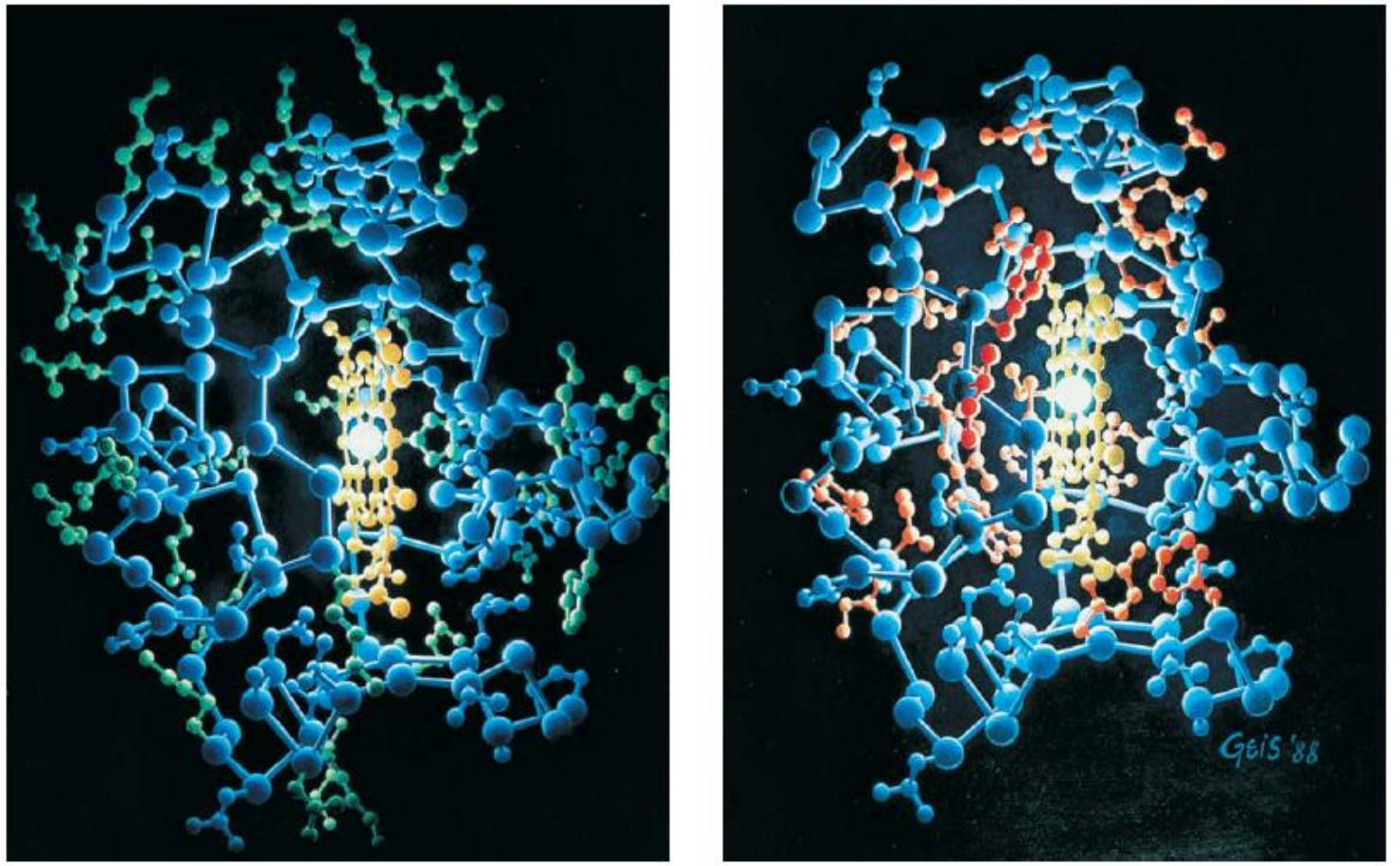
第三章 蛋白质
Proteins



(a) (b)

FIG. 6-27 Side chain distribution in horse heart cytochrome *c*. In these paintings, based on an X-ray structure determined by Richard Dickerson, the protein is illuminated by its single iron atom centered in a heme group. Hydrogen atoms are not shown. In (*a*) the hydrophilic side chains are green, and in (*b*) the hydrophobic side chains are orange.

本章主要内容 (14学时)

一．蛋白质概述 ←

二．氨基酸 (重点) ←····

三．多肽（重点）

四．蛋白质的结构（重点）

五．蛋白质结构与功能（重点）

六．蛋白质的性质（重点）

七．蛋白质的分离纯化与鉴定（重点）

一、蛋白质概述

- 构成生物体最基本的结构物质和功能物质。
- 一切生命过程，都离不开蛋白质的参与。
- Protein来自希腊文“Proteios”

  --- “首要地位”

  --- 1838年，荷兰化学家G. J. Mulder

Proteins are at **the center of action** in biological process. They are **essential** structural and functional components of cells.

1. 蛋白质研究史上的重大事件

- **1890年，结晶出第一个蛋白质, egg albumin (F Hofmeister)**

The Hofmeister series is a classification of ions in order of their ability to salt out （盐析）or salt in （盐溶）proteins.

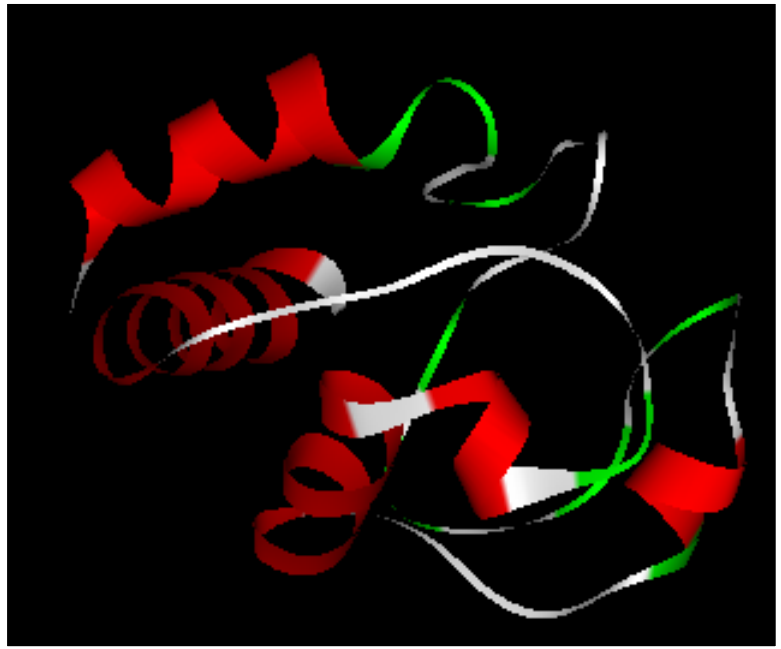
- **1902年，蛋白质是氨基酸通过肽键连接而成的多肽链（F Hofmeister, E Fischer）**

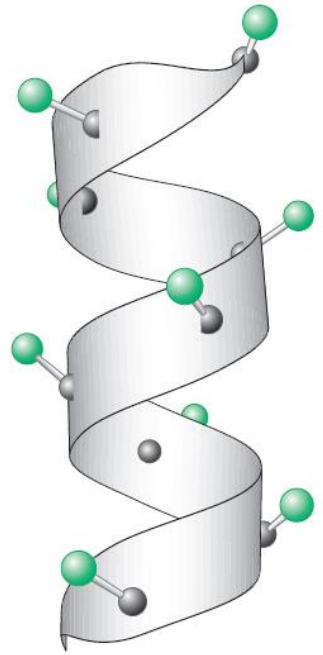
- **F Hofmeister made the proposal based on the biuret reaction (双缩脲反应) in proteins.**
- **E Fischer had a wealth of chemical details supporting the peptide-bond model.**

1. 蛋白质研究史上的重大事件

- 1951年，L Pauling提出蛋白质的α-helix模型，1954年获诺贝尔化学奖。

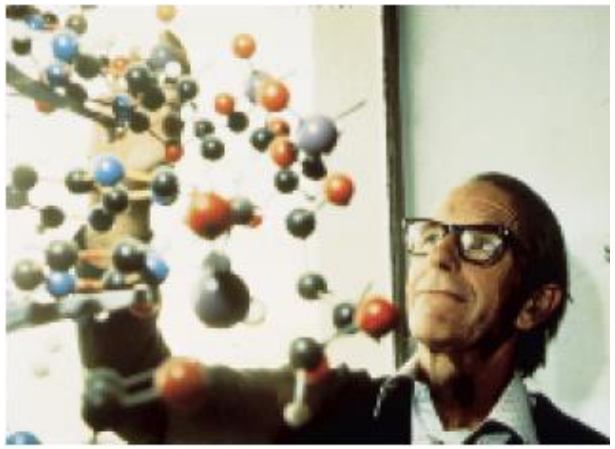
Linus Pauling, 1901–1994





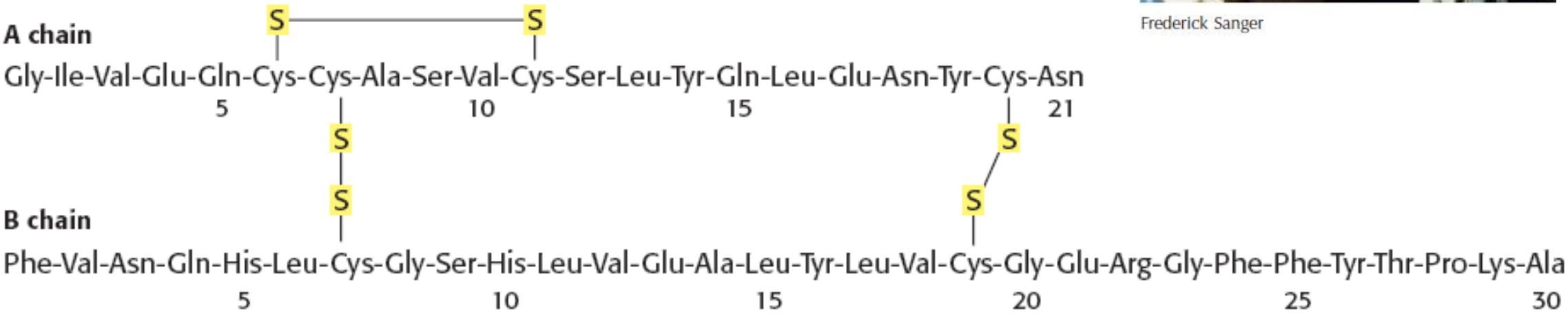
1. 蛋白质研究史上的重大事件

- 1955年，F Sanger测定出牛胰岛素的一级结构，1958年获诺贝尔化学奖。



Frederick Sanger

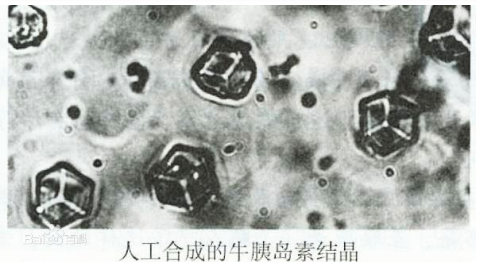
你知道当时测序的主要手段吗？
现在又会用哪种技术？



1965年，中国科学家首次人工合成胰岛素

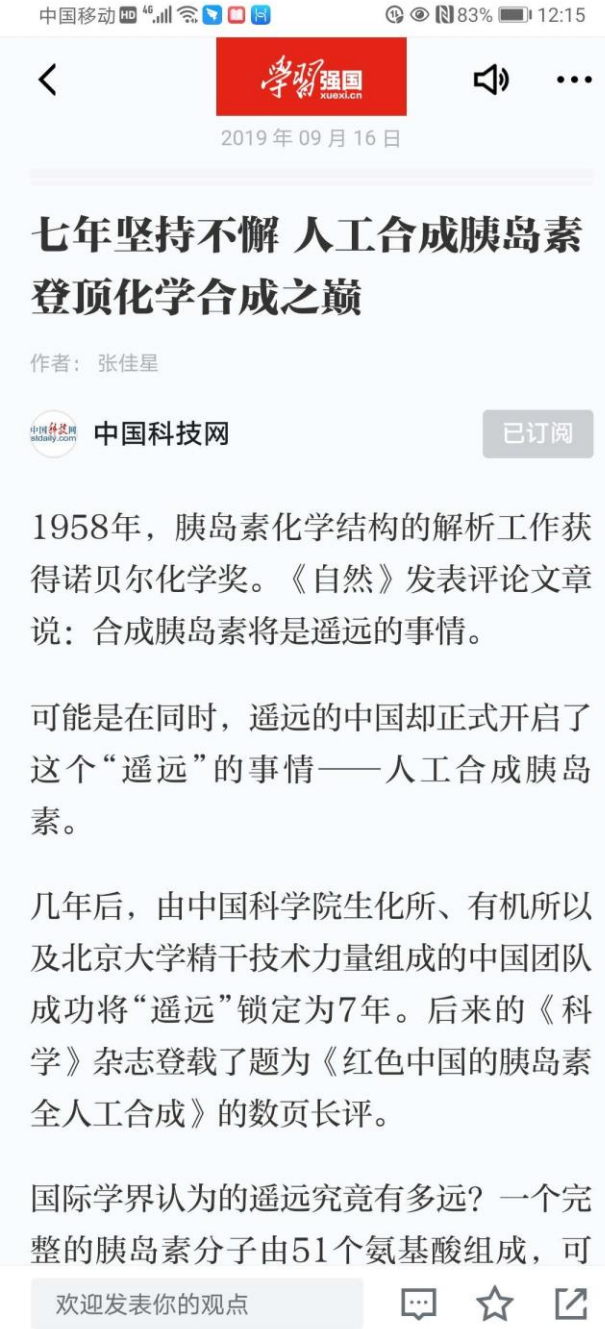
七年坚持不懈 人工合成胰岛素登顶化学合成之巅

https://article.xuexi.cn/articles/index.html?study_style_id=feeds_default&study_comment_disable=0&art_id=13179104125348139972&source=share&share_to=wx_single



