

蛋白質概論

組成

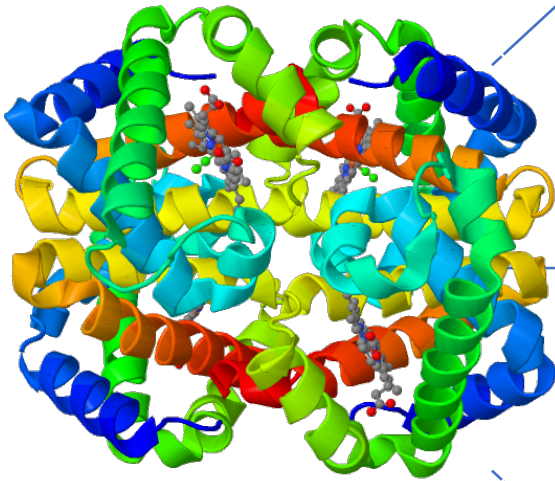
- 如何合成？

結構

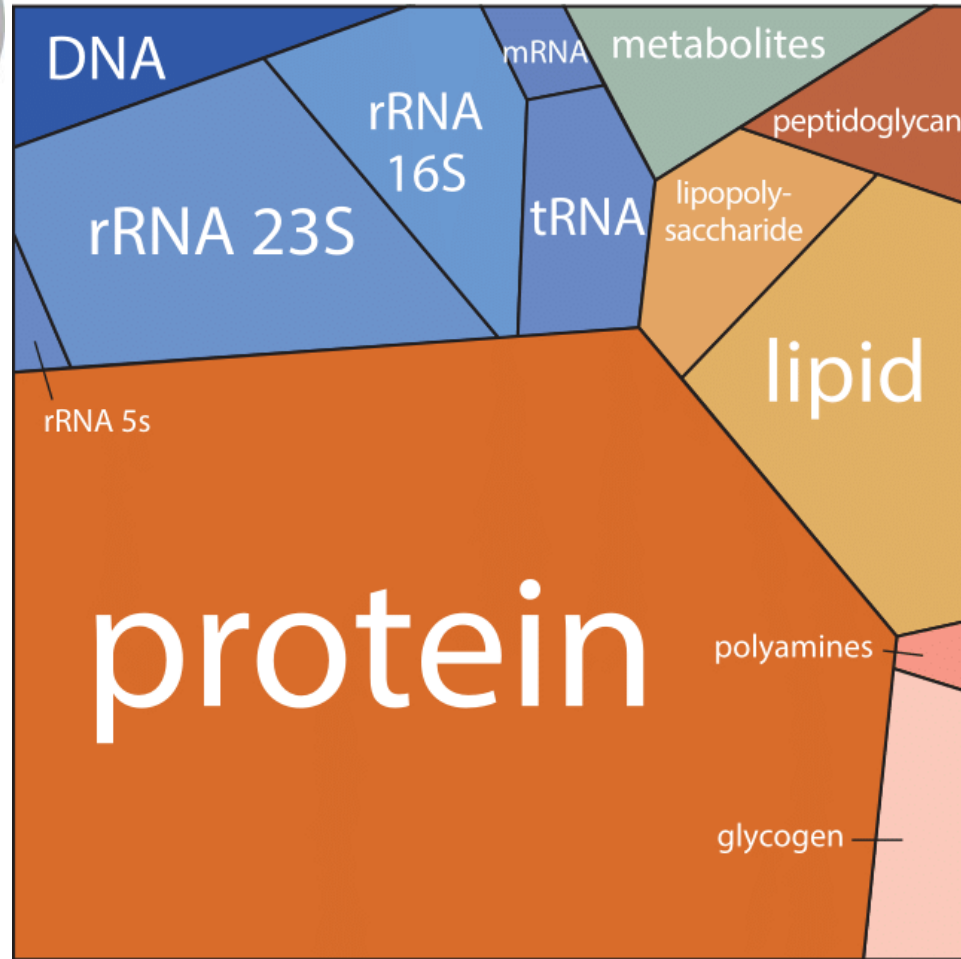
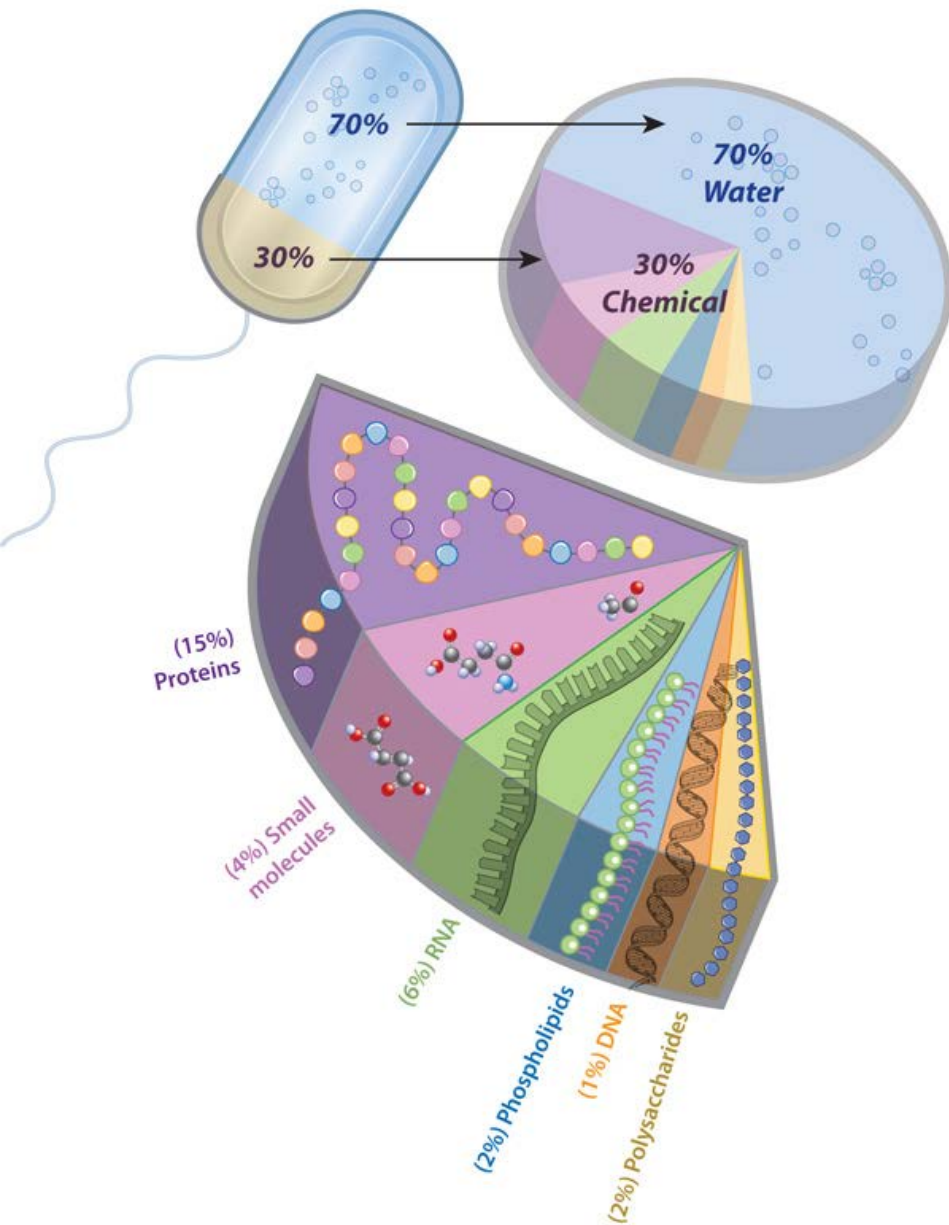
- 結構種類？
- 如何分析？

功能

- 氧結合蛋白
- 免疫球蛋白



細胞中的大分子組成： 以大腸桿菌為例



<http://book.bionumbers.org/what-is-the-macromolecular-composition-of-the-cell/>

<https://www.nature.com/scitable/topicpage/what-is-a-cell-14023083/>

蛋白質的功能多樣性

- 蛋白質有什麼功能？



螢光素酶

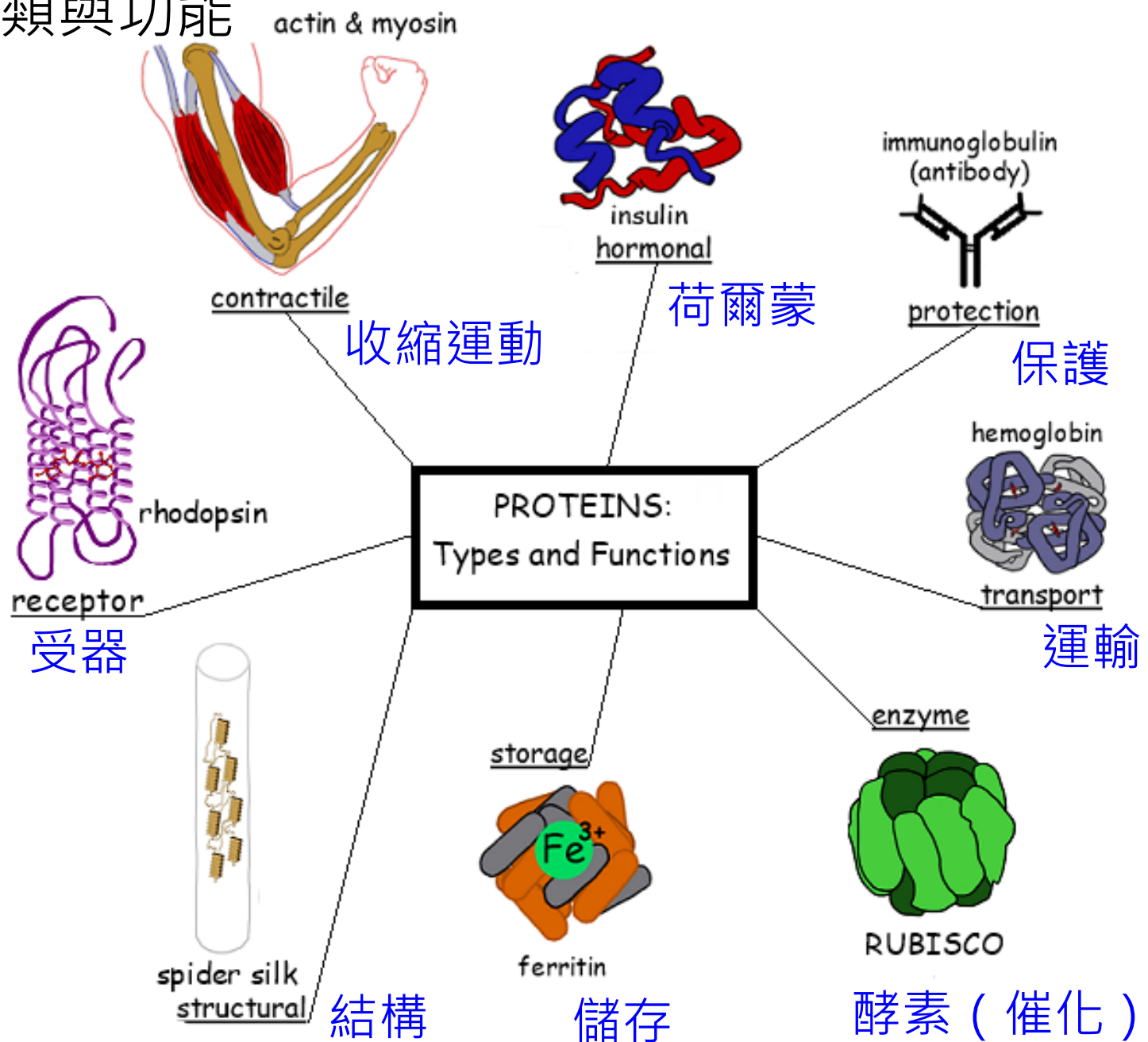


血紅蛋白



角蛋白

蛋白質種類與功能



蛋白質的組成

- 蛋白質僅由胺基酸所組成？



複合蛋白：胺基酸 + 其他成份

TABLE 3-4 Conjugated Proteins

Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1 -Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides 黃素核苷酸	Succinate dehydrogenase
Metalloproteins	Iron Zinc Calcium Molybdenum Copper	Ferritin Alcohol dehydrogenase Calmodulin Dinitrogenase Plastocyanin

免疫球蛋白
/抗體

酪蛋白

輔基(prosthetic group)參考資料：

https://en.wikibooks.org/wiki/Structural_Biochemistry/Enzyme/Prosthetic_Group

簡單蛋白：僅由胺基酸所組成

脂蛋白

醣蛋白

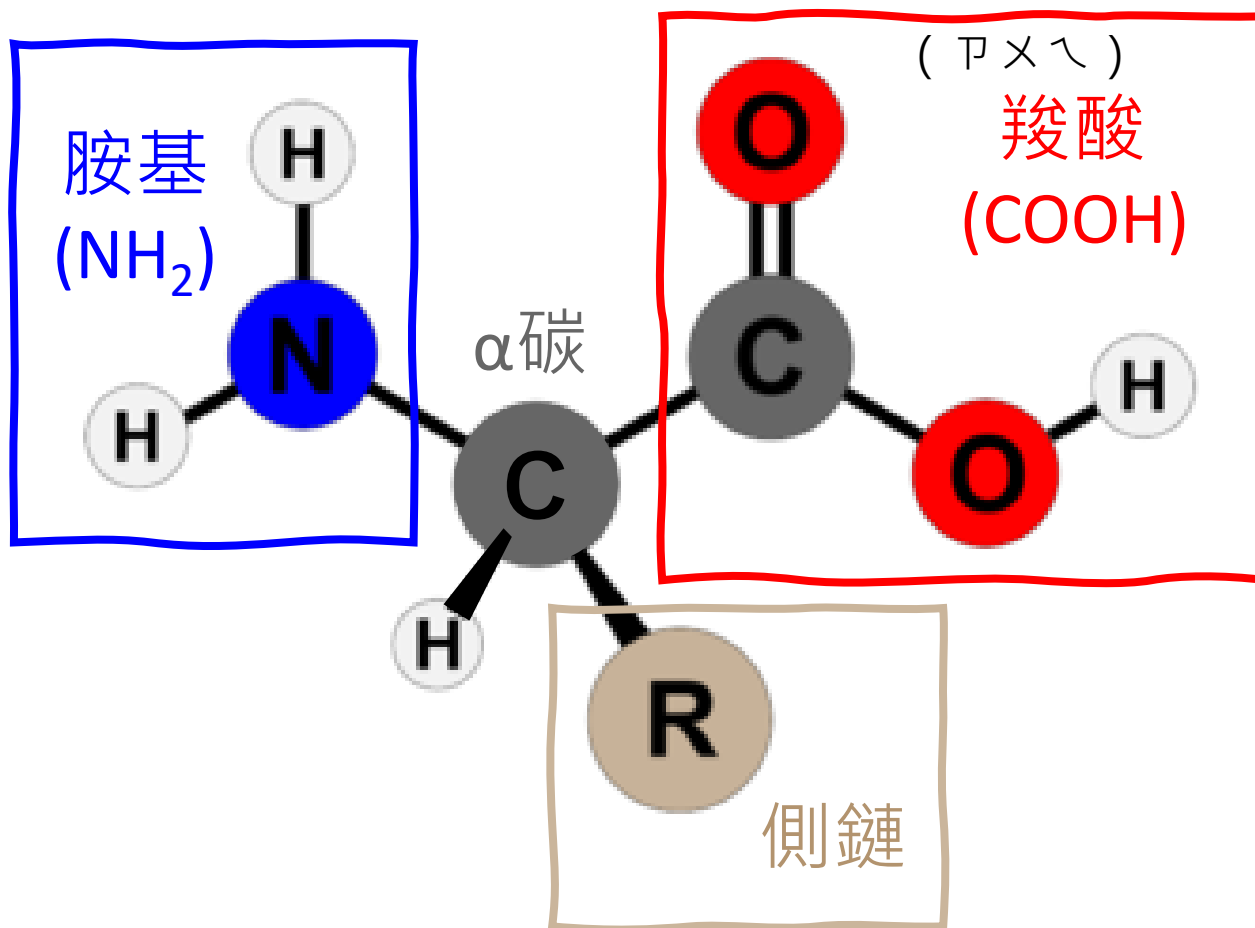
磷蛋白

血基質蛋白

核黃蛋白

金屬蛋白

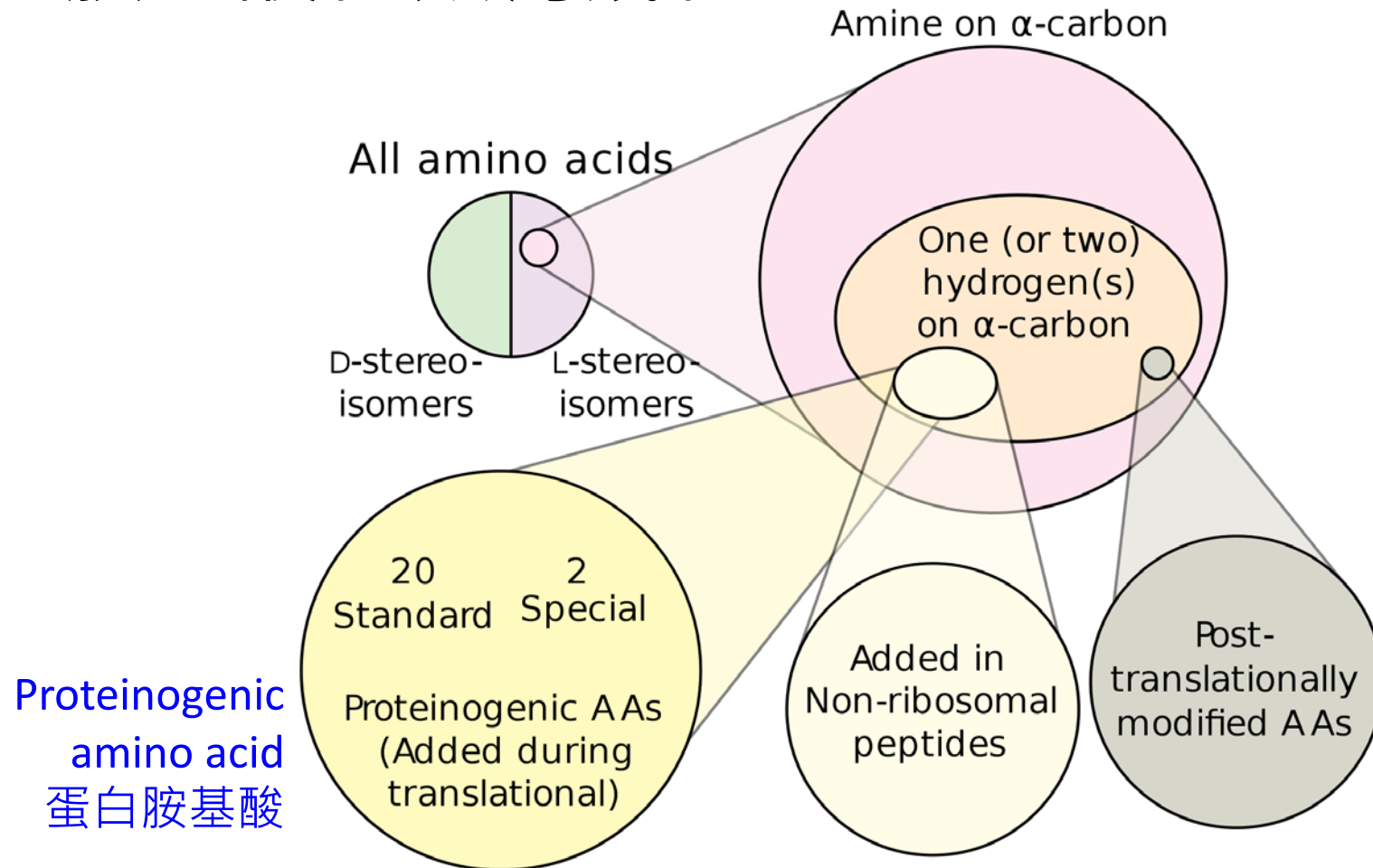
胺基酸的共同結構



胺基酸側鏈構造決定不同種類

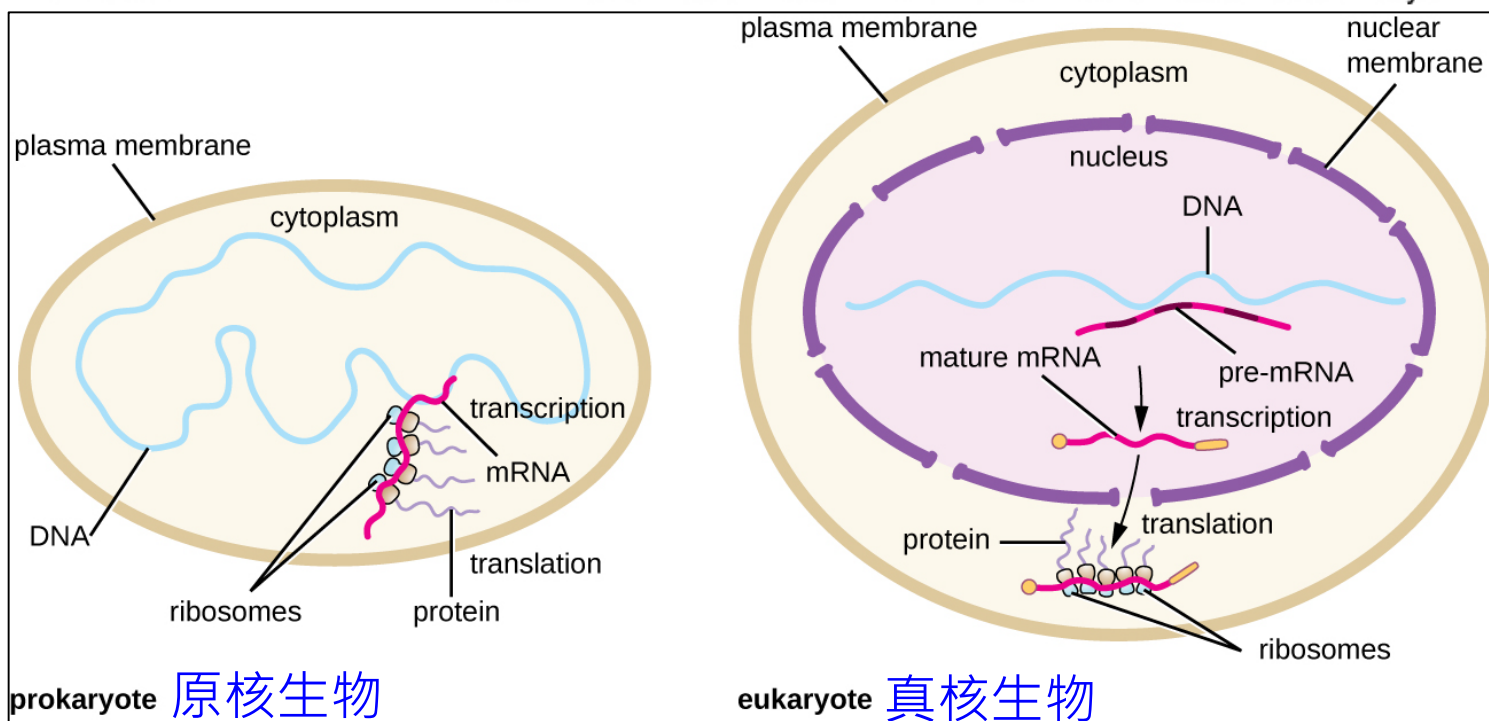
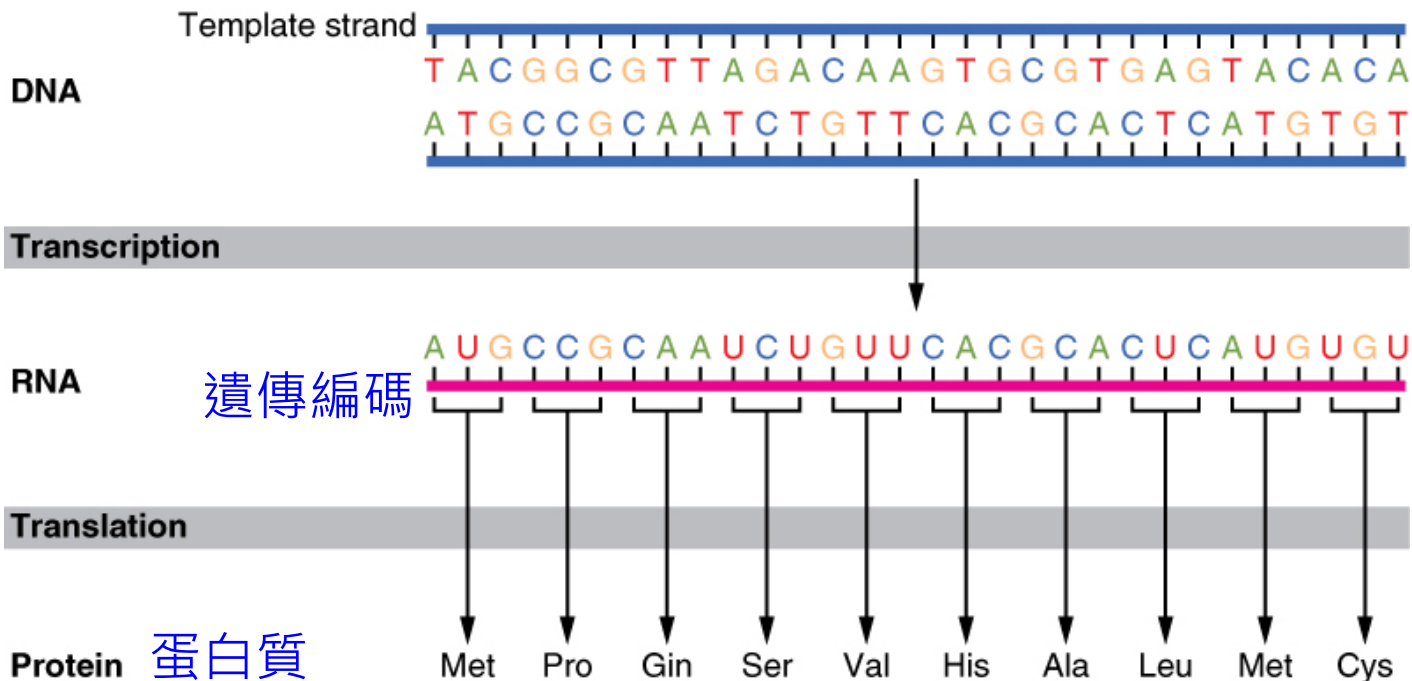
(可由遺傳編碼轉譯出的)

