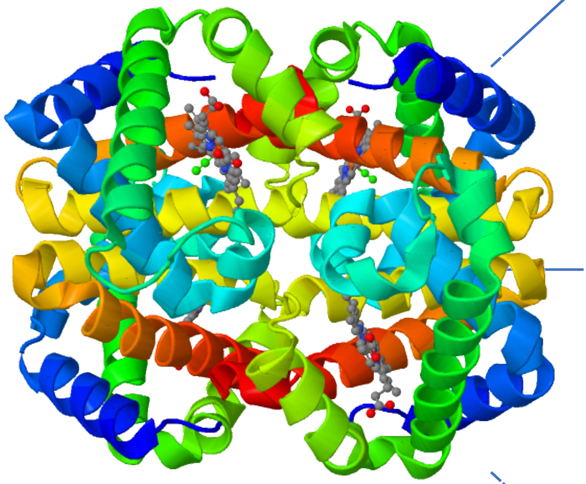
蛋白質概論



組成

- 如何合成？
- 如何分析？

結構

- 結構種類？
- 如何分析？

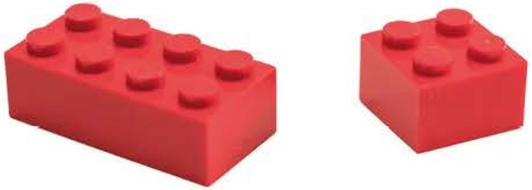
功能

- 氧結合蛋白
- 免疫球蛋白
- 分子馬達


PATISSERIE

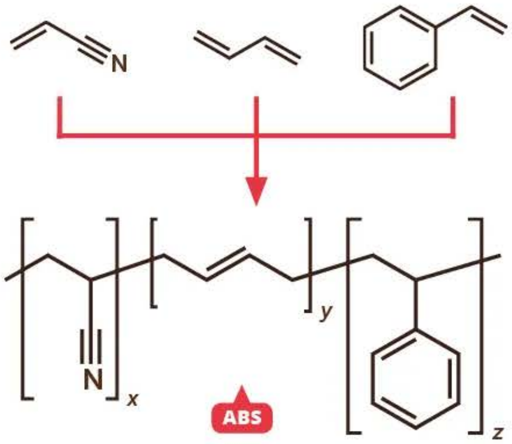
WHAT ARE LEGO BRICKS MADE OF?

LEGO BRICKS

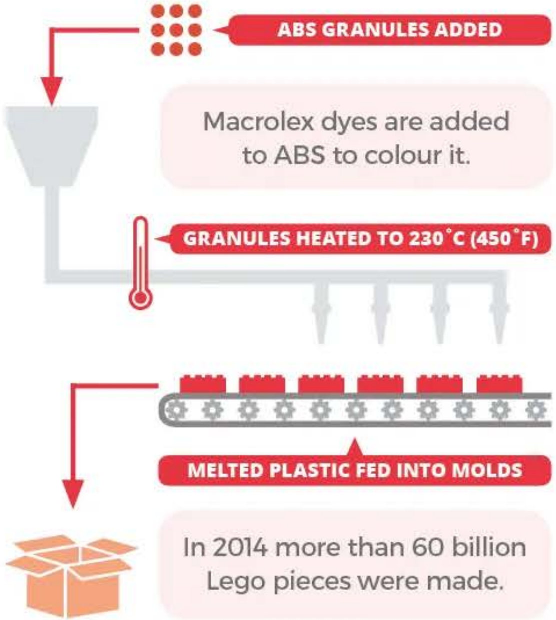


醋酸纖維素

Up until 1963, cellulose acetate was used to make Lego bricks and parts. Lego bricks are now made from acrylonitrile butadiene styrene (ABS). ABS is less subject to warping and colour fading.



HOW LEGO IS MADE



ABS GRANULES ADDED

Macrolex dyes are added to ABS to colour it.

GRANULES HEATED TO 230˚C (450˚F)

MELTED PLASTIC FED INTO MOLDS

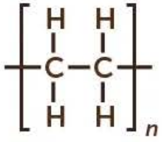
In 2014 more than 60 billion Lego pieces were made.

OTHER LEGO PARTS

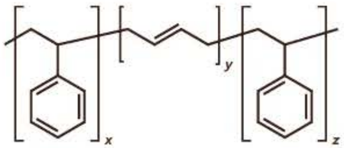
聚碳酸酯

ABS is opaque, so a polycarbonate polymer has to be used for transparent Lego parts. For leaves, bushes and trees, Lego has recently started using polyethene derived from sugar cane.

聚乙烯



POLYETHENE

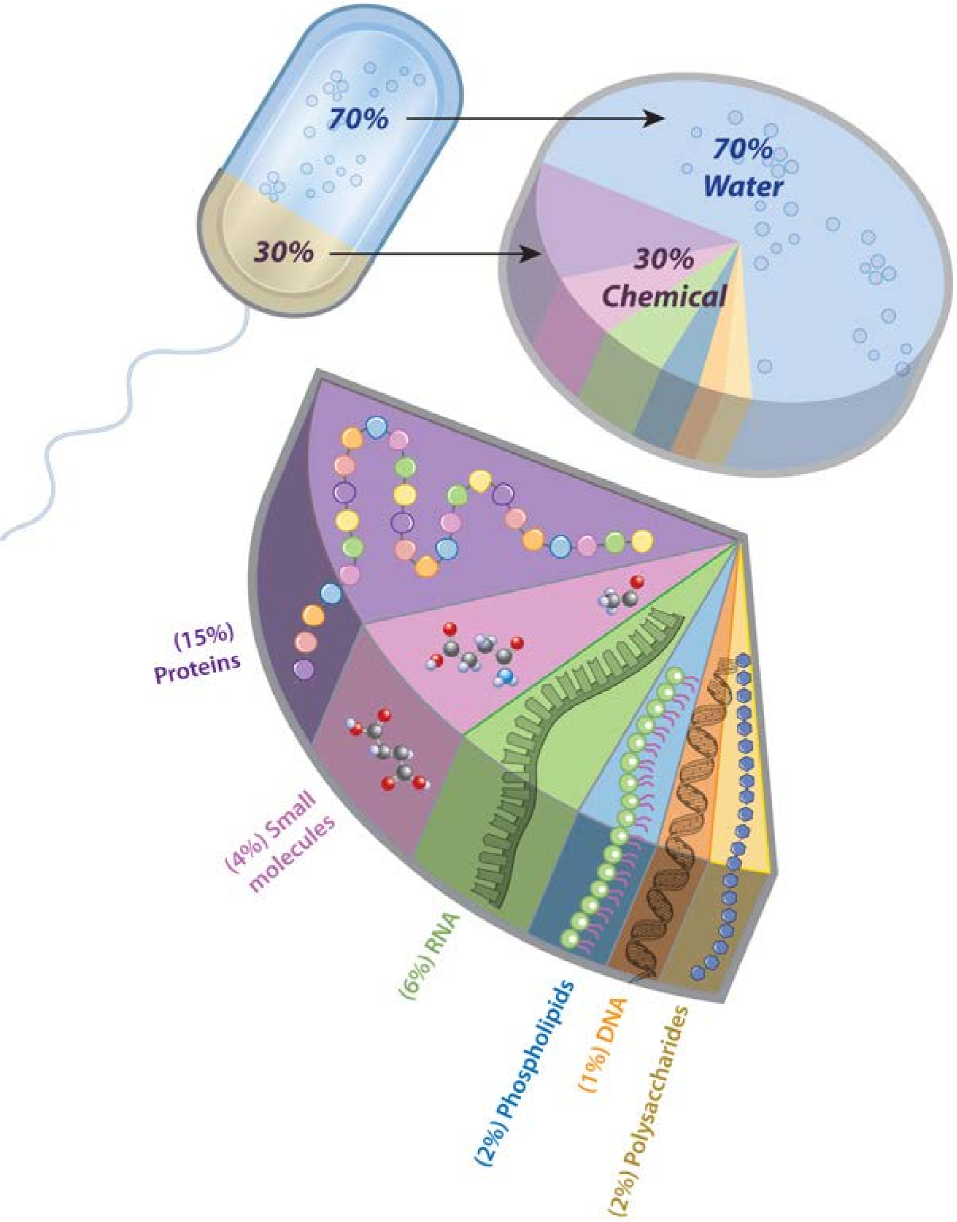


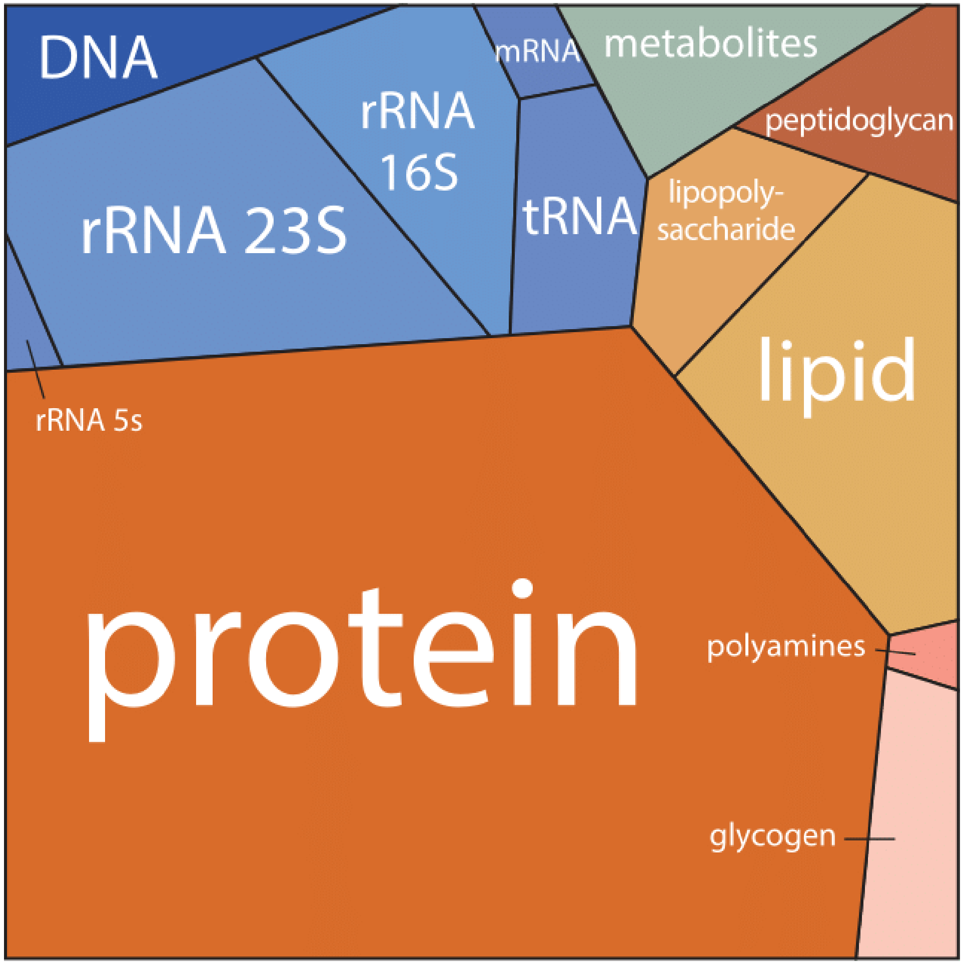
SBS

Tyres and elastic materials are made from styrene butadiene styrene (SBS).

苯乙烯丁二烯苯乙烯

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/

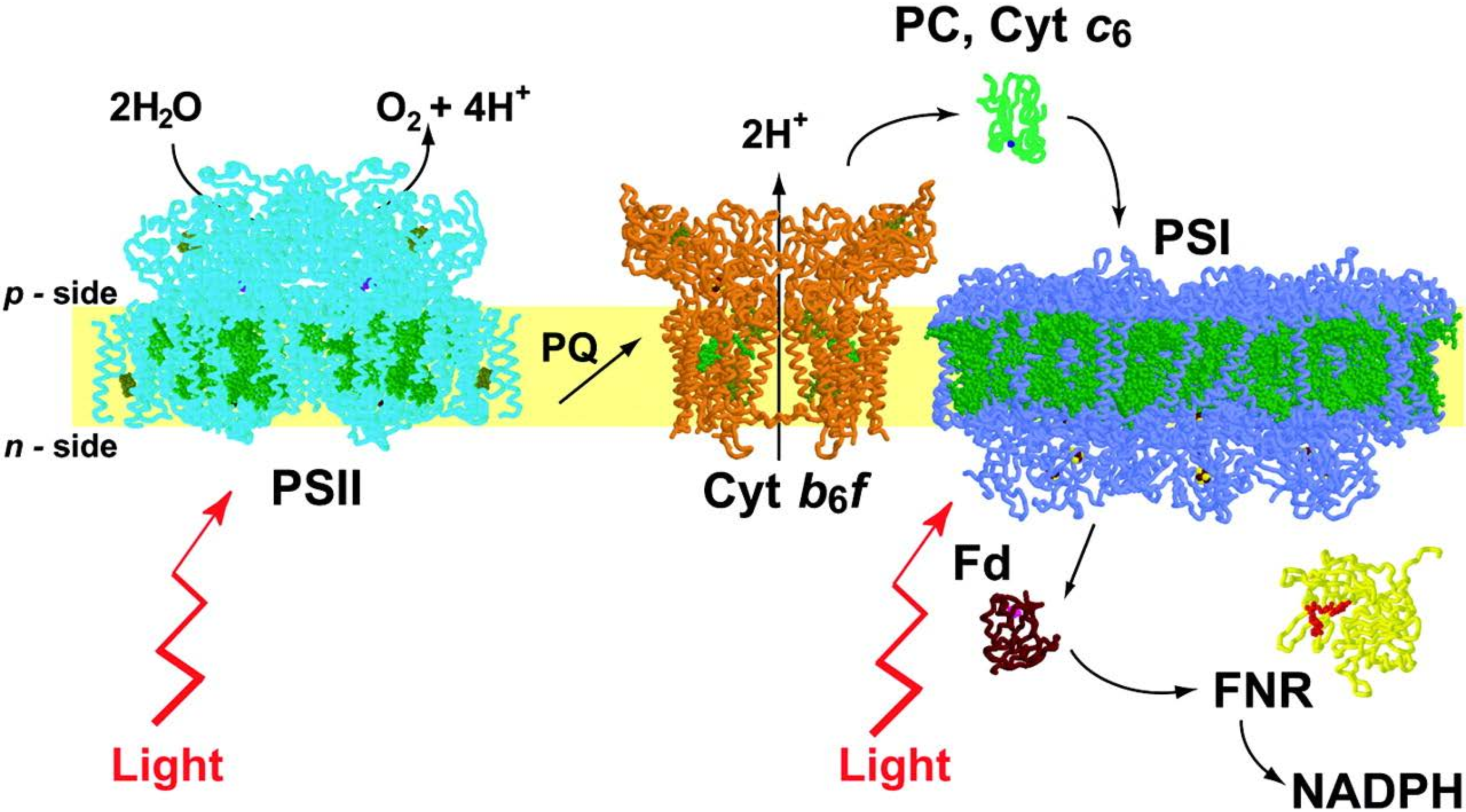
蛋白質的功能多樣性

- 蛋白質有什麼功能？

螢光素酶

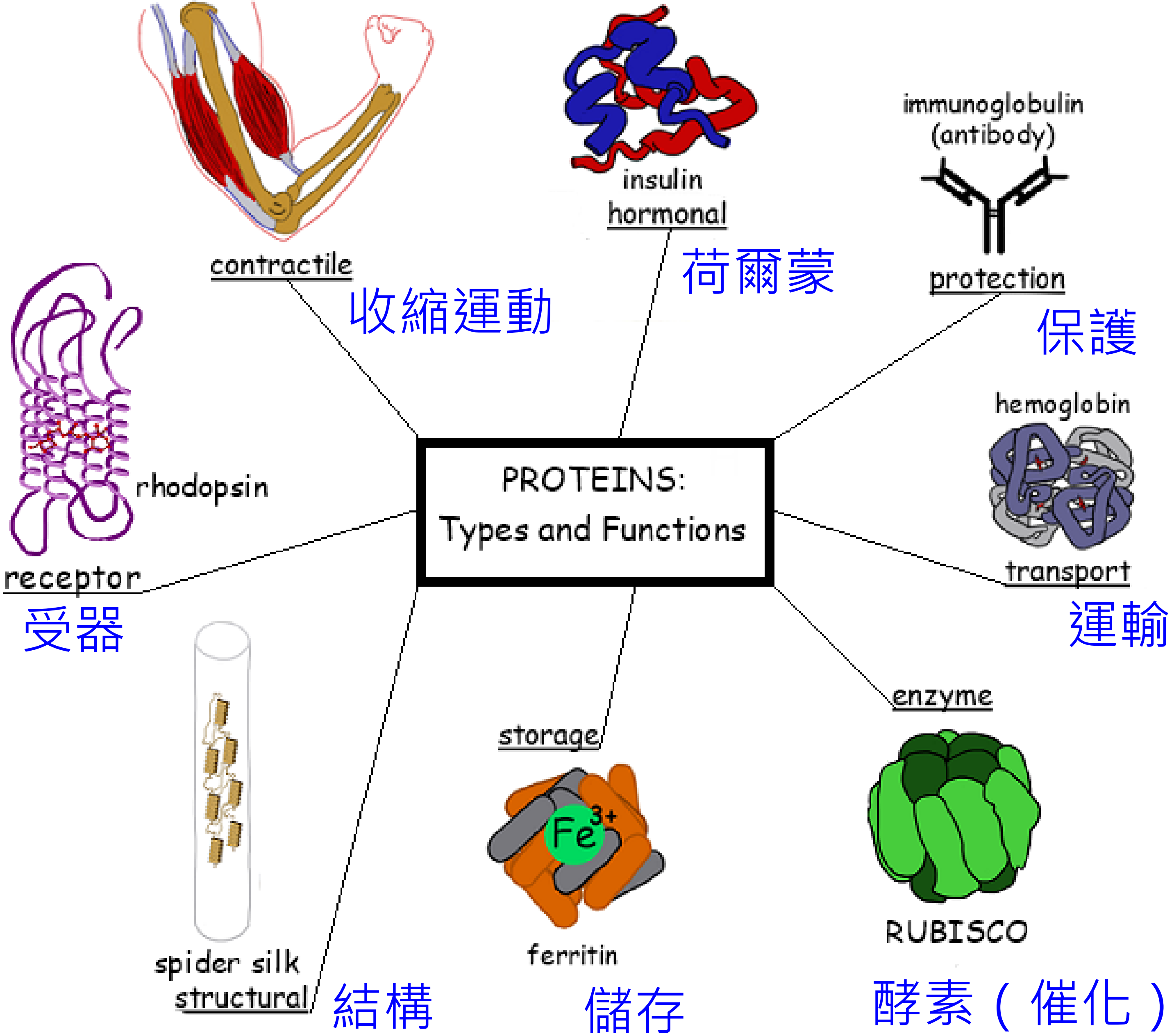
血紅蛋白

角蛋白



Kurisu, G., Zhang, H., Smith, J. L., & Cramer, W. A. (2003). Structure of the cytochrome b6f complex of oxygenic photosynthesis: tuning the cavity. *Science*, *302*(5647), 1009-1014.

蛋白質種類與功能



https://alevelbiology.co.uk/notes/functions-of-proteins/

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

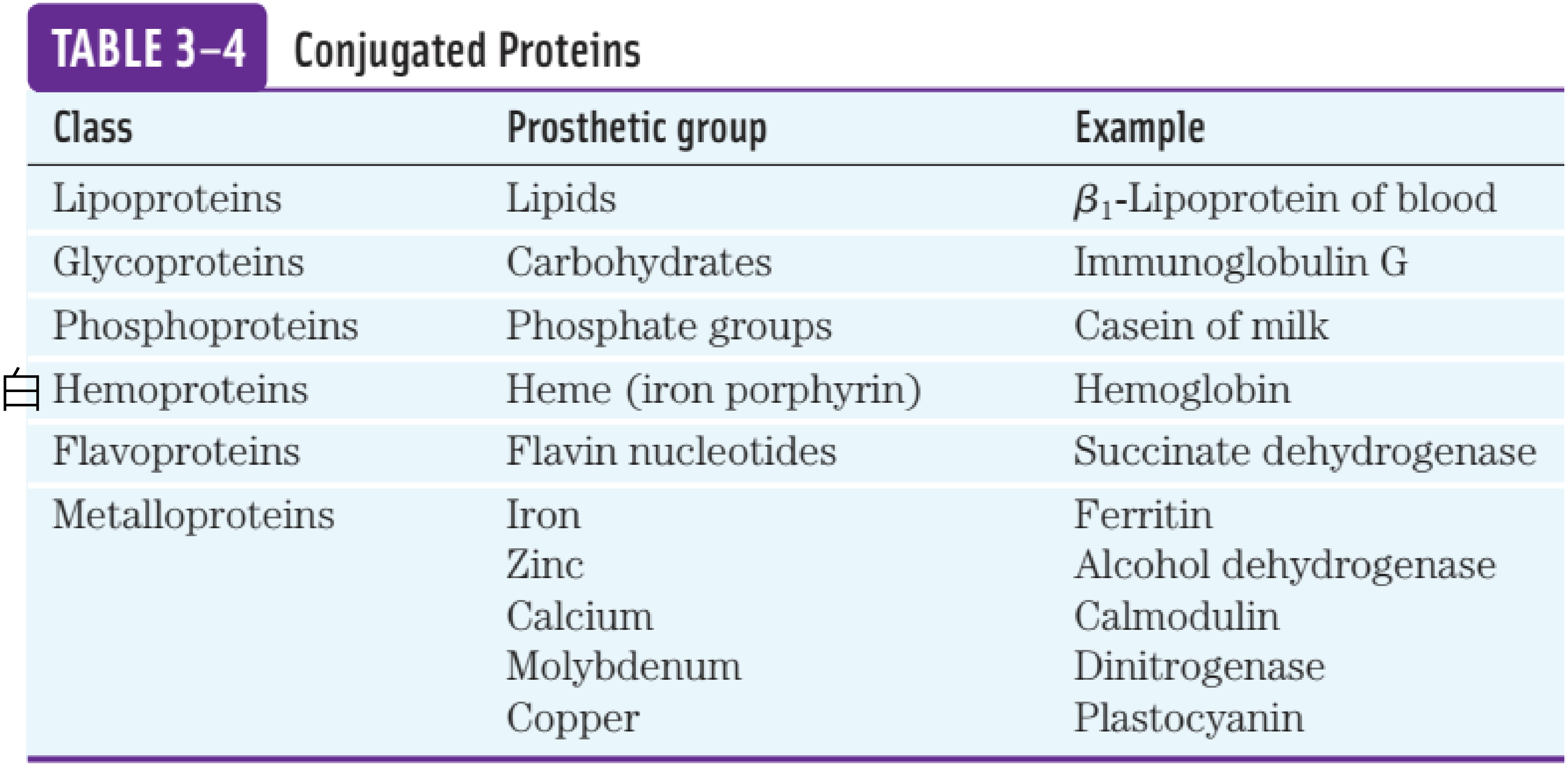


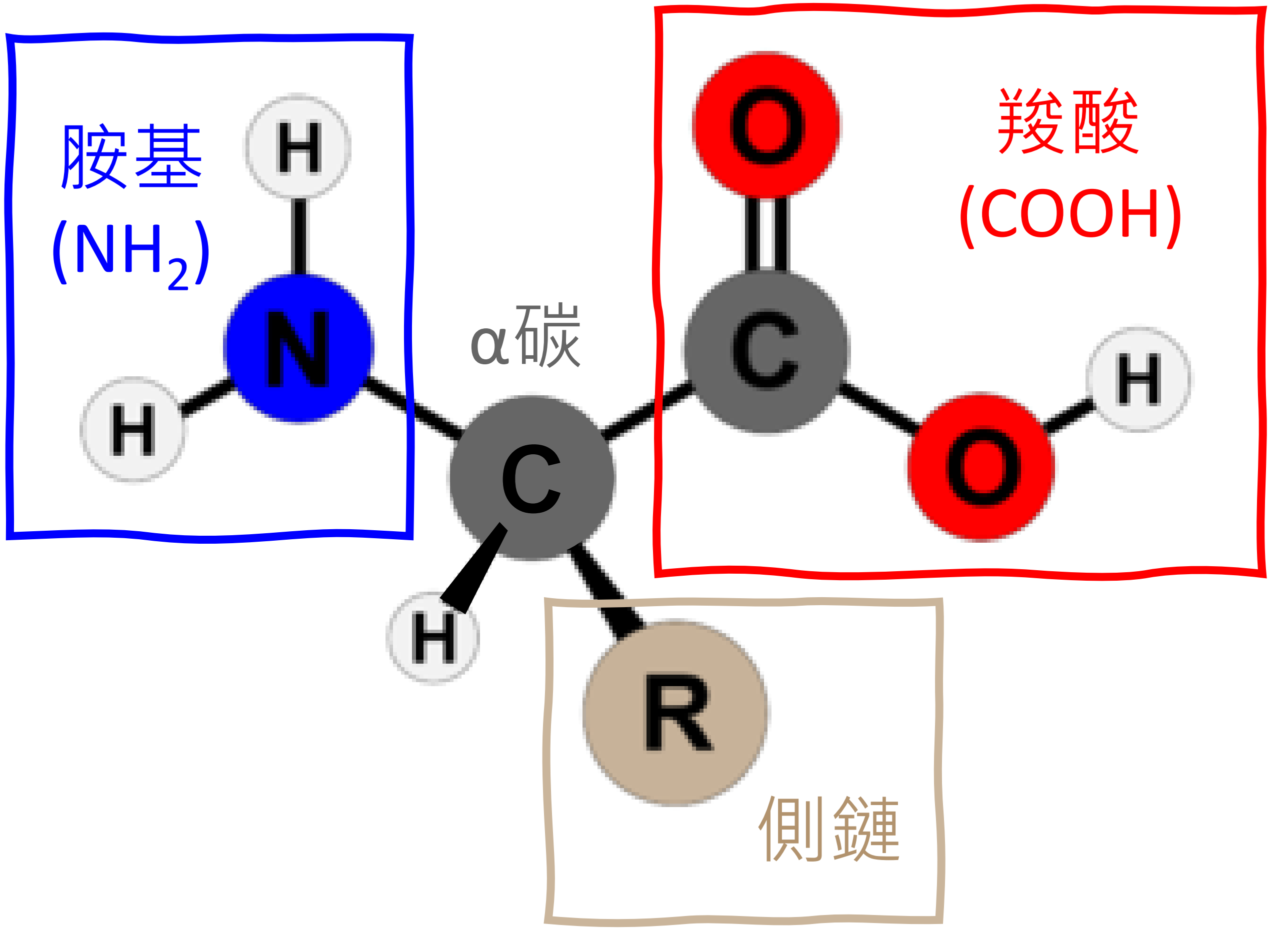
TABLE 3–4 Conjugated Proteins

	Class	Prosthetic group	Example
脂蛋白	Lipoproteins	Lipids	β_1-Lipoprotein of blood
醣蛋白	Glycoproteins	Carbohydrates	Immunoglobulin G
磷蛋白	Phosphoproteins	Phosphate groups	Casein of milk
血基質蛋白	Hemoproteins	Heme (iron porphyrin)	Hemoglobin
核黃蛋白	Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
金屬蛋白	Metalloproteins	Iron Zinc Calcium Molybdenum Copper	Ferritin Alcohol dehydrogenase Calmodulin Dinitrogenase Plastocyanin

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

胺基酸的共同結構

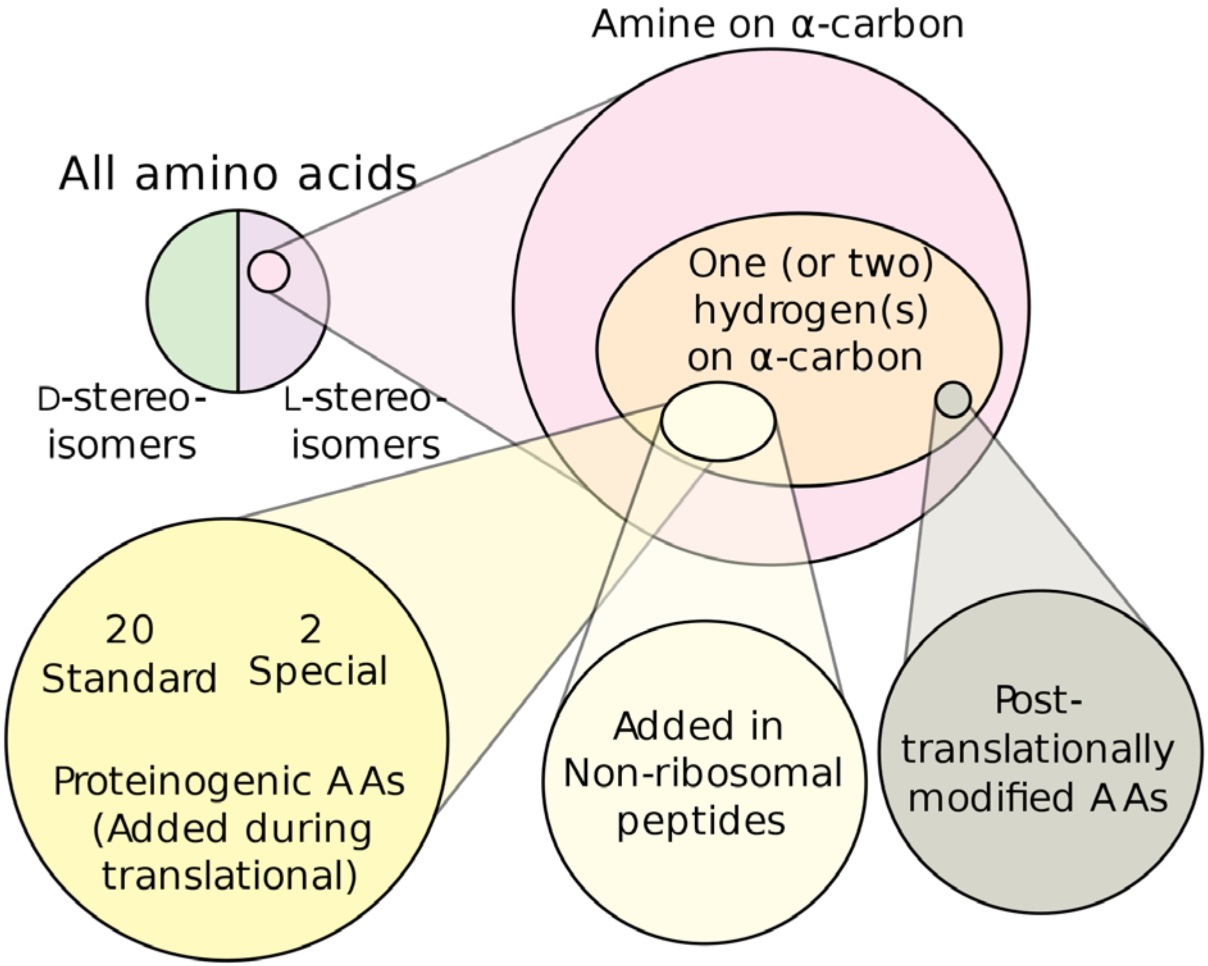


Modified from https://en.wikipedia.org/wiki/Amino_acid

胺基酸側鏈構造決定不同種類

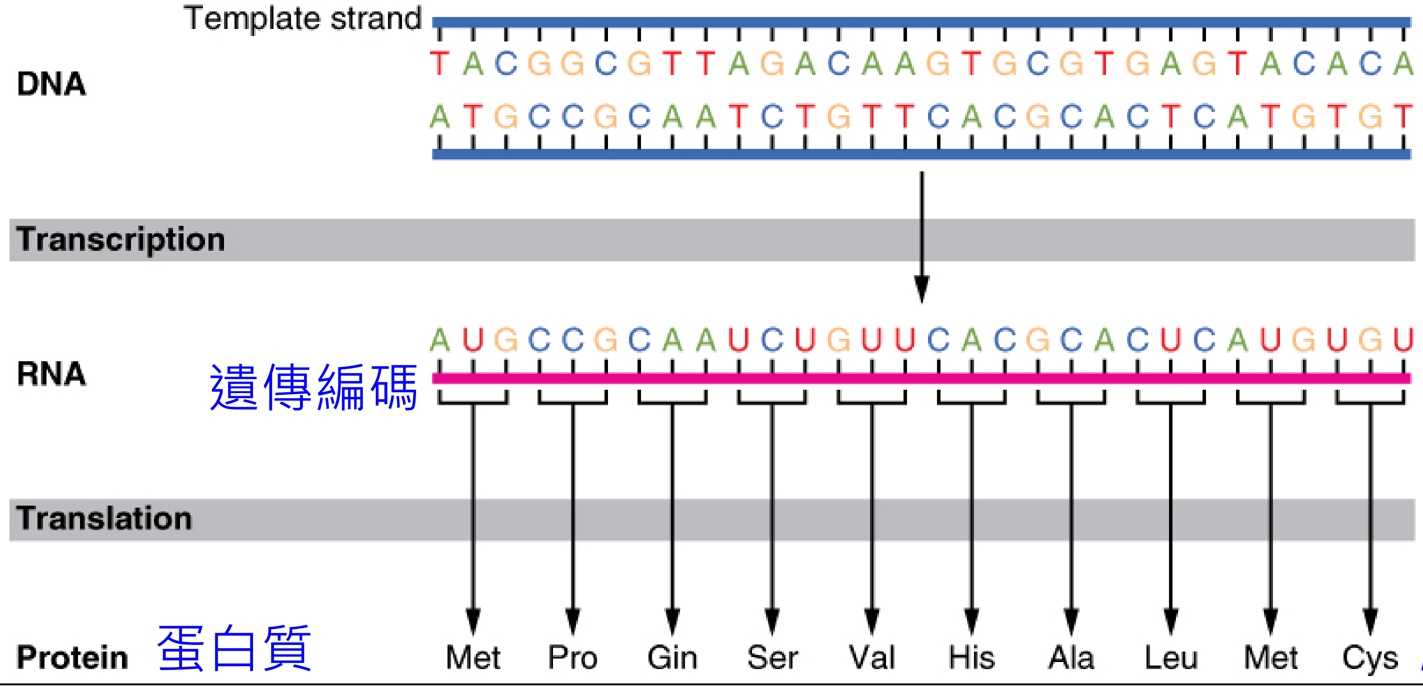
（可由遺傳編碼轉譯出的）

- 胺基酸種類有幾種？



Proteinogenic amino acid
蛋白胺基酸

https://en.wikipedia.org/wiki/Proteinogenic_amino_acid



Template strand

DNA

T A C G G C G T T A G A C A A G T G C G T G A G T A C A C A

A T G C C G C A A T C T G T T C A C G C A C T C A T G T G T

轉錄 **Transcription**

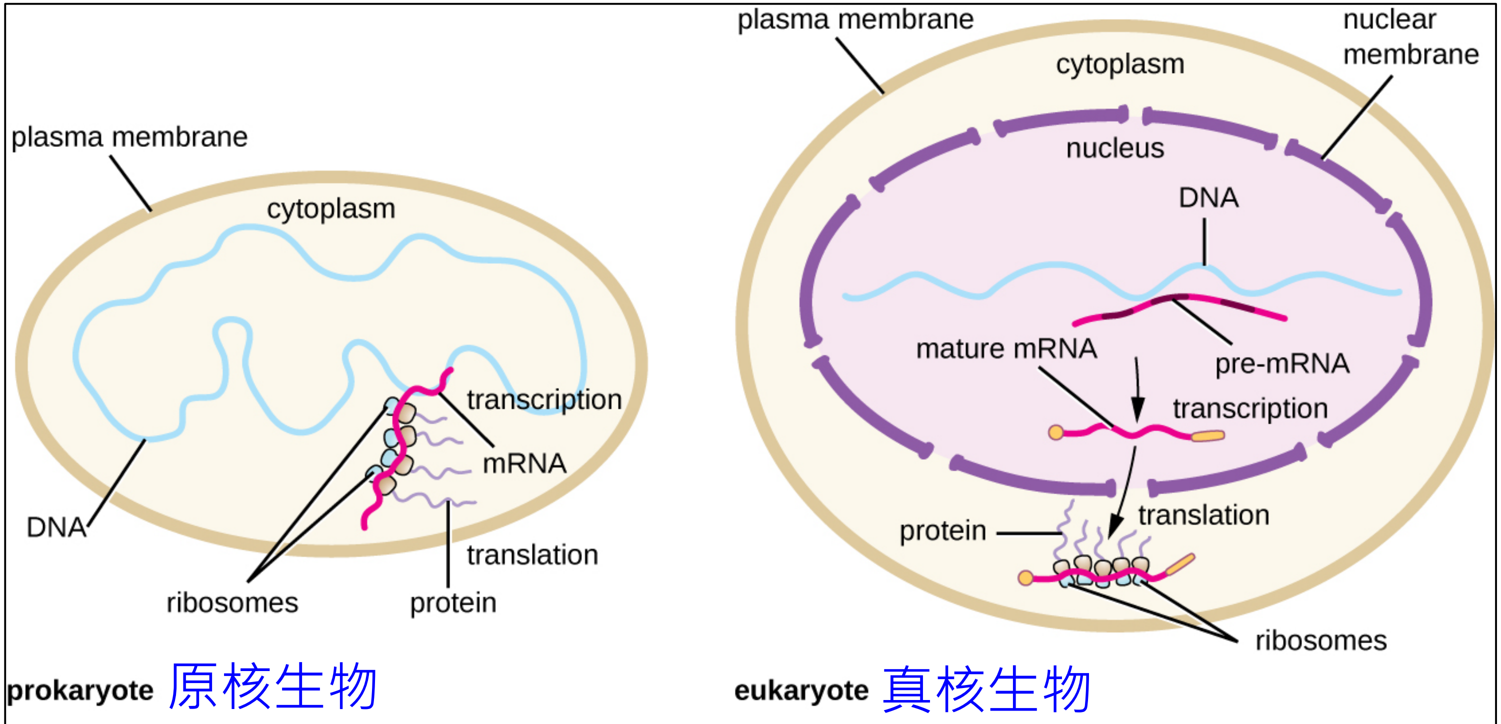
RNA 遺傳編碼

A U G C C G C A A U C U G U U C A C G C A C U C A U G U G U

轉譯 **Translation**

Protein 蛋白質

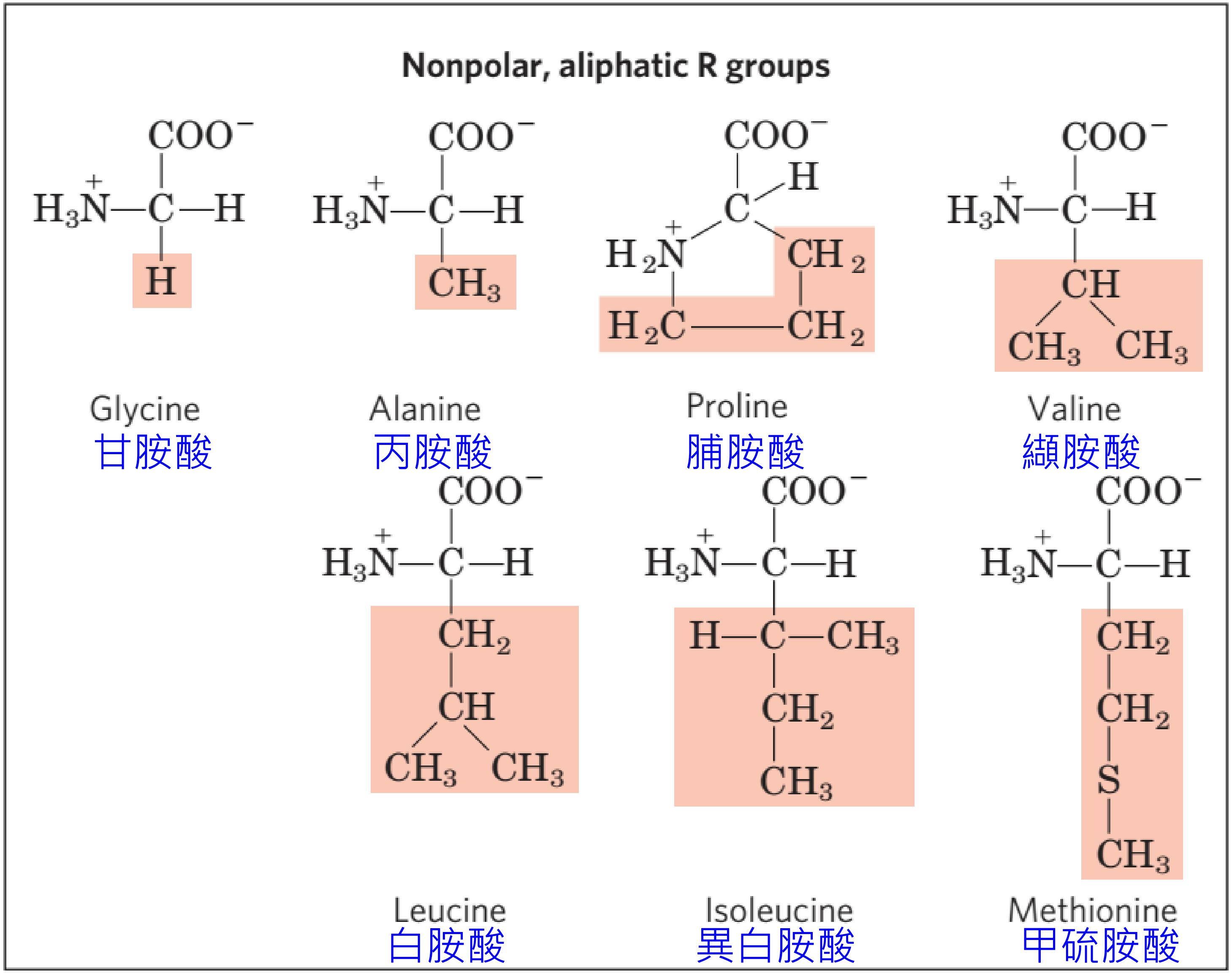
Met Pro Gin Ser Val His Ala Leu Met Cys 胺基酸



