1 蛋白質抽取 Protein Extraction

1.1 如何開始？ How to start?

5W principles 基本原則

1.2 材料來源 Materials & sources

材料取得與保存

1.3 均質及抽取 Homogenization & extraction

確實做好第一步

1.4 鹽析及沉澱法 Salting-out & precipitation

最經濟方便的方法

1.1 如何開始？ How to start?

先考慮以下諸點：

	5W
a. 要純化那一個蛋白質？	What？
b. 為何要純化此蛋白質？	Why？
c. 由何種材料純化？	Where from？
d. 由那一個生長期？	When？
e. 如何純化此蛋白質？	How？

5W & 3 Stages

酵素純化三階段 Three stages

(1) **粗蛋白質** (crude protein)：

採樣 → 均質打破細胞 → 抽出全部蛋白
(多用鹽析沉澱法)

(2) **部分純化** (partially purified)：

初步的純化，使用各種管柱層析法

(3) **均質蛋白** (homogeneous)：

目標酵素的進一步精製純化，可用製備式電泳或 HPLC 等

純化階段及分析方法

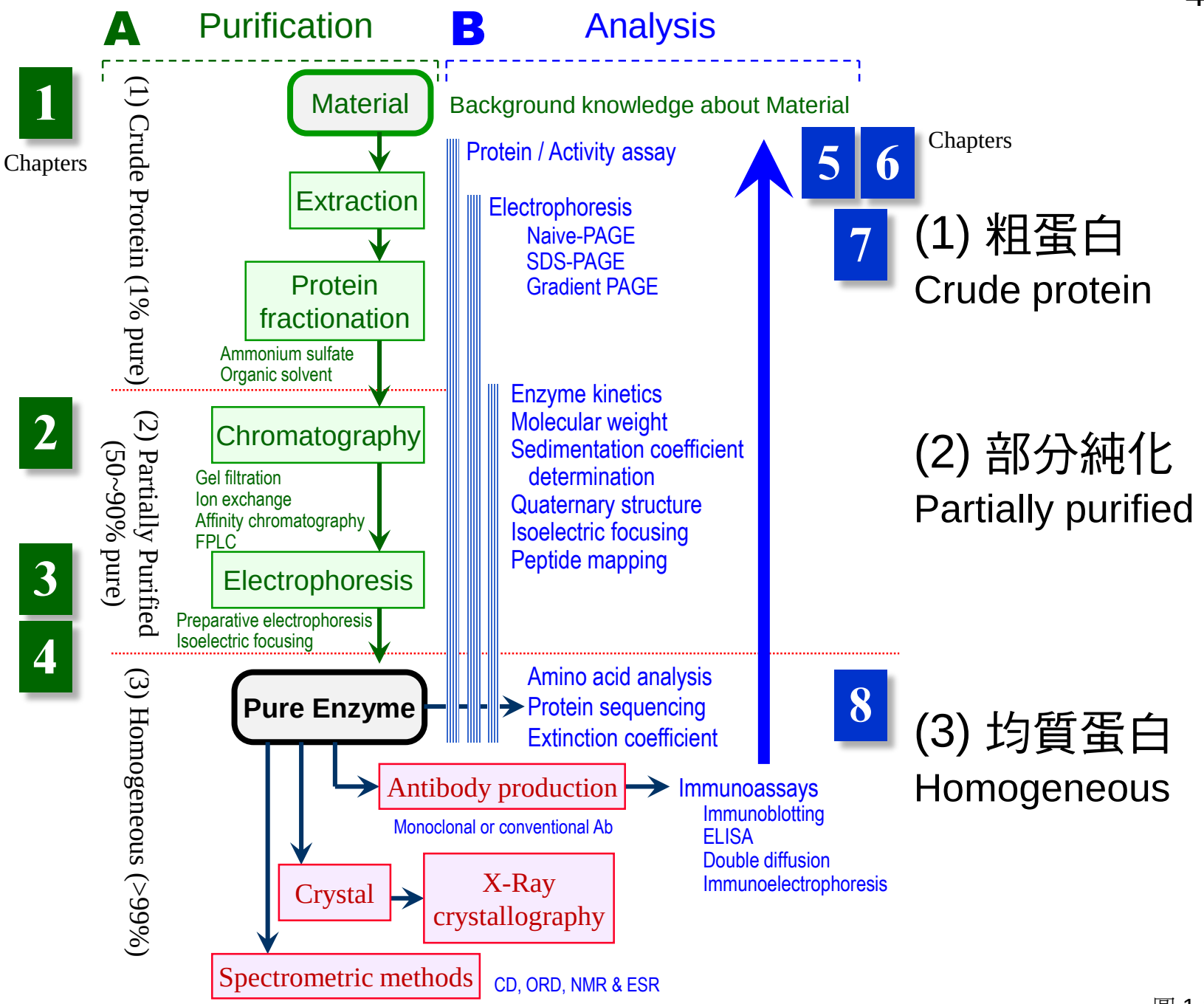


圖 1.1

純化或分析方法原理 4 Chapters

Cell

Cell homogenization 1

Organelle isolation? 胞器

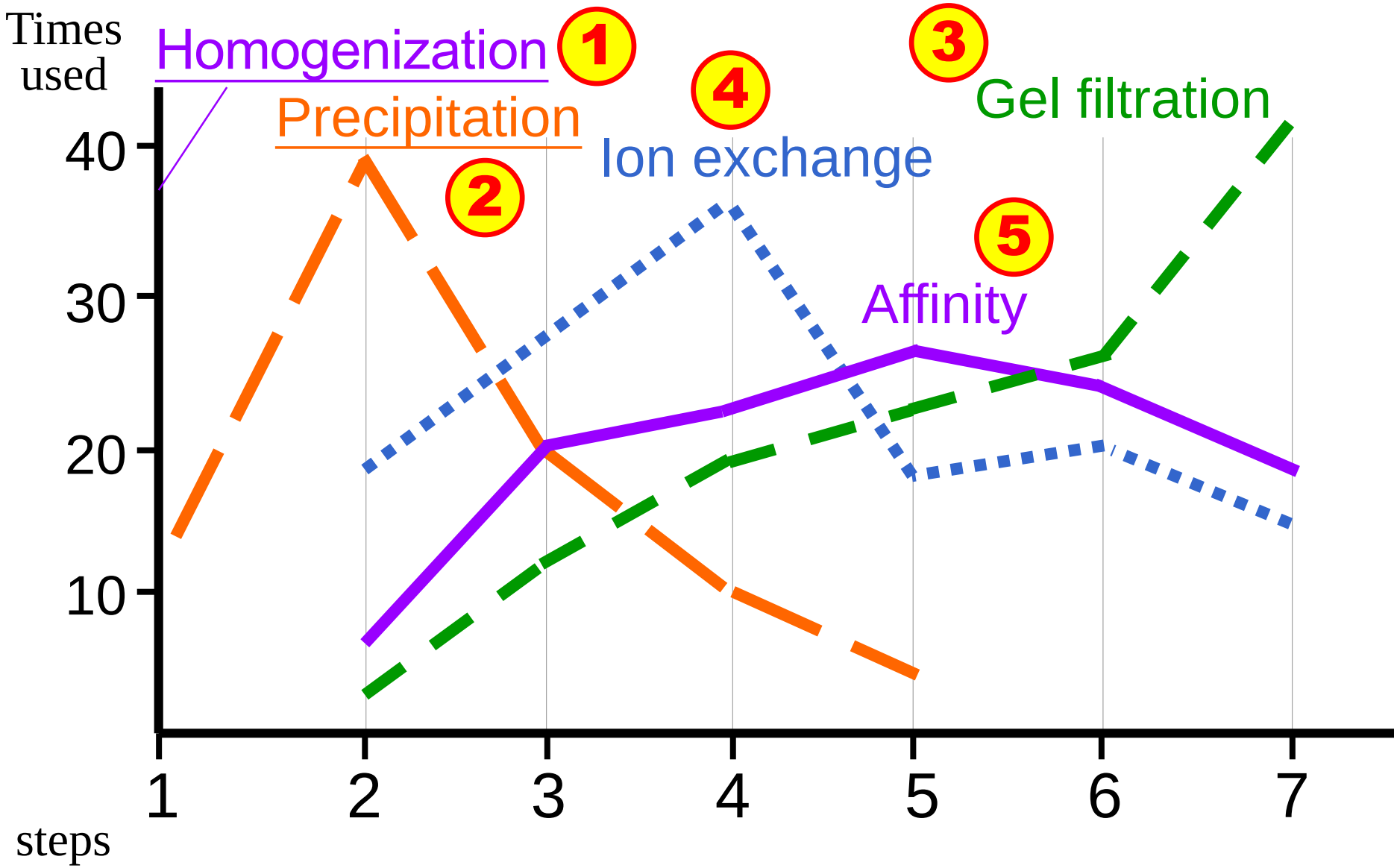
Small molecules	Macro molecules			細胞碎片 Cell debris
Amino acid, monosaccharide, nucleotide, fatty acid	Nucleic acid	Protein	Polysaccharides	

