# Bioquímica Avançada (QBQ-5751) 2023

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## Aminoácidos e Peptídeos

Princípios de termodinâmica
e Forças Intermoleculares

→

Estrutura covalente de **proteínas**
(Estrutura e função /evolução química)

↓

Estrutura 3D de proteínas

↓

*Folding* e dinâmica de proteínas

←

... Enzimas

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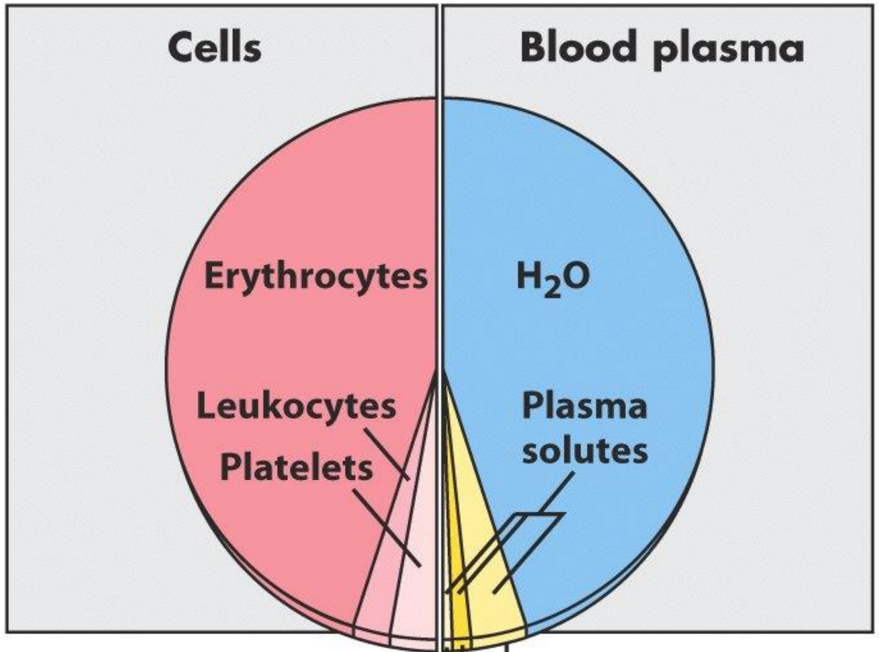
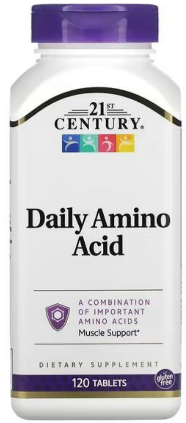



**Inorganic components (10%)**
NaCl, bicarbonate, phosphate, $CaCl_2$, $MgCl_2$, KCl, $Na_2SO_4$

**Organic metabolites and waste products (20%)**
glucose, amino acids, lactate, pyruvate, ketone bodies,citrate, urea, uric acid

**Plasma proteins (70%)**
Major plasma proteins: serum albumin, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL), immunoglobulins (hundreds of kinds), fibrinogen, prothrombin, many specialized transport proteins such as transferrin

## Funções + Aplicações de aminoácidos → Pesquisa, Terapêutica, Alimentação, Nutrição, Indústria






- Fontes de energia metabólica
- Blocos construtivos das proteínas e peptídeos:
  *20 $\alpha$-aminoácidos usuais/ "essenciais"aos humanos (9): F, H, I, L, K, M, T, W, V*
  *aminoácidos não usuais*
- Intermediários biossintéticos: síntese de aminoácidos, nucleotídeos, compostos nitrogenados
- Ações biológicas: algumas já citadas
- Marcador de envelhecimento
- Suplementos alimentares
- Compostos neuroativos
- Reagentes para sínteses orgânicas (heterociclos e $\beta$-lactamas)
- Síntese química de aminoacil-resinas, peptídeos e proteínas
- Preparação de colunas quirais p/ cromatografia
- Cosmética

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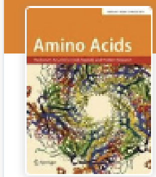


# Amino Acids

The Forum for Amino Acid, Peptide and Protein Research

Editorial board Aims & scope Journal updates

*Amino Acids* publishes contributions from all fields of amino acid and protein research: analysis, separation (proteomics), synthesis, biosynthesis, evolution, folding, structure, stability, dynamics, medicinal chemistry, catabolism, cross linking amino acids, racemization/enantiomers, modification of amino acids such as phosphorylation, methylation, acetylation, hydroxylation and glycosylation. The *journal* also welcomes papers on new roles for amino acids and proteins in physiology and pathophysiology, biology, amino acid analogues, polyamines, labeled amino acids, peptides, stable and radioactive isotopes of amino acids, excitatory amino acids, unusual amino acids, and peptides. Applications include medicine (gastroenterology, nephrology, psychiatry, neurology, oncology), biochemistry, structural biology, agriculture and plants, food chemistry, nutrition, microbiology, neurochemistry, pharmacology, and sports. We also encourage the submissions of computational papers and interdisciplinary research such as economic, social sciences and humanities. — show all

**Editor-in-Chief**

Ellen I. Closs

**Publishing model**

Hybrid (Transformative Journal). How to publish with us, including Open Access

| | | |
|---|---|---|
| **3.789 (2021)**<br>Impact factor | **34 days**<br>Submission to first decision (Median) | **1,068,917 (2021)**<br>Downloads |
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2023: > 3.9

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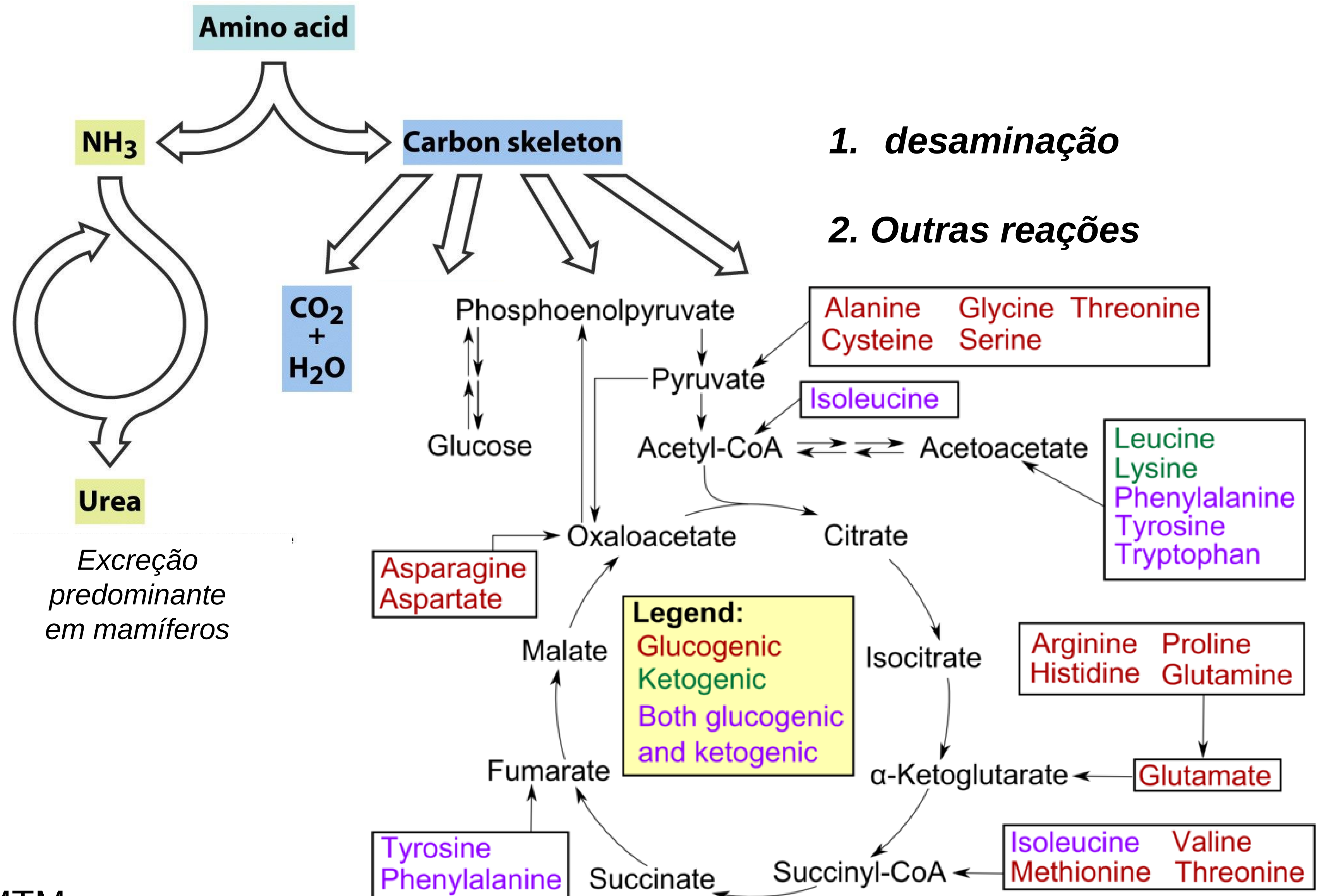
# Aminoácidos: <u>blocos construtivos</u> de proteínas → Metabolismo de *proteínas endógenas e da dieta*

## ***metabolismo de aminoácidos*** *(degradação e biossíntese)*

***1. desaminação***

***2. Outras reações***

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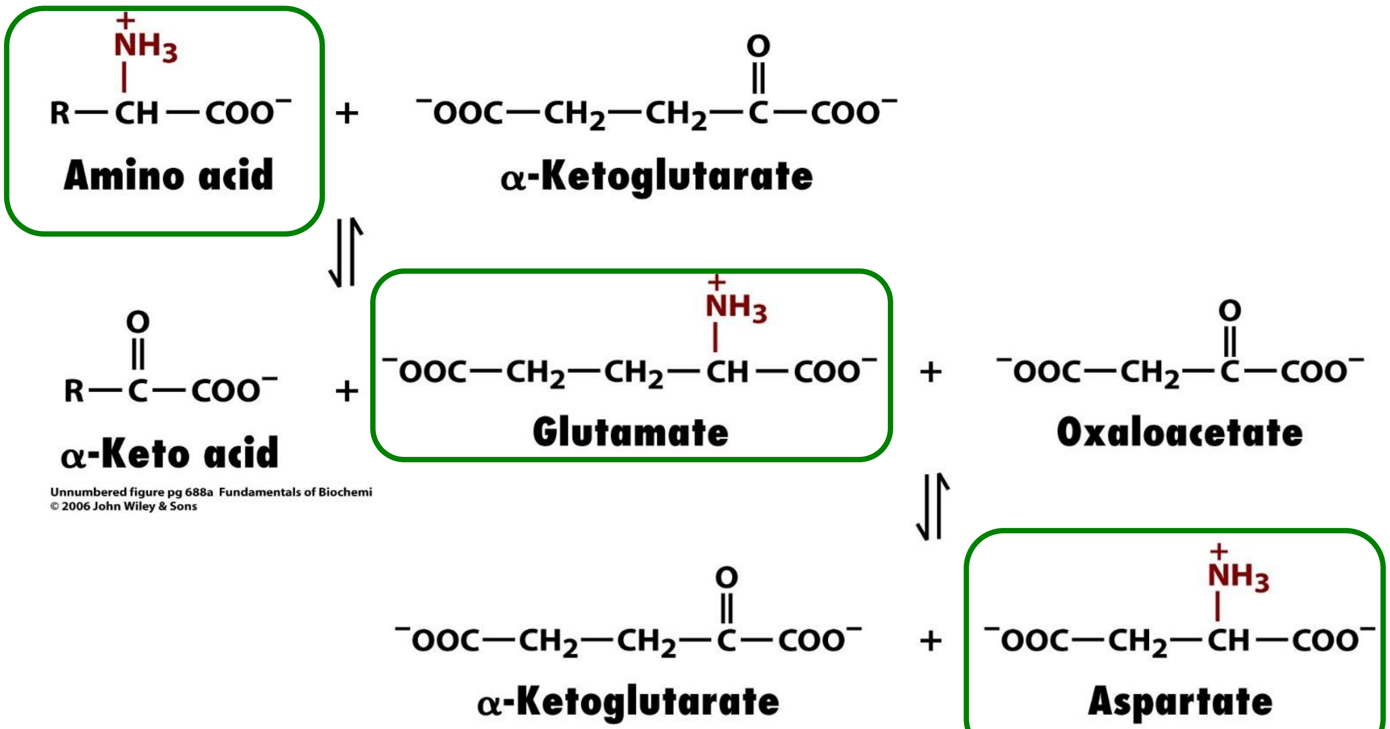
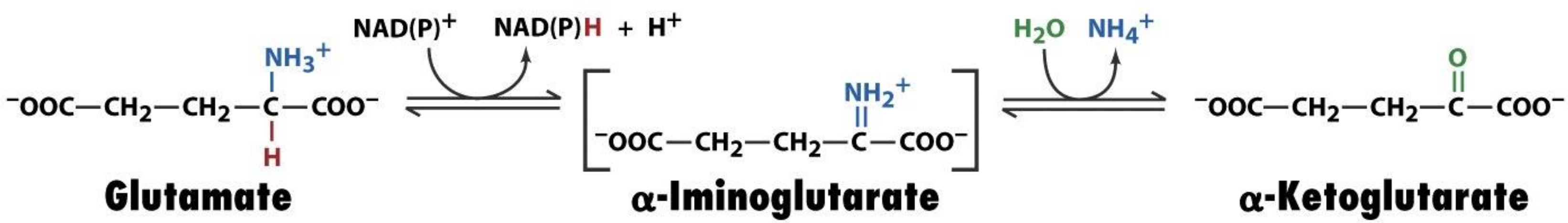
## *Desaminação* → reações catalisadas por transaminases





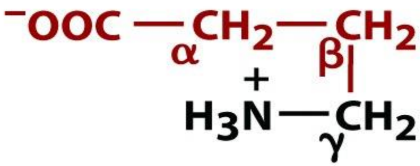



## *Desaminação* → reação catalisada por glutamato desidrogenase (GDH)



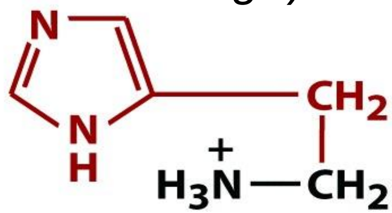
# Aminoácidos:
## <u>blocos construtivos</u> de outros compostos nitrogenados bioativos

(*vasodilatador; estimula secreção de ácido no estômago*)

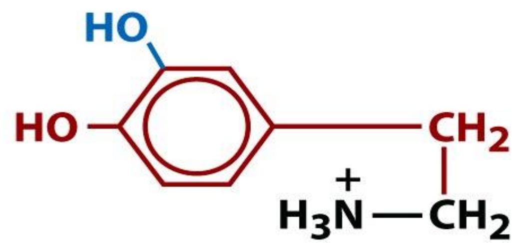



γ-Aminobutyric acid (GABA)

(*inibe eplepsia e hipertensão*)

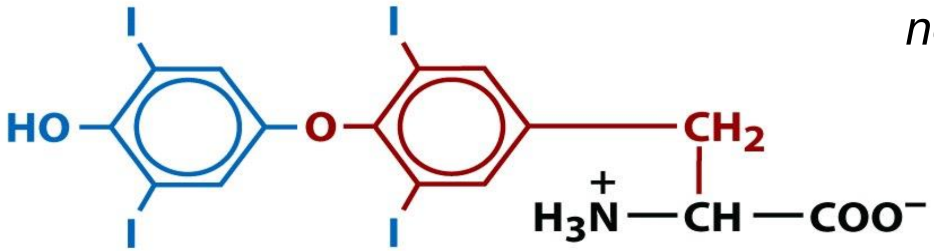



Histamine




Dopamine

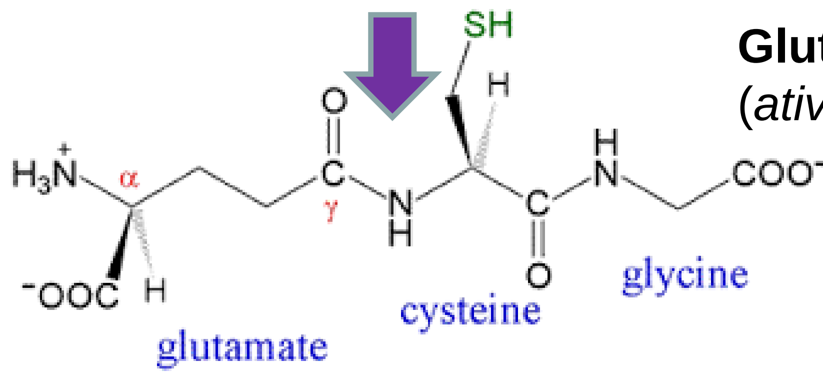
(*estimula os neuroreceptores D1-D5; altera pressão arterial*)




(*hormônio tireoideano/ tetraiodotironina = T4*)