• 胺基酸種類有幾種？



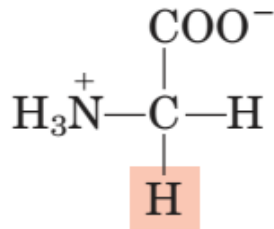
轉錄

轉譯

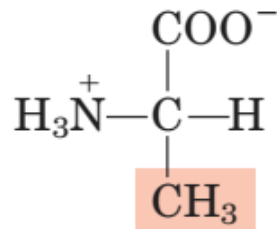


側鏈為非極性、疏水

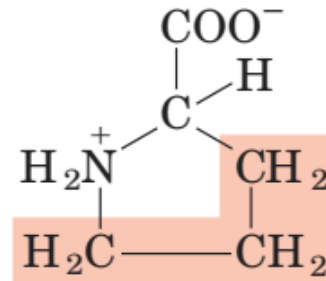
Nonpolar, aliphatic R groups



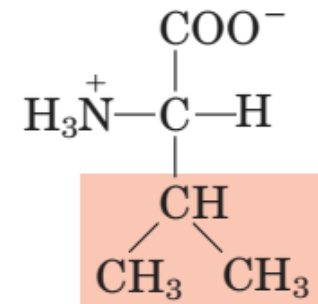
Glycine
甘胺酸



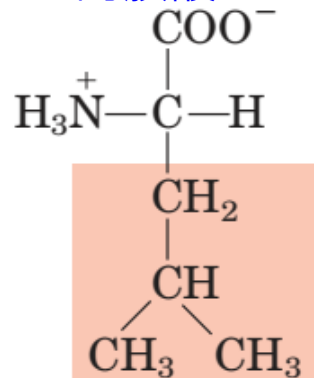
Alanine
丙胺酸



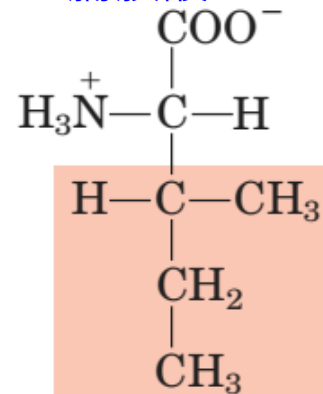
Proline
脯胺酸



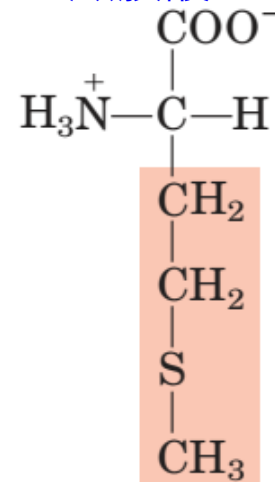
Valine
纈胺酸



Leucine
白胺酸

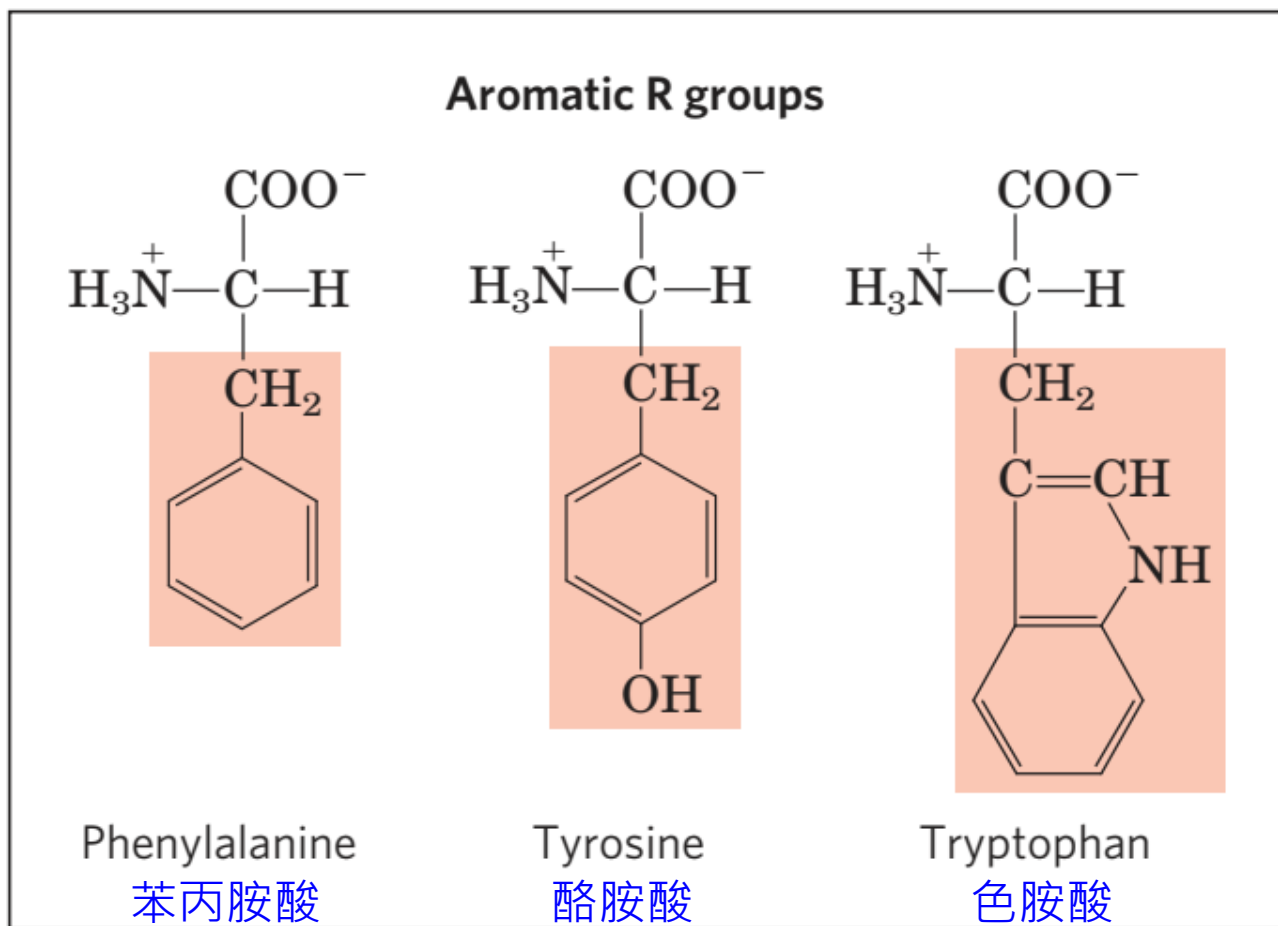


Isoleucine
異白胺酸



Methionine
甲硫胺酸

側鏈為芳香族



疏水

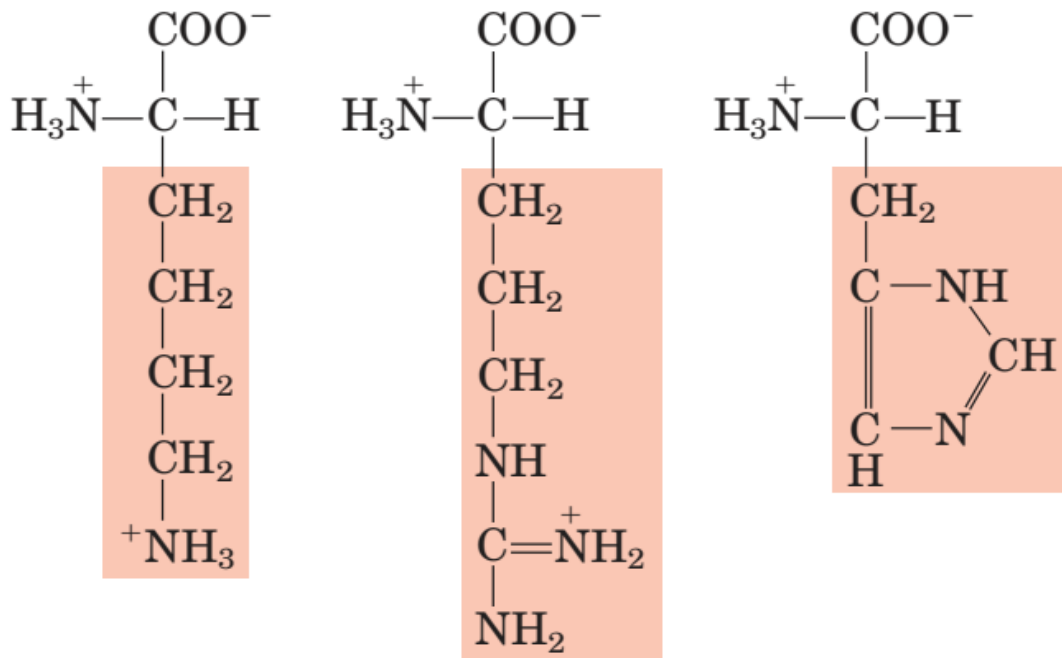
有極性

疏水

側鏈帶正電

側鏈帶負電

Positively charged R groups

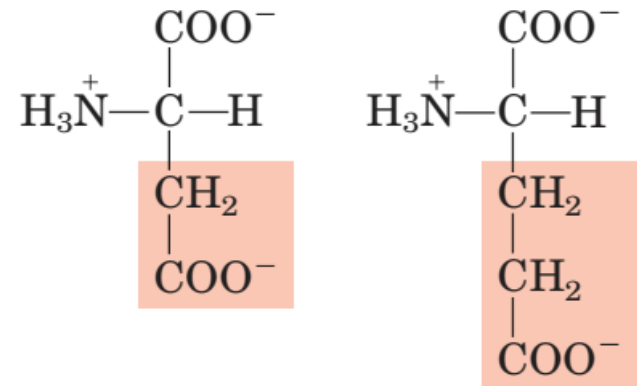


Lysine
離胺酸

Arginine
精胺酸

Histidine
組胺酸

Negatively charged R groups

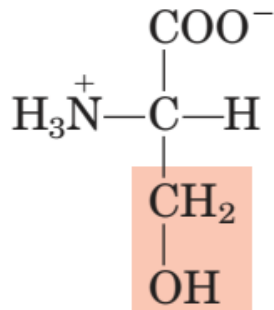


Aspartate
天門冬胺酸

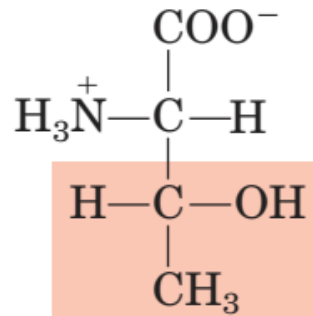
Glutamate
麩胺酸

側鏈不帶電、但具有極性

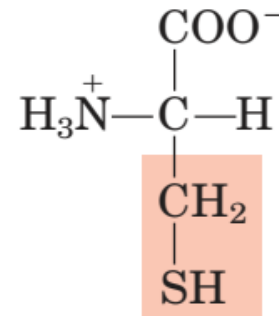
Polar, uncharged R groups



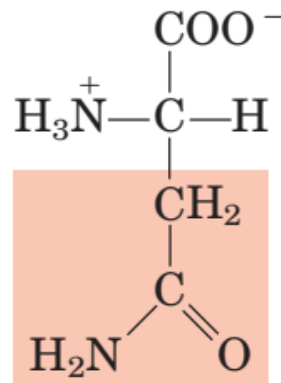
Serine
絲胺酸



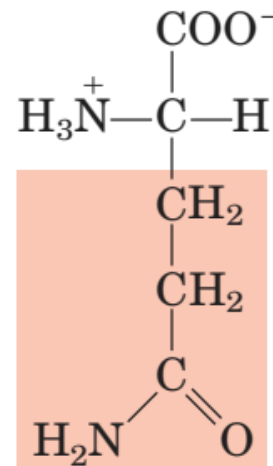
Threonine
蘇胺酸



Cysteine
半胱胺酸



Asparagine
天門冬醯胺酸



Glutamine
麩醯胺酸

討論：為什麼要有那麼多種胺基酸？

- 側鏈具備不同特性：

- 1) 大小

- 2) 極性

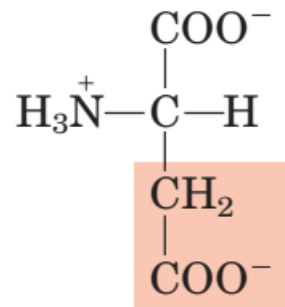
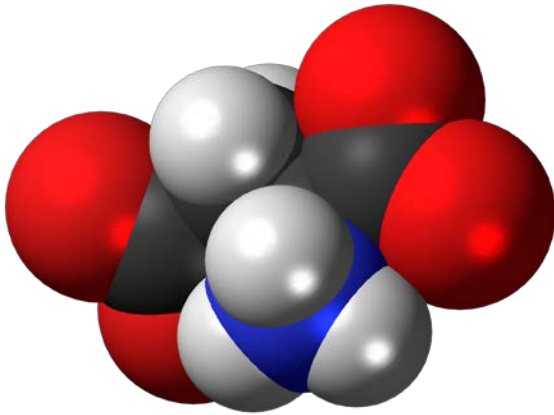
- 3) 帶電性

- 4) 靈活性 (flexibility)

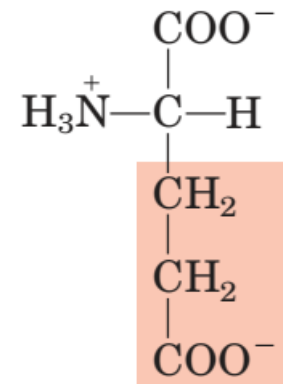
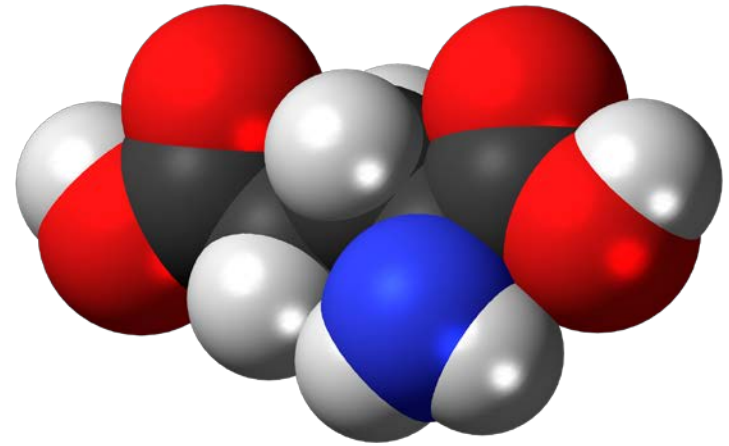
- 5) 結構形態...等。

- 不同胺基酸組合而成的蛋白質具備有不同特性。

(1)大小



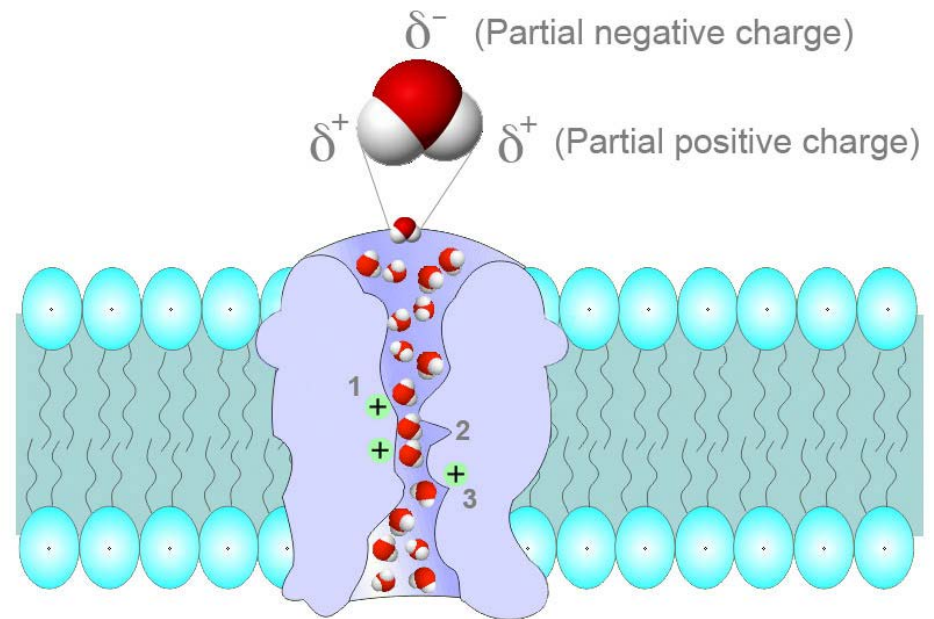
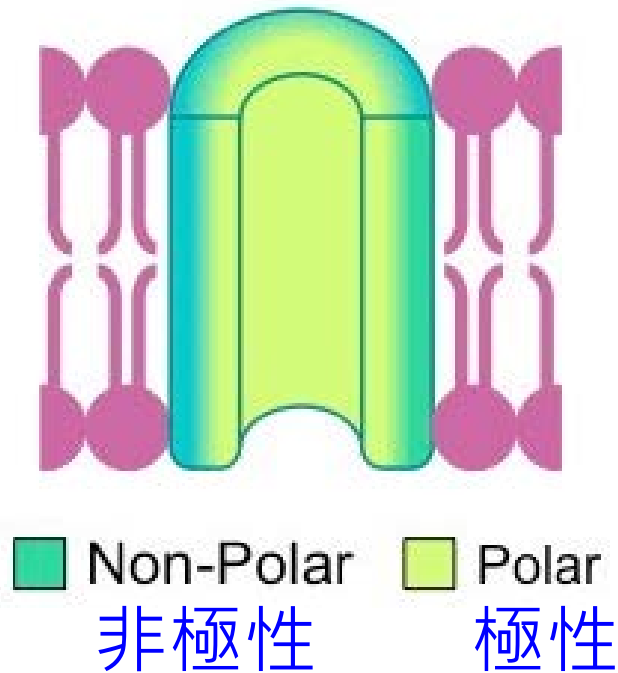
Aspartate



Glutamate

(2) 極性

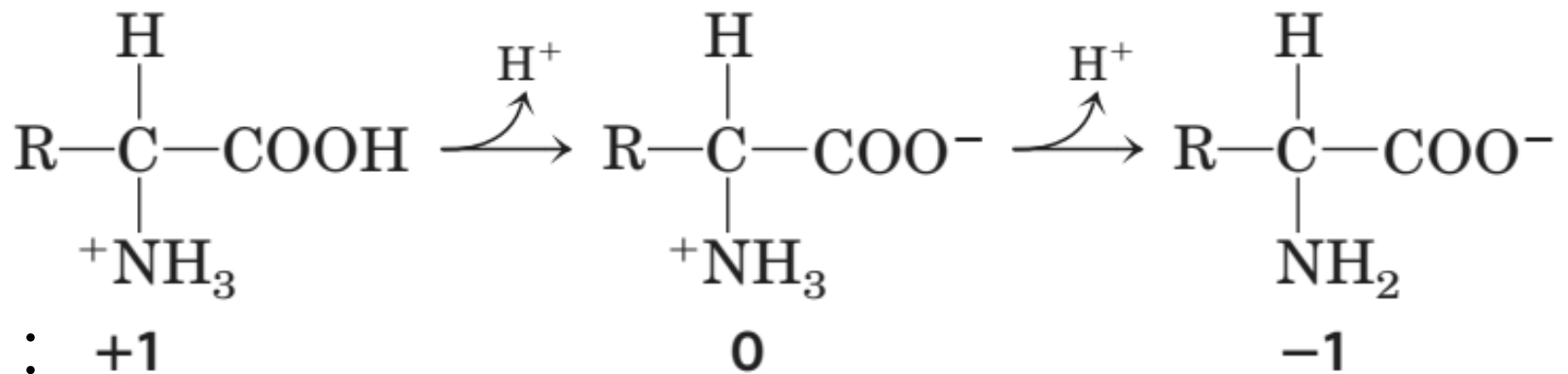
- 以通道蛋白質為例：

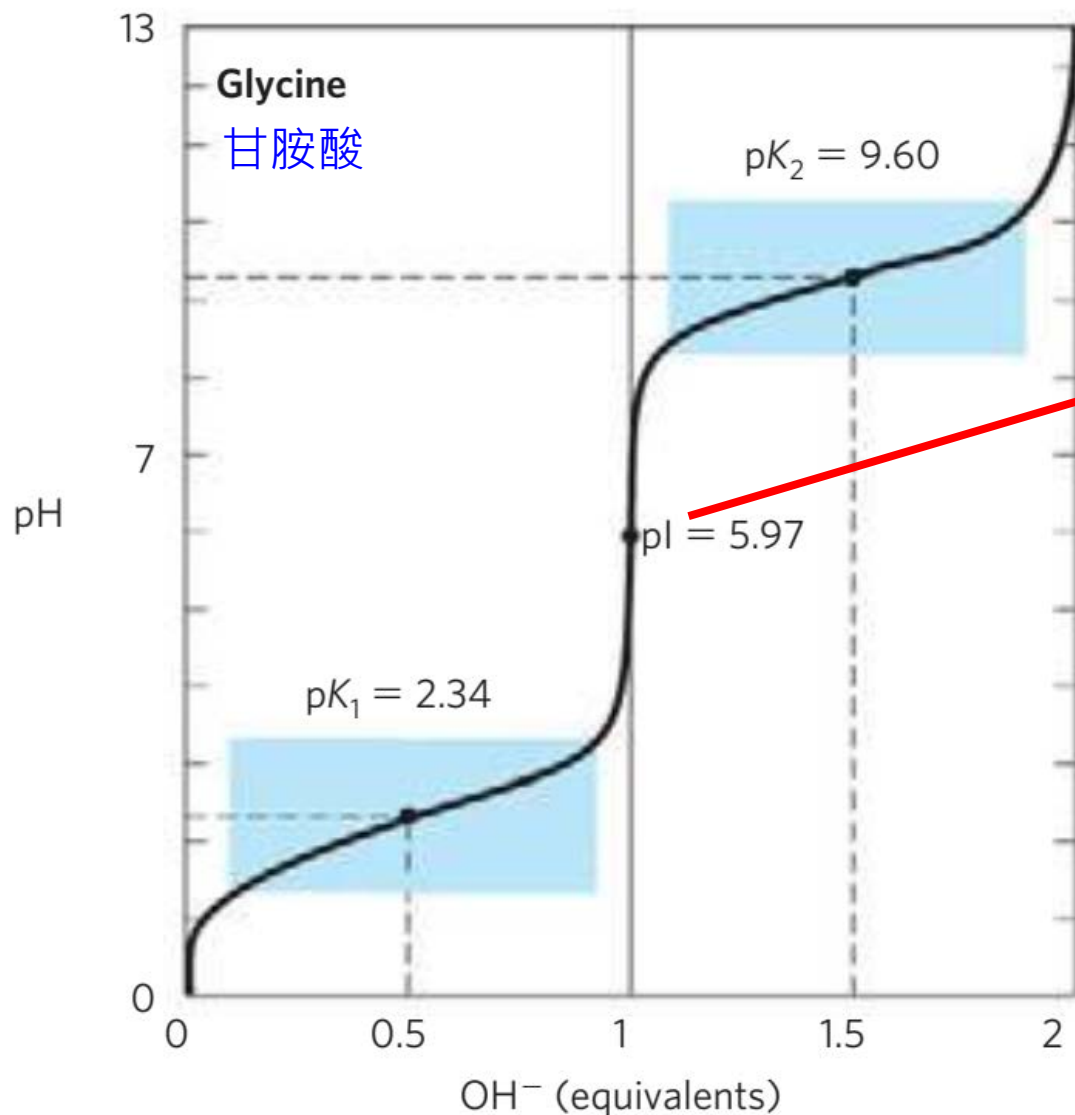
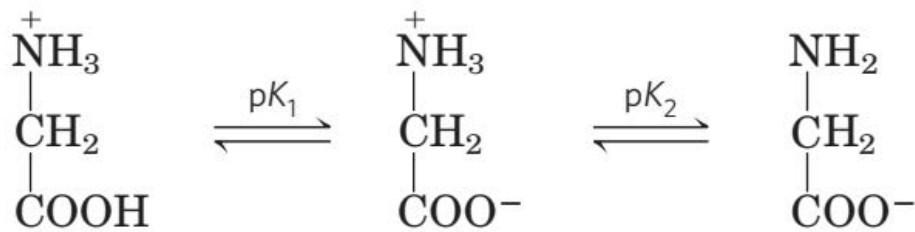