人工合成的牛胰岛素结晶

我国人工胰岛素合成缘何未获诺奖？（熊卫民，博文）



中国移动 83% 12:15

学习强国 xuexi.cn

2019 年 09 月 16 日

七年坚持不懈 人工合成胰岛素登顶化学合成之巅

作者： 张佳星

中国科技网 已订阅

1958年，胰岛素化学结构的解析工作获得诺贝尔化学奖。《自然》发表评论文章说：合成胰岛素将是遥远的事情。

可能是在同时，遥远的中国却正式开启了这个“遥远”的事情——人工合成胰岛素。

几年后，由中国科学院生化所、有机所以及北京大学精干技术力量组成的中国团队成功将“遥远”锁定为7年。后来的《科学》杂志登载了题为《红色中国的胰岛素全人工合成》的数页长评。

国际学界认为的遥远究竟有多远？一个完整的胰岛素分子由51个氨基酸组成，可

欢迎发表你的观点

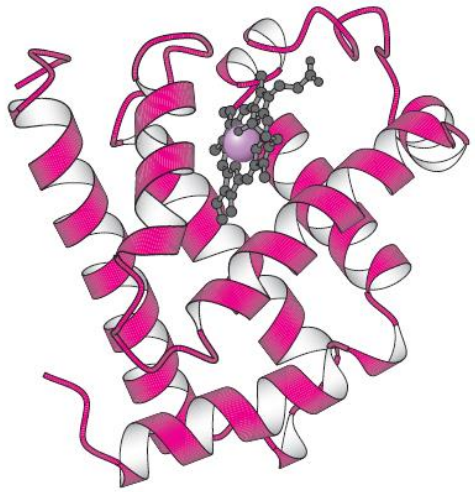
1. 蛋白质研究史上的重大事件

- 1960年前后，蛋白质第一个三维结构。

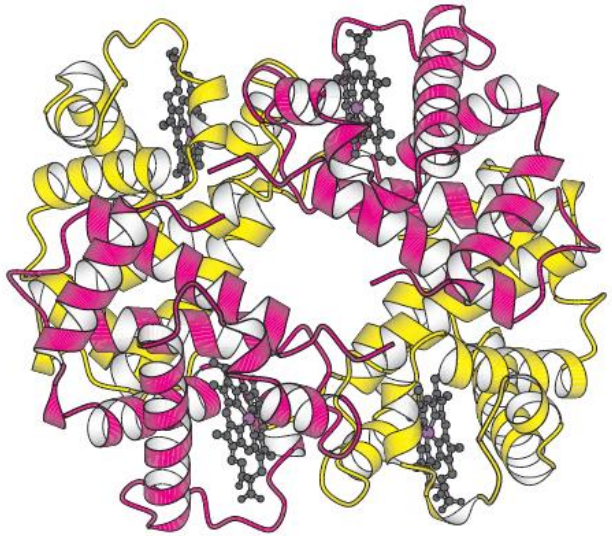
  myoglobin（肌红蛋白）(J Kendrew)

  hemoglobin（血红蛋白）(M Perutz)

- 1962 Nobel Prize



肌红蛋白

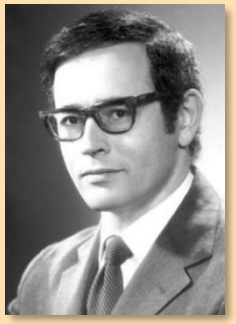


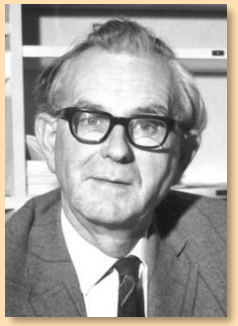
血红蛋白

Max Perutz, 1914–2002 (left)
John Kendrew, 1917–1997 (right)

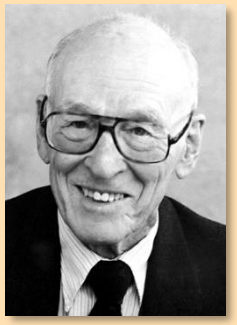
1. 蛋白质研究史上的重大事件

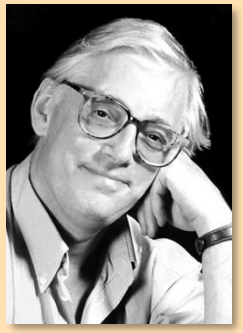
- 先后多位科学家获得诺贝尔奖。


GM Edelman

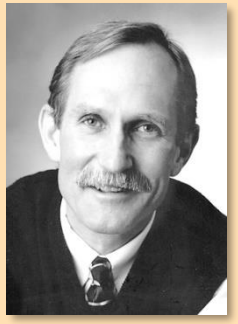

RR Porter

1972，抗体结构


PD Boyer

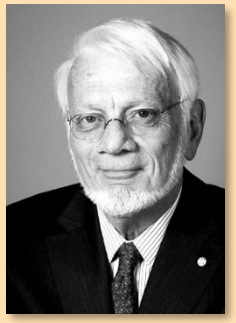

JE Walker

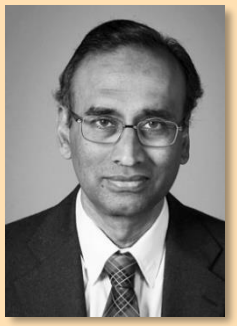
1997，ATP合成机理


P Agre


R MacKinnon

2003，离子通道结构与机理

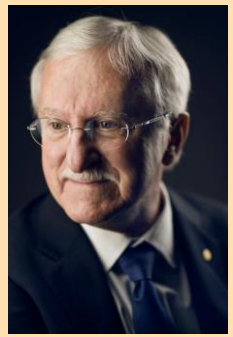

V Ramakrishnan

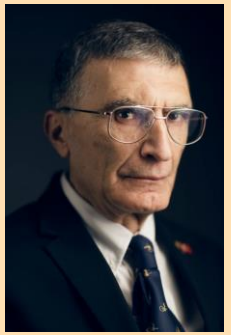

TA Steitz


AE Yonath

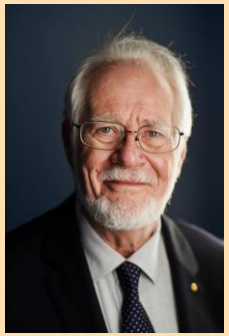
2009，核糖体结构与功能


T Lindahl

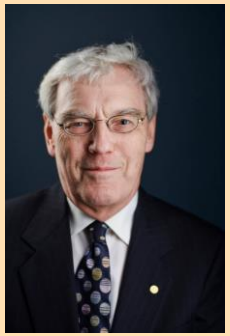

P Modrich


A Sancar

2015，DNA修复机制


J Dubochet


J Frank


R Henderson

2017，溶液中生物分子高清结构的测定


FH Arnold


GP Smith


SGP Winter

2018，酶定向进化；肽及抗体的噬菌体展示


E Charpentier


JA Doudna

2020，基因组编辑


D Julius

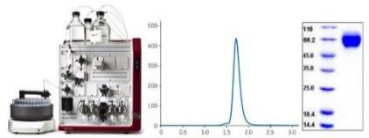

A Patapoutian

2021，感知疼痛和温度的机制

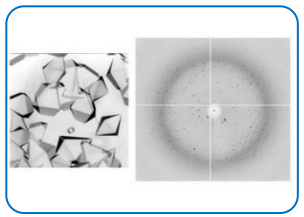
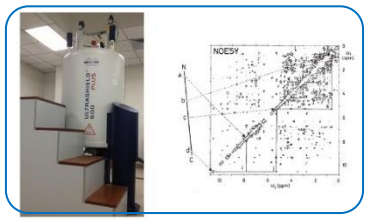
https://www.nobelprize.org/

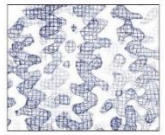
现代药学中药物先导化合物发现的主要途径

Recombinant Protein

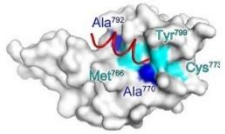
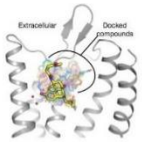
Target Gene →  →  →  → 

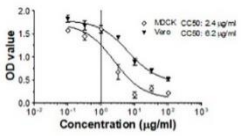
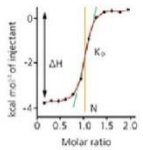
Cryo-EM, X-Ray Diffraction and NMR

High-through-put Screening

   → **Lead Compound**

2. 蛋白质的主要功能

a. 构成生命体，特别是动物体的基本结构材料（structural proteins）

b. 催化活性 --- 酶（enzyme）

c. 物质的转运（transport proteins）

d. 协调运动，调节机体活动（motile and regulatory proteins）

e. 营养物（nutrient proteins）

f. 免疫保护（defense proteins）

g. 支架（adapter proteins）

h. 控制生长和分化（control proteins）

3. 蛋白质的化学组成

(1) 元素组成

- 都含有C、H、O、N
- 其他主要是S、P、Fe、Cu、Zn、 Mg 和I等。

元素	百分含量	平均 (%)
C	50~55	52
H	6.9~7.7	7
O	21~24	23
N	15.0~17.6	16
S	0.3~2.3	2
P	0.4~0.9	0.6

3. 蛋白质的化学组成

(2) 蛋白质含量计算

- 蛋白质占生物组织中所有含氮物质的绝大部分。
- 可将生物组织的含氮量近似地看作蛋白质的含氮量。
- 蛋白质粗略含量的计算公式：

蛋白质含量(克%)= 每克生物样品中含氮克数 × 6.25 × 100

3. 蛋白质的化学组成

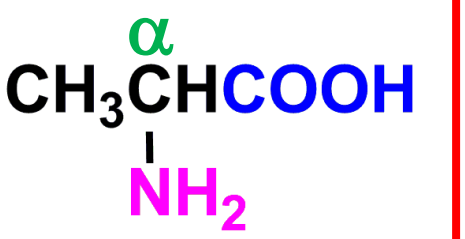
(3) 氨基酸是蛋白质的基本结构单元

- 完全水解: 各种氨基酸的混合物。
- 大多数的蛋白质都是由20 种氨基酸组成

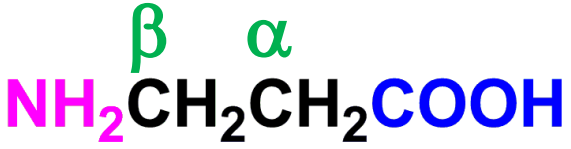
--- 基本氨基酸 (standard amino acids)