Thyroxine

**Glutathione**
(*atividade redutora*)

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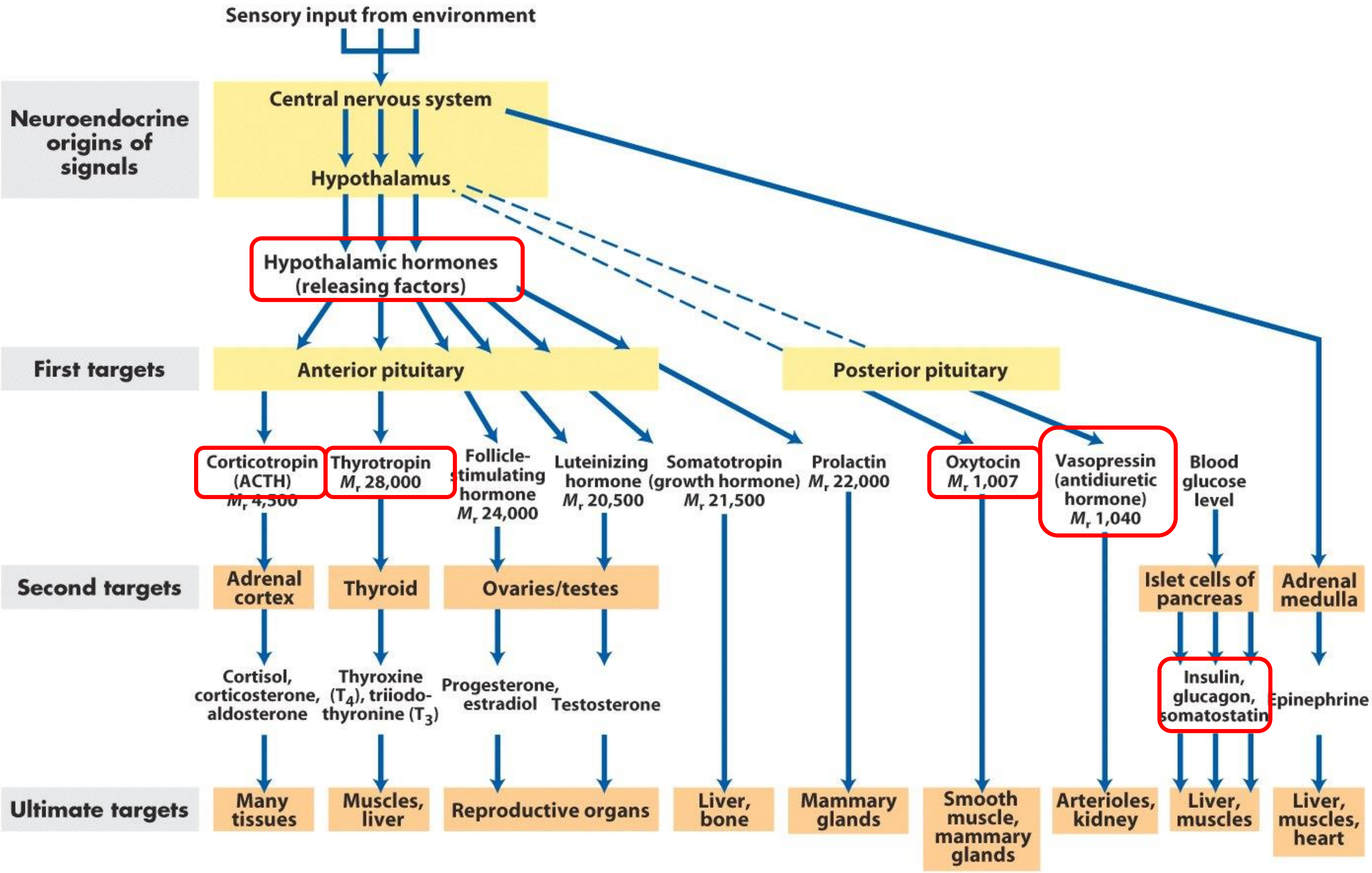
## Aminoácidos: blocos construtivos de fatores liberadores de hormônios/ hormônios peptídicos em prol do controle de funções vitais

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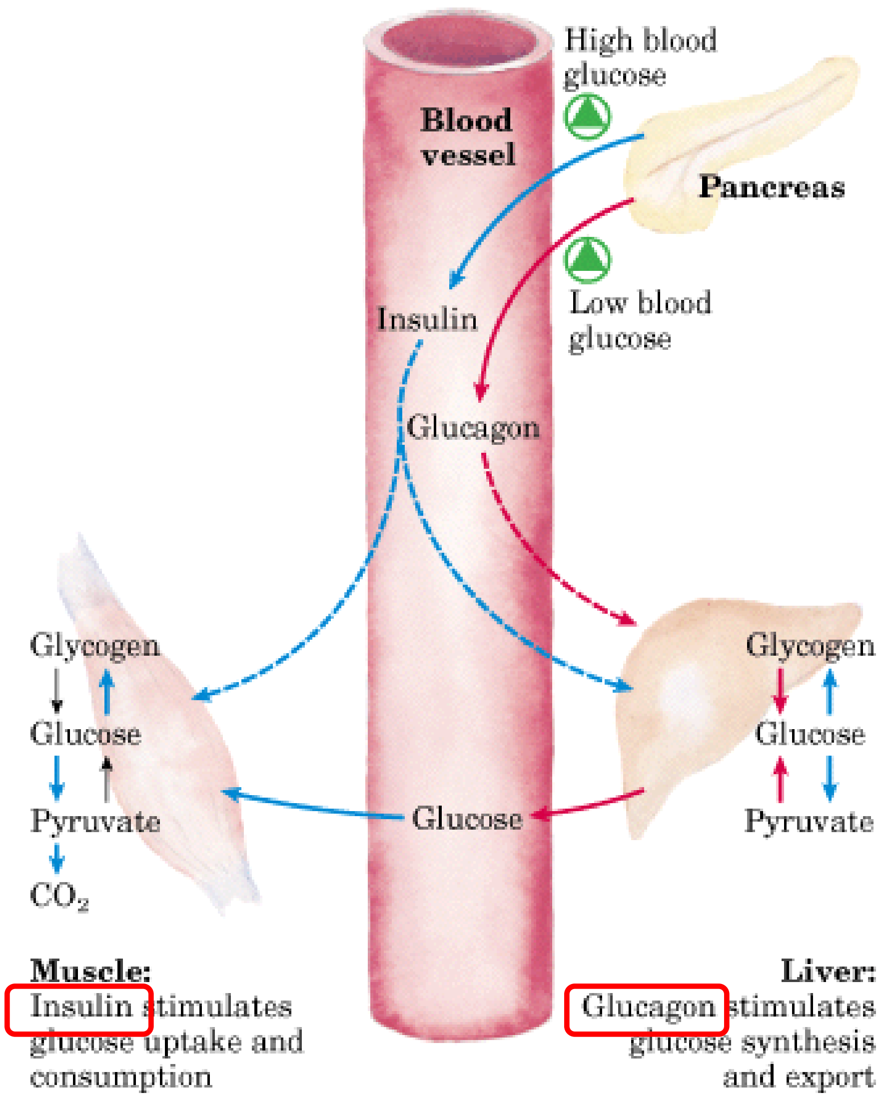
**Aminoácidos: blocos construtivos dos hormônios peptídicos envolvidos no controle da glicemia → metabolismo de mamíferos**

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# Aminoácidos: <u>blocos construtivos</u> de inibidores sintéticos de proteases→ ciência terapêutica

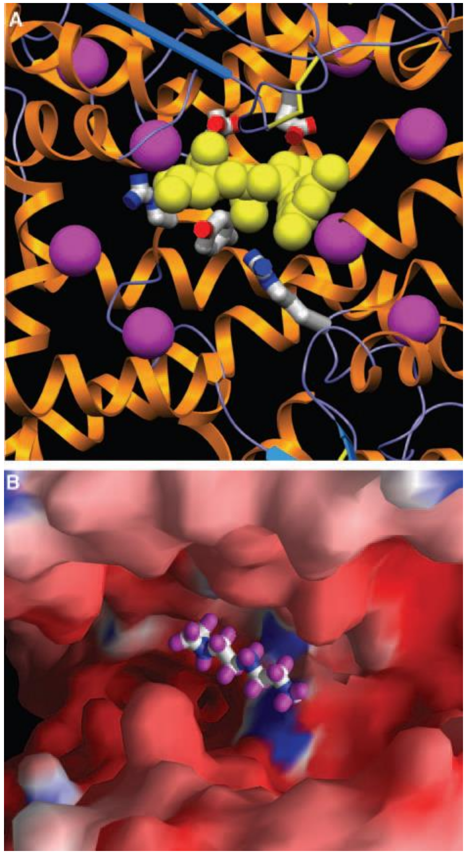


**Figure 1. A novel ACE2 inhibitor identified by molecular docking.** A, The active site residues of ACE2 are displayed. ACE2-strands are colored blue and -helices are colored gold. Yellow spheres indicate the position of a known ACE2 inhibitor used as a guide for targeting small molecules. Magenta spheres mark the boundary of the scoring grid used to calculate energy scores for molecularly docked small molecules. B, The top scoring compound, N-(2-aminoethyl)-1 aziridine-ethanamine (NAAE),is shown in the ACE2 active site. ACE2

Angiotensin-converting enzyme (**ACE**) inhibitors: are widely used for cardiovascular diseases, including high blood pressure, heart failure, heart attack and kidney failure, and have combined annual sales in excess of US $6 billion.

**Para desenvolver um inibidor é preciso saber:**

**Protease: especificidade/modo de ação/estrutura**
**Proteólise e estruturas de proteínas**
**Geração de fragmentos/peptídeos**
**Identificação e estruturas**
**Síntese/purificação/caracterização de peptídeos**
**Inibição da função catalítica**
**Busca de análogos não peptídicos**
**Ação inibitória específica**

↓

**Química, estrutura, propriedades de aminoácidos**

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http://www.haircareresources.com/amino-acid.html

**Aminoácidos:**
**componentes de produtos da cosmética**

..... Certain amino acids have been determined to contribute to the thinning and thickening of hair.

...Some of the amino acids that the body needs to *produce healthier hair* are methionine, cysteine, cystine, and tyrosine.

**Methionine**

Methionine, an essential amino acid, is a powerful antioxidant. This amino acid is a good source of sulfur which helps in the prevention of hair disorders as well as skin and nail disorders..... Methionine prevents the premature loss of hair......

**Cysteine**

The non essential amino acid cysteine is important for the proper growth of hair. Cysteine has been found to increase the growth of hair by 100%.

Cysteine also acts as a powerful antioxidant and protects the body from damage brought about by radiation.

*Gutiérrez-Preciado et al, (2010)* ***Nature Education*** **3(9):29**

## An Evolutionary Perspective on Amino Acids

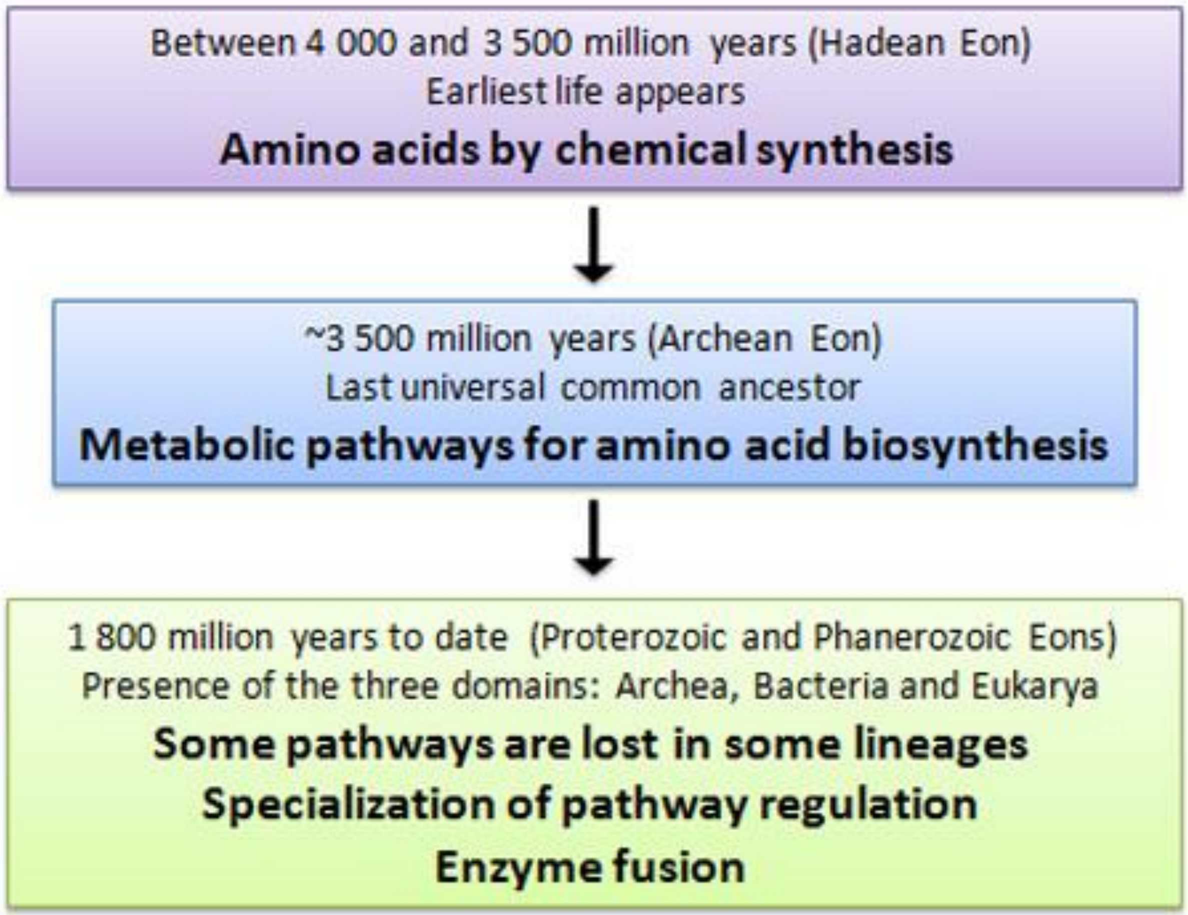


**Figure 1: Major events in the evolution of amino acid synthesis**

The way amino acids are synthesized has changed during the history of Earth. The Hadean Eon represents the time from which Earth first formed. The subsequent Archean Eon (approximately 3,500 million years ago) is known as the age of bacteria and archaea. The Proterozoic Eon was the gathering up of oxygen in Earth's atmosphere, and the Phanerozoic Eon coincides with the major diversification of animals, plants, and fungi.

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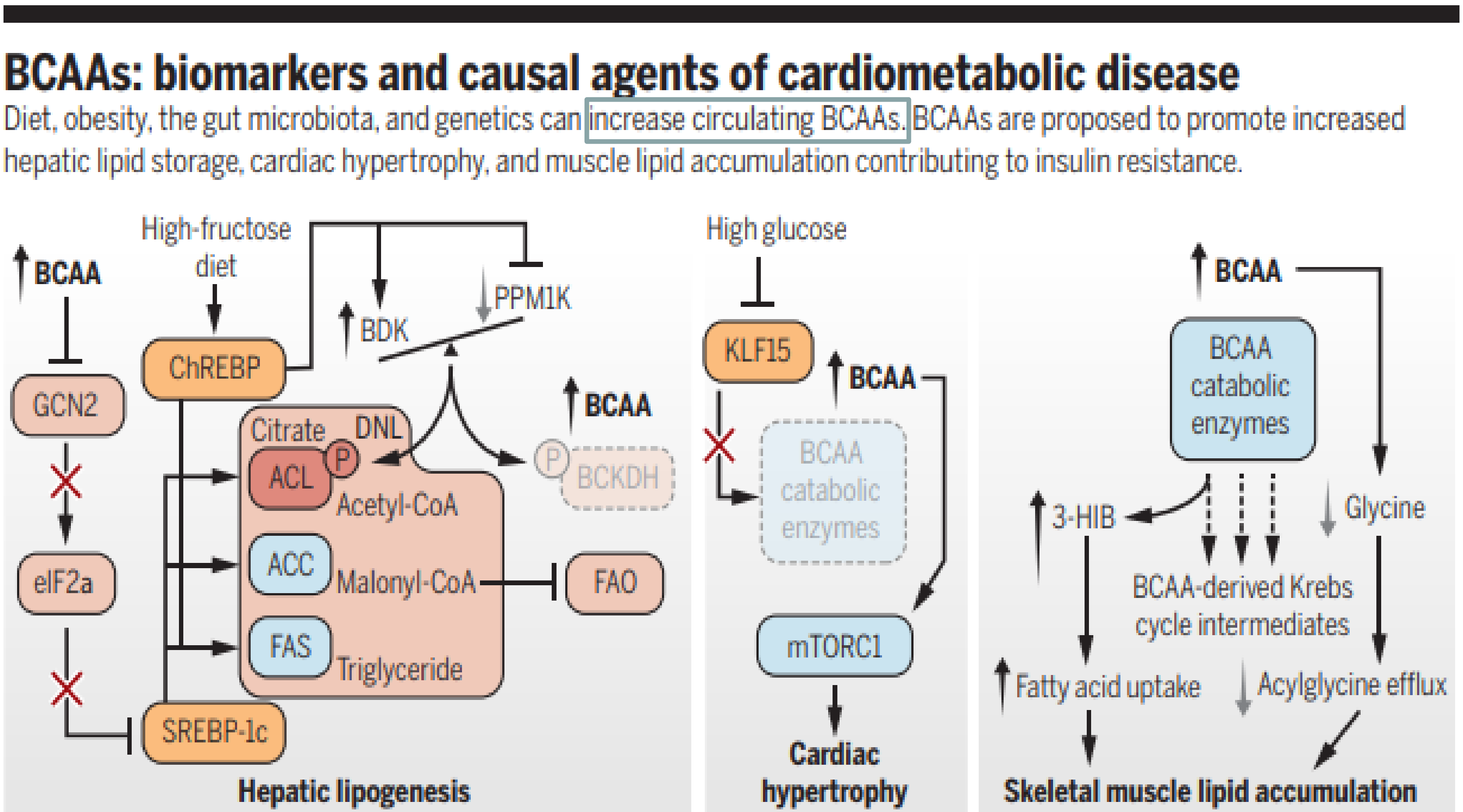
P.J. White & C.B. Newgard, *Science* **2019**, 363 (6427), 582-583

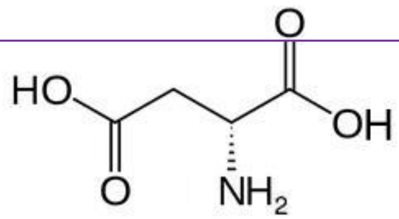
**Branched-Chain Amino Acids in disease:** **Leu, Ile, Val**



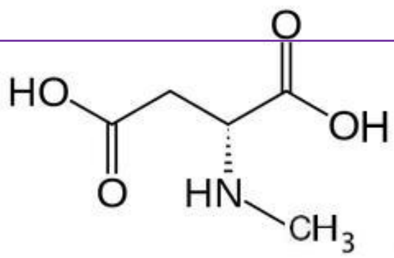
Kaji Y[1] et al. **Invest Ophthalmol Vis Sci. 2007, 48(9),3923-7** MTM

**Localization of D-beta-aspartic acid-containing proteins in human eyes**

Biologically uncommon **D-beta-aspartic acid (D-beta-Asp)** has been detected in proteins from various human tissues in elderly donors. Previous studies have identified D-beta-Asp residues at four different specific sites in alpha-crystallin from aged human lenses and an **increased amount of D-beta-Asp residues with age**. D-beta-Asp is formed as a result of racemization and accumulates with age; therefore, it is thought to be a potential marker of aging. To reveal the role of the D-beta-Asp formation in the aging process of eyes, immunohistochemical localization of D-beta-Asp was investigated in ocular samples of various ages.