水通道蛋白

(3) 帶電性

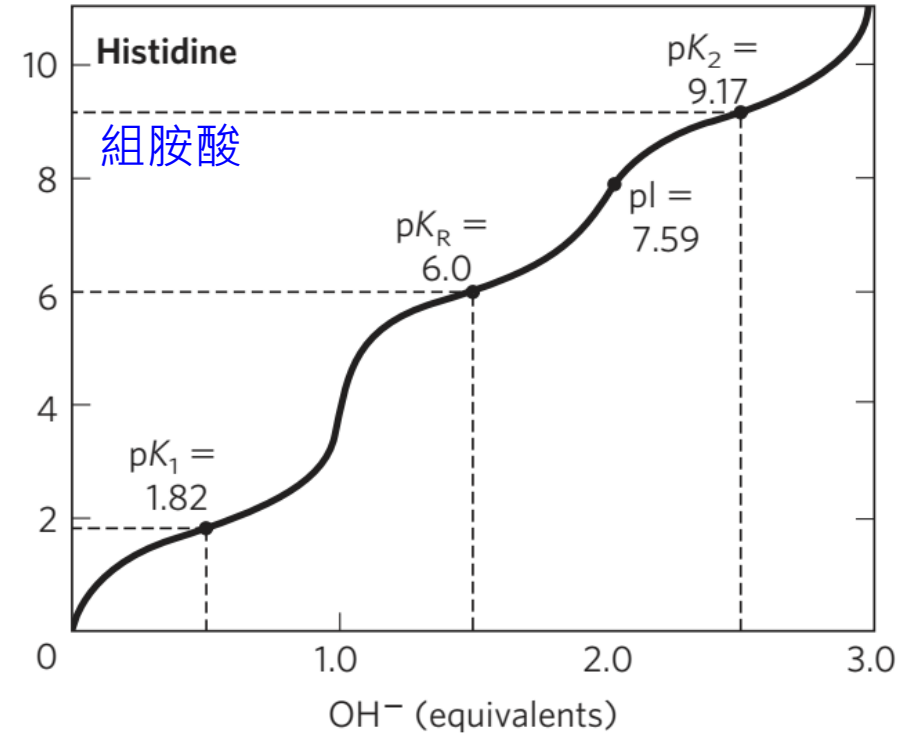
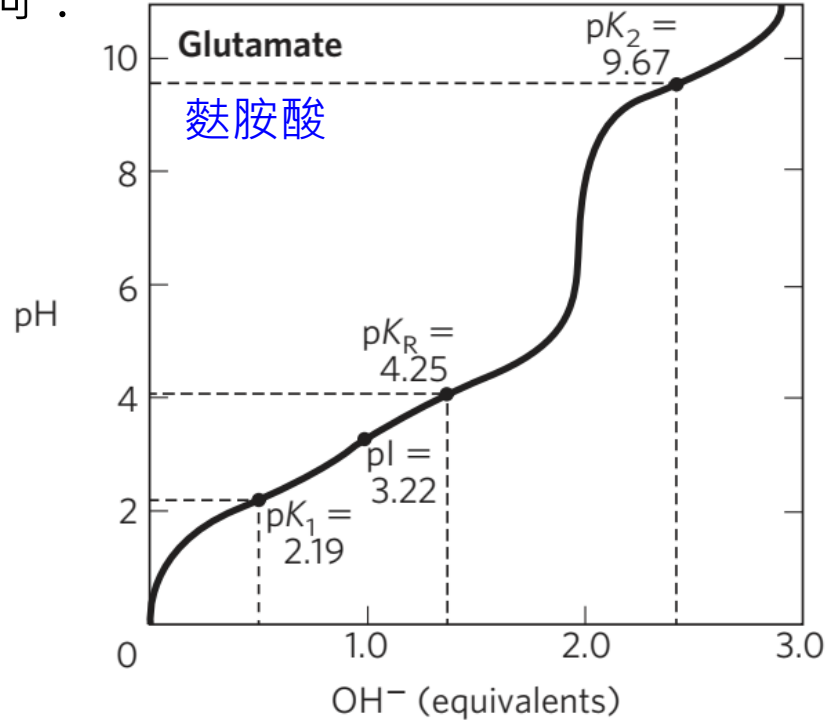
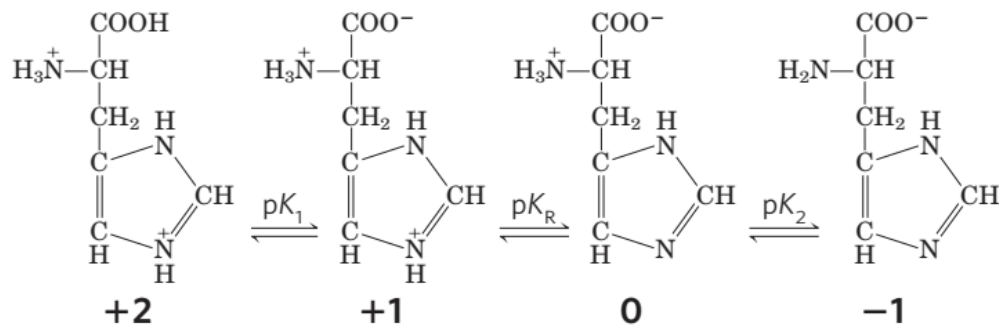
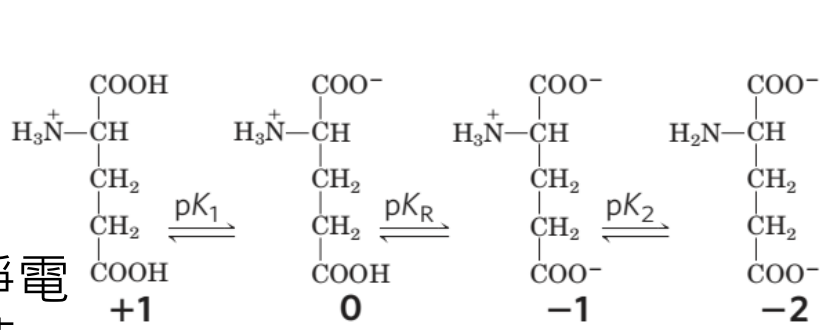
- 胺基酸依據所在的酸鹼度(pH值)環境會有不同的帶電狀態：





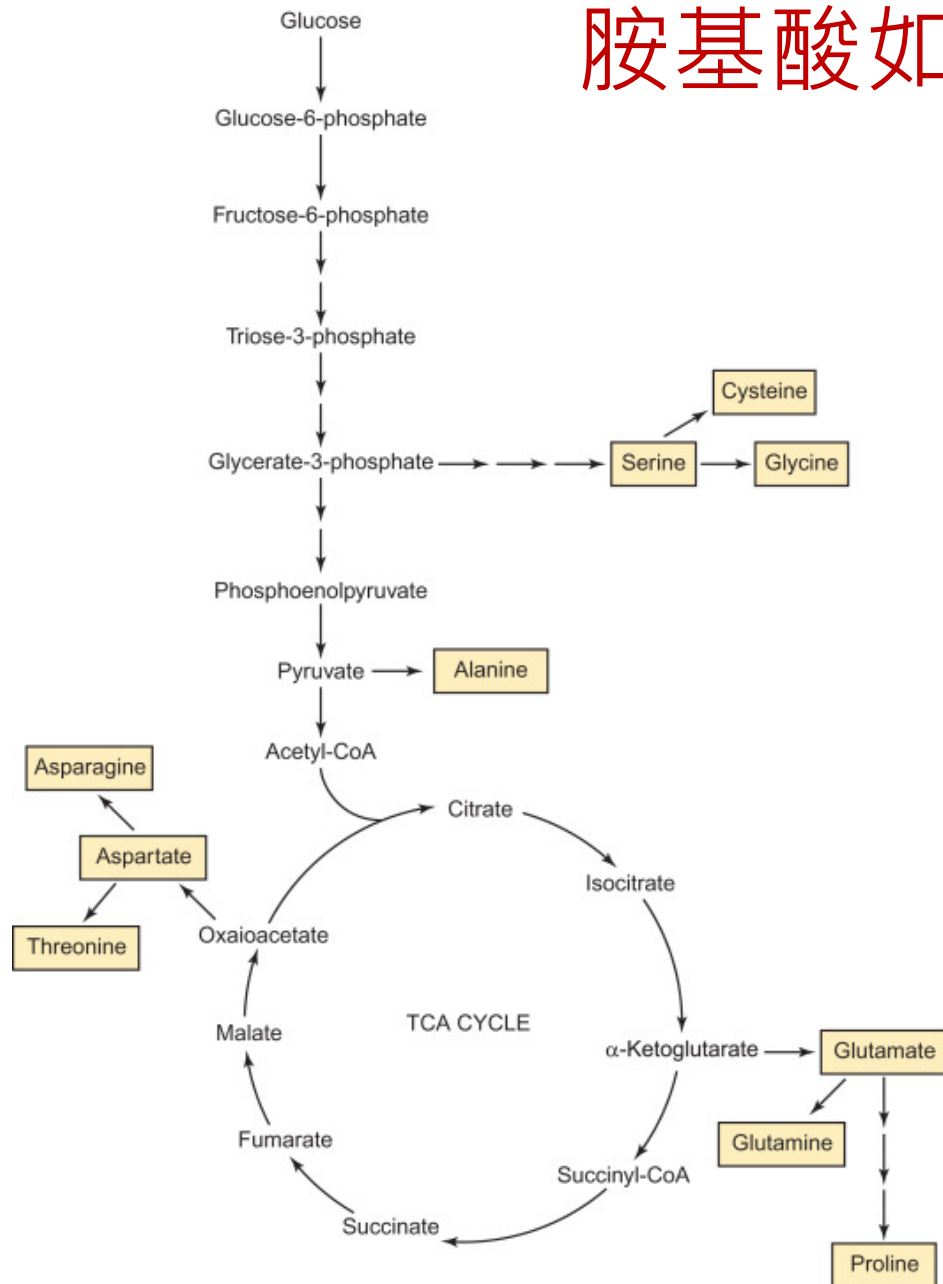
等電點(pI)：蛋白質在特定pH值時所帶正、負電荷數量相等，蛋白質分子的淨電荷為零，此pH值稱為pI。

淨電荷：



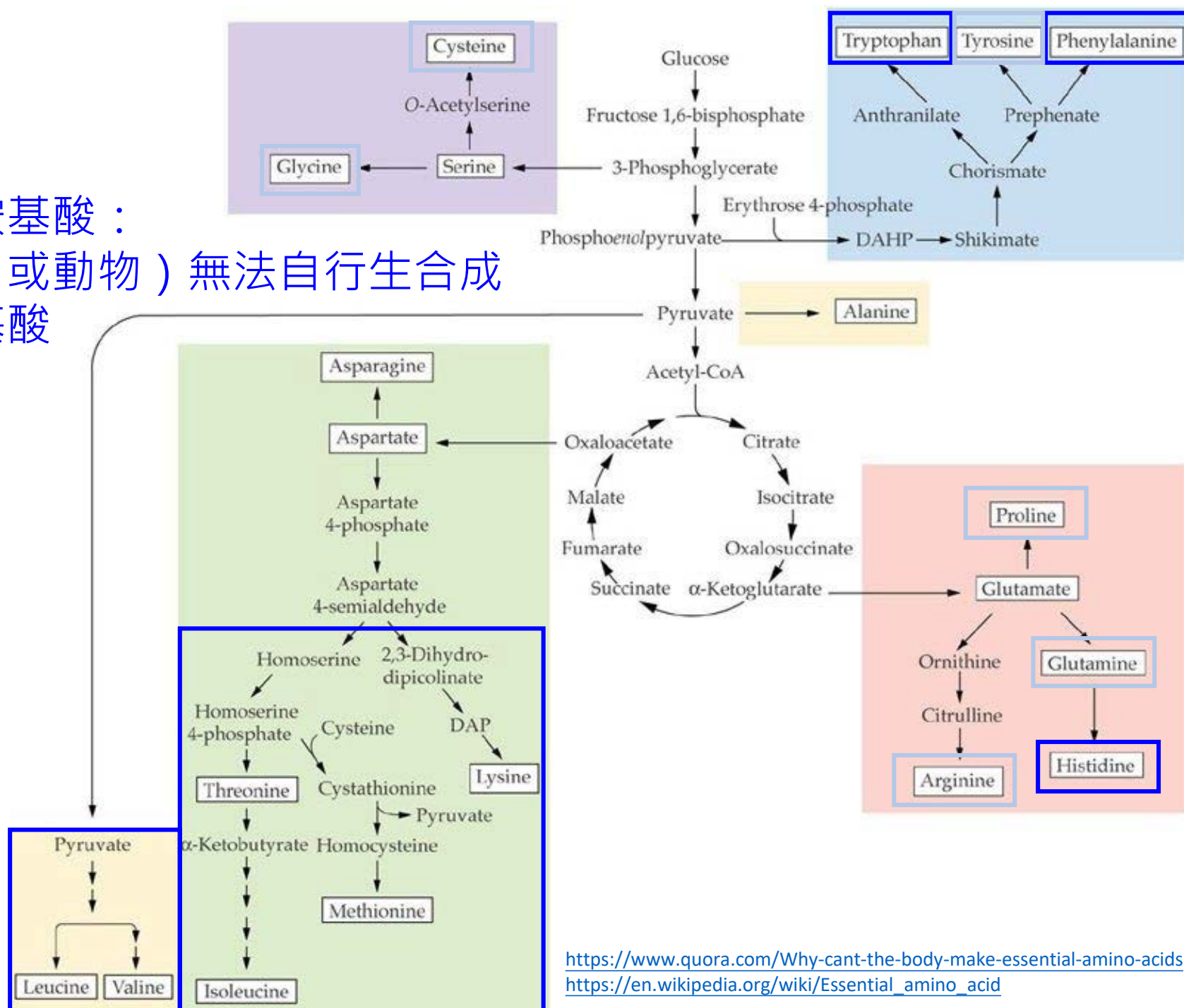
胺基酸如何合成？

人類細胞合成胺基酸途徑



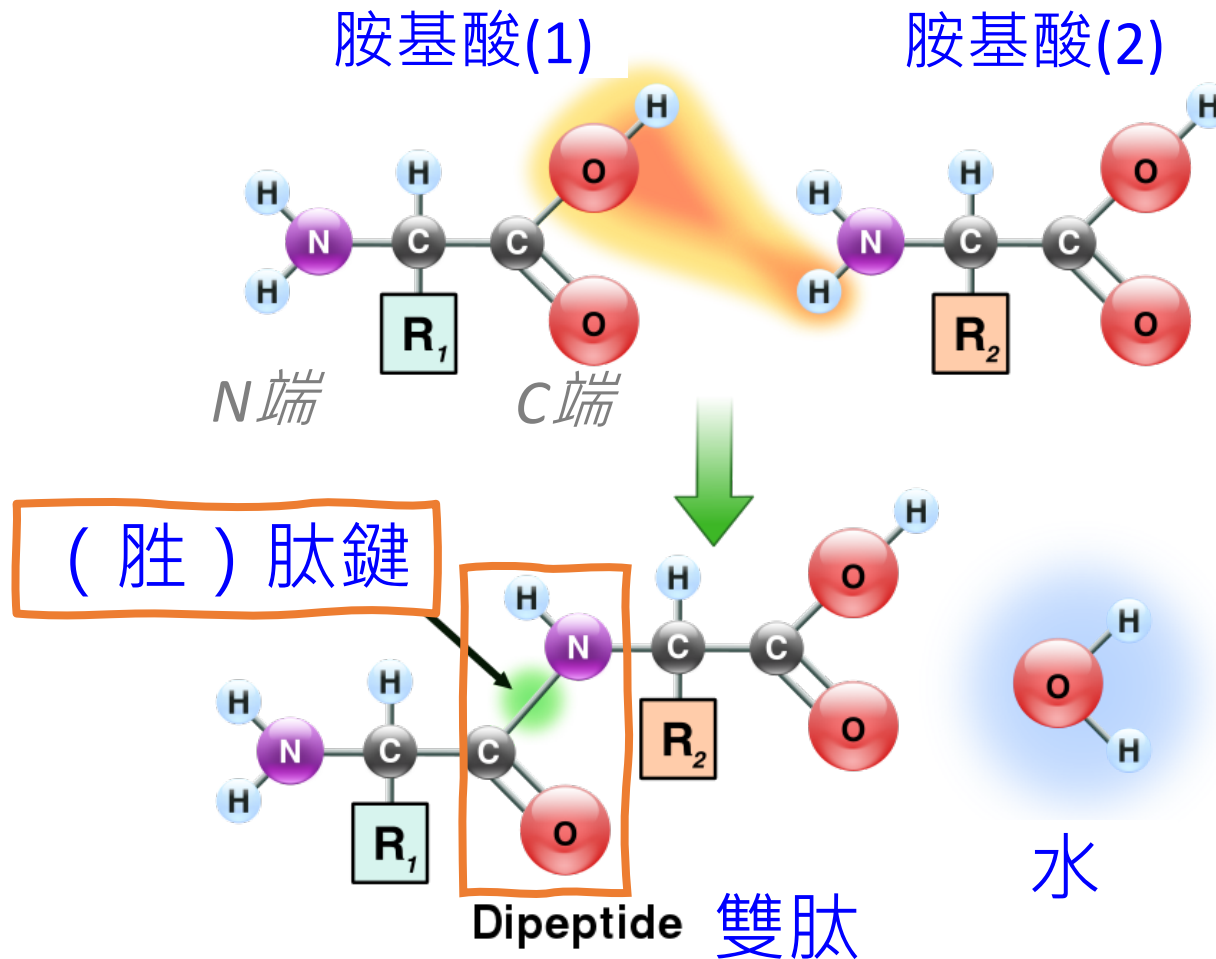
TCA: 三羧酸循環 (tricarboxylic acid cycle)

必需胺基酸：
人類（或動物）無法自行生合成的胺基酸



蛋白質如何合成？

- 胺基酸 → 胜肽(peptide) → 蛋白質(protein)



單肽(monopeptide)

雙肽(dipeptide)

三肽(tripeptide)

四肽(tetrapeptide)

五肽(pentapeptide)

...

寡肽(oligopeptide)

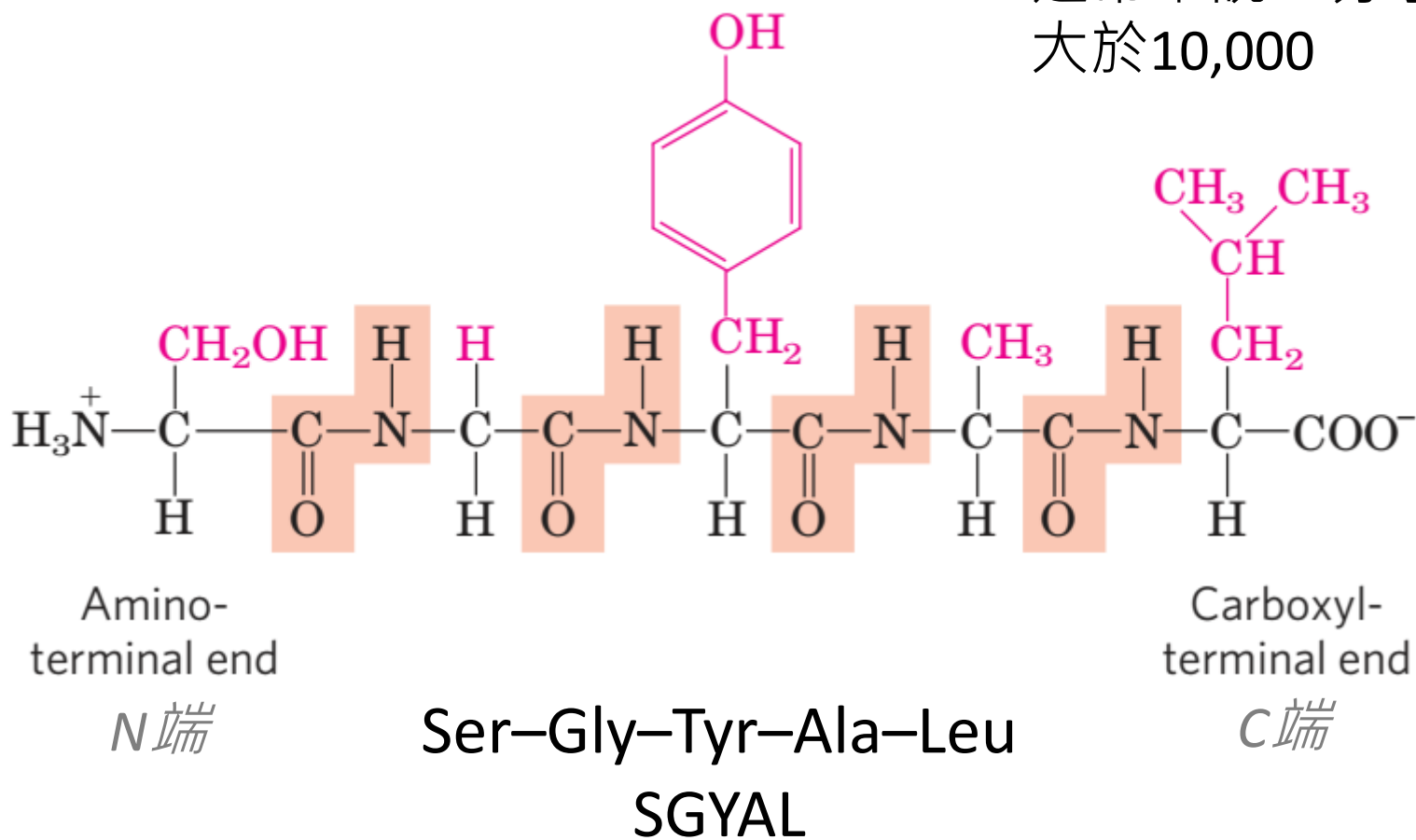
2-20個胺基酸

多肽(polypeptide)

通常來說，分子量
小於10,000

蛋白質(protein)

通常來說，分子量
大於10,000



蛋白質分子量與胺基酸個數的換算

- 胺基酸平均分子量為多少？

<https://bionumbers.hms.harvard.edu/search.aspx>

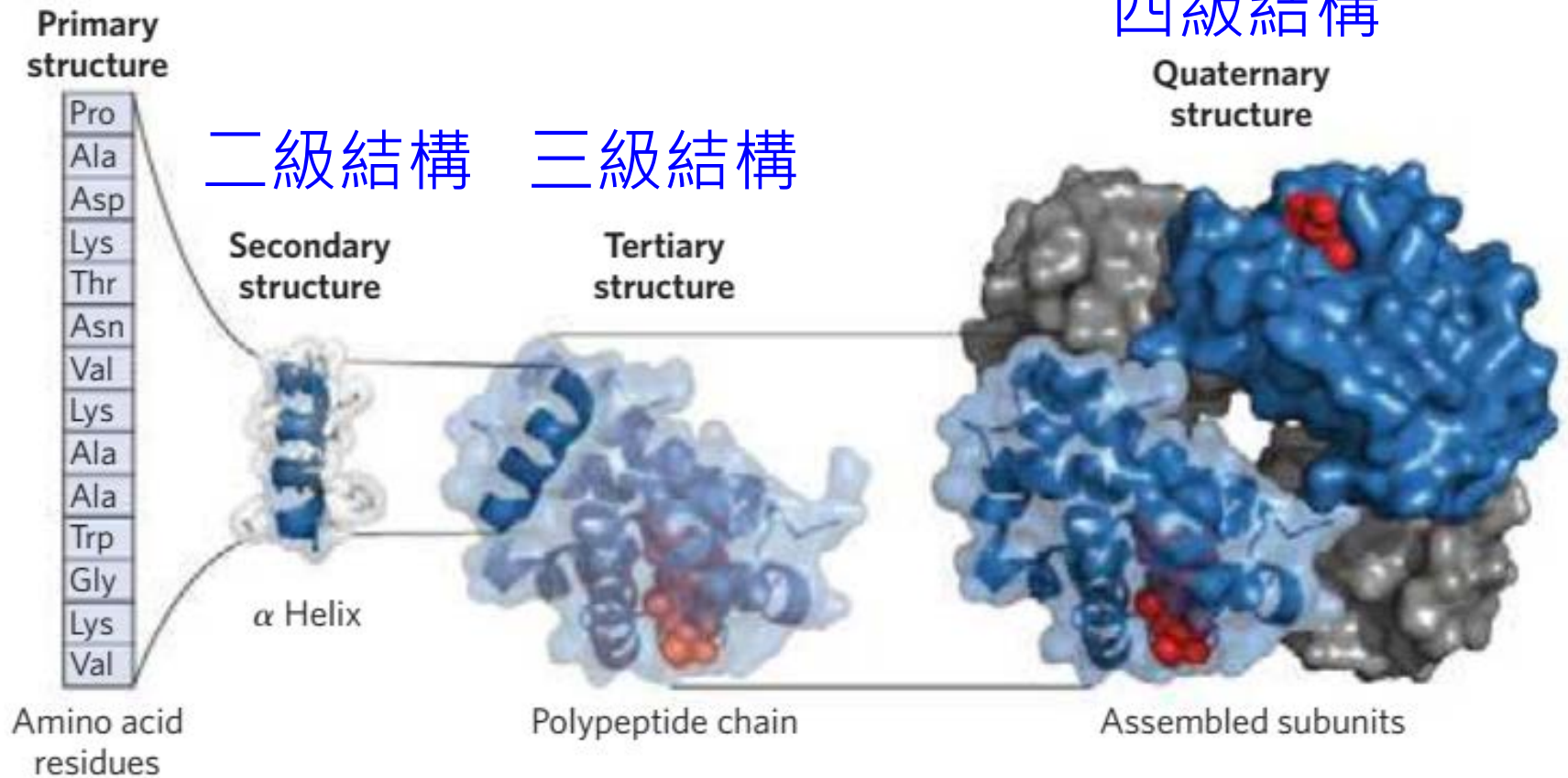


Or, Google “average amino acid molecular weight”.