prokaryote 原核生物

eukaryote 真核生物

側鏈為非極性、疏水



Nonpolar, aliphatic R groups

Glycine
甘胺酸

Alanine
丙胺酸

Proline
脯胺酸

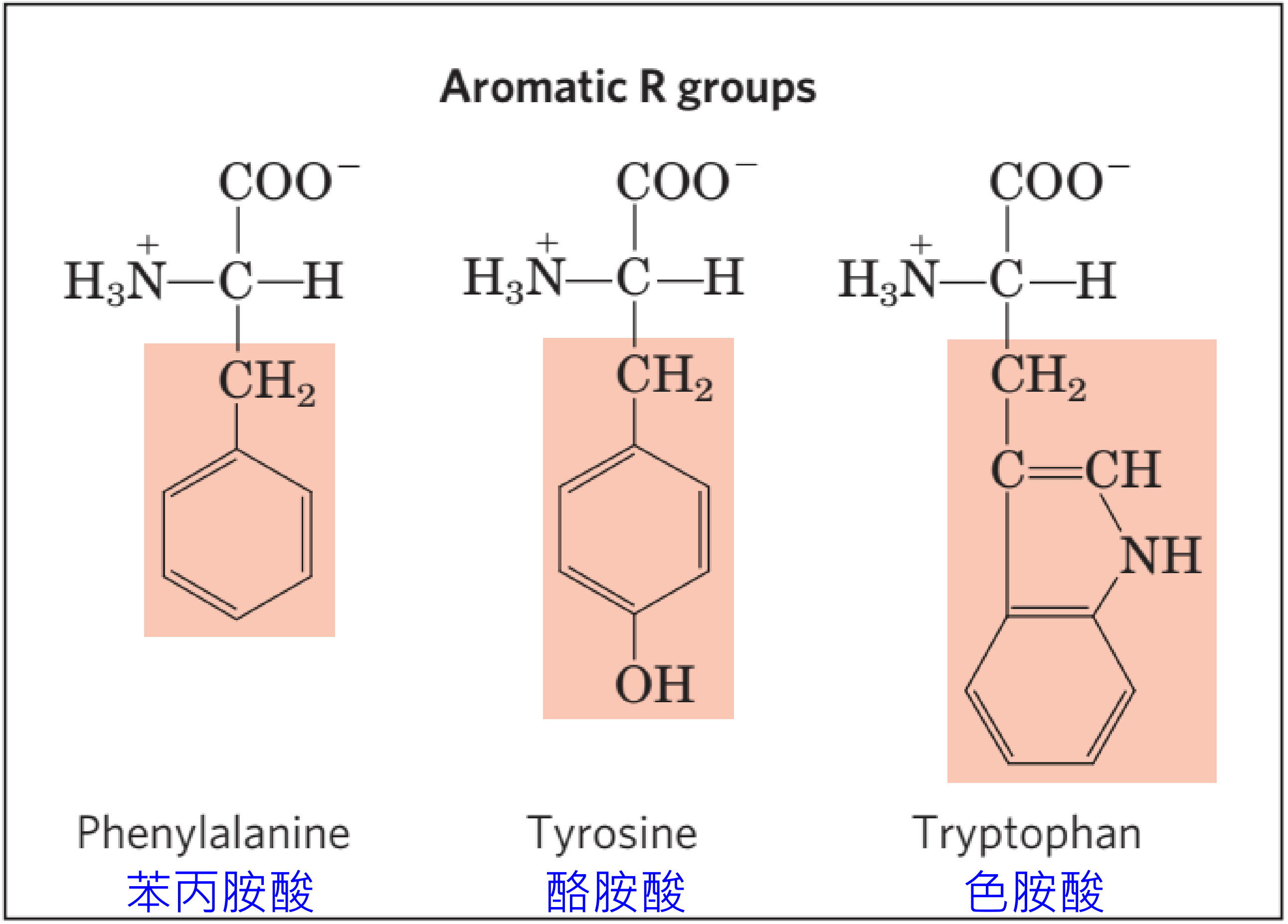
Valine
纈胺酸

Leucine
白胺酸

Isoleucine
異白胺酸

Methionine
甲硫胺酸

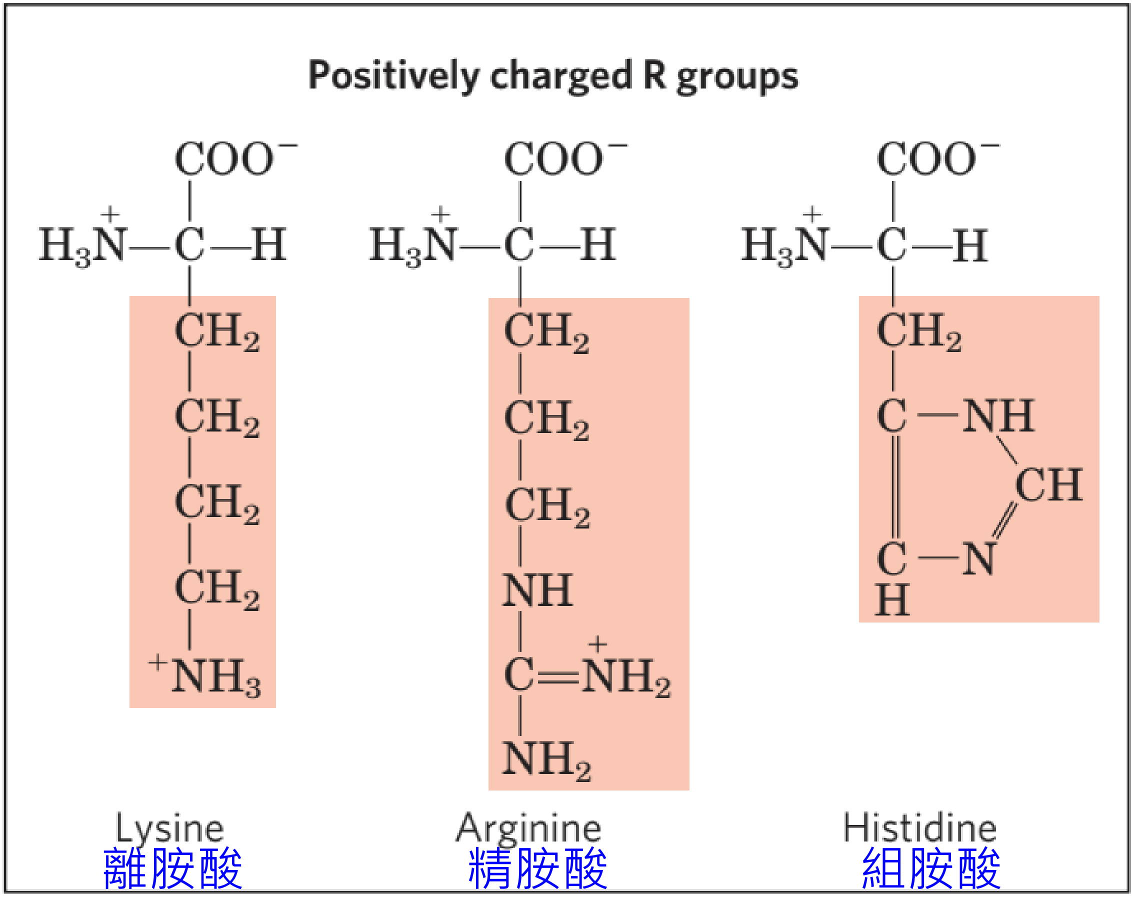
側鏈為芳香族

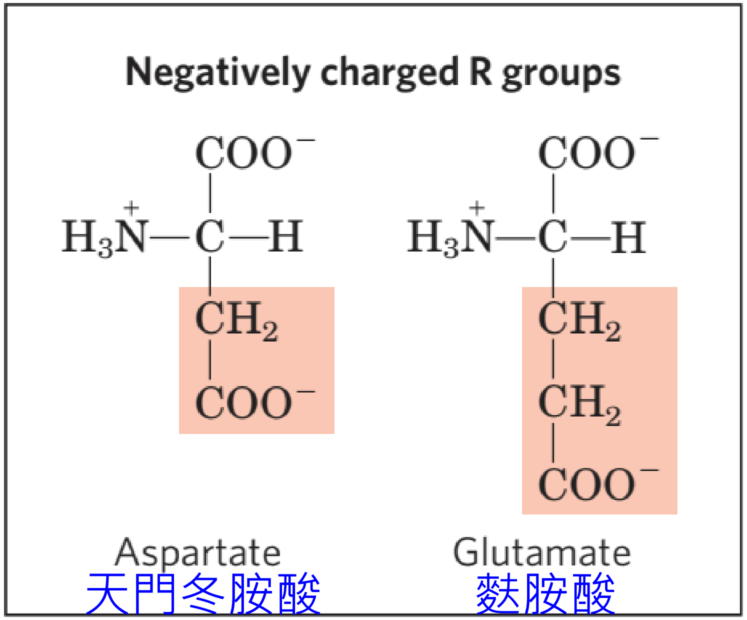


Phenylalanine	Tyrosine	Tryptophan
苯丙胺酸	酪胺酸	色胺酸
疏水	有極性	疏水

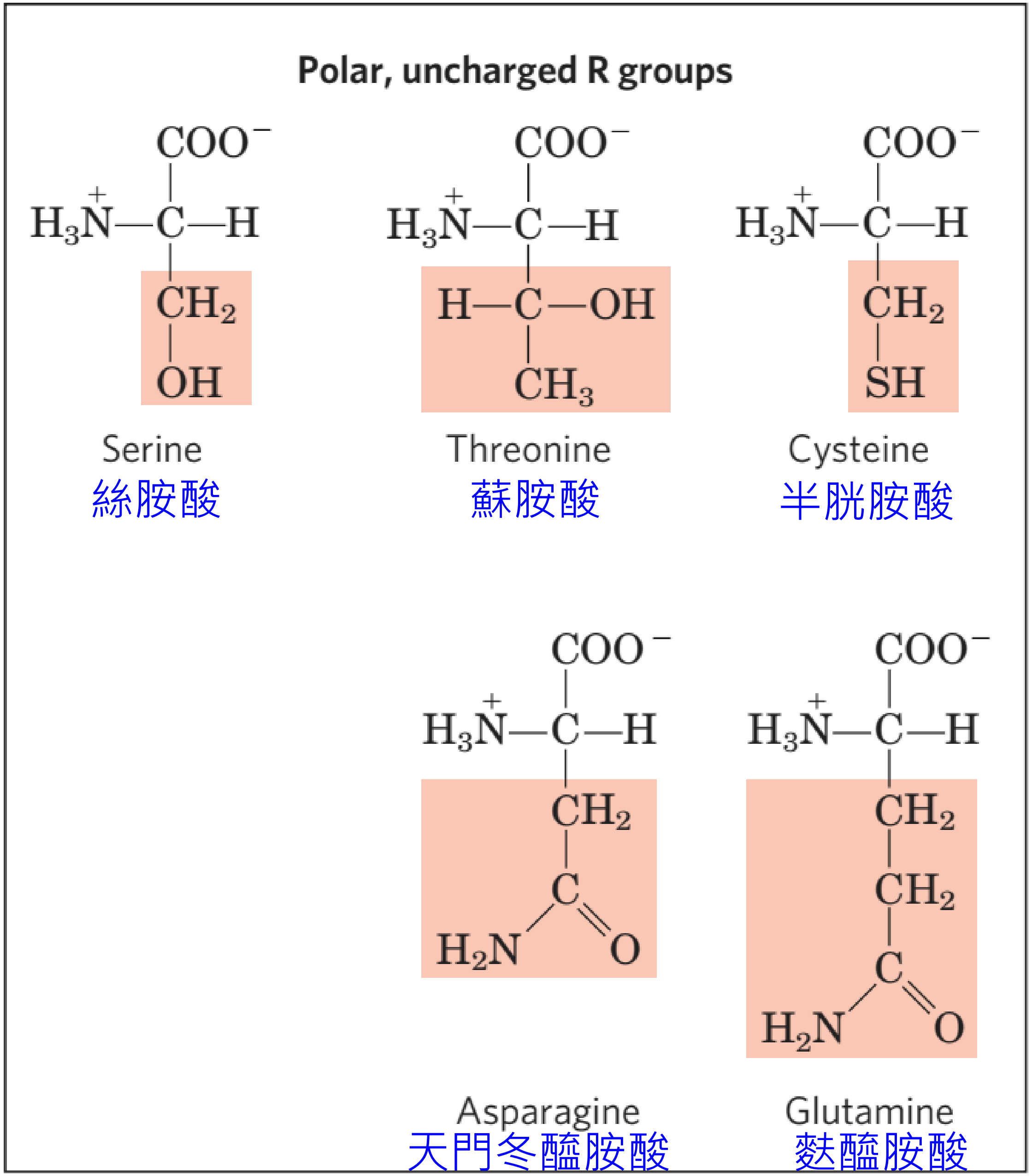
側鏈帶正電

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

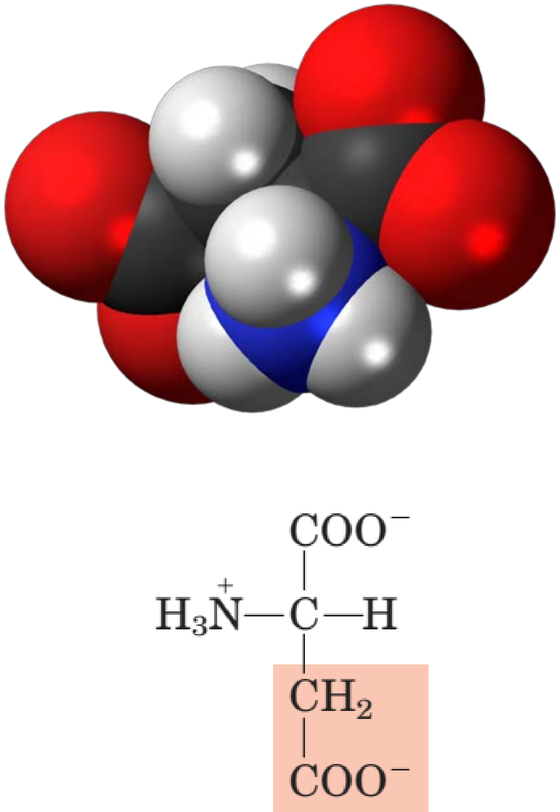
Asparagine
天門冬醯胺酸

Glutamine
麩醯胺酸

討論：為什麼要有那麼多種胺基酸？

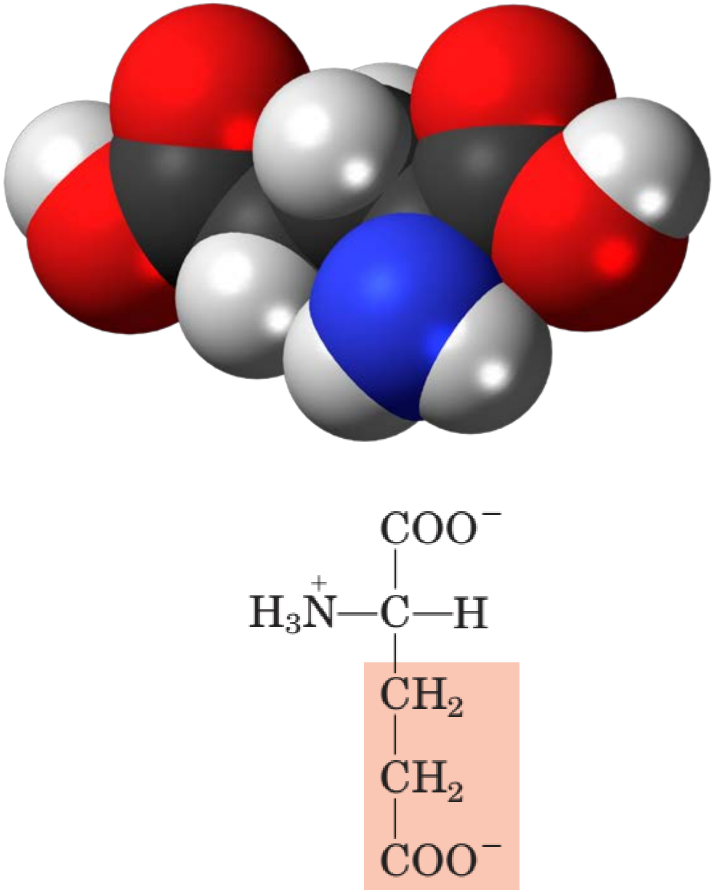
- 側鏈具備不同特性：
  1) 大小
  2) 極性
  3) 帶電性
  4) 靈活性（flexibility）
  5) 結構形態…等。
- 不同胺基酸組合而成的蛋白質具備有不同特性。

(1)大小



$$\begin{array}{rcl} & \mathrm{COO^-} & \\ & | & \\ \mathrm{H_3\overset{+}{N}}- & \mathrm{C} & -\mathrm{H} \\ & | & \\ & \mathrm{CH_2} & \\ & | & \\ & \mathrm{COO^-} & \end{array}$$

Aspartate

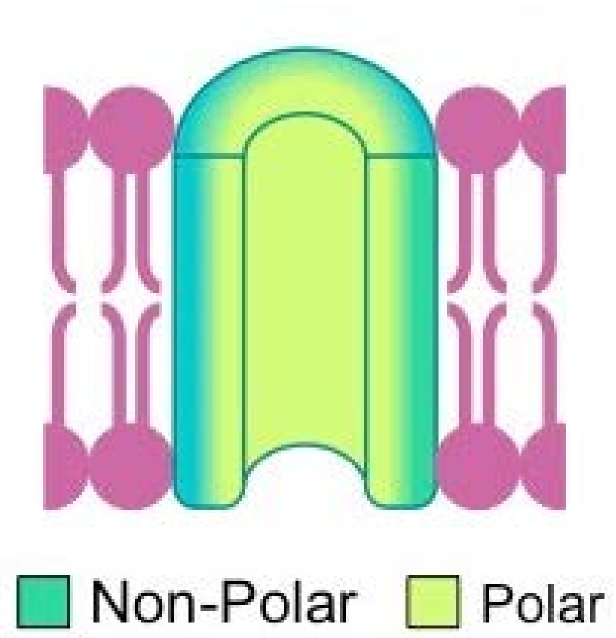


$$\begin{array}{rcl} & \mathrm{COO^-} & \\ & | & \\ \mathrm{H_3\overset{+}{N}}- & \mathrm{C} & -\mathrm{H} \\ & | & \\ & \mathrm{CH_2} & \\ & | & \\ & \mathrm{CH_2} & \\ & | & \\ & \mathrm{COO^-} & \end{array}$$

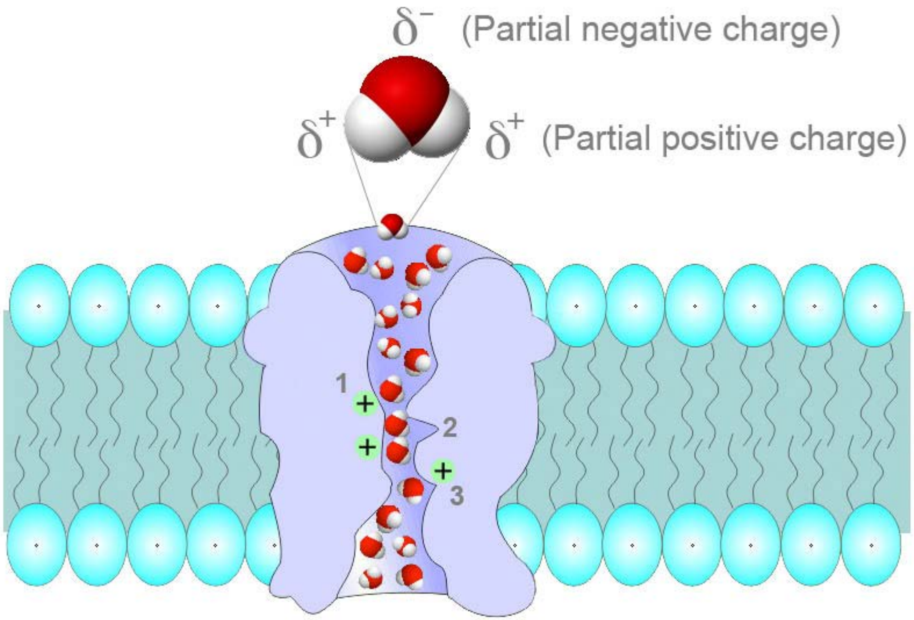
Glutamate

(2) 極性

- 以通道蛋白質為例：



非極性　　極性

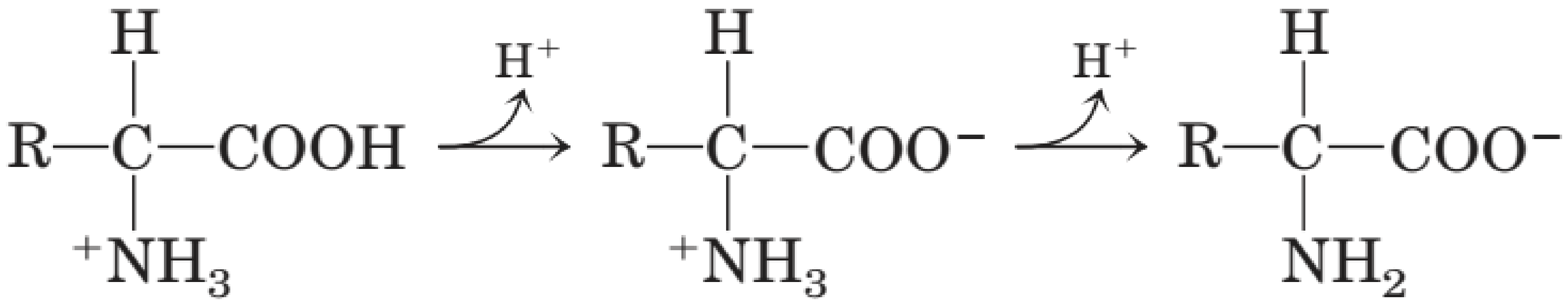


水通道蛋白

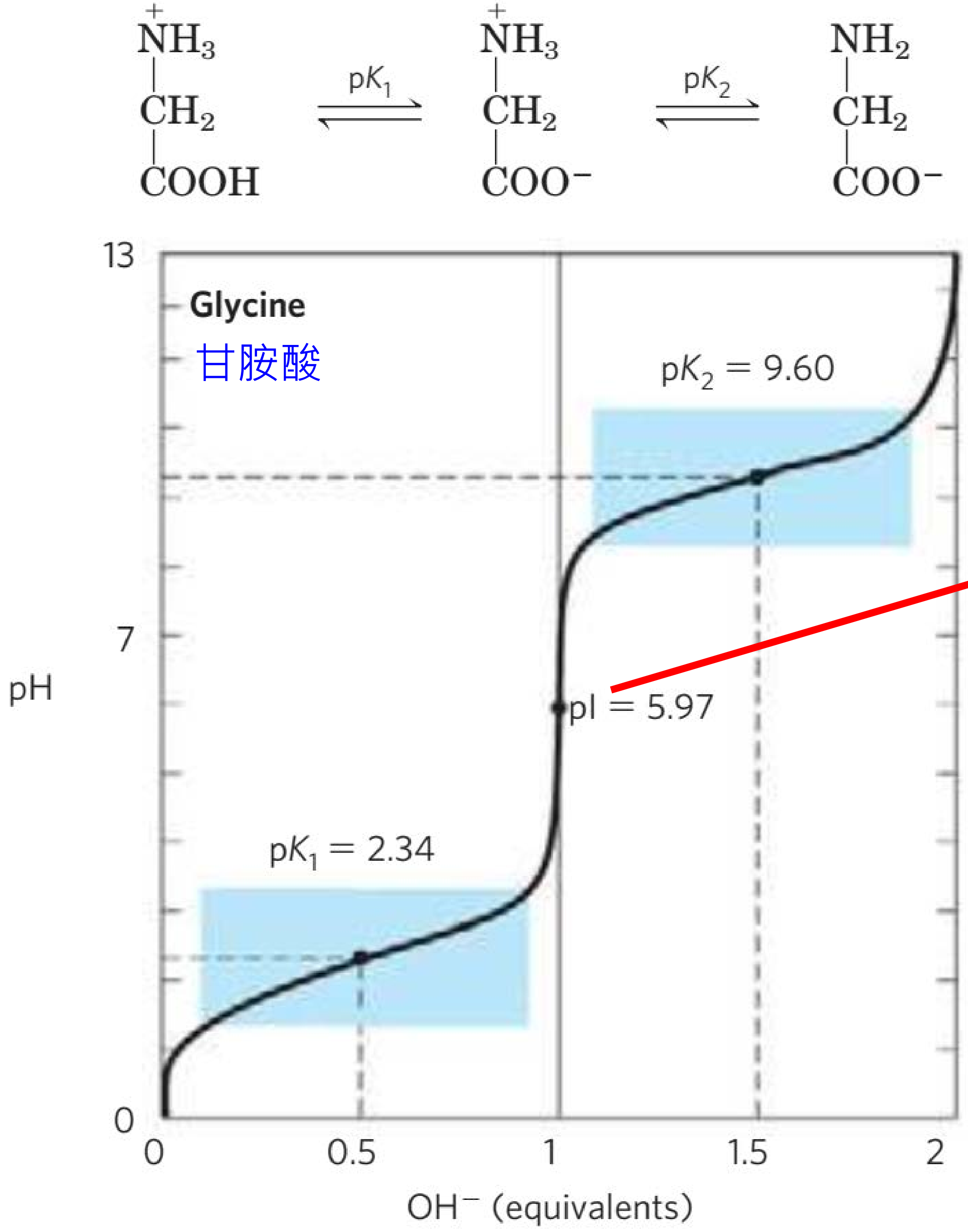
https://ib.bioninja.com.au/standard-level/topic-1-cell-biology/13-membrane-structure/membrane-proteins.html
https://askabiologist.asu.edu/venom/protein-channels

(3) 帶電性

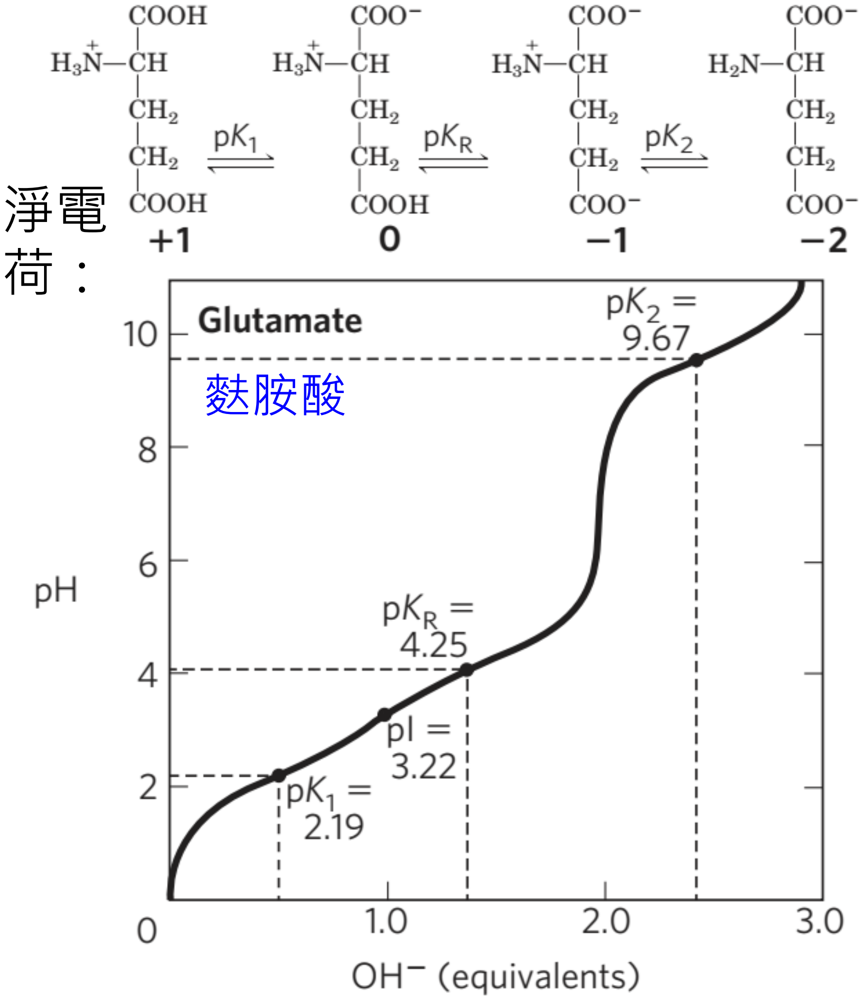
- 胺基酸依據所在的酸鹼度(pH值)環境會有不同的帶電狀態：

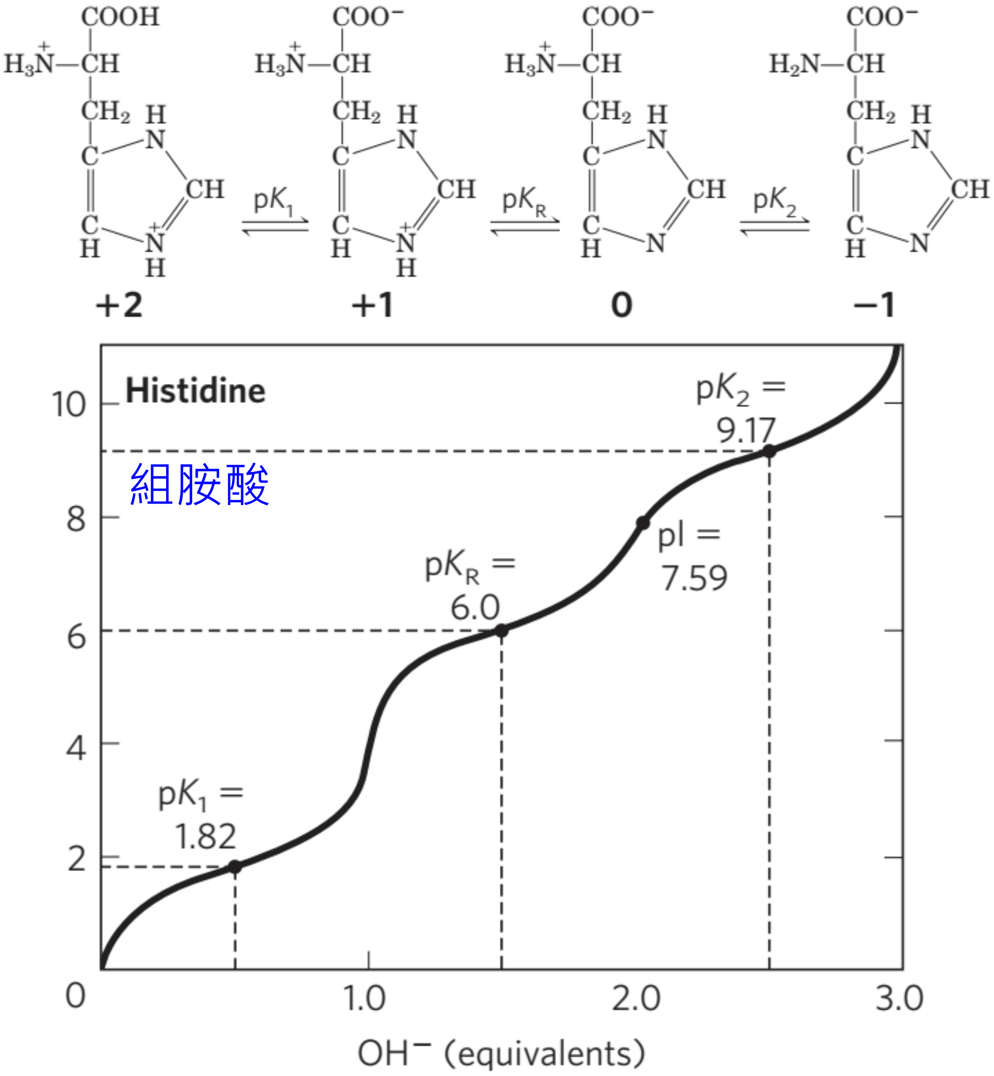


淨電荷： **+1** **0** **−1**



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。

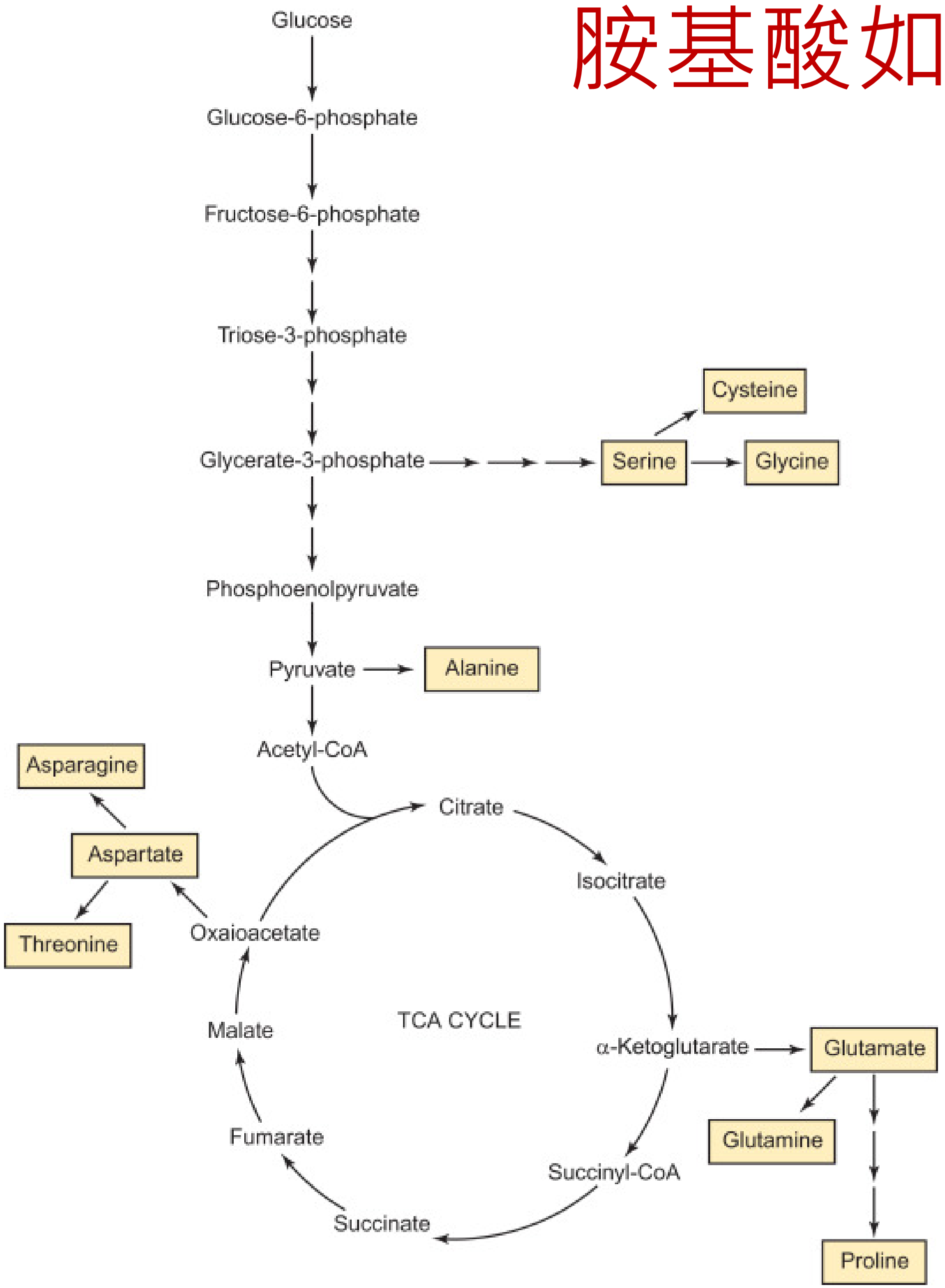




胺基酸等電點參考資料：http://www.chem.ucalgary.ca/courses/351/Carey5th/Ch27/ch27-1-4-2.html

胺基酸如何合成？

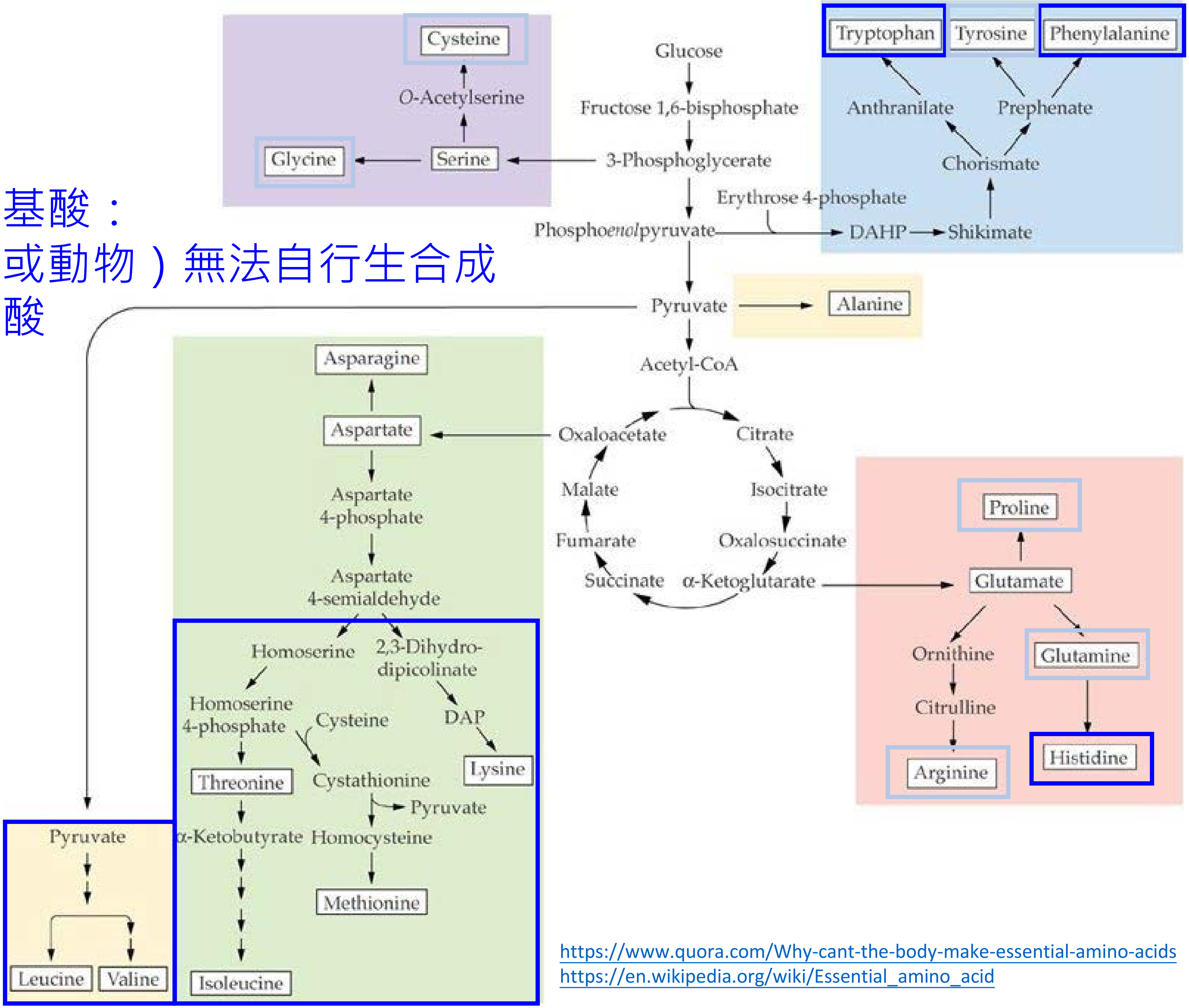
人類細胞合成胺基酸途徑



TCA: 三羧酸循環（tricarboxylic acid cycle）

https://www.sciencedirect.com/topics/nursing-and-health-professions/nonessential-amino-acid

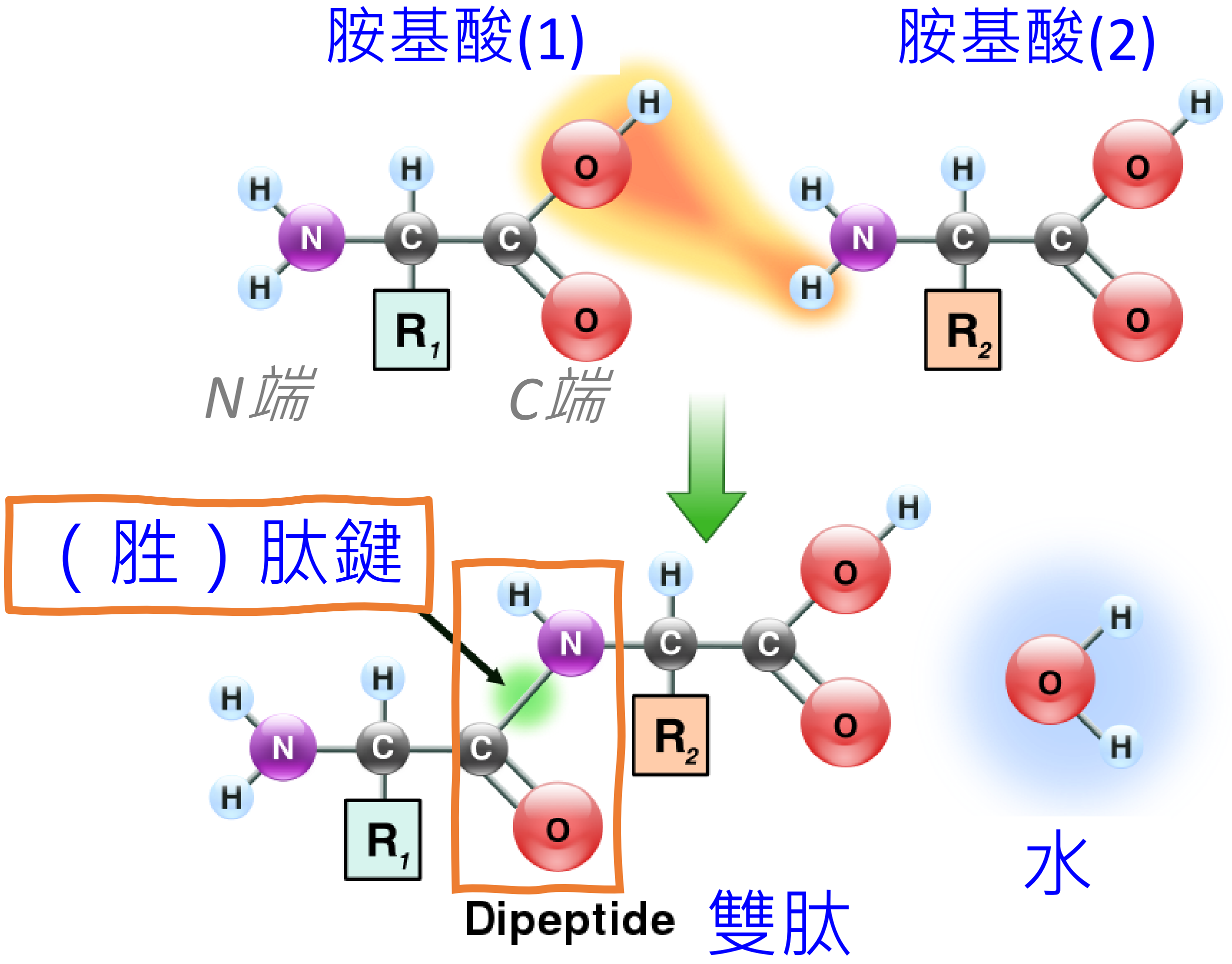
必需胺基酸：
人類（或動物）無法自行生合成
的胺基酸



https://www.quora.com/Why-cant-the-body-make-essential-amino-acids
https://en.wikipedia.org/wiki/Essential_amino_acid

蛋白質如何合成？

- 胺基酸→胜肽(peptide)→蛋白質(protein)



胺基酸(1)

胺基酸(2)

N端

C端

（胜）肽鍵

Dipeptide 雙肽

水

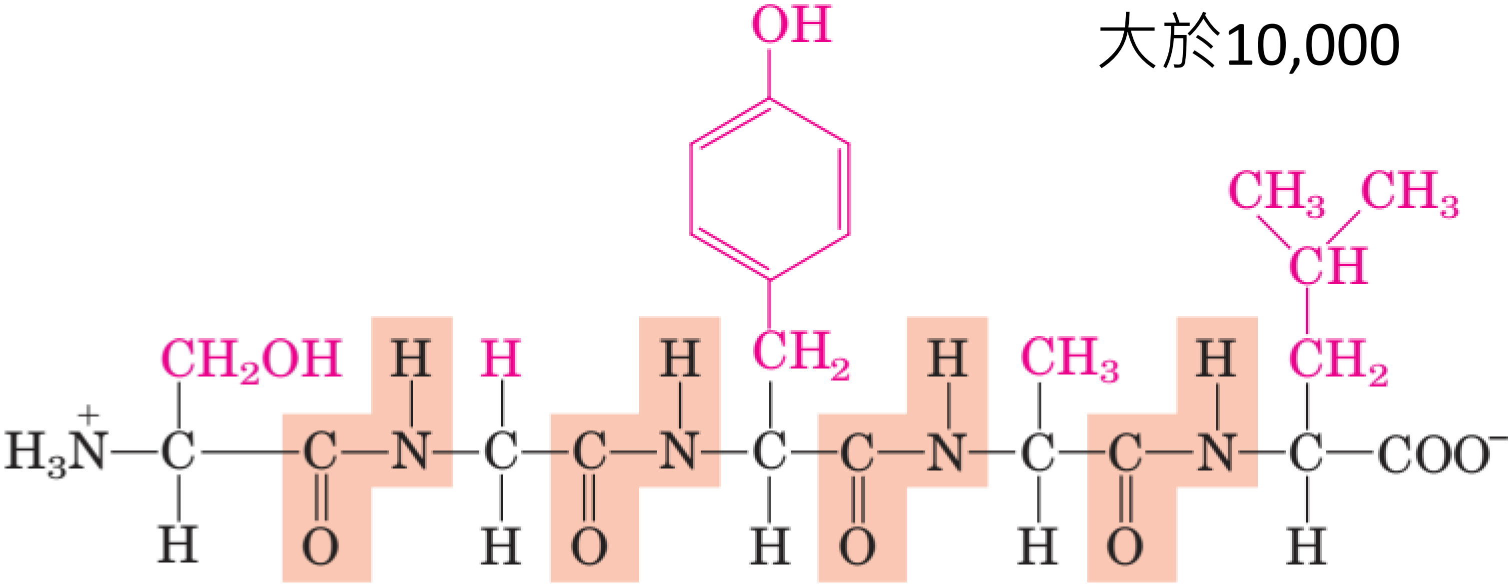
Modified from https://en.wikipedia.org/wiki/Amino_acid

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



Amino-
terminal end
N端

Carboxyl-
terminal end
C端

Ser–Gly–Tyr–Ala–Leu
SGYAL

蛋白質分子量與胺基酸個數的換算

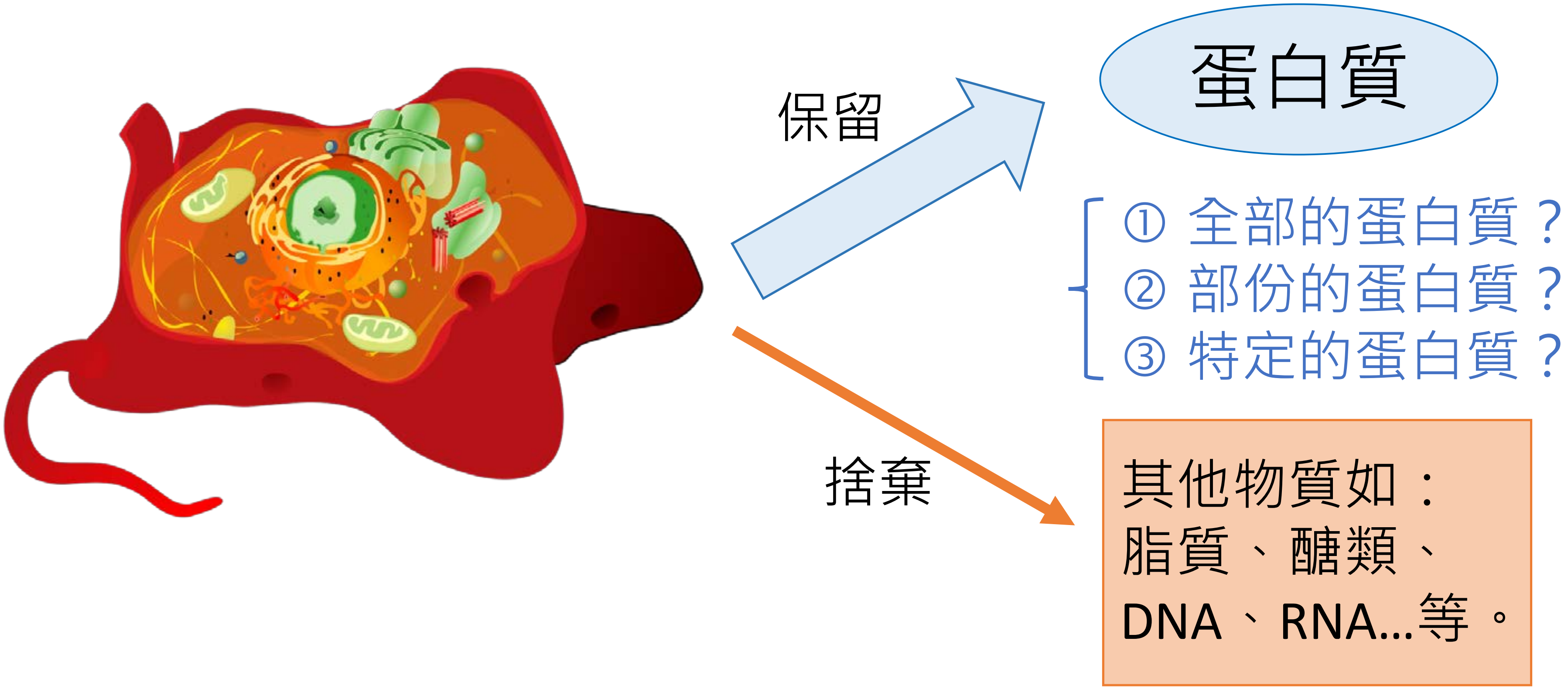
- 胺基酸平均分子量為多少？
  https://bionumbers.hms.harvard.edu/search.aspx

B10NUMB3R5

THE DATABASE OF USEFUL BIOLOGICAL NUMBERS

蛋白質組成如何分析？

1. 蛋白質分離與純化



保留

蛋白質

① 全部的蛋白質？
② 部份的蛋白質？
③ 特定的蛋白質？

捨棄

其他物質如：
脂質、醣類、
DNA、RNA...等。

蛋白質分離與純化方法

1) 沒有「一招打天下」的蛋白質純化方法。
2) 純化蛋白質所需要的量依照後續分析方法靈敏度而定。
3) 污染問題：不純物干擾蛋白質分析、高含量蛋白干擾微量蛋白質分析…
4) 蛋白質穩定度問題：活性、功能是否跟活體下一樣？

Lee, C. H. (2017). A simple outline of methods for protein isolation and purification. Endocrinology and Metabolism, 32(1), 18.