Ammonium sulfate precipitation 硫酸銨沈澱法

Molecular size	Molecular charge	Molecular polarity	Affinity
2 Gel filtration, SDS-PAGE, Ultrafiltration 3	Ion exchange, Chromatofocusing, Disc-PAGE, Isoelectric focusing	Reverse phase chromatography, HIC, Salting-out	Affinity chromatography, Hydroxyapatite

Useful basic protein properties in planning isolation steps

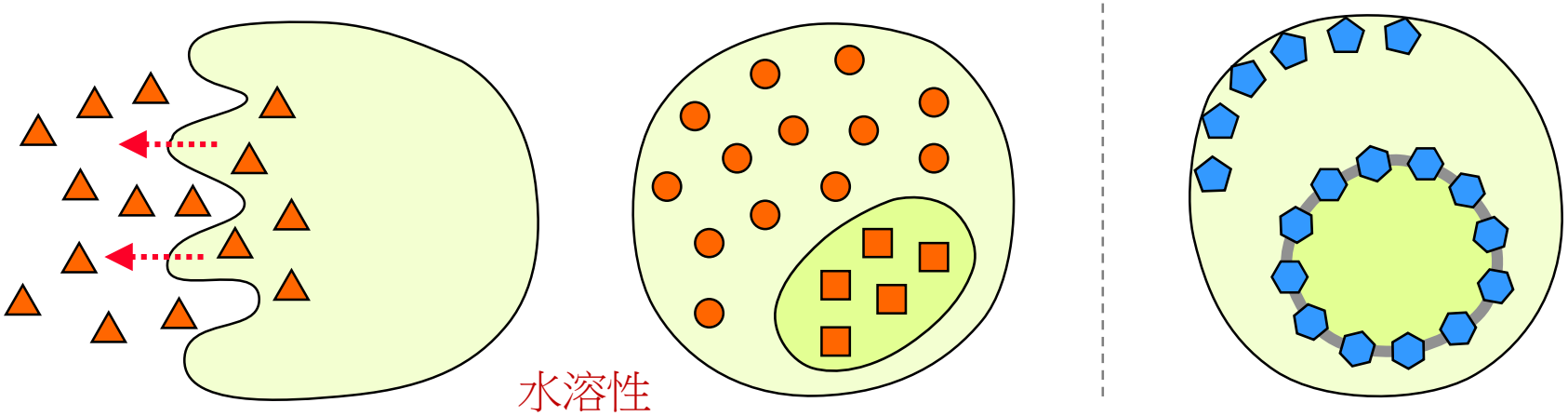
純化方法應用次序 Which step first?