二、氨基酸

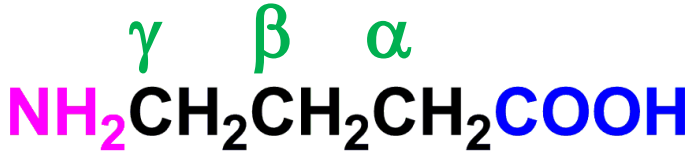
- 什么是氨基酸？什么又是α-氨基酸？



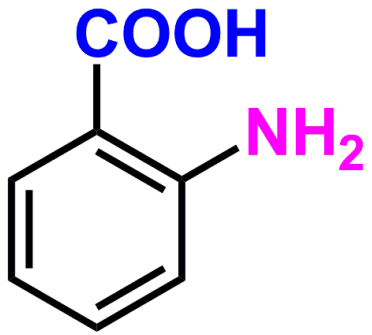
α-amino acid



β-amino acid



γ-amino acid

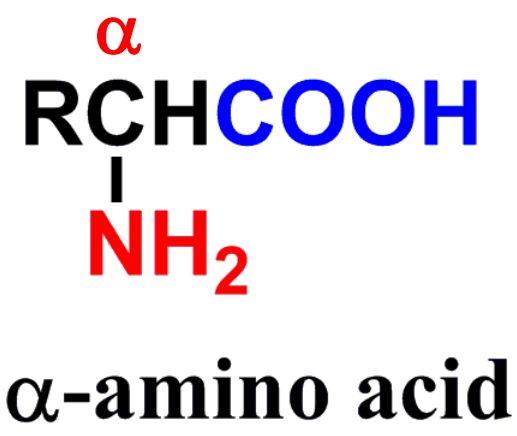


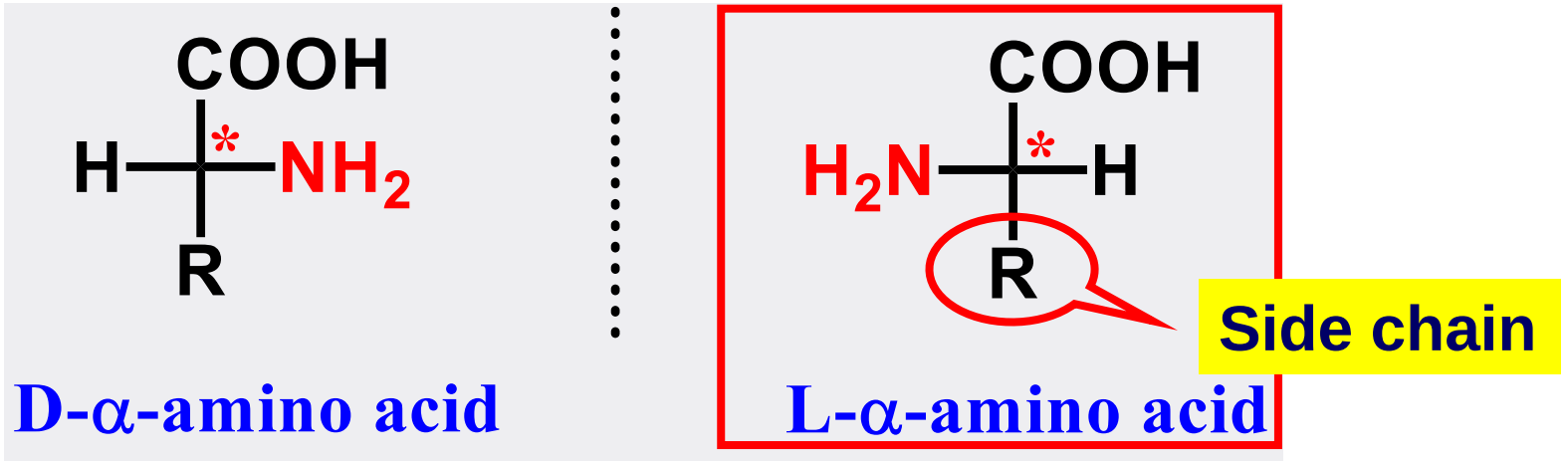
o-amino benzoic acid

- 组成肽和蛋白质的结构单元分子
  --- α- amino Acid

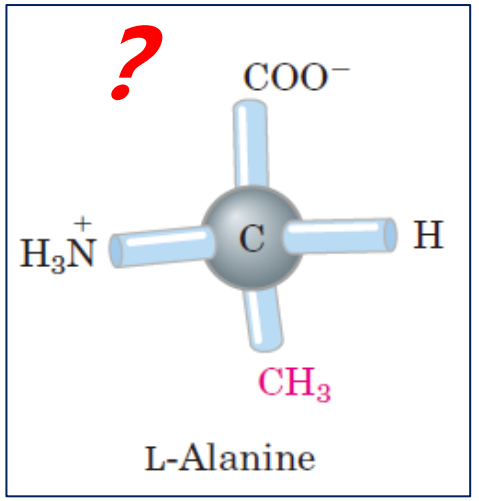
二、氨基酸

1. Configuration (构型)



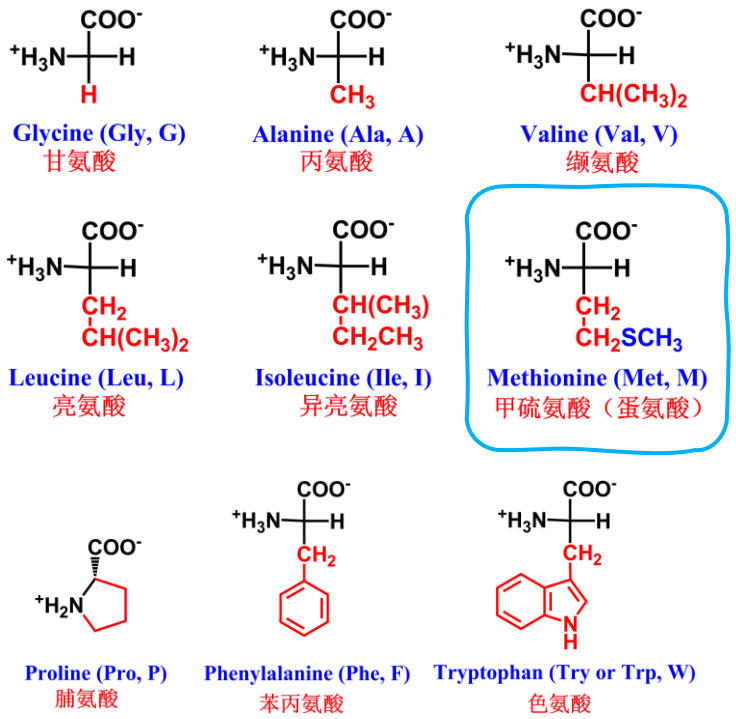


- When R ≠ H, α-C is a chiral carbon, there are two stereoisomers (D and L- α- amino acid).
- The amino acids in natural proteins are the L- α - amino acids.



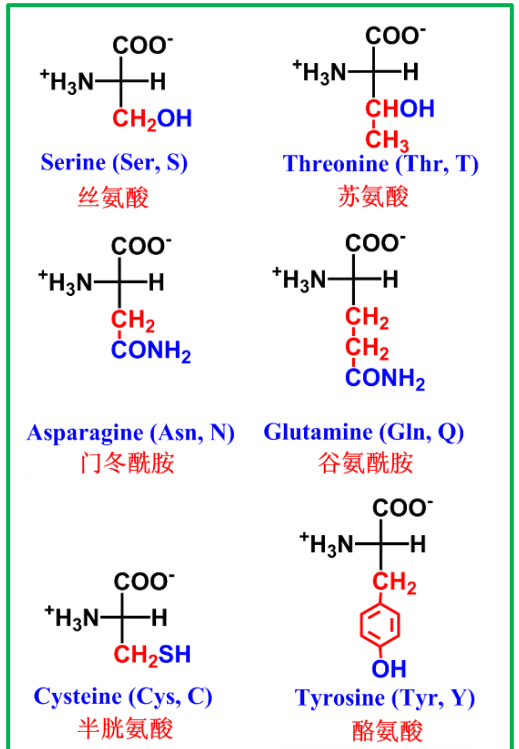
2. 20 standard amino acids

Nonpolar side chain (9)

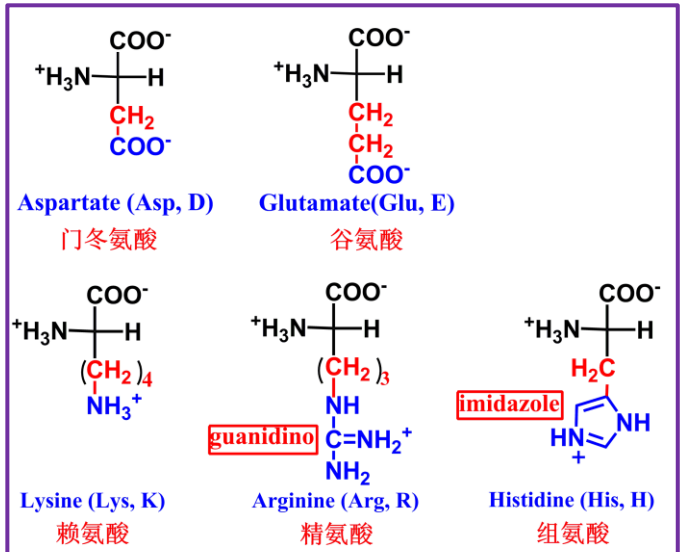


Polar side chain (11)

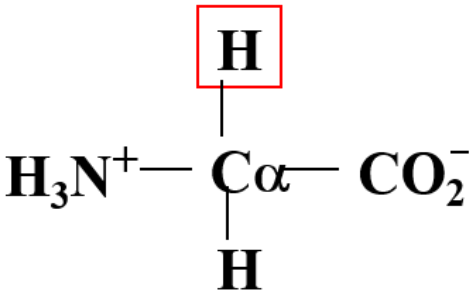
Uncharged polar side chain (6)



Charged polar side chain (5)

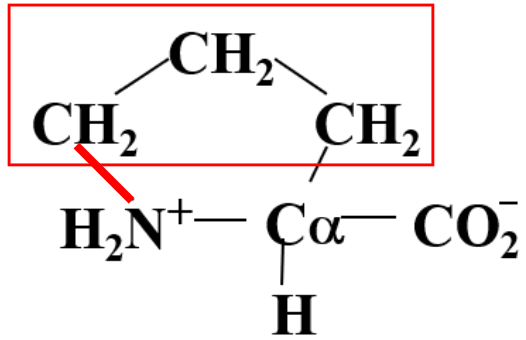


Glycine and Proline



Glycine, Gly, G, 甘氨酸
(not chiral)

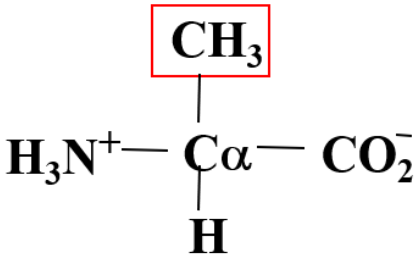
- Simplest amino acid with a hydrogen atom as the R group
- Not chiral since the Cα is not asymmetric



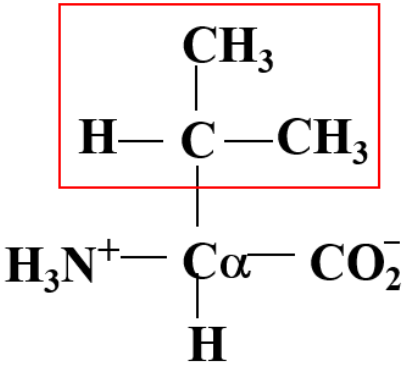
Proline, Pro, P，脯氨酸
(non-polar/hydrophobic)

- Side chain bonded covalently to the peptide backbone nitrogen
- Cα-N bond constrained
- Aliphatic

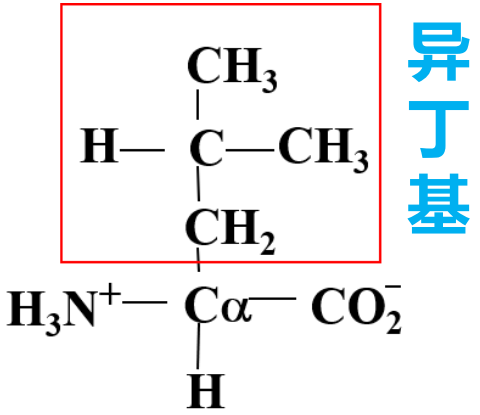
Aliphatic Amino Acids



Alanine, Ala, A
丙氨酸

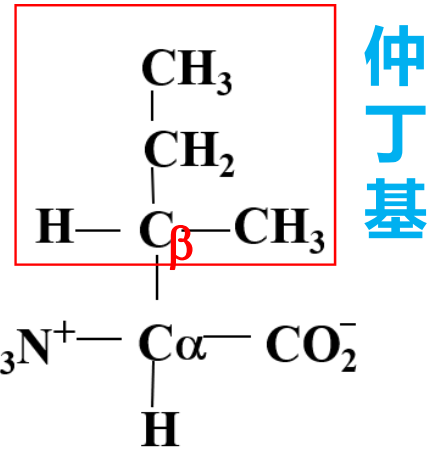


Valine, Val, V
缬氨酸



异丁基

Leucine, Leu, L
亮氨酸

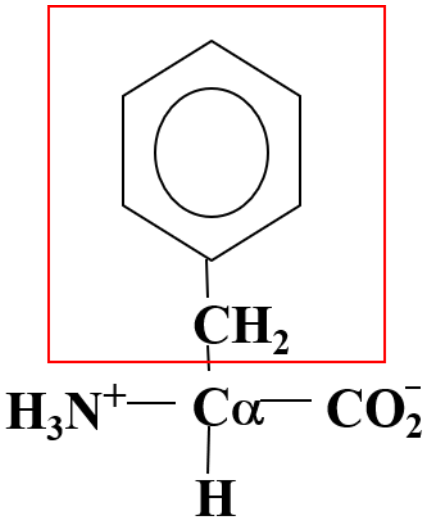


仲丁基

Isoleucine, Ile, I
异亮氨酸
(Two chiral centers)

- non-polar/hydrophobic, No reactive groups; inert
- Interact with each other---- hydrophobic bond
- Cβ of Isoleucine is also chiral

Aromatic Amino Acids

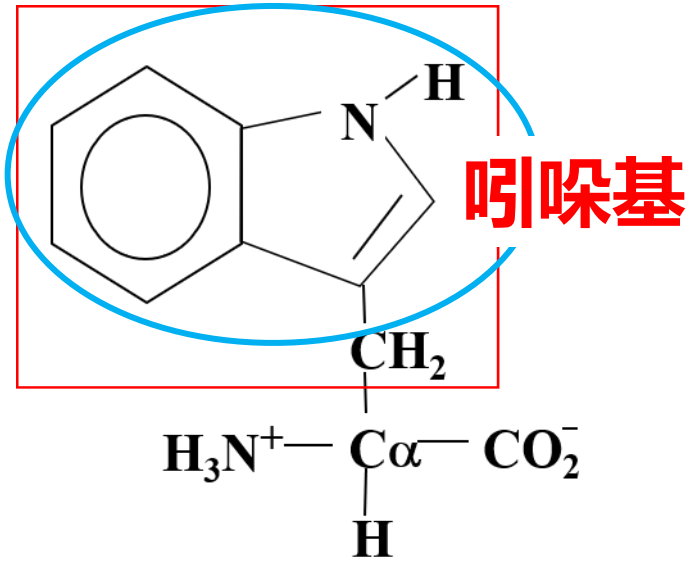


Phenylalanine, Phe, F

苯丙氨酸

(non-polar/hydrophobic)

- Aromatic ring with reactivity comparable to benzene
- Hydrophobic contacts

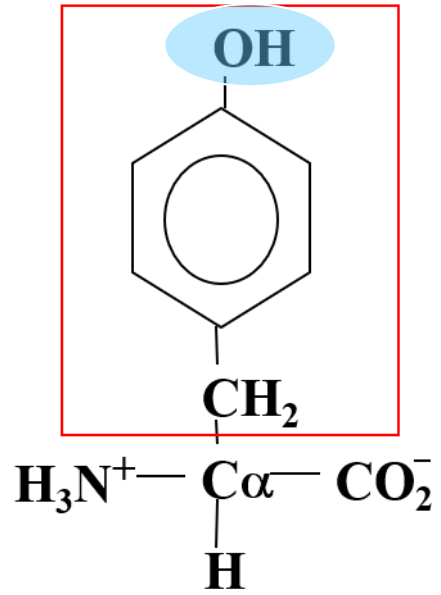


Tryptophan, Trp, W

色氨酸

(non-polar/hydrophobic)