蛋白質的結構

- 蛋白質的四級結構

一級結構

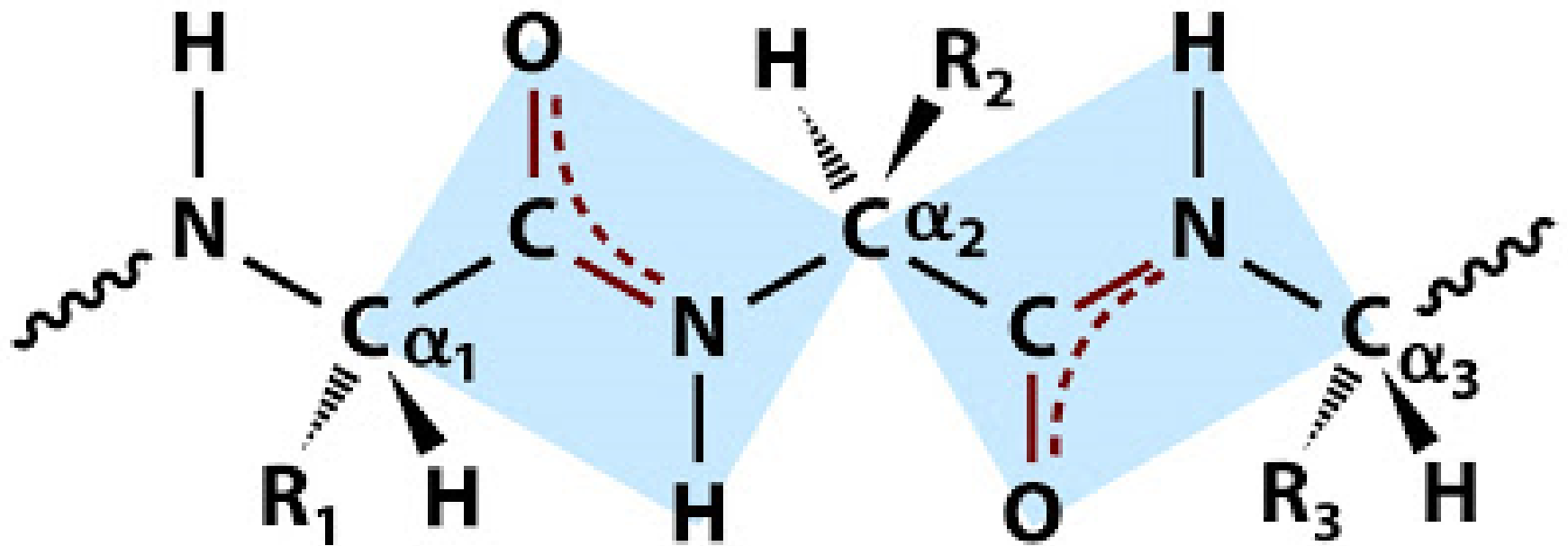


蛋白質的一、二級結構

- 1) 一級結構：胜肽中胺基酸的組成與排列次序（N端→C端）。
 - 可由蛋白質（胜肽）定序得知。
- 2) 二級結構：蛋白質中區域性的結構，因氫鍵作用產生空間上穩定的胺基酸排列。
 - 常見（有規則）二級結構為 α -螺旋、 β -褶片與 β -轉角。
 - 無規則捲曲(random coil)也多屬穩定結構。

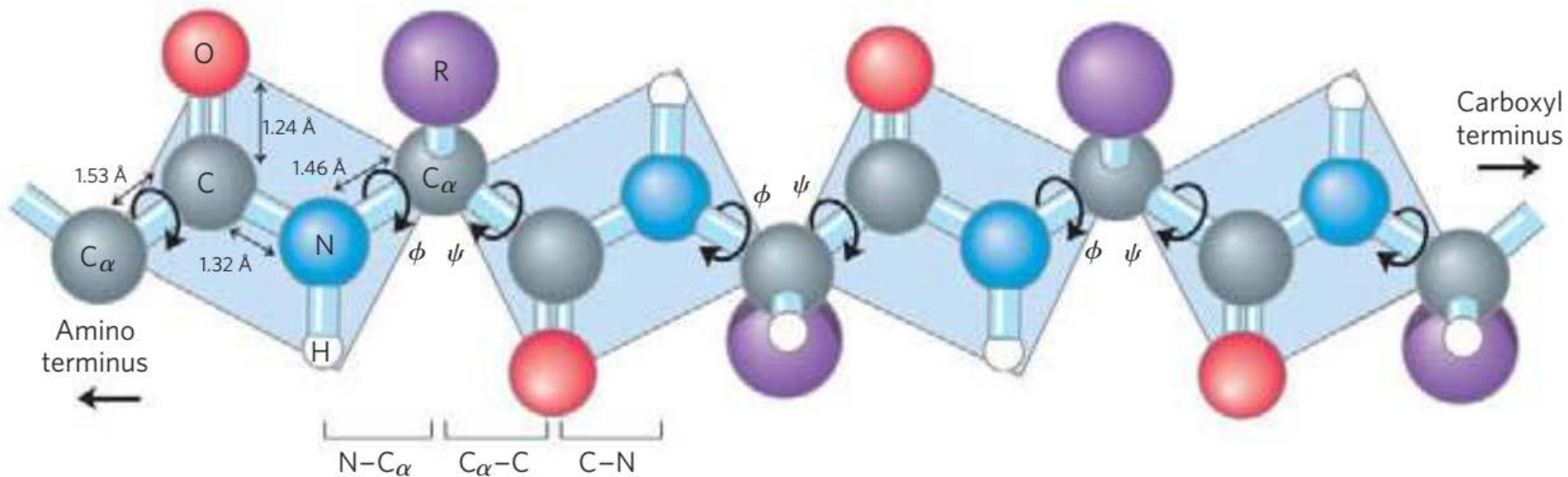
如何形成二級結構？

- 肽鍵因共振具有部份雙鍵特性，形成不易旋轉的平面結構。



胜肽中可旋轉的共價鍵

- C_{α} 上的 ϕ (phi) 與 ψ (psi) 角



Ramachandran Principle :
蛋白質中的 ϕ (phi)與 ψ (psi)角

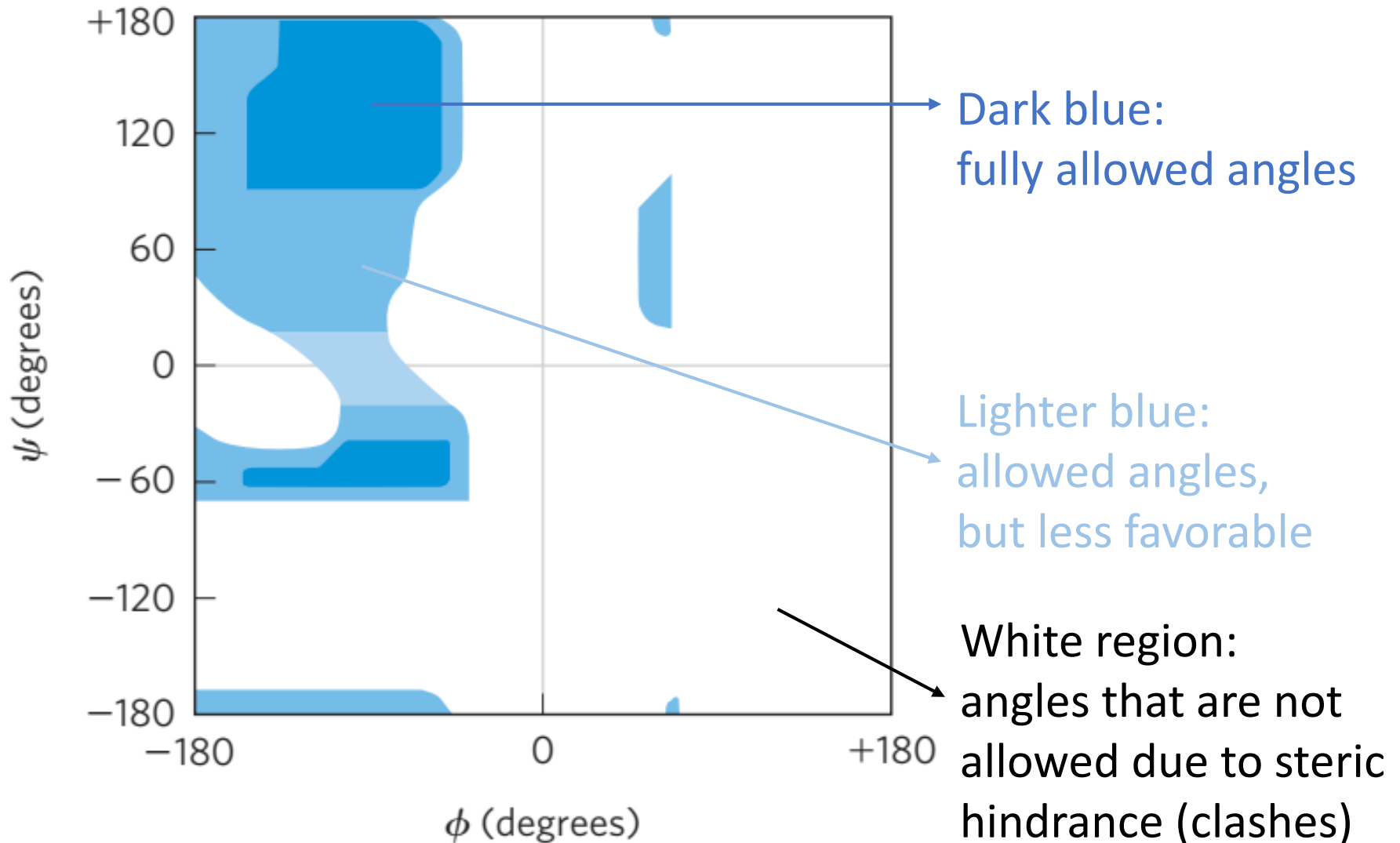
An animated atomic model:

<http://bioinformatics.org/molvis/phipsi/>

Ramachandran Plot

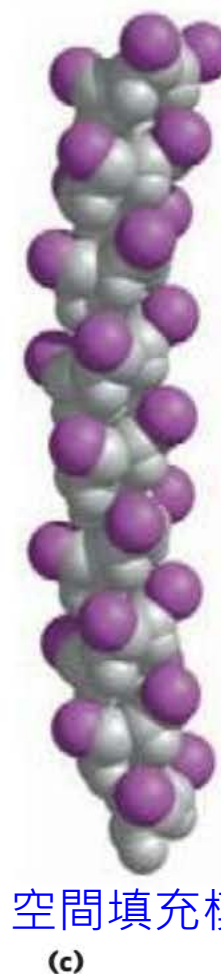
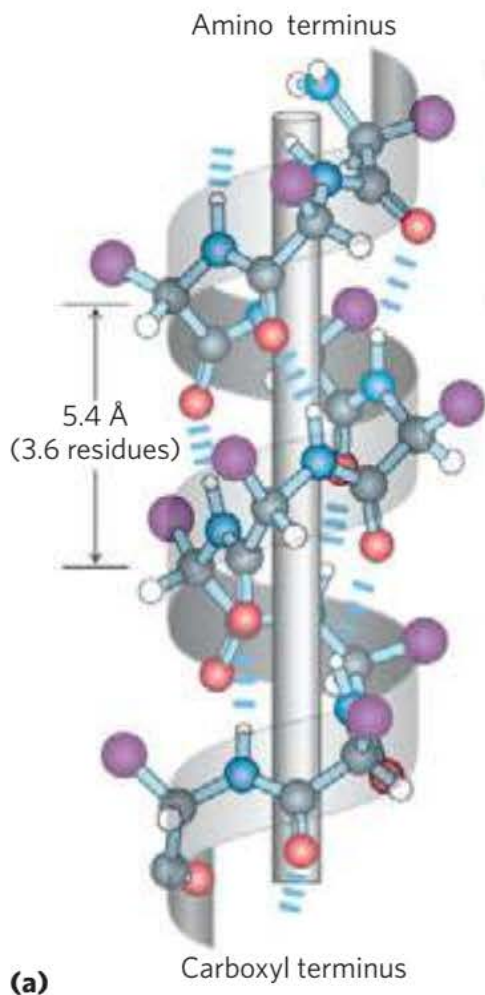
- Ramachandran plot for L-Ala residues

丙氨酸

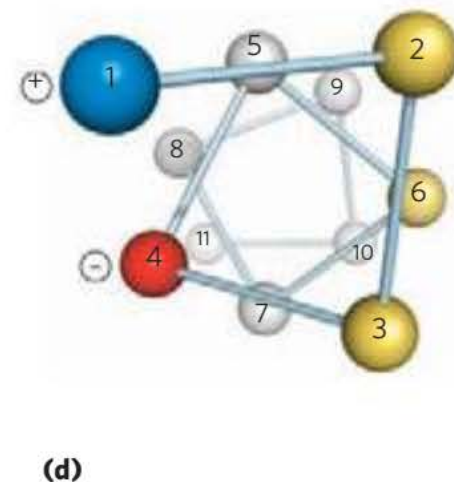




α -螺旋結構



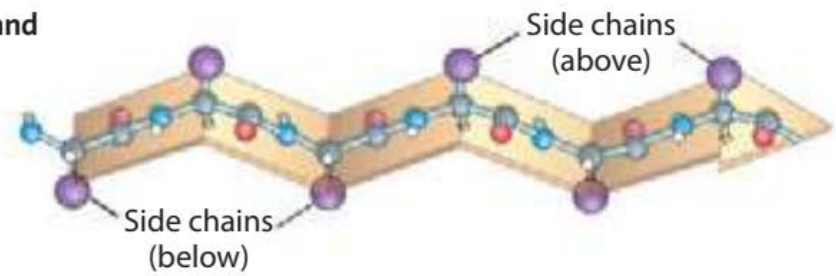
黃色：疏水性側鏈
藍色：正電荷側鏈
紅色：負電荷側鏈



β -褶片結構

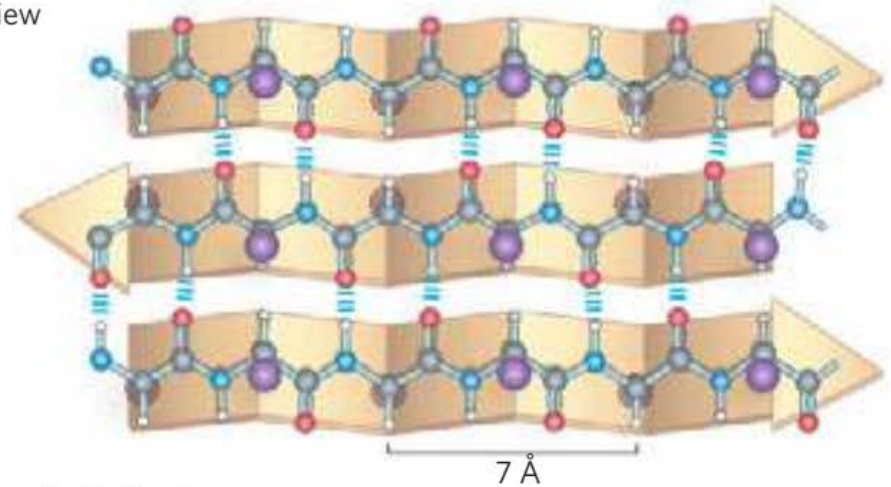
(a) β strand

Side view



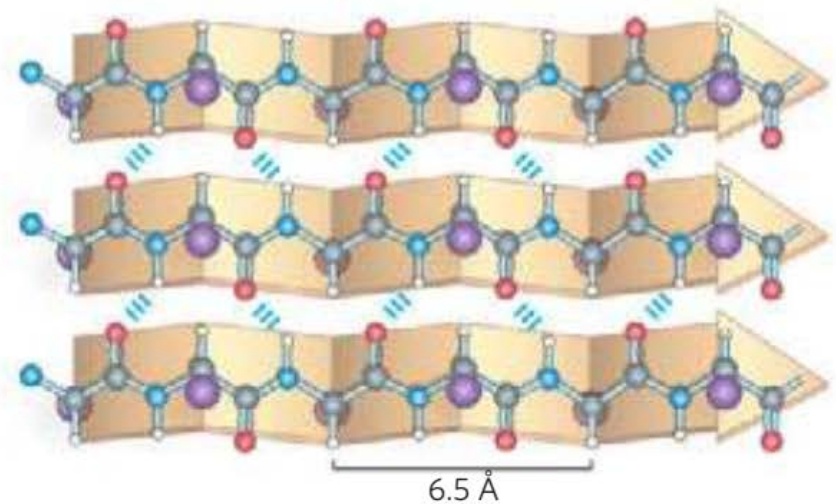
(b) Antiparallel β sheet

Top view

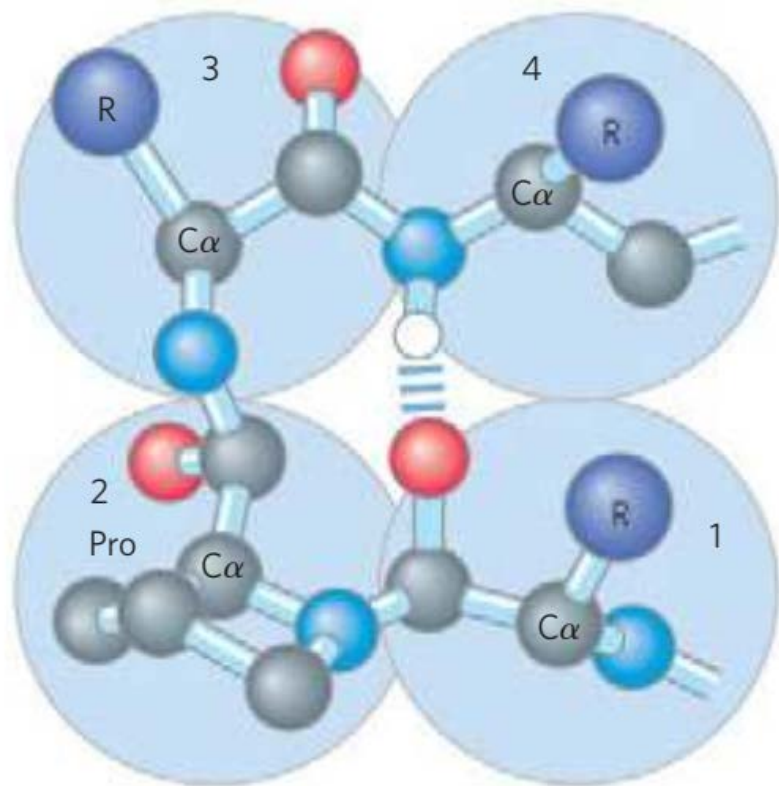


(c) Parallel β sheet

Top view

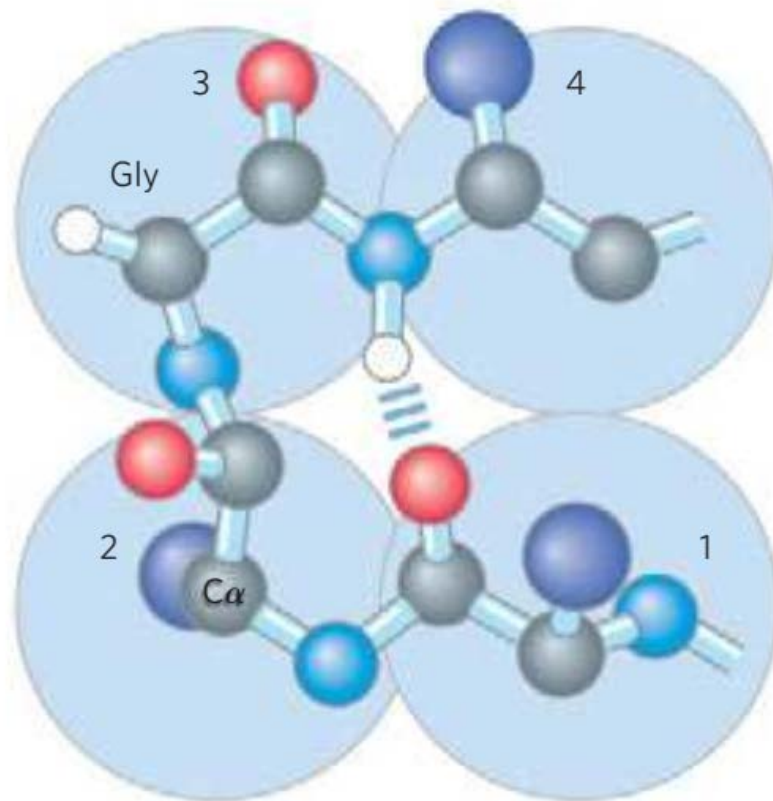


β -轉角結構



Type I β turn

較為普遍

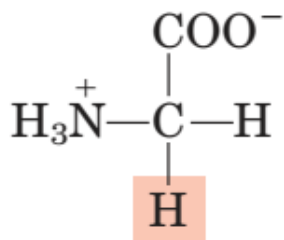
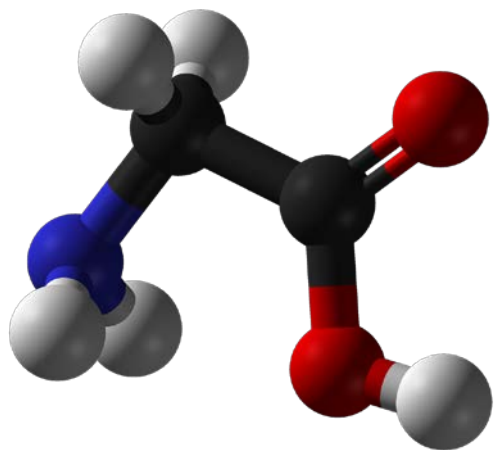


Type II β turn

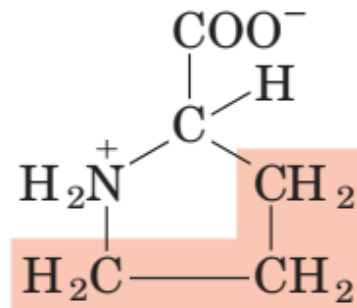
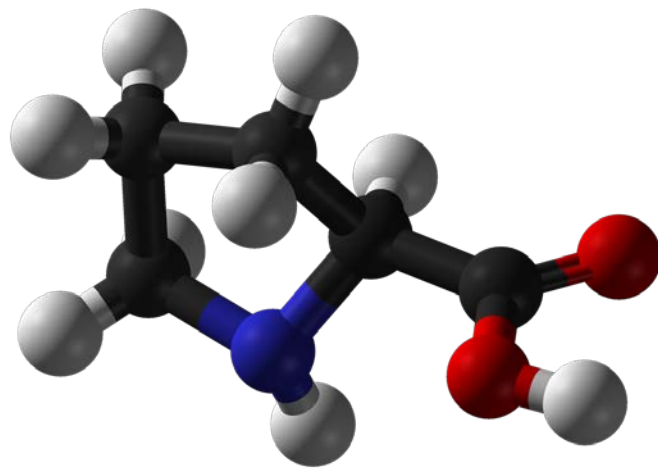
- Not all H atoms are shown in these depictions.