Adapted from Bonnerjera et al. (1986) Bio/Technology, 4:954-958

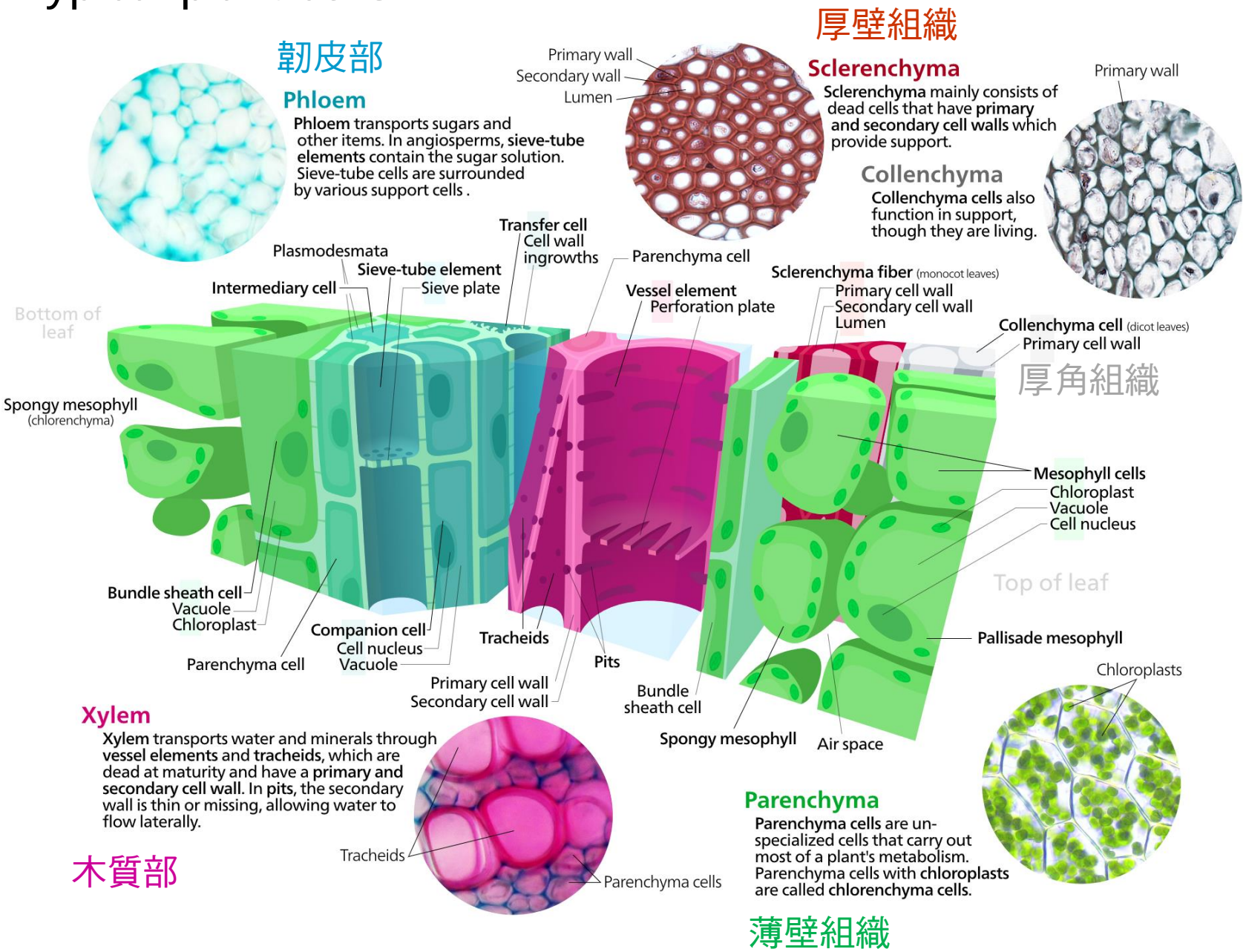
1.2 選擇材料 Choosing starting material

Which organism?	動物、植物、微生物
Which tissue?	根莖葉花果或組織培養
Which organelle?	細胞核、液泡、葉綠体
Secreted enzyme?	外泌酵素的穩定性較佳
Membrane protein?	脂溶性蛋白質較難純化