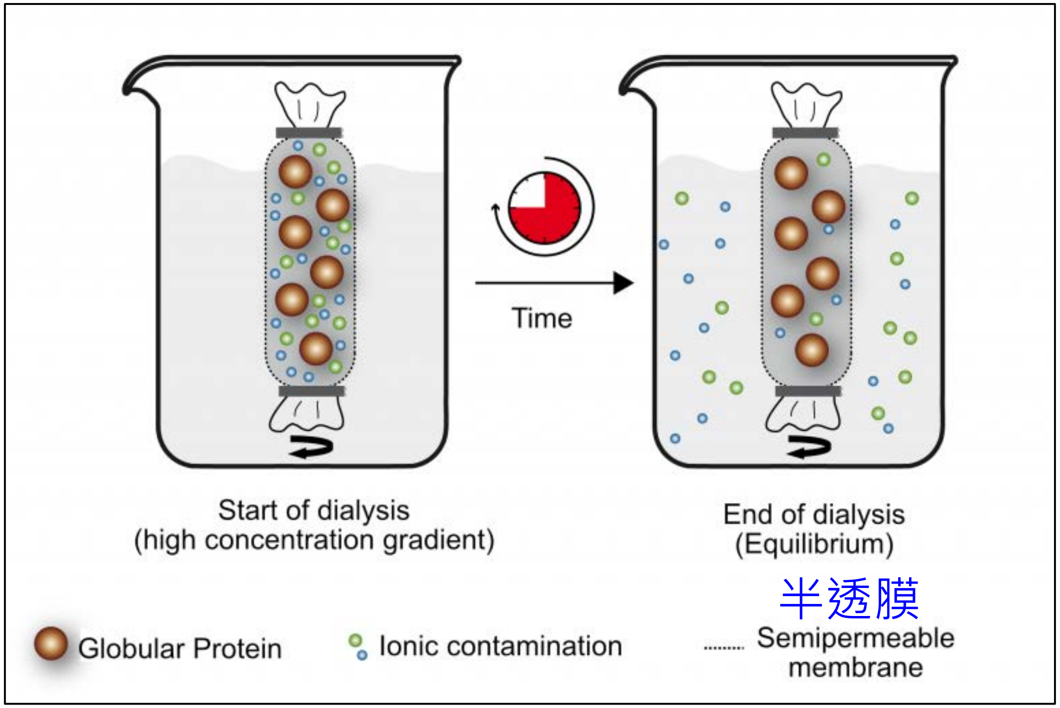
蛋白質分離與純化原理

- 可利用蛋白質的下列特性將蛋白質分離：
  A. 分子量大小。
  B. 帶電特性。
  C. 溶解度。
  D. 蛋白質與特定物質間的吸附作用。

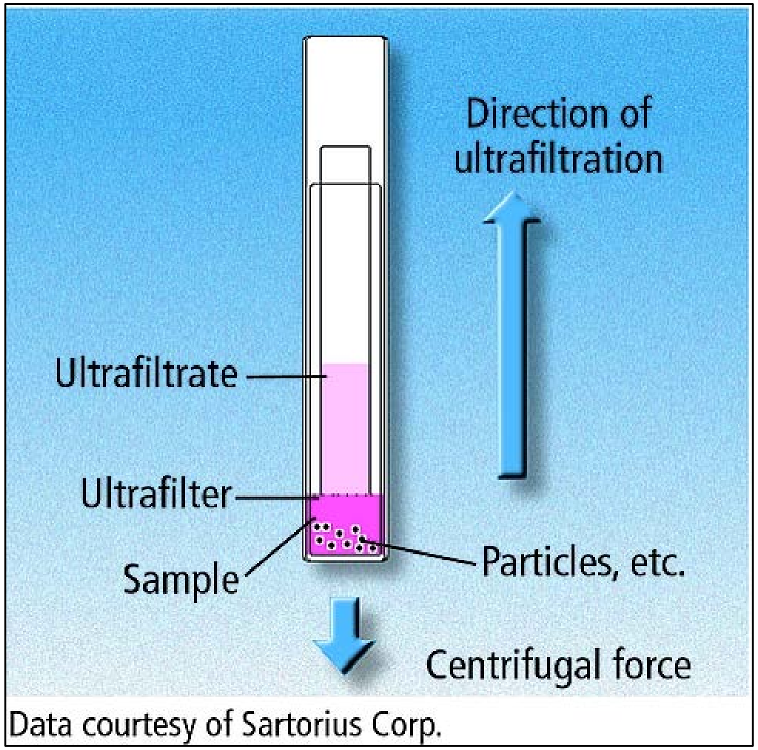
A. 依據分子量大小分離

透析(dialysis)

超過濾(ultrafiltration)

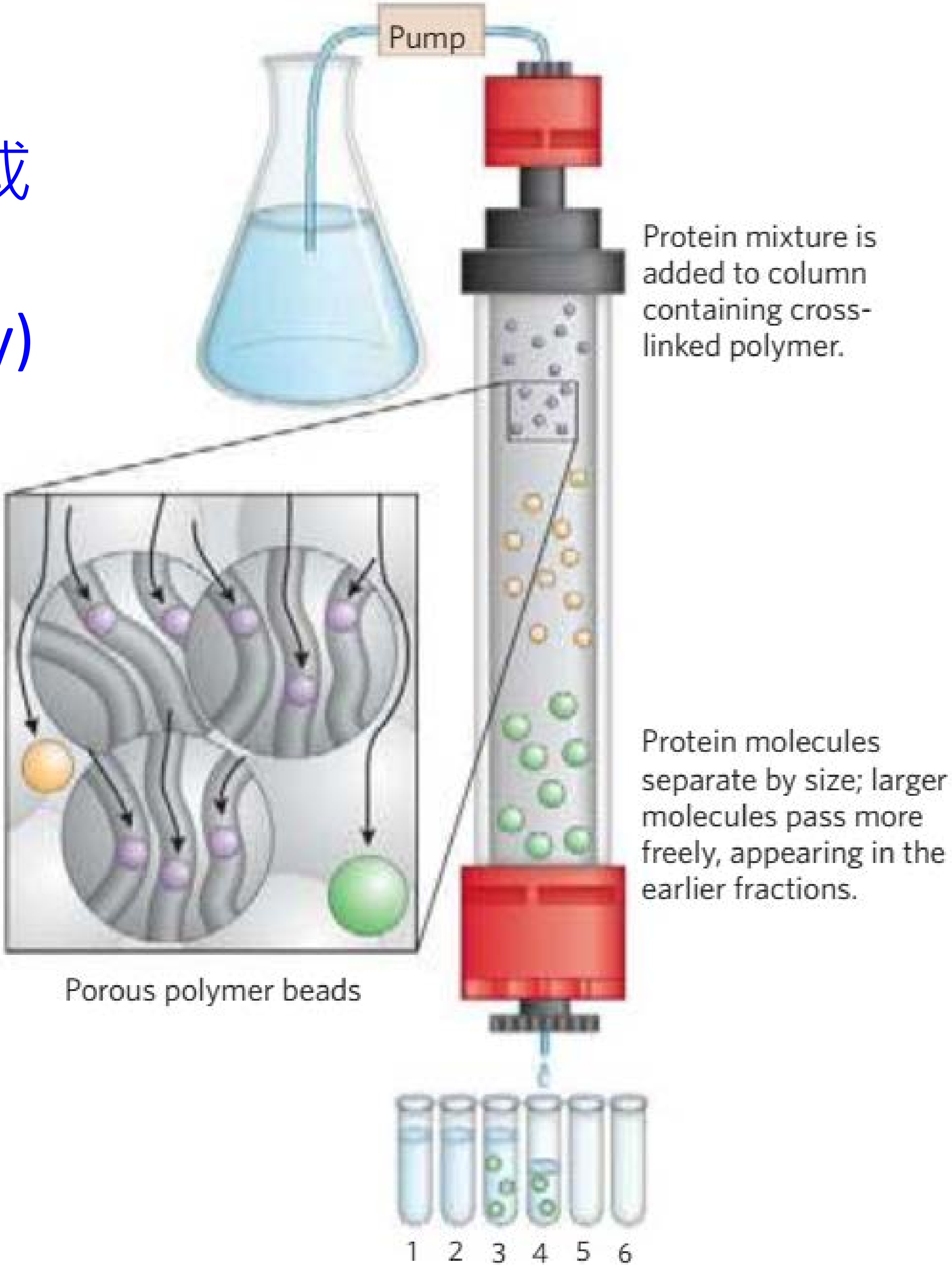


https://www.scienova.com/dialyse/

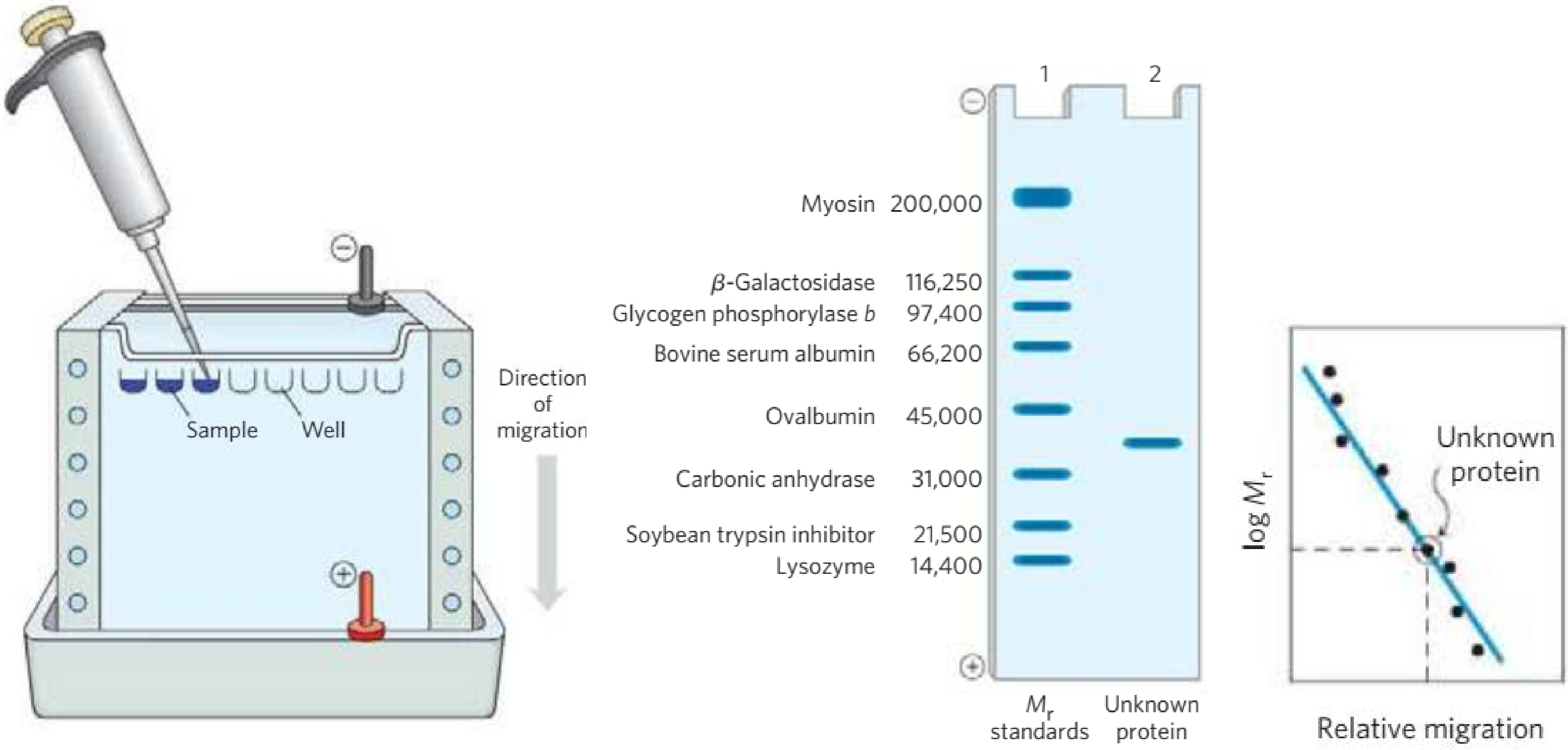


Data courtesy of Sartorius Corp.

分子篩(molecular sieve)或粒徑篩析層析法(size-exclusion chromatography)



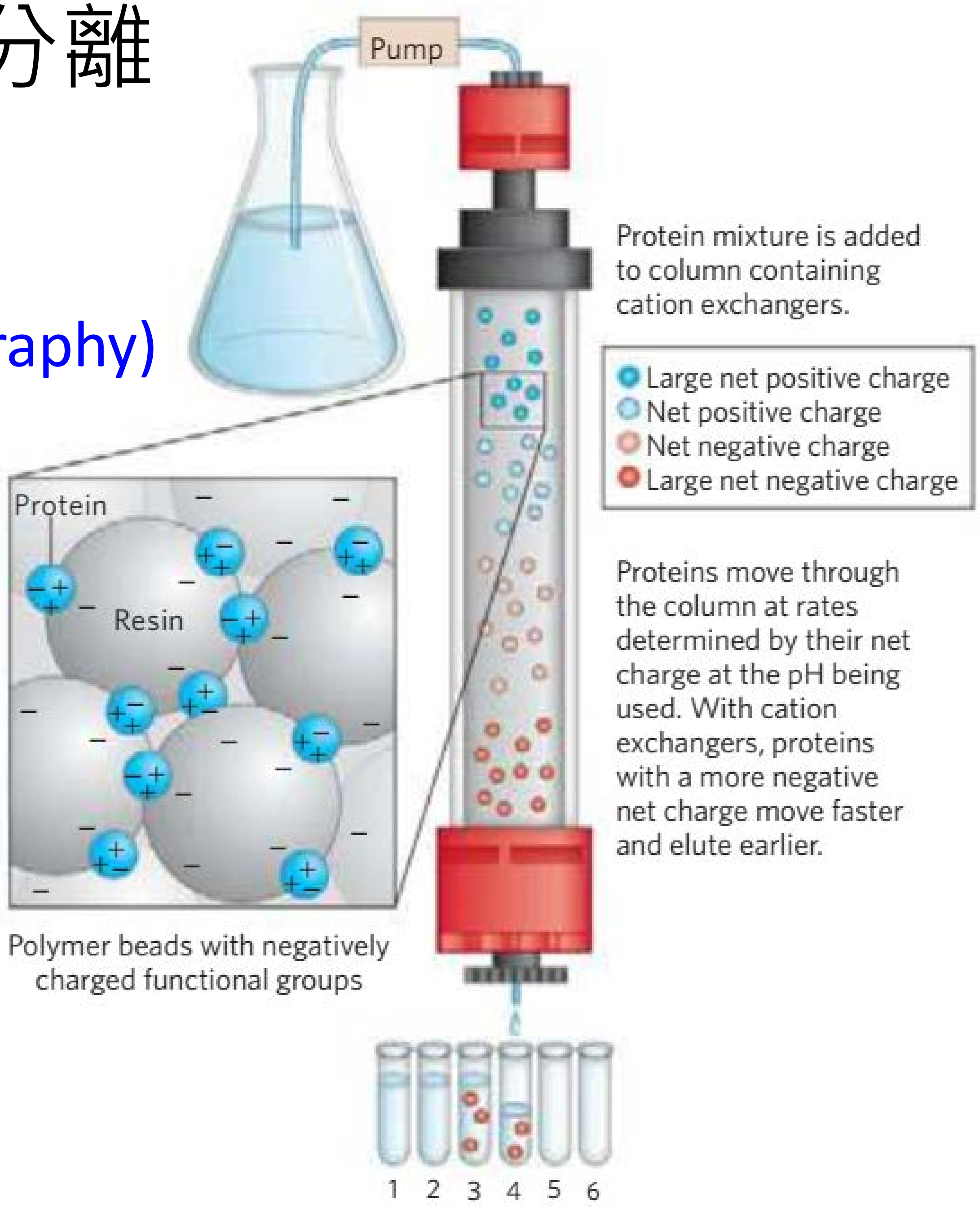
十二烷基硫酸鈉聚丙烯醯胺膠體電泳：(sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE)



SDS-PAGE可用於估量蛋白質分子量

B. 利用帶電特性分離

離子交換層析法
(ion exchange chromatography)



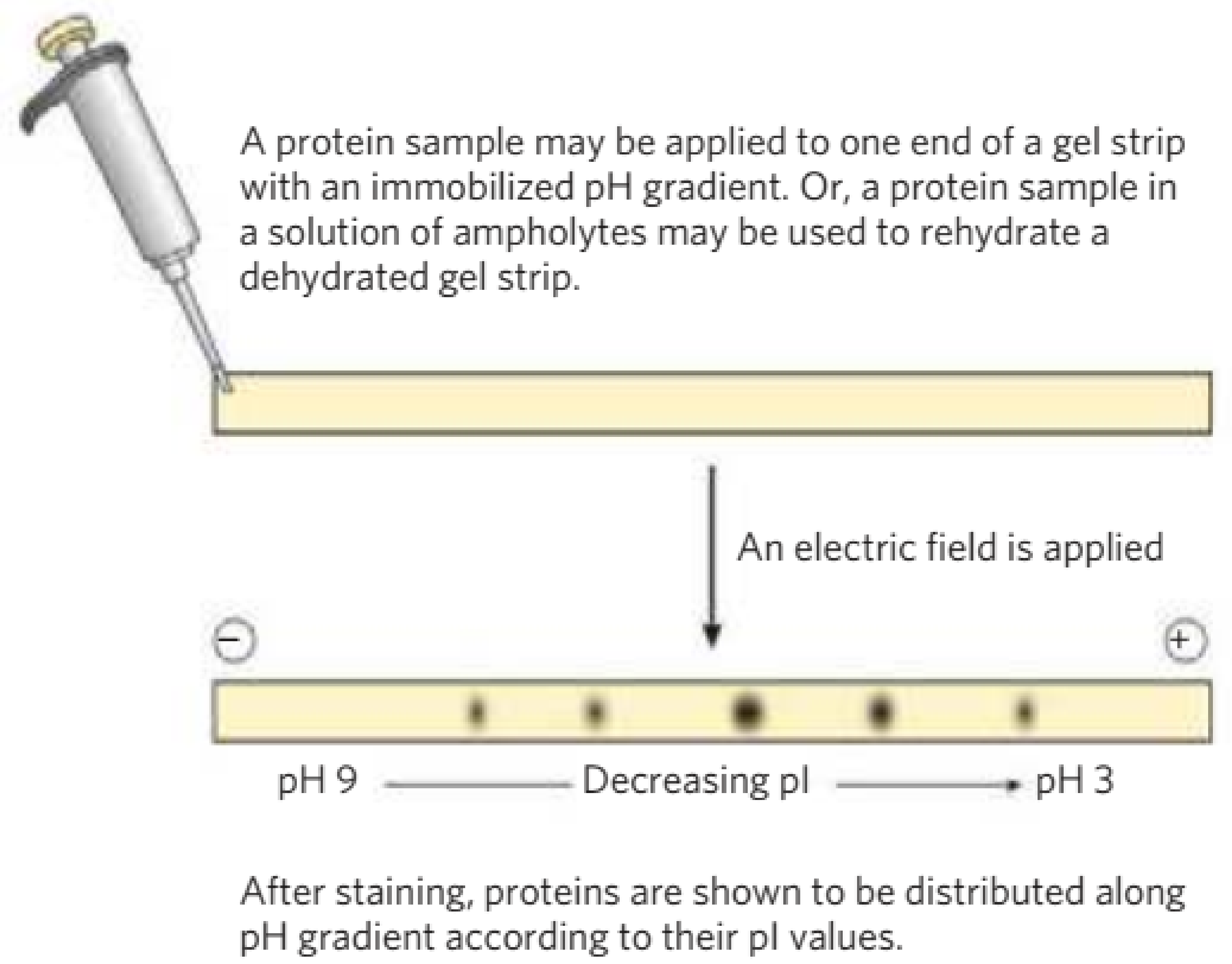
Protein mixture is added to column containing cation exchangers.

- ● Large net positive charge
- ○ Net positive charge
- ○ Net negative charge
- ● Large net negative charge

Proteins move through the column at rates determined by their net charge at the pH being used. With cation exchangers, proteins with a more negative net charge move faster and elute earlier.

Polymer beads with negatively charged functional groups

等電點聚焦法 (isoelectric focusing)

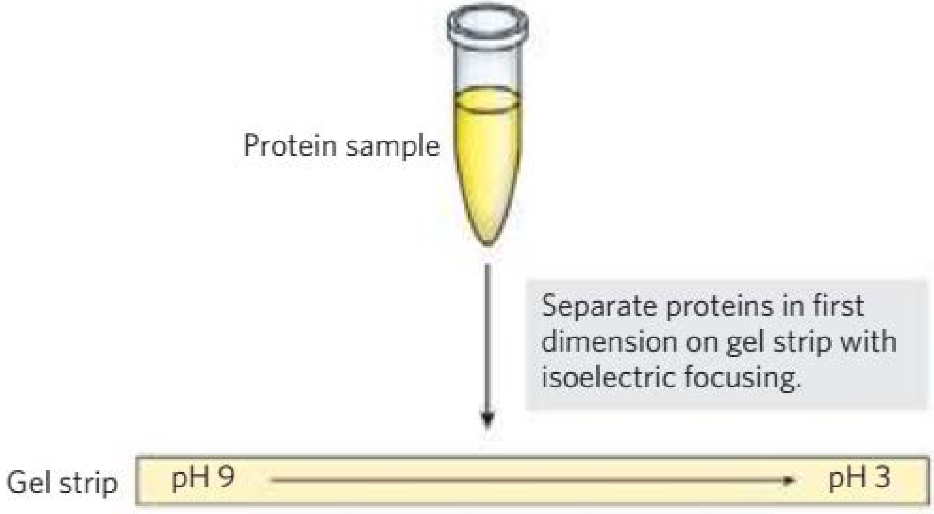


A protein sample may be applied to one end of a gel strip with an immobilized pH gradient. Or, a protein sample in a solution of ampholytes may be used to rehydrate a dehydrated gel strip.

An electric field is applied

After staining, proteins are shown to be distributed along pH gradient according to their pI values.

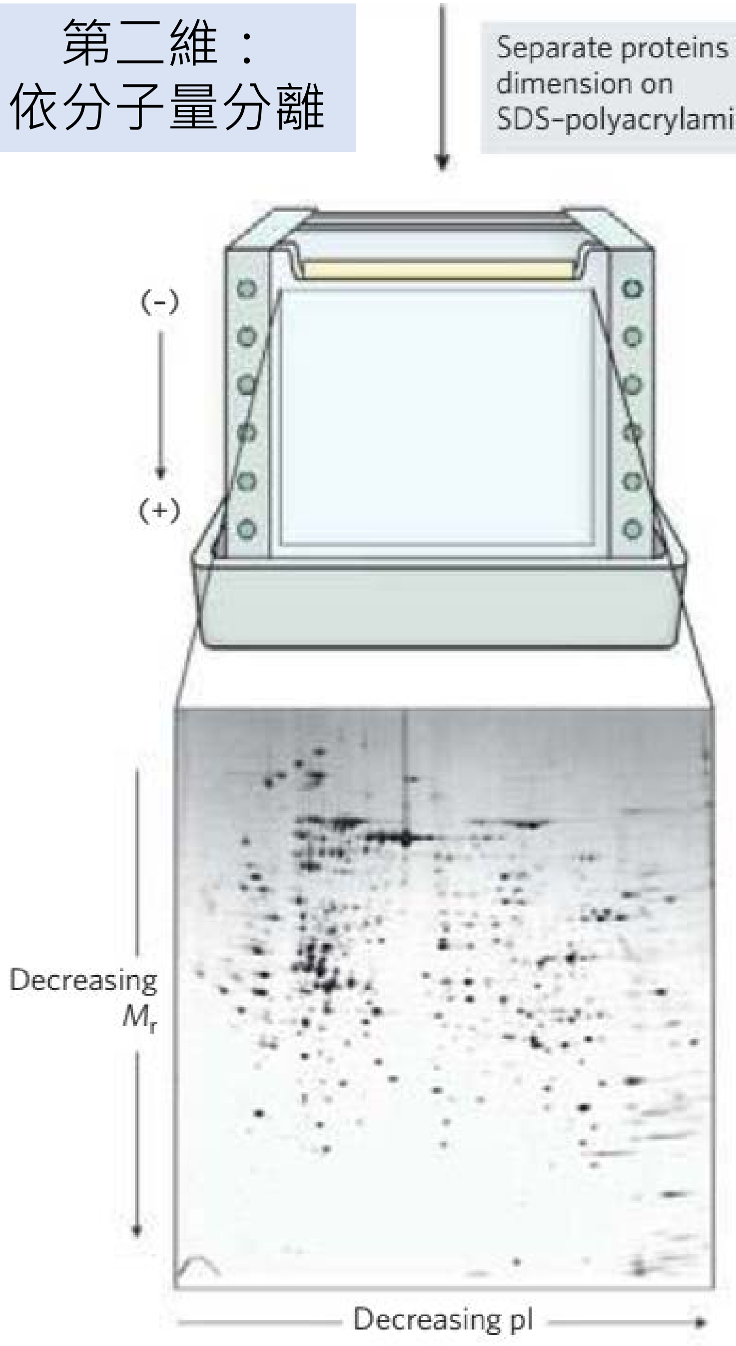
二維電泳
(two-dimensional [2D] electrophoresis)



Separate proteins in first dimension on gel strip with isoelectric focusing.

第一維：依等電點分離

第二維：
依分子量分離

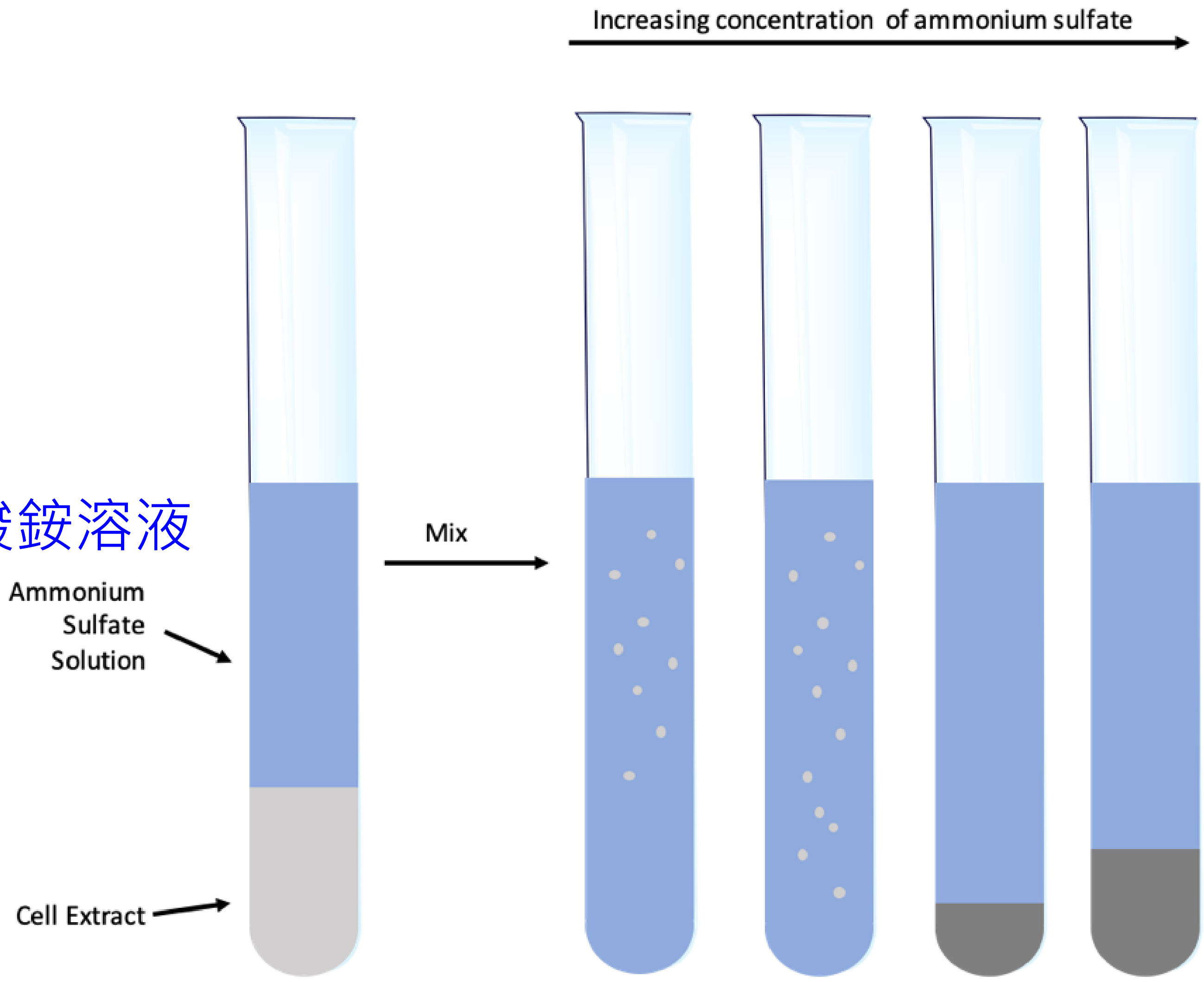


Separate proteins in second dimension on SDS-polyacrylamide gel.

C. 利用溶解度分離

鹽析法
(salting out)

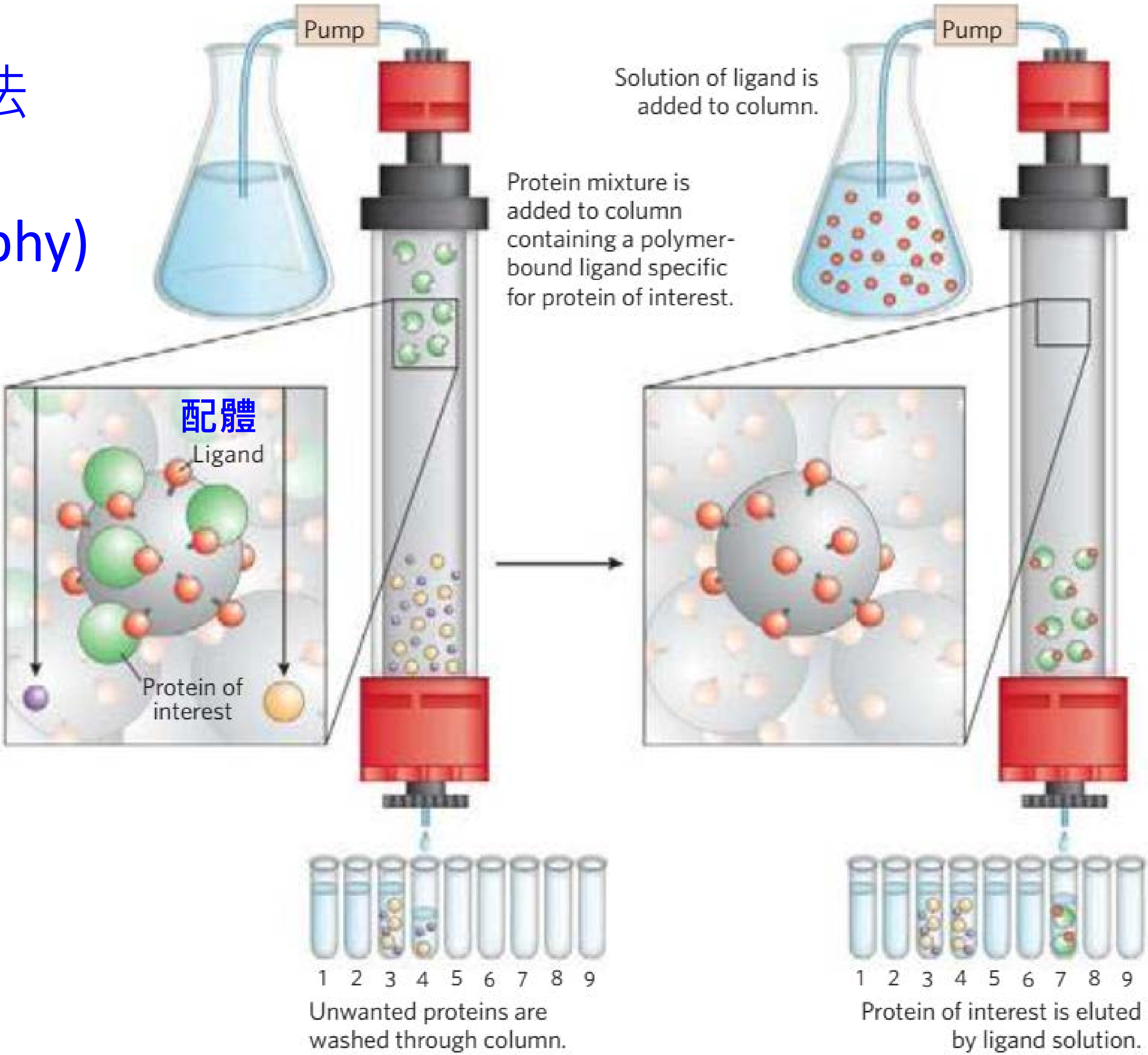
硫酸銨溶液



https://masteringcollegebiochemistry.wordpress.com/2019/01/24/protein-purification-salting-out/

D. 利用專一性吸附作用分離

親和性層析法
(affinity chromatography)

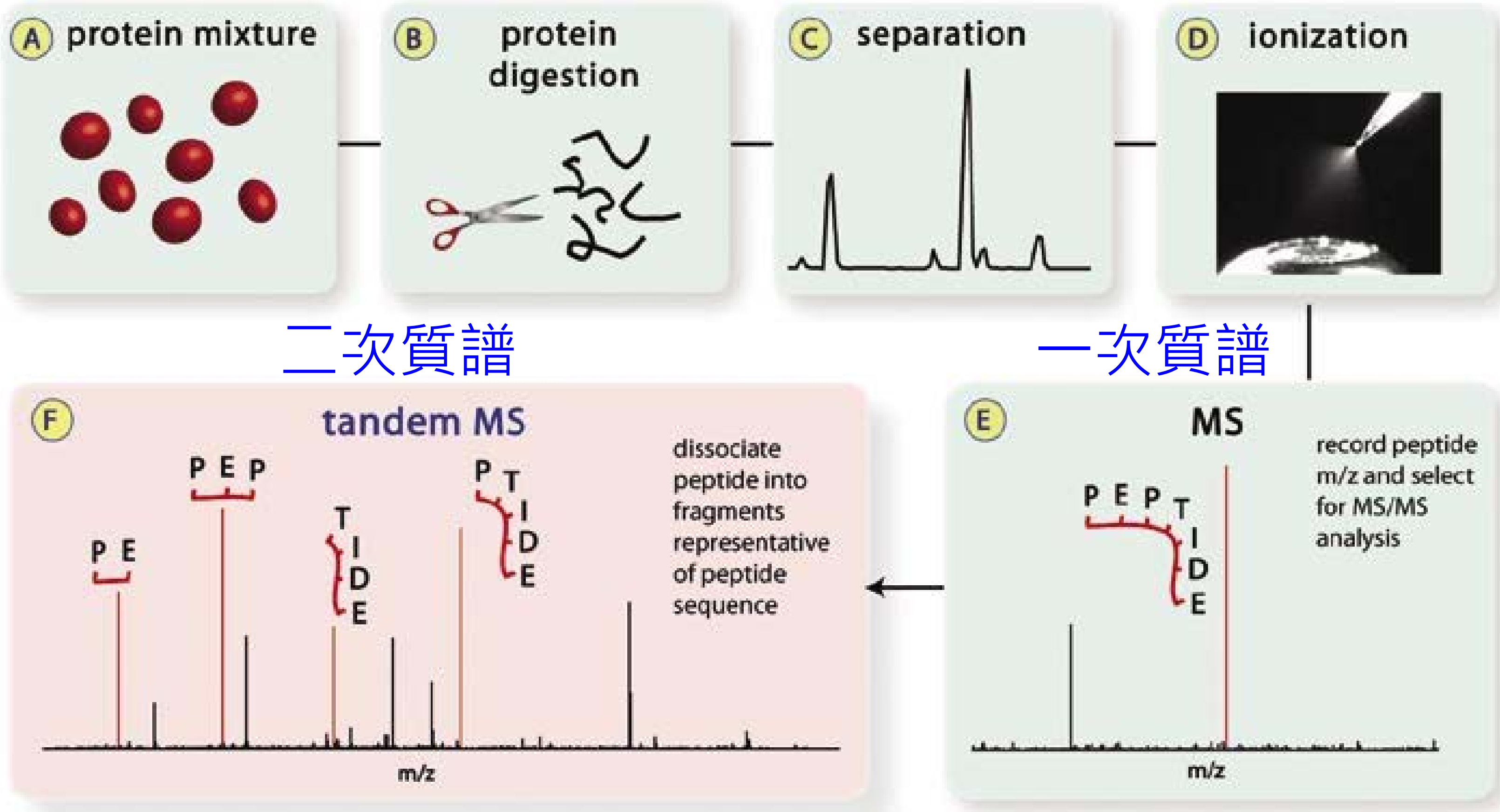


蛋白質組成如何分析？

2. 蛋白質（胜肽）定序：質譜法(mass spectrometry)

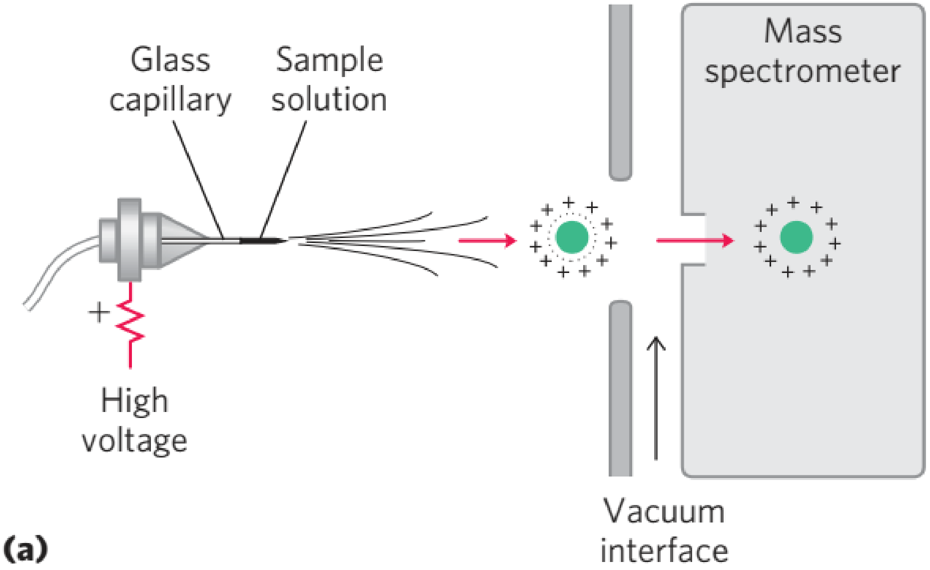
蛋白質混合物　蛋白質分解　胜肽分離　游離化

二次質譜　一次質譜

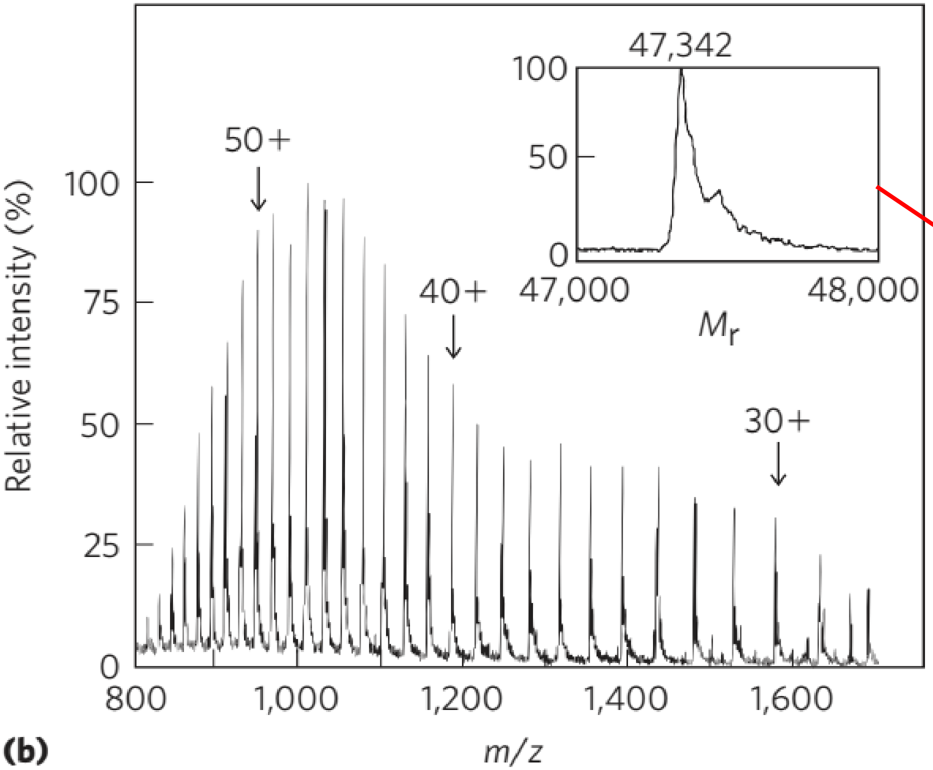


https://www.future-science.com/doi/full/10.2144/05384TE01

游離化與一次質譜



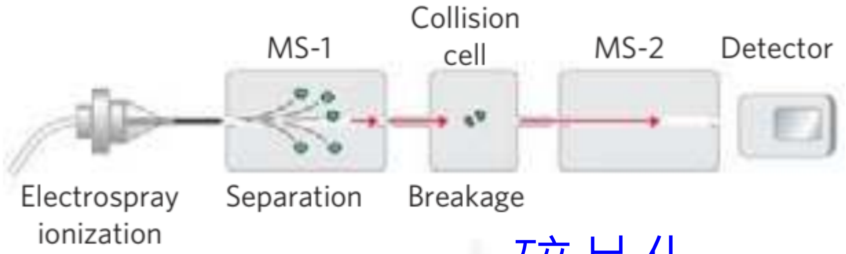
電灑游離法 (electrospray ionization)