胺基酸對二級結構靈活性（flexibility）的影響

- 哪一個胺基酸會讓 ϕ 與 ψ 角轉動最靈活？
- 哪一個胺基酸會限制住兩旁的 ϕ 與 ψ 角範圍？
- 這兩個胺基酸適合作為 α -螺旋中的胺基酸嗎？

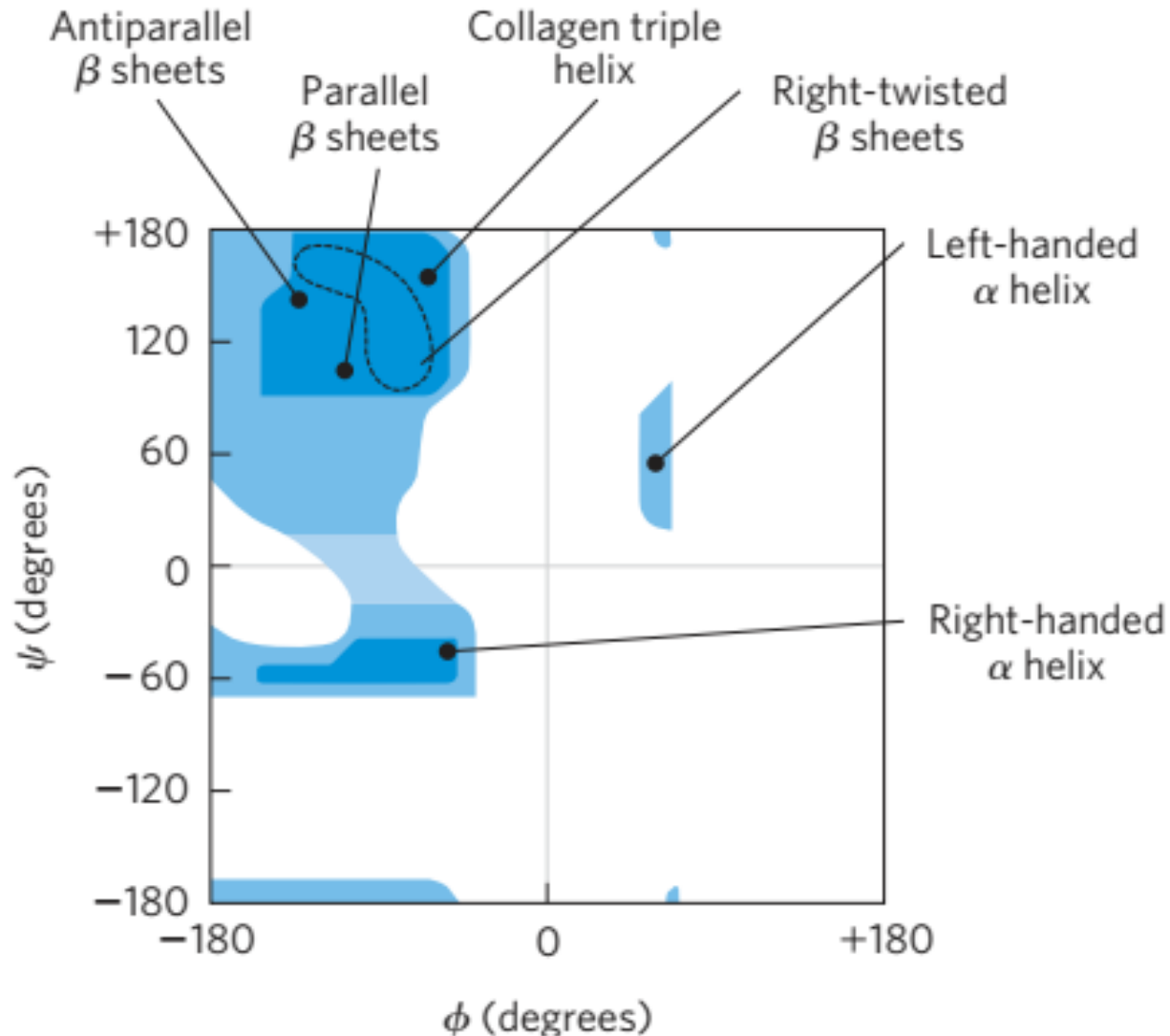


甘胺酸
Glycine



脯胺酸
Proline

蛋白質二級結構具有固定範圍的 ϕ 與 ψ 角



蛋白質的三、四級結構

- 3) 三級結構：（單一多肽的）蛋白質整體結構。
- 具有二級結構的多肽因**胺基酸側鏈間交互作用**而摺疊扭轉成特有的立體形狀。
- 4) 四級結構：有些具有生物功能的蛋白質是由**兩個以上**的多肽（次單元）所組成，這種由次單元組成的蛋白質結構稱之。

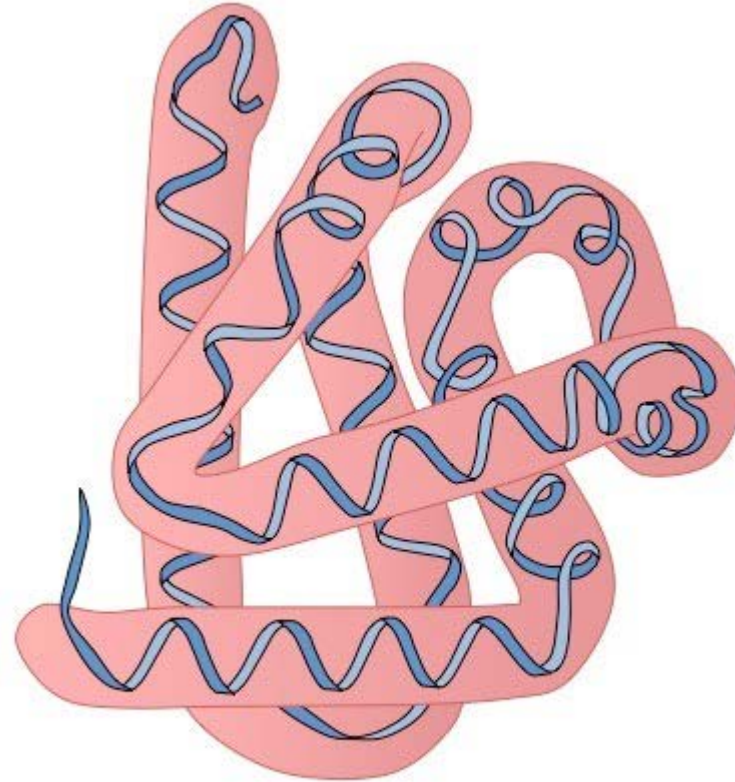
蛋白質三級結構的兩大分類



Fibrous Protein

纖維蛋白

例如：膠原蛋白、肌凝蛋白、肌動蛋白、角蛋白等。



Globular Protein

球形蛋白

例如：酵素、血紅蛋白、胰島素、免疫球蛋白等。

介於蛋白質二級與三級結構中的 模體與結構域

- 模體(motif, fold)為兩個以上的蛋白質二級結構相連所形成的摺疊模式。
- 結構域(domain)為構成蛋白質中結構單元，具有獨立的摺疊模式，常具有特定的功能。

Motifs 模體

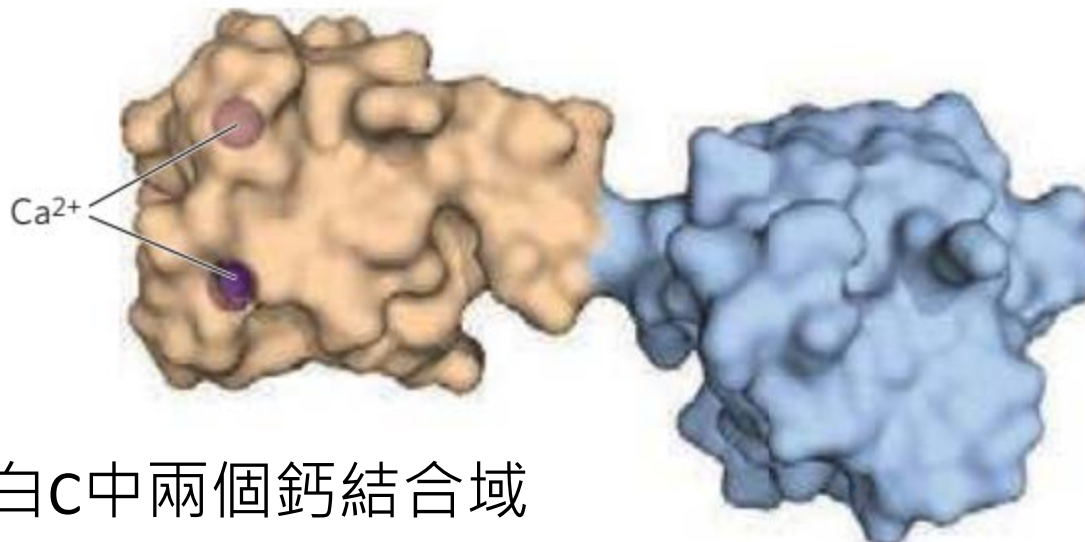


(a) β - α - β Loop



(b) β Barrel

Structural domains 結構域



肌鈣蛋白c中兩個鈣結合域

如何維持蛋白質結構？

- 維持蛋白質結構的作用力

- 共價鍵結

- ✓ 例如一級結構中的肽鍵與三、四級結構中的雙硫鍵。

- 非共價作用力

- ✓ 例如二、三、四級結構中的氫鍵、離子鍵、凡得瓦力與疏水作用。

- ✓ 為弱的作用力，因此大部分蛋白質只能在溫和的環境（溫度、pH值）中發揮功能。

“A native protein conformation is only marginally stable.”

- 蛋白質的變性(denaturation)

- 蛋白質維持結構的作用力受破壞，失去特有的結構與生物功能。

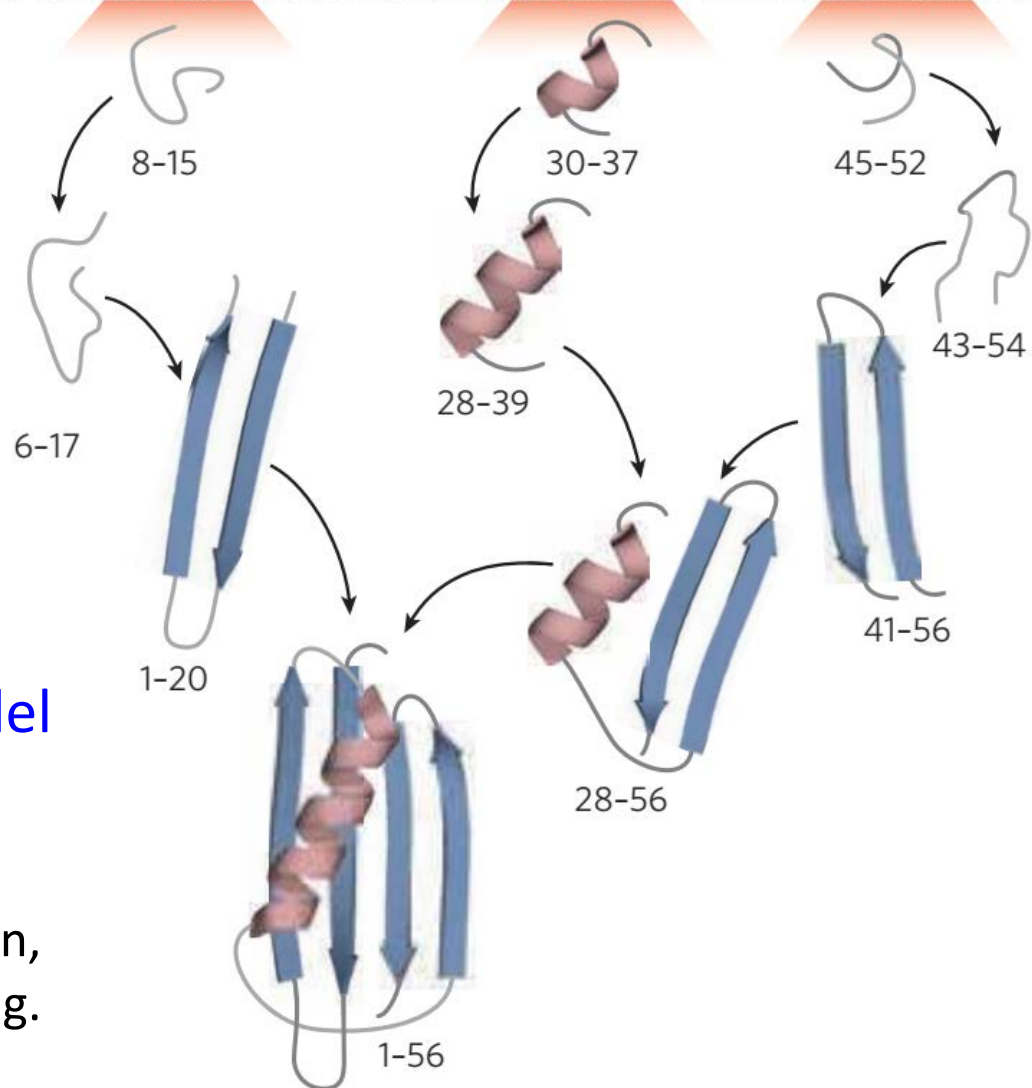
蛋白質的摺疊途徑

- 分層式途徑

二級結構 → 模體 →
結構域 → 三級結構

Amino acid sequence of a 56-residue peptide

MTYKLIL**NGKTLKG**ETTTEAVDAATAEKV**FKQYANDN**GV DGEWT**YDDATKT**FTVTE



Nucleation-condensation model

A hierarchical pathway is shown,
based on computer modeling.

與蛋白質摺疊缺失有關的疾病

- 蛋白質構形病(proteopathy)
 - 為與蛋白質摺疊缺失有關的疾病的統稱。

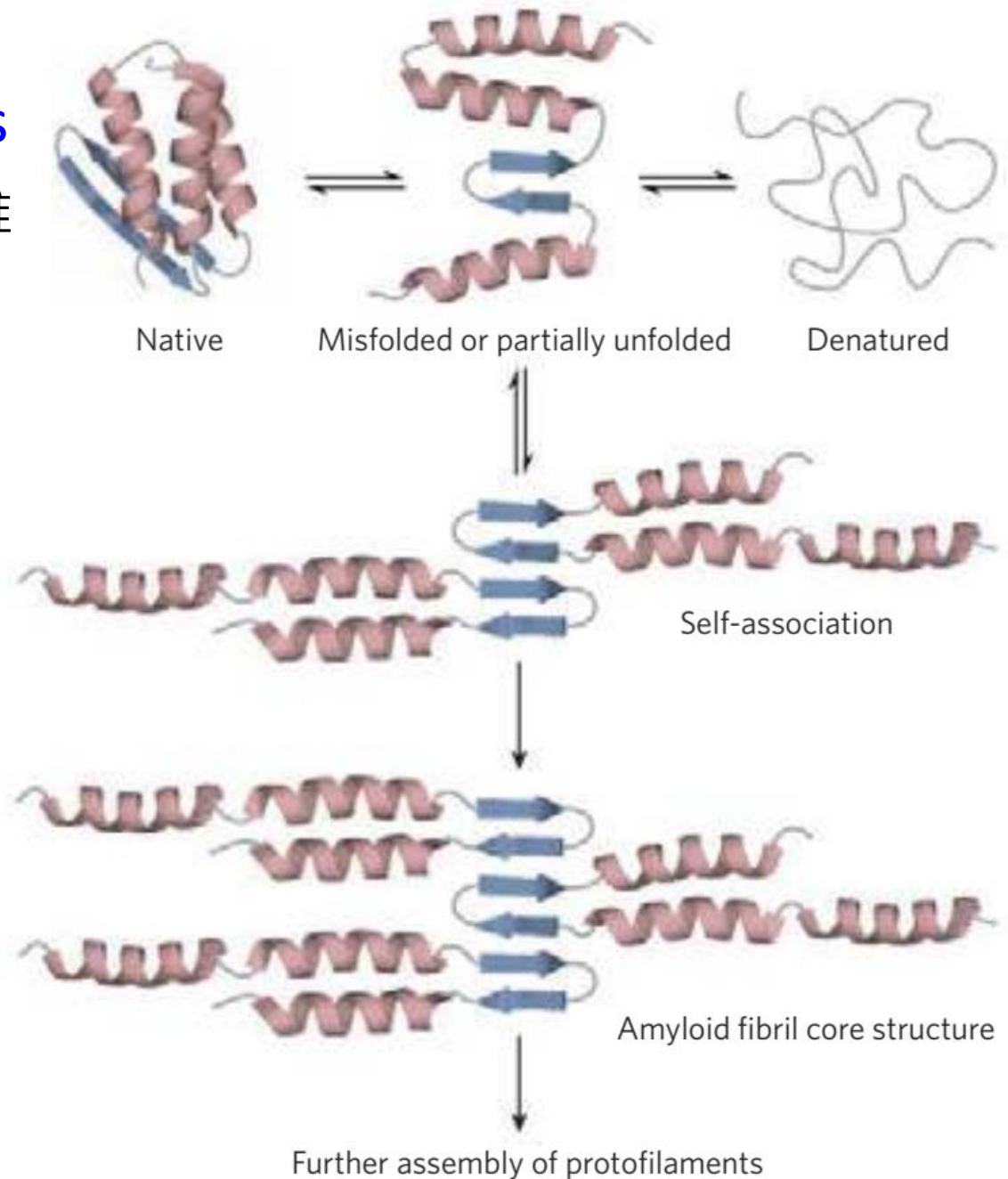
https://en.wikipedia.org/wiki/Proteopathy#List_of_proteopathies

1. 類澱粉蛋白變性症(amyloidosis)

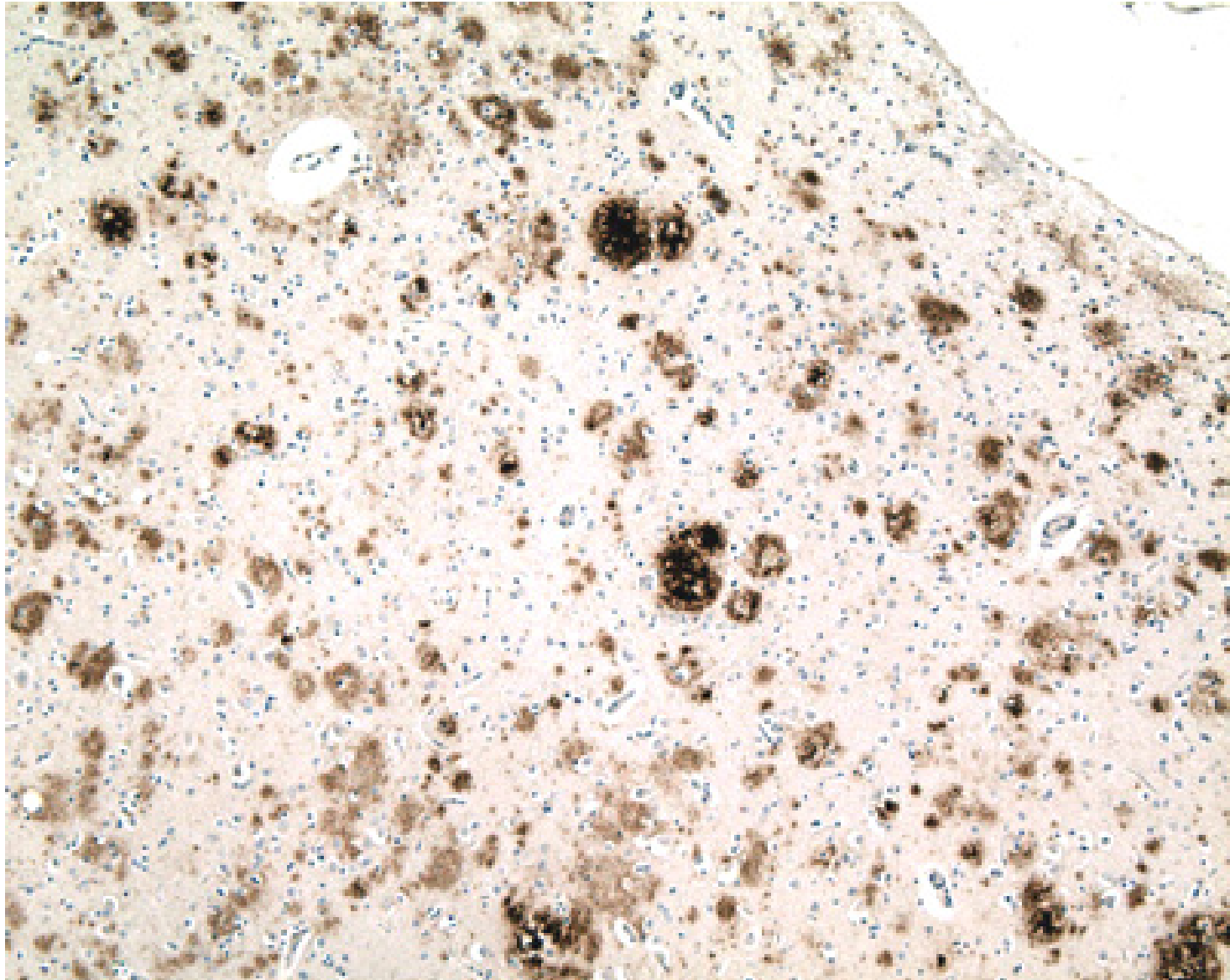
- 原本由細胞分泌出的可溶性蛋白因為摺疊缺失使得這些蛋白質轉換成不可溶的類澱粉蛋白纖維(amyloid fiber)。
- 如阿茲海默症、帕金森氏症等都與這一類的蛋白質摺疊缺失有關。

Formation of disease-causing amyloid fibrils

類澱粉蛋白纖維



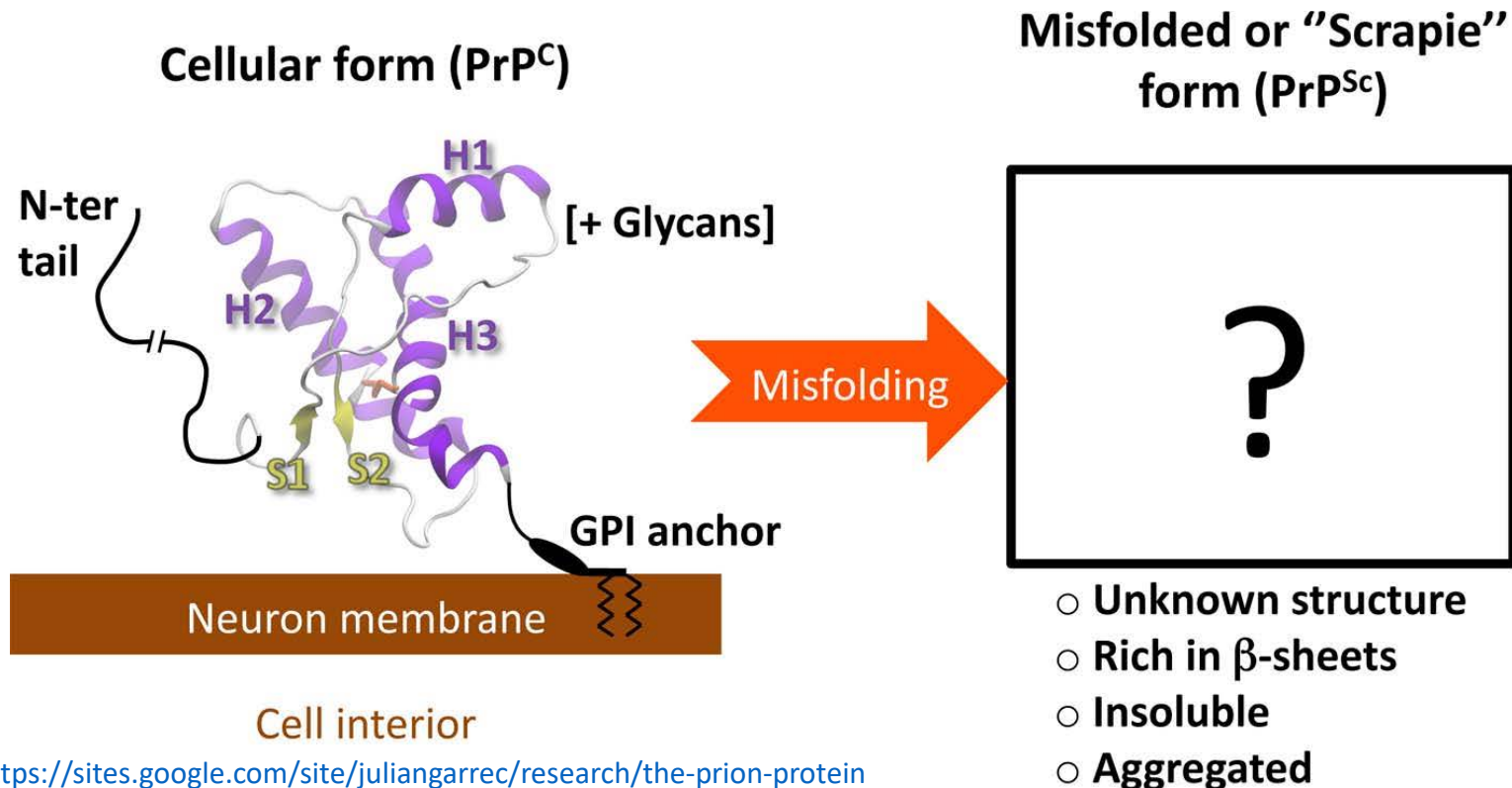
- 阿茲海默症病人大腦皮質的切片，棕色部份為 β 類澱粉蛋白質 (amyloid beta) 。



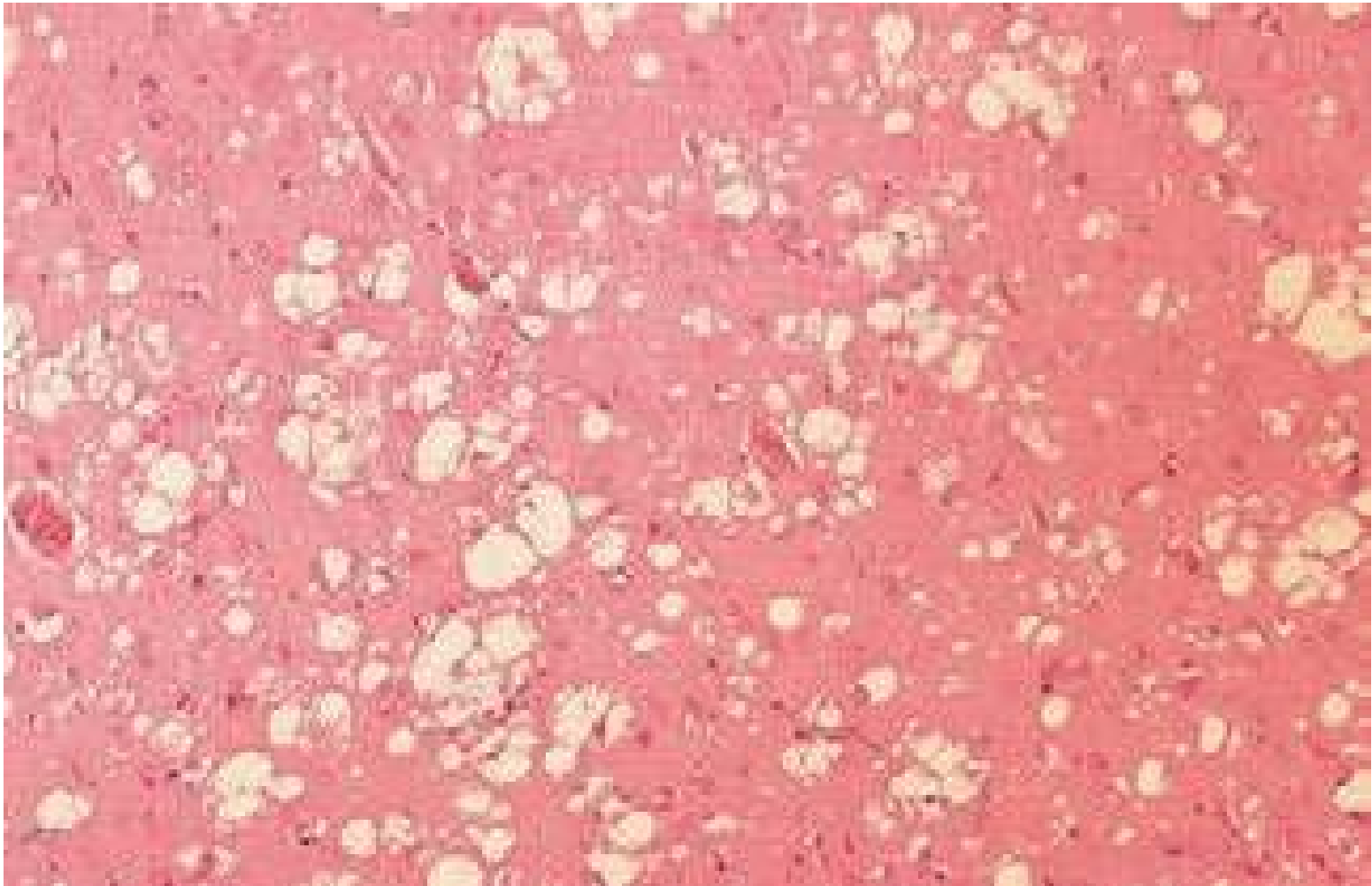
2. 普粒子疾病(Prion disease)

又稱傳染性海綿狀腦病(transmissible spongiform encephalopathies, TSEs)

- 普粒子(prion: proteinaceous infectious particle)
 - 正常細胞的普粒子(PrP^{C})接受外來不正常的普粒子(PrP^{Sc})會誘導成 PrP^{Sc} 。
 - 綿羊「搔癢症」、「狂牛症」、人類庫賈氏病(Creutzfeldt-Jakob disease, CJD)

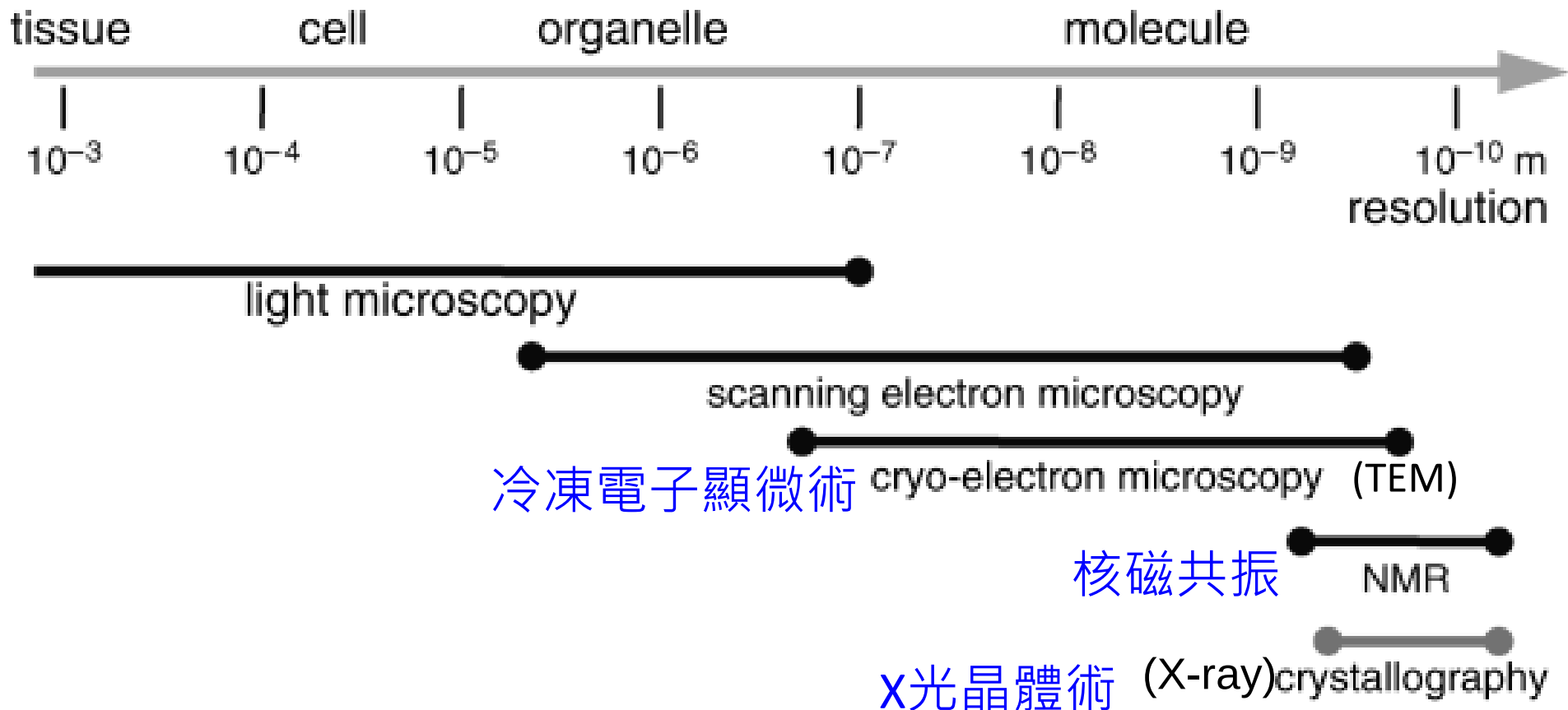


Stained section of cerebral cortex from autopsy of a patient with Creutzfeldt-Jakob disease shows spongiform (vacuolar) degeneration, the most characteristic neurohistological feature.