D-Aspartic Acid




N-Methyl-D-Asparate (NMDA)

Melville GW et al., **PLoS ONE 12(8): e0182630 (2017)**

**The effects of d-aspartic acid supplementation in resistance-trained men over a three months training period: A randomised controlled trial.**

**D-aspartic acid (D-AA)** is an amino acid regulator of testosterone synthesis and may act on a stimulatory receptor (NMDA). D-AA shows promise in aiding male fertility. Healthy men supplementing D-AA experience only temporary increases in testosterone, which limits its use.

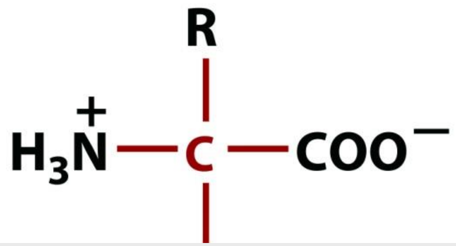
D-AA works in the central brain region to cause a release of hormones, such as luteinizing hormone, follicle-stimulating hormone, and growth hormone.

## Aminoácidos modificados e não usuais

**Código genético: 20 α-aminoácidos usuais**
**α-Aminoácidos modificados (não usuais) em peptídeos/proteínas?**

**modificação pós-tradução**

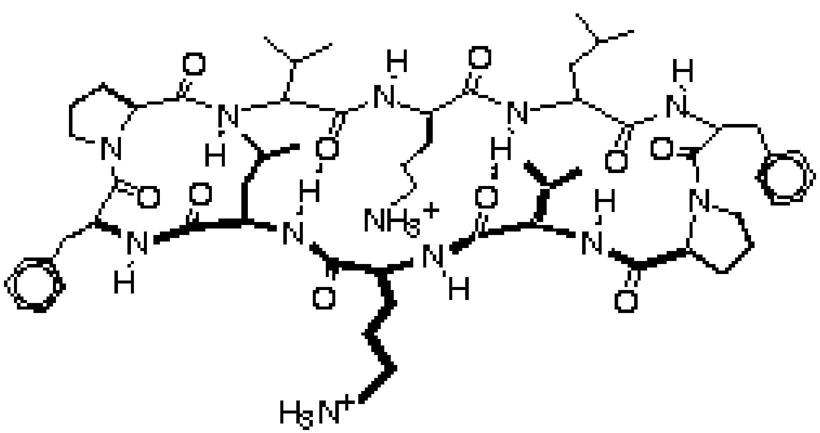
**Exceção: Sec ou SeCys**




**$CH_2$-Se-H**

21º. Aminoácido proteico

**D-Phe: peptídeo antibiótico gramicidina A**



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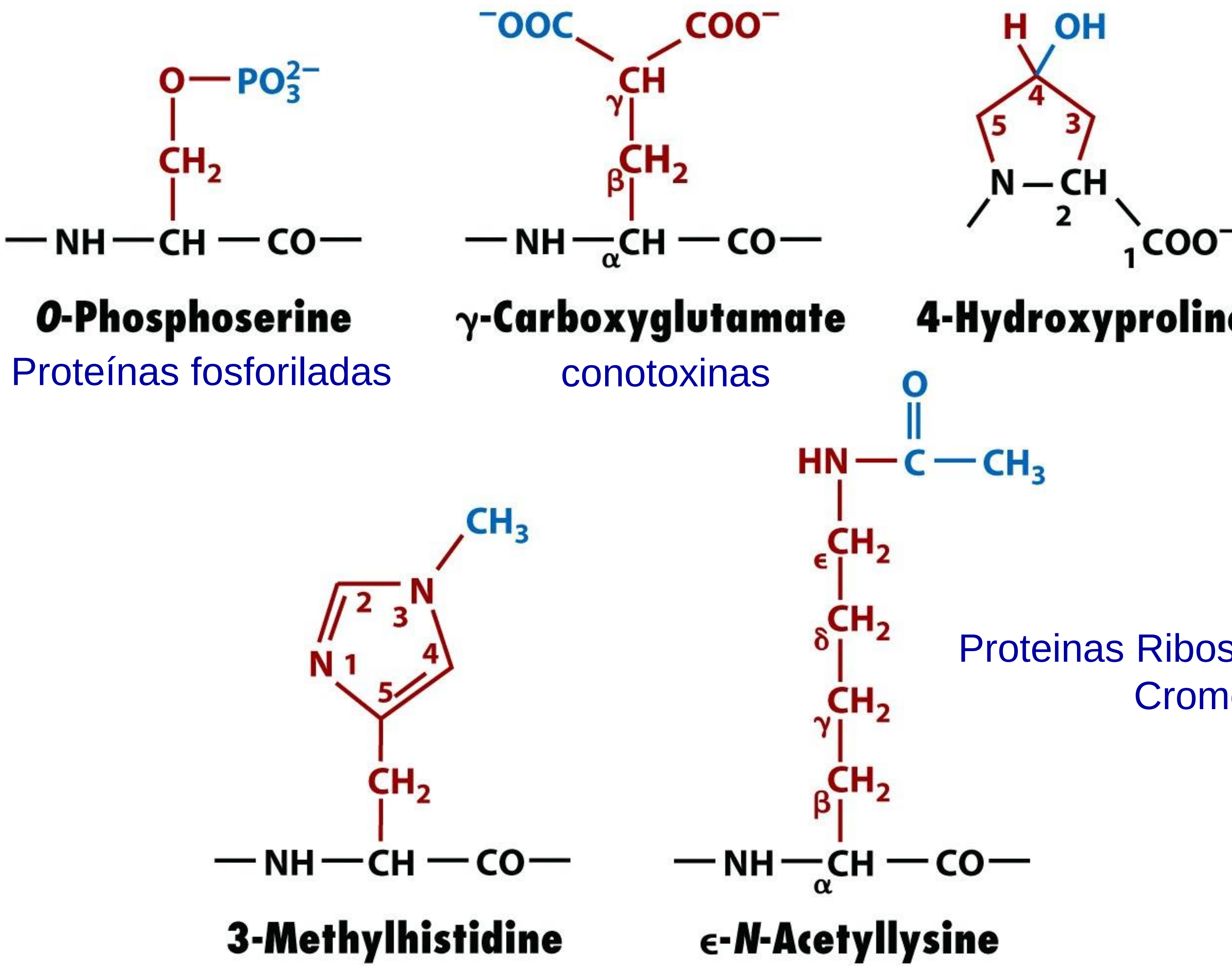



4-Hydroxyproline: colágeno

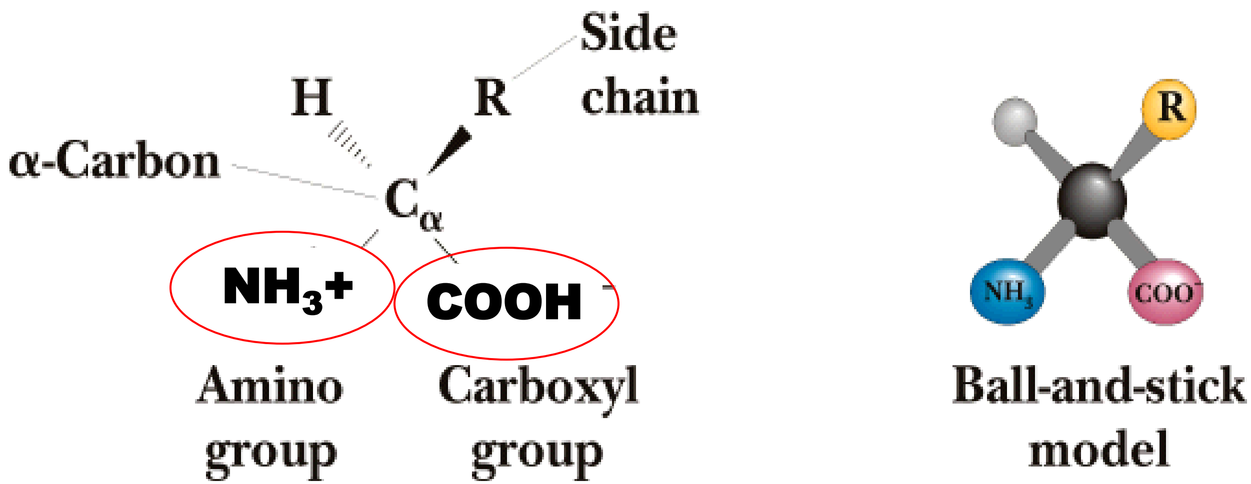
O-Phosphoserine: Proteínas fosforiladas

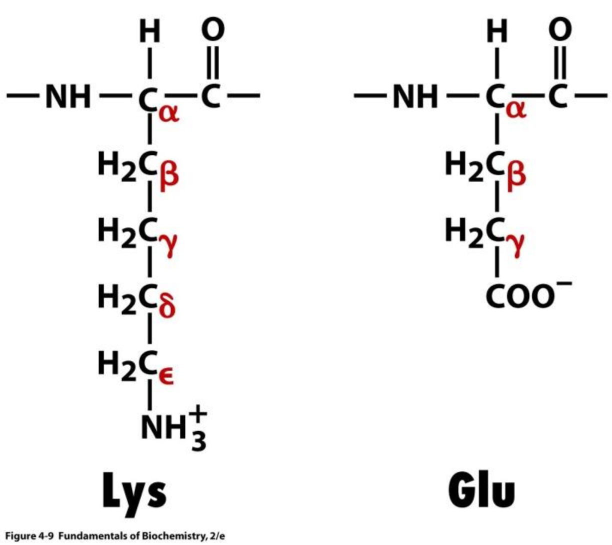
γ-Carboxyglutamate: conotoxinas

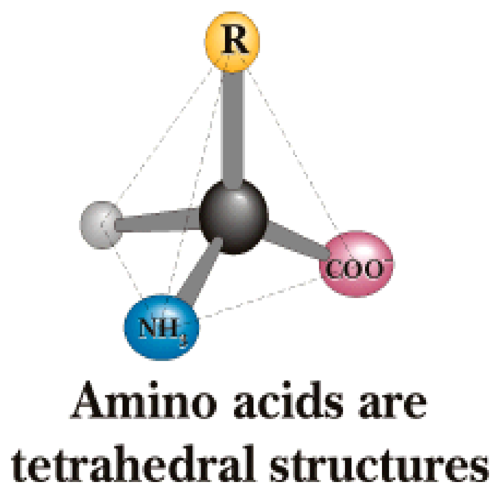
Proteinas Ribossomais
Cromossomais

Figure 4-15 Fundamentals of Biochemistry, 2/e

# Estrutura geral dos α- aminoácidos

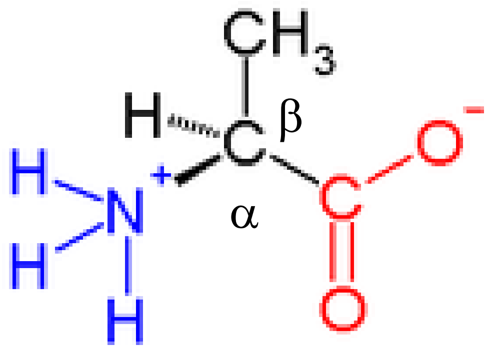


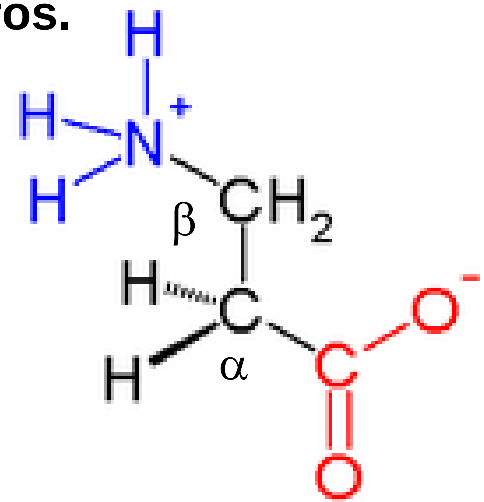




- 700 diferentes aminoácidos na natureza (plantas, bactérias e fungos)

**A maioria é α-aminoácidos, mas existem outros.**

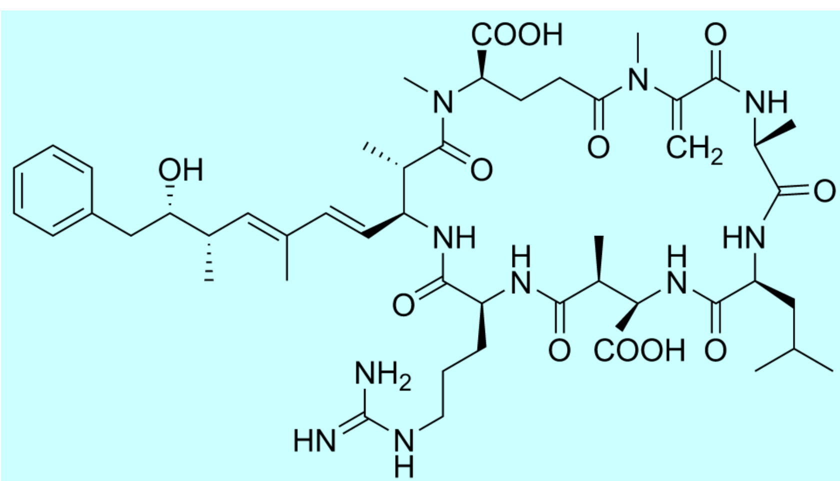


L-α-alanine

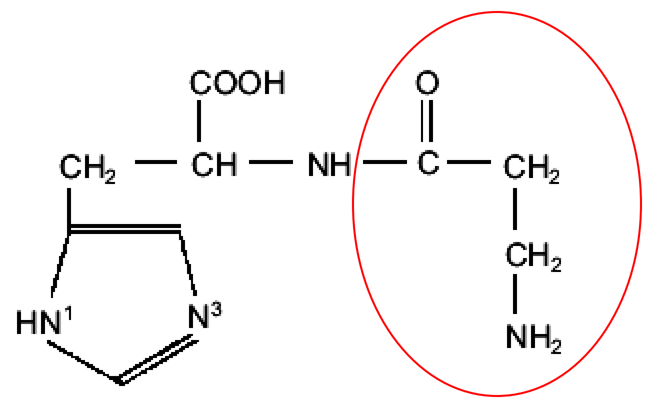


β-alanine

**em MICROCISTINA**
**(toxina de alga *Microcystis aeruginosa* )**



**em CARNOSINA**
**(dipeptídeo de glia e neurônios)**



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# Propriedades físico-químicas dos α-aminoácidos

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- Capacidade de formar ligações de H
- Capacidade de se envolver em ligações S-S
- Reatividade química
- Acidez X Basicidade
- Polaridade
- Aromaticidade
- Volume
- Capacidade de absorver/emitir luz
- Capacidade de desviar luz plano polarizada
- Quiralidade
- Flexibilidade conformacional