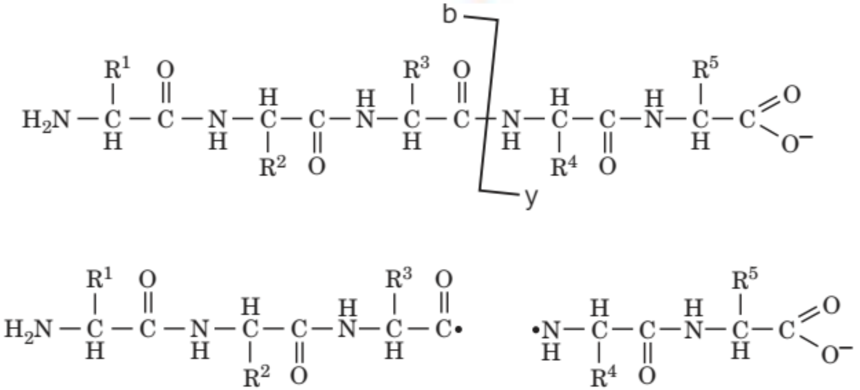
電灑游離法所得出之一次質譜

Computer-generated transformation of the mass spectrum

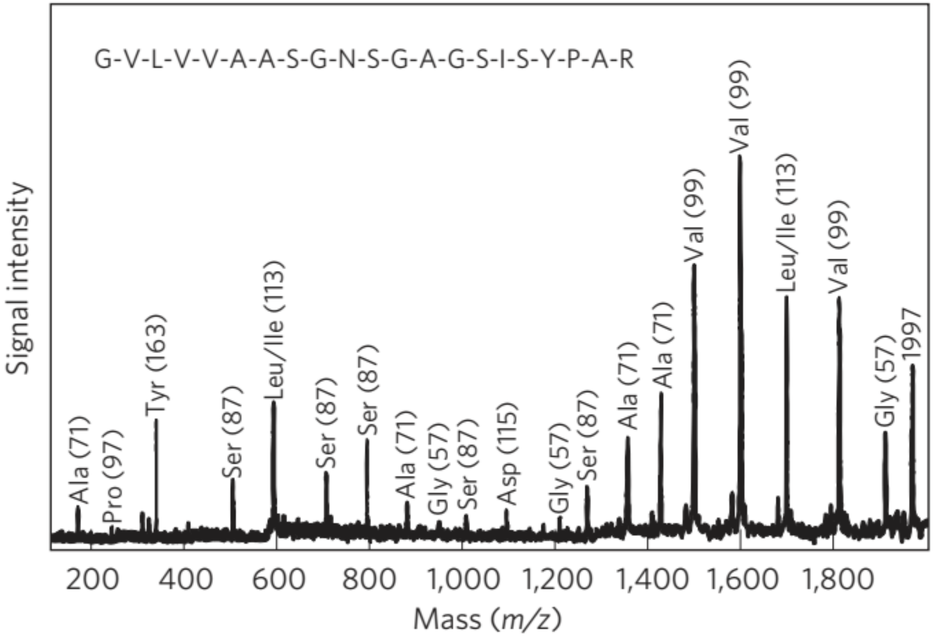
利用串連質譜法(tandem mass spectrometry)取得二次質譜



碎片化 (fragmentation)



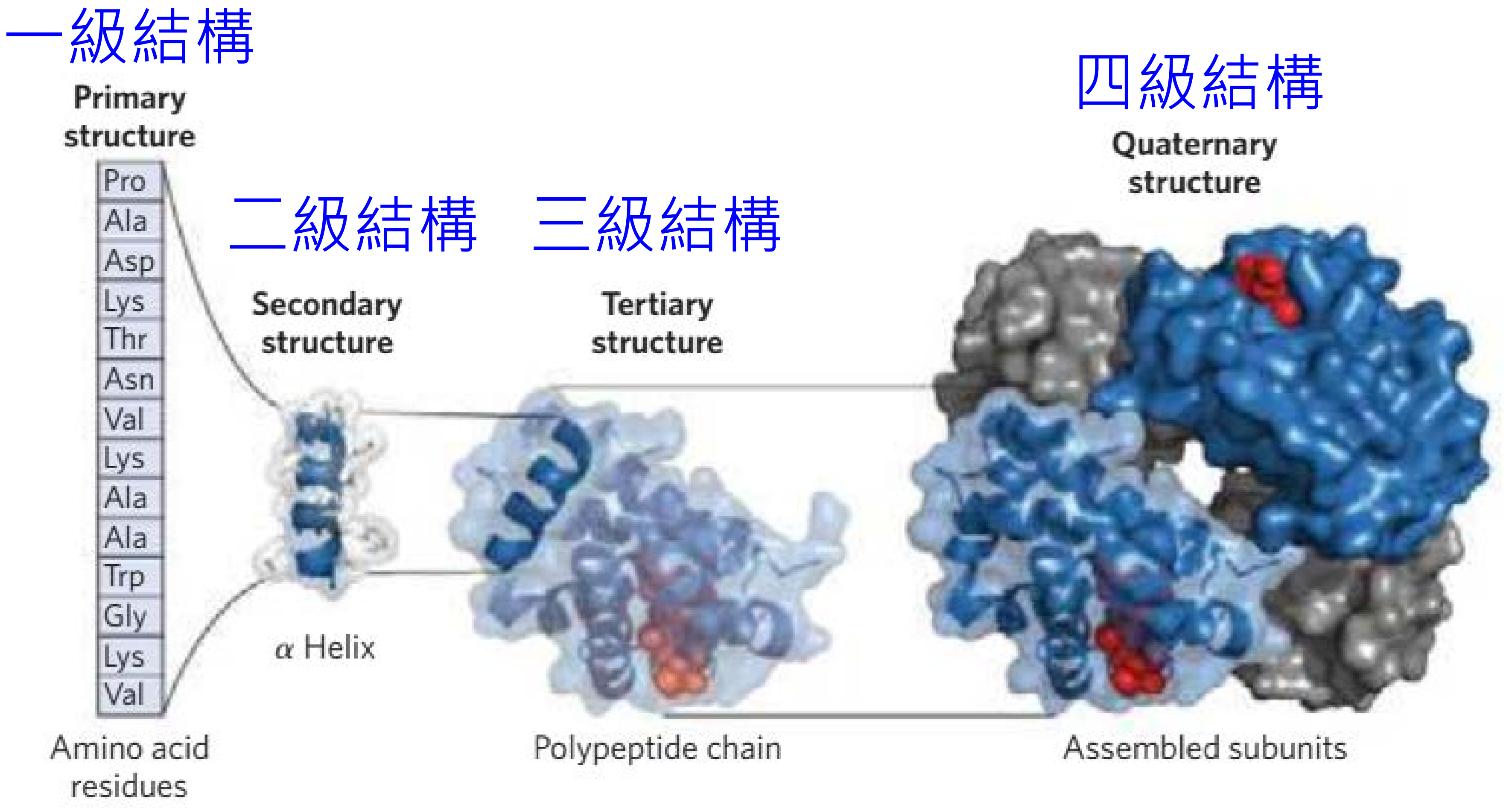
二次質譜



The labeled peaks are y-type ions derived from amino acid residues. The number in parentheses over each peak is the molecular weight of the amino acid ion. The successive peaks differ by the mass of a particular amino acid in the original peptide. The deduced sequence is shown at the top.

蛋白質的結構

- 蛋白質的四級結構



一級結構

二級結構

三級結構

四級結構

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-摺片與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。

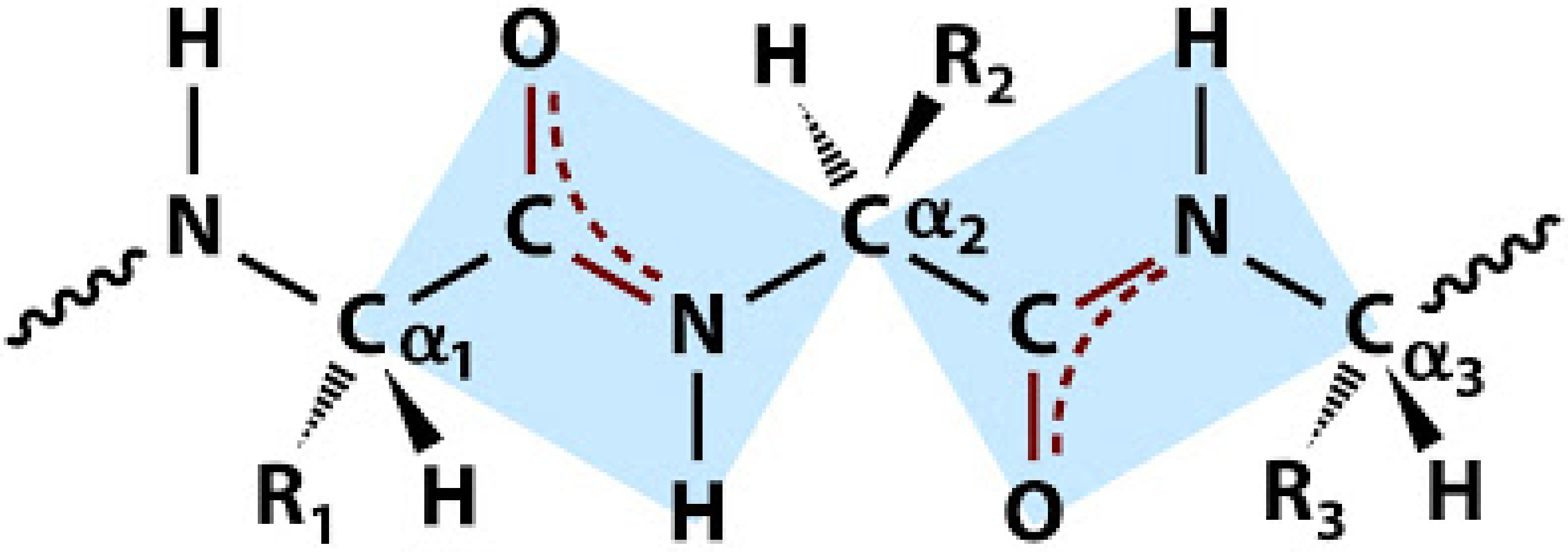
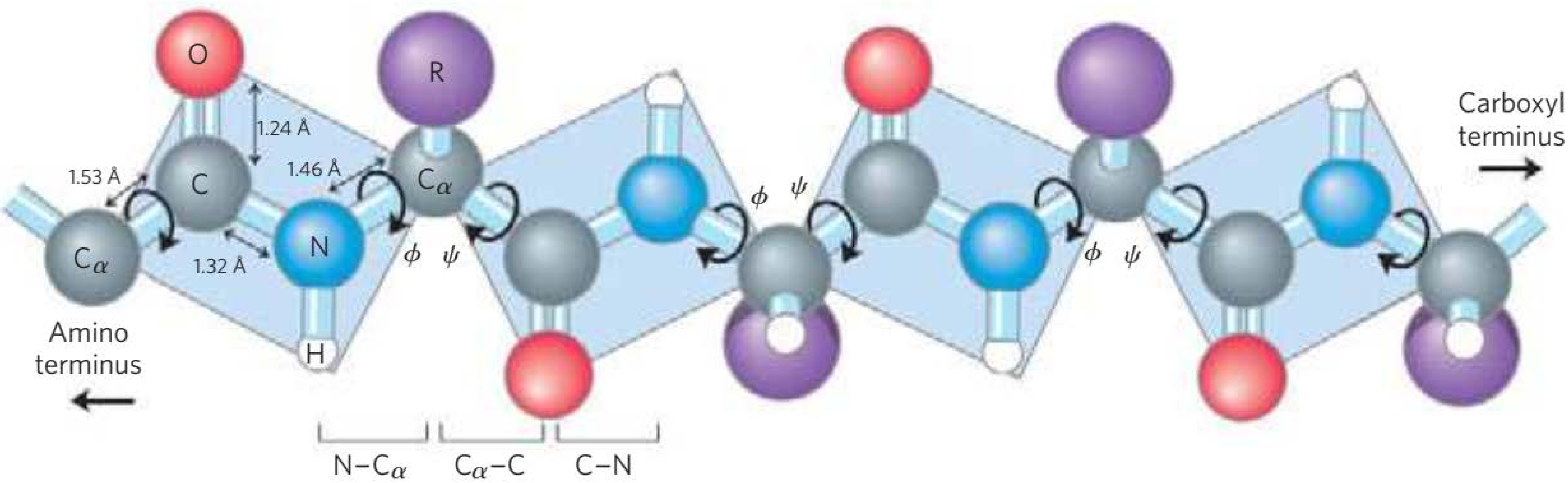


Figure 4-6 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

胜肽中可旋轉的共價鍵

- C_α上的ϕ(phi)與ψ(psi)角



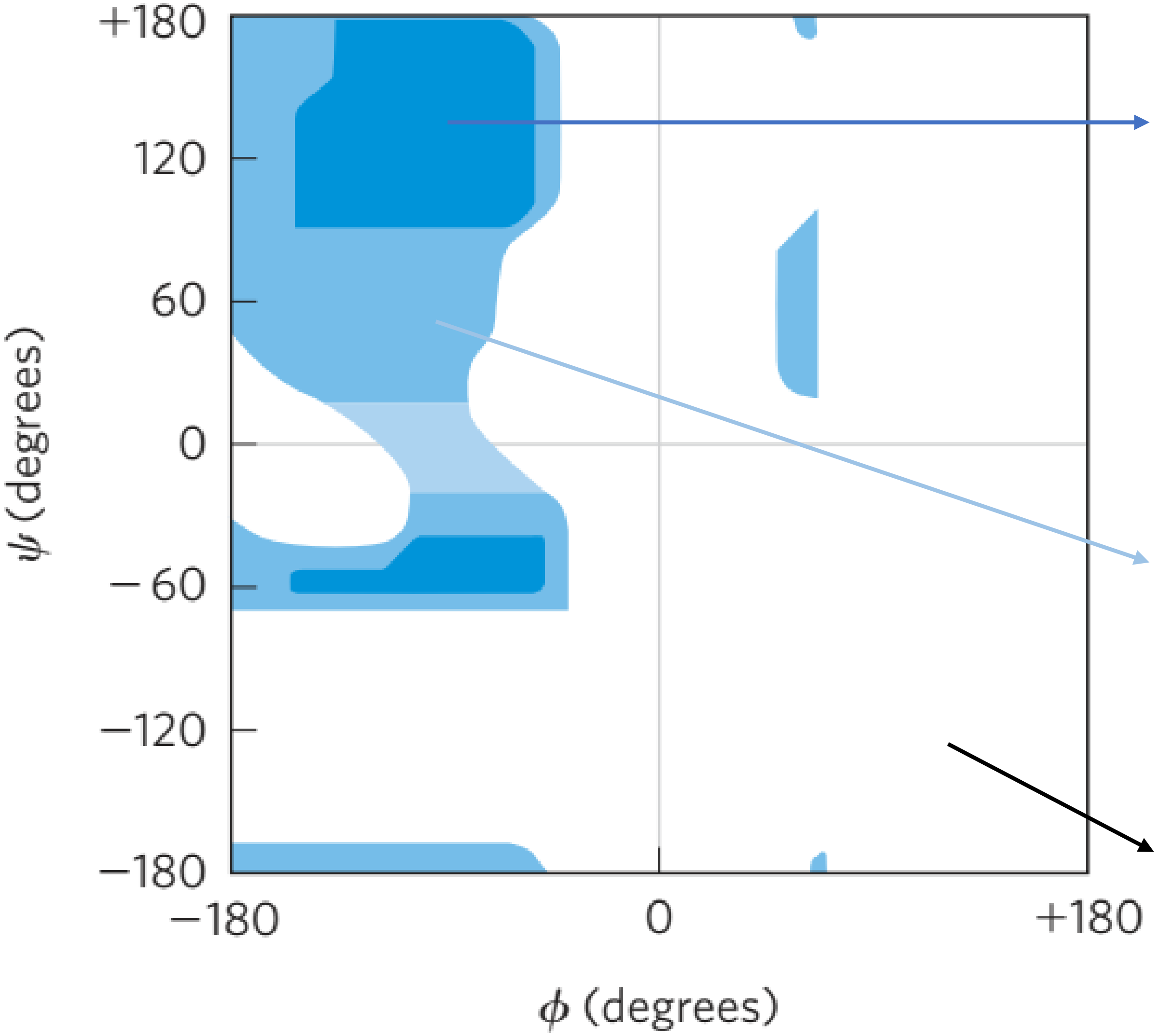
Ramachandran Principle：
蛋白質中的ϕ(phi)與ψ(psi)角

An animated atomic model:

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

Ramachandran Plot

- Ramachandran plot for L-Ala residues
丙胺酸

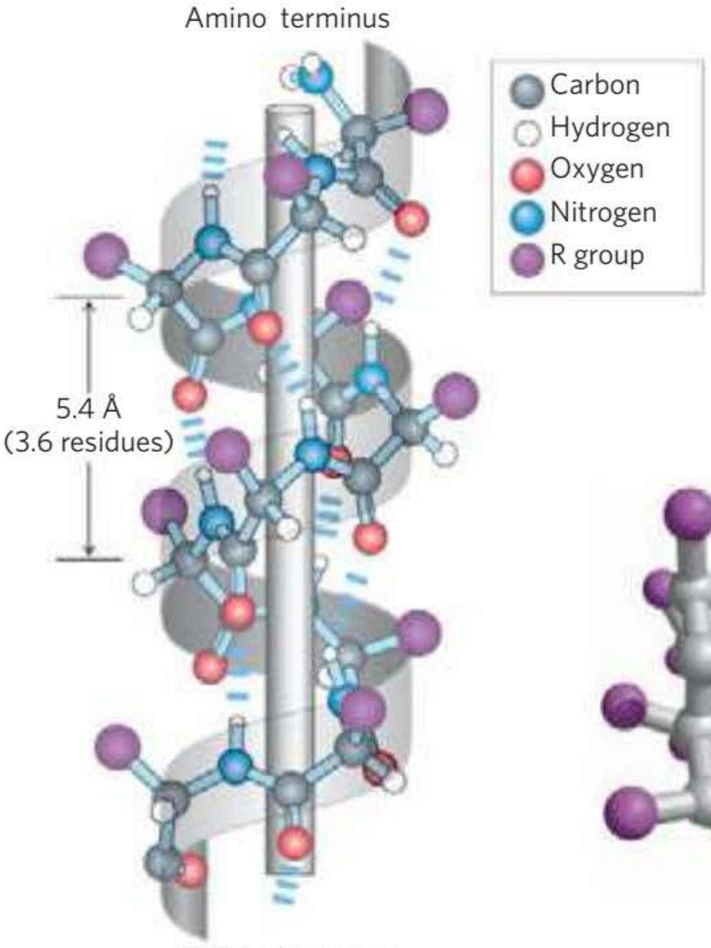


Dark blue:
fully allowed angles

Lighter blue:
allowed angles,
but less favorable

White region:
angles that are not
allowed due to steric
hindrance (clashes)

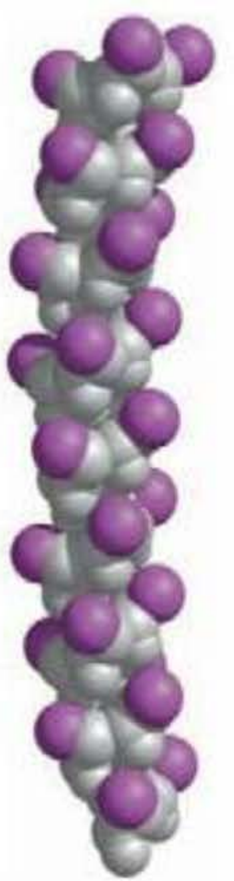
α-螺旋結構



(a)

球棒模型

(b)



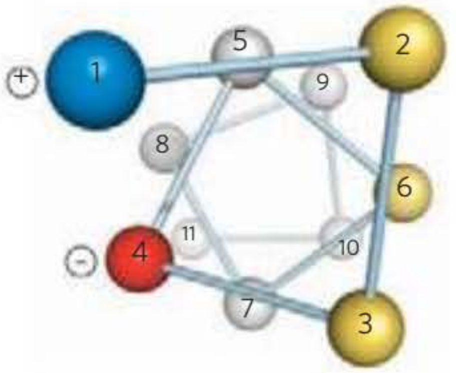
空間填充模型

(c)

黃色：疏水性側鏈

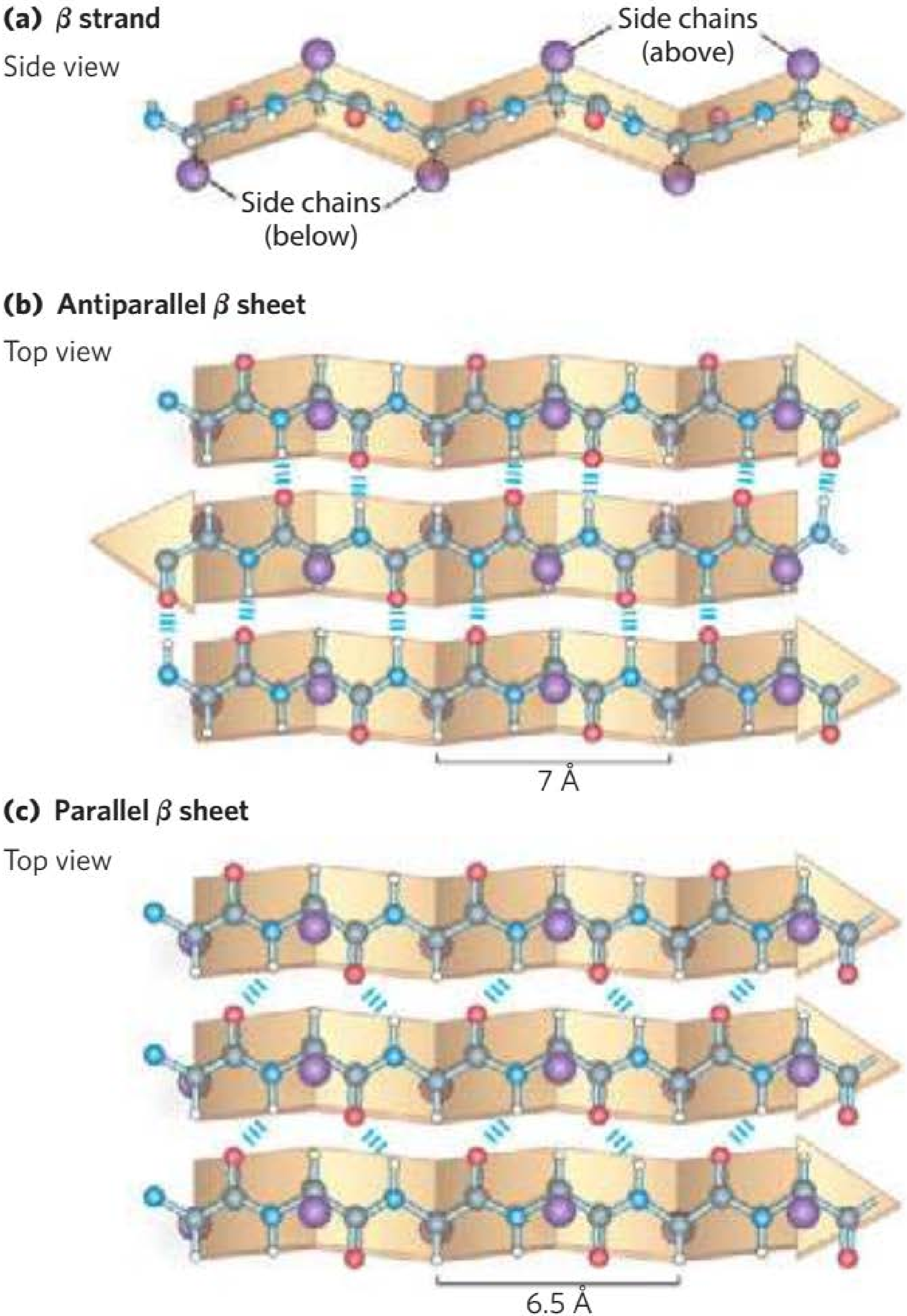
藍色：正電荷側鏈

紅色：負電荷側鏈

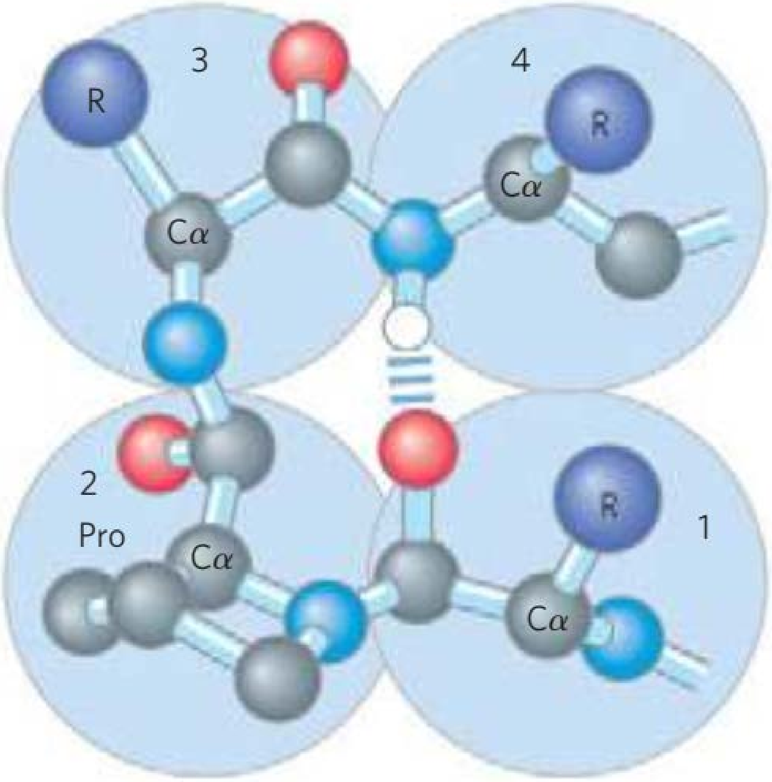


(d)

β-褶片結構

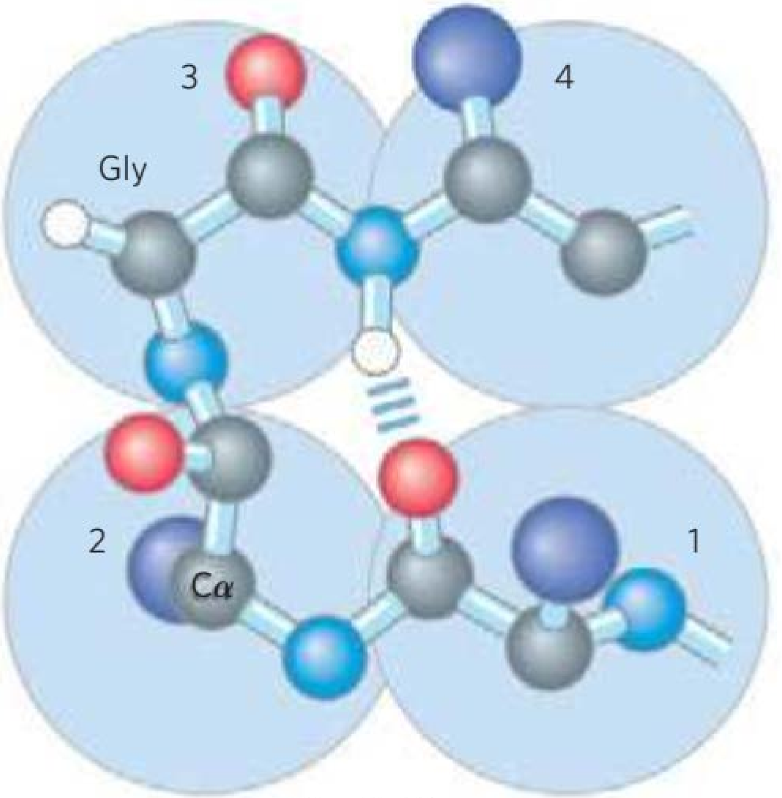

(a) ***β* strand**
Side view
Side chains (above)
Side chains (below)
(b) Antiparallel *β* sheet
Top view
7 Å
(c) Parallel *β* sheet
Top view
6.5 Å

β-轉角結構



Type I β turn

較為普遍

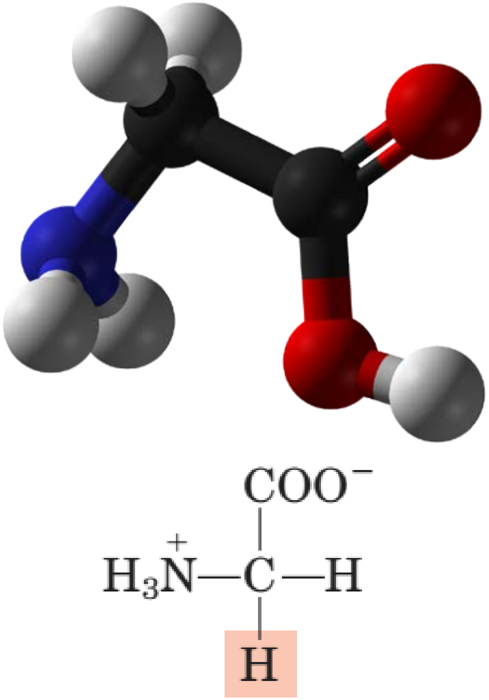


Type II β turn

- Not all H atoms are shown in these depictions.

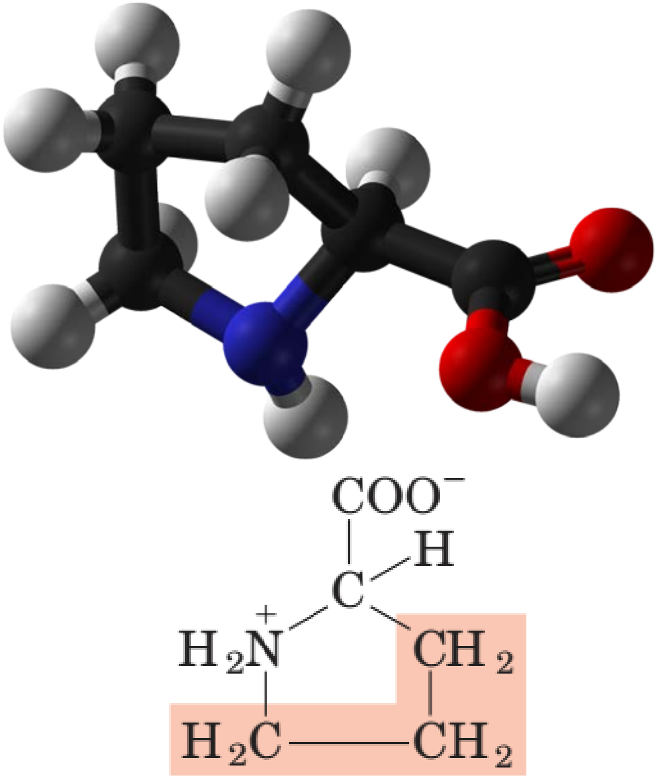
胺基酸對二級結構靈活性（ flexibility ）的影響

- 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

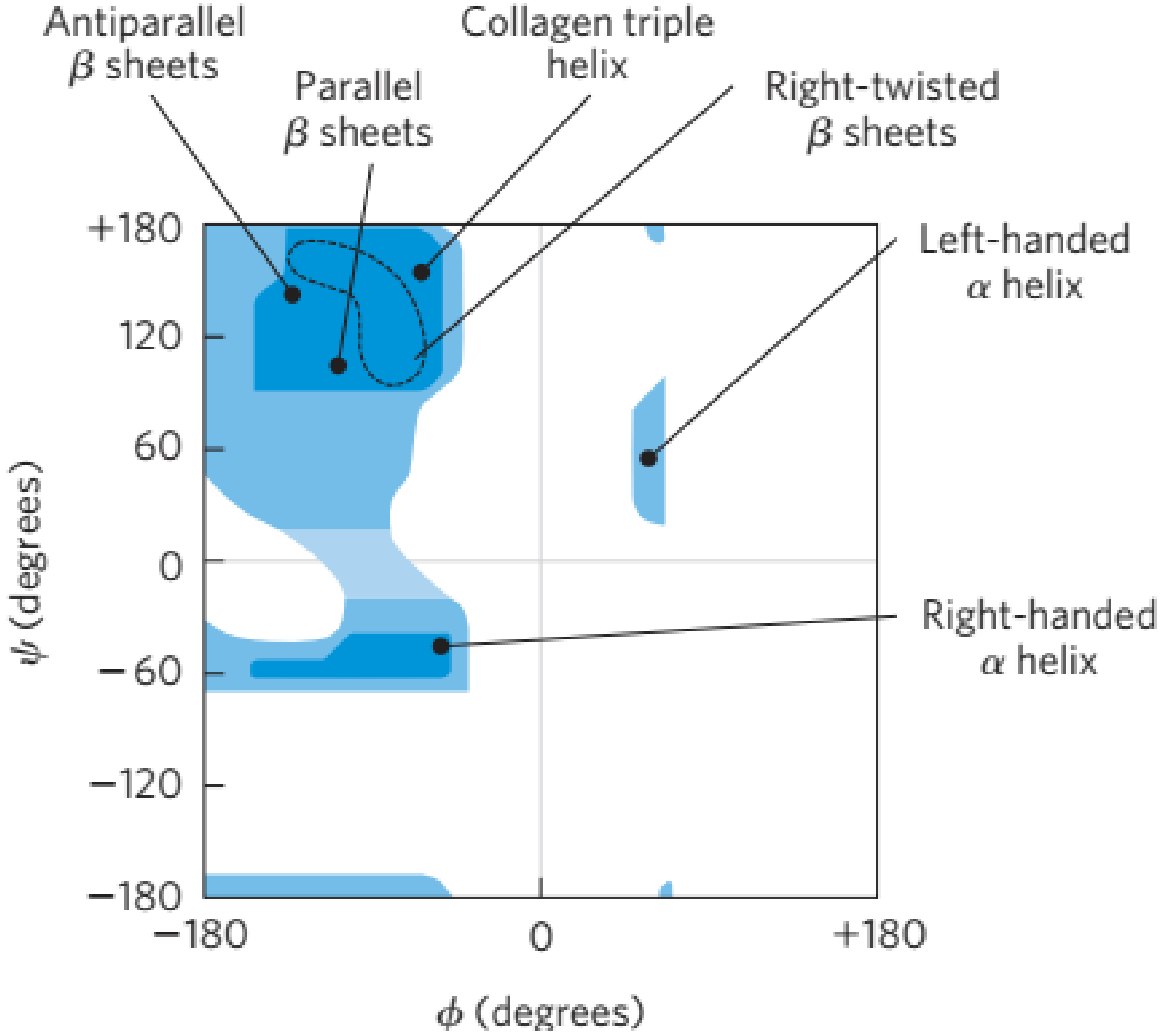
Glycine



脯胺酸

Proline

蛋白質二級結構具有固定範圍的φ與ψ角

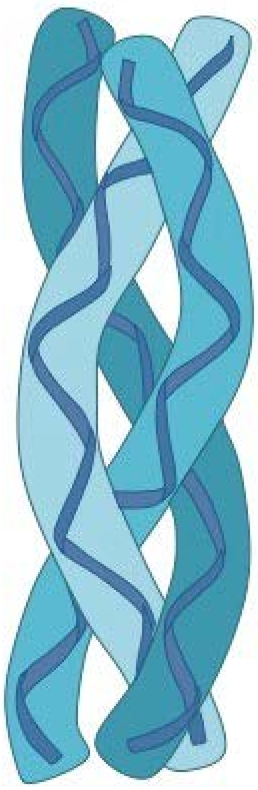


蛋白質的三、四級結構

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。

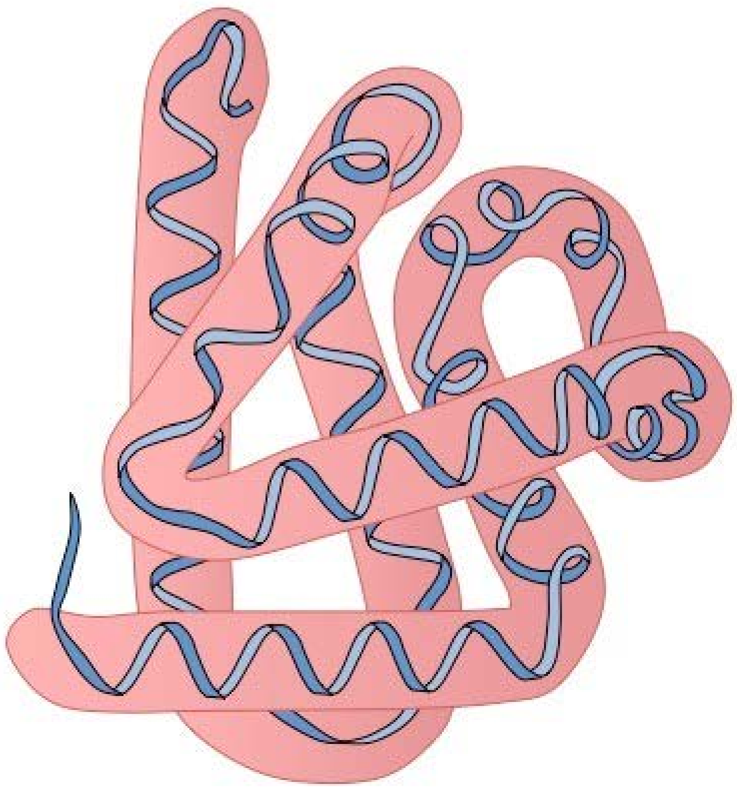
蛋白質三級結構的兩大分類



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。

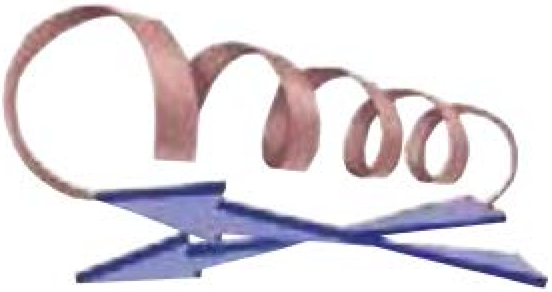
https://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/24-proteins/fibrous-vs-globular-protein.html

蛋白質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。

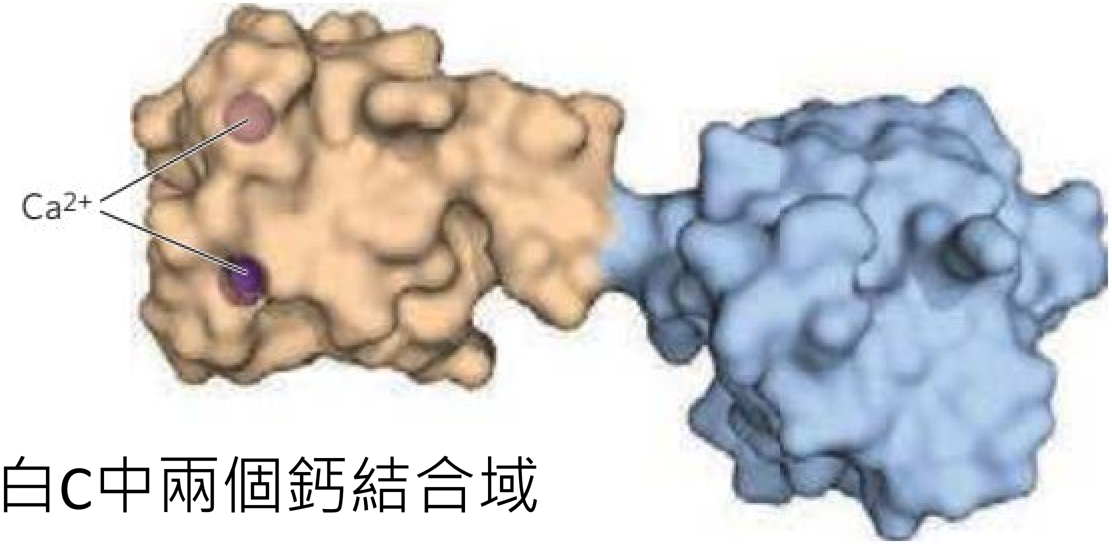
Motifs 模體



(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

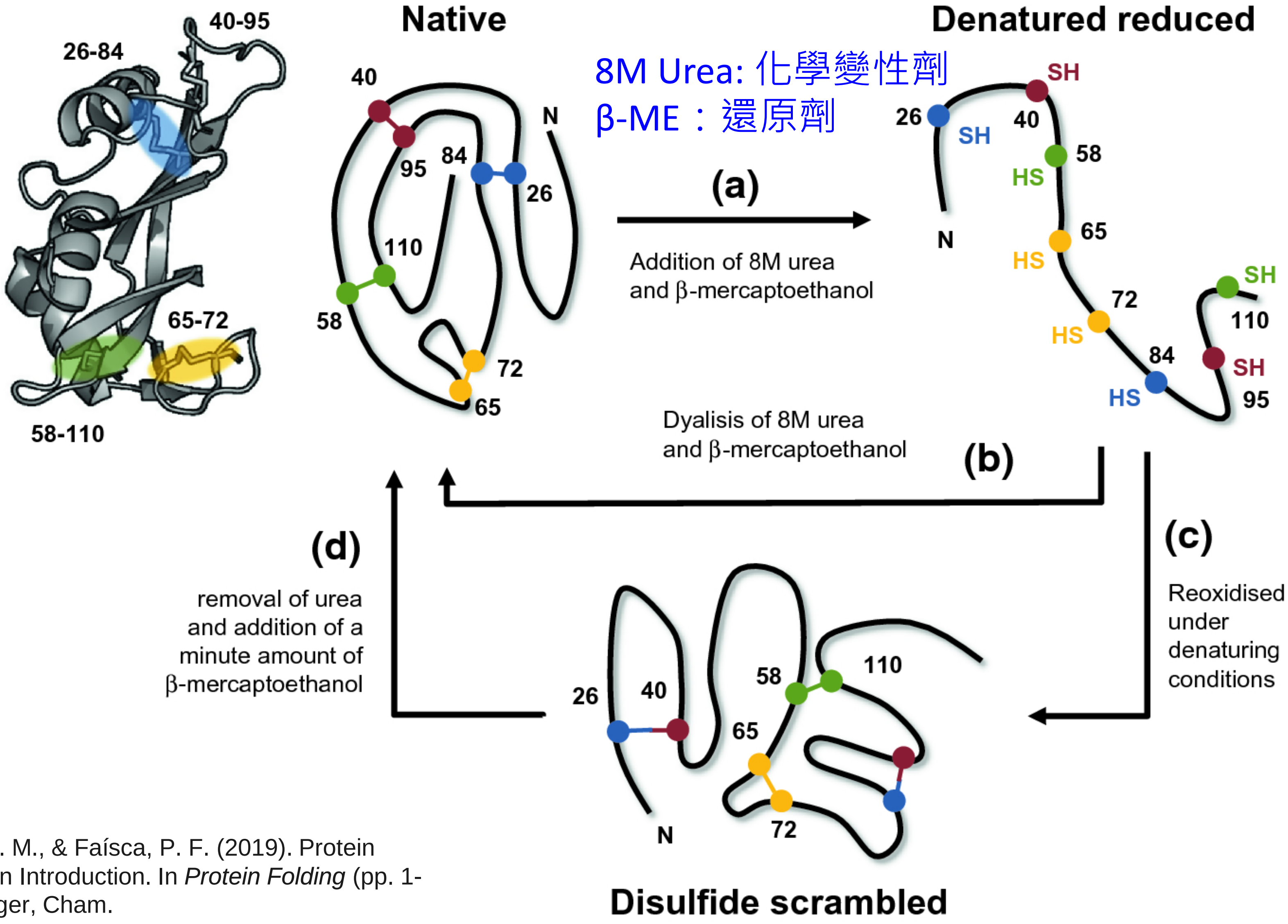
    ✓例如二、三、四級結構中的氫鍵、離子鍵、凡得瓦力與疏水作用。

    ✓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。

蛋白質結構由何者決定？

- Anfinsen等人的實驗：



8M Urea: 化學變性劑
β-ME：還原劑

Gomes, C. M., & Faísca, P. F. (2019). Protein Folding: An Introduction. In *Protein Folding* (pp. 1-63). Springer, Cham.