- Fluorescence sensitive to the side chain environment
- Indole-N particpates in H-bonds as a donor



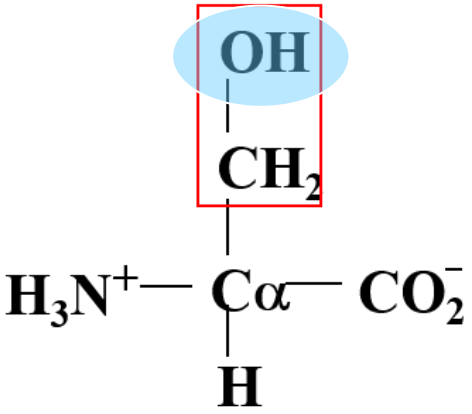
Tyrosine, Tyr, Y

酪氨酸

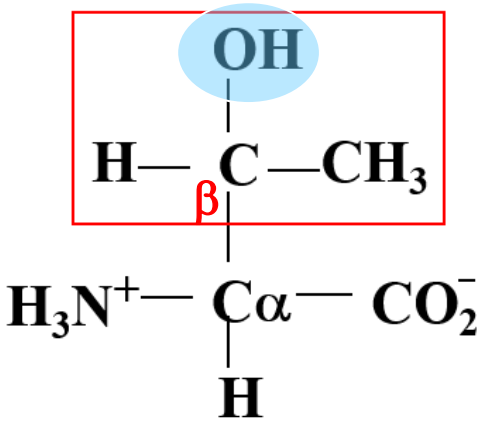
(polar)

- Hydroxyl group, site of phosphorylation in proteins

Hydroxyl Amino Acids



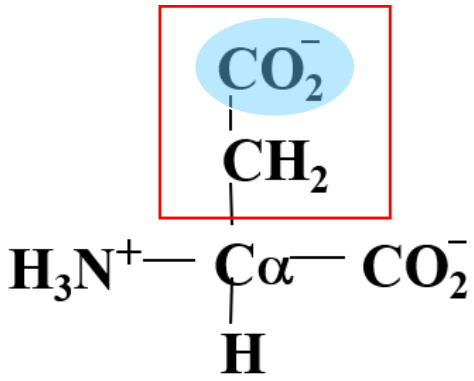
Serine, Ser, S，丝氨酸
(polar)



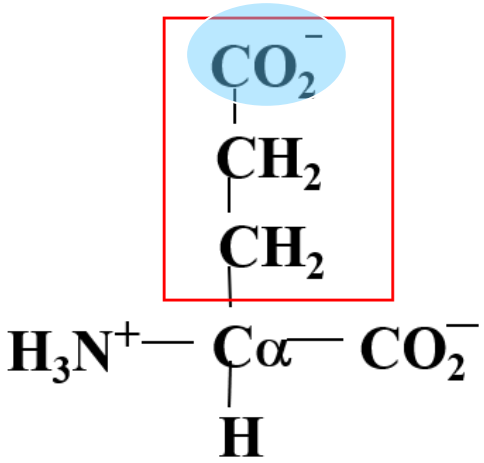
Threonine, Thr, T，苏氨酸
(polar, two chiral centers)

- Participates in hydrogen bonding in proteins
- Site of phosphorylation of proteins by protein kinases
- Cβ of Threonine is also chiral

Acidic Amino Acids



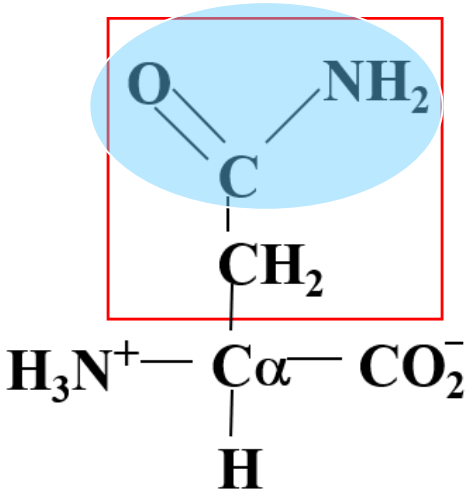
Aspartic acid, Asp, D
天冬氨酸



Glutamic acid, Glu, E
谷氨酸

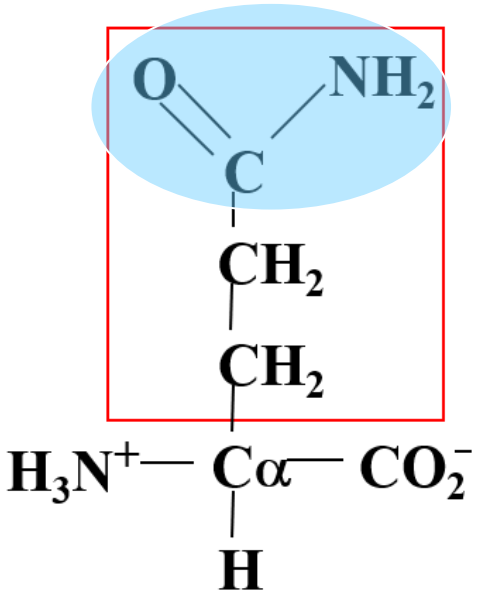
- They are ionized and negatively charged at physiological pH
- participate in electrostatic interactions
- Hydrogen bond acceptors
- metal chelators

Amides Amino Acids



Asparagine, Asn, N

天冬酰胺

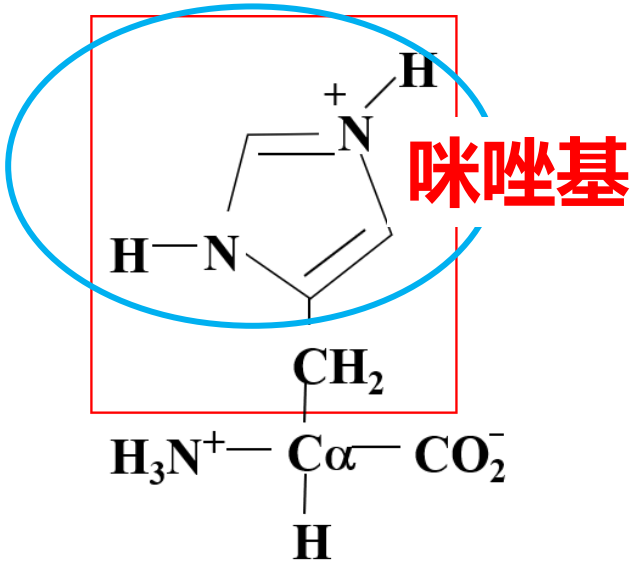


Glutamine, Gln, Q

谷氨酰胺

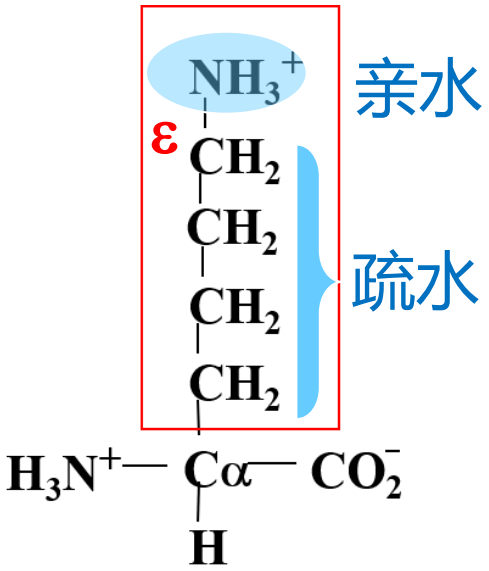
- Amide forms of Asp and Glu.
- Both hydrogen bond donors and acceptors

Basic Amino Acids



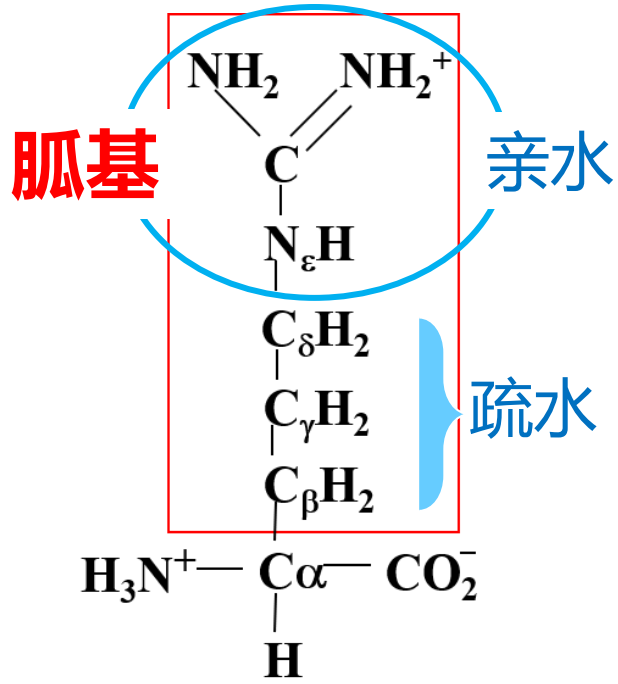
Histidine, His, H
组氨酸

- Imidazole side chain
- Often utilized in enzymes due to versatility (H-bond donor or acceptor, nucleophile or electrophile)



Lysine, Lys, K
赖氨酸

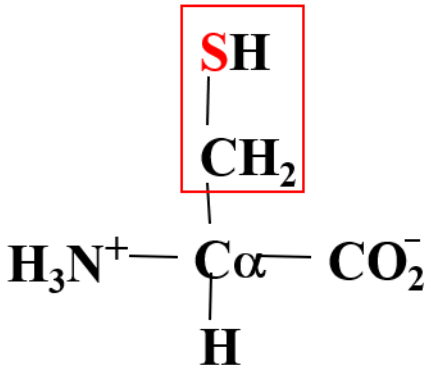
- Protonated
- Hydrophobic chain with a terminal NH_2
- Modification site in pro.



Arginine, Arg, R
精氨酸

- Protonated
- Planar terminal δ-guanido group

Sulfur-containing Amino Acids

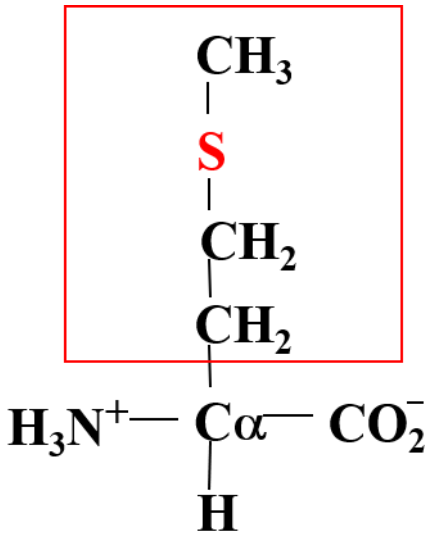


Cysteine, Cys, C

半胱氨酸

(polar)

- Thiol group, most reactive side chain
- Readily oxidized
- Also forms complexes with metal ions such as zinc



Methionine, Met, M

甲硫氨酸

(non-polar/hydrophobic)

- Susceptible to oxidation
- Can be replaced in proteins by seleno-methionine in which the sulfur is replaced by a selenium atom

? *这两个氨基酸的手性碳构型相同吗?*

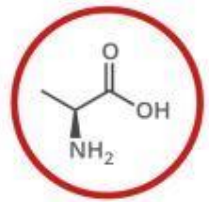
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

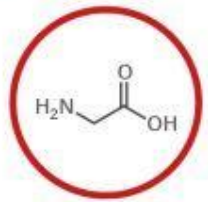
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL

Chemical Structure — single letter code

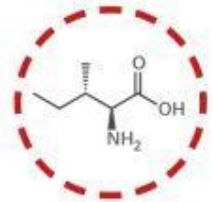
NAME (A) — three letter code — DNA codons



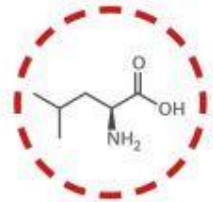
ALANINE (A) — *Ala* — GCT, GCC, GCA, GCG



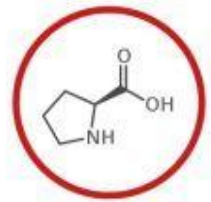
GLYCINE (G) — *Gly* — GGT, GGC, GGA, GGG



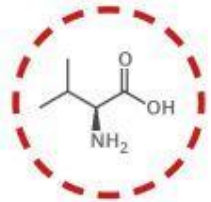
ISOLEUCINE (I) — *Ile* — ATT, ATC, ATA



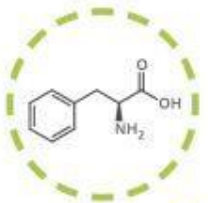
LEUCINE (L) — *Leu* — CTT, CTC, CTA, CTG, TTA, TTG



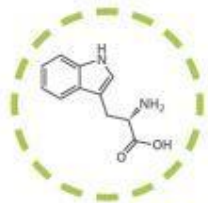
PROLINE (P) — *Pro* — CCT, CCC, CCA, CCG