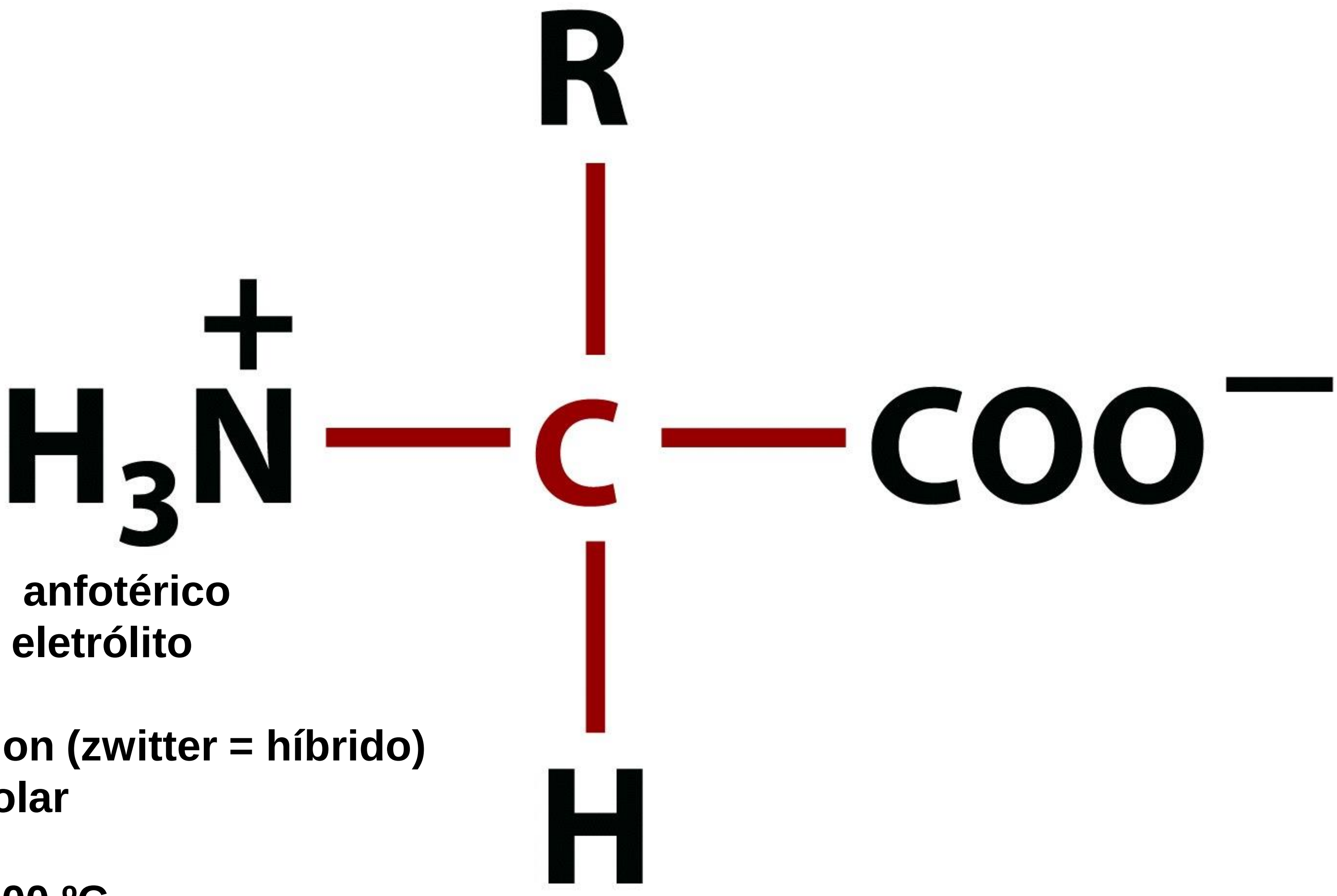
**Solubility**

Amino acids are generally soluble in water and insoluble in non-polar organic solvents such as hydrocarbons.

**Melting points**

The amino acids are crystalline solids with surprisingly high melting points. It is difficult to pin the melting points down exactly because the amino acids tend to decompose before they melt. Decomposition and melting tend to be in the 200 - 300°C range.

# Propriedades ácido-base




**Caráter  anfotérico**
**Caráter eletrólito**

**Zwitterion (zwitter = híbrido)**
**Íon dipolar**

**PF = ~300 ºC**
**(compostos iônicos típicos)**

**Solúveis em solventes polares**

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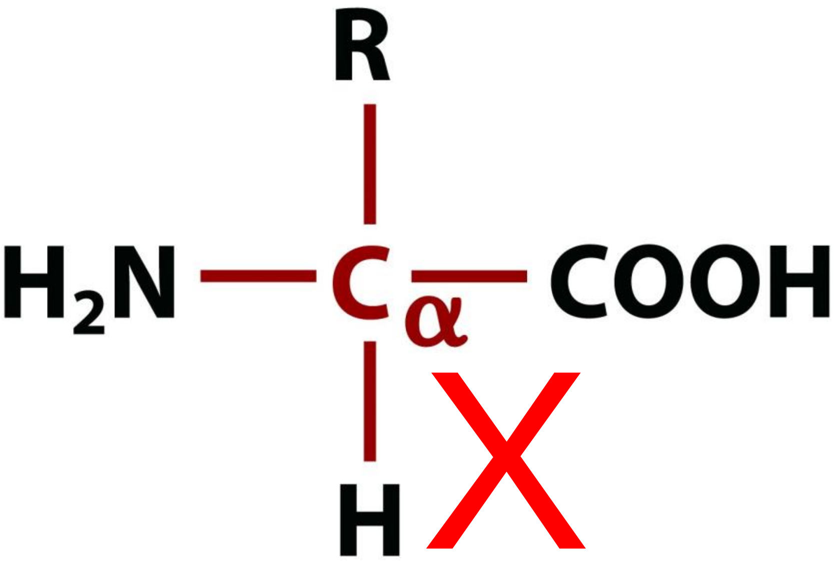
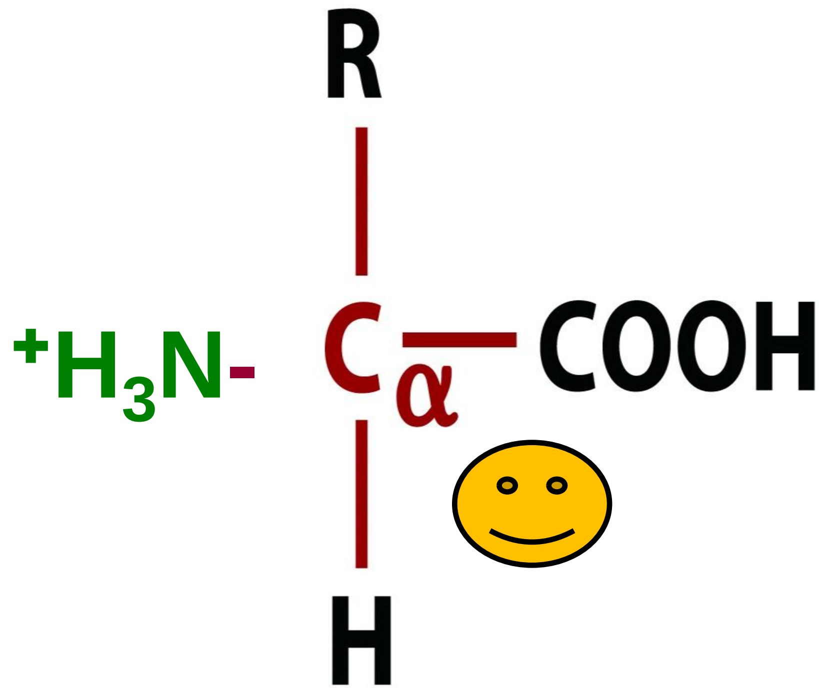
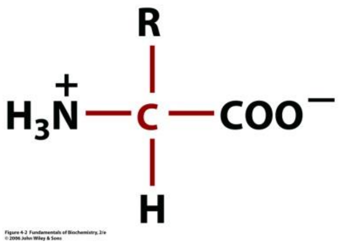
## Por isso,





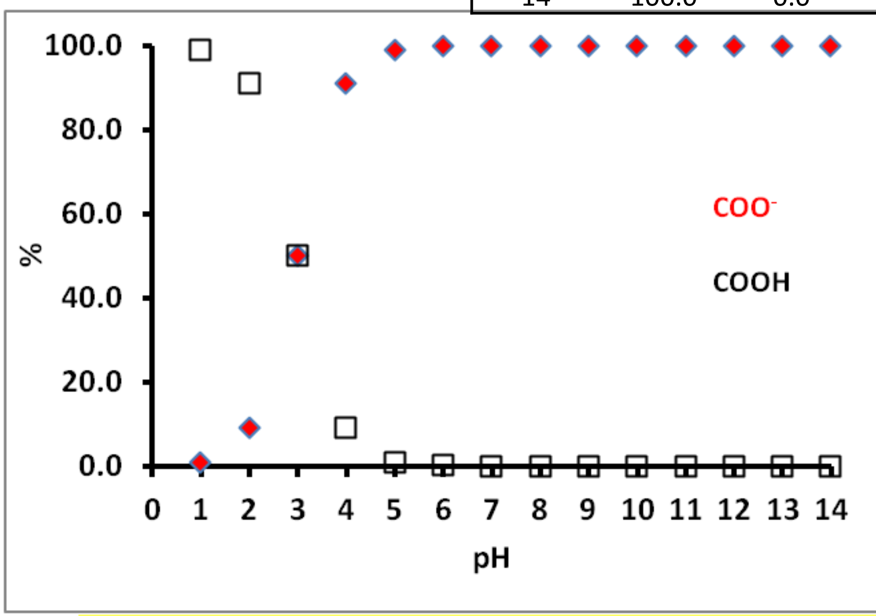



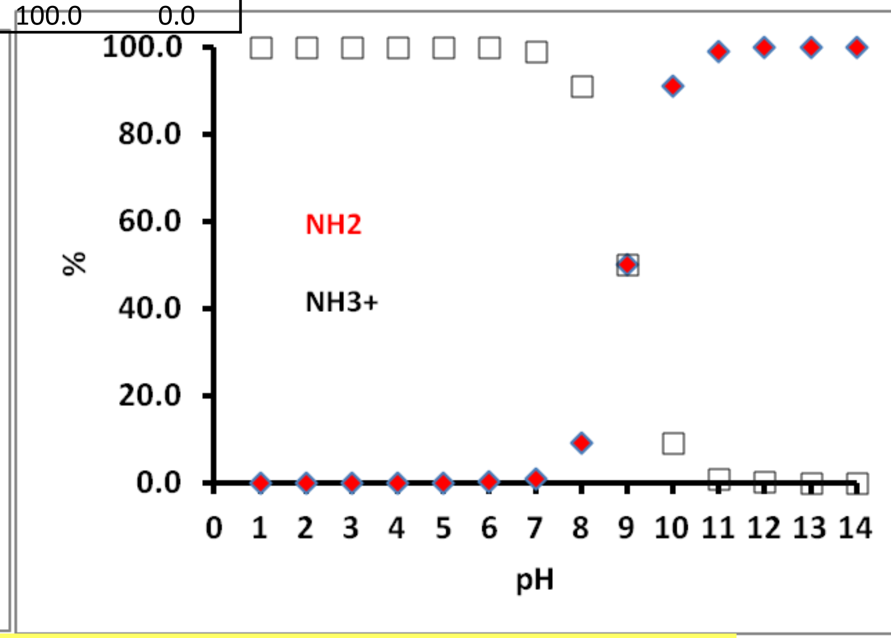


- COOH
p$K_a$ = 3

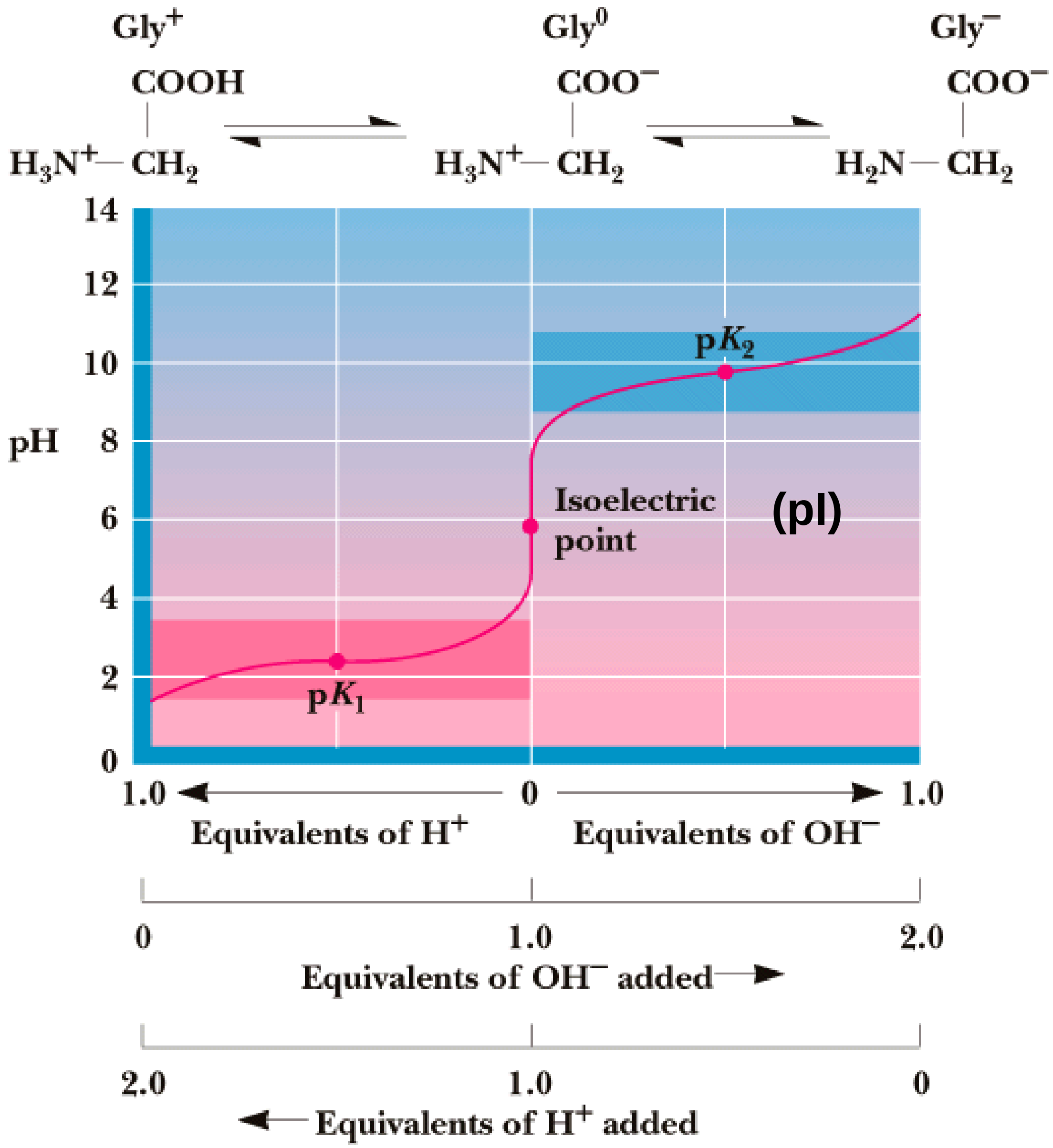
- $NH_3^+$
p$K_a$ = 9

| pH | %B - COO- | %A - COOH | %B - NH2 | %A -NH3+ |
|---|---|---|---|---|
| 1 | 1.0 | 99.0 | 0.0 | 100.0 |
| 2 | 9.1 | 90.9 | 0.0 | 100.0 |
| 3 | 50.0 | 50.0 | 0.0 | 100.0 |
| 4 | 90.9 | 9.1 | 0.0 | 100.0 |
| 5 | 99.0 | 1.0 | 0.0 | 100.0 |
| 6 | 99.9 | 0.1 | 0.1 | 99.9 |
| 7 | 100.0 | 0.0 | 1.0 | 99.0 |
| 8 | 100.0 | 0.0 | 9.1 | 90.9 |
| 9 | 100.0 | 0.0 | 50.0 | 50.0 |
| 10 | 100.0 | 0.0 | 90.9 | 9.1 |
| 11 | 100.0 | 0.0 | 99.0 | 1.0 |
| 12 | 100.0 | 0.0 | 99.9 | 0.1 |
| 13 | 100.0 | 0.0 | 100.0 | 0.0 |
| 14 | 100.0 | 0.0 | 100.0 | 0.0 |




**$pH = pKa + \log [A^-]/[HA]$**; qdo [A-] = [HA]: **pH = pKa**


Gly+
COOH
H3N+— CH2
Gly0
COO−
H3N+— CH2
Gly−
COO−
H2N — CH2
14
12
10
pH
8
6
4
2
0
pK2
Isoelectric
point
(pI)
pK1
1.0
0
1.0
Equivalents of H+
Equivalents of OH−
0
1.0
2.0
Equivalents of OH− added
2.0
1.0
0
Equivalents of H+ added