Anffisen法則

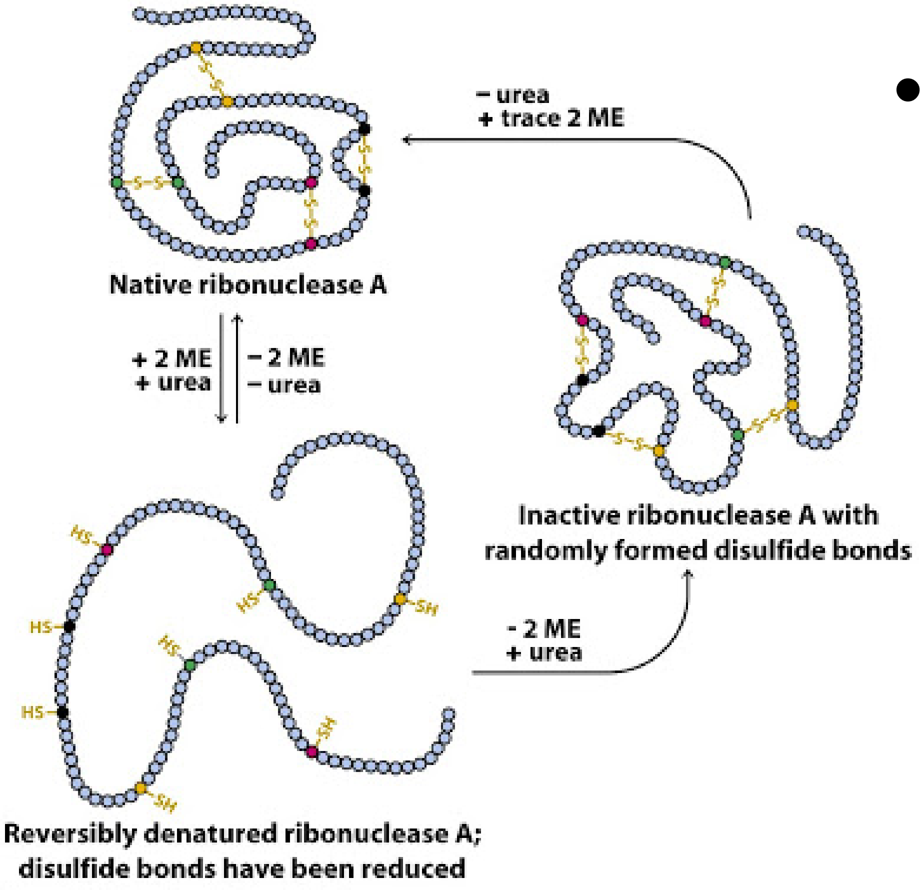


Figure 4-29 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

- 胺基酸序列（一級結構）決定蛋白質的結構。
  - 後續實驗指出，並非所有蛋白質都可以在試管中復性(renaturation)，只有少部份小形、穩定的蛋白可以。

https://greatexperimentsblog.blogspot.com/2017/11/anfinsens-dogma.html

蛋白質如何摺疊出有生物活性的結構？

- Levinthal悖論(1969)
  - 假設一個蛋白質有100個胺基酸，一個胺基酸可以有10種不同φ與ψ角的立體結構 → 這個蛋白質有10^{100}種不同結構的可能性。
  - 如果測試每一種可能性要花10^{-13}秒，那麼需要10^{77}年才能把所有構形全部試過。

  - 這個悖論同時也是現今從頭開始(*de novo*)蛋白質結構預測的最大問題。

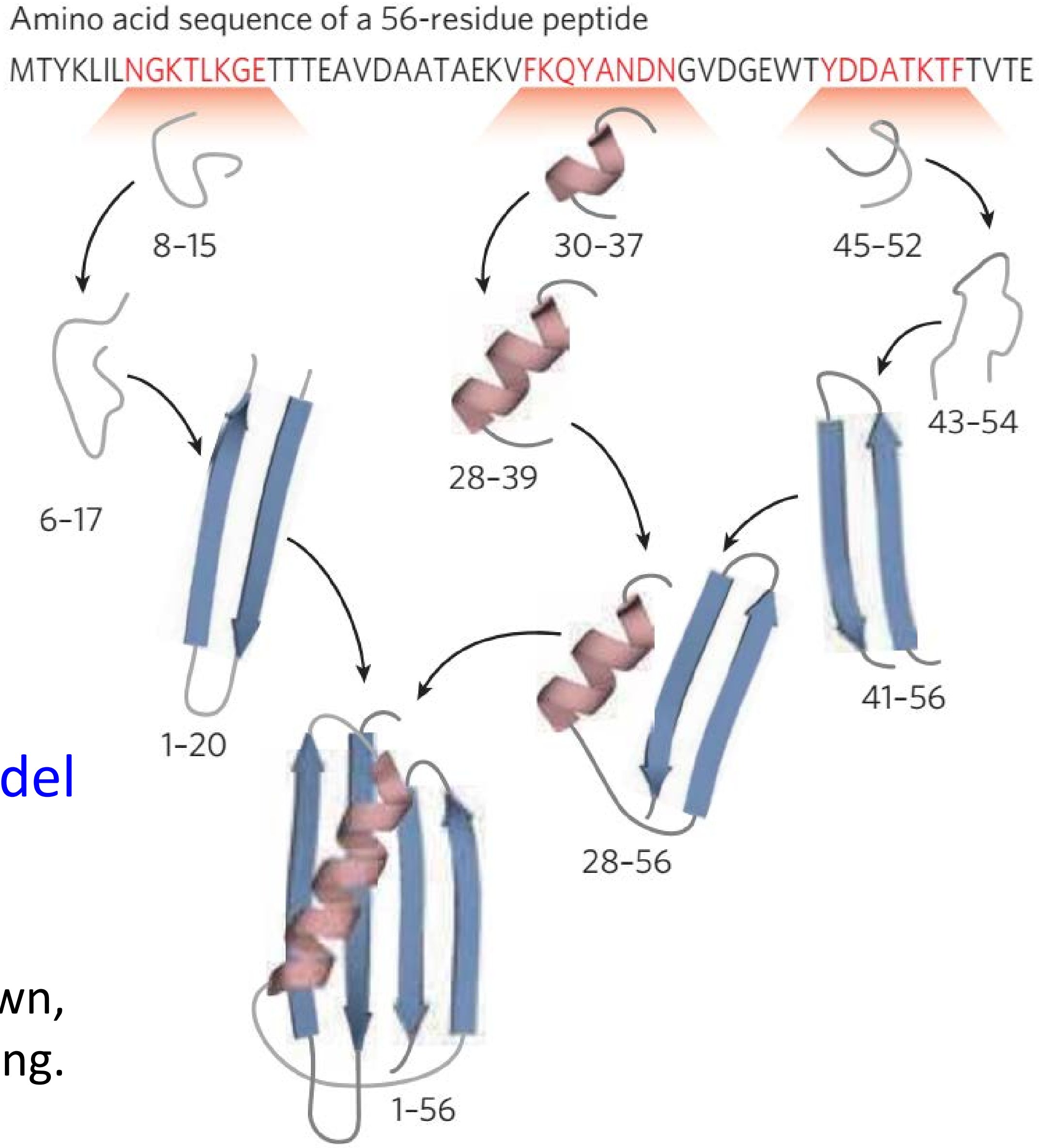
蛋白質的摺疊途徑

- 分層式途徑

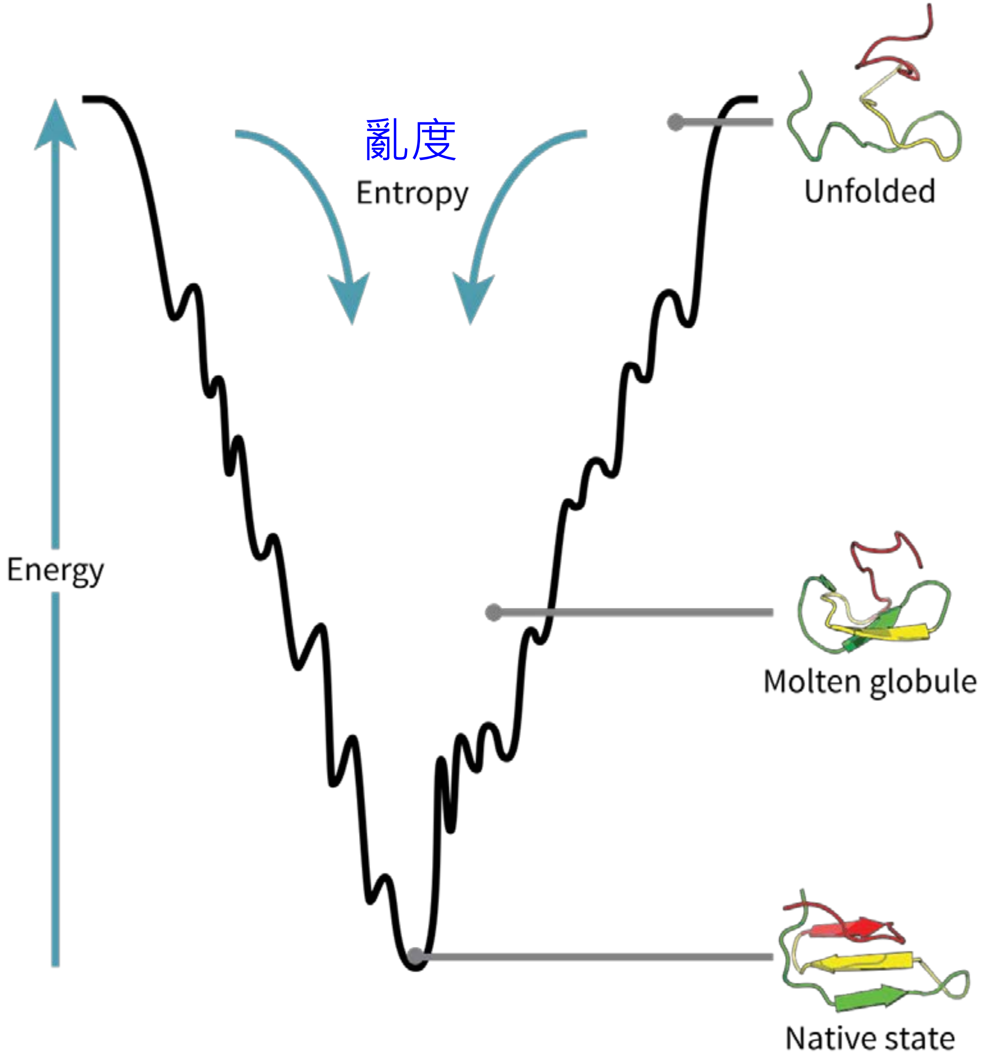
二級結構→模體→結構域→三級結構

Nucleation-condensation model

A hierarchical pathway is shown, based on computer modeling.



蛋白質摺疊的折疊漏斗(folding funnel)理論



A typical protein has multiple possible folding intermediates with significant stability on the multiple pathways leading to the native structure.

半成形蛋白球

https://en.wikipedia.org/wiki/Folding_funnel

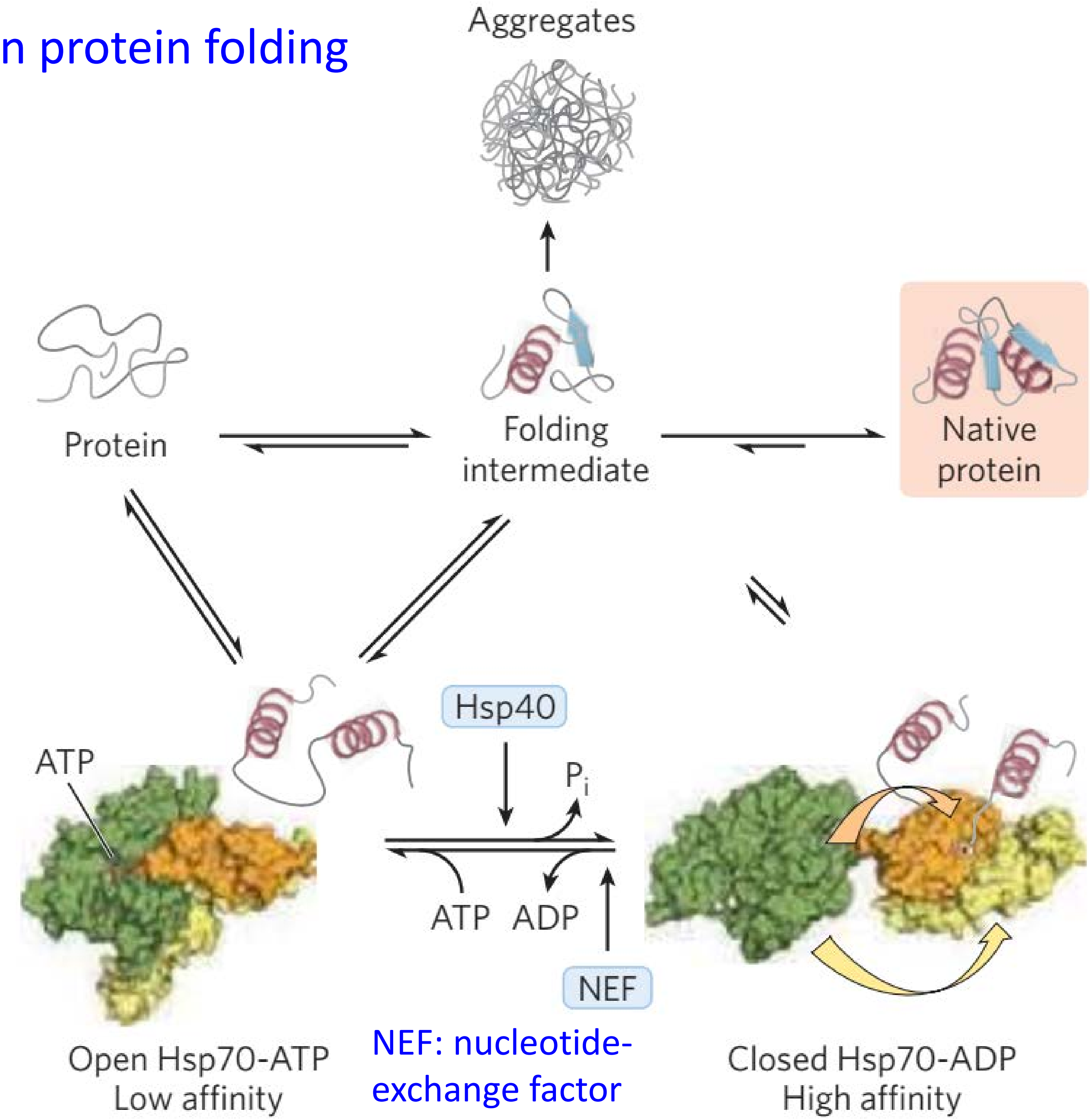
利用其他蛋白質協助蛋白質摺疊

- 蛋白質在合成後，並非所以蛋白直接能自發地摺疊成正確的構形，需要許多其他蛋白質的協助。

A. 伴護蛋白(chaperone)有兩大家族(family)：

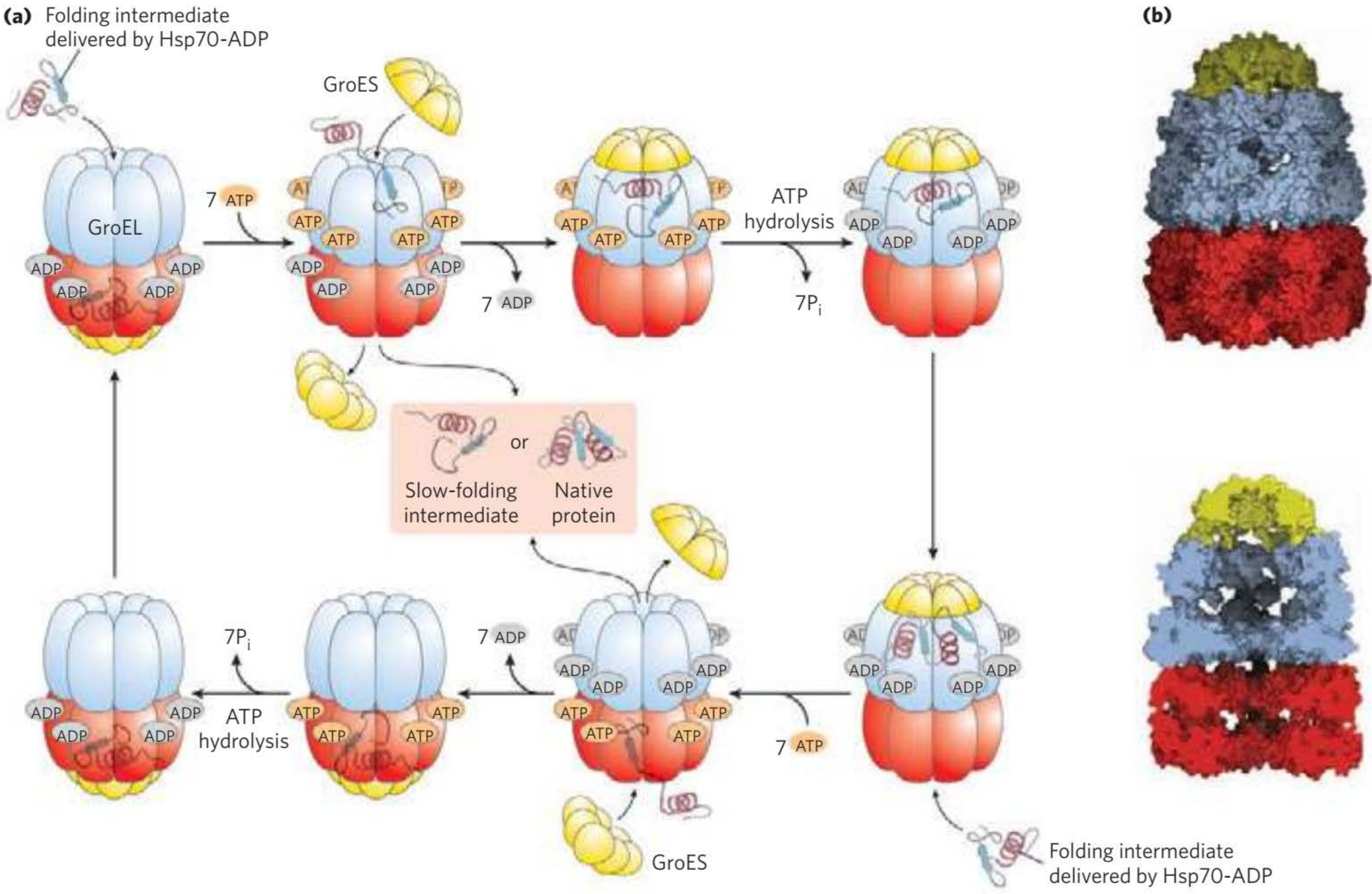
1. Hsp70（熱休克蛋白70）
    - 會與未摺疊或部份摺疊的蛋白結合，避免未摺疊或部份摺疊的蛋白質黏集而被降解。
2. Chaperonin（伴侶素）
    - 扮演主動角色，例如Hsp60。

Chaperones in protein folding



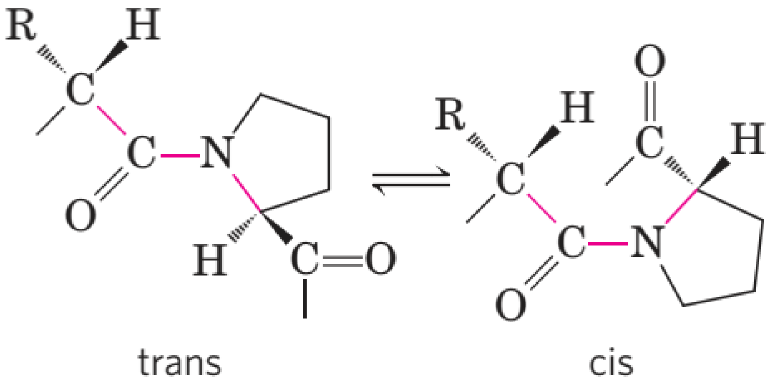
NEF: nucleotide-exchange factor

Chaperonins in protein folding



其他協助蛋白質摺疊的蛋白質

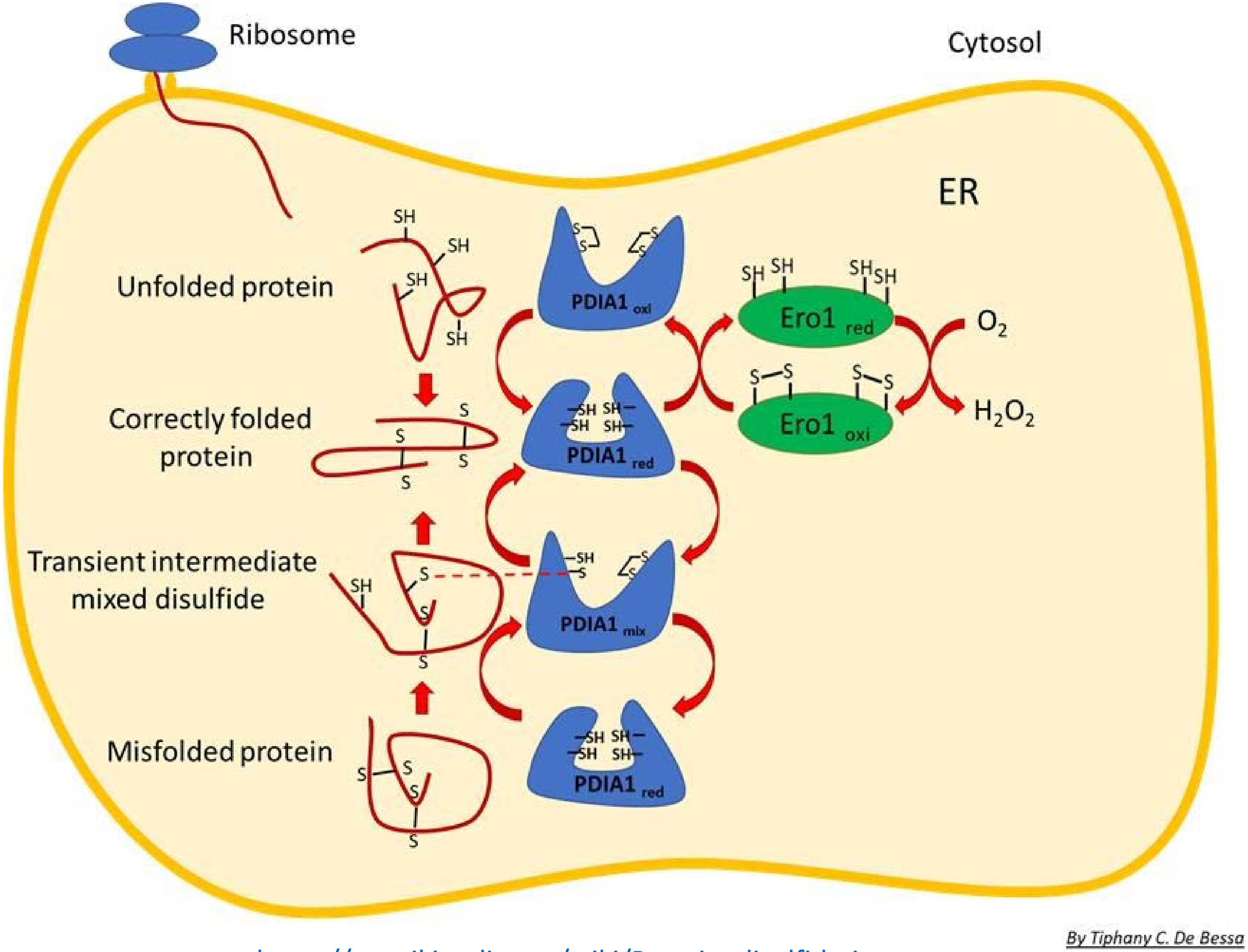
B. Peptide proyl cis-trans isomerase (PPI)負責脯胺酸參與肽鍵時的異構化反應。



反式 **Proline isomers** 順式

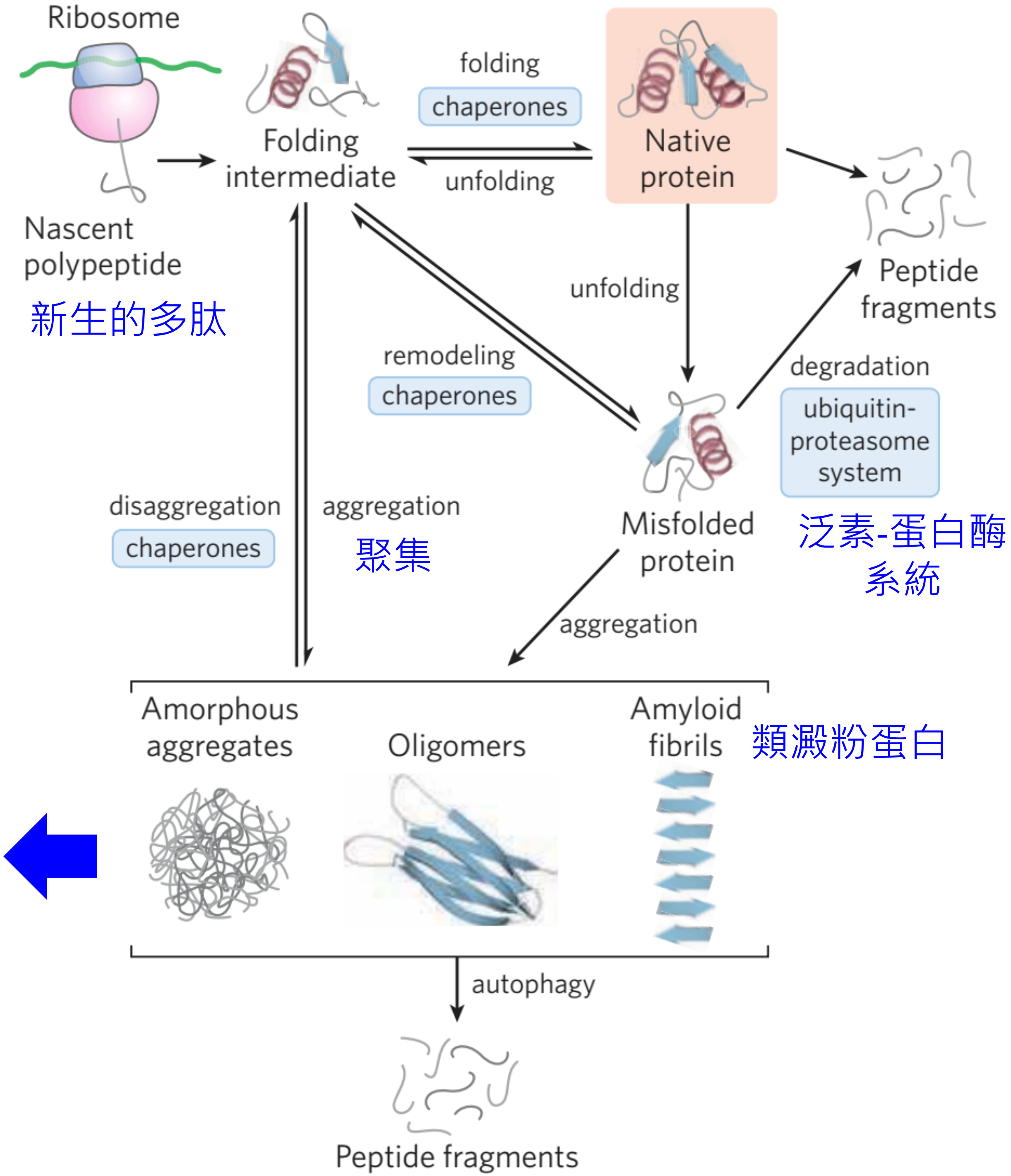
順式脯胺酸常出現在β-轉角結構

C. Protein disulfide isomerase (PDI)負責雙硫鍵的正確配對。



https://en.wikipedia.org/wiki/Protein_disulfide-isomerase

蛋白內穩態(Proteostasis)與摺疊缺失



- 未能正常分解掉的凝結蛋白會造成疾病與老化。

與蛋白質摺疊缺失有關的疾病

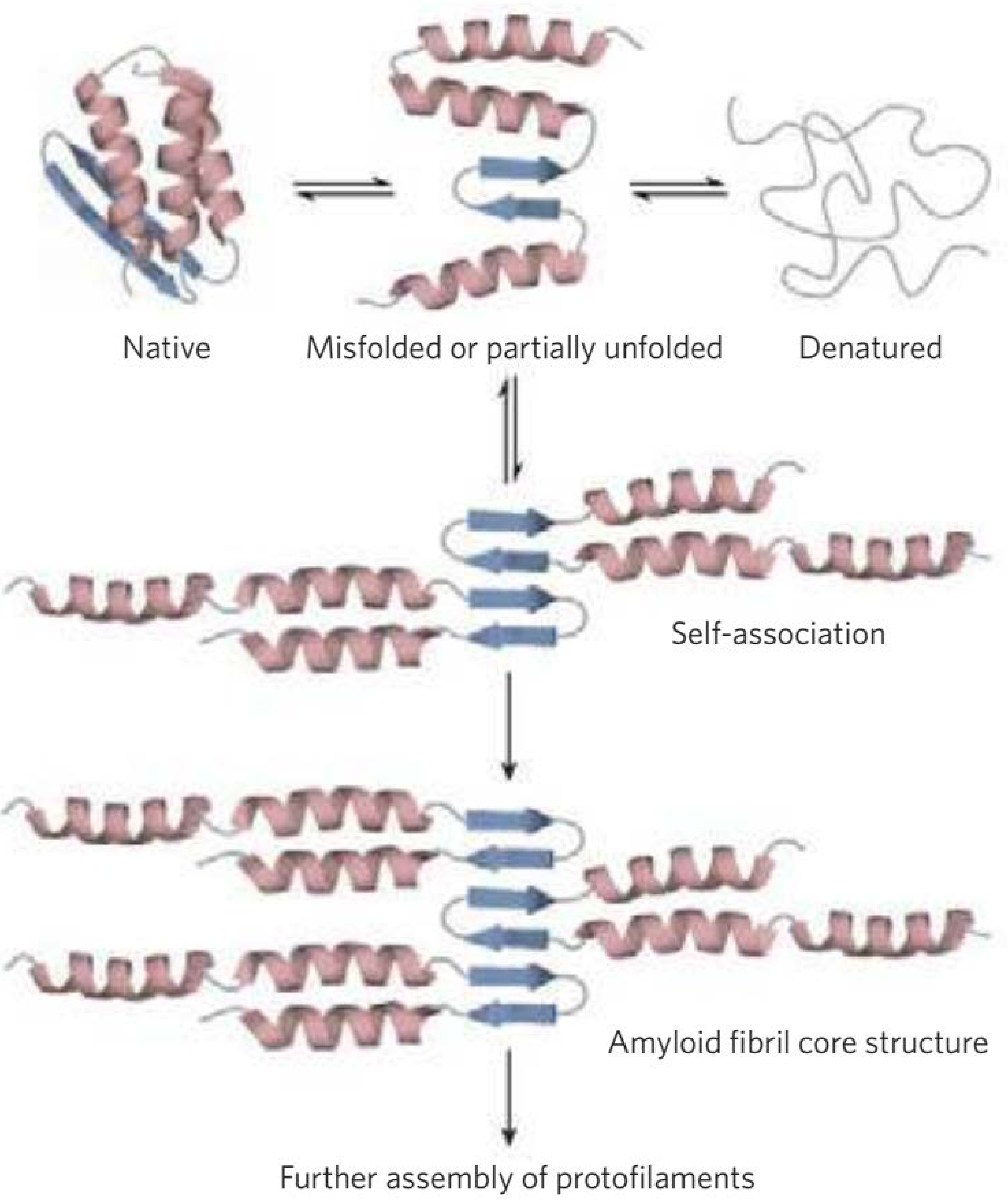
- 蛋白質構形病(proteopathy)
  - 為與蛋白質摺疊缺失有關的疾病的統稱。

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

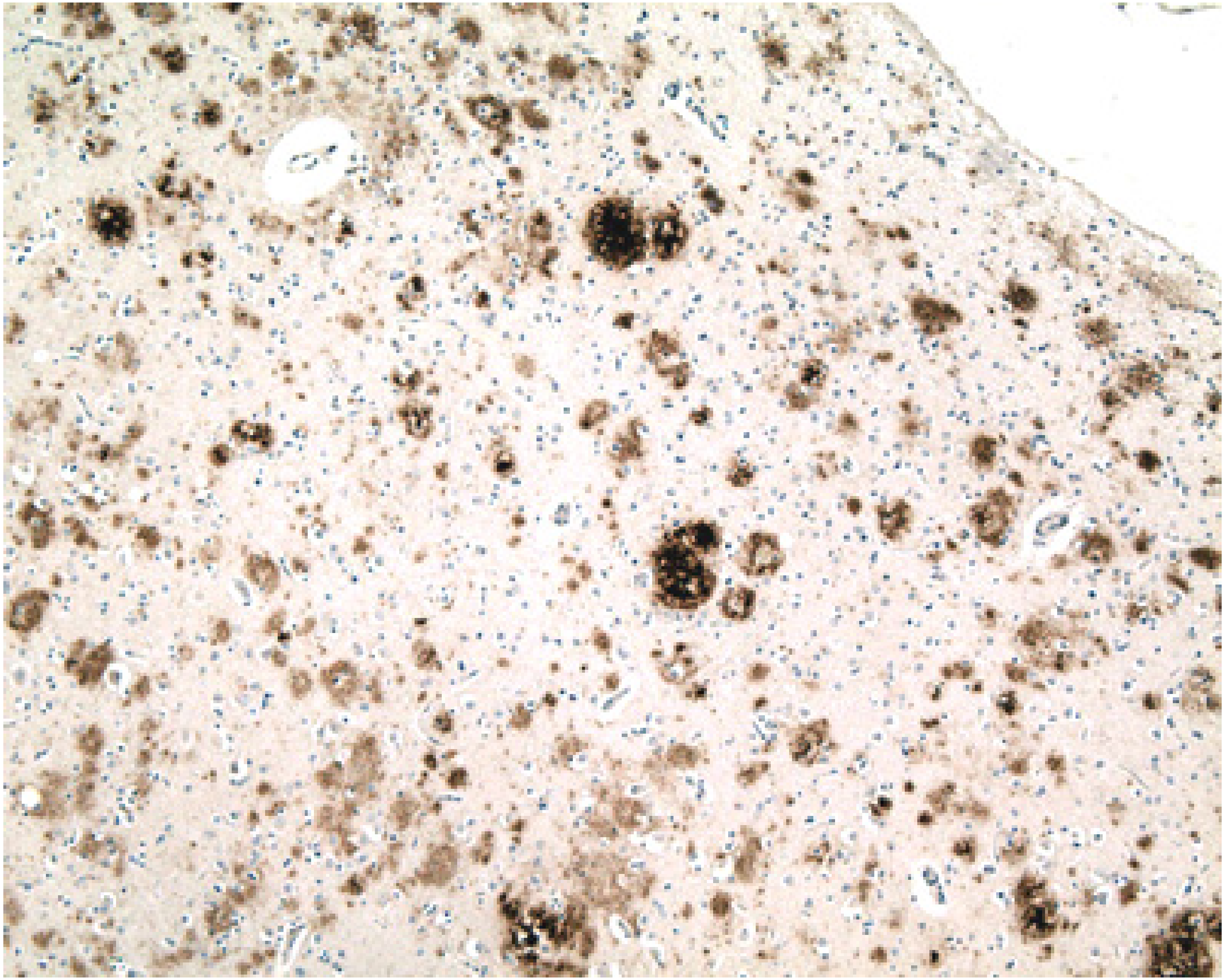
1. 類澱粉蛋白變性症(amyloidosis)
   - 原本由細胞分泌出的可溶性蛋白因為摺疊缺失使得這些蛋白質轉換成不可溶的類澱粉蛋白纖維(amyloid fiber)。
   - 如阿茲海默症、帕金森氏症等都與這一類的蛋白質摺疊缺失有關。

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)。

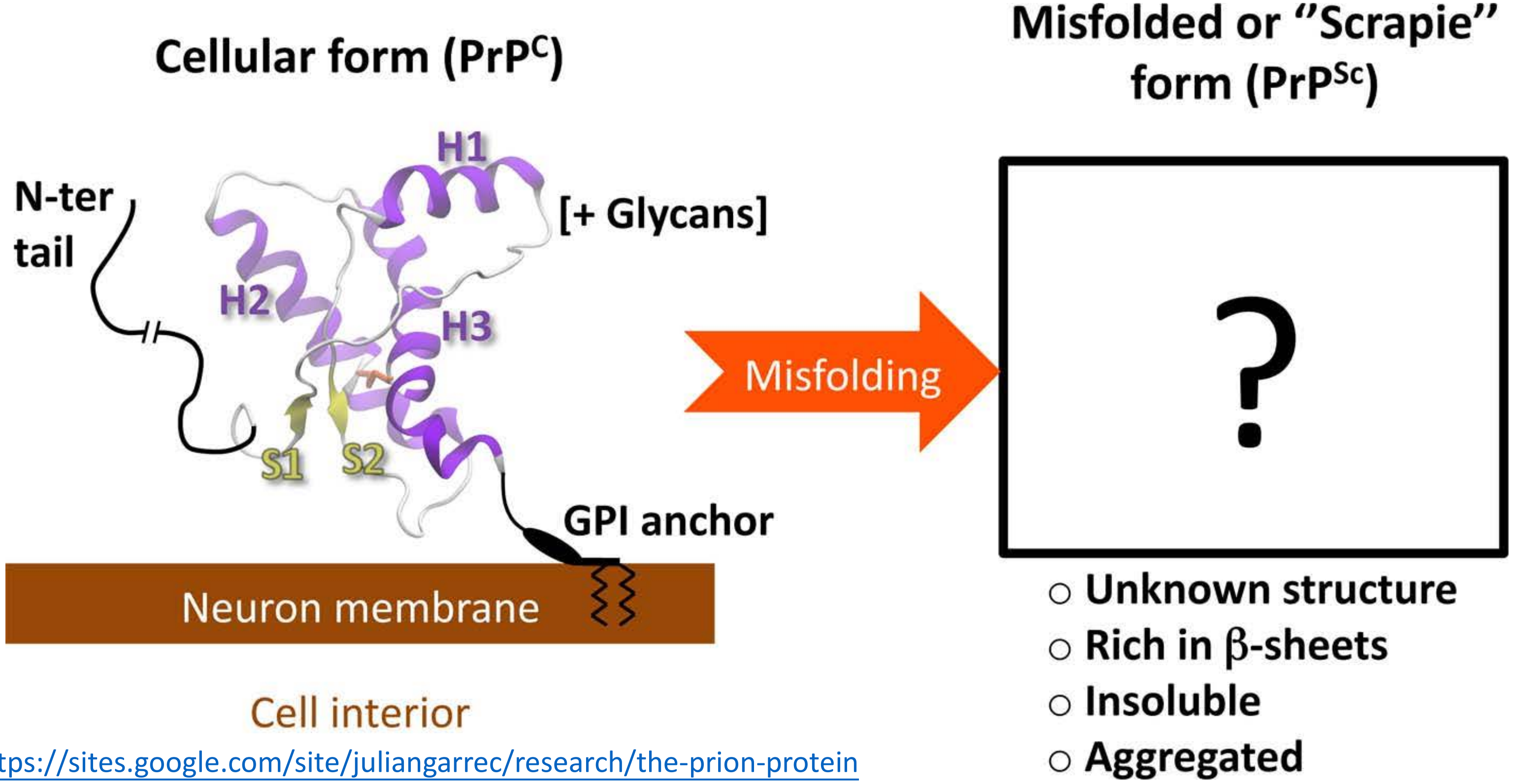


https://commons.wikimedia.org/wiki/File:Proteopathy_Abeta_deposits_in_Alzheimer_disease.jpg

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)



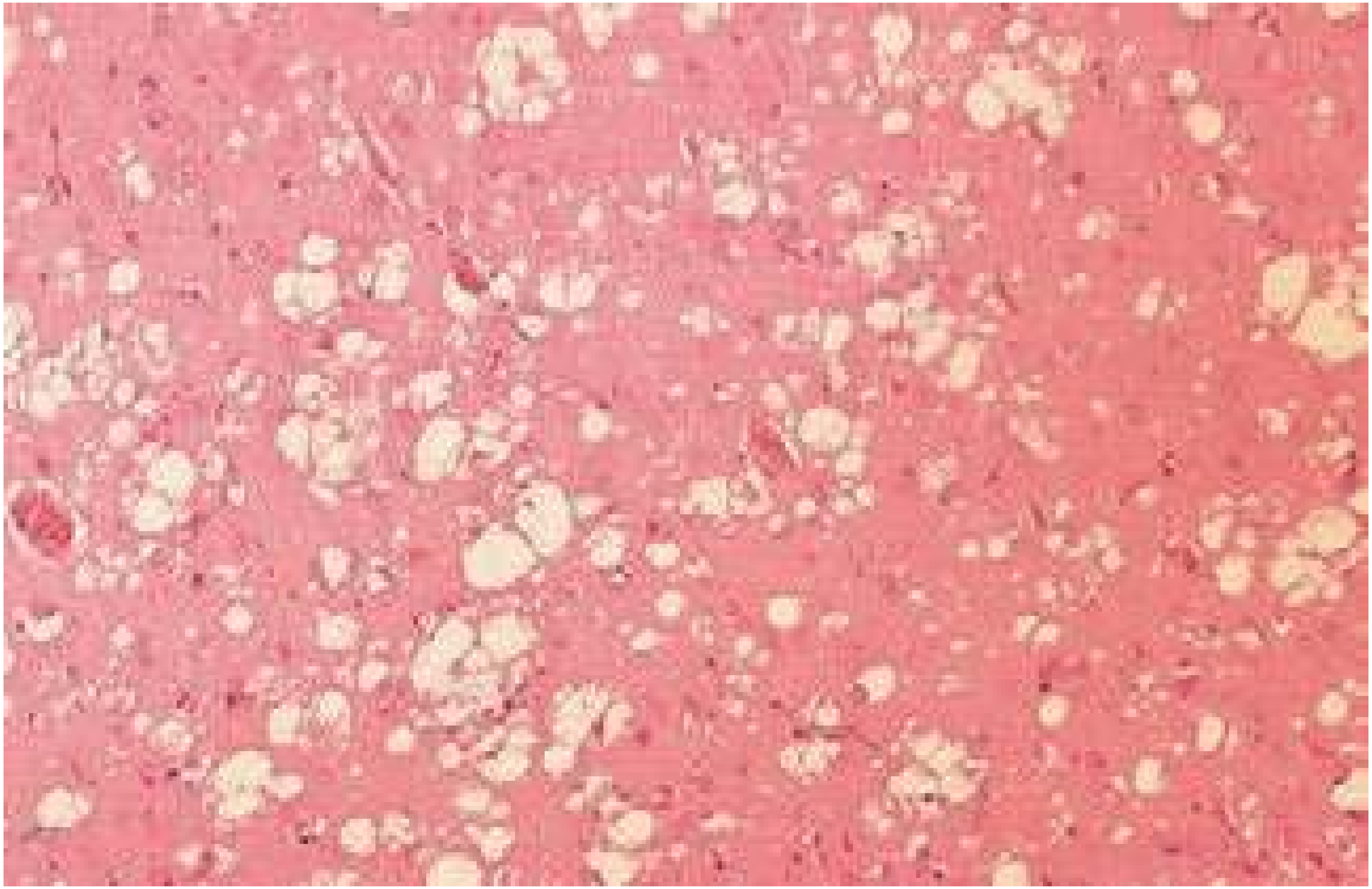
Cellular form (PrP^{C})

Misfolded or "Scrapie" form (PrP^{Sc})