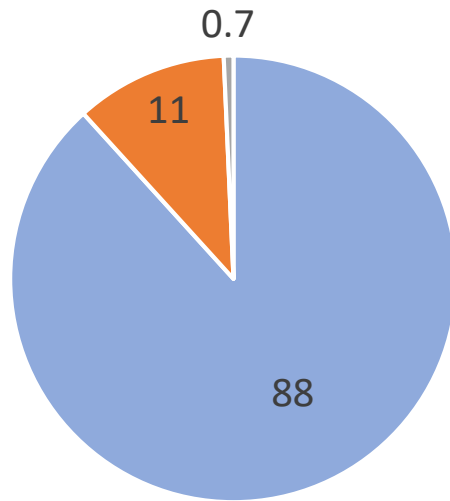
蛋白質結構如何分析？

- Resolution range of the different methods for structure determination of biological macromolecules



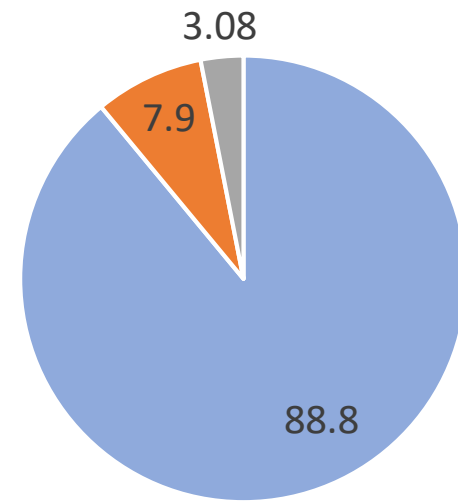
不同分析方法得出的蛋白質結構在蛋白質結構資料庫(Protein Data Bank, PDB)所佔比例

2014 July



- X-ray
- Nuclear Magnetic Resonance
- Electron Microscopy

2020 May

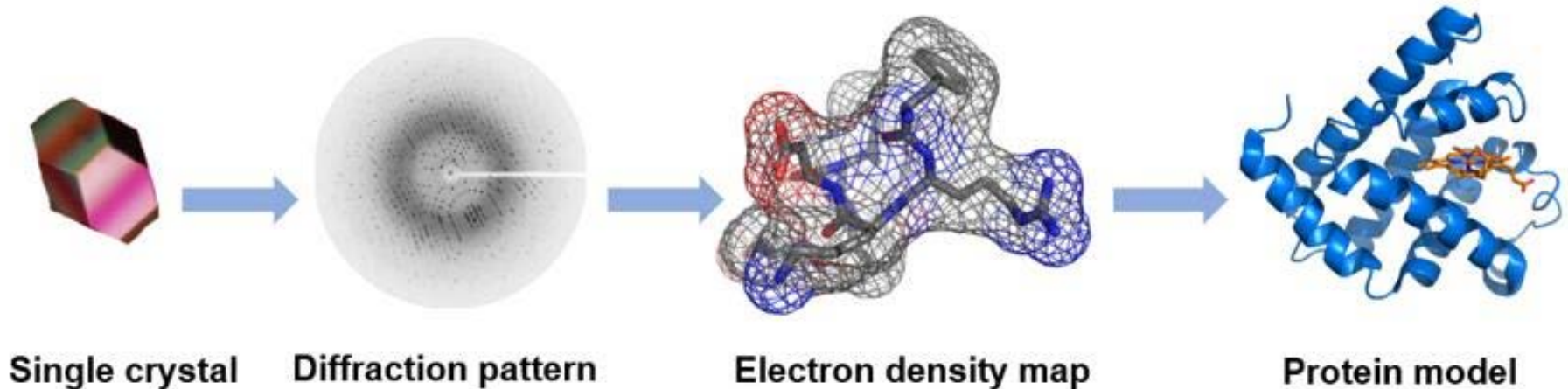


- X-ray
- Nuclear Magnetic Resonance
- Electron Microscopy

Click <https://www.rcsb.org/stats/summary> for the latest stats.

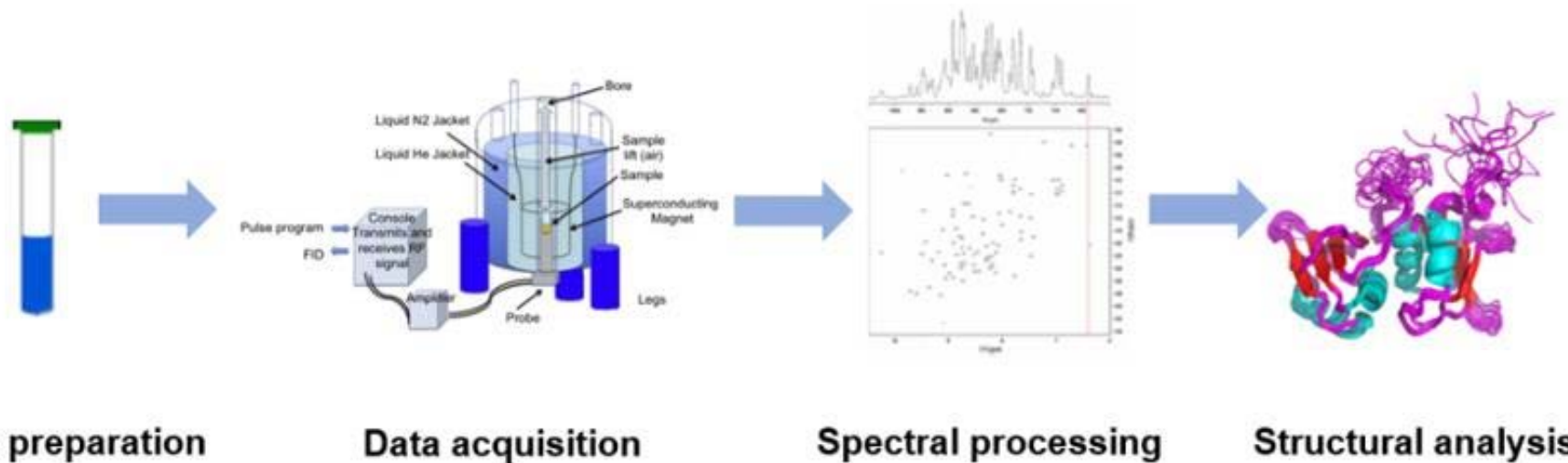
(1) X光晶體學(X-ray crystallography)

- 利用X光決定蛋白質結晶中原子的排列位置。
- 單晶體X光繞射(single crystal X-ray diffraction)四步驟：
 1. 蛋白質結晶：trial-and-error。
 2. 利用X光照射以不同角度照射結晶形成繞射圖。
 3. 分析繞射圖中單位晶胞(unit cell)的電子密度分佈(electron density distribution)。
 4. 利用電子密度推估原子排列方式。



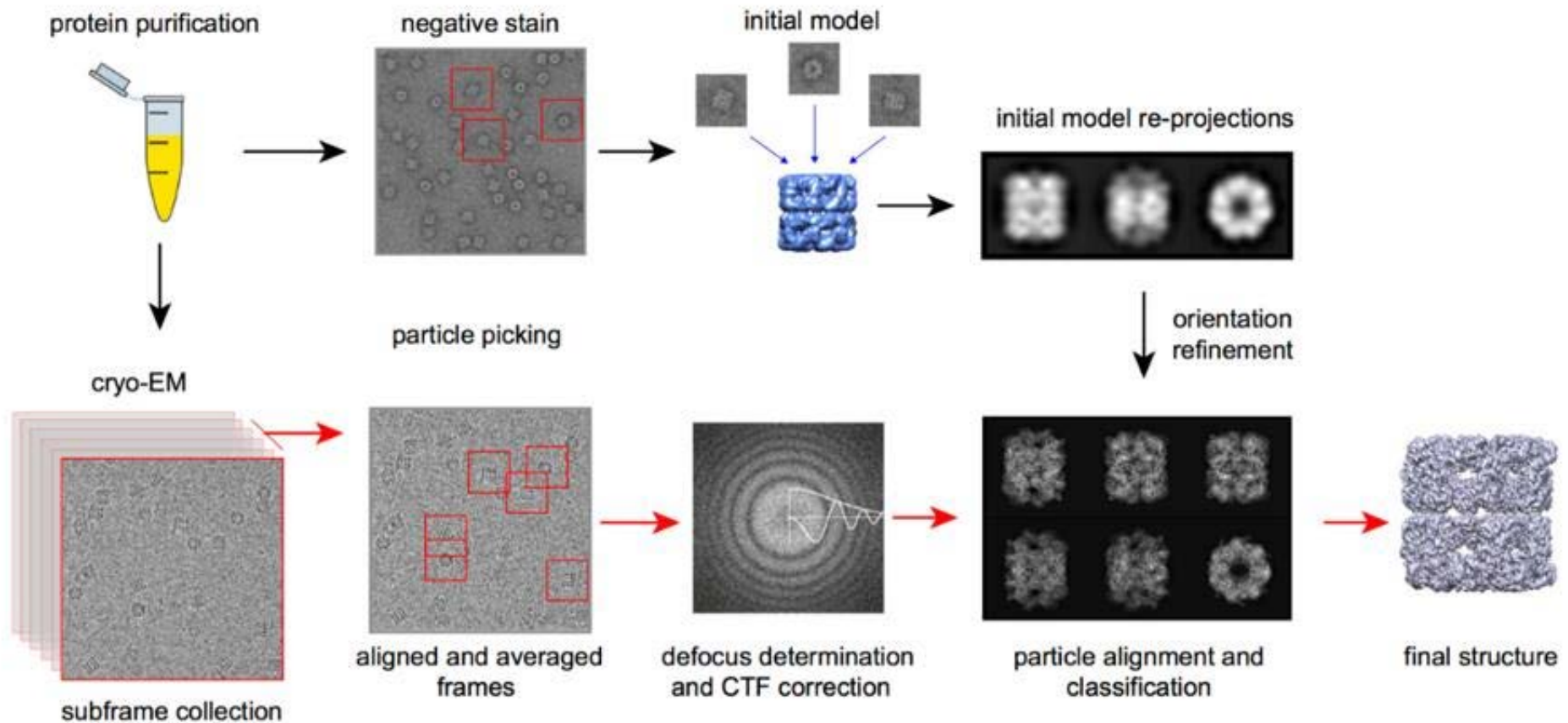
(2)核磁共振 (nuclear magnetic resonance, NMR)

- 原子因周圍的電子雲環境造成共振頻率改變產生核磁共振的的差異，稱為化學位移，因此，藉由不同原子的共振譜可以得知蛋白質胺基酸之間的關係。

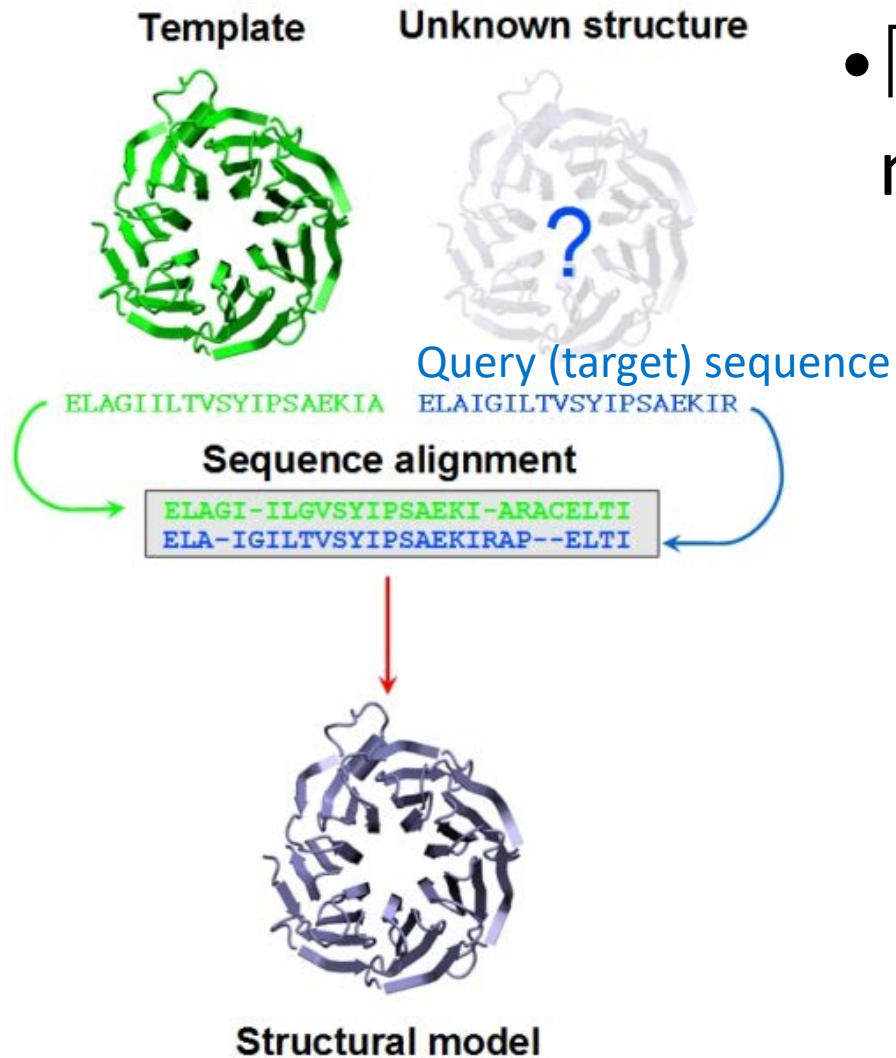


(3) 冷凍電子顯微術 (cryo-electron microscopy)

- 單顆粒分析(single particle analysis)



電腦輔助蛋白質結構預測



- 同源建模(homology modelling)

- 利用胺基酸序列相似(具備> 35%胺基酸序列一致性)且已經解析的蛋白質作為模板(template)，再經由電腦運算的方式得出未知蛋白質結構模型。

延伸閱讀：

<https://www.unil.ch/pmf/en/home/menus/technologies/homology-modeling.html>

https://proteopedia.org/wiki/index.php/Practical_Guide_to_Homology_Modeling

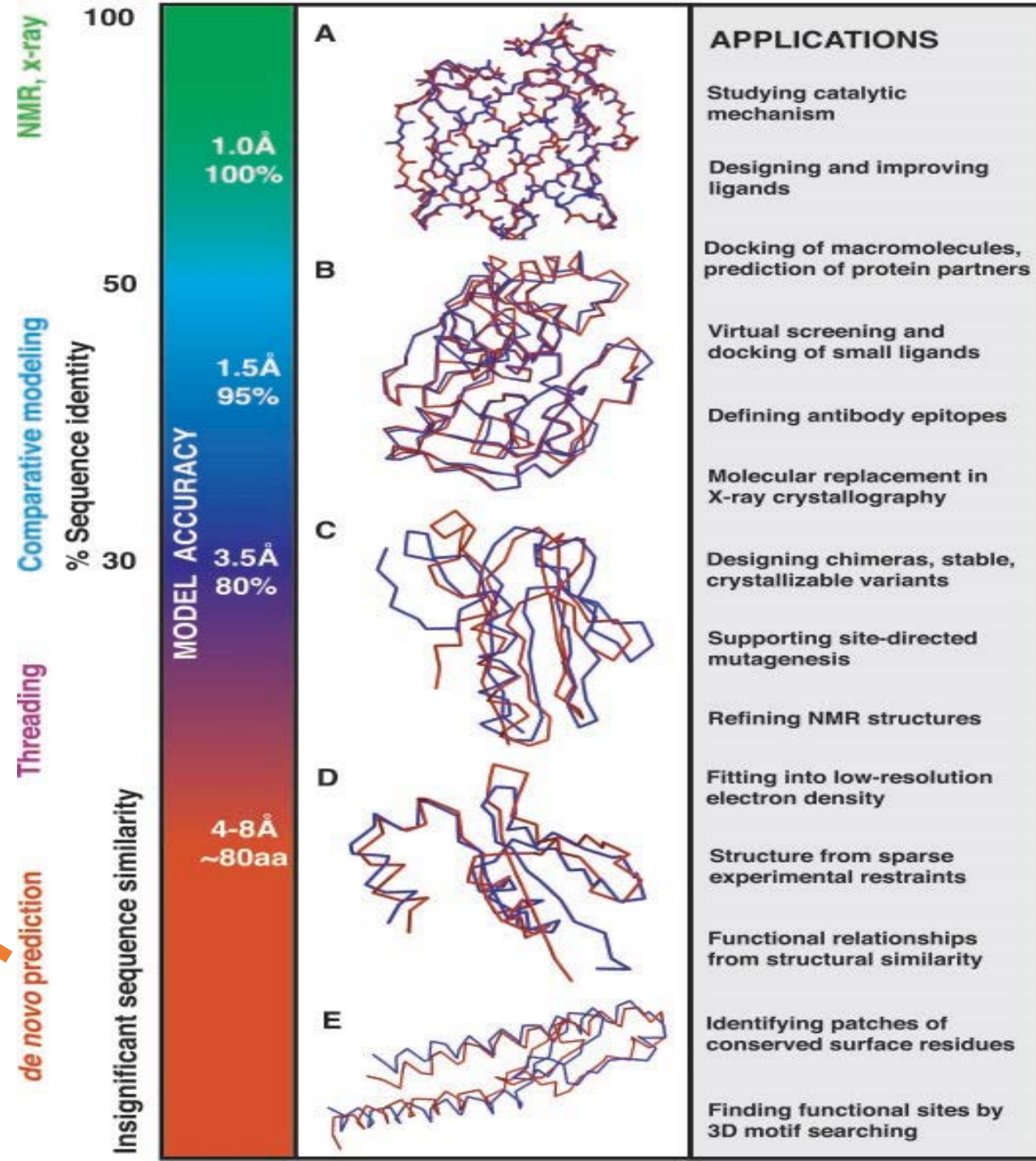
- Accuracy and application of protein structure models.

紅色：預測結構；
藍色：真實結構

同源建模 ←

從頭開始結構預測
(如：AlphaFold)

Baker, D. (2001). Protein Structure Prediction and Structural Genomics. Science, 294(5540), 93–96.

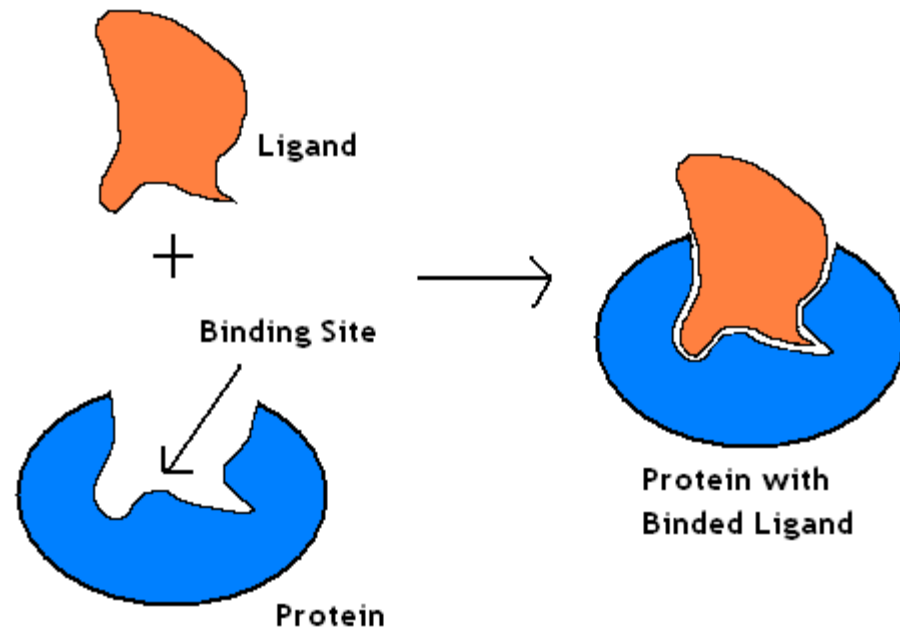


蛋白質的功能

- 蛋白質立體結構與功能的關係
 - 蛋白質在活體中為動態分子。
 - 蛋白質的功能與其蛋白質跟其他分子（配體 ligand）進行交互作用產生構形改變 (conformational change) 息息相關。
 - 進行交互作用的分子種類。
 - 構形改變後造成的影響：訊息傳遞、化學反應、分子移動、通道開闢...等。

配體與結合位點

- 配體、配位基(ligand)
 - 能與蛋白質產生可逆(reversibly)結合的分子。
 - 可以是任何分子，包含其他的蛋白質。
- 結合位點(binding site)：
 - 蛋白質上與配體相結合的位點。
 - 大小、形狀、電荷、疏水/親水性互補。
 - 專一性(specificity)：蛋白質可以從各種分子中選擇性結合單一或少數分子。



不同蛋白質-配體交互作用機制

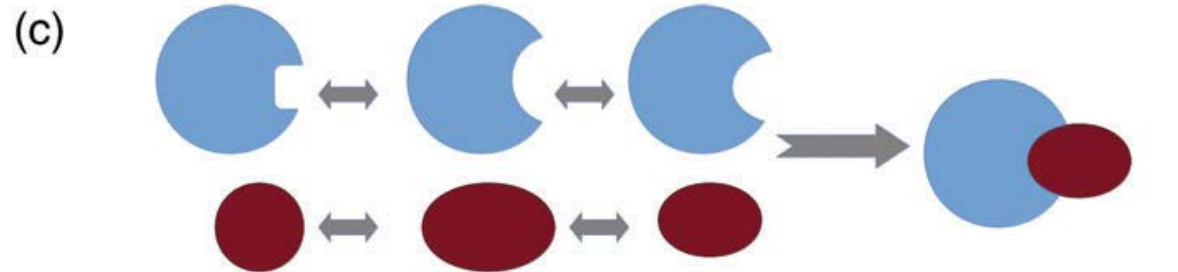
鎖與鑰
(lock-key)