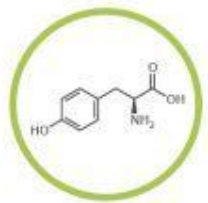
VALINE (V) — *Val* — GTT, GTC, GTA, GTG



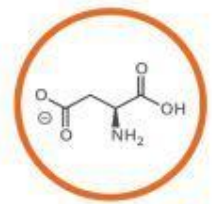
PHENYLALANINE (F) — *Phe* — TTT, TTC



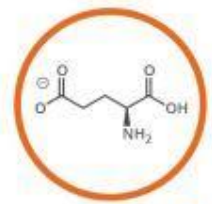
TRYPTOPHAN (W) — *Trp* — TGG



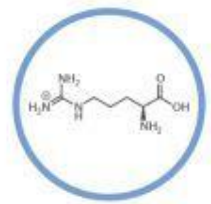
TYROSINE (Y) — *Tyr* — TAT, TAC



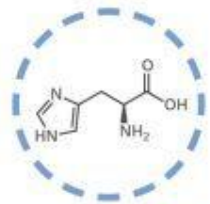
ASPARTIC ACID (D) — *Asp* — GAT, GAC



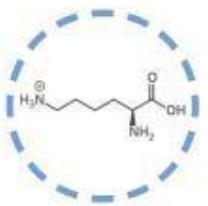
GLUTAMIC ACID (E) — *Glu* — GAA, GAG



ARGININE (R) — *Arg* — CGT, CGC, CGA, CGG, AGA, AGG



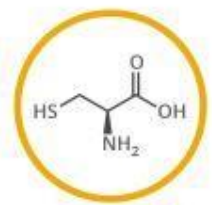
HISTIDINE (H) — *His* — CAT, CAC



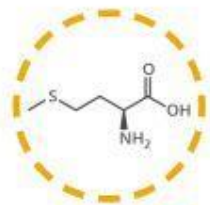
LYSINE (K) — *Lys* — AAA, AAG

SERINE (S) — *Ser* — TCT, TCC, TCA, TCG, AGT, AGC

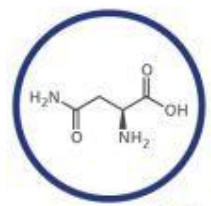
THREONINE (T) — *Thr* — ACT, ACC, ACA, ACG



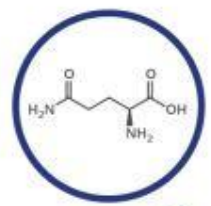
CYSTEINE (C) — *Cys* — TGT, TGC



METHIONINE (M) — *Met* — ATG



ASPARAGINE (N) — *Asn* — AAT, AAC



GLUTAMINE (Q) — *Gln* — CAA, CAG

Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

二、氨基酸

3. Essential amino acids

- **The amino acids that must obtain from our diets because we either cannot synthesize them at all or cannot synthesize them in adequate amounts.**

- **9 essential amino acids:**

  Met, Val, Lys, Ile, Phe, Leu, Trp, Thr, His(婴儿)

(记忆口诀：甲携来一本亮色书)

二、氨基酸

4. Nonstandard Amino Acids

- **More than 200 different amino acids are found in living organisms.**

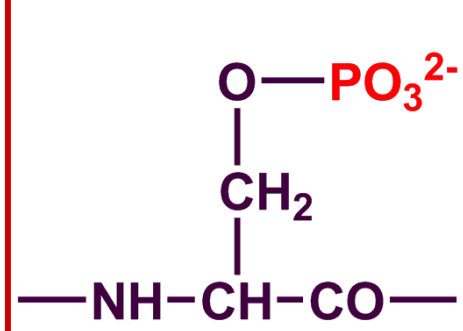
(1) amino acid derivatives in proteins

--- Unusual amino acids in proteins

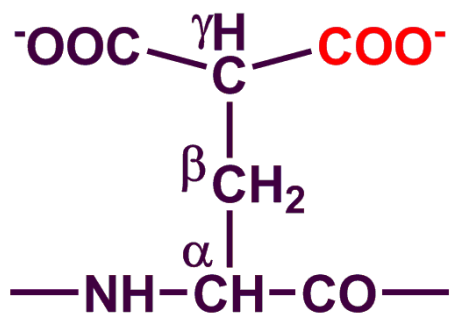
--- In almost cases, they result from posttranslational modifications

--- Modification: hydroxylation, methylation, acetylation, carboxylation, phosphorylation

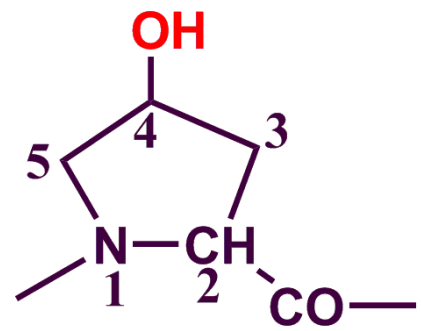
Some modified amino acids in proteins



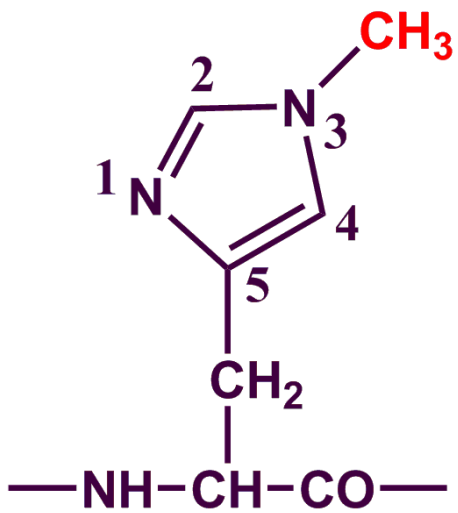
O-phosphoserine



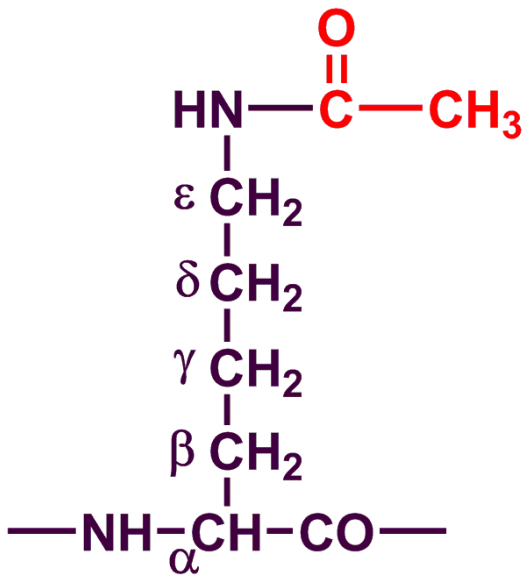
γ-carboxyglutamate



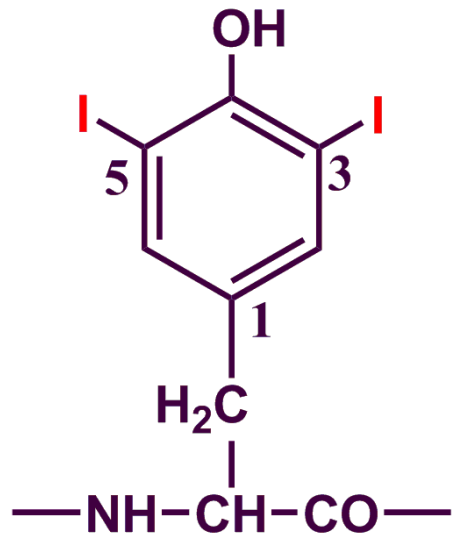
4-hydroxyproline



3-methylhistidine

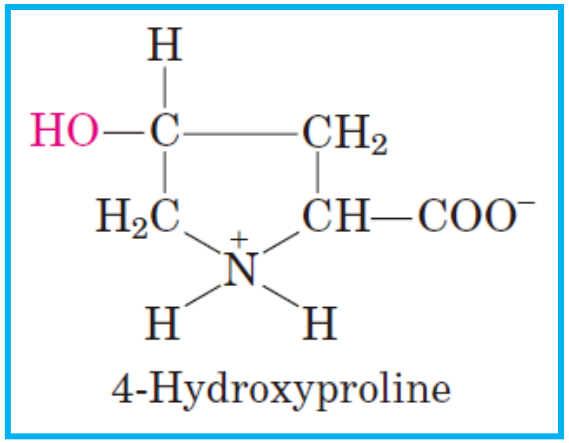


ε-*N*-acetyllysine



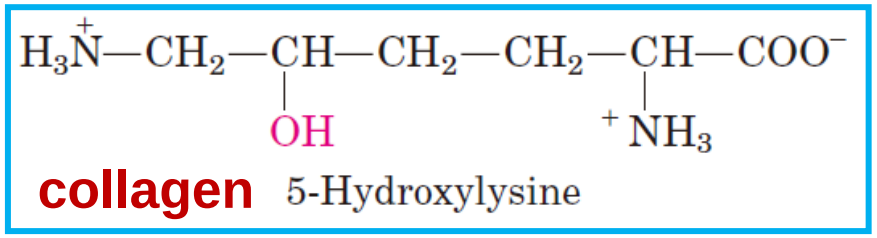
3, 5-diiodotyrosine

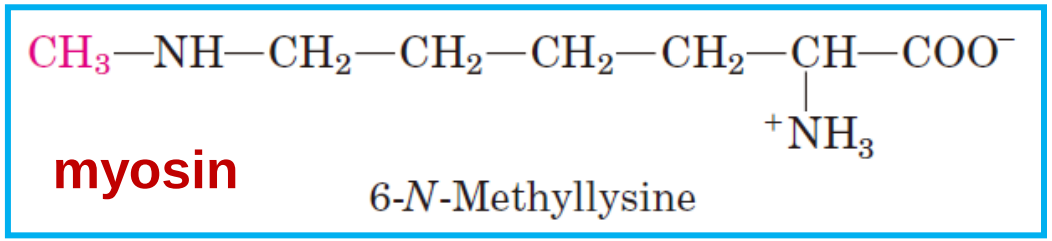
Uncommon amino acids also have important functions

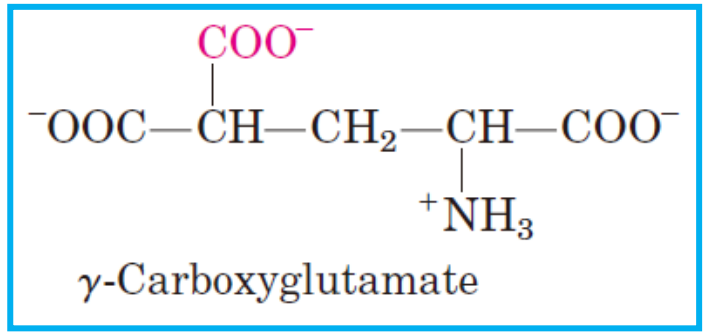


- plant cell wall proteins
- collagen

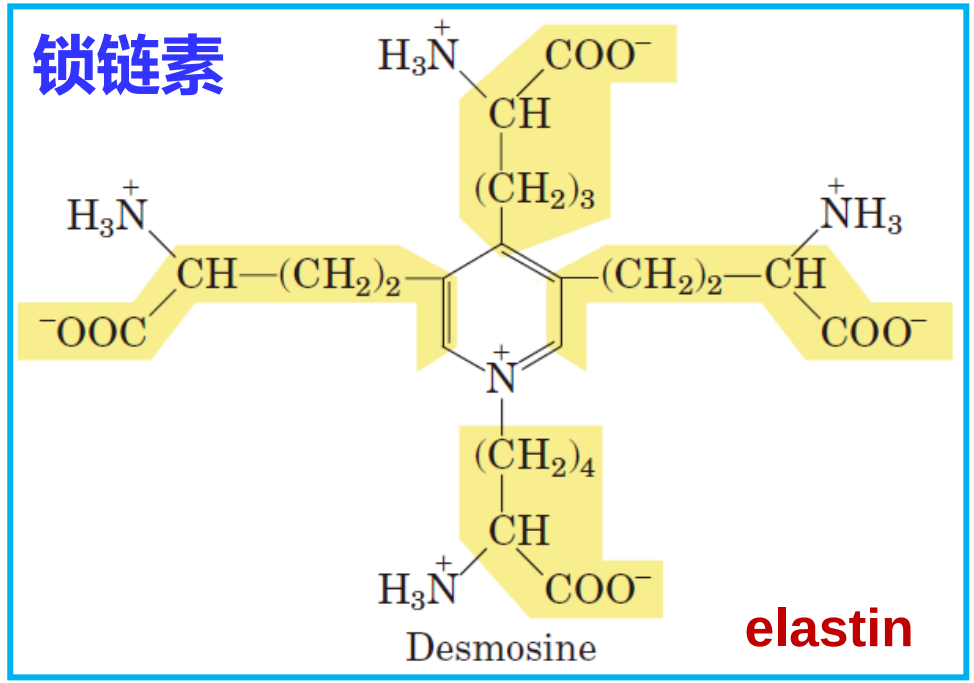
你知道吗?