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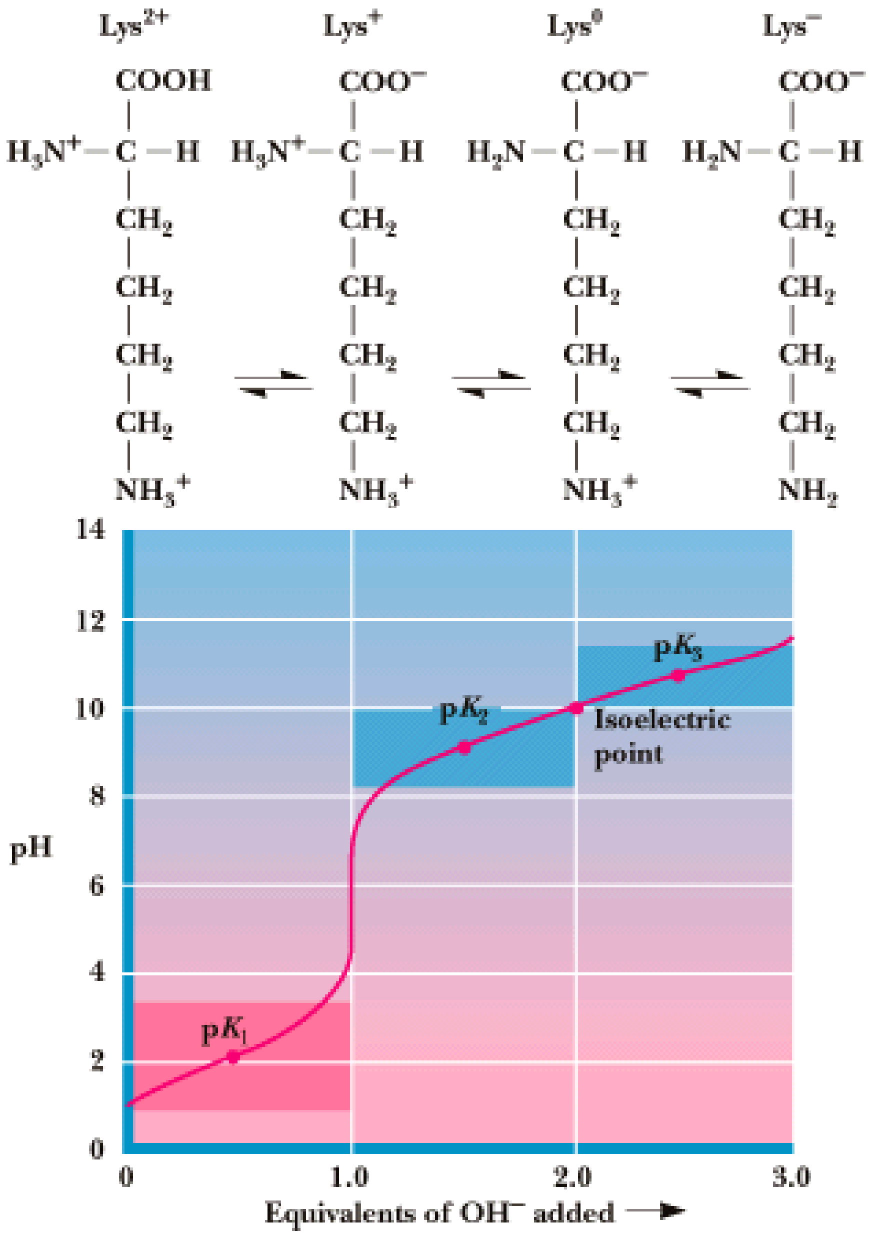



**Cálculo de pI**
**Capacidade tamponante**

## $pK_a$ Values of Polyfunctional Amino Acids

| Amino Acid | α-$CO_2H$ $pK_a^1$ | α-$NH_3$ $pK_a^2$ | Side Chain $pK_a^3$ | pI |
|---|---|---|---|---|
| Arginine | 2.1 | 9.0 | 12.5 | 10.8 |
| Aspartic Acid | 2.1 | 9.8 | 3.9 | 3.0 |
| Cysteine | 1.7 | 10.4 | 8.3 | 5.0 |
| Glutamic Acid | 2.2 | 9.7 | 4.3 | 3.2 |
| Histidine | 1.8 | 9.2 | 6.0 | 7.6 |
| Lysine | 2.2 | 9.0 | 10.5 | 9.8 |
| Tyrosine | 2.2 | 9.1 | 10.1 | 5.7 |

# Estrutura, polaridade, volume, aromaticidade

## Usuais apolares (9): G, A, V, L, I, M, P, F, W

Ala

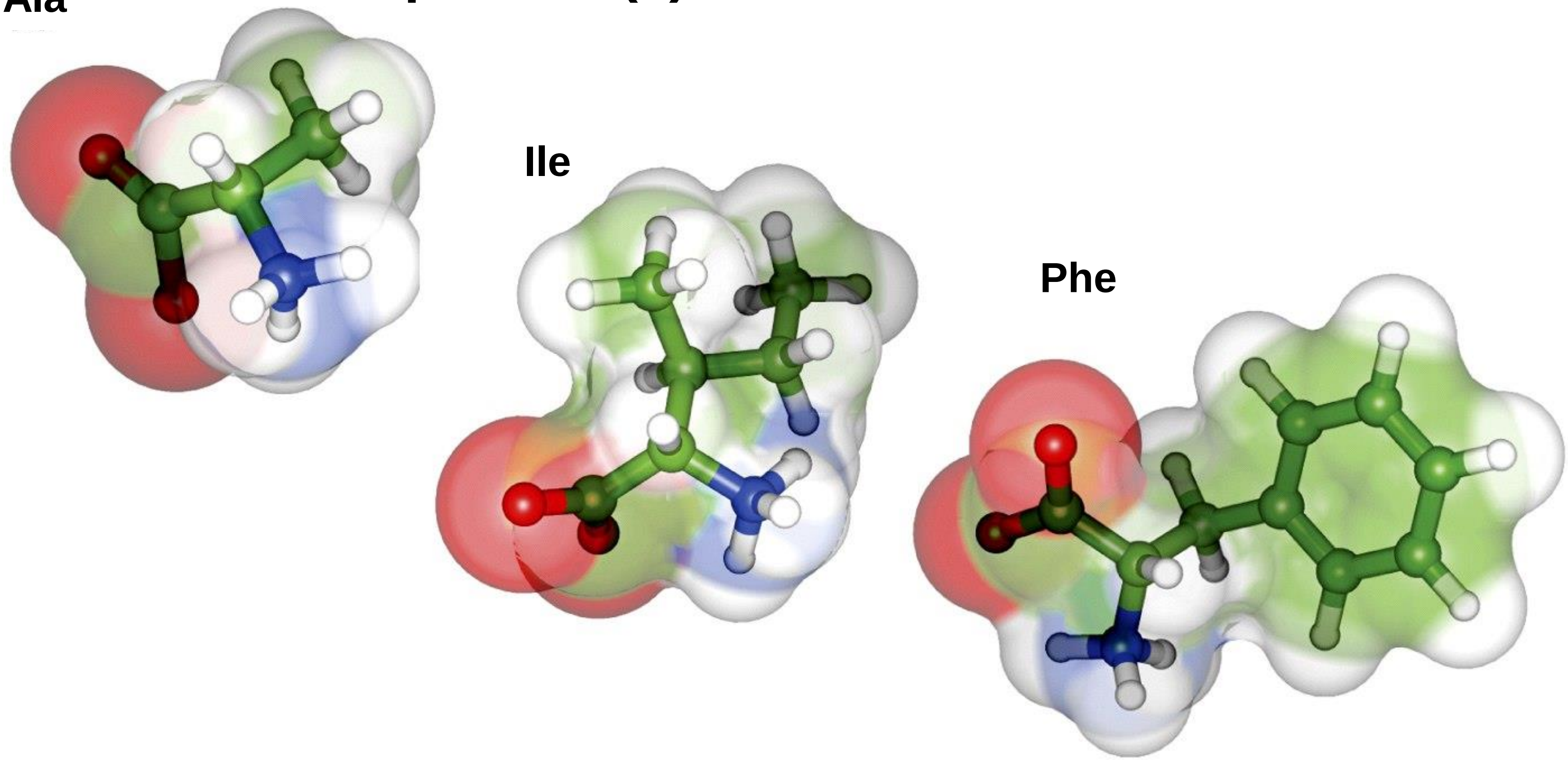
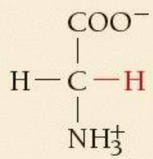
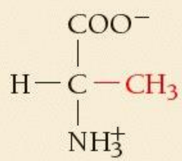
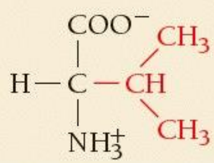



Figure 4-4 Fundamentals of Biochemistry, 2/e

**Table 4-1** ***Key to Structure.*** **Covalent Structures and Abbreviations of the "Standard" Amino Acids of Proteins, Their Occurrence, and the p*K* Values of Their Ionizable Groups**

| Name, Three-letter Symbol, and One-letter Symbol | Structural Formula[a] | Residue Mass (D)[b] | Average Occurrence in Proteins (%)[c] | $pK_1$ α-COOH[d] | $pK_2$ $\alpha\text{-}NH_3^+$[d] | $pK_R$ Side Chain[d] |
|---|---|---|---|---|---|---|
| ***Amino acids with nonpolar side chains*** | | | | | | |
| Glycine<br>Gly<br>G |  | 57.0 | 7.2 | 2.35 | 9.78 | |
| Alanine<br>Ala<br>A |  | 71.1 | 7.8 | 2.35 | 9.87 | |
| Valine<br>Val<br>V |  | 99.1 | 6.6 | 2.29 | 9.74 | |

[a]The ionic forms shown are those predominating at pH 7.0 (except for that of histidine[f]) although residue mass is given for the neutral compound. The $C_\alpha$ atoms, as well as those atoms marked with an asterisk, are chiral centers with configurations as indicated according to Fischer projection formulas (Section 4-2). The standard organic numbering system is provided for heterocycles.

[b]The residue masses are given for the neutral residues. For the molecular masses of the parent amino acids, add 18.0 D, the molecular mass of $H_2O$, to the residue masses. For side chain masses, subtract 56.0 D, the formula mass of a peptide group, from the residue masses.

[c]Calculated from a database of nonredundant proteins containing 300,688 residues as compiled by Doolittle, R.F. *in* Fasman, G.D. (Ed.), *Predictions of Protein Structure and the Principles of Protein Conformation,* Plenum Press (1989).

[d]Data from Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research* (3rd ed.), pp. 1–31, Oxford Science Publications (1986).



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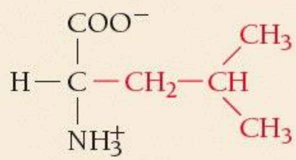
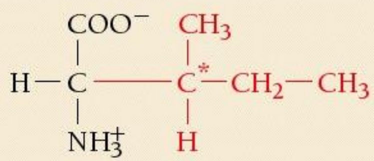
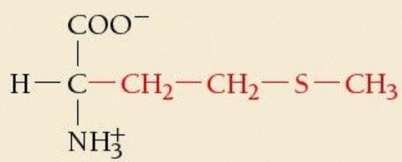
**Table 4-1** *Key to Structure.* **Covalent Structures and Abbreviations of the "Standard" Amino Acids of Proteins, Their Occurrence, and the p*K* Values of Their Ionizable Groups**

| Name, Three-letter Symbol, and One-letter Symbol | Structural Formula[a] | Residue Mass (D)[b] | Average Occurrence in Proteins (%)[c] | $pK_1$ $\alpha$-COOH[d] | $pK_2$ $\alpha$-$NH_3^+$[d] | $pK_R$ Side Chain[d] |
|---|---|---|---|---|---|---|
| ***Amino acids with nonpolar side chains*** | | | | | | |
| Leucine<br>Leu<br>L |  | 113.2 | 9.1 | 2.33 | 9.74 | |
| Isoleucine<br>Ile<br>I |  | 113.2 | 5.3 | 2.32 | 9.76 | |
| Methionine<br>Met<br>M |  | 131.2 | 2.2 | 2.13 | 9.28 | |

[a]The ionic forms shown are those predominating at pH 7.0 (except for that of histidine[f]) although residue mass is given for the neutral compound. The $C_\alpha$ atoms, as well as those atoms marked with an asterisk, are chiral centers with configurations as indicated according to Fischer projection formulas (Section 4-2). The standard organic numbering system is provided for heterocycles.

[b]The residue masses are given for the neutral residues. For the molecular masses of the parent amino acids, add 18.0 D, the molecular mass of $H_2O$, to the residue masses. For side chain masses, subtract 56.0 D, the formula mass of a peptide group, from the residue masses.

[c]Calculated from a database of nonredundant proteins containing 300,688 residues as compiled by Doolittle, R.F. *in* Fasman, G.D. (Ed.), *Predictions of Protein Structure and the Principles of Protein Conformation,* Plenum Press (1989).

[d]Data from Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research* (3rd ed.), pp. 1–31, Oxford Science Publications (1986).

**Table 4-1 part 2 Fundamentals of Biochemistry, 2/e**


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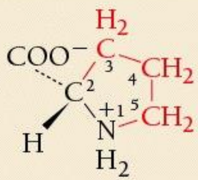
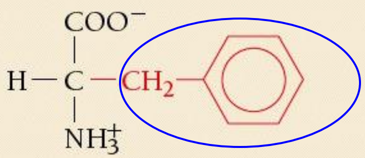
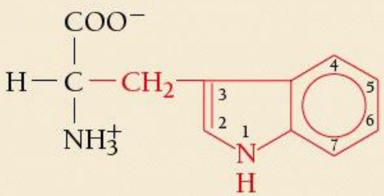
**Table 4-1** *Key to Structure.* **Covalent Structures and Abbreviations of the "Standard" Amino Acids of Proteins, Their Occurrence, and the p*K* Values of Their Ionizable Groups**

| Name, Three-letter Symbol, and One-letter Symbol | Structural Formula[a] | Residue Mass (D)[b] | Average Occurrence in Proteins (%)[c] | $pK_1$ α-COOH[d] | $pK_2$ α-$NH_3^+$[d] | $pK_R$ Side Chain[d] |
|---|---|---|---|---|---|---|
| ***Amino acids with nonpolar side chains*** | | | | | | |
| Proline<br>Pro<br>P |  | 97.1 | 5.2 | 1.95 | 10.64 | |
| Phenylalanine<br>Phe<br>F |  | 147.2 | 3.9 | 2.20 | 9.31 | |
| Tryptophan<br>Trp<br>W |  | 186.2 | 1.4 | 2.46 | 9.41 | |

[a]The ionic forms shown are those predominating at pH 7.0 (except for that of histidine[f]) although residue mass is given for the neutral compound. The $C_\alpha$ atoms, as well as those atoms marked with an asterisk, are chiral centers with configurations as indicated according to Fischer projection formulas (Section 4-2). The standard organic numbering system is provided for heterocycles.

[b]The residue masses are given for the neutral residues. For the molecular masses of the parent amino acids, add 18.0 D, the molecular mass of $H_2O$, to the residue masses. For side chain masses, subtract 56.0 D, the formula mass of a peptide group, from the residue masses.

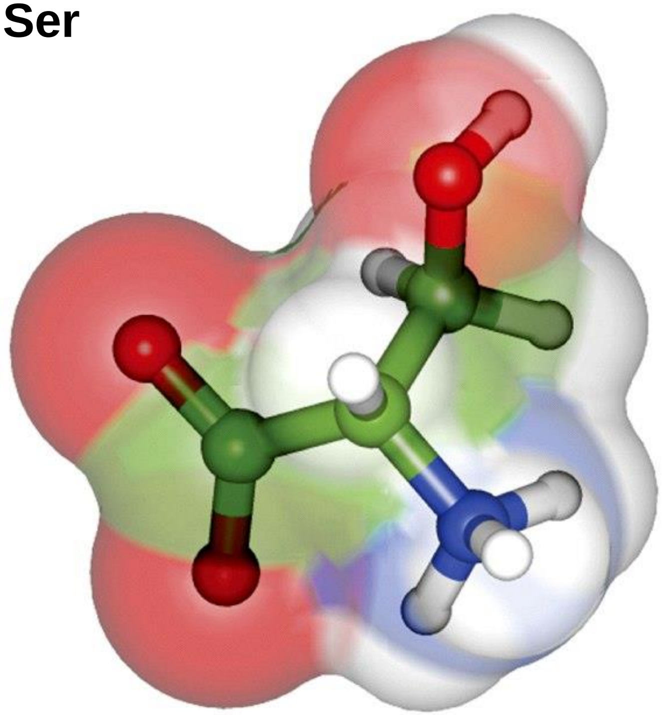
[c]Calculated from a database of nonredundant proteins containing 300,688 residues as compiled by Doolittle, R.F. *in* Fasman, G.D. (Ed.), *Predictions of Protein Structure and the Principles of Protein Conformation,* Plenum Press (1989).

[d]Data from Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research* (3rd ed.), pp. 1–31, Oxford Science Publications (1986).