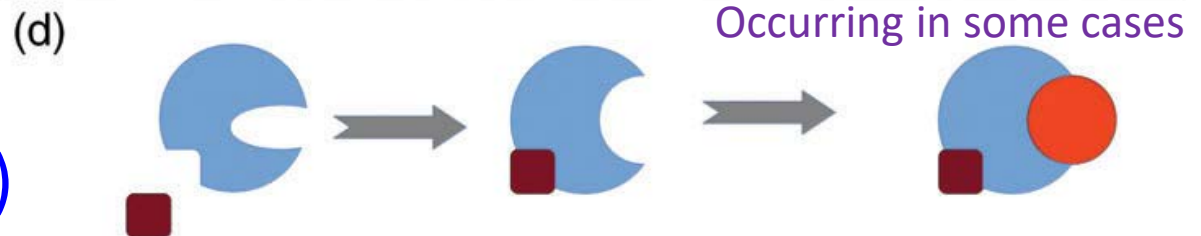
誘導配合
(induced-fit)



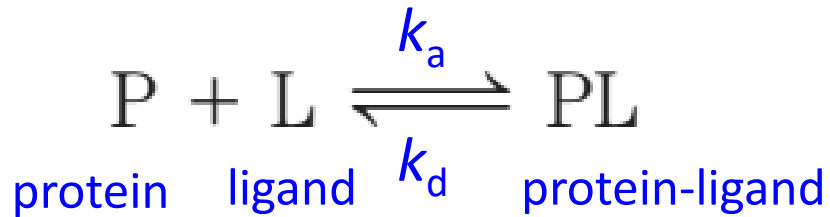
構形選擇
(conformational selection)



異位模式
(allosteric model)



蛋白質與配體結合的親和力(affinity)

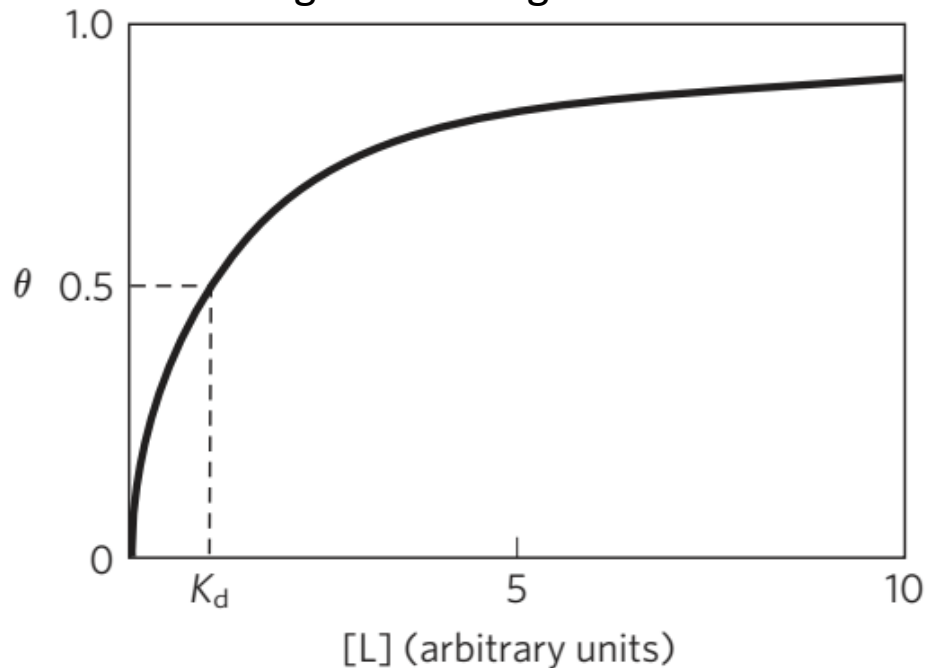


$$K_d = \frac{[\text{P}][\text{L}]}{[\text{PL}]} = \frac{k_d}{k_a}$$

↓
dissociation constant 解離常數

$$\theta = \frac{\text{binding sites occupied}}{\text{total binding sites}} = \frac{[\text{PL}]}{[\text{PL}] + [\text{P}]} = \frac{[\text{L}]}{[\text{L}] + K_d}$$

Ligand-binding curve



Oxygen-binding curve

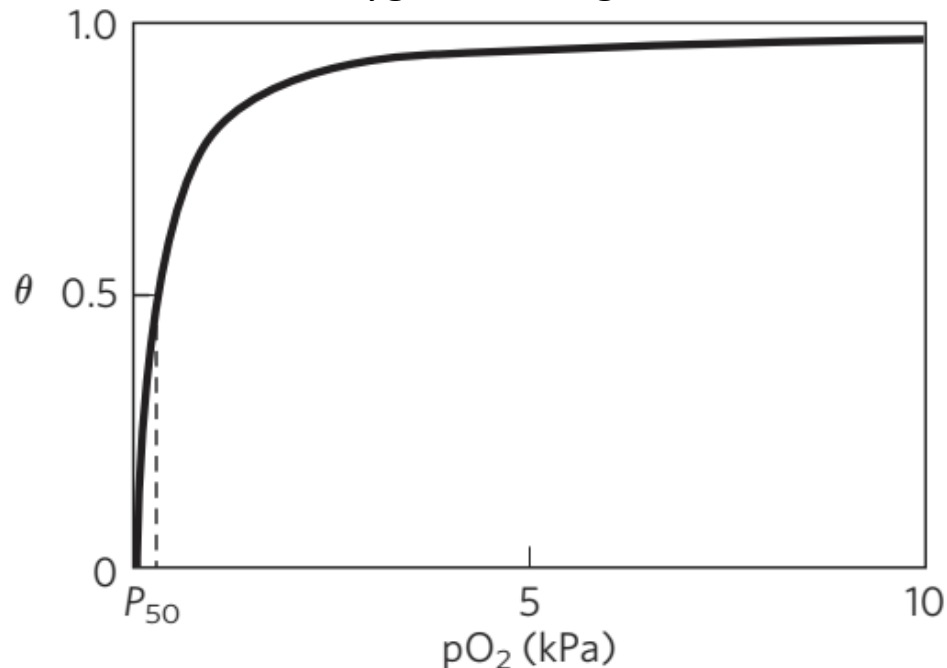
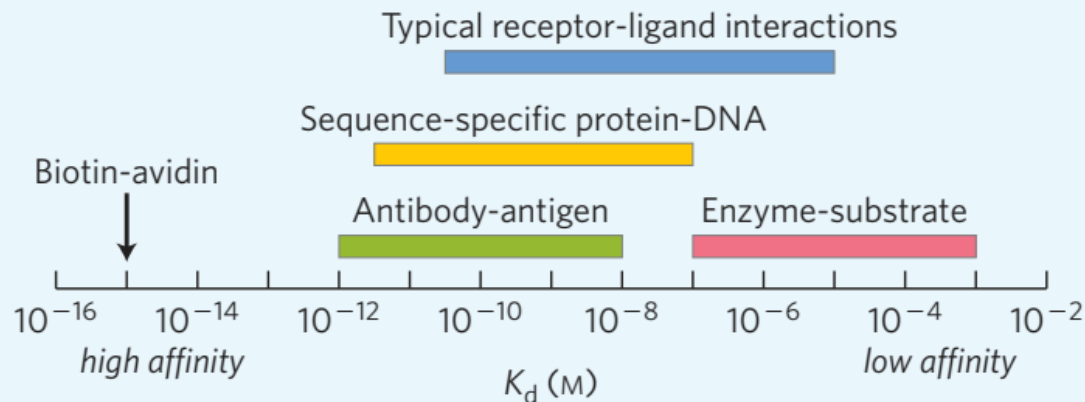


TABLE 5-1 Some Protein Dissociation Constants

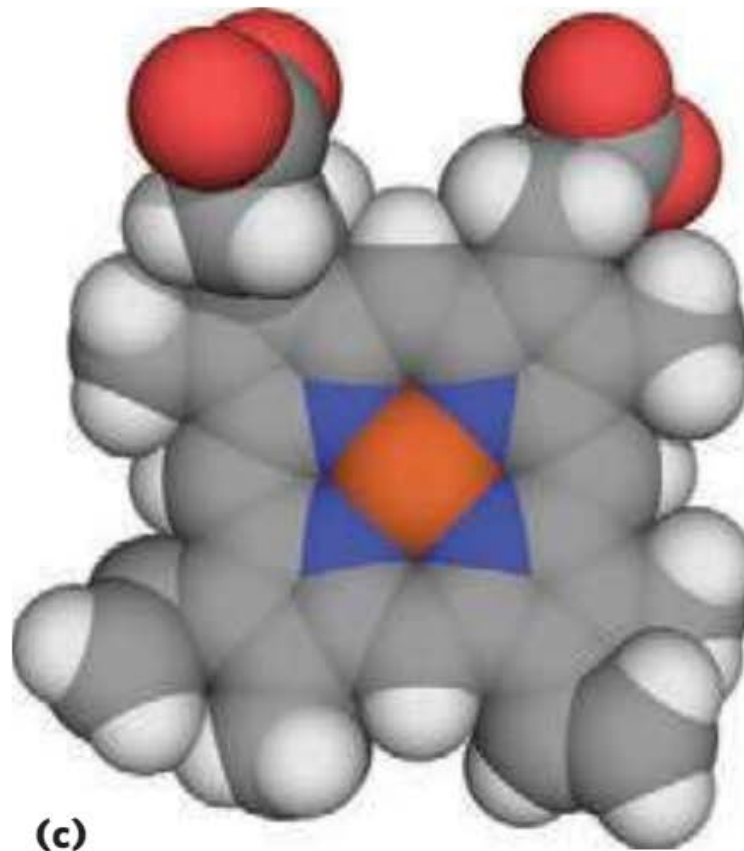
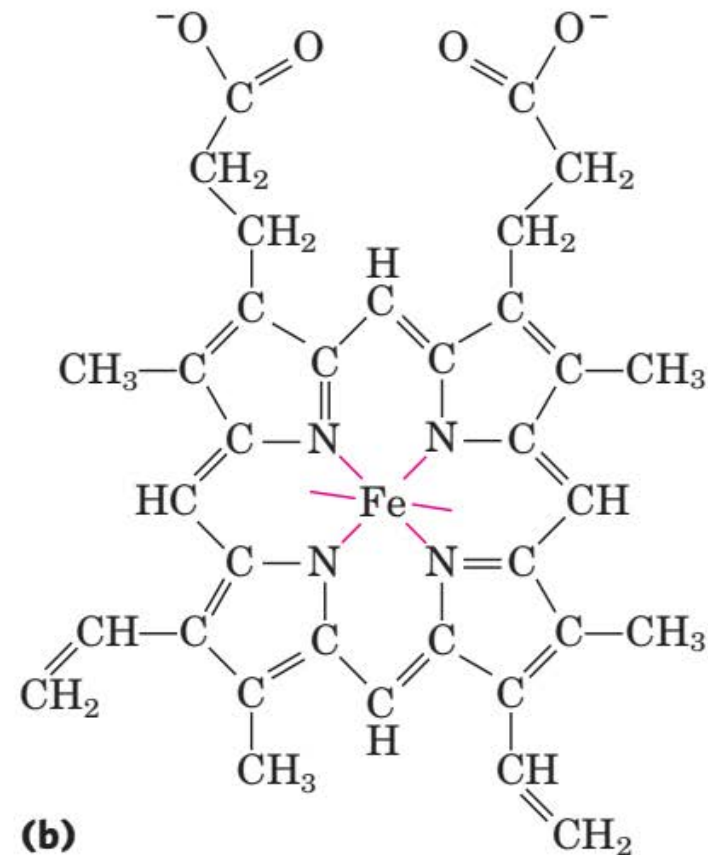
Protein	Ligand	K_d (M)*
Avidin (egg white) 親和素	Biotin 生物素	1×10^{-15}
Insulin receptor (human)	Insulin 胰島素	1×10^{-10}
Anti-HIV immunoglobulin (human) [†]	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (<i>E. coli</i>)	Ni^{2+}	1×10^{-7}
Calmodulin (rat) [‡]	Ca^{2+}	3×10^{-6}
		2×10^{-5}



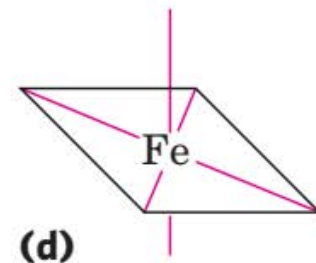
Color bars indicate the range of dissociation constants typical of various classes of interactions in biological systems. A few interactions, such as that between the protein avidin and the enzyme cofactor biotin, fall outside the normal ranges. The avidin-biotin interaction is so tight it may be considered irreversible. Sequence-specific protein-DNA interactions reflect proteins that bind to a particular sequence of nucleotides in DNA, as opposed to general binding to any DNA site.

氧結合蛋白：肌紅蛋白與血紅蛋白

- 血基質(heme)：與氧氣結合的輔基
(prosthetic group)

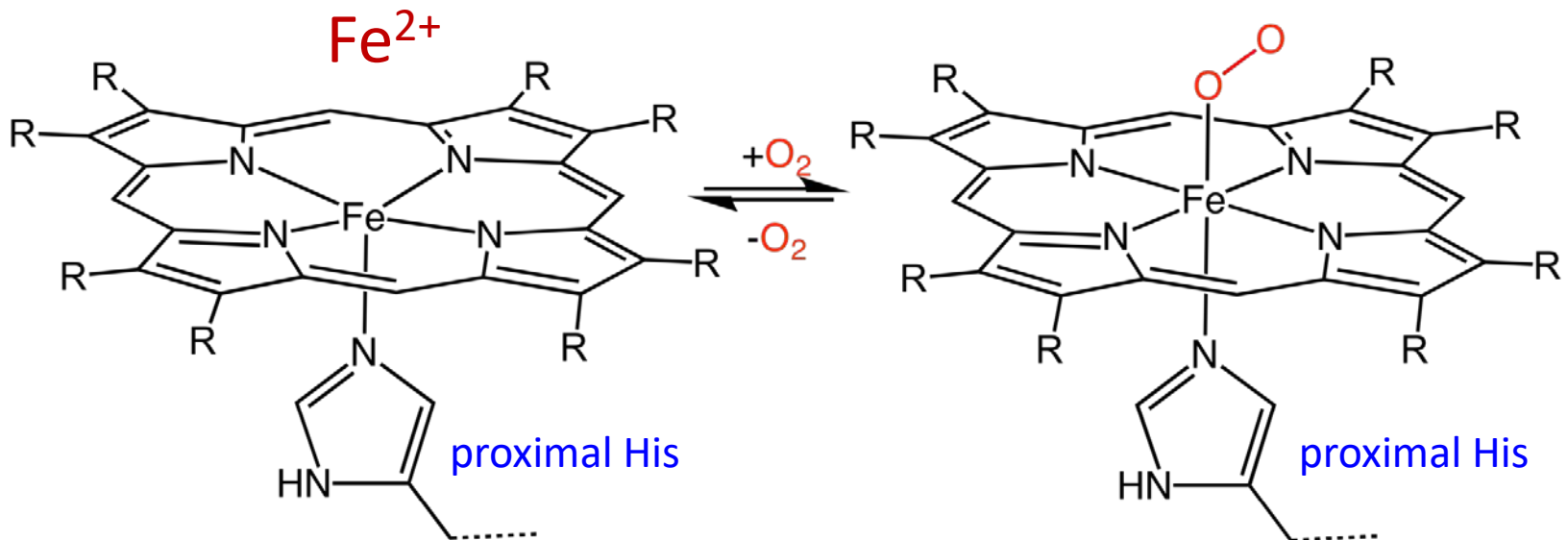
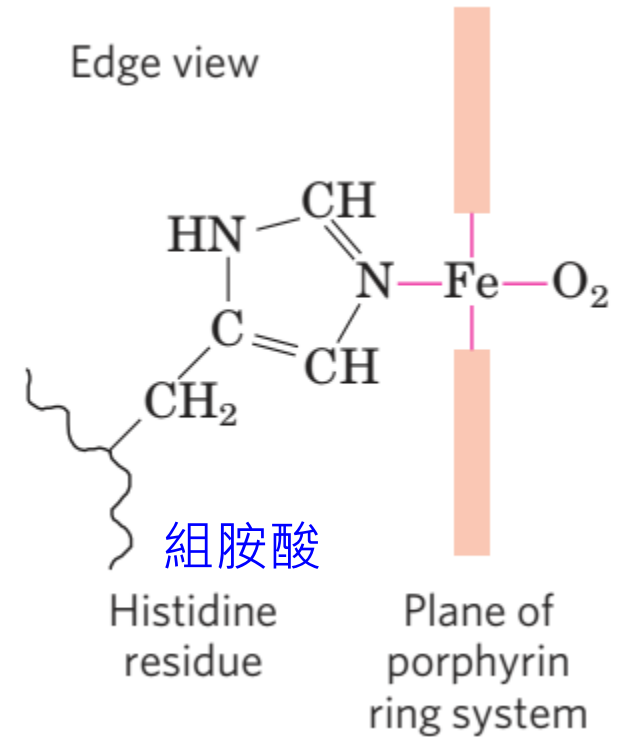


鐵原子具備有
六個配位鍵



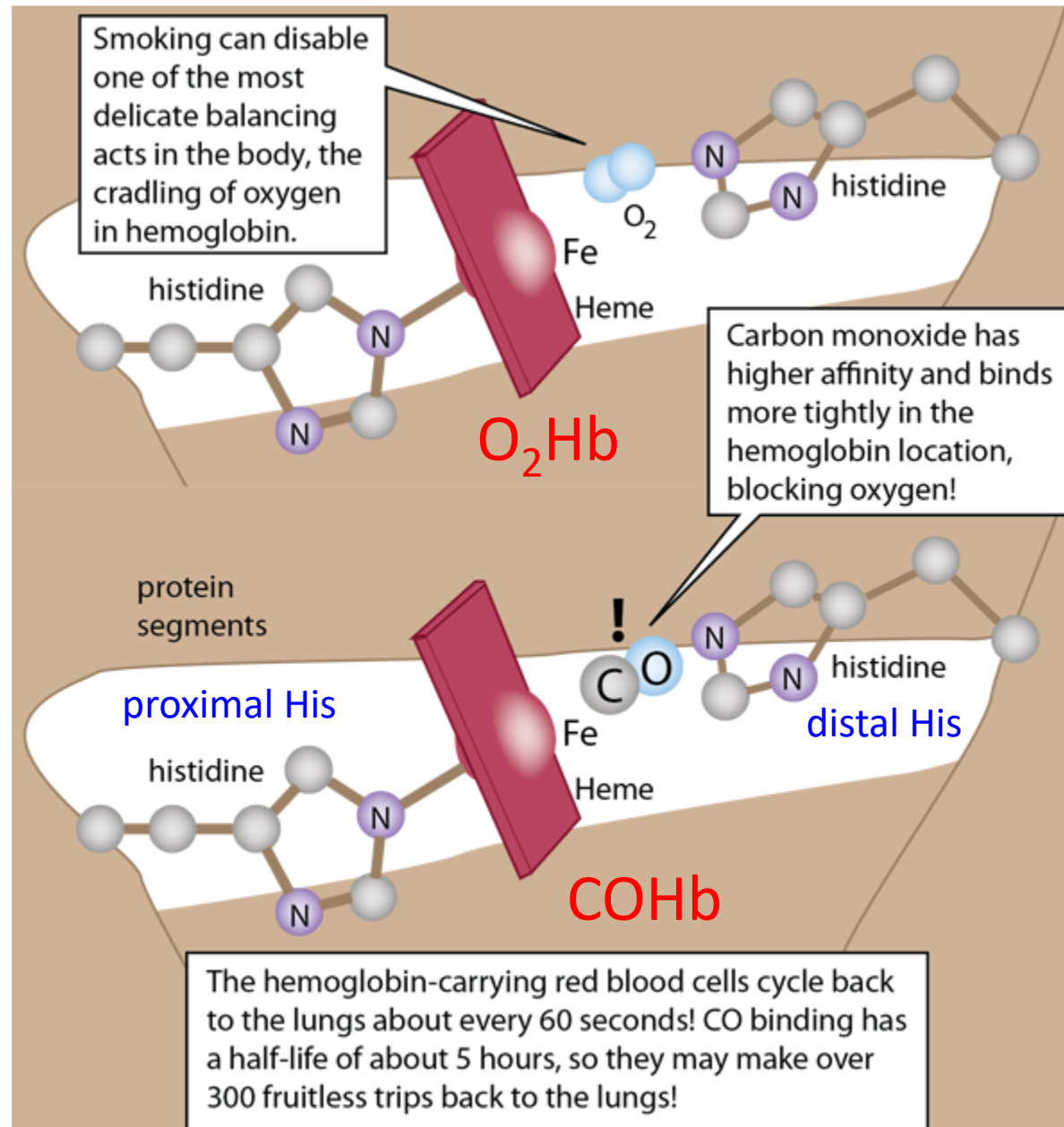
鐵離子與氧分子結合

- 由於氧結合蛋白的結構，鐵離子只有一個配位鍵會接上氧 → 可保持鐵離子維持在二價(Fe^{2+})。
- 深紫 (缺氧) → 亮紅 (含氧)



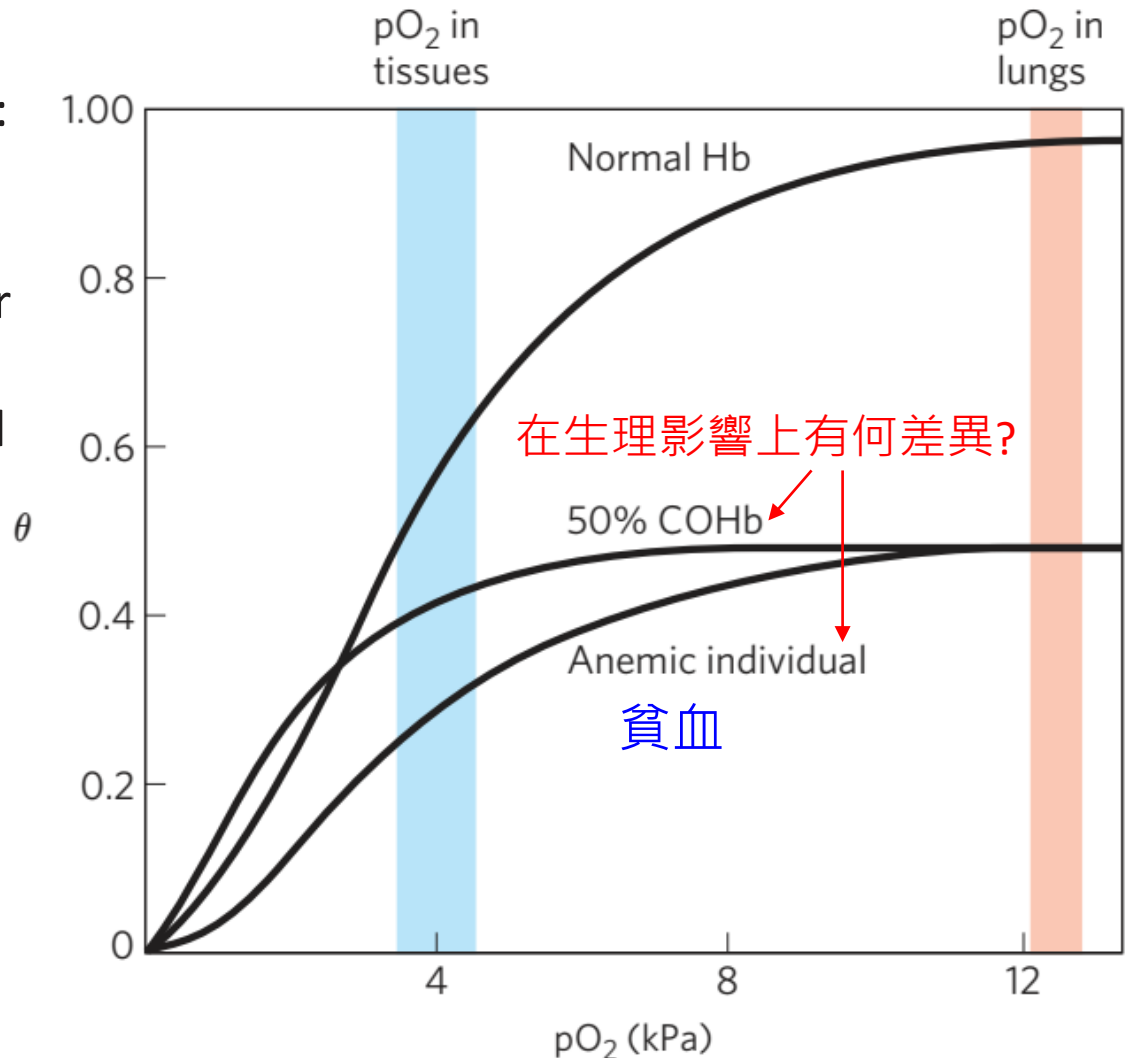
其他小分子也會與血基質結合

一氧化碳(CO)、
一氧化氮(NO)
等...