- ○ **Unknown structure**
- ○ **Rich in β-sheets**
- ○ **Insoluble**
- ○ **Aggregated**

https://sites.google.com/site/juliangarrec/research/the-prion-protein

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



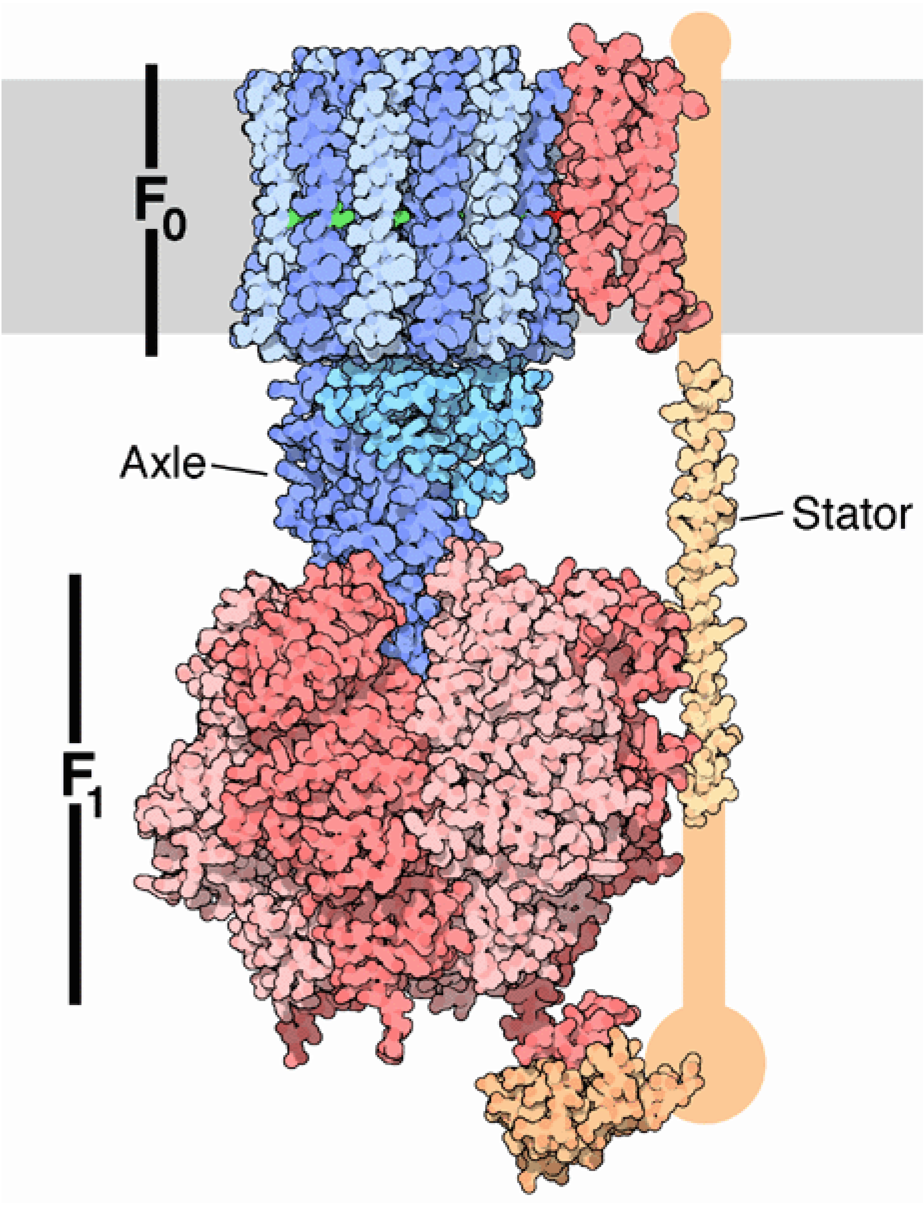
蛋白質結構如何分析？

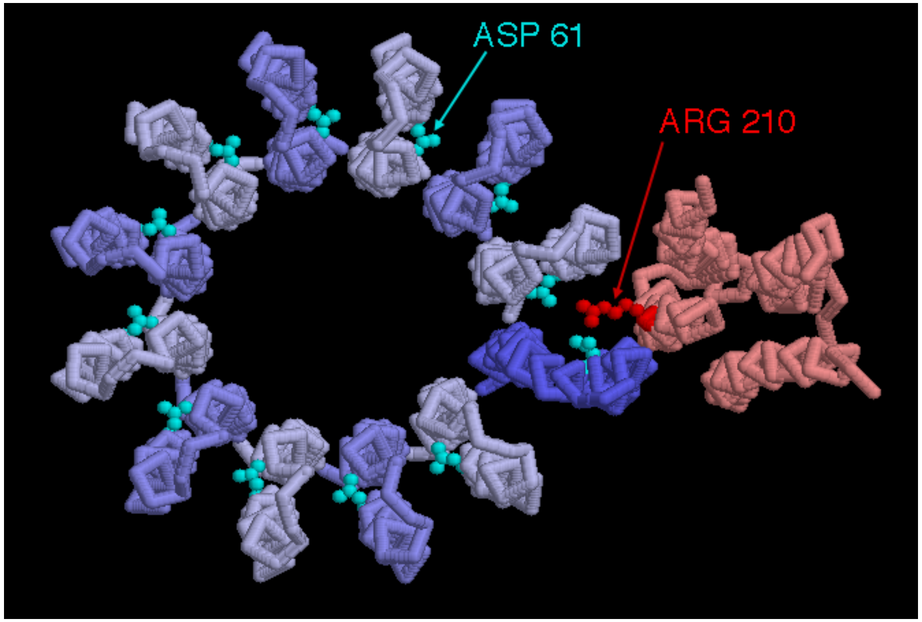
- 蛋白質結構分析的目的：
  - 了解蛋白質功能或與其他蛋白質交互作用關係
  - 藥物設計

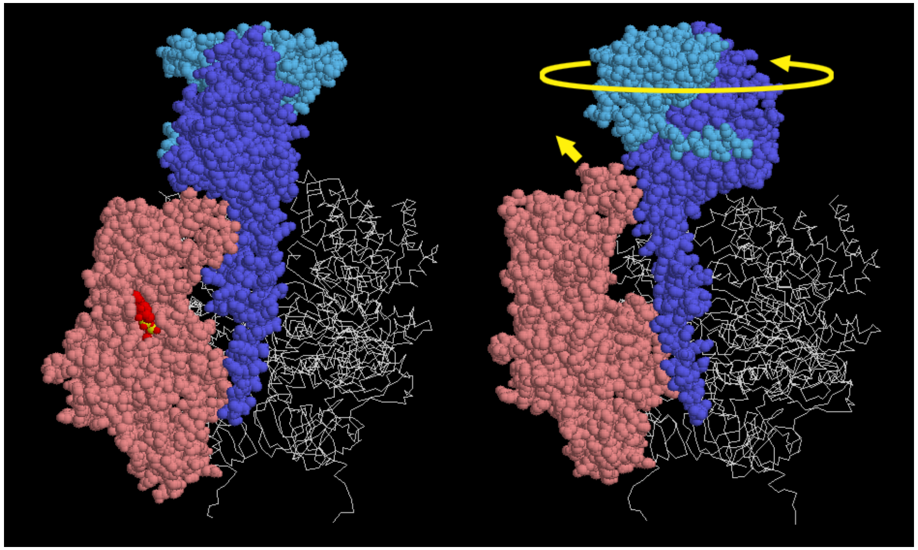
- An artist’s depiction of receptor mimicry. A SARS antibody (purple) mimics the function of the virus’s natural receptor (teal), prematurely triggering (and thus deactivating) the mechanism that enables the SARS virus to infect a cell.

https://als.lbl.gov/antibody-uses-mimicry-to-block-sars-coronavirus/

以ATP 合成酶為例：

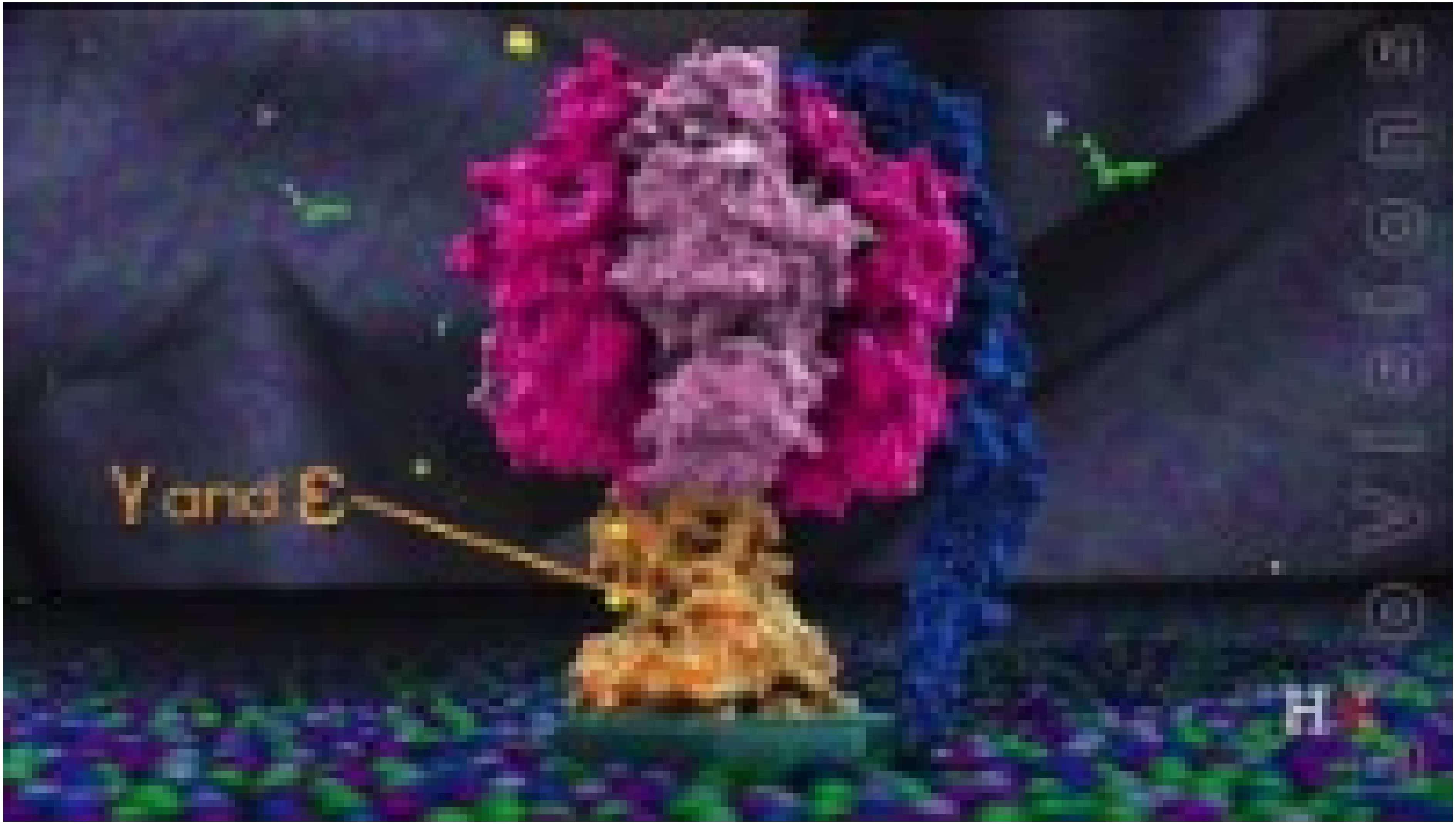






Protein Data Base (PDB), Molecule of Month, https://pdb101.rcsb.org/motm/72

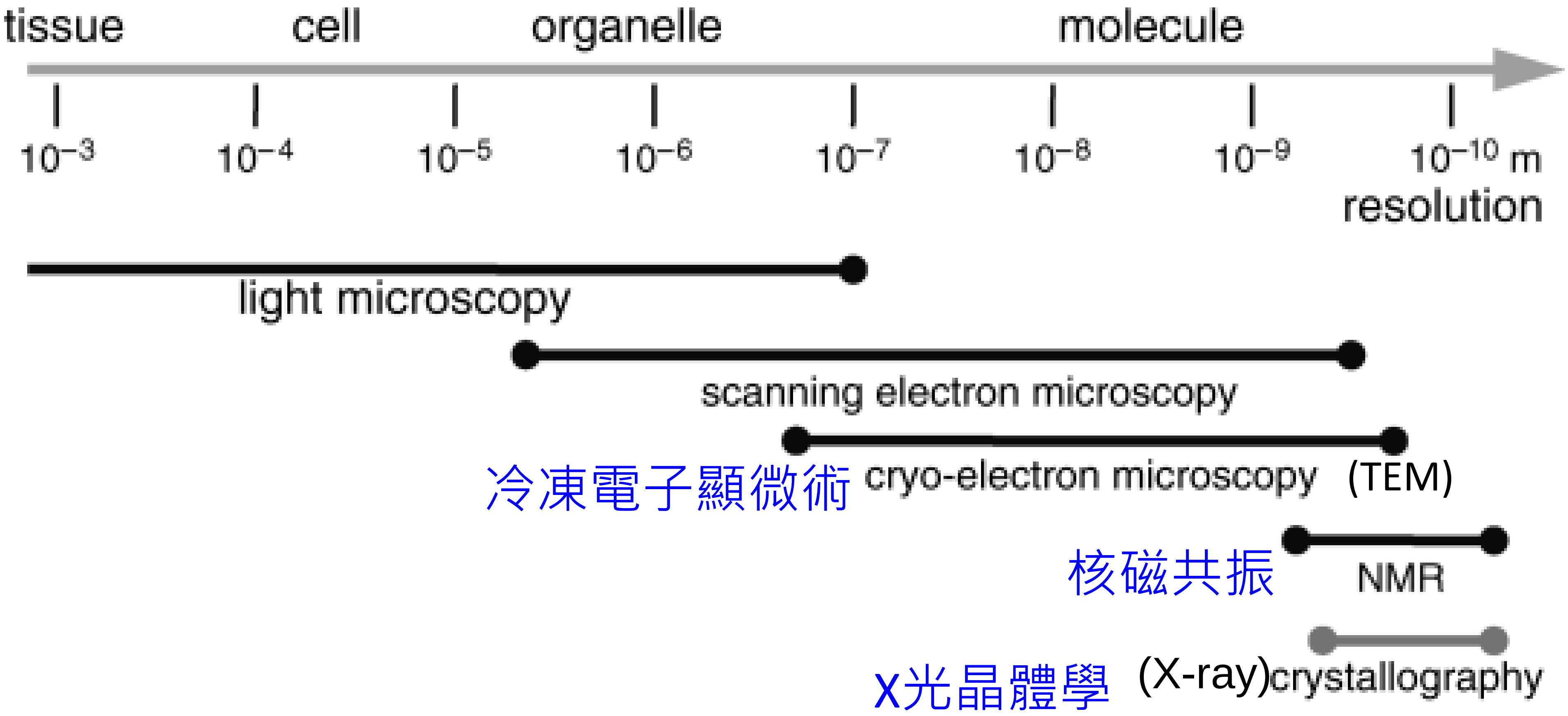
ATP synthase in action



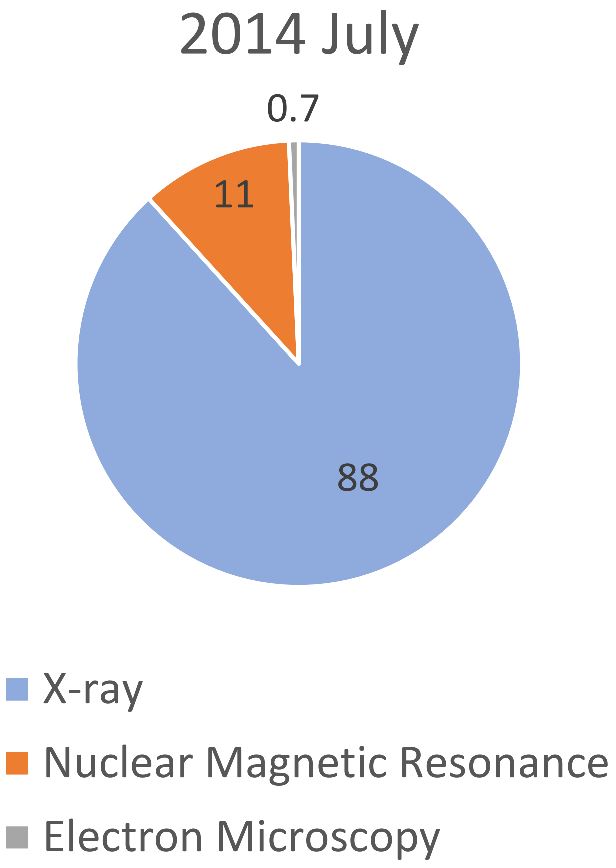
https://youtu.be/kXpzp4RDGJI

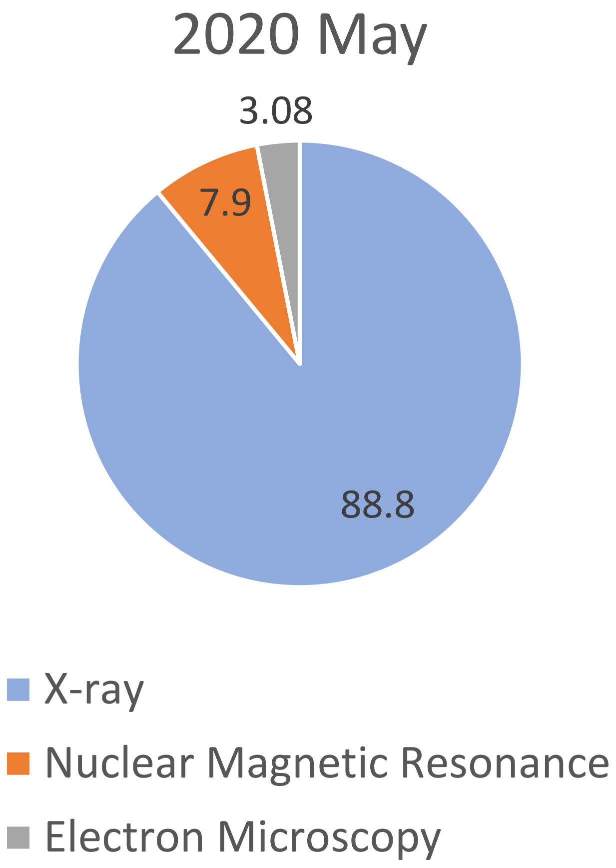
蛋白質結構解析方法

- 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



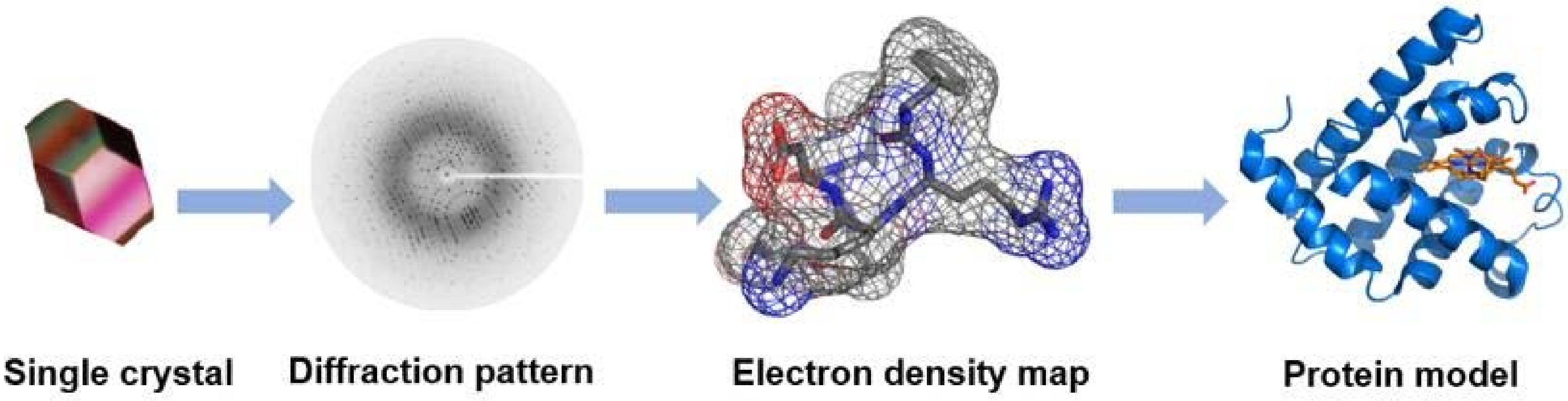


Modified from https://www.rcsb.org/stats/summary

(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：

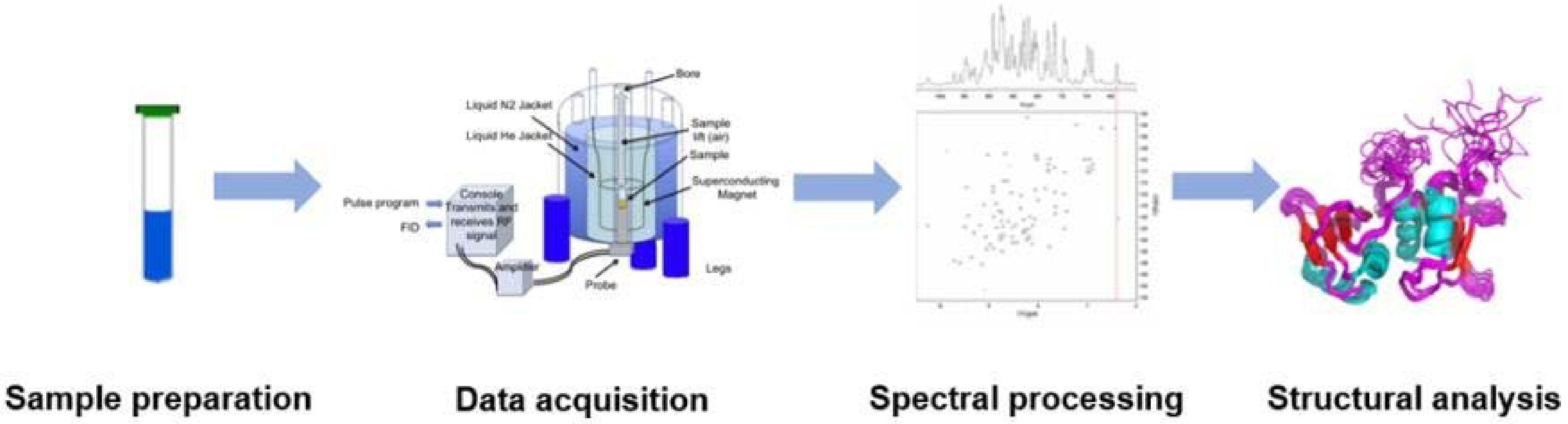
1. 蛋白質結晶：trial-and-error。
2. 利用X光照射以不同角度照射結晶形成繞射圖。
3. 分析繞射圖中單位晶胞(unit cell)的電子密度分佈(electron density distribution)。
4. 利用電子密度推估原子排列方式。



https://www.creative-biostructure.com/comparison-of-crystallography-nmr-and-em_6.htm

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

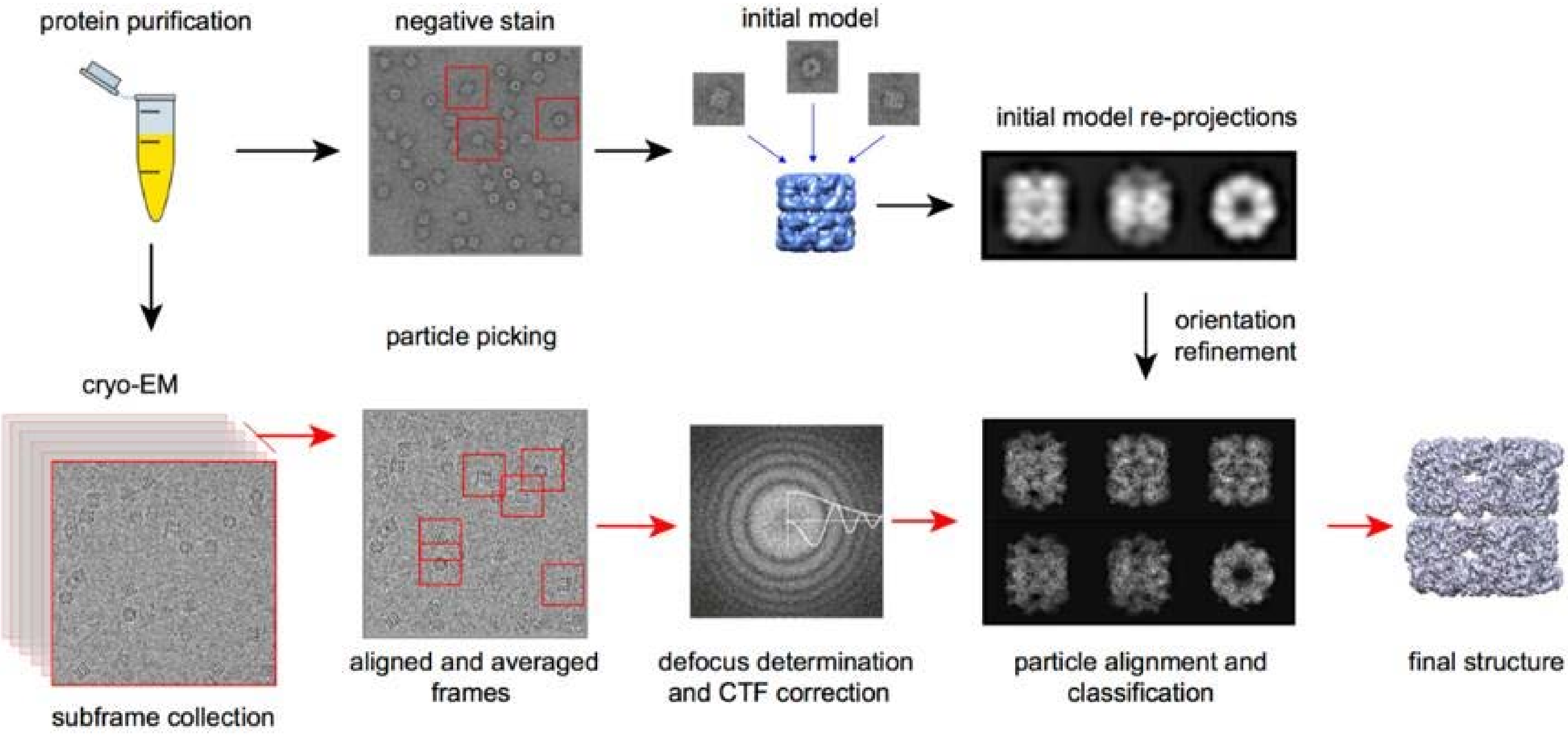
- 原子因周圍的電子雲環境造成共振頻率改變產生核磁共振的的差異，稱為化學位移，因此，藉由不同原子的共振譜可以得知蛋白質胺基酸之間的關係。



https://www.creative-biostructure.com/comparison-of-crystallography-nmr-and-em_6.htm

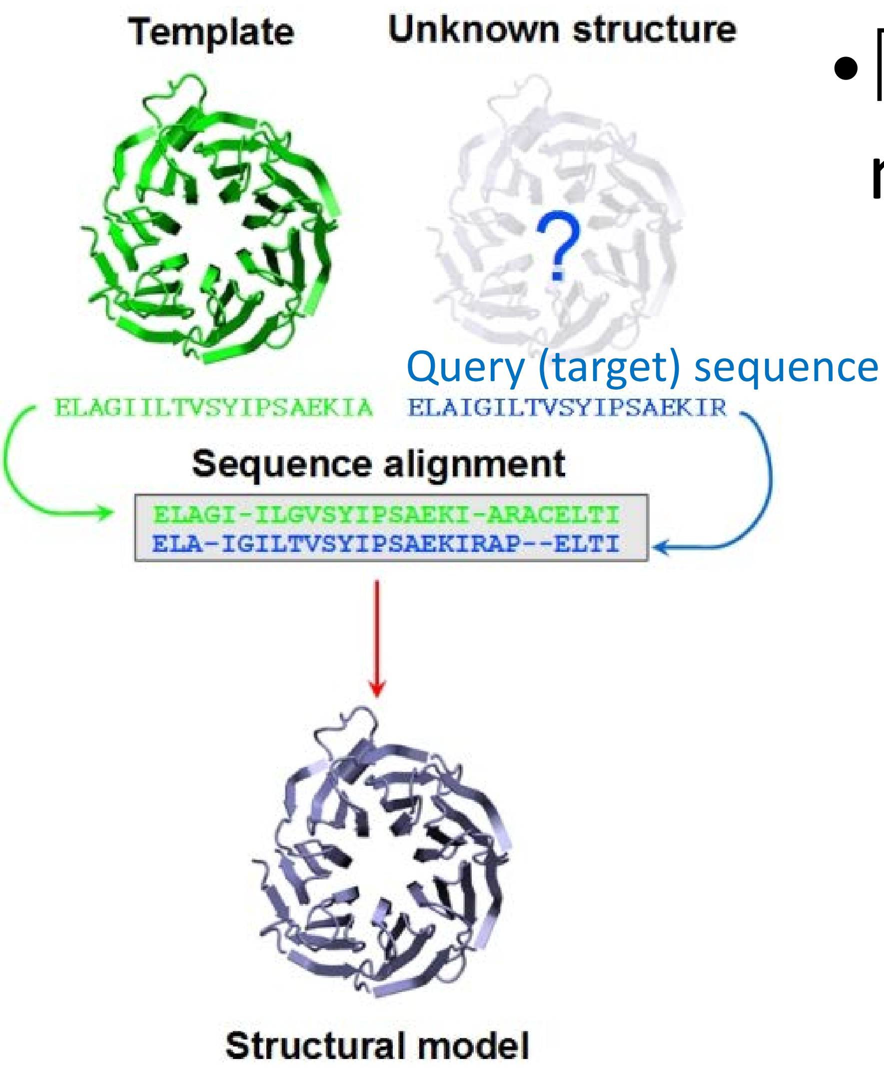
(3) 冷凍電子顯微術 (cryo-electron microscopy)

- 單顆粒分析(single particle analysis)



https://www.creative-biostructure.com/comparison-of-crystallography-nmr-and-em_6.htm

電腦輔助蛋白質結構預測



https://www.unil.ch/pmf/en/home/menuinst/technologies/homology-modeling.html

- 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。

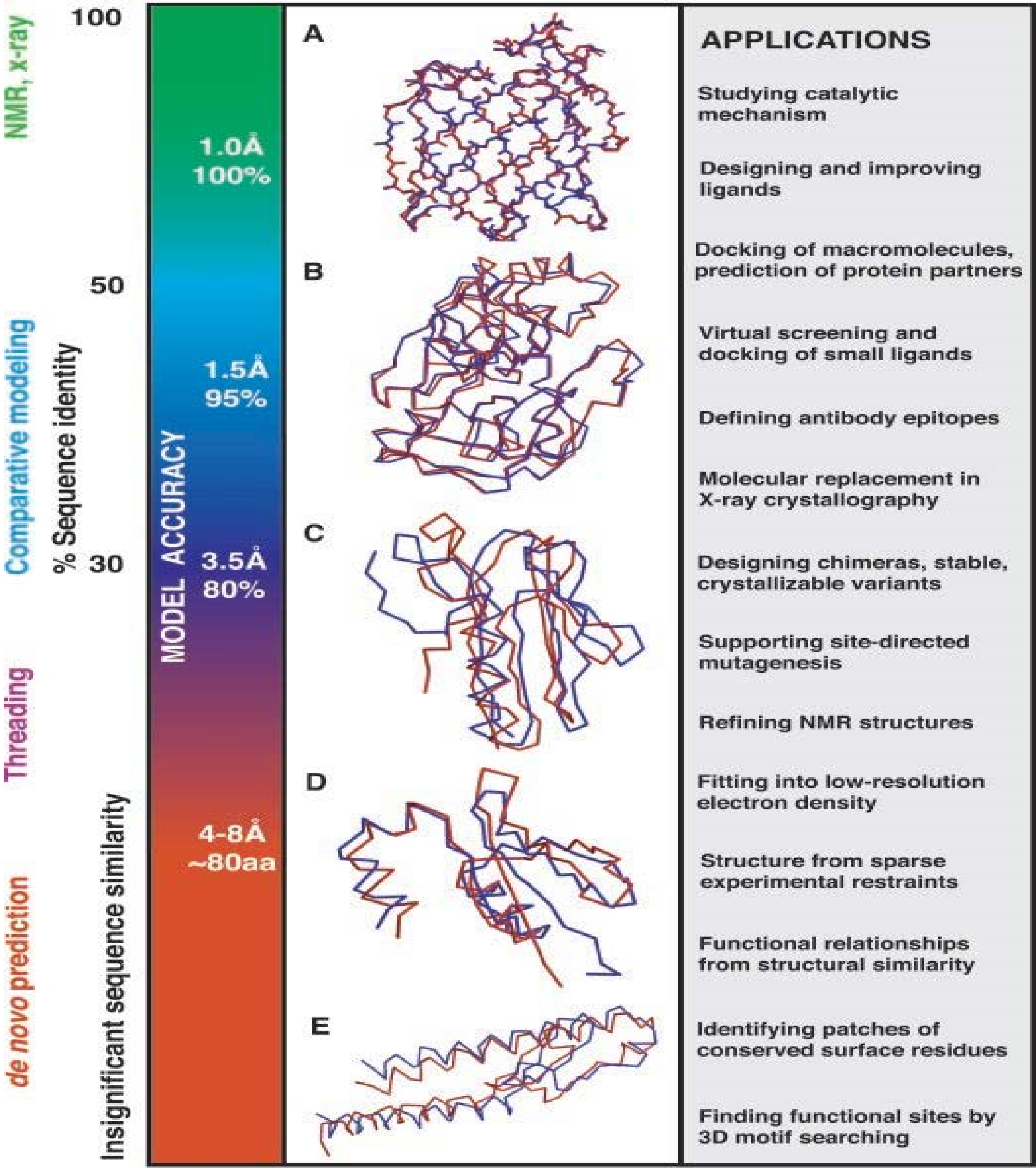
延伸閱讀：
https://proteopedia.org/wiki/index.php/Practical_Guide_to_Homology_Modeling

- Accuracy and application of protein structure models.

Predicted structures in red, actual structures in blue.

同源建模

從頭開始結構預測
→現代科學挑戰



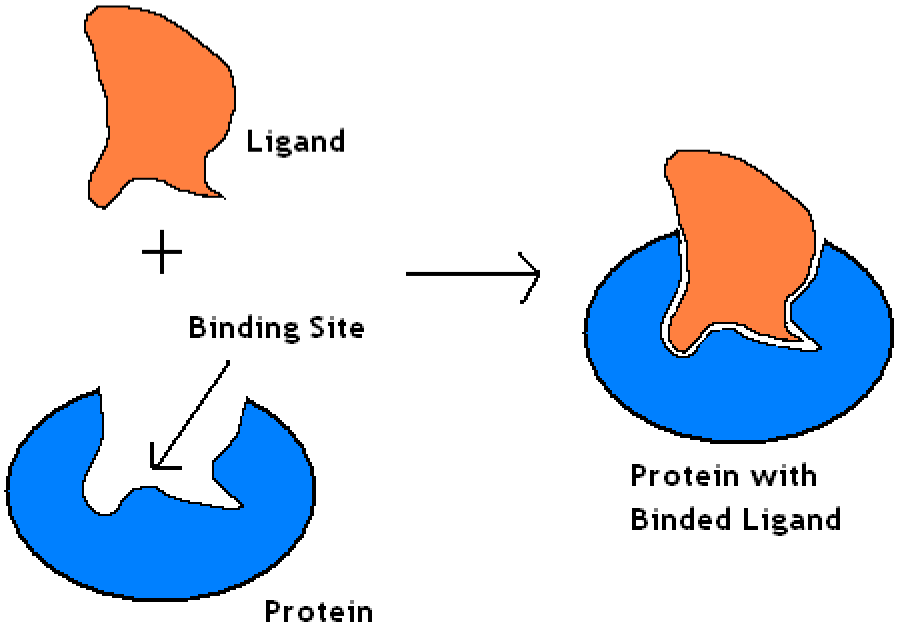
Baker, D. (2001). Protein Structure Prediction and Structural Genomics. Science, 294(5540), 93–96.

蛋白質的功能

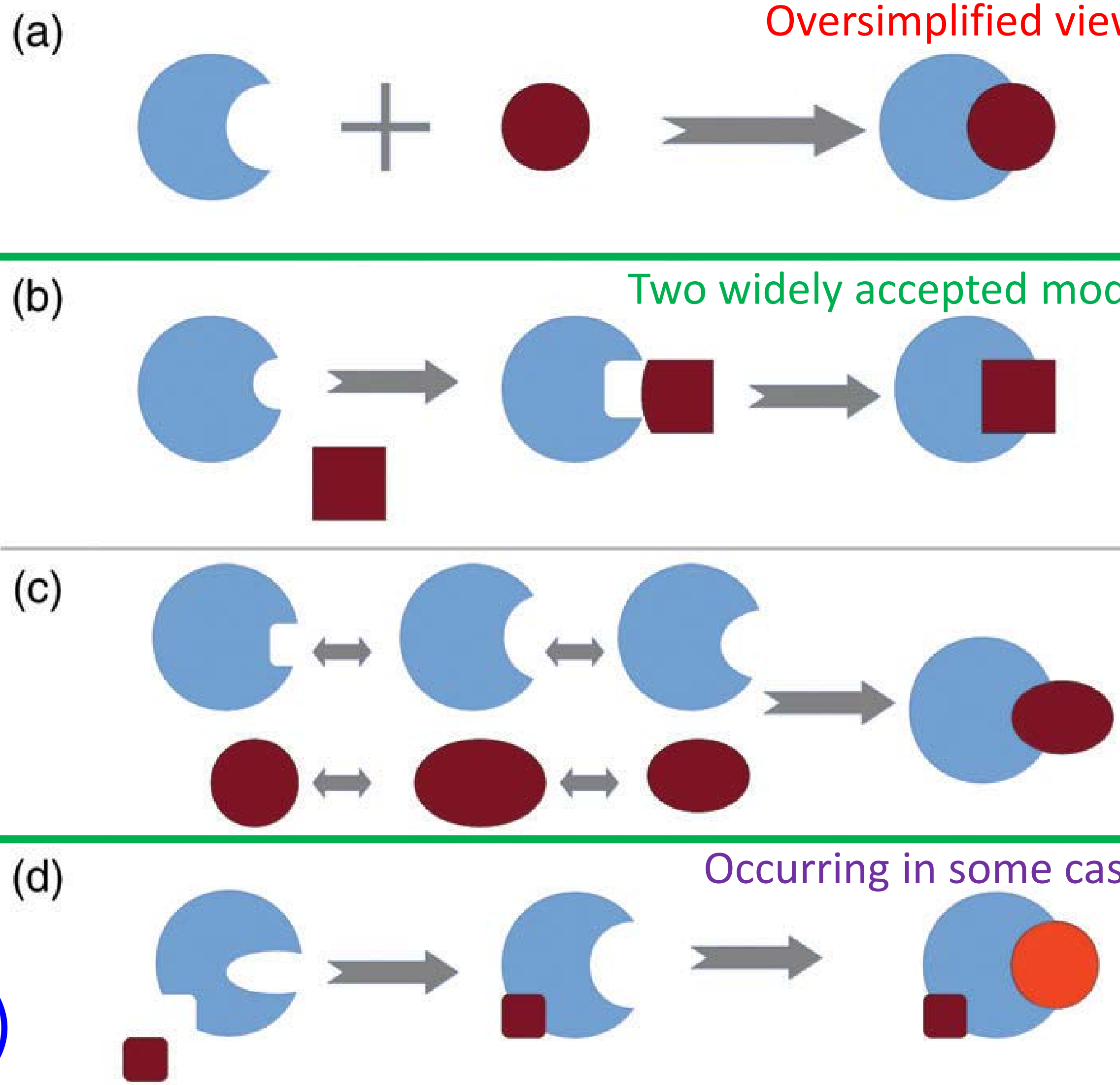
- 蛋白質立體結構與功能的關係
  - 蛋白質在活體中為動態分子。
  - 蛋白質的功能與其蛋白質跟其他分子（配體 ligand）進行交互作用產生構形改變 (conformational change)息息相關。
    - 進行交互作用的分子種類。
    - 構形改變後造成的影響：訊息傳遞、化學反應、分子移動、通道開闔...等。

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)

Oversimplified view

Two widely accepted models

誘導配合
(induced-fit)

構形選擇
(conformational selection)

Occurring in some cases

異位模式
(allosteric model)

Iglesias, J., Saen-oon, S., Soliva, R., & Guallar, V. (2018). Computational structure-based drug design: Predicting target flexibility. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, *8*(5), e1367.