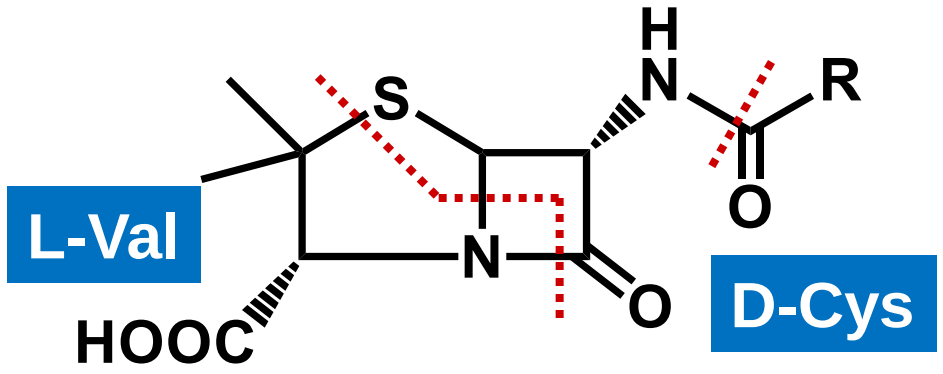
prothrombin



4. Nonstandard Amino Acids

(2) D-amino acid

- 非蛋白氨基酸
- Components of relatively short (<20 residues) bacterial polypeptides（细菌多肽）.
- Important constituents of bacterial cell walls
- Compoments of many bacterially produced peptide antibiotics（抗生素）



Penicillin

Cell

CellPress
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Article

An antibiotic from an uncultured bacterium binds to an immutable target

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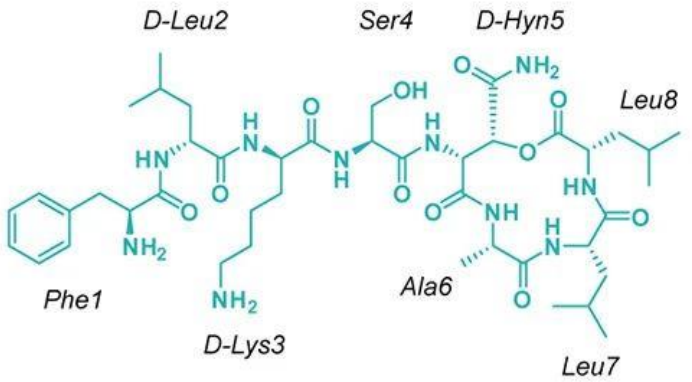
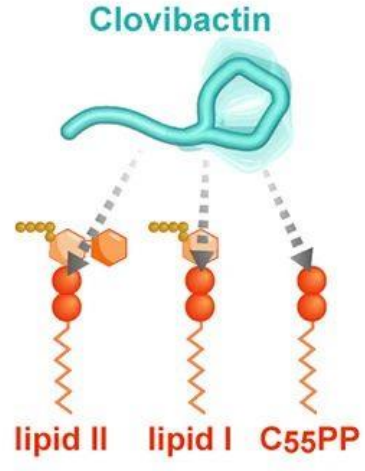
- 2023年8月22日，荷兰乌得勒支大学、德国波恩大学及NovoBiotic公司的研究人员
- **新技术—iChip**：能分离出单个细菌并在原位土壤中继续培养形成菌落，从而让那些无法培养的细菌能在实验室中生长。
- **新型抗生素Clovibactin**：抗菌谱广，有效杀灭耐药的革兰氏阳性菌，甚至是多重耐药的“超级细菌”，细菌不产生耐药性。机制独特。

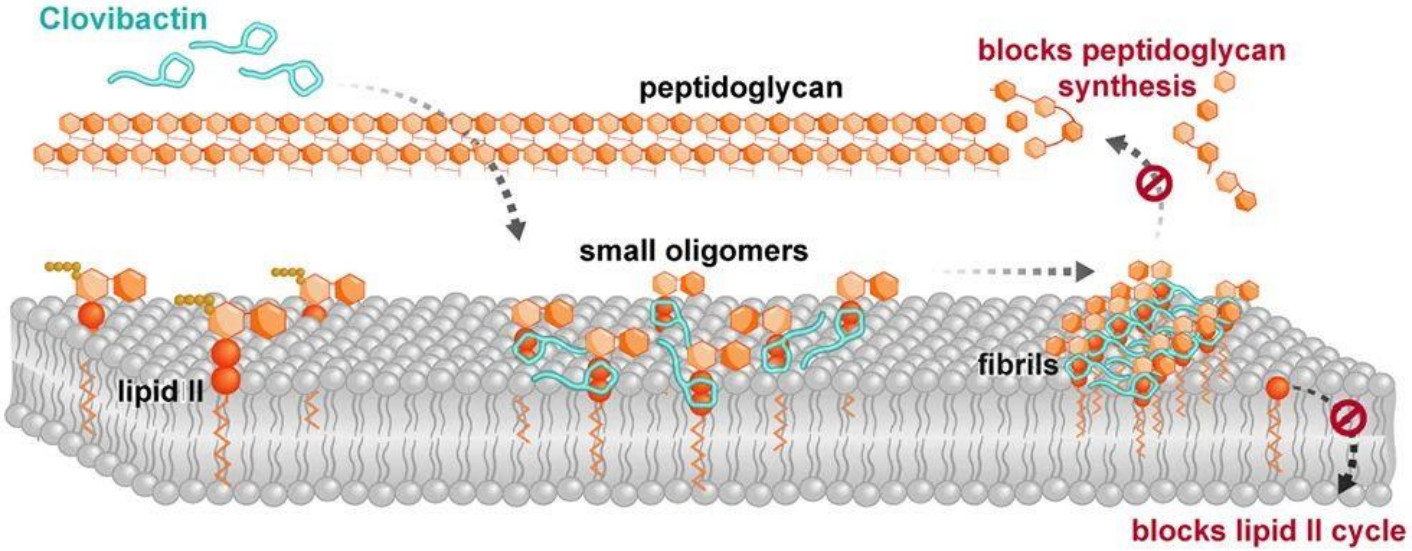
Clovibactin from unculturable bacteria



Multi-target mechanism accounts for lack of resistance



A supramolecular structure blocks bacterial peptidoglycan synthesis

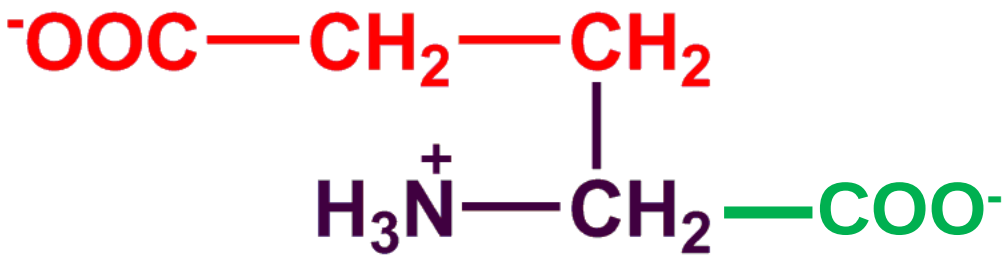


4. Nonstandard Amino Acids

(3) Biologically active amino acids

- 非蛋白氨基酸（about 300 additional AA）
- Transport nitrogen, such as Orn (鸟氨酸), Cit (瓜氨酸) in the biosynthesis of urea
- β-alanine as a component of pantothenic acid (泛酸)
- Chemical messengers （化学信使）

Example 1

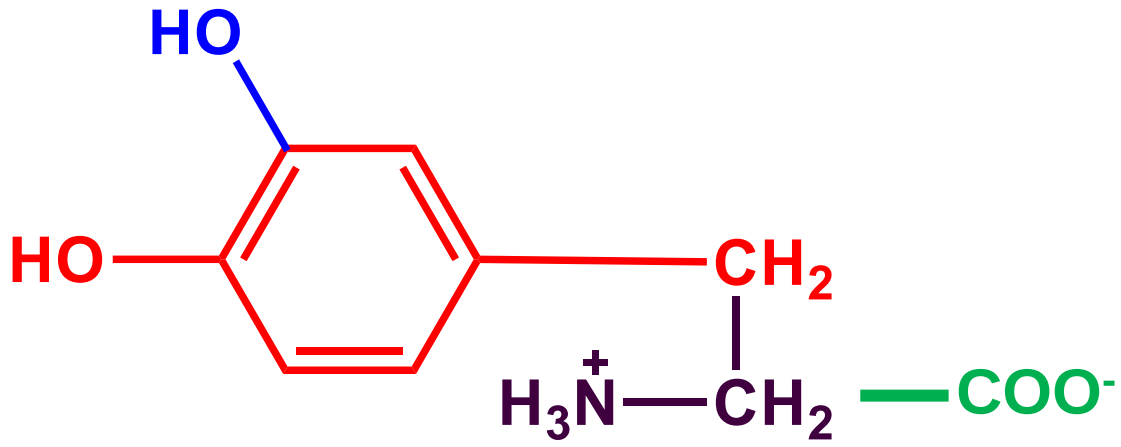


γ-aminobutyric acid (GABA)

- A glutamine（谷氨酸）decarboxylation（脱羧） product
- 重要的抑制性神经递质。
- Neurotransmitters (神经递质)

  --- substances released by nerve cells to alter the behavior of their neighbors.

Example 2

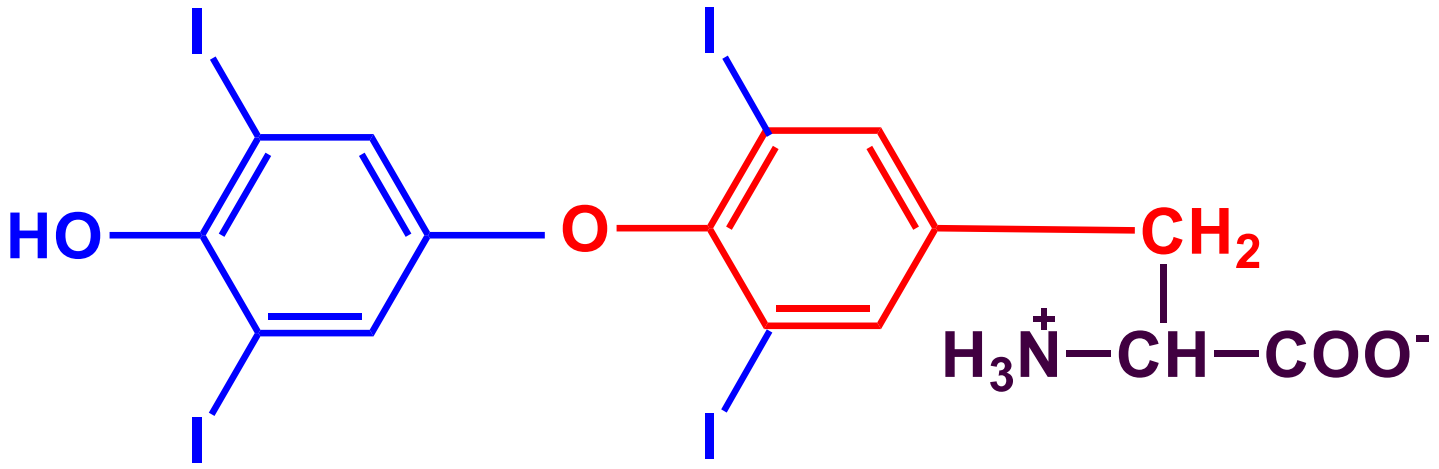


Dopamine

Arvid Carlsson

- A tyrosine (酪氨酸) derivative
- 重要的兴奋性神经递质。传递亢奋和欢愉信息，与情欲、开心、上瘾等有关。
- Arvid Carlsson，确定多巴胺的作用，获2000年诺贝尔医学奖。

Example 3



Thyroxine

- Tyrosine derivative
- An iodine-containing thyroid（甲状腺）hormone that generally stimulates vertebrate（脊椎动物） metabolism

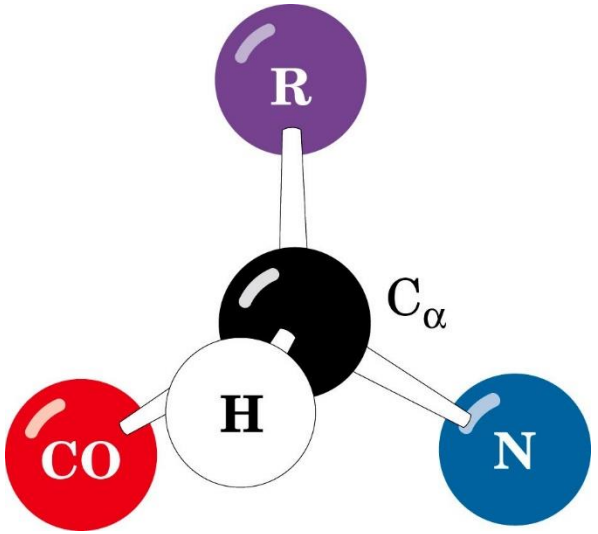
二、氨基酸

5. Properties of Amino Acids

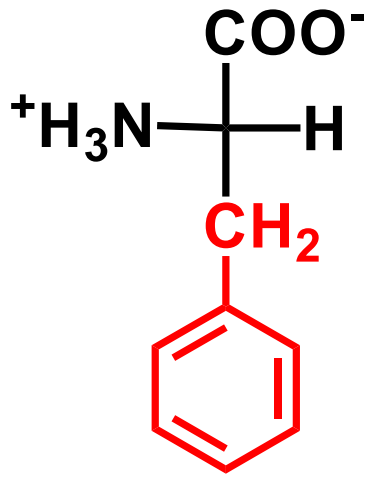
(1) optical activity

- 除 Glycine（R = H）外，氨基酸均含有一个手性α-碳原子，因此都具有旋光性。

- **比旋光度 ([α])：** 氨基酸重要物理常数之一，是鉴别各种氨基酸的重要依据。(见教材p49, 表3.3)

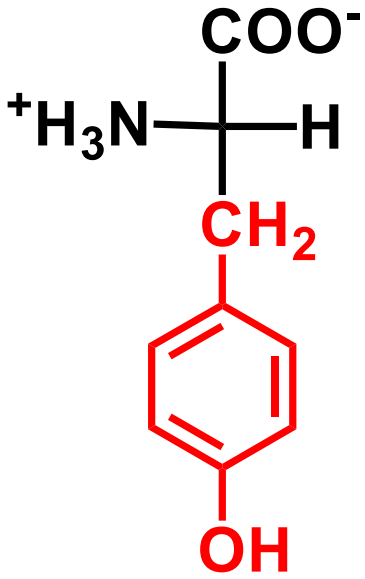


(2) UV absorption



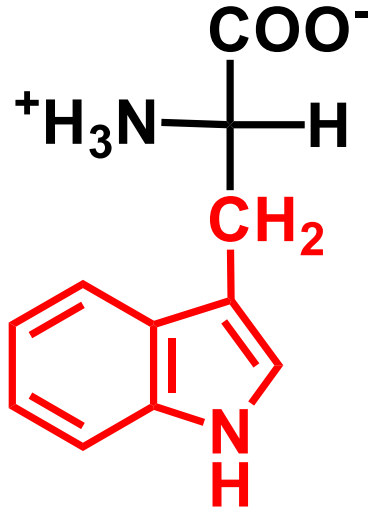
Phenylalanine (Phe, F)