# Estrutura, polaridade, volume, aromaticidade

## Usuais polares não carregados (6): S,T, N, Q, Y, C

Ser



Gln

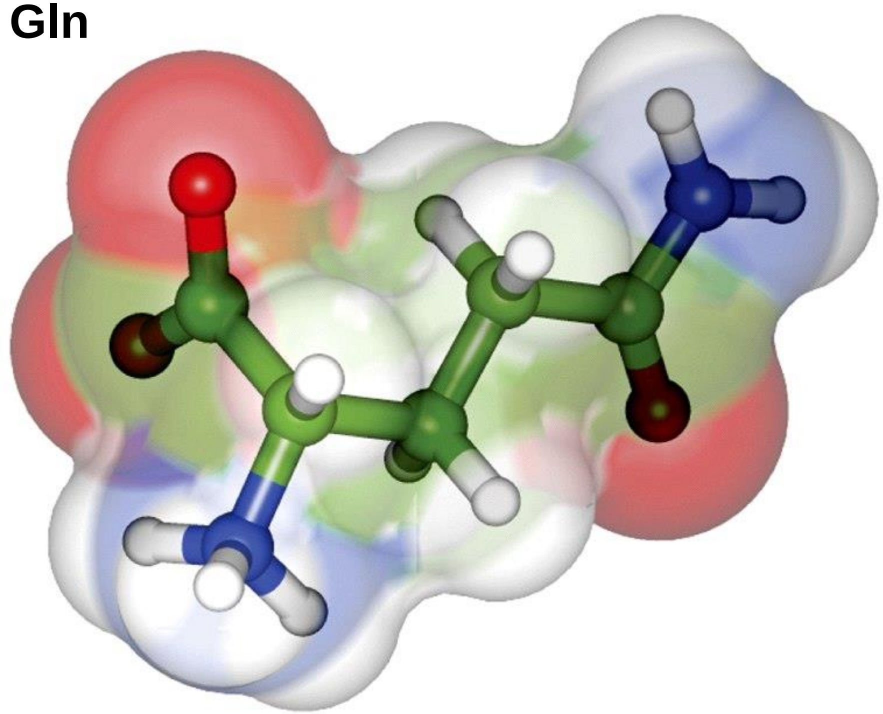
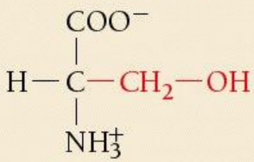
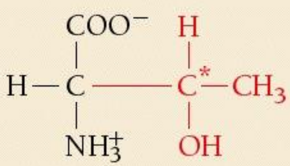
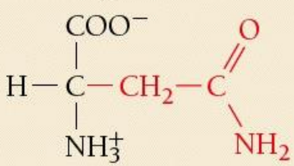


Figure 4-5 Fundamentals of Biochemistry, 2/e

**Table 4-1 (continued)**

| Name, Three-letter Symbol, and One-letter Symbol | Structural Formula[a] | Residue Mass (D)[b] | Average Occurrence in Proteins (%)[c] | $pK_1$ α-COOH[d] | $pK_2$ α-$NH_3^+$[d] | $pK_R$ Side Chain[d] |
|---|---|---|---|---|---|---|
| ***Amino acids with uncharged polar side chains*** | | | | | | |
| Serine<br>Ser<br>S |  | 87.1 | 6.8 | 2.19 | 9.21 | |
| Threonine<br>Thr<br>T |  | 101.1 | 5.9 | 2.09 | 9.10 | |
| Asparagine[e]<br>Asn<br>N |  | 114.1 | 4.3 | 2.14 | 8.72 | |

[e]The three- and one-letter symbols for asparagine *or* aspartic acid are Asx and B, whereas for glutamine *or* glutamic acid they are Glx and Z. The one-letter symbol for an undetermined or "nonstandard" amino acid is X.

[f]Both neutral and protonated forms of histidine are present at pH 7.0, since its $pK_R$ is close to 7.0.

**Table 4-1 part 4 Fundamentals of Biochemistry, 2/e**


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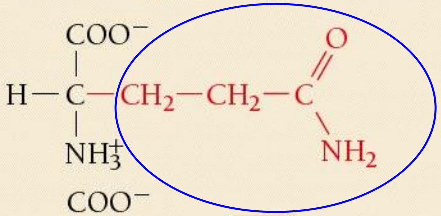
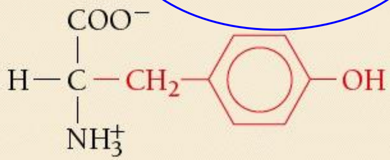
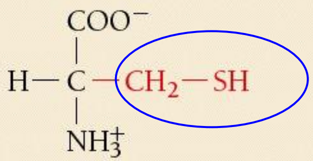
**Table 4-1** (continued)

| Name, Three-letter Symbol, and One-letter Symbol | Structural Formula[a] | Residue Mass (D)[b] | Average Occurrence in Proteins (%)[c] | $pK_1$ α-COOH[d] | $pK_2$ $\alpha\text{-}NH_3^{+}$[d] | $pK_R$ Side Chain[d] |
|---|---|---|---|---|---|---|
| ***Amino acids with uncharged polar side chains*** | | | | | | |
| Glutamine[e] Gln Q |  | 128.1 | 4.3 | 2.17 | 9.13 | |
| Tyrosine Tyr Y |  | 163.2 | 3.2 | 2.20 | 9.21 | 10.46 (phenol) |
| Cysteine Cys C |  | 103.1 | 1.9 | 1.92 | 10.70 | 8.37 (sulfhydryl) |

[e]The three- and one-letter symbols for asparagine *or* aspartic acid are Asx and B, whereas for glutamine *or* glutamic acid they are Glx and Z. The one-letter symbol for an undetermined or "nonstandard" amino acid is X.

[f]Both neutral and protonated forms of histidine are present at pH 7.0, since its $pK_R$ is close to 7.0.

**Table 4-1 part 5 Fundamentals of Biochemistry, 2/e**

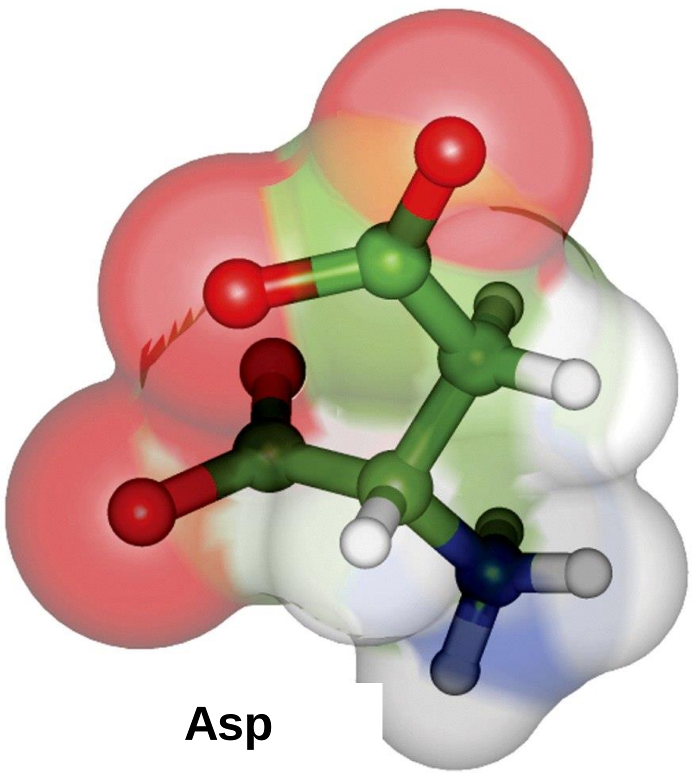

# Natureza dos R dos α-aminoácidos:

**determinantes para a estrutura tridimensional das proteínas**

MTM

# Estrutura, polaridade, volume, aromaticidade

## Usuais polares carregados (5): K, R, H, D, E



Asp

Figure 4-7a Fundamentals of Biochemistry, 2/e

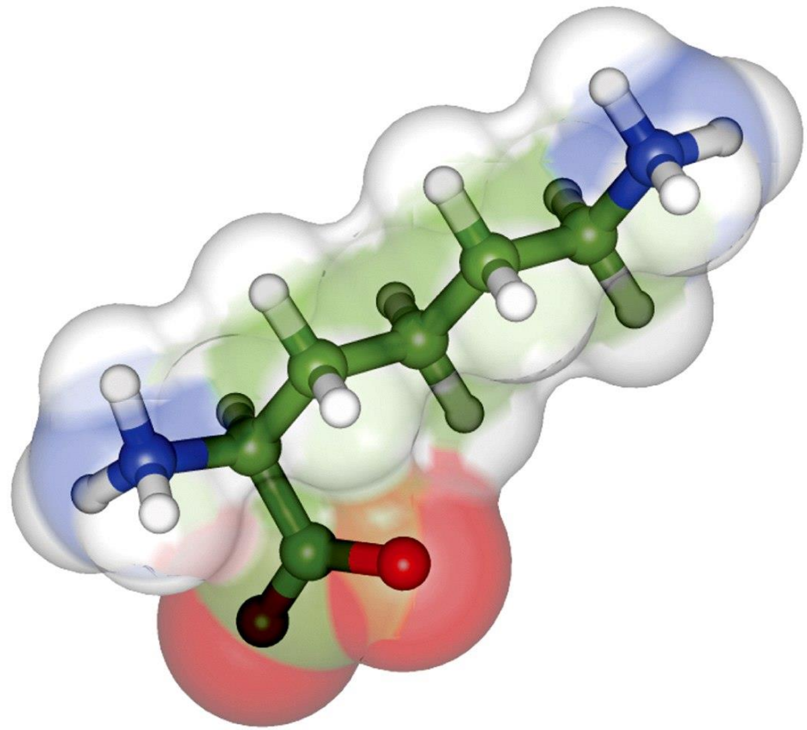
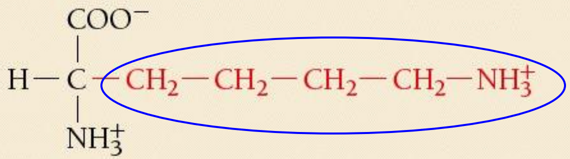
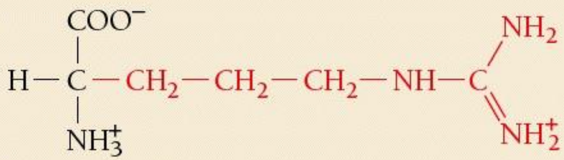
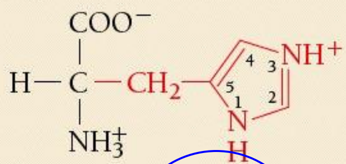
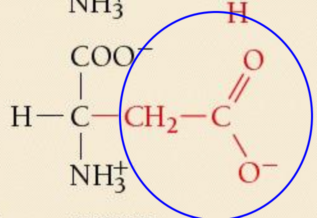
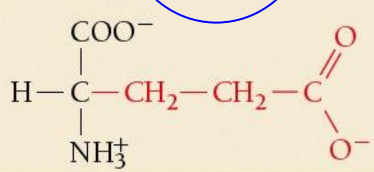



Figure 4-7b Fundamentals of Biochemistry, 2/e


Lys

**Table 4-1** (continued)

| Name, Three-letter Symbol, and One-letter Symbol | Structural Formula[a] | Residue Mass (D)[b] | Average Occurrence in Proteins (%)[c] | $pK_1$ α-COOH[d] | $pK_2$ α-$NH_3^+$[d] | $pK_R$ Side Chain[d] |
|---|---|---|---|---|---|---|
| ***Amino acids with charged polar side chains*** | | | | | | |
| Lysine<br>Lys<br>K |  | 128.2 | 5.9 | 2.16 | 9.06 | 10.54 (ε-$NH_3^+$) |
| Arginine<br>Arg<br>R |  | 156.2 | 5.1 | 1.82 | 8.99 | 12.48 (guanidino) |
| Histidine[f]<br>His<br>H |  | 137.1 | 2.3 | 1.80 | 9.33 | 6.04 (imidazole) |
| Aspartic acid[e]<br>Asp<br>D |  | 115.1 | 5.3 | 1.99 | 9.90 | 3.90 (β-COOH) |
| Glutamic acid[e]<br>Glu<br>E |  | 129.1 | 6.3 | 2.10 | 9.47 | 4.07 (γ-COOH) |

[e]The three- and one-letter symbols for asparagine *or* aspartic acid are Asx and B, whereas for glutamine *or* glutamic acid they are Glx and Z. The one-letter symbol for an undetermined or "nonstandard" amino acid is X.

[f]Both neutral and protonated forms of histidine are present at pH 7.0, since its $pK_R$ is close to 7.0.



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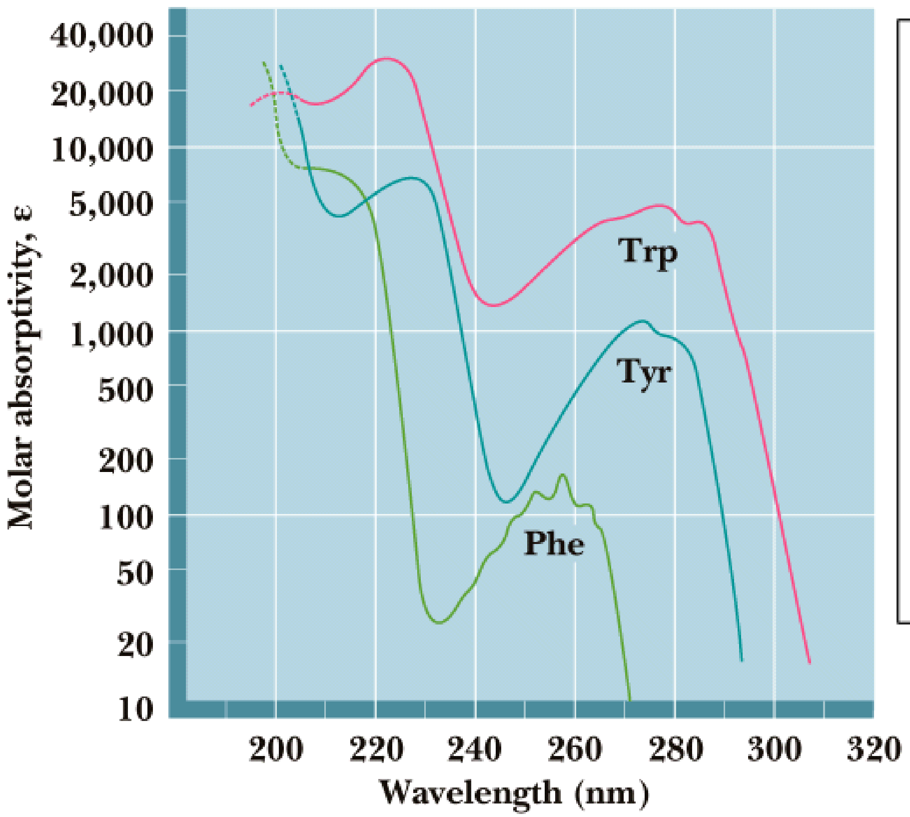
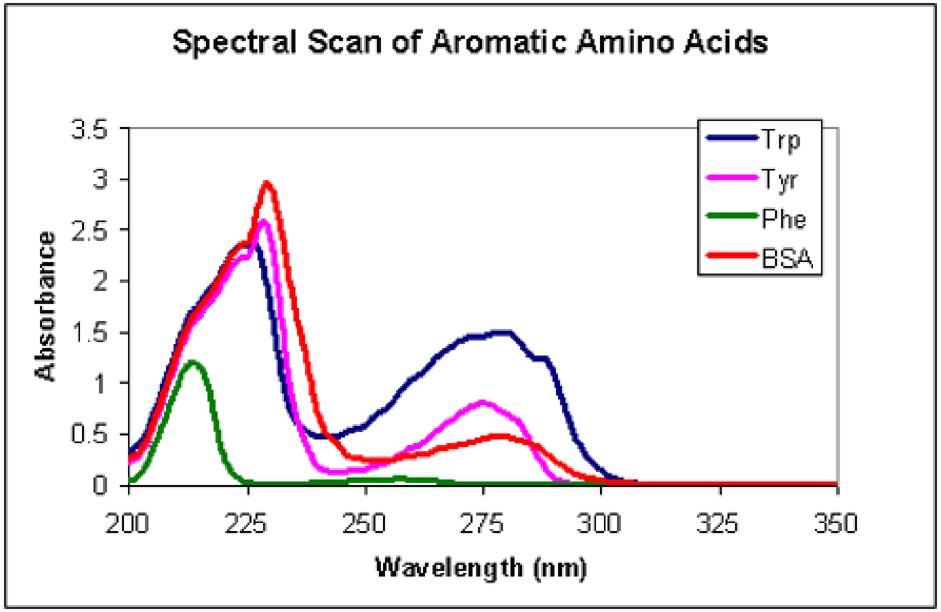
# Absorção de luz

**Todos: absorvem em 200-220 nm; alguns absorvem em 250-280 nm**

# Importância: detecção e dosagem

**(deles e dos biopolímeros formados por eles)**

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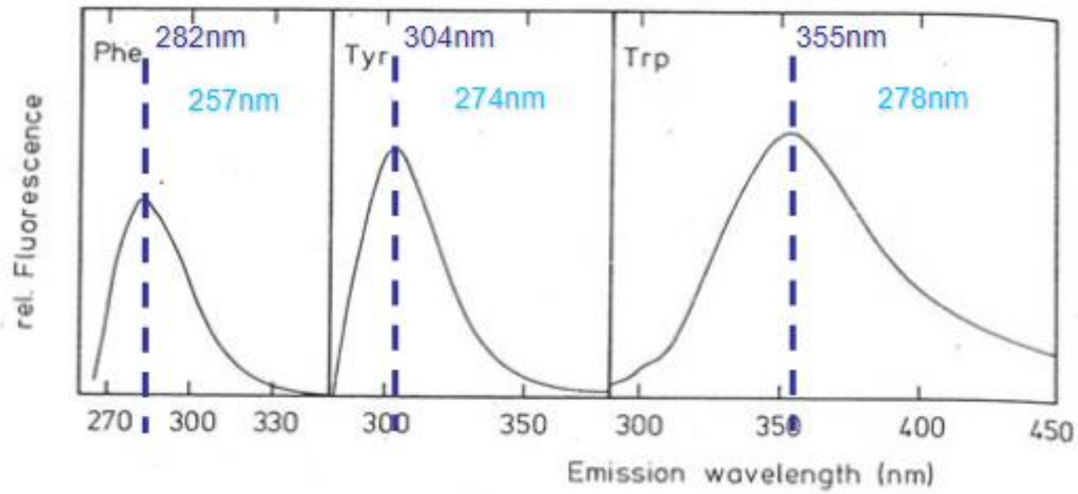
# Fluorescência de aminoácidos




| | Absorbance | | Fluorescence | | Sensitivity |
|---|---|---|---|---|---|
| Amino acid | $\lambda_{max}$ (nm) | $\varepsilon_{max}$ ($M^{-1}cm^{-1}$) | $\lambda_{max}$ (nm) | $\varphi_F{}^b$ | $\varepsilon_{max} \times \varphi_F{}^b$ ($M^{-1}cm^{-1}$) |
| Tryptophan | 280 | 5600 | 355 | 0.13 | 730 |
| Tyrosine | 275 | 1400 | 304 | 0.14 | 200 |
| Phenylalanine | 258 | 200 | 282 | 0.02 | 4 |