蛋白質與配體結合的親和力(affinity)

$$\mathrm{P} + \mathrm{L} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \mathrm{PL}$$

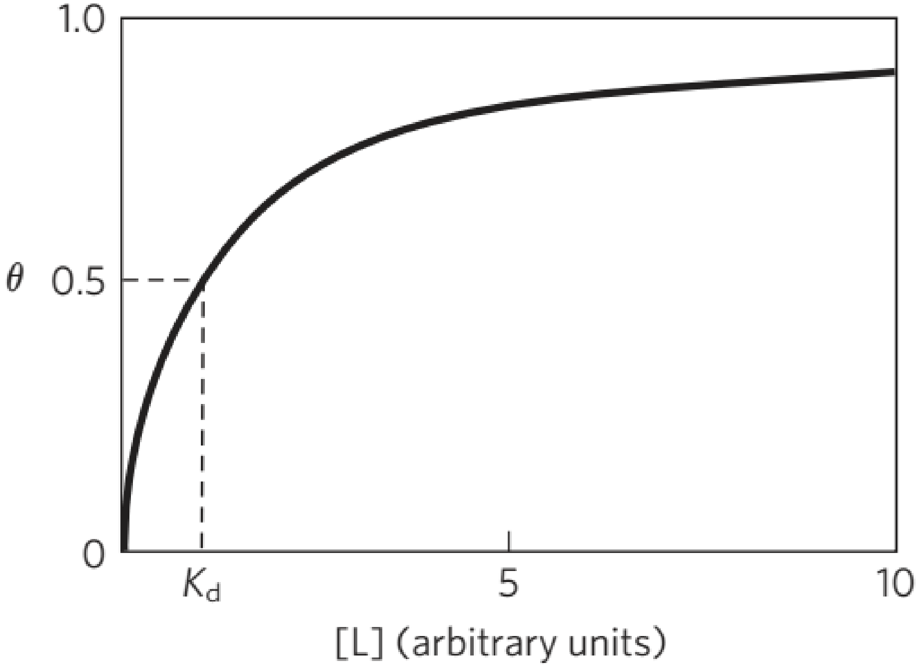
protein ligand protein-ligand

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

K_d ↓ dissociation constant 解離常數

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

Ligand-binding curve



Oxygen-binding curve

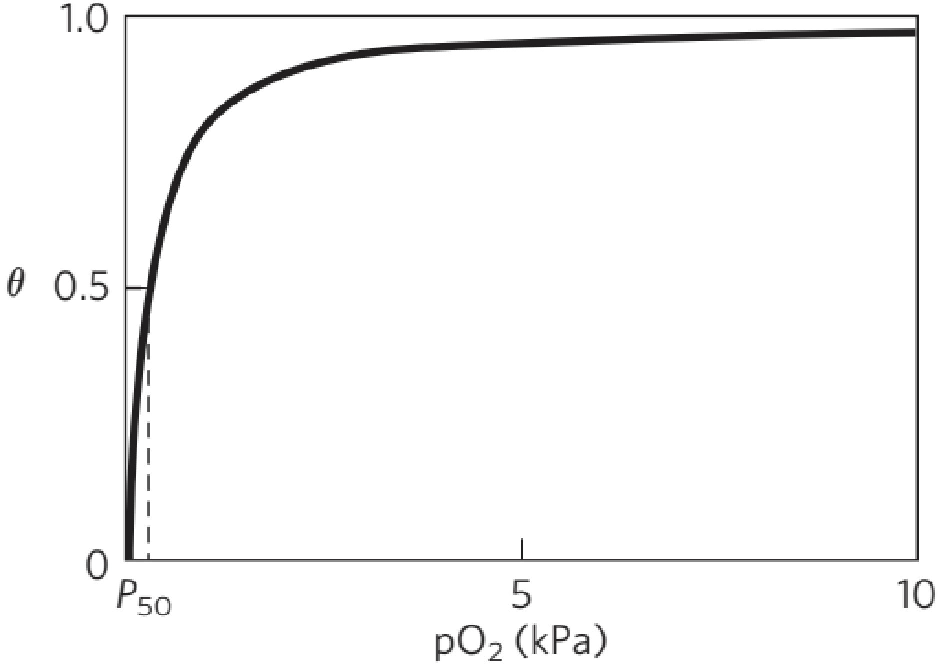
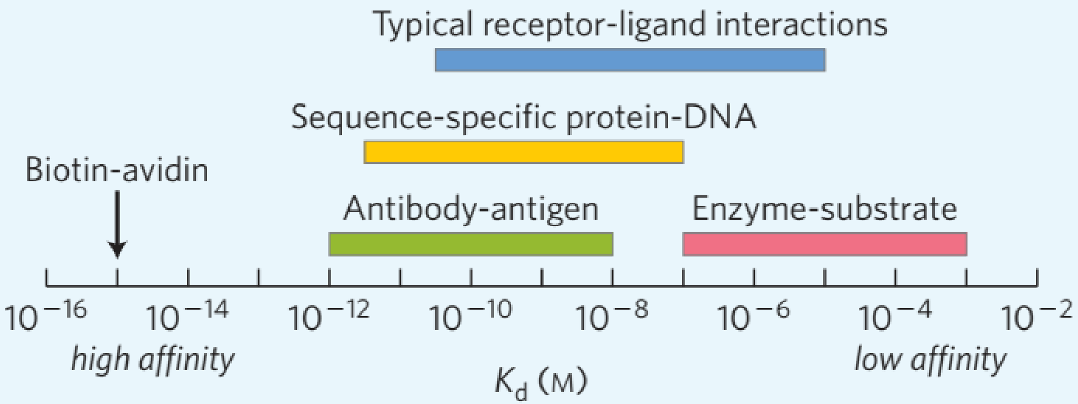


TABLE 5–1 Some Protein Dissociation Constants

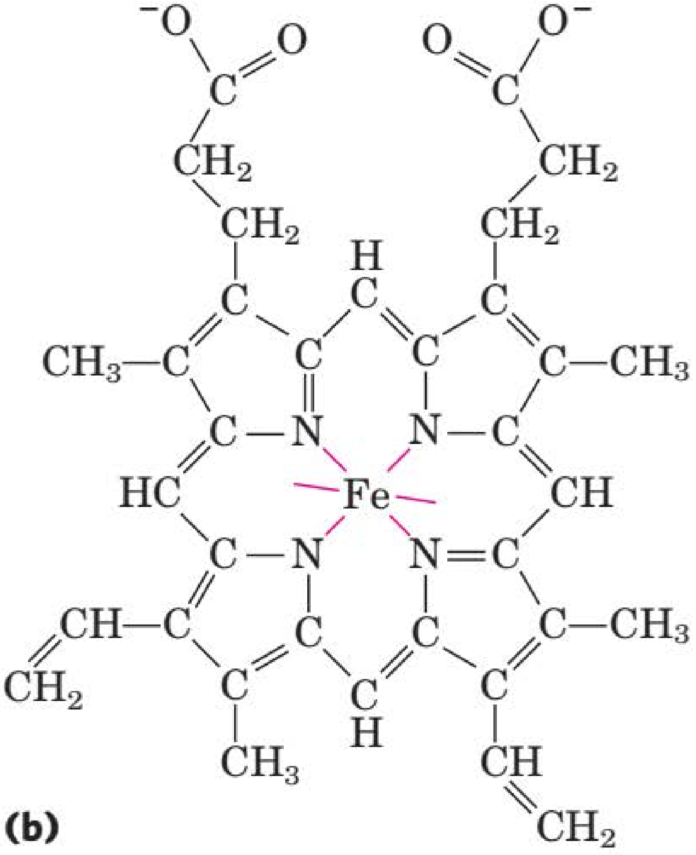
Protein	Ligand	K_d (M)*
Avidin (egg white) 親和素	Biotin 生物素	1×10^{-15}
Insulin receptor (human)	Insulin 胰島素	1×10^{-10}
Anti-HIV immunoglobulin (human)[†]	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)[‡]	Ca^{2+}	3×10^{-6}
		2×10^{-5}



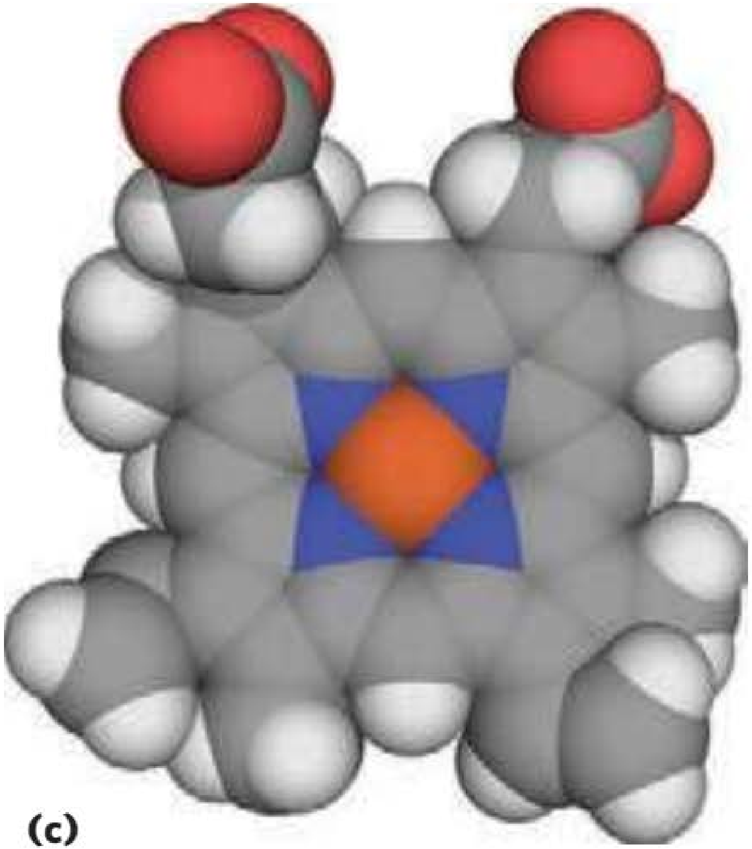
Color bars indicate the range of dissociation constants typical of various classes of interactions in biological systems. A few interactions, such as that between the protein avidin and the enzyme cofactor biotin, fall outside the normal ranges. The avidin-biotin interaction is so tight it may be considered 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)

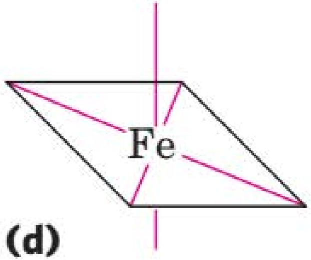


(b)



(c)

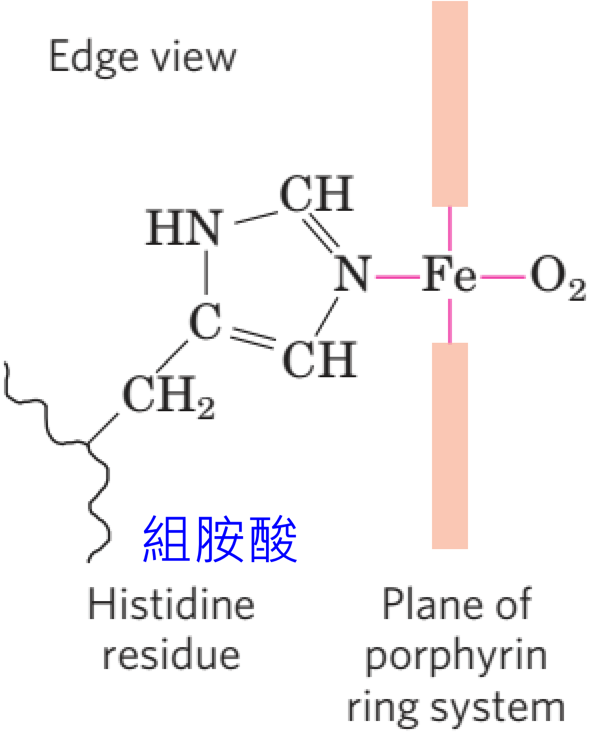
鐵原子具備有六個配位鍵

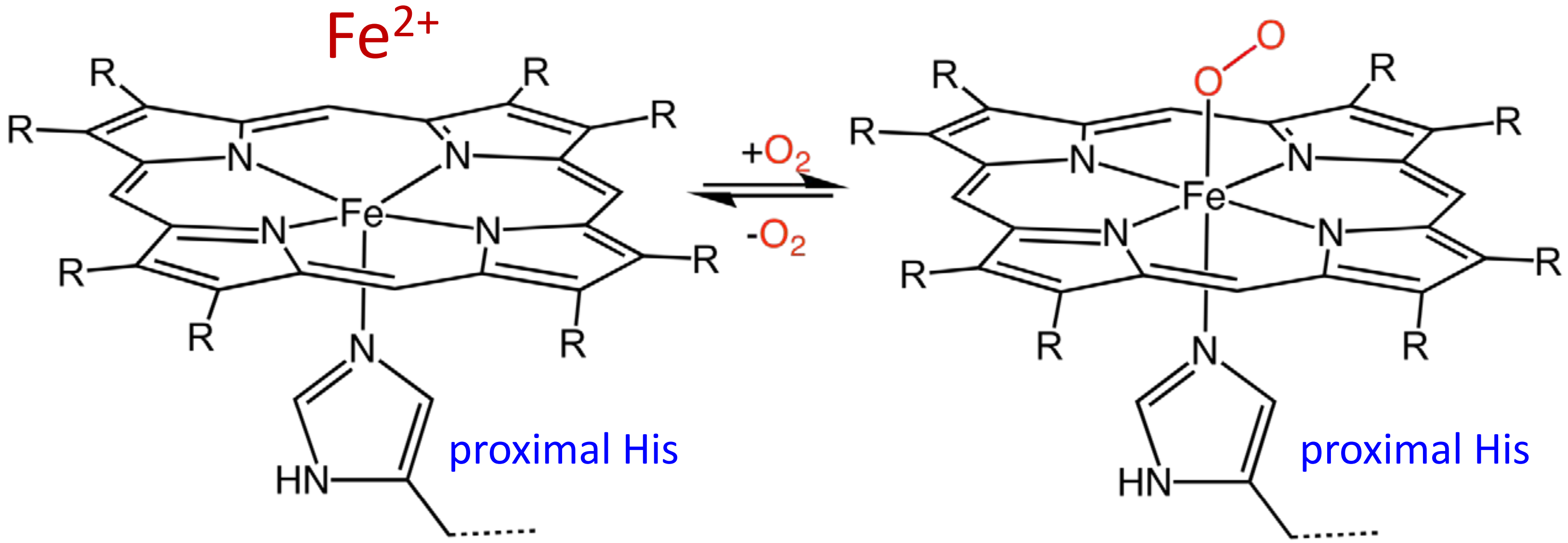


(d)

鐵離子與氧分子結合

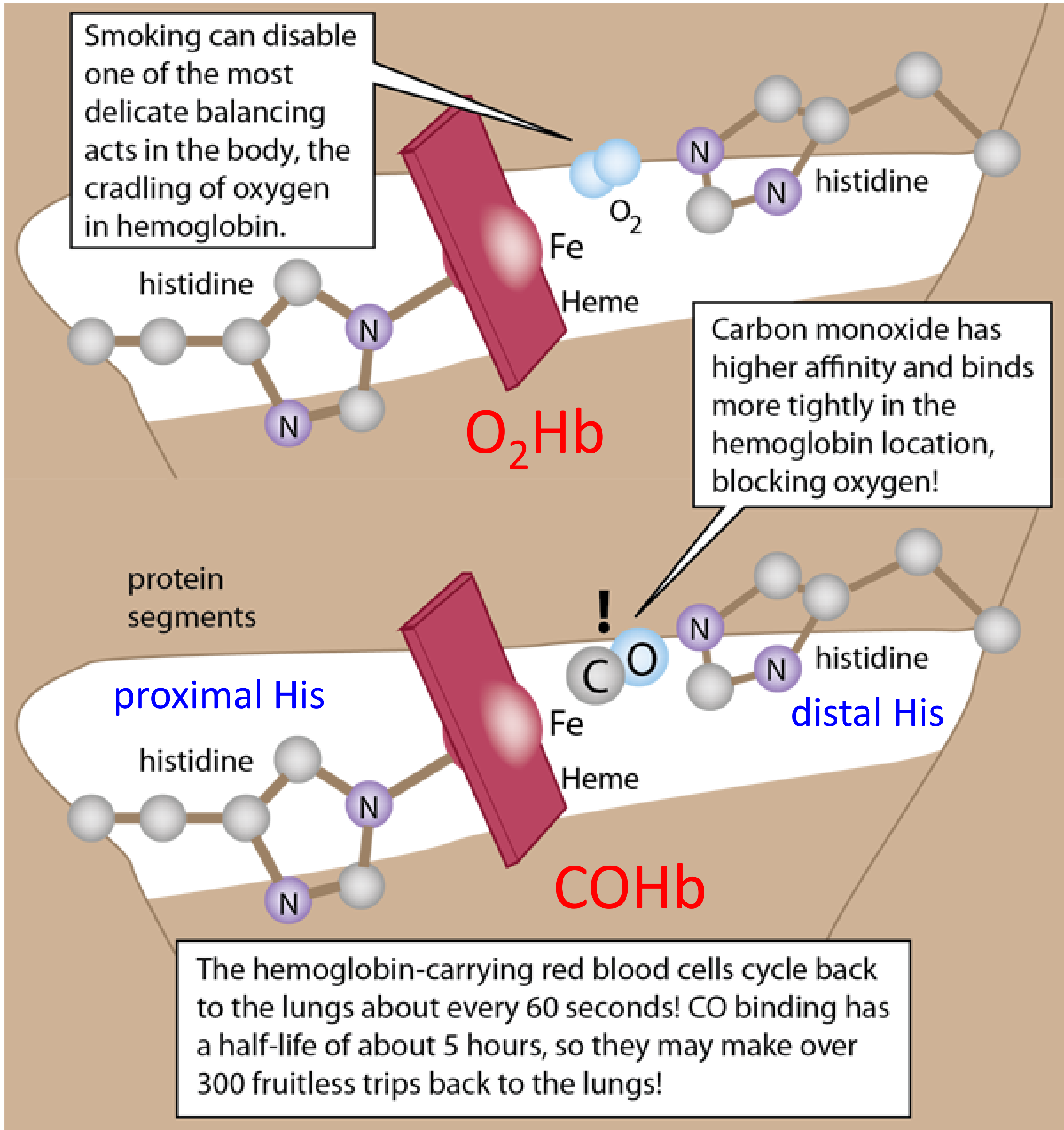
- 由於氧結合蛋白的結構，鐵離子只有一個配位鍵會接上氧→可保持鐵離子維持在二價(Fe^{2+})。
- 深紫（缺氧）→亮紅（含氧）





其他小分子也會與血基質結合

一氧化碳(CO)、
一氧化氮(NO)
等…



Smoking can disable one of the most delicate balancing acts in the body, the cradling of oxygen in hemoglobin.

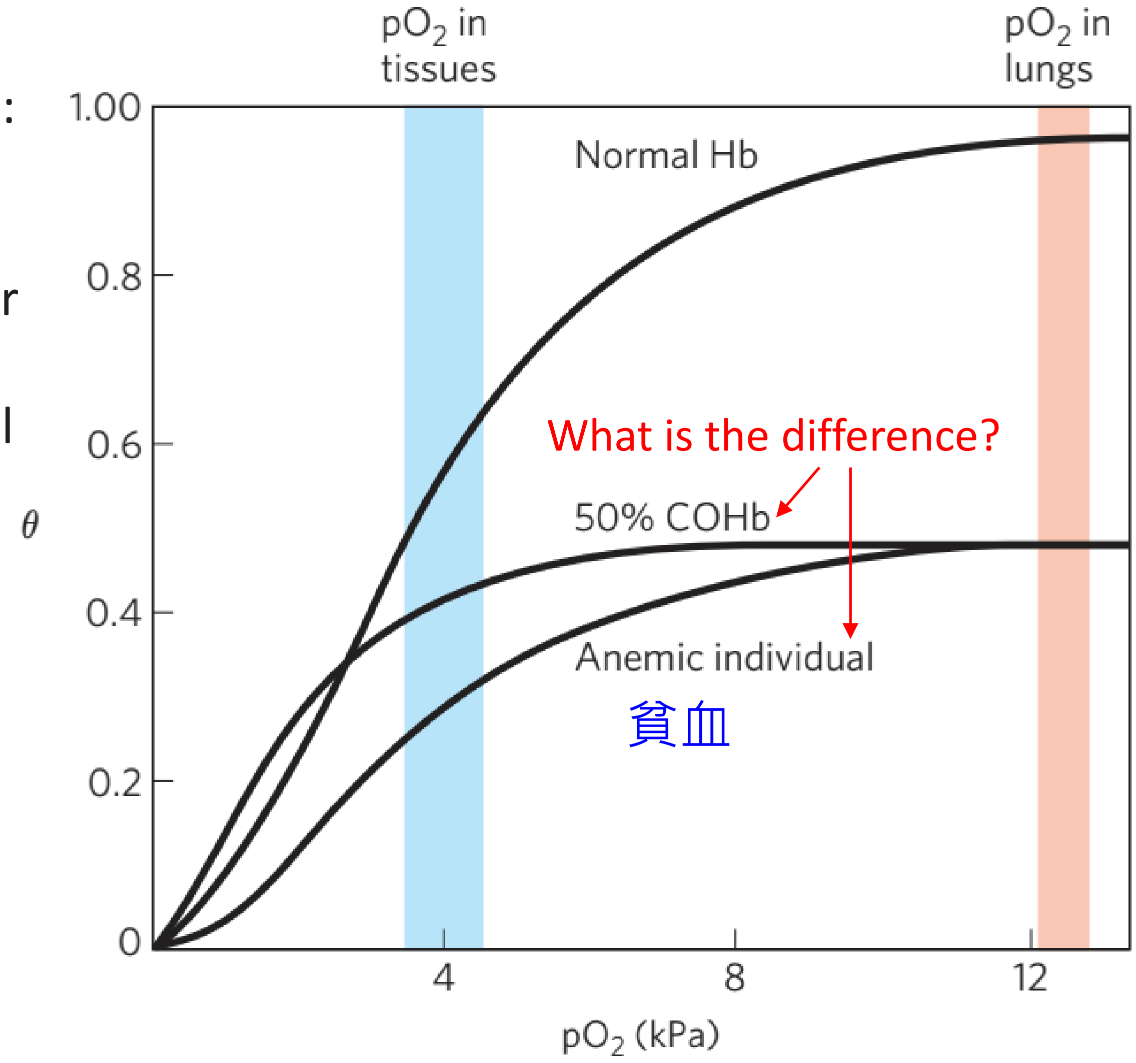
Carbon monoxide has higher affinity and binds more tightly in the hemoglobin location, blocking oxygen!

The hemoglobin-carrying red blood cells cycle back to the lungs about every 60 seconds! CO binding has a half-life of about 5 hours, so they may make over 300 fruitless trips back to the lungs!

http://hyperphysics.phy-astr.gsu.edu/hbase/Organic/hemo.html

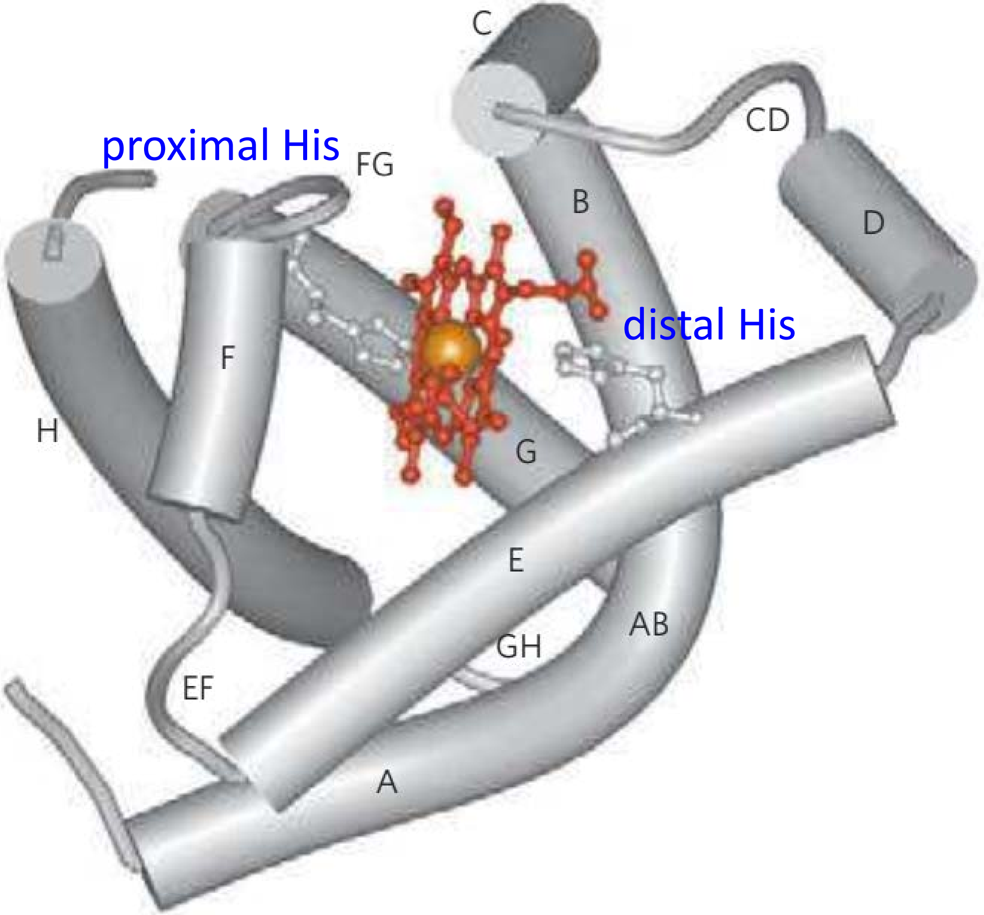
CO與血紅蛋白結合造成身體缺氧 (oxygen deprivation)

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

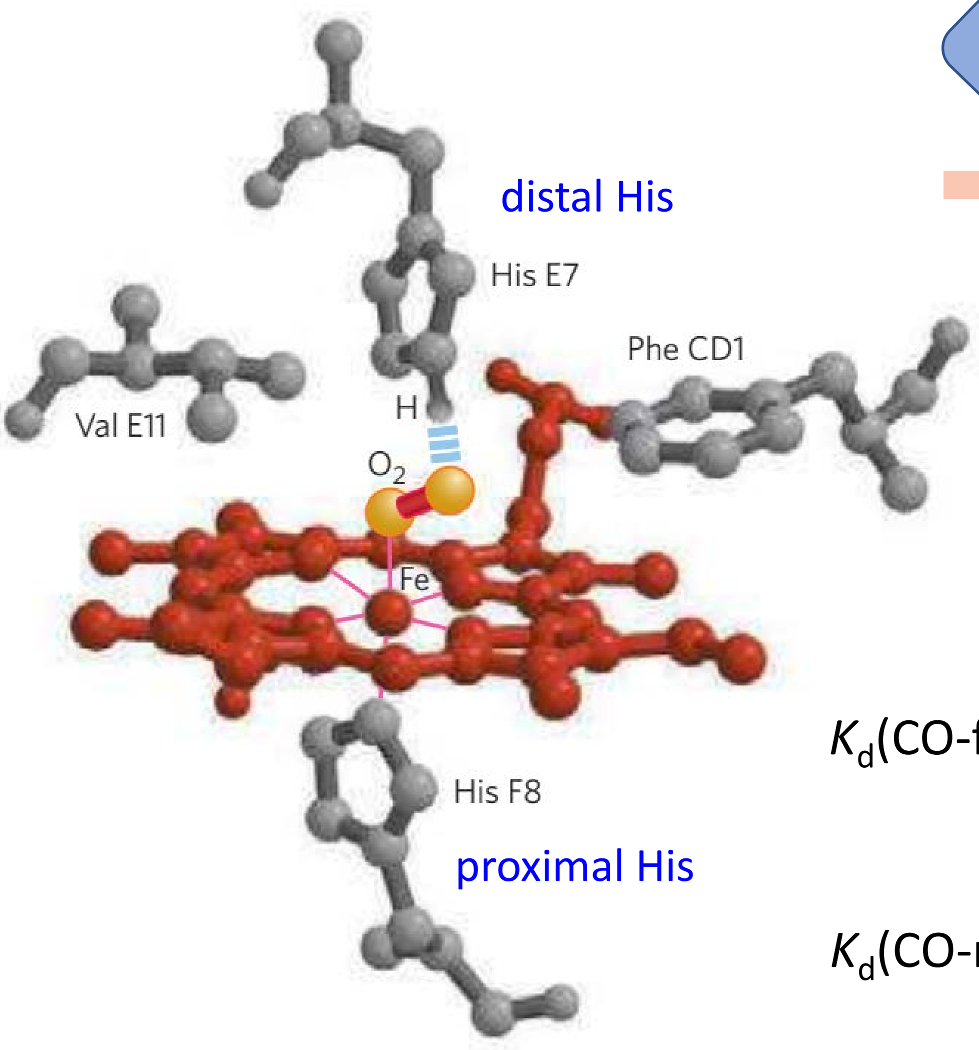
- 肌紅蛋白 (myoglobin, Mb)
  - 單一多肽組成
  - 共有153個胺基酸
  - 78%胺基酸位於α-螺旋

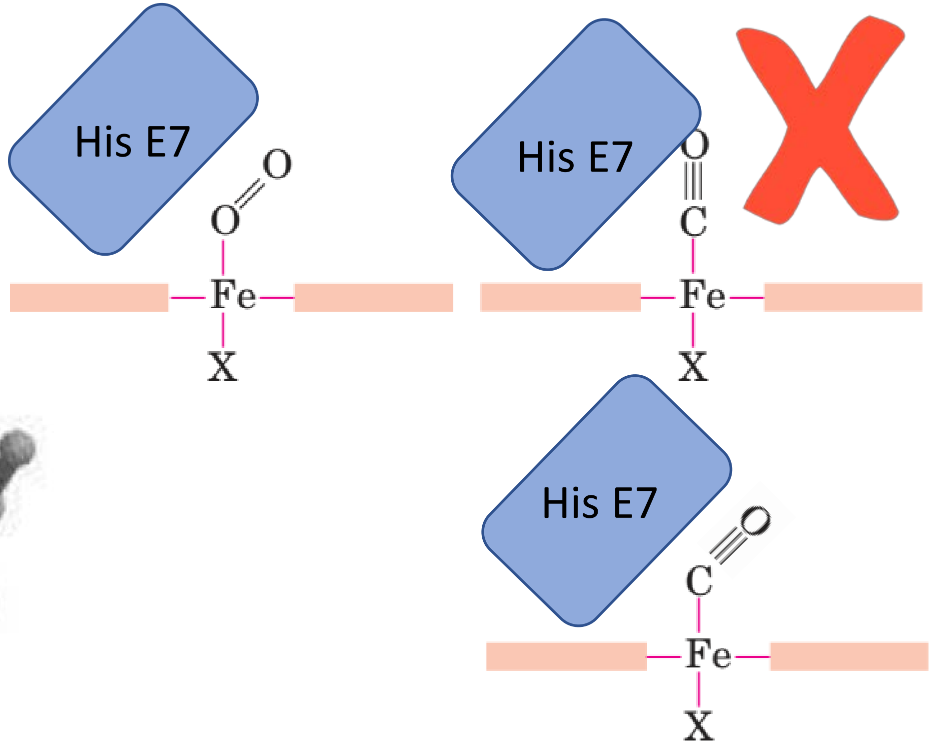


(PDB ID 1MBO) The eight -helical segments are labeled A through H. Nonhelical residues in the bends that connect them are labeled AB, CD, EF, and so forth.

蛋白質結構影響配體結合的親和力

- 立體效應(steric effect)





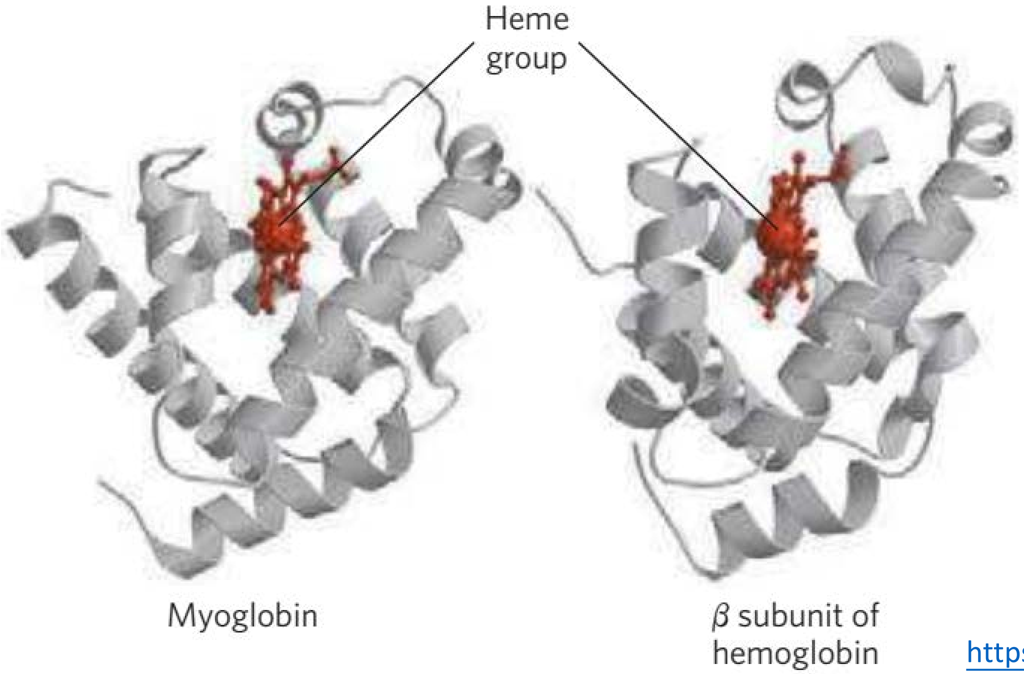
K_d(CO-free heme) ≈ 20,000 x K_d(O_2-free heme)

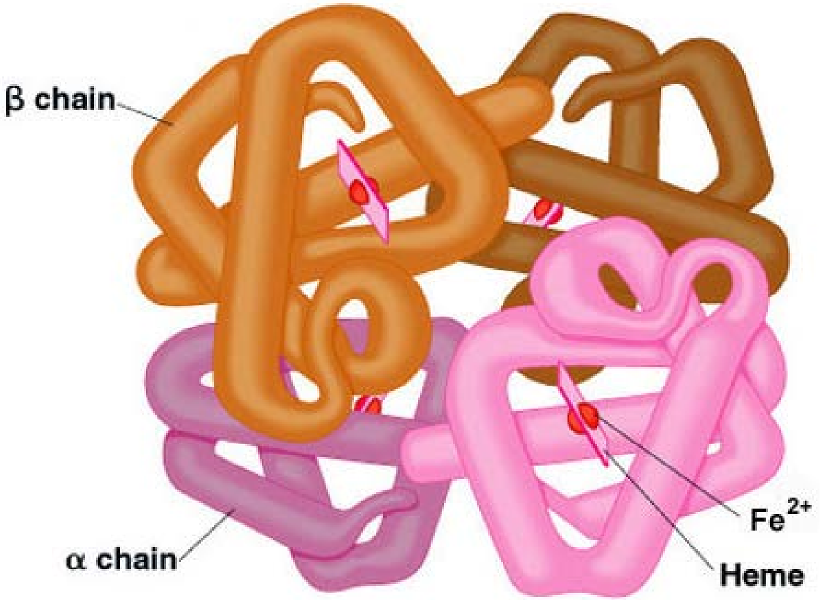
Steric effect of distal His
→ Reduction in CO binding

K_d(CO-myoglobin) ≈ 200 x K_d(O_2-myoglobin)

血紅蛋白次單元與肌紅蛋白結構相似

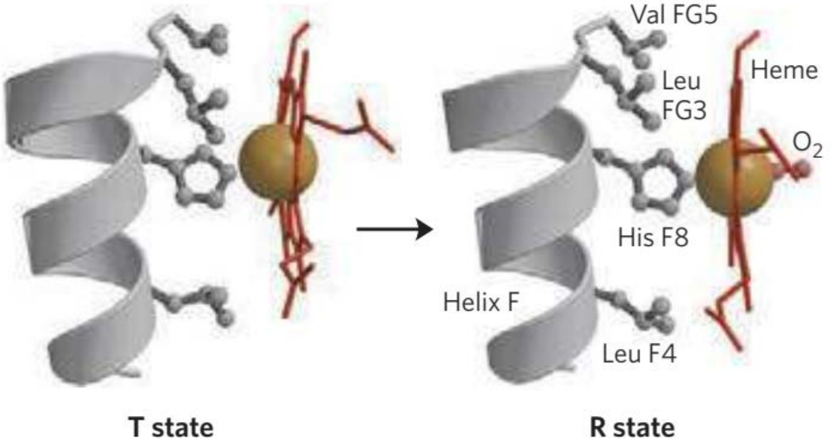
- 血紅蛋白(hemoglobin)
  - 成人血紅蛋白由α與β兩種球蛋白(globin)組成，兩種多肽50%以上胺基酸不同，但是結構卻很相似。
  - 四級結構由兩個α鏈與與兩個β鏈所組成。

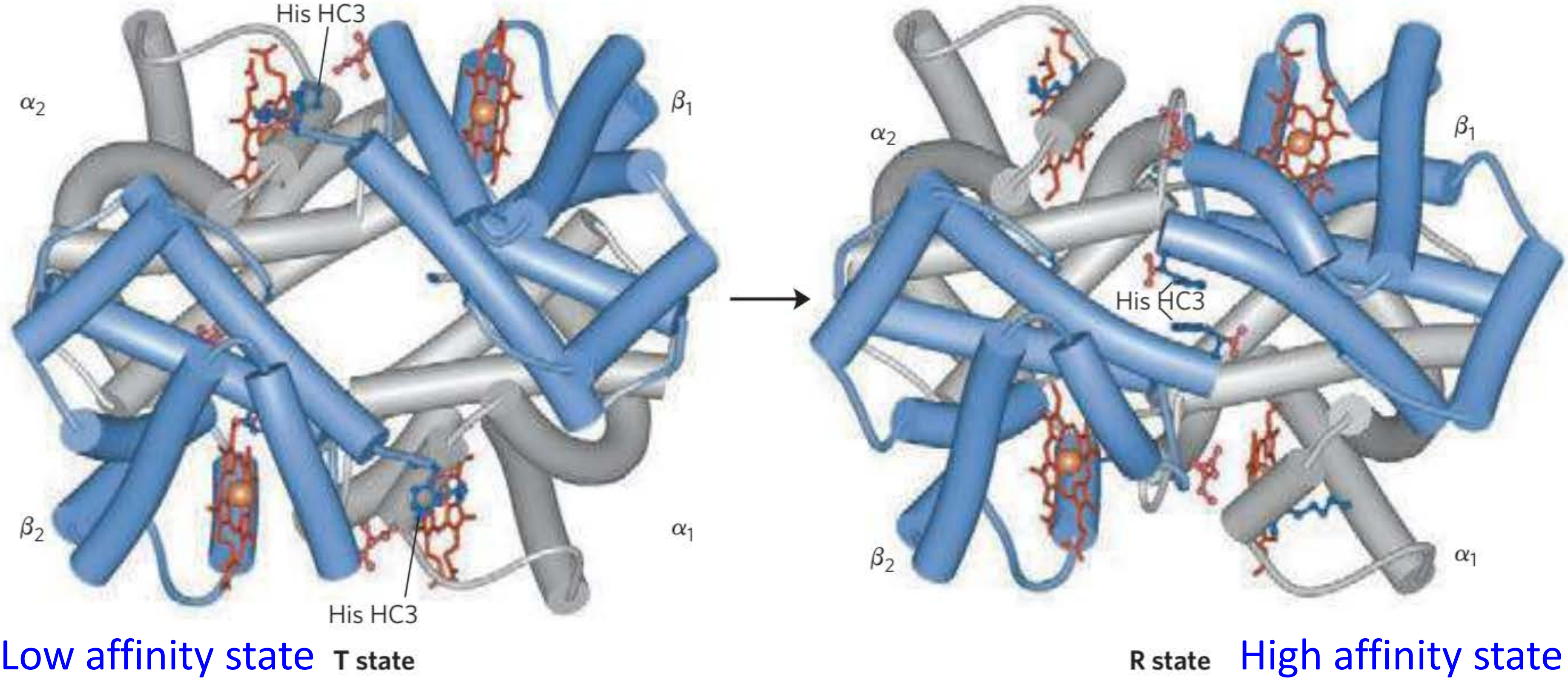




https://themedicalbiochemistrypage.org/hemoglobin-and-myoglobin/

血紅蛋白構型改變造成氧親和力不同



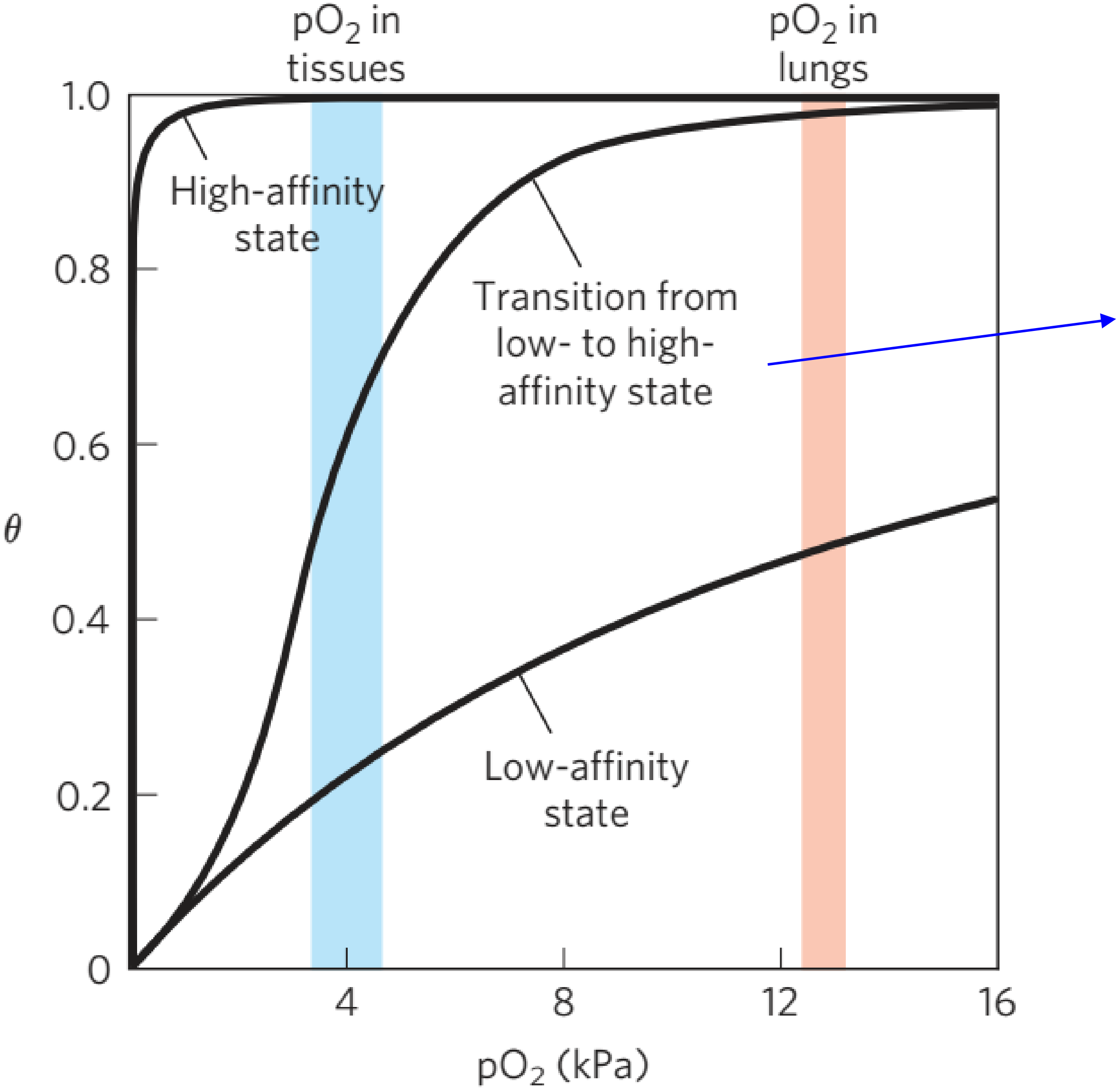


Low affinity state

High affinity state

血紅蛋白的氧協同結合效應

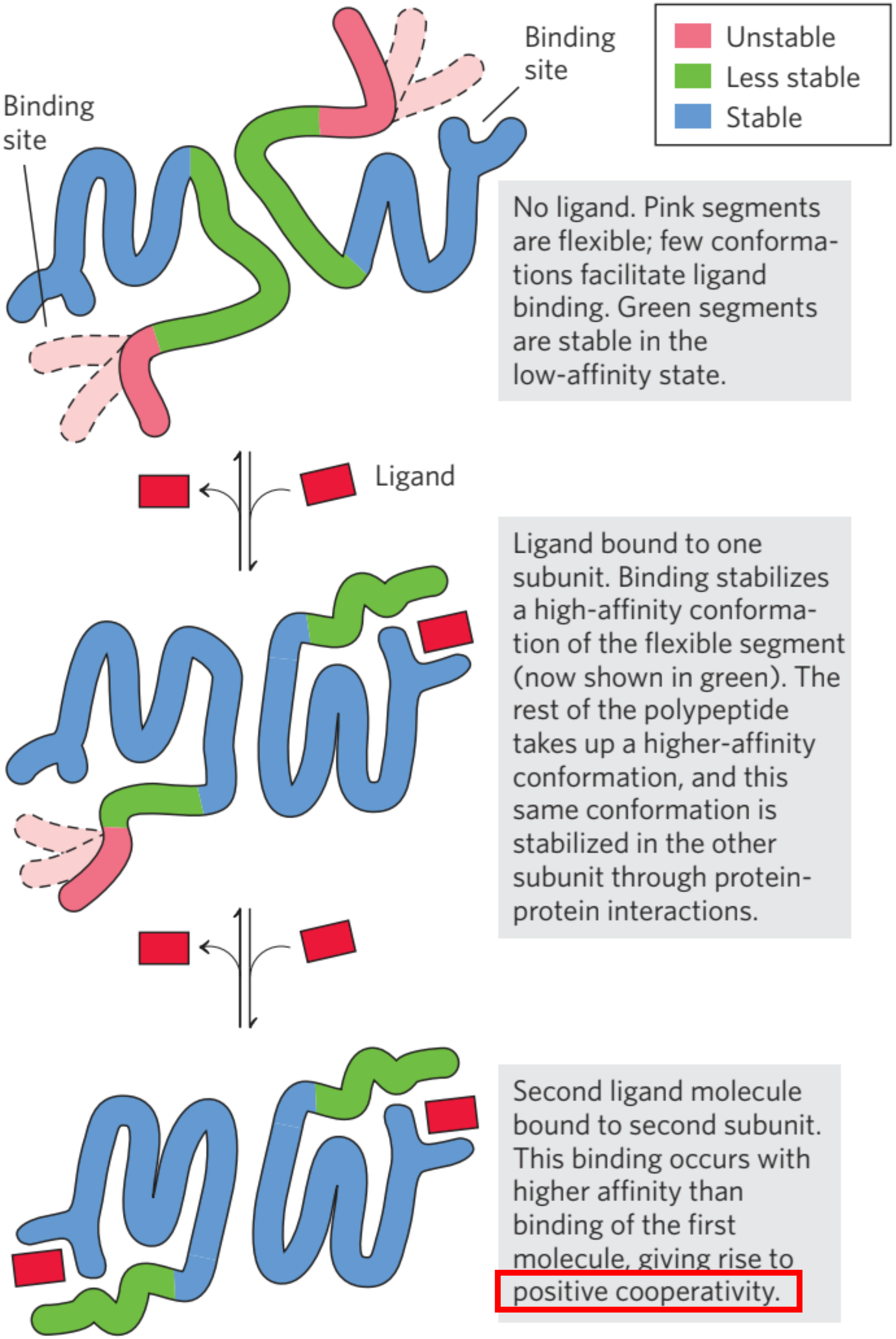
(cooperative binding)



A sigmoid binding curve can be viewed as a hybrid curve reflecting a transition from a low-affinity to a high-affinity state.

正向協同作用

- 異位蛋白(allosteric protein)接上配體後，影響鄰近蛋白的穩定性，提高其配體親和力。

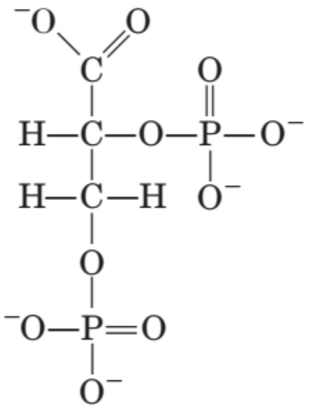


No ligand. Pink segments are flexible; few conformations facilitate ligand binding. Green segments are stable in the low-affinity state.