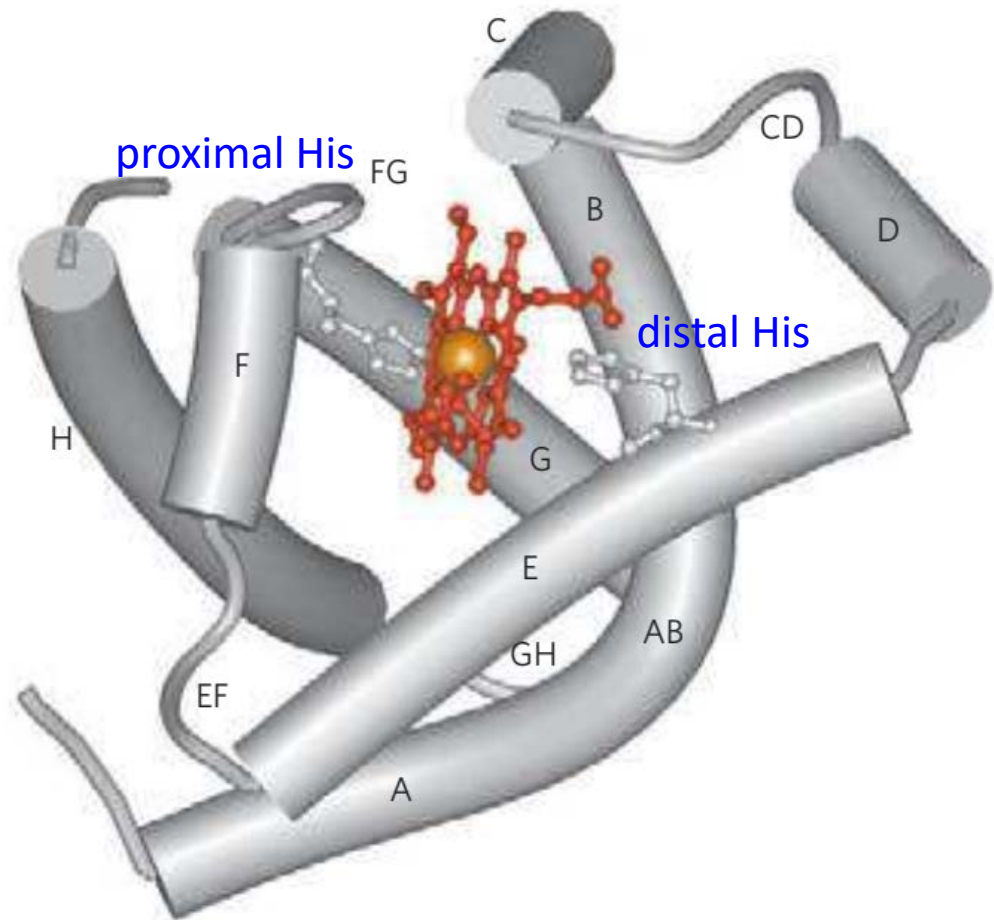
CO與血紅蛋白結合造成身體缺氧 (oxygen deprivation)

Several oxygen-binding curves:
for normal hemoglobin,
hemoglobin from an anemic
individual with only 50% of her
hemoglobin functional, and
hemoglobin from an individual
with 50% of his hemoglobin
subunits complexed with CO.
The pO_2 in human lungs
and tissues is indicated.



肌紅蛋白有單一結合位點與氧結合

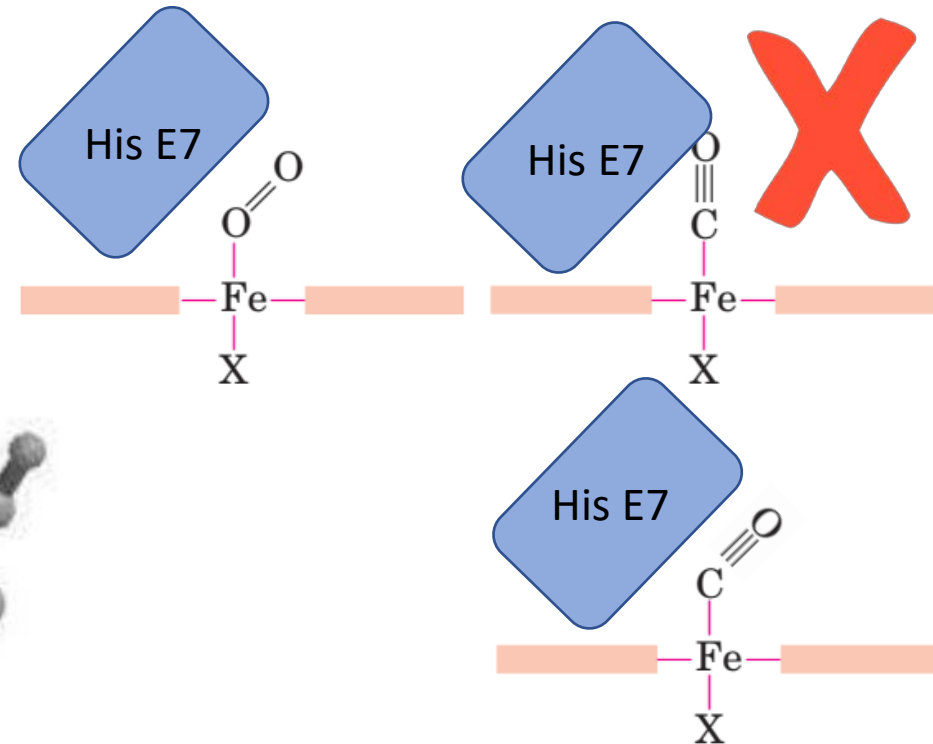
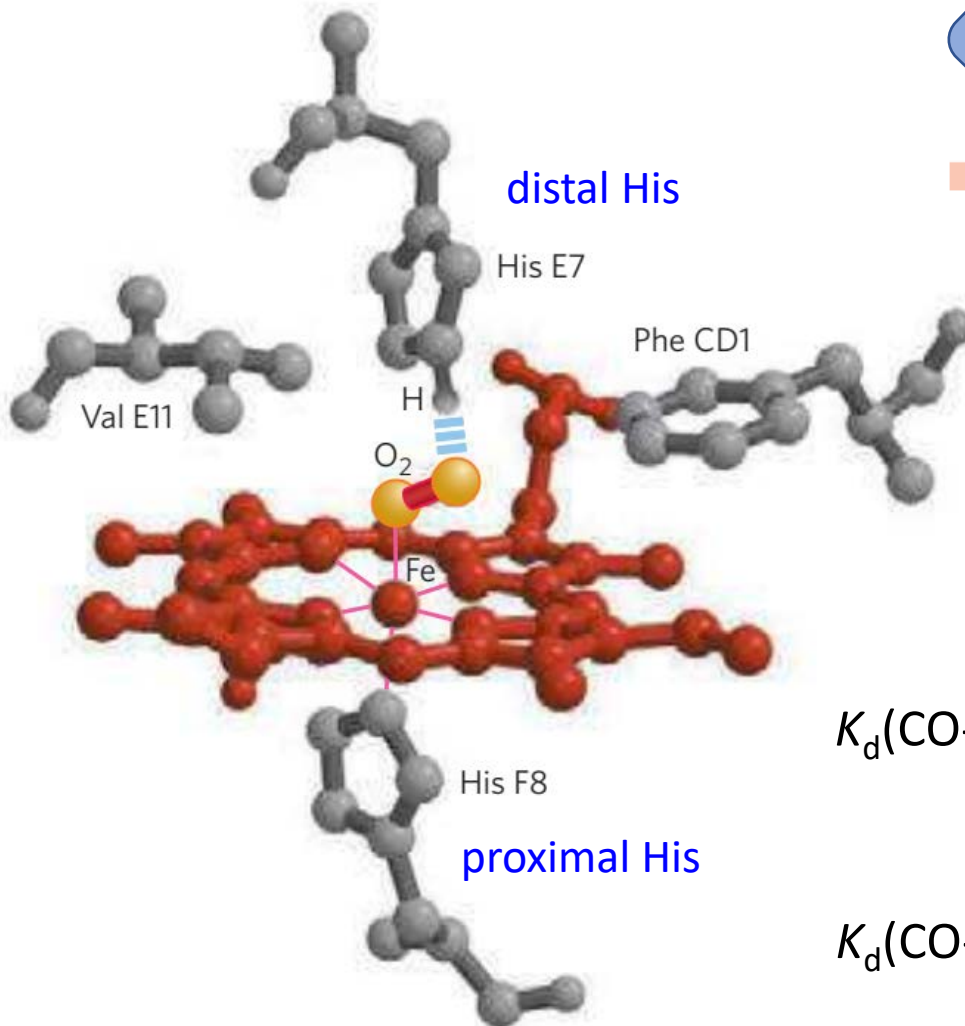
- 肌紅蛋白 (myoglobin, Mb)
 - 單一多肽組成
 - 共有153個胺基酸



(PDB ID 1MBO) The eight α -helical segments are labeled A through H. Nonhelical residues in the bends that connect them are labeled AB, CD, EF, and so forth.

蛋白質結構影響配體結合的親和力

- 立體效應(steric effect)



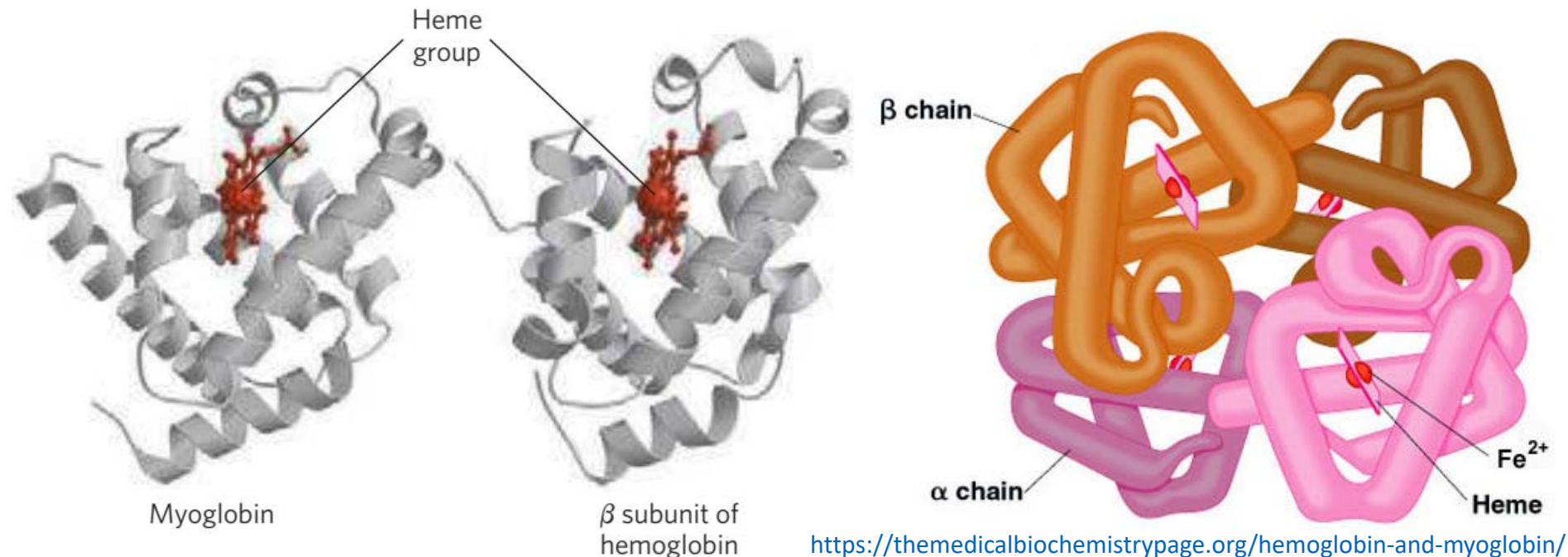
$$K_d(\text{CO-free heme}) \approx 1/20,000 \times K_d(\text{O}_2\text{-free heme})$$

↓ Steric effect of distal His
→ Reduction in CO binding

$$K_d(\text{CO-myoglobin}) \approx 1/200 \times K_d(\text{O}_2\text{-myoglobin})$$

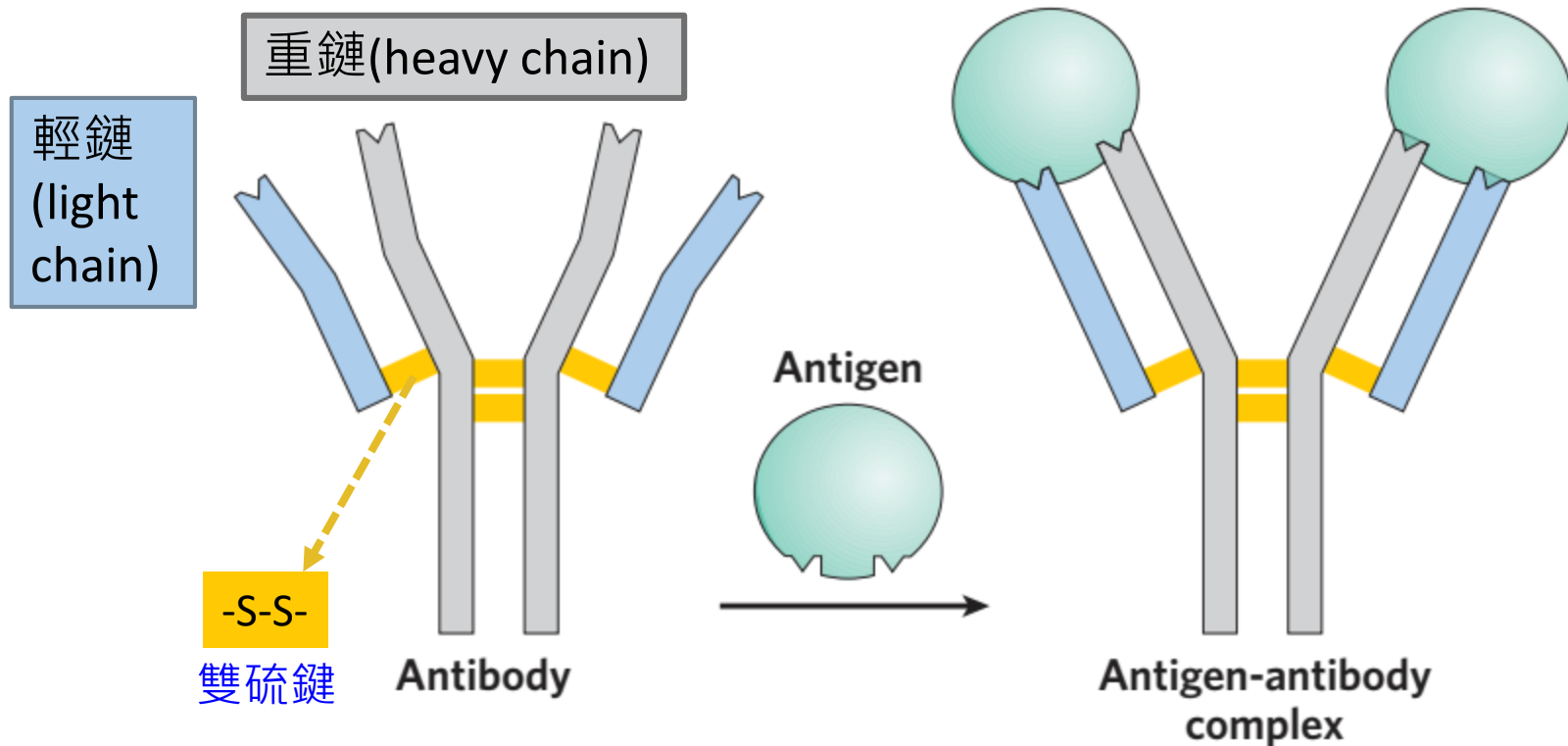
血紅蛋白次單元與肌紅蛋白結構相似

- 血紅蛋白(hemoglobin)
 - 成人血紅蛋白由 α 與 β 兩種球蛋白(globin)組成，兩種多肽50%以上胺基酸不同，但是結構卻很相似。
 - 四級結構由兩個 α 鏈與與兩個 β 鏈所組成。



免疫球蛋白：互補交互作用

- 免疫球蛋白(immunoglobulin)又稱為抗體(antibody)
- 免疫球蛋白G(IgG)與抗原(antigen)的結合：

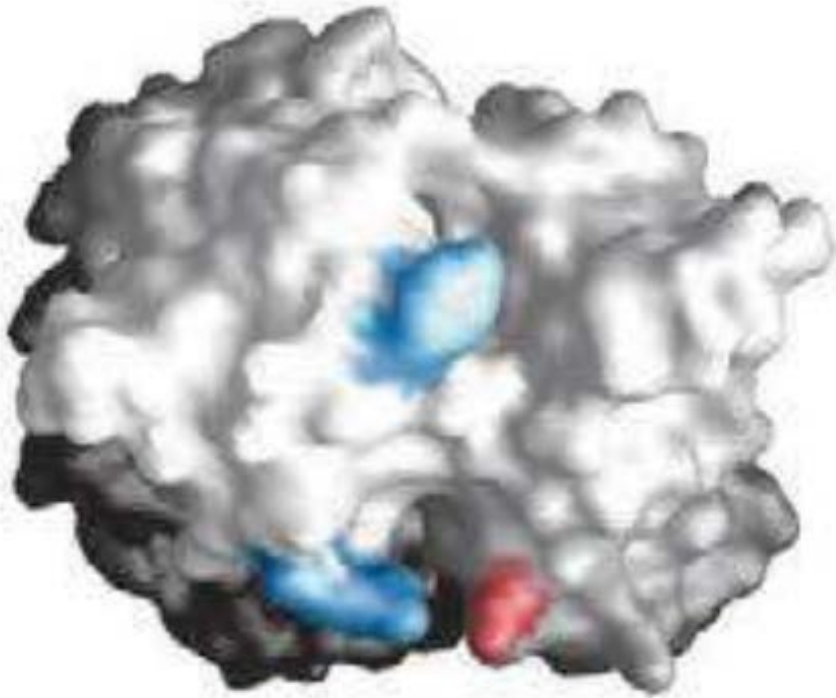


To generate an optimal fit for the antigen, the binding sites of IgG often undergo slight conformational changes. Such **induced fit** is common to many protein-ligand interactions.

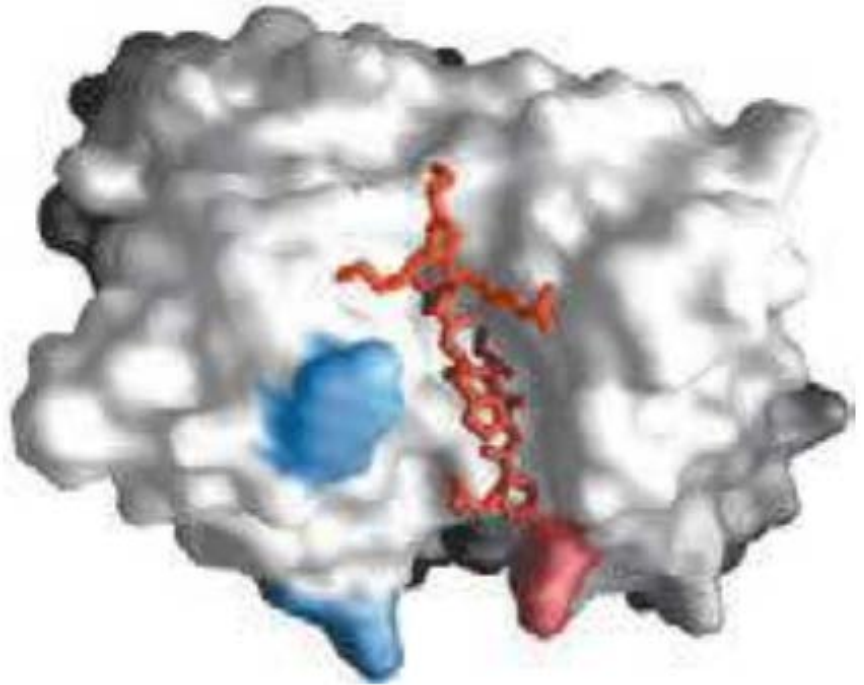
誘導配合

IgG與抗原結合的誘導配合

- 抗體與抗原的結合依賴非共價作用力
 - 例如：靜電作用、氫鍵、凡得瓦力與疏水作用。



(a) Conformation with
no antigen bound

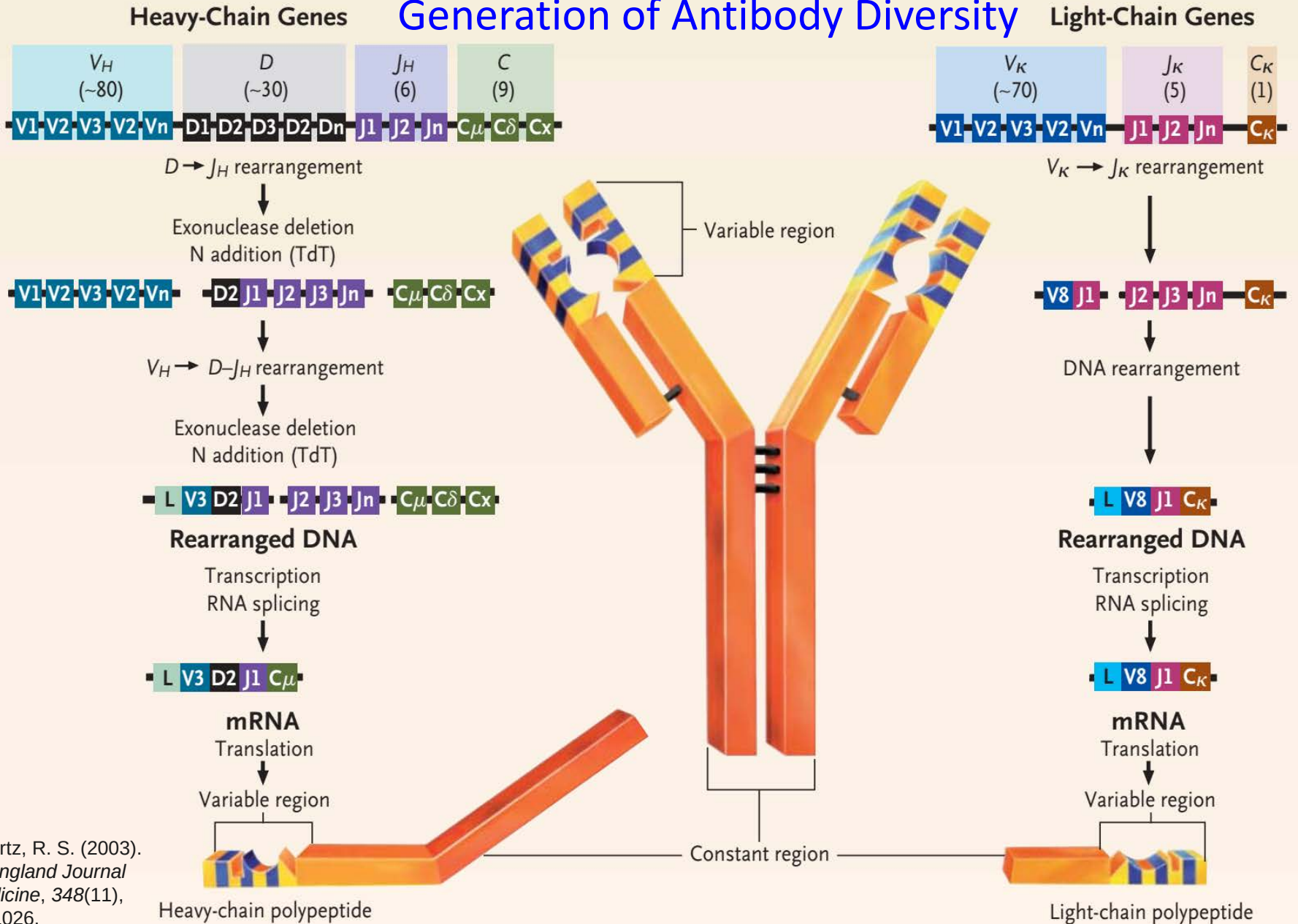


(c) Antigen bound
(shown)

Two residues in the heavy chain (blue) and one in the light chain (red) are colored to provide visual points of reference.

免疫球蛋白如何辨識多樣的抗原？

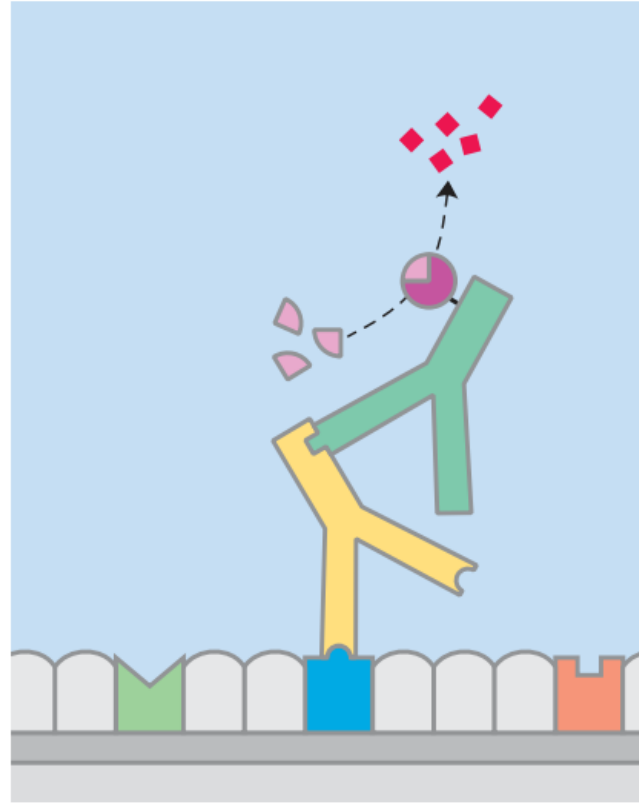
Generation of Antibody Diversity



Schwartz, R. S. (2003). *New England Journal of Medicine*, 348(11), 1017-1026.

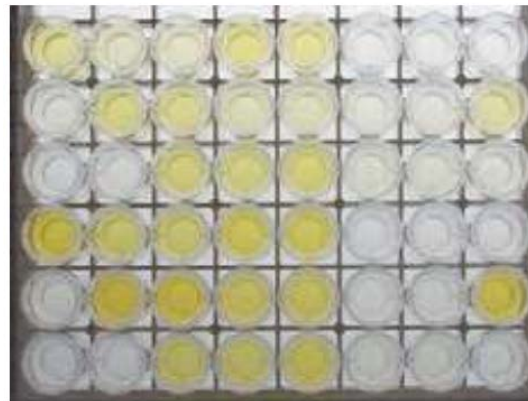
利用抗體的生物技術方法與原理

- 1 Coat surface with sample (antigens). 
- 2 Block unoccupied sites with nonspecific protein. 
- 3 Incubate with primary antibody against specific antigen. 
- 4 Incubate with secondary antibody-enzyme complex that binds primary antibody. 
- 5 Add substrate. 
- 6 Formation of colored product indicates presence of specific antigen. 



1. 酵素結合免疫吸附分析法， Enzyme-linked immunosorbent assay (ELISA)

Test for the presence of
herpes simplex virus (HSV)
antibodies in blood samples.



2. 免疫墨點法 (即西方墨點法)

Western blot

Purification of a protein
kinase.