# Quiralidade → Atividade óptica



Aminoácidos são substâncias opticamente ativas pq desviam a luz plano-polarizada

**dextrorrotatórias: *d* (dexter = direita) = + ( sentido horário)**

**levorrotatórias: *l* (laeves = esquerda) = - (sentido anti-horário)**

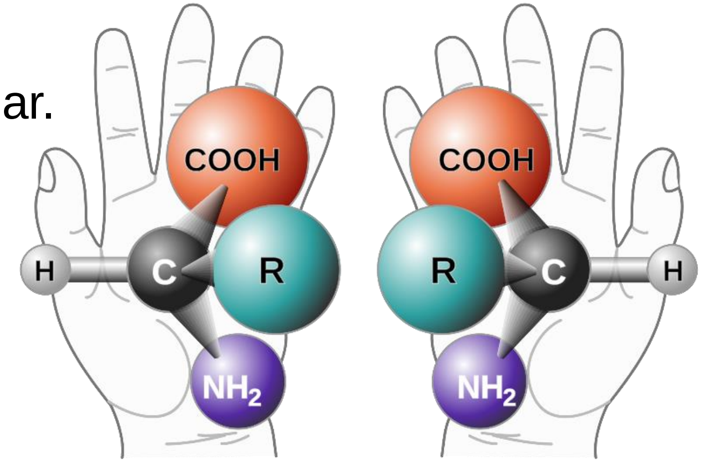
**Porque isso ocorre? Possuem assimetria/quiralidade**

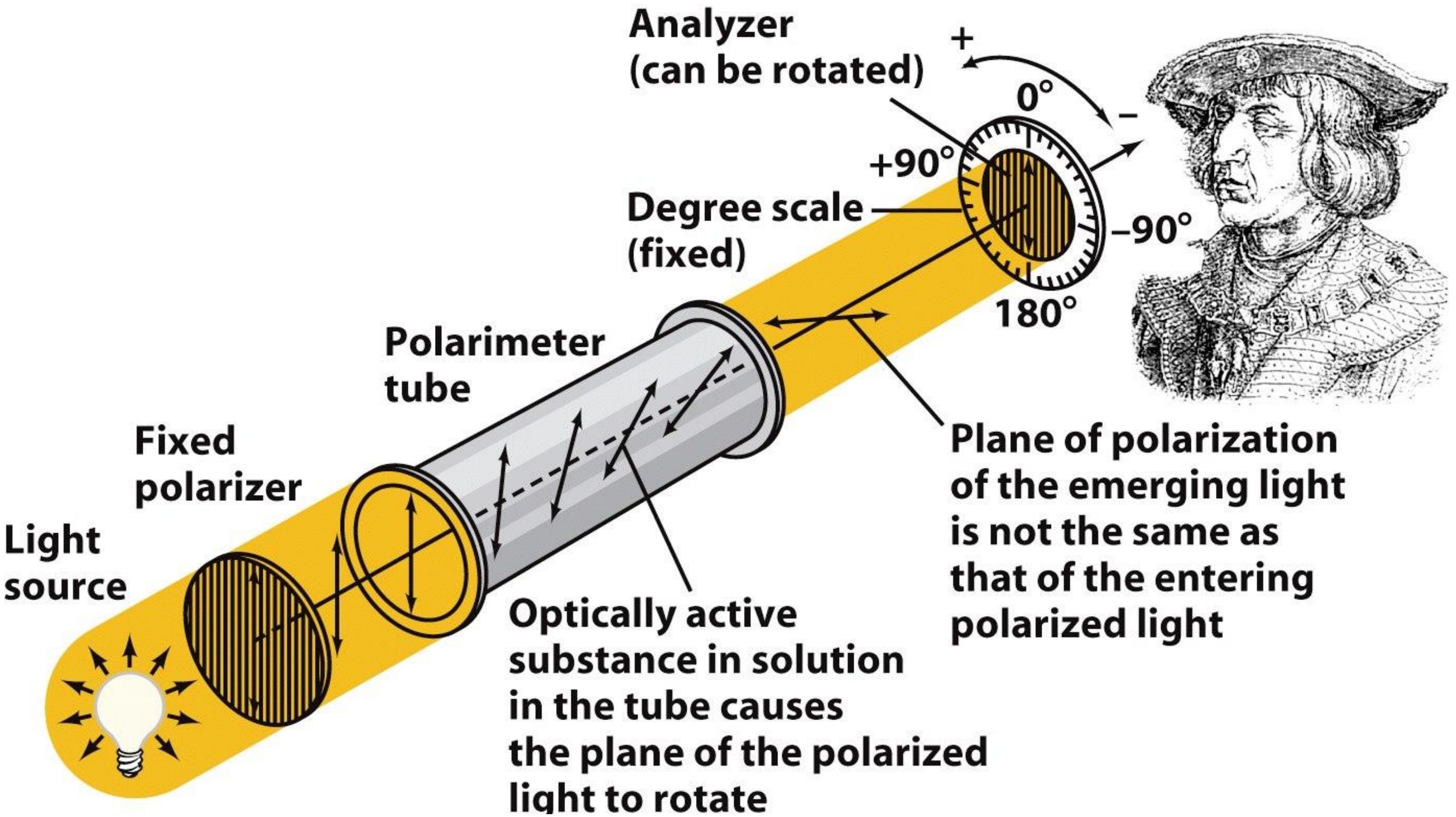
característica típica de substâncias que contém { centro(s) assimétrico(s)/quirálico(s) / carbono(s) assimétrico(s) / **C***

impossibilita a sobreposição à sua imagem especular.

**no. de possíveis estereoisômeros = $2^n$**

(2 possíveis configurações; n = no. de C*)**:**






$[\alpha]_D^{25}$= % rotação observada (º) / caminho óptico (dm) X concentração (g/ml)

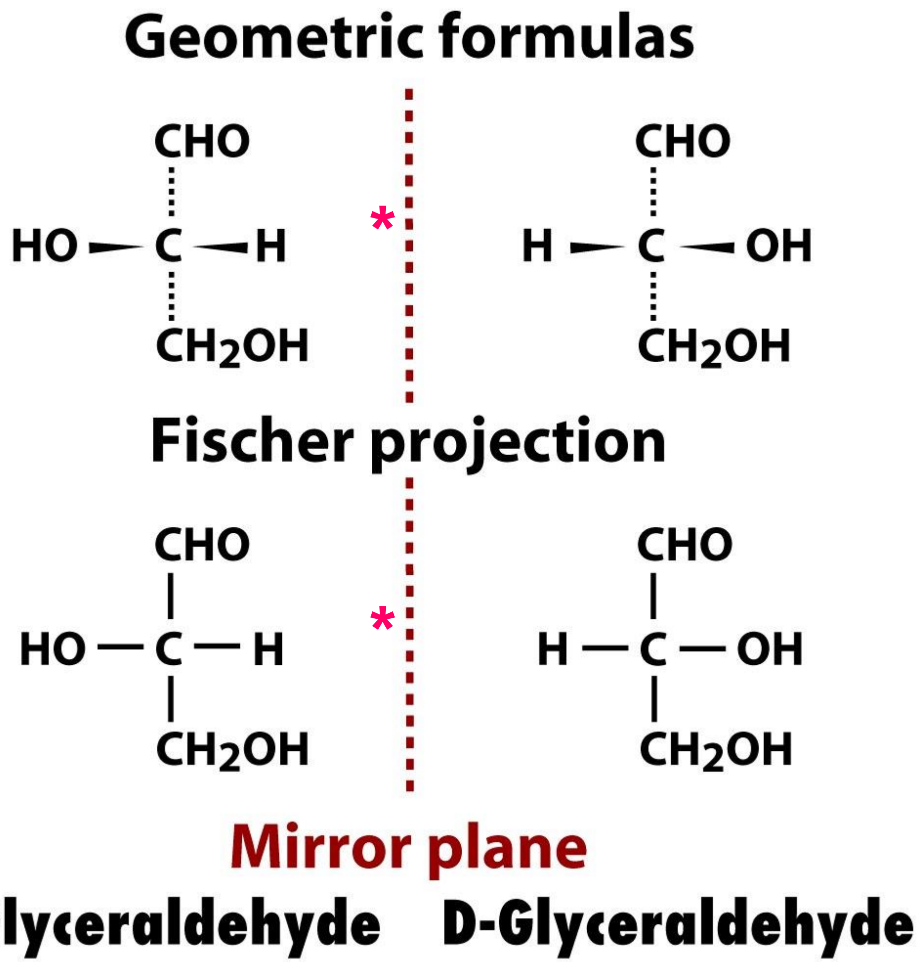
*D = linha D do espectro do Na: λ= 598,3nm*

| | | | |
|---|---|---|---|
| *L*- Pro | -86,2º | *D*- Pro | + 86,2 º |
| *L*- Leu | -10,4 º | *D*- Leu | +10,4 º |
| *L*- Arg | +12,5 º | *D*- Arg | -12,5 º |

**Nomenclatura para especificar configuração absoluta:**

(*disposição espacial dos grupos ao redor do C*)*

**sistema *D, L*; *Emil Fischer*, 1891: configuração de um composto de referência**



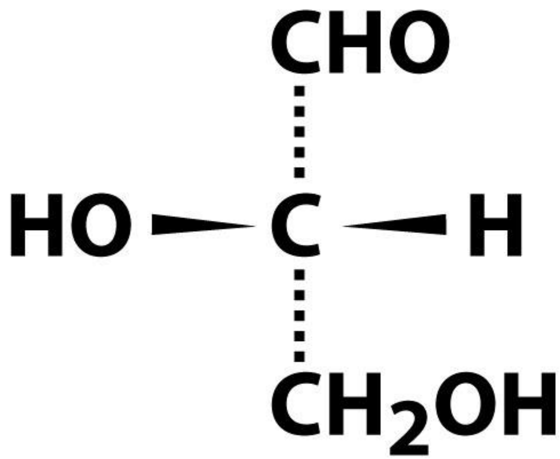

**(50% de chance de erro)**

Figure 4-12 Fundamentals of Biochemistry, 2/e

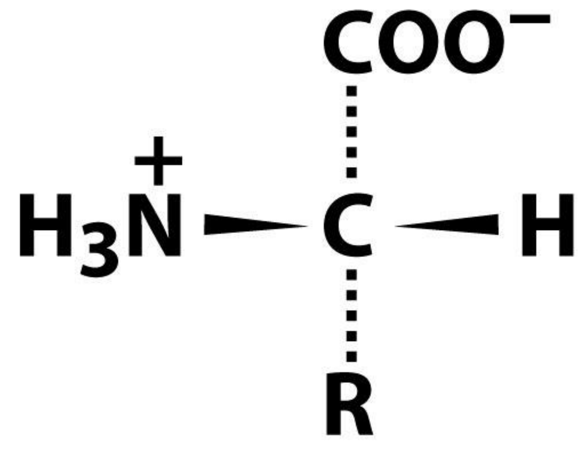


MTM

<u>**Configuração absoluta dos aminoácidos segundo sistema D,L:**</u>




**L-Glyceraldehyde**




**L-α-Amino acid**

Unnumbered figure pg 87 Fundamentals of Biochemistry, 2/e


**(forma – ou l )**

**1949: configuração absoluta confirmada por cristalografia de raios X**

**(*configuração dos grupos ao redor do C* está relacionada com aquela dos grupos do L-gliceraldeído*: reações químicas convertem os diferentes grupos com os quais são comparados em relação à configuração)**

↓

**Todos os aminoácidos proteicos: L**

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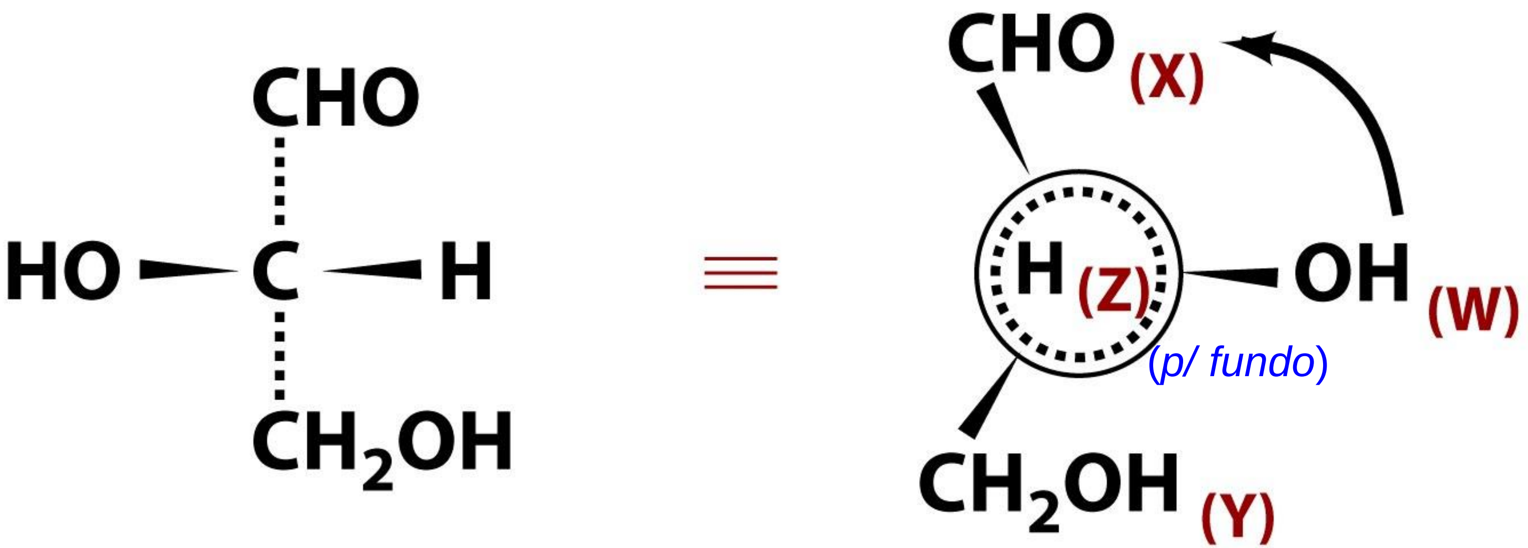
**Nomenclatura para especificar configuração absoluta:**

**sistema R (rectus), S (sinistre)**; ***Cahn-Ingold-Prelog, 1956*:**

**ordem de prioridade dos átomos: nos. atômicos dos grupos ligados a um C***

**SH > OH > $NH_2$ > COOH > CHO > $CH_2OH$ > $C_6H_5$ > $CH_3$ > $^2H$ > $^1H$**

**mais adequada para moléculas com mais de um C*.**




**L-Glyceraldehyde** **(S)-Glyceraldehyde**

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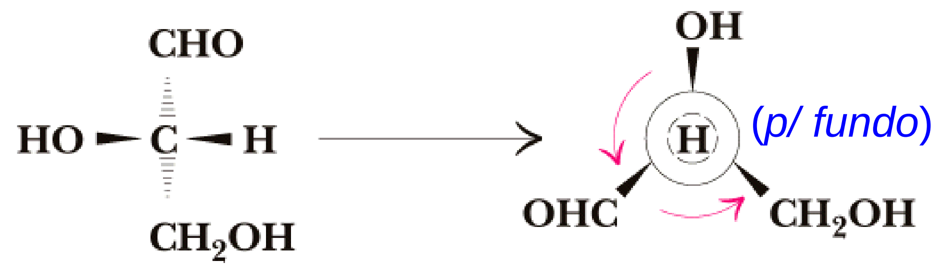
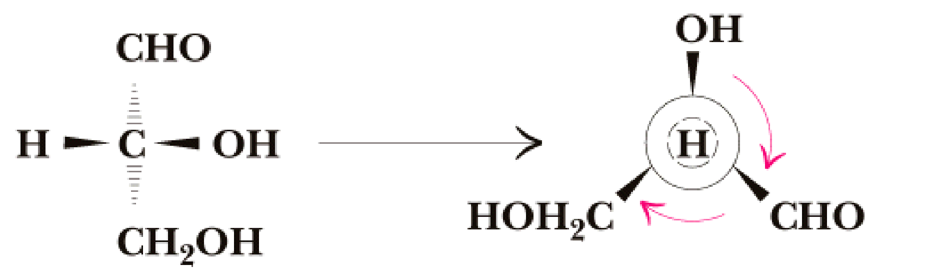
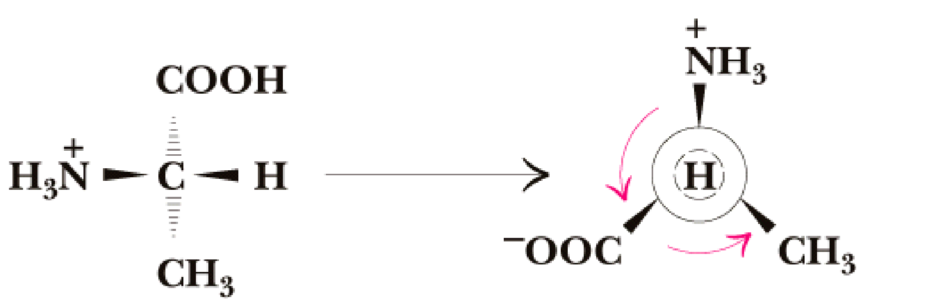
# Sistemas L,D e S, R aplicados aos aminoácidos