Ligand bound to one subunit. Binding stabilizes a high-affinity conformation of the flexible segment (now shown in green). The rest of the polypeptide takes up a higher-affinity conformation, and this same conformation is stabilized in the other subunit through protein-protein interactions.

Second ligand molecule bound to second subunit. This binding occurs with higher affinity than binding of the first molecule, giving rise to positive cooperativity.

2,3-二磷酸甘油酸的負向協同作用

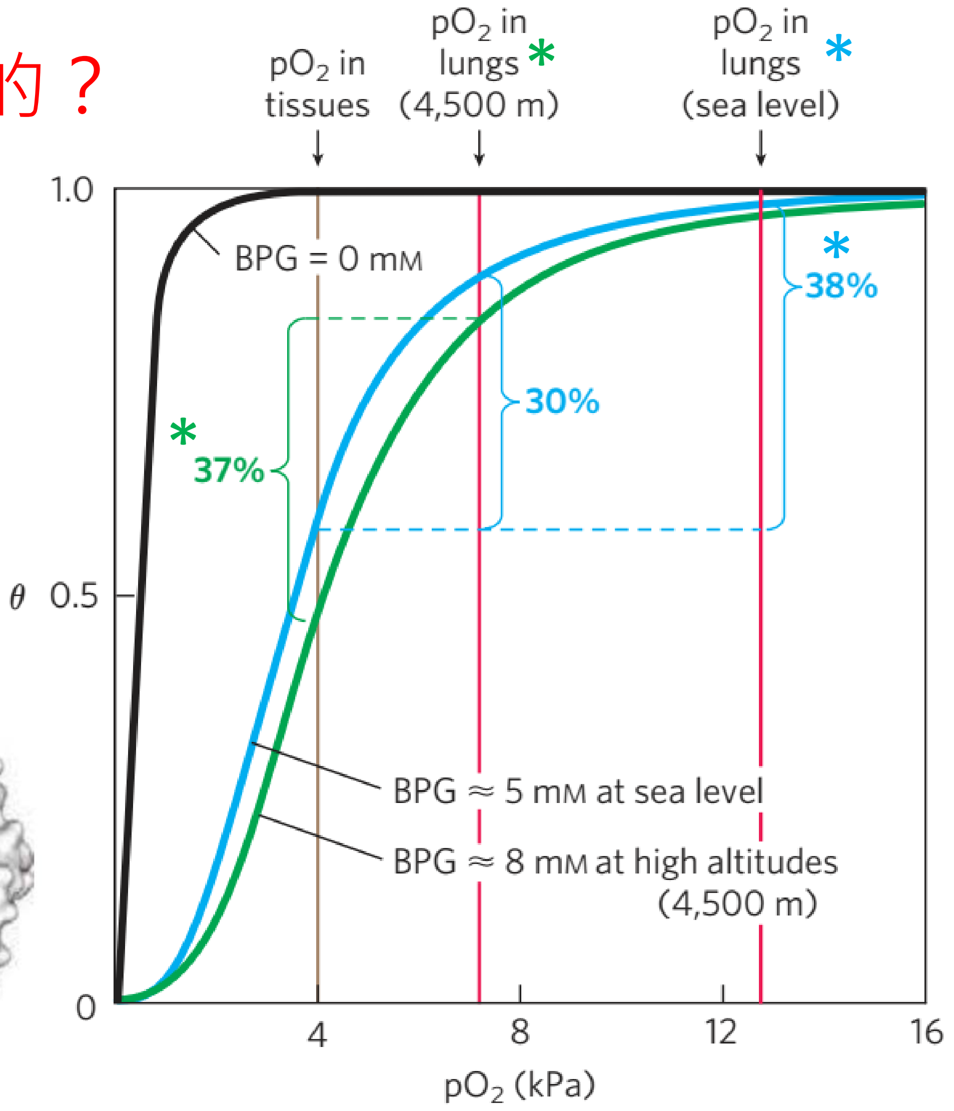


2,3-Bisphosphoglycerate

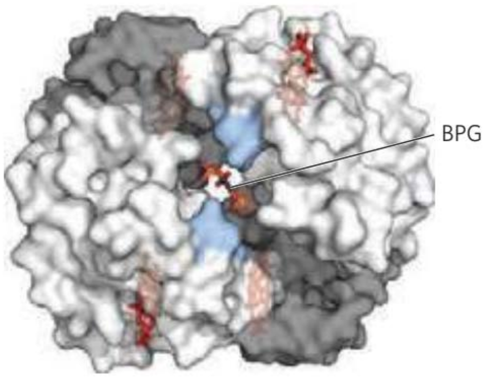
2,3-二磷酸甘油酸(BPG)

- Effect of BPG on oxygen binding to hemoglobin

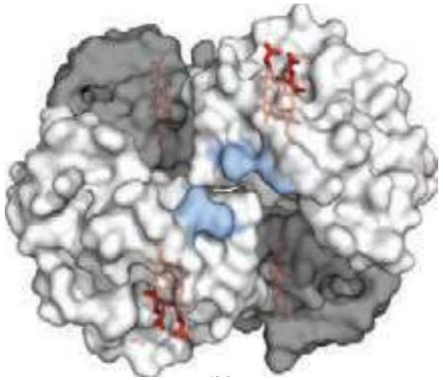
目的？



Binding pocket for BPG disappears.



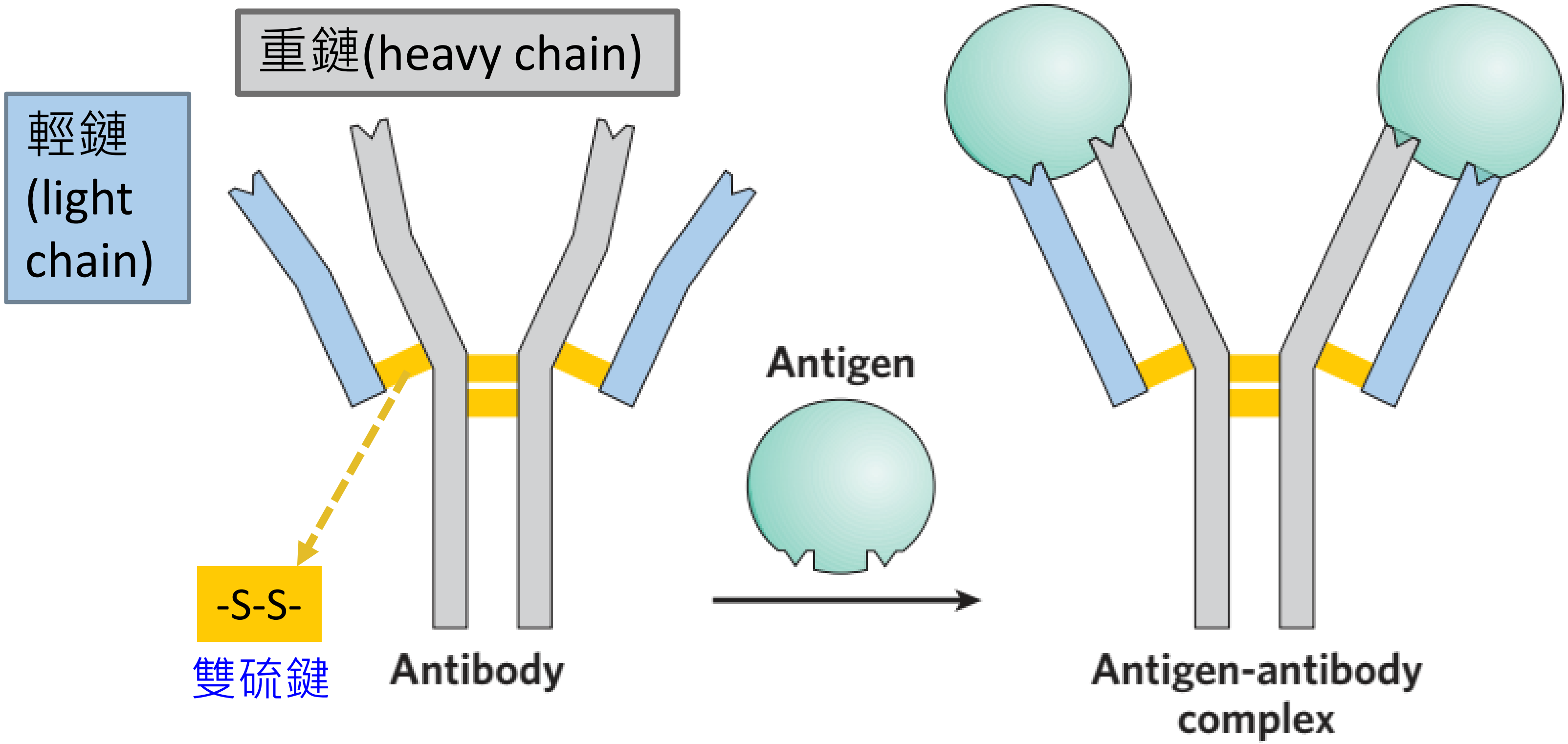
T state



R state

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：

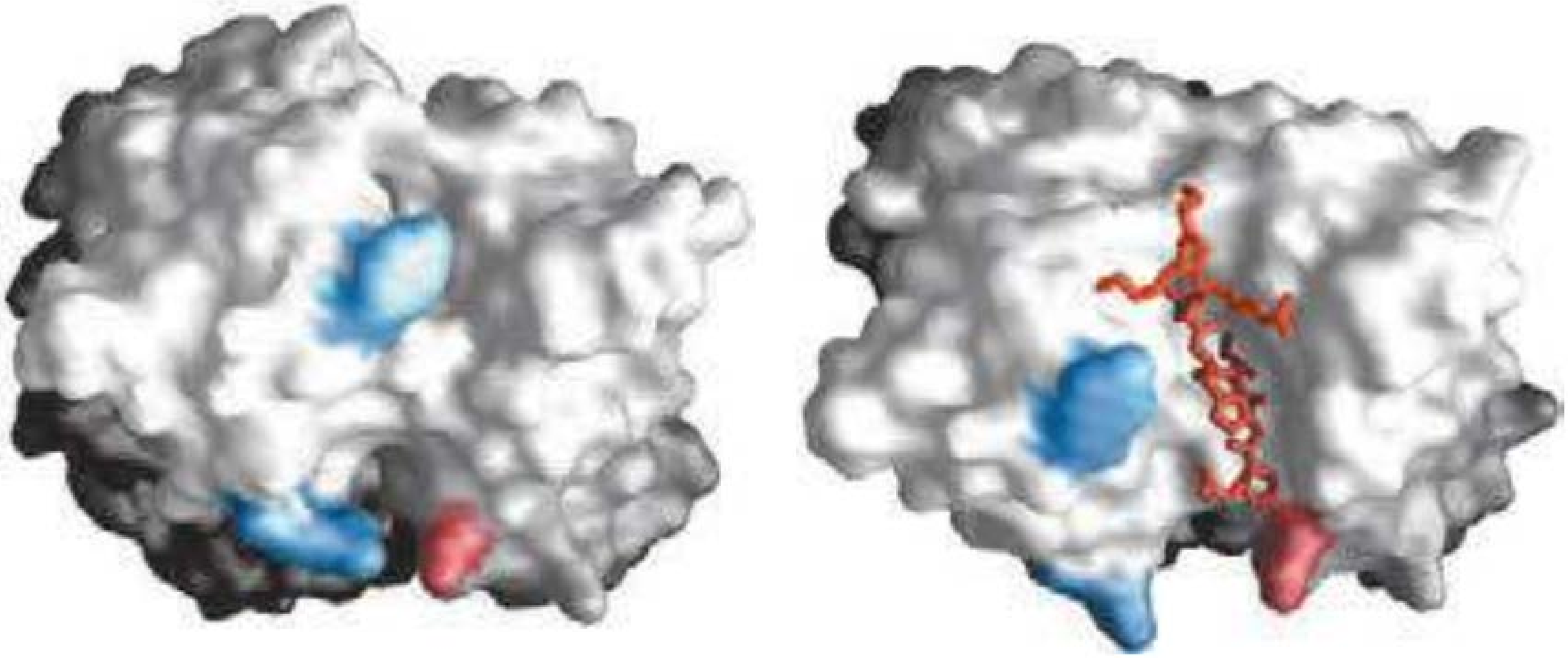


To generate an optimal fit for the antigen, the binding sites of IgG often undergo slight conformational changes. Such induced fit is common to many protein-ligand interactions.

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。



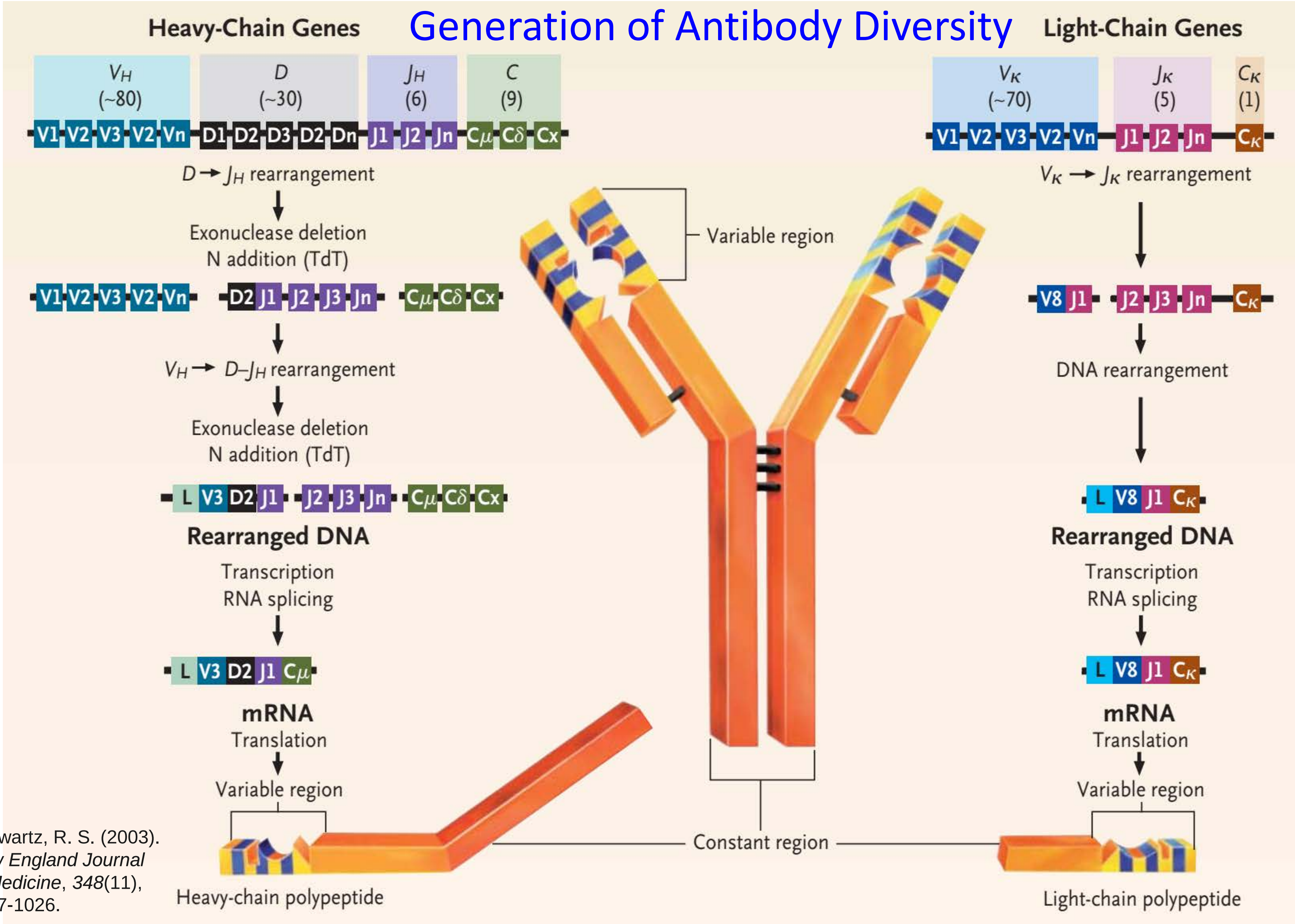
(a) Conformation with no antigen bound

(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.

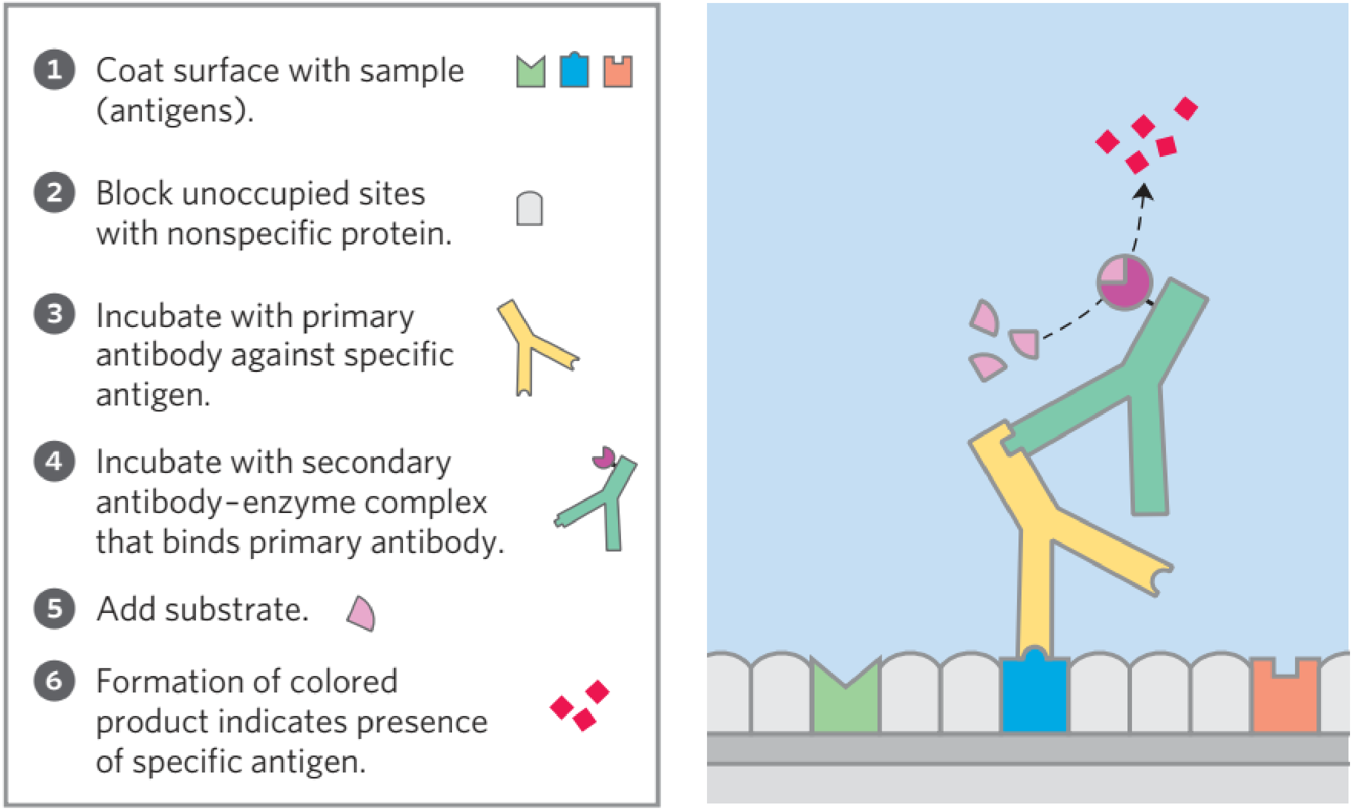
免疫球蛋白如何辨識多樣的抗原？

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



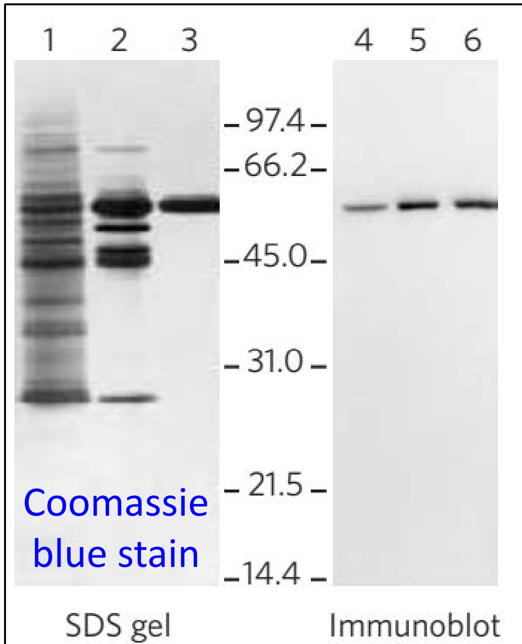
1. Coat surface with sample (antigens).
2. Block unoccupied sites with nonspecific protein.
3. Incubate with primary antibody against specific antigen.
4. Incubate with secondary antibody–enzyme complex that binds primary antibody.
5. Add substrate.
6. Formation of colored product indicates presence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

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

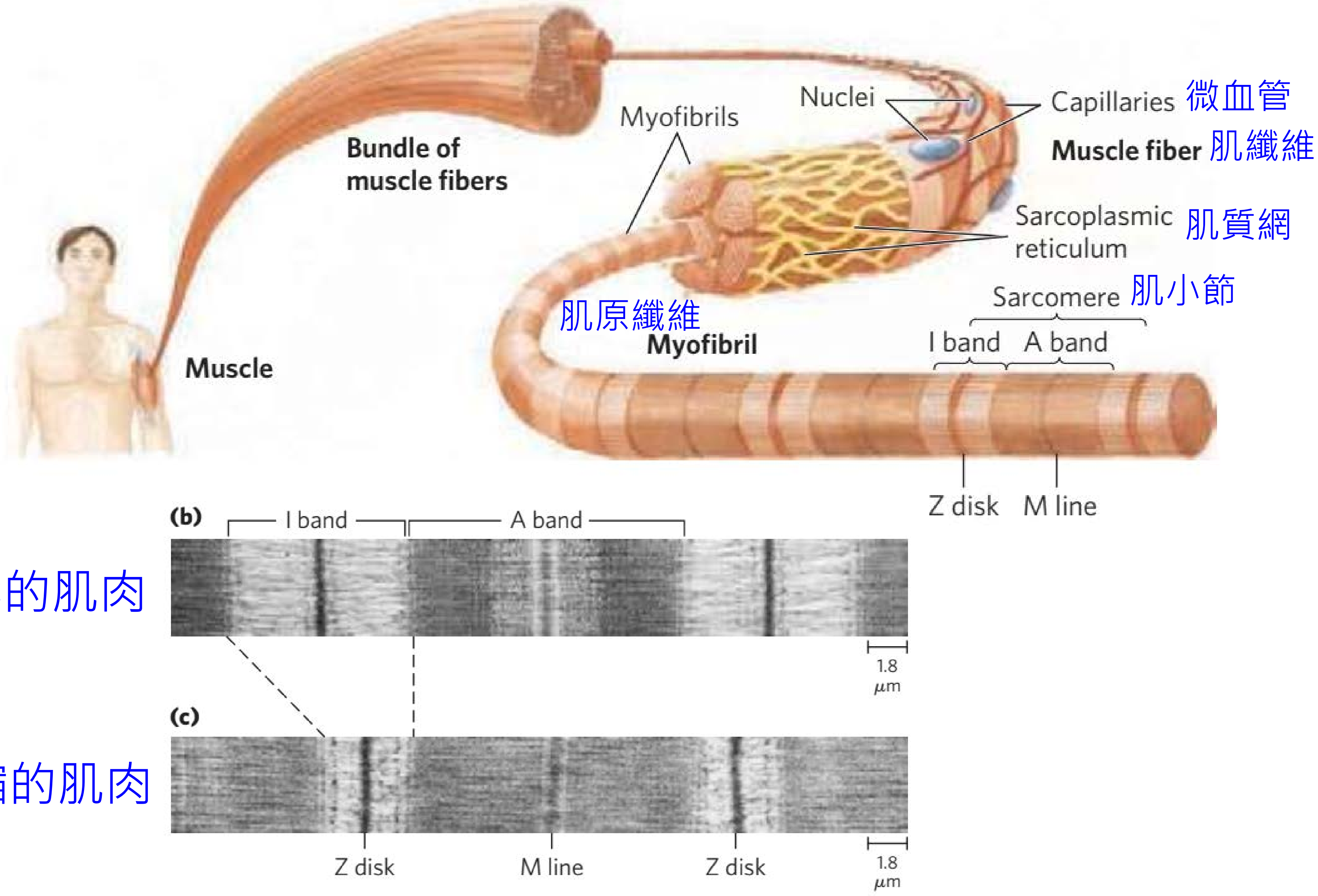
Test for the presence of herpes simplex virus (HSV) antibodies in blood samples.

2. 免疫墨點法
（即西方墨點法）
Western blot

Purification of a protein kinase.



分子馬達：肌凝蛋白與肌動蛋白



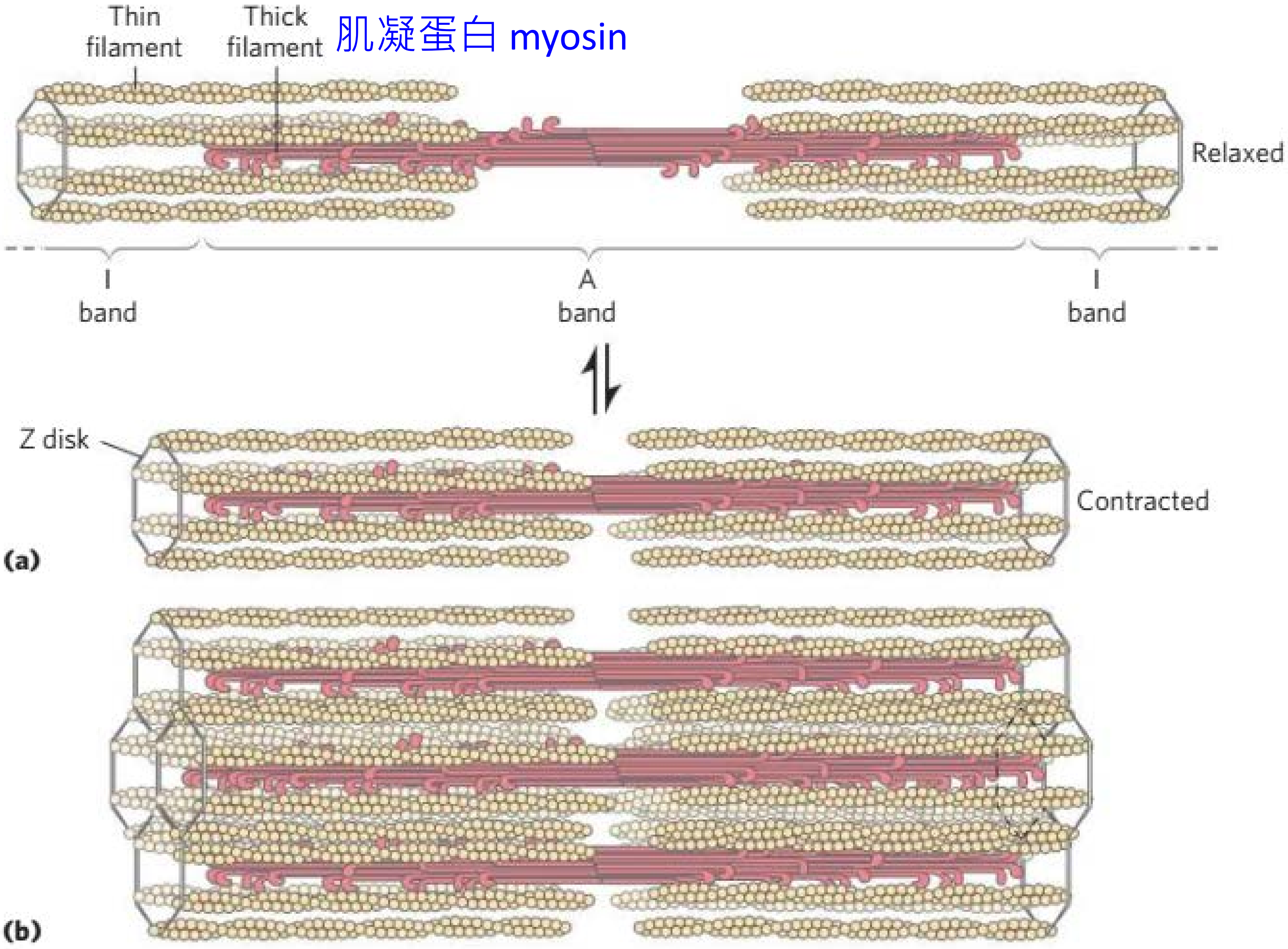
放鬆的肌肉

收縮的肌肉

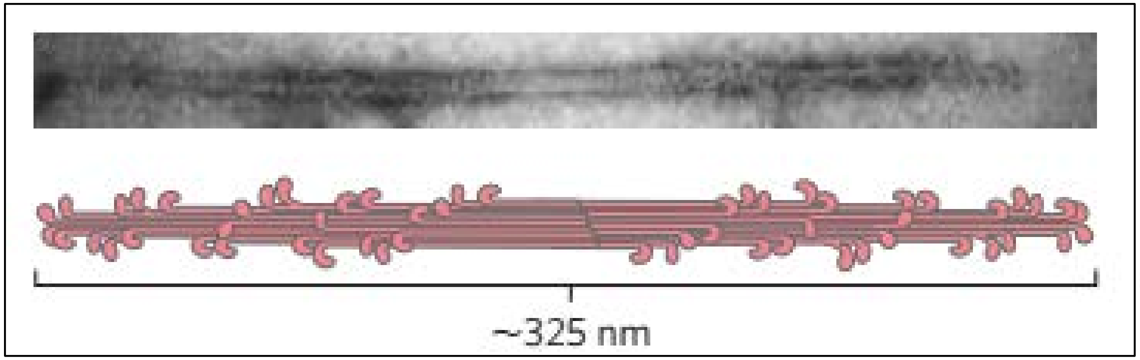
Muscle contraction

肌動蛋白 actin

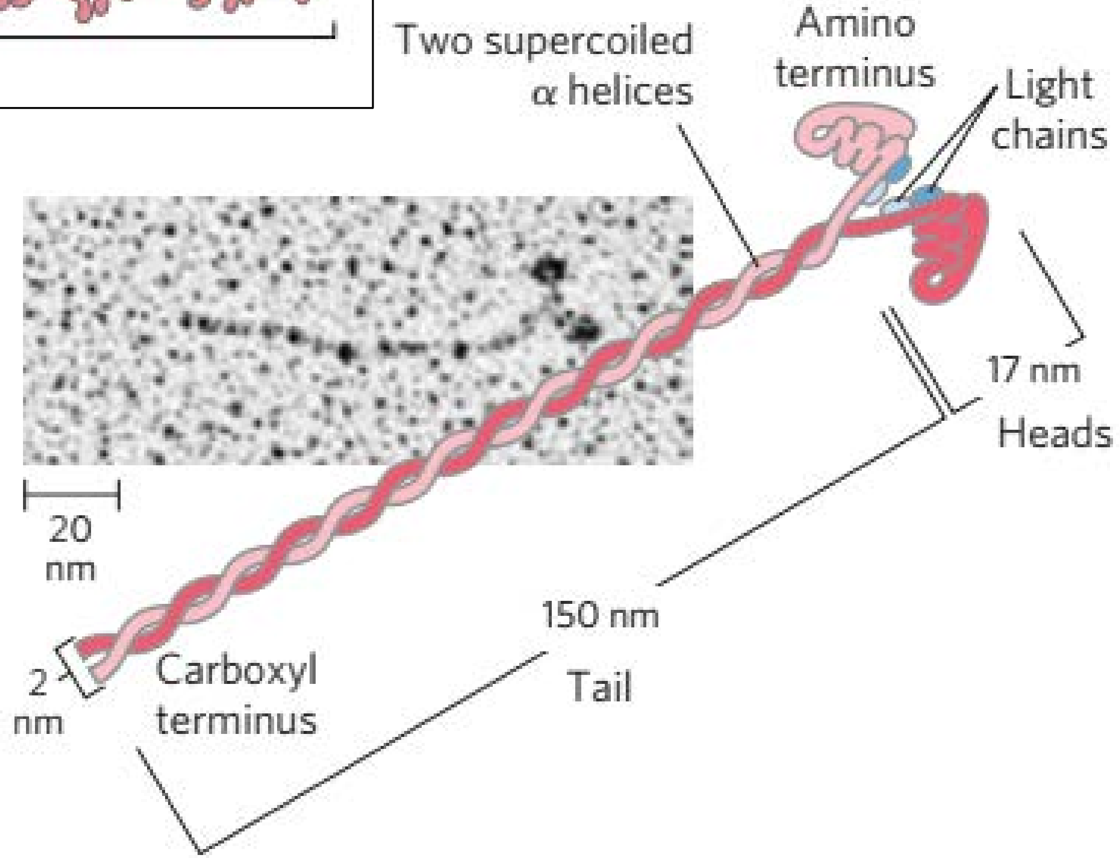
肌凝蛋白 myosin



肌凝蛋白的結構



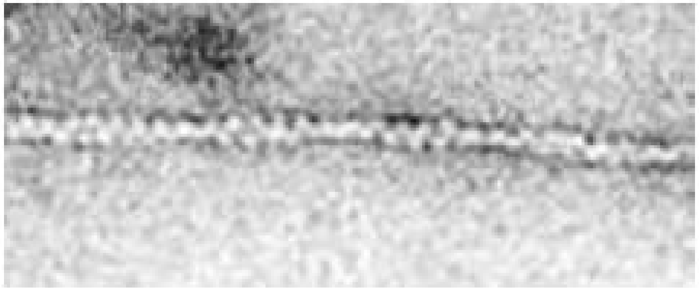
- 兩重鏈（粉紅色）
  - 頭（N端）
  - 尾（C端）：α-螺旋
- 四輕鏈（藍色）

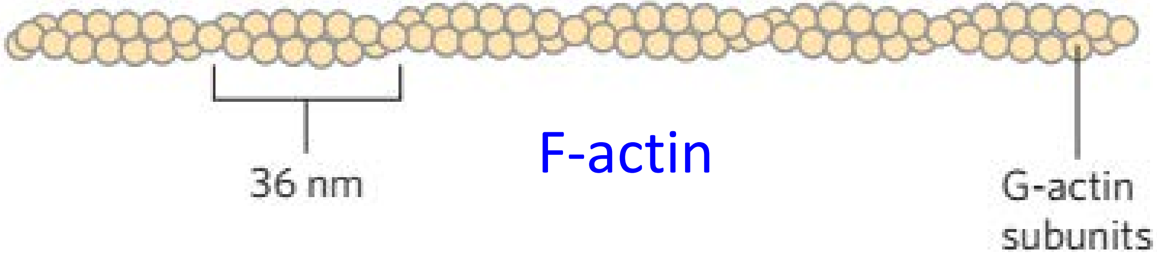


肌動蛋白的結構

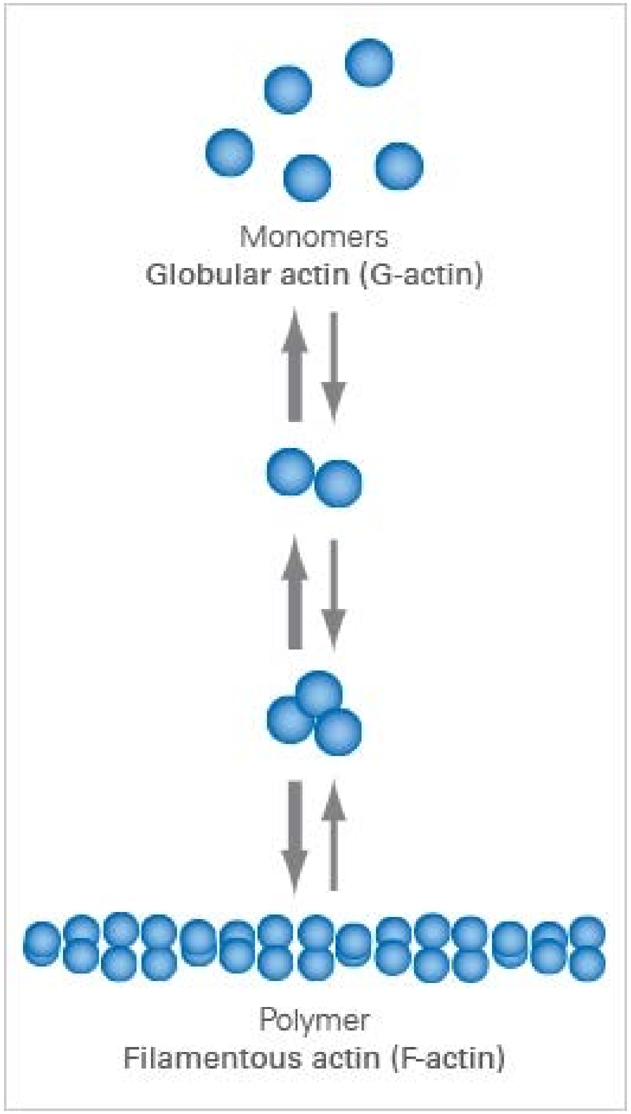
Two forms of actins:

- Monomeric, globular G-actin
- Polymeric, filamentous F-actin



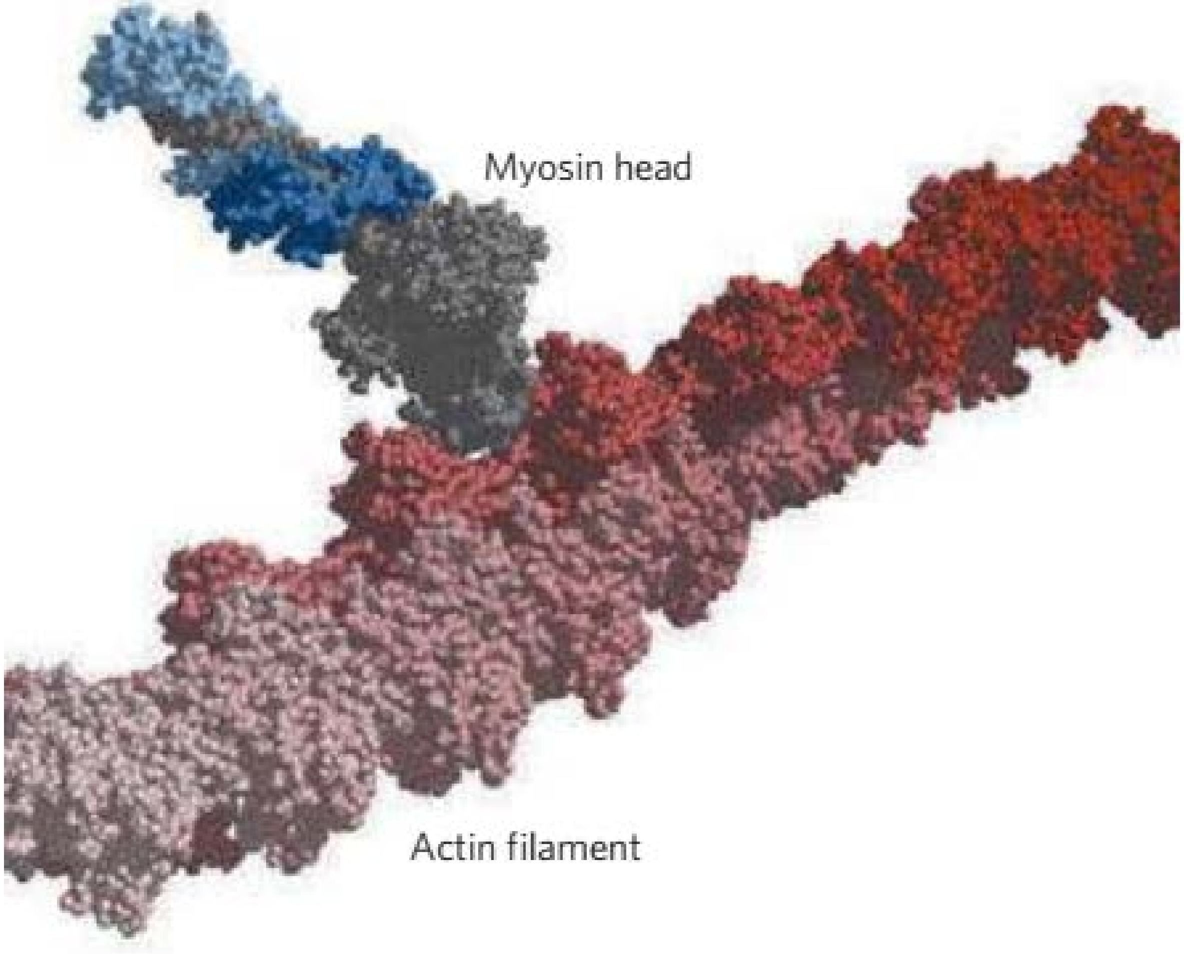


F-actin

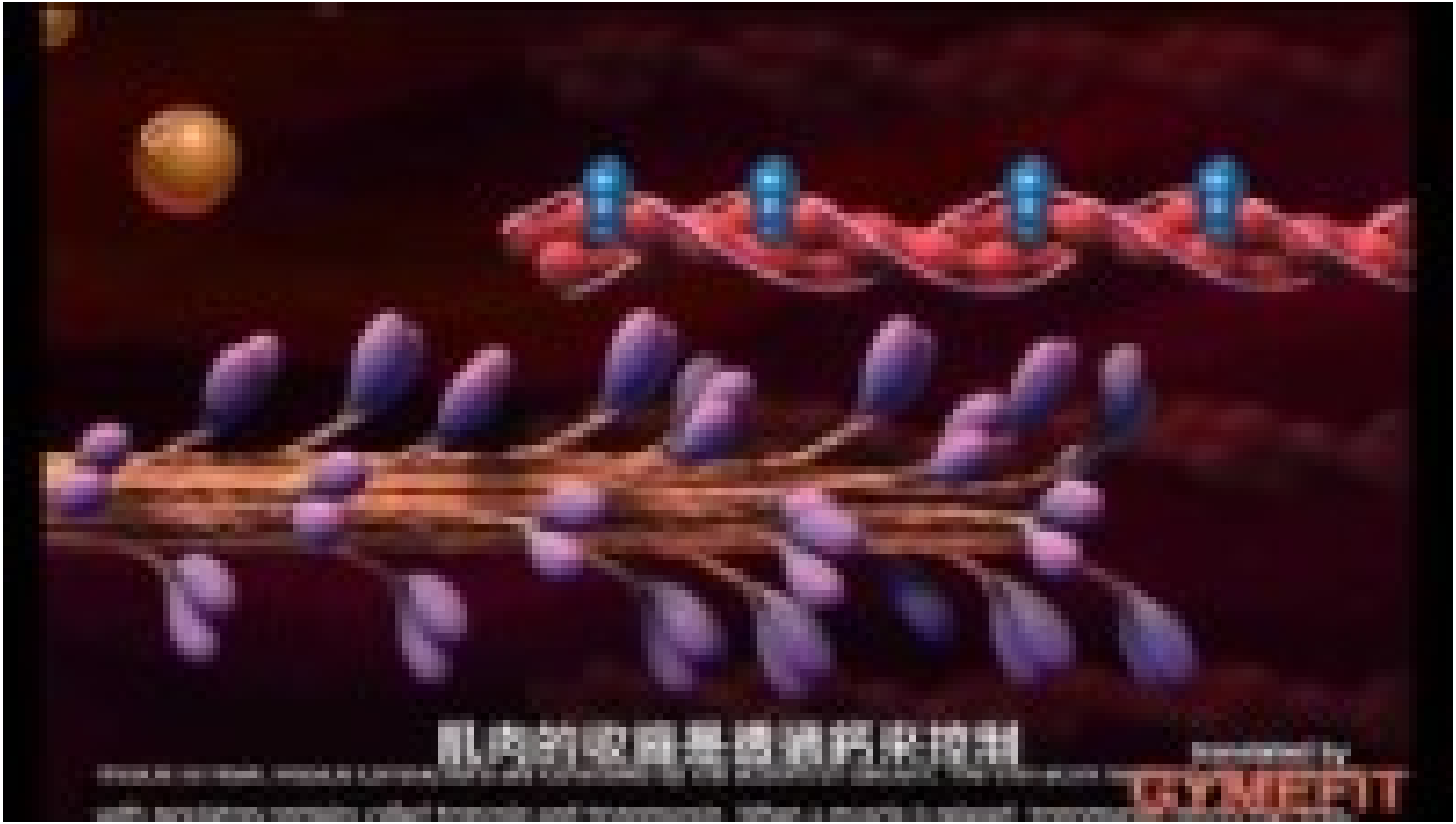


https://ibidi.com/content/80-f-actin-a-crucial-protein

- Space-filling model of an actin filament (shades of red) with one myosin head (gray and two shades of blue) bound to an actin monomer within the filament



肌肉收縮的機制



https://youtu.be/uyZFOAxJGM0