典型植物細胞

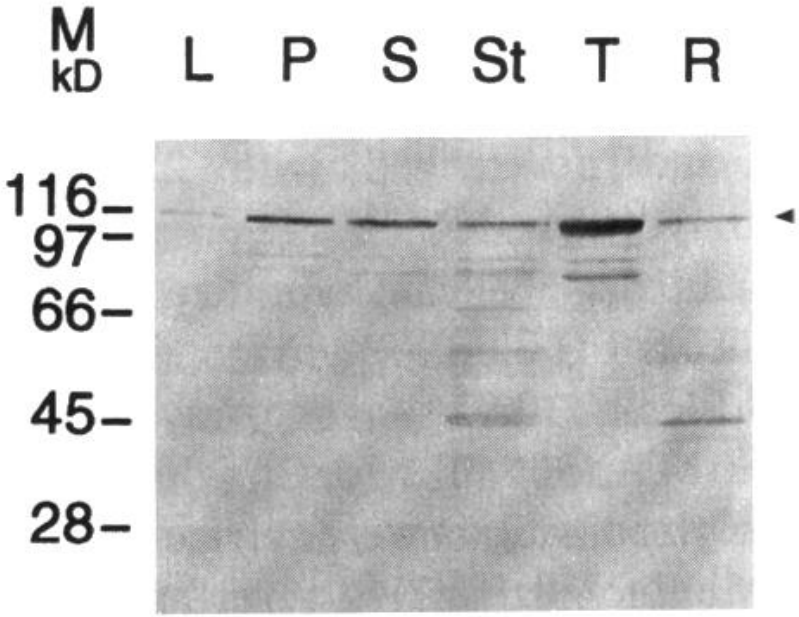
Typical plant cells



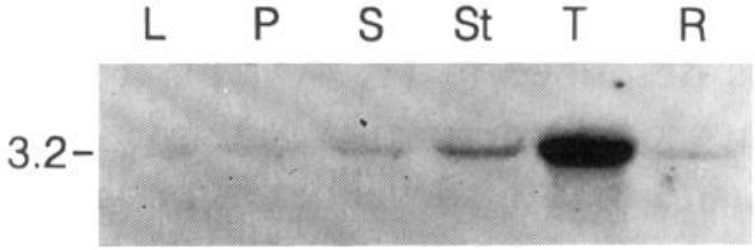
(Wikipedia, by Kelvinsong - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=25593329)

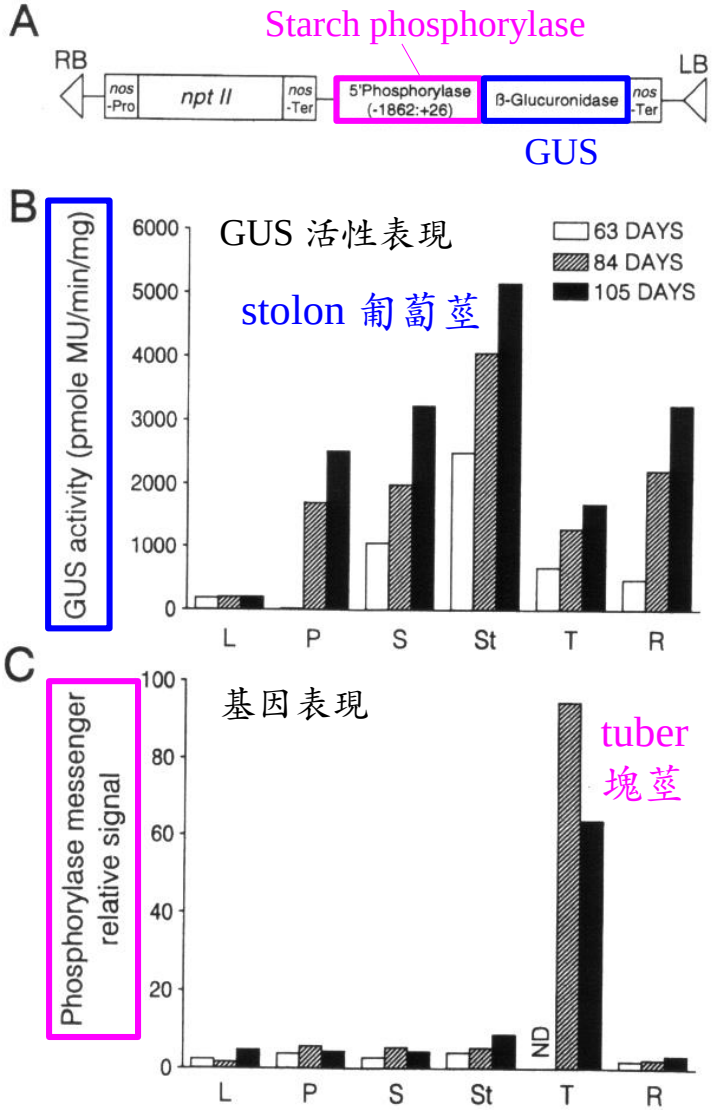
■ 各器官中表現不同 Expression in organs

SP protein expressed (potato organs)



SP RNA expression (potato organs)





St Pierre,B.; Bertrand,C.; Camirand,A.; Cappadocia,M.; Brisson,N. (1996) Plant Molecular Biology 30: 1087-1098
The starch phosphorylase gene is subjected to different modes of regulation in starch-containing tissues of potato

植物材料問題 Special problems of plant materials

細胞壁 Cell wall 較難打破

葉綠体 Chloroplast 特有的酵素與基因

液　泡 Vacuole 有許多干擾物質

蛋白酶 (proteases)