苯丙氨酸



Tyrosine (Tyr, Y)

酪氨酸



Tryptophan (Try, W)

色氨酸

UV 特征吸收：λ_{max} = 260－280 nm

(2) UV absorption

- **Tyr: λ_{max} = 275 nm**

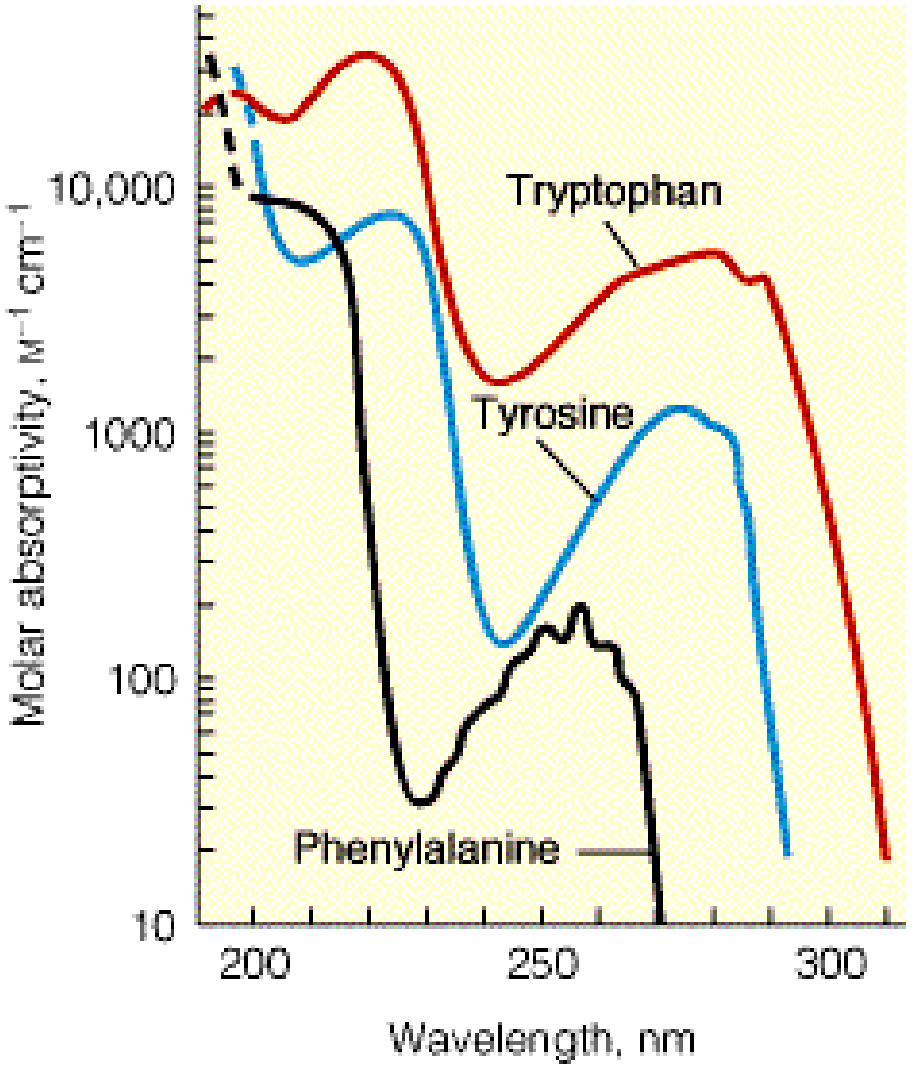
  ε_{275} = 1.4x10^3

- **Phe: λ_{max} = 257 nm**

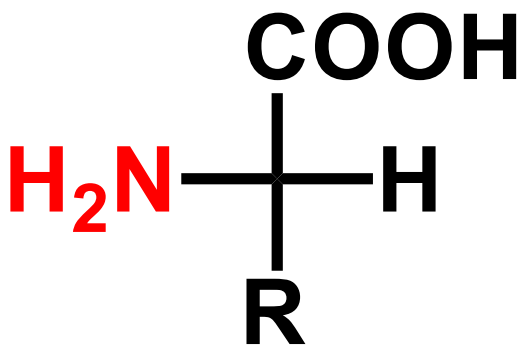
  ε_{257} = 2.0x10^2

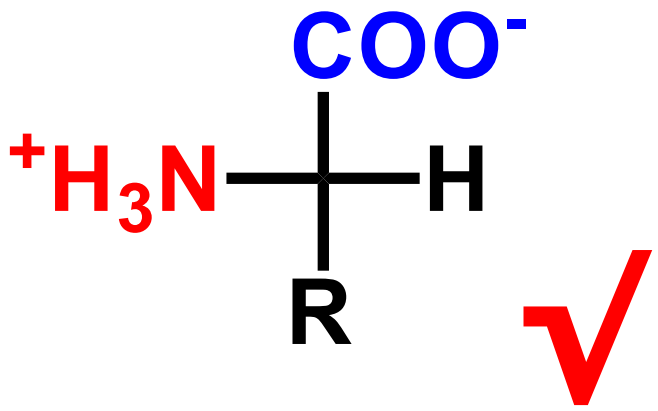
- **Trp: λ_{max} = 280 nm**

  ε_{280} = 5.6x10^3



存在形式？





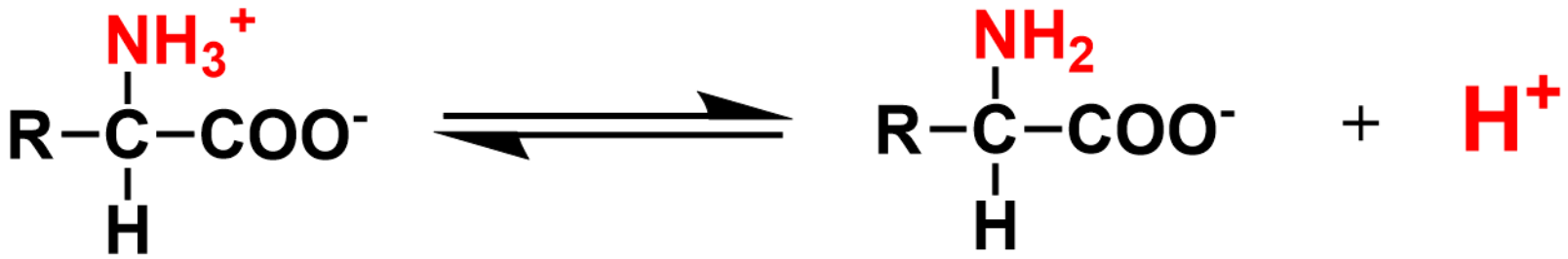
√

氨基酸在结晶状态或在接近中性的溶液中，是以**两性离子** (dipolar ion or zwitterion) 的形式存在。是一种内盐 (inner salt)。

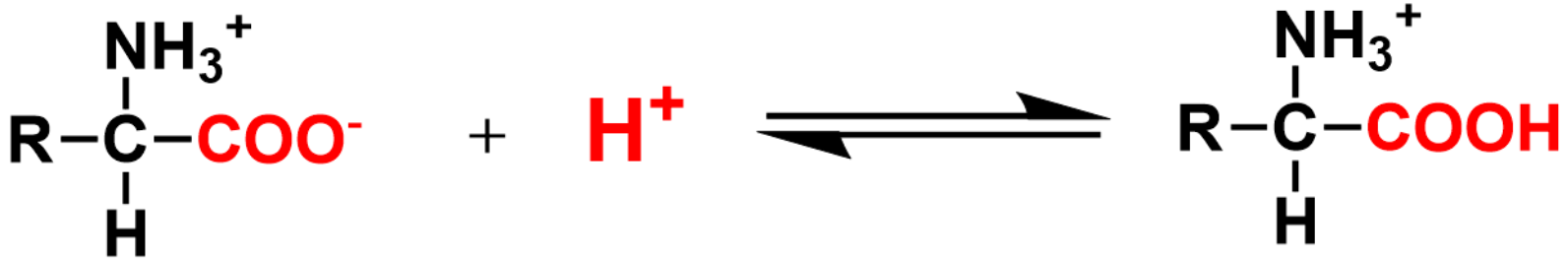
(3) Acid-Base Properties

氨基酸既是酸又是碱

- Act as an acid (proton donor)



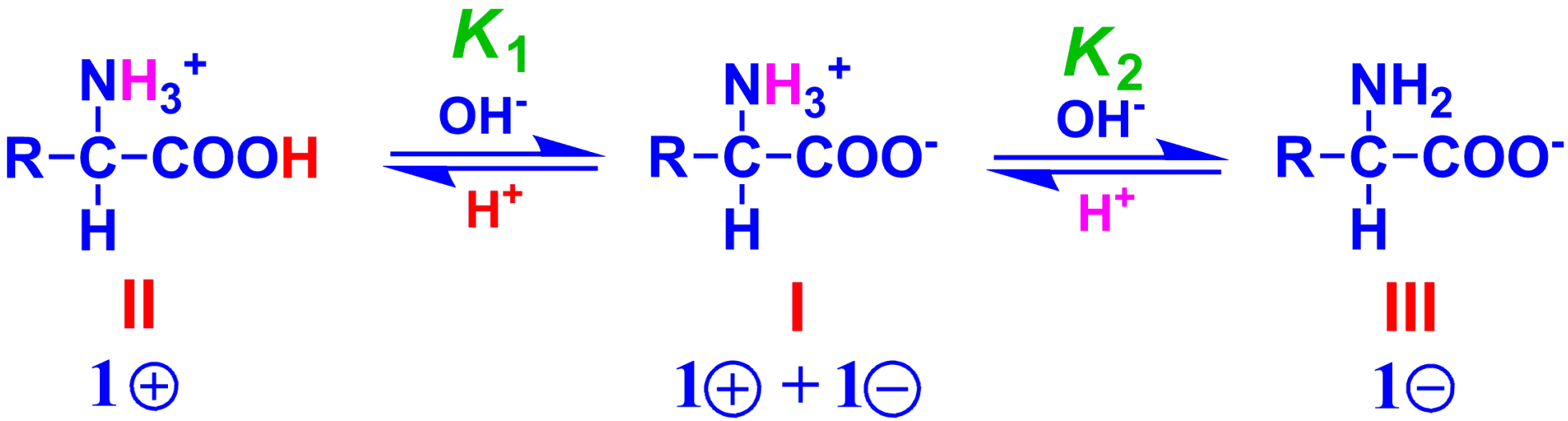
- Act as a base (proton acceptor)



- Amino acids are ampholytes (两性电解质)

(4) Isoelectric Point

低pH (酸)　　高pH (碱)



