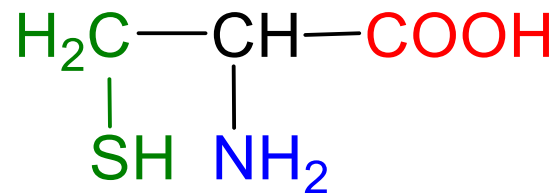
# IR spektroskopija



# NMR spektroskopija



# MS masena spektrometrija



NIST Chemistry WebBook (<http://webbook.nist.gov/chemistry>)