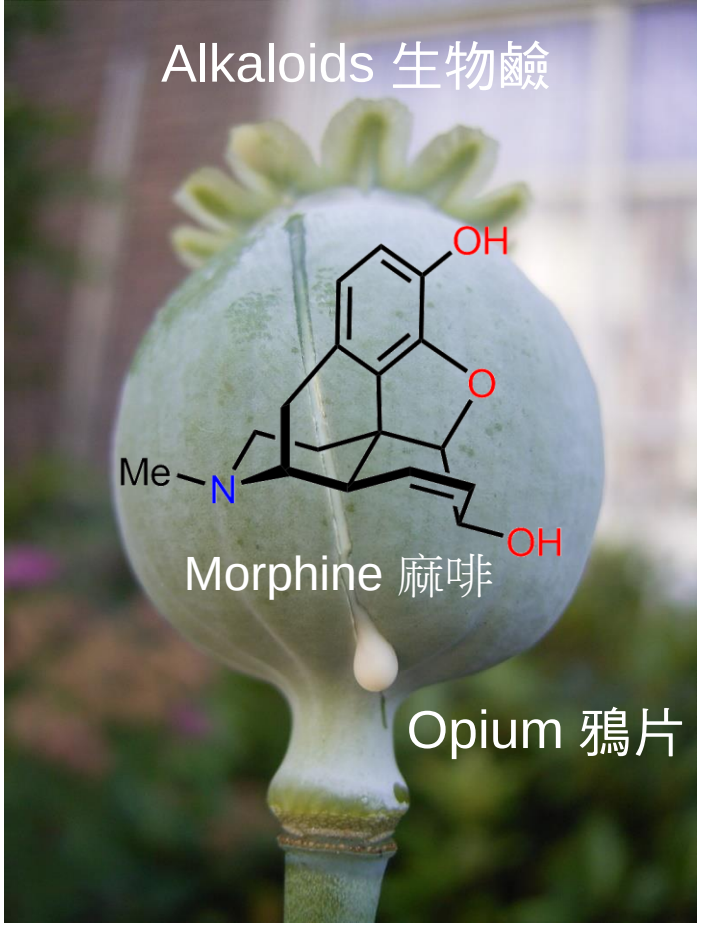
二次代謝物 生物鹼 (alkaloid)

多酚化合物 (polyphenols)