L-Glyceraldehyde (*S*)-Glyceraldehyde



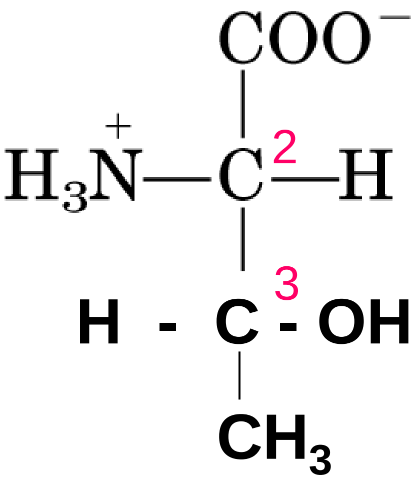

D-Glyceraldehyde (*R*)-Glyceraldehyde




L-Alanine (*S*)-Alanine

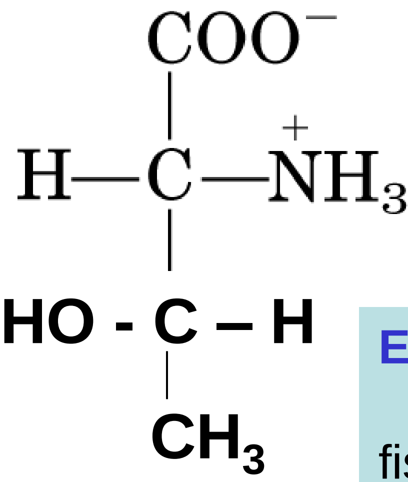
**Com exceção de Cys, todos os 20 L-aminoácidos de pept/prot são S!**

**$2^n = 2^2 = 4$ isômeros**



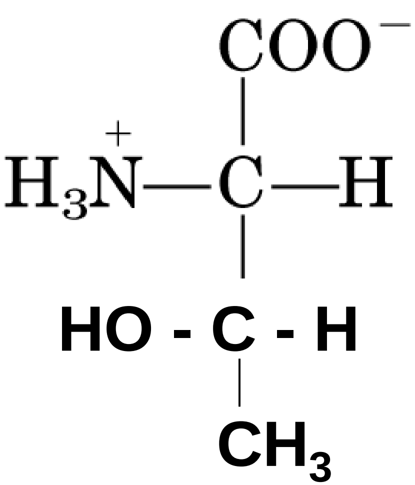

**L-Thr**

**(2*S*, 3*R*)-Thr**

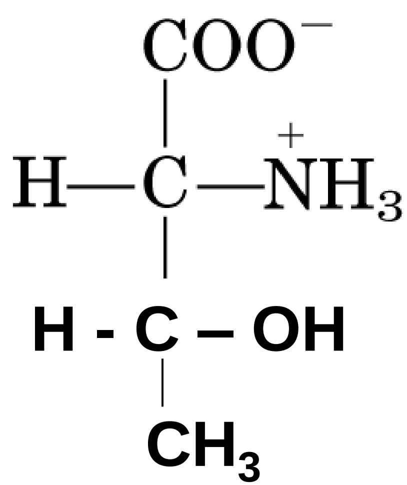



**D-Thr**

**Enantiômeros:** imagens especulares não sobreponíveis; fisica- e quimicamente indistinguíveis.




**L-*allo*-Thr**




**D-*allo*-Thr**

**Diastereisômeros:**
imagens especulares entre si, mas não de L- ou D-Thr
fisica- e quimicamente distinguíveis (*PF, espectros, reatividade química diferentes*).

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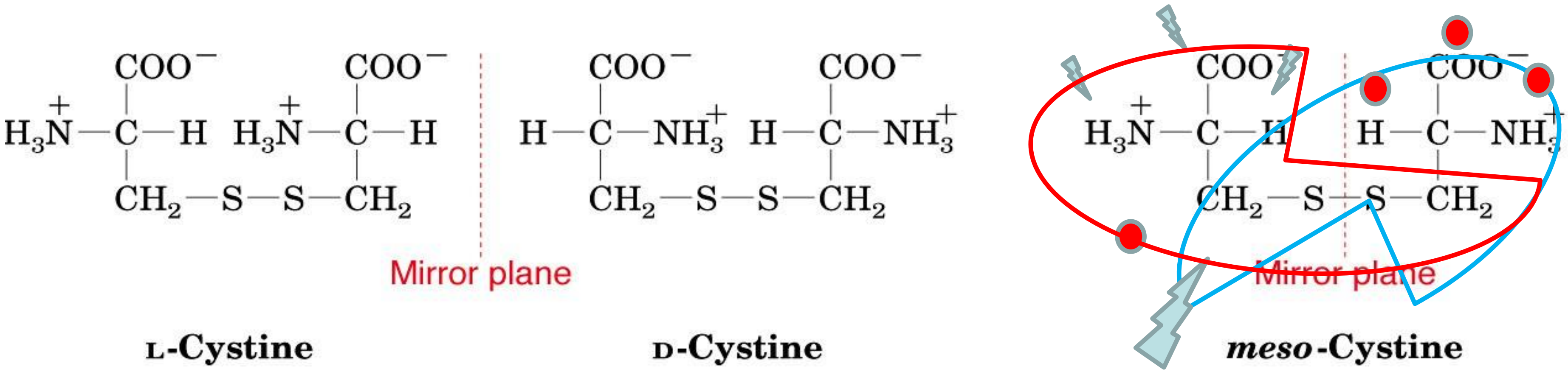



L-Cystine D-Cystine *meso*-Cystine

**L-Cystine: Optical rotation**: -218° to -224° (c = 2, 1 N HCl, 20°C) EMD Millipore

-215 to -230 deg (C=1, 1mol/L HCl) [a]20/D Chemical Industry Co., Ltd. Tokyo

**D-Cystine: Optical rotation**: [α]20/D +212° (c = 1, 1 M HCl, 20°C) Merck

**Isômero meso** ou **allo** ou **internamente compensado:**

**Oticamente inativo**

2 centros assimétricos quimicamente idênticos

uma parte da molécula não se sobrepõe à outra parte → as partes são imagens especulares uma da outra

A molécula é sobreponível à sua imagem especular

## <u>Como separar/analisar/quantificar aminoácidos</u>? MTM

## 1) Separação por diferença de polaridade:

**<u>Cromatografia em papel</u> (fase estacionária = celulose)**

**<u>Cromatografia em camada delgada</u> (CCD; TLC): sílica, celulose, alumina**

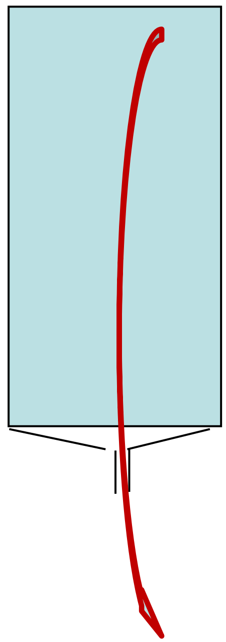
**<u>Revelação:</u> reação química que gera produtos coloridos ou fluorescentes**

**comparação com aminoácidos de concentração conhecida e entre Rfs**

**<u>Cromatografia em coluna</u>** (preenchida com material não iônico <u>polar ou apolar</u>)

(eluente = fase móvel)

(Coluna = fase estacionária)



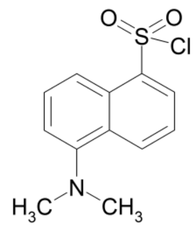
**comparação da amostra com mistura-padrão de concentração conhecida, seus tr** (tempos de retenção**)** **e áreas dos seus picos**

**<u>Neste caso</u>: Derivatização ou revelação dos aminoácidos**

**<u>pré-coluna</u>: antes da cromatografia**
**<u>pós-coluna</u>: depois da cromatografia**
**Que reagentes?**

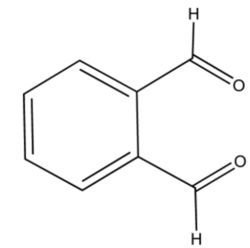
cloreto de dansila

produto fluorescente



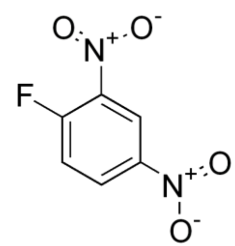
o-ftaldeído (OPA)

produto fluorescente



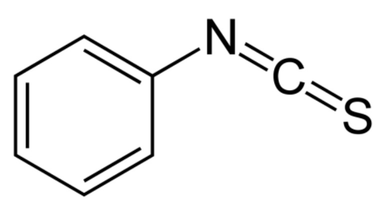
2,4 dinitrofluorobenzeno (DTNB; reagente de Sanger)

produto colorido



fenilisotiocianato (PITC)

produto que absorve em 254 nm



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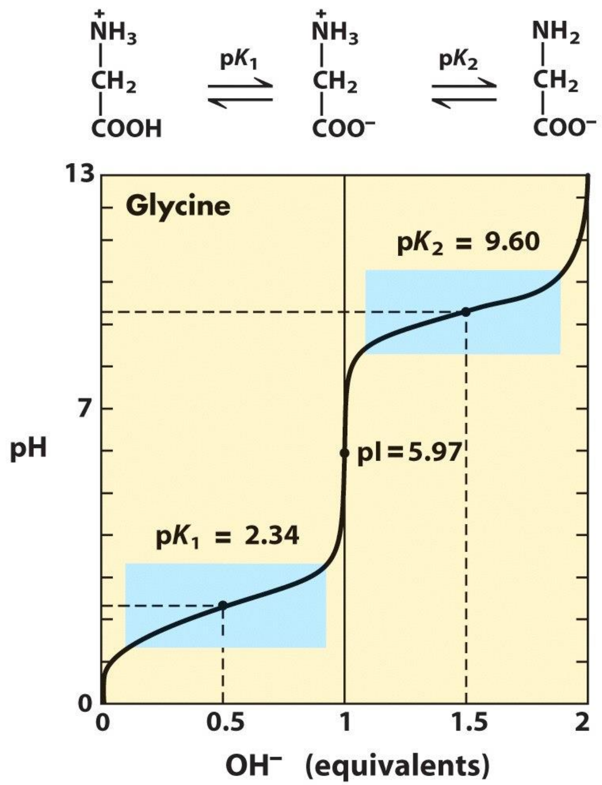
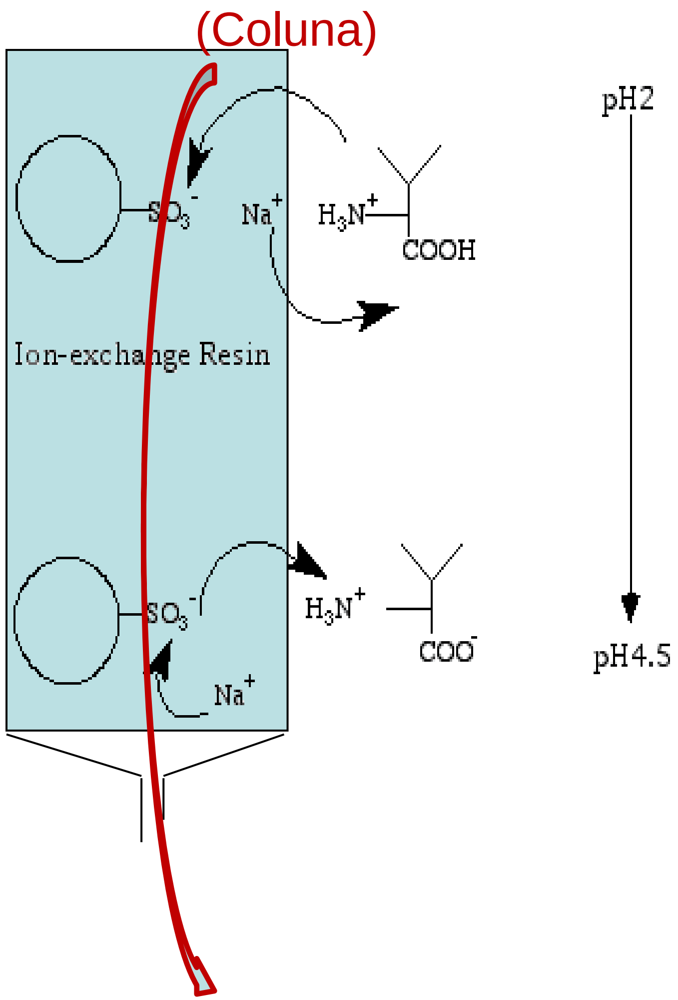
# 2) Separação por diferença de <u>carga líquida</u> em dado pH:

**propriedades ácido-base dos aminoácidos**

- cromatografia dos aminoácidos <u>em coluna</u> (preenchida com material iônico)
- uso de NaCl ou KCl para eluir os aminoácidos retidos na coluna
- comparação com aminoácidos-padrão de concentração conhecida e entre volume do eluente e áreas dos picos

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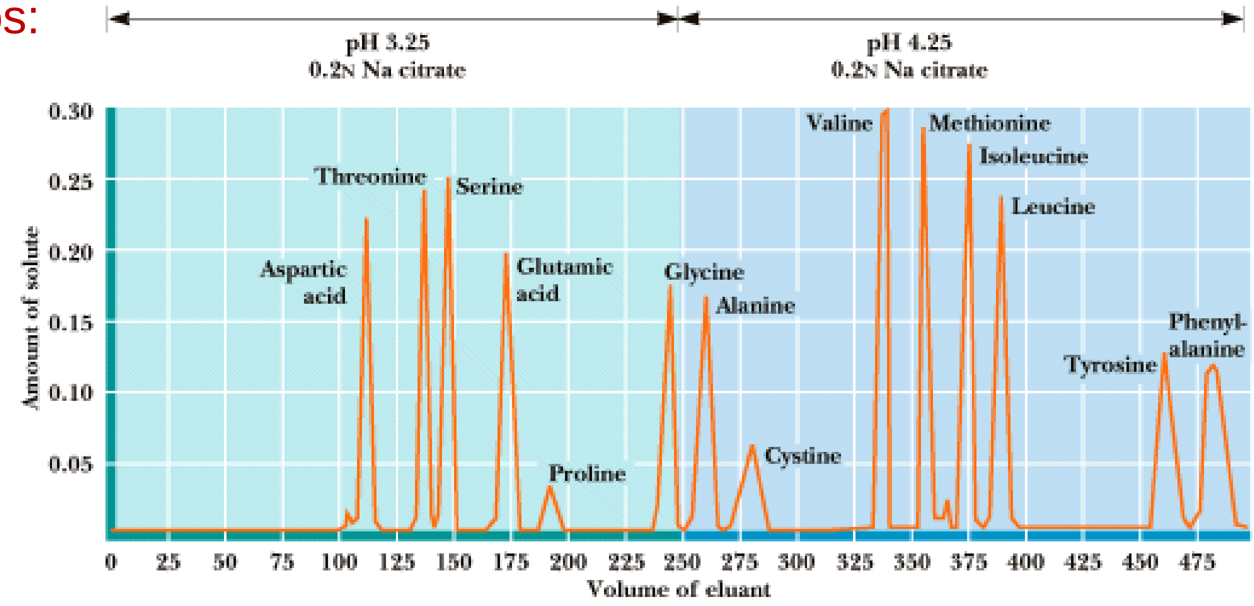
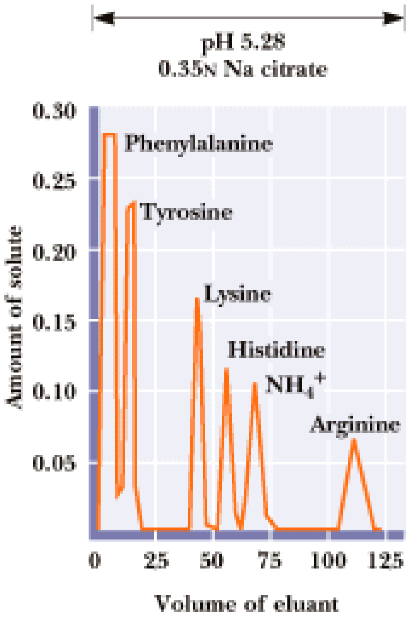
cromatografia de troca iônica catiônica: sem pré-derivatização da mistura de aminoácidos:




Comparação com padrões de concentração conhecida!

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