如果在某一 pH 值下，氨基酸所带正电荷的数目与负电荷的数目正好相等，即净电荷为零，则称该 pH 值为该氨基酸的等电点 (pI)。

(4) Isoelectric Point

a. 等电点时，几乎全部以两性离子 I 的形式存在，并伴以极少量、严格相等的 II 和 III。

b. 处于等电点的氨基酸，其溶解度最小。

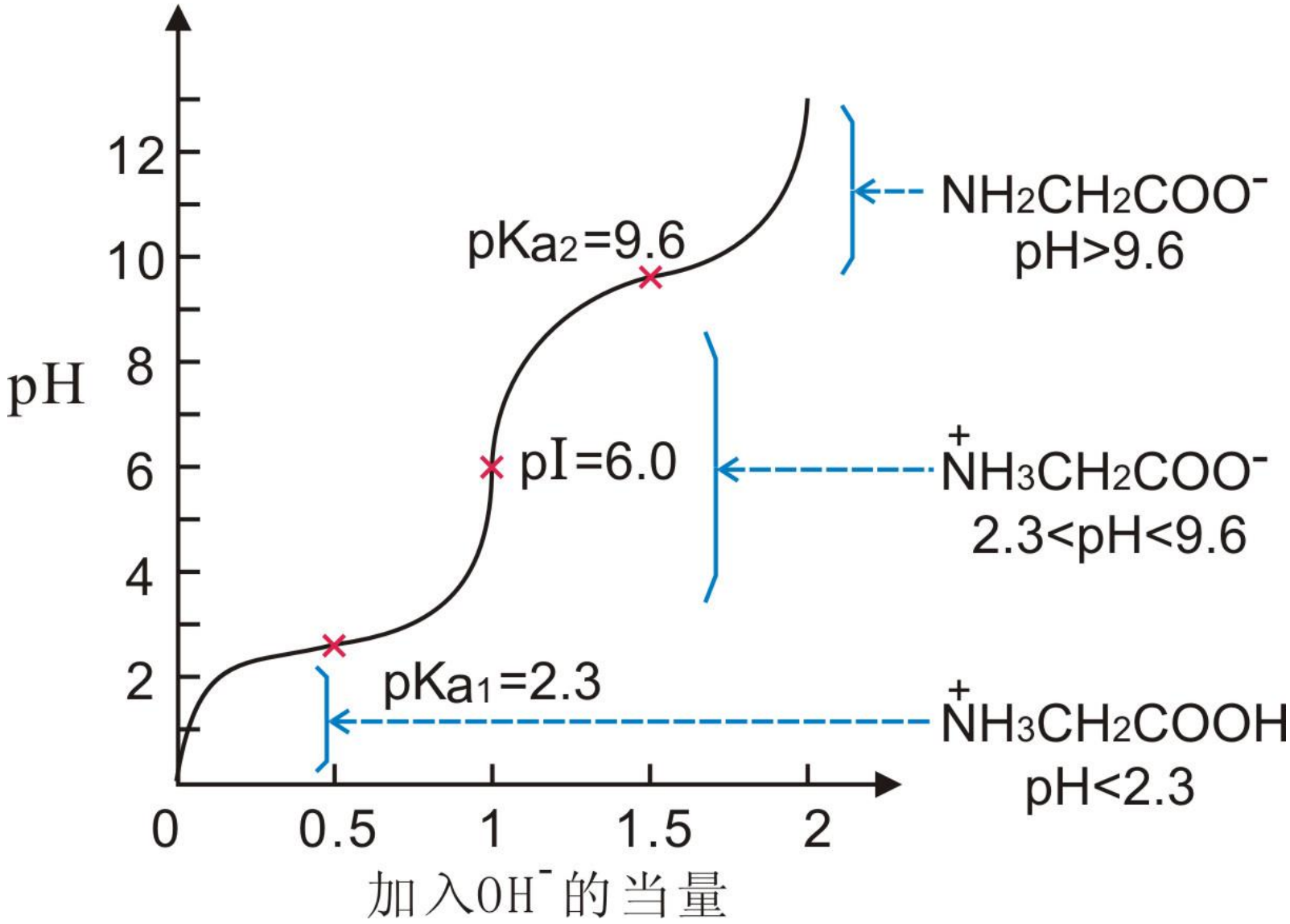
c. PI 与解离常数 p*K*间的关系

Neutral amino acids: $pI = (pK_1 + pK_2)/2$

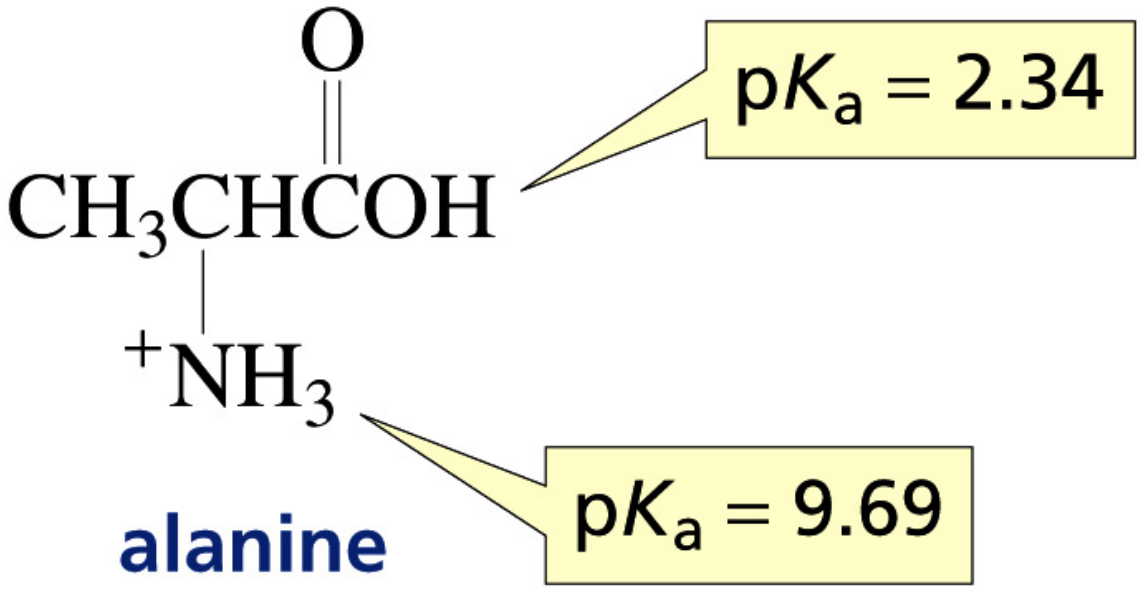
Acidic amino acids : $pI = (pK_1 + pK_{RCOOH})/2$

Basic amino acids : $pI = (pK_2 + pK_{RNH2})/2$

Titration Curve and pI

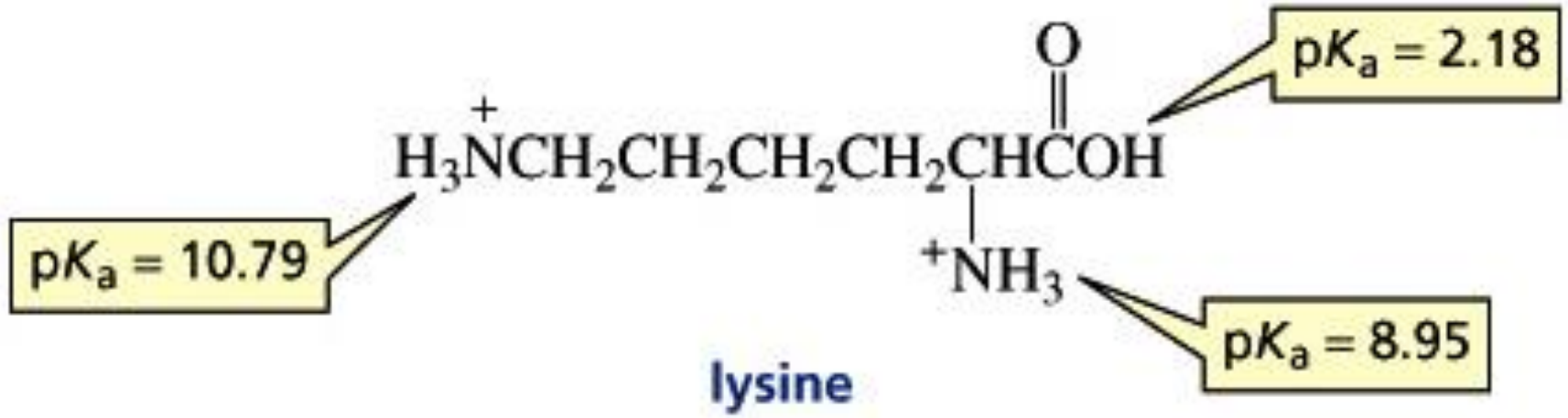


Calculation of pI (1)



$$pI = \frac{2.34 + 9.69}{2} = \frac{12.03}{2} = 6.02$$

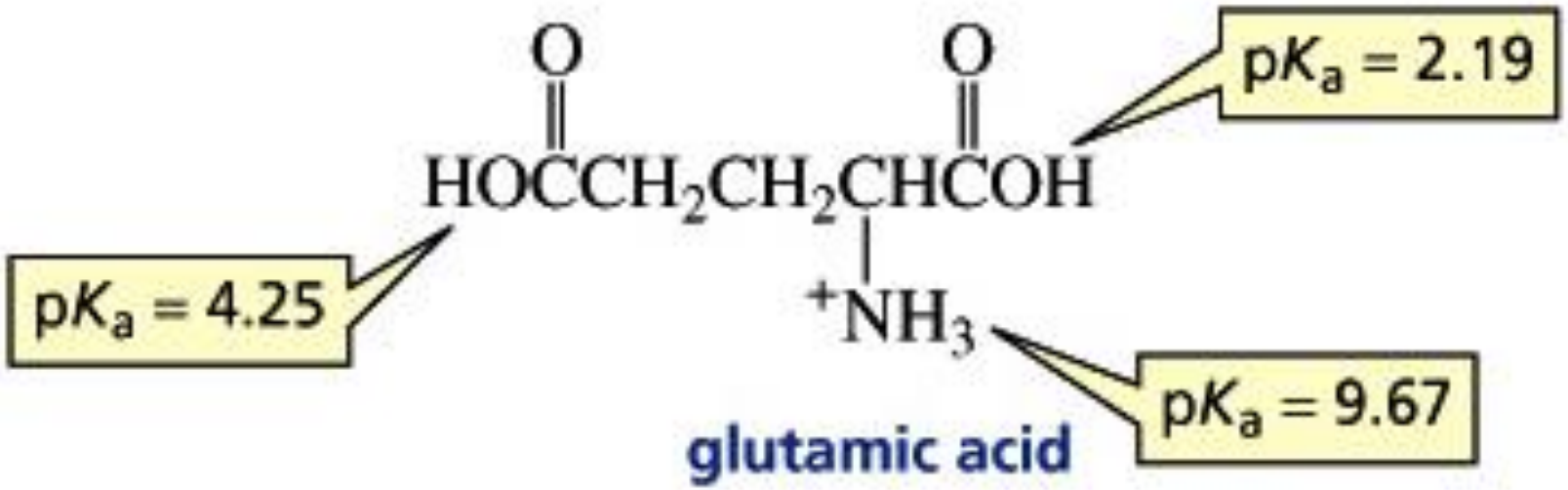
Calculation of pI (2)



lysine

$$pI = \frac{8.95 + 10.79}{2} = \frac{19.74}{2} = 9.87$$

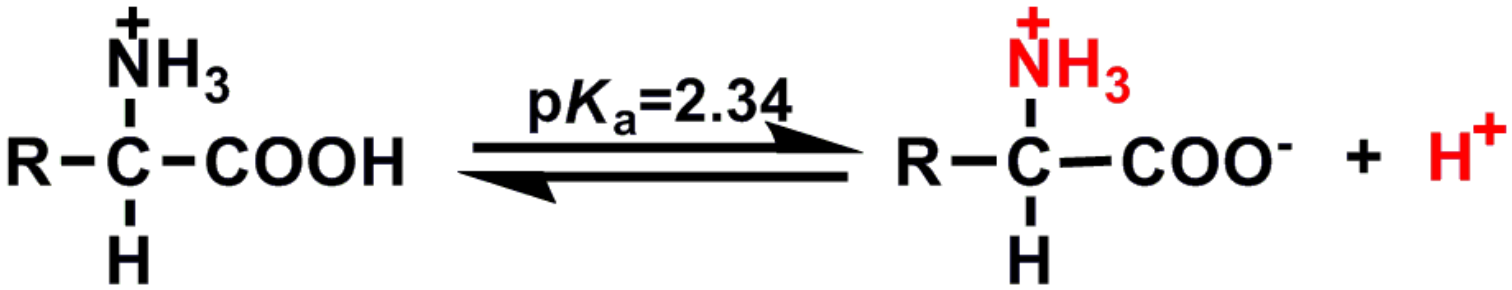
Calculation of pI (3)



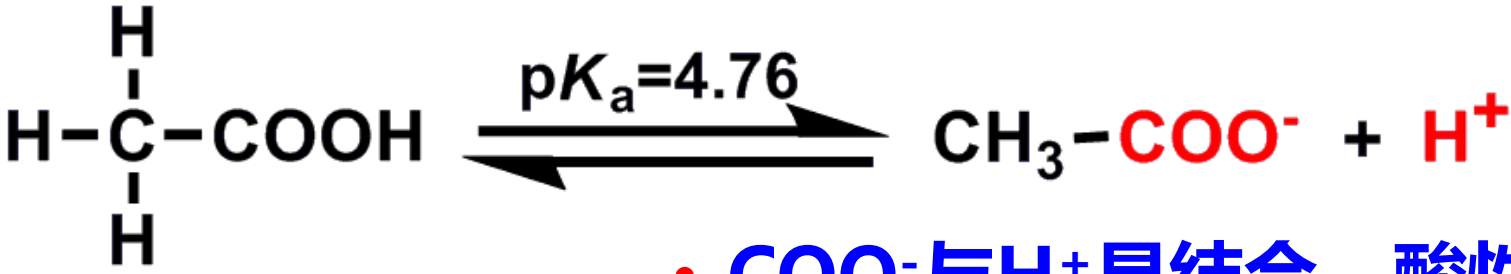
$$pI = \frac{2.19 + 4.25}{2} = \frac{6.44}{2} = 3.22$$

思考：为什么α-氨基酸中羧基的酸性比醋酸的酸性强100倍以上？

1. 诱导效应　2. 场效应



- NH_3^+强拉电子基，COOH容易解离H^+
- COO^-与H^+不易结合，碱性弱而酸性强



- COO^-与H^+易结合，酸性强

课后思考题（抽两位同学下堂课回答，记表现分）：

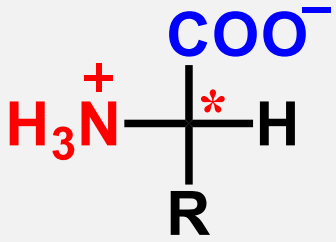
1. α-氨基酸中氨基的碱性比普通的有机胺中氨基的碱性强还是弱？为什么？

2. 试比较α-氨基酸、β-氨基酸和γ-氨基酸的等电点高低，并解释原因。

请将作答PPT于本周二下午5点前提交课堂派“作业”。

本次课主要内容小结

- 氨基酸结构



L-α-amino acid

20种基本氨基酸

- 15种中性氨基酸
- 2种酸性氨基酸
- 3种碱性氨基酸

必需氨基酸

非基本氨基酸

- 氨基酸的一般性质

--- 手性（旋光活性）

--- 紫外吸收（特征吸收）

--- 两性离子（既是酸又是减）

--- 等电点（定义、计算、特性）

--- α-氨基酸中羧基、氨基的酸碱性与普通有机羧酸、胺的差异

课后复习要点及习题

- 掌握氨基酸的基本结构特征。
- 掌握20种基本氨基酸的结构及侧链性质。
- 熟悉非基本氨基酸的种类、结构特点及功能。
- 教材相关习题。
- 课后思考题（2题，抽学生下堂课回答）。
- 附加题：Thr分子中有几个手性碳？画出其所有立体异构体的Fischer投影式结构，并通过查阅资料指出哪种结构是蛋白质中存在的形式。

预 习

- **氨基酸**
  - --- 侧链的化学性质
  - --- 氨基酸的分离与分析
- **多肽**
  - --- 肽键、氨基酸顺序、氨基酸残基
  - --- 肽的pI计算
  - --- 肽链的水解