Flavonoid 類黃酮

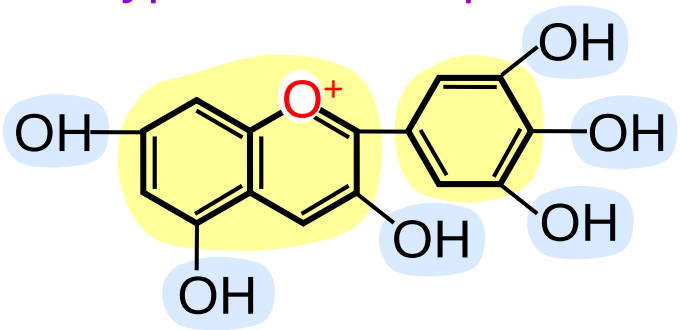
Tannin 單寧

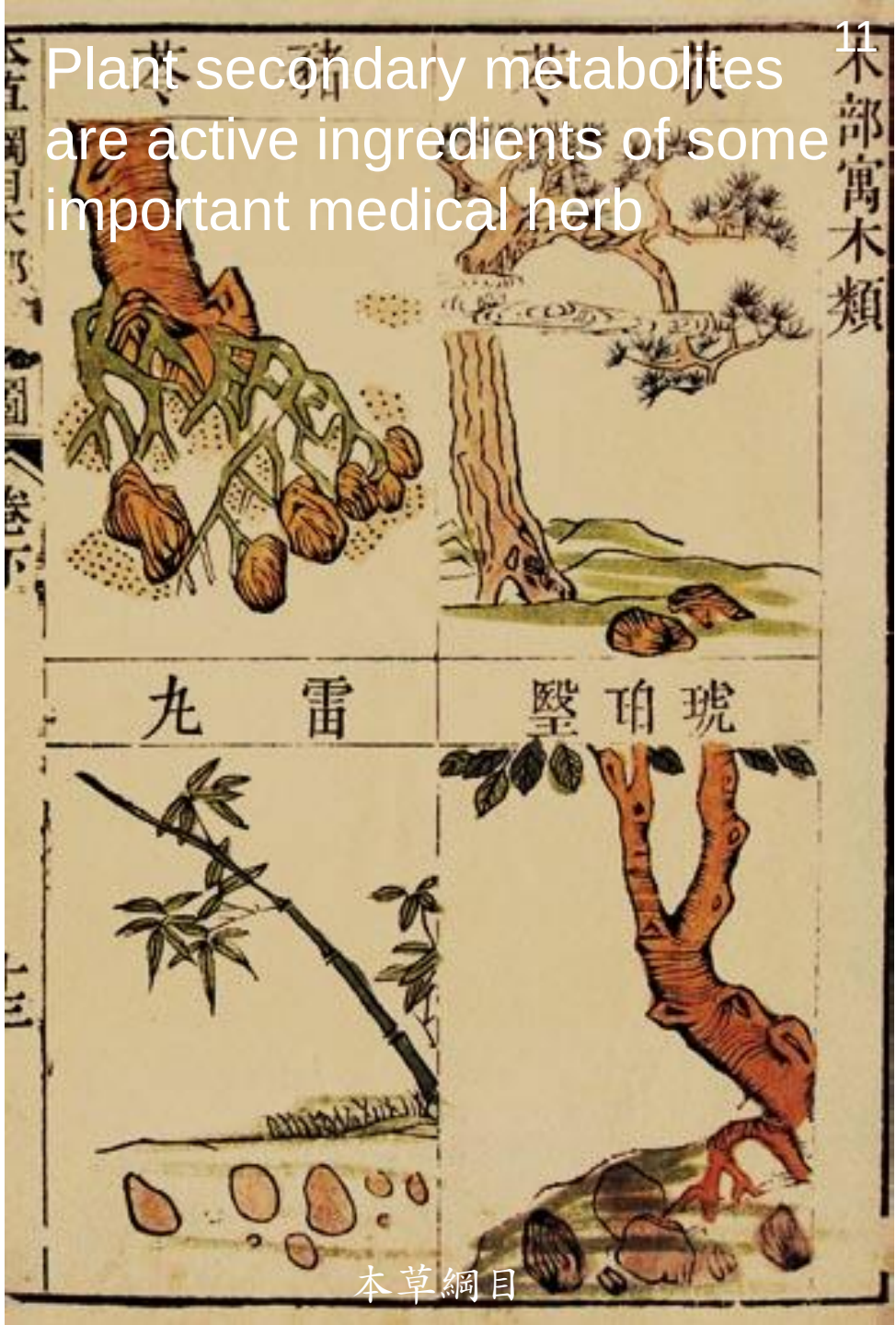
植物二次代謝物有重要藥理作用



Wikipedia (Public Domain, by KGM007)

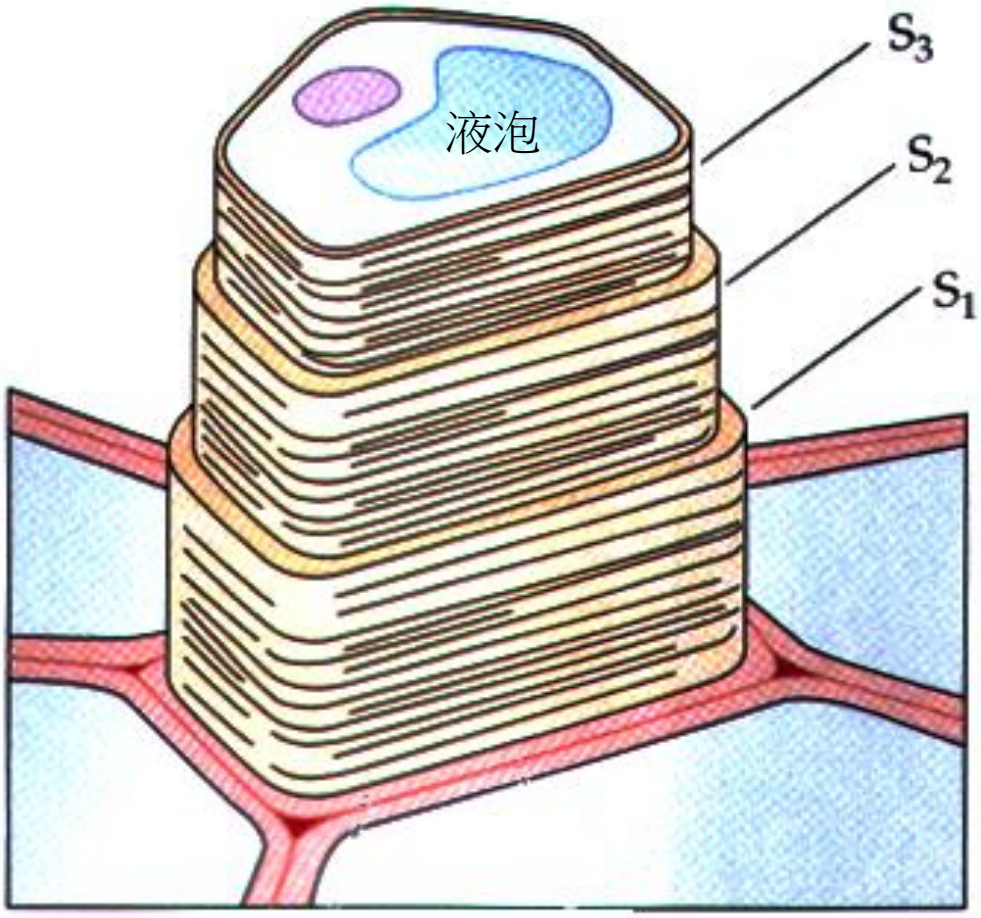
Polyphenolic compounds

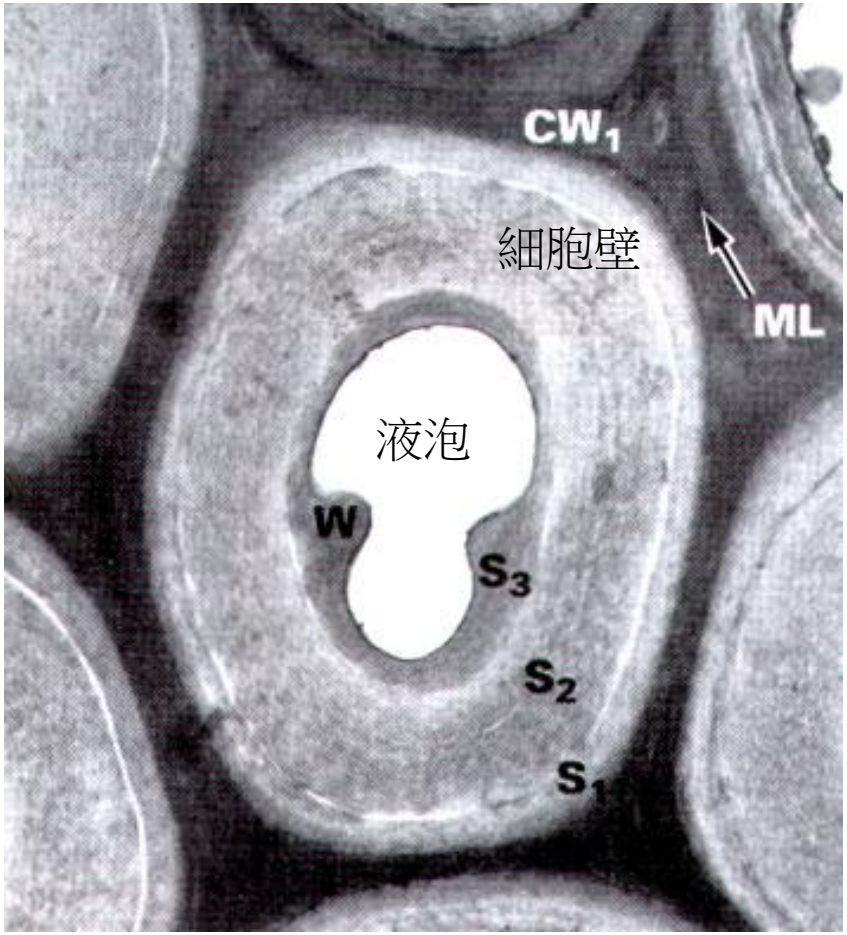




植物的細胞壁 Plant cell wall is a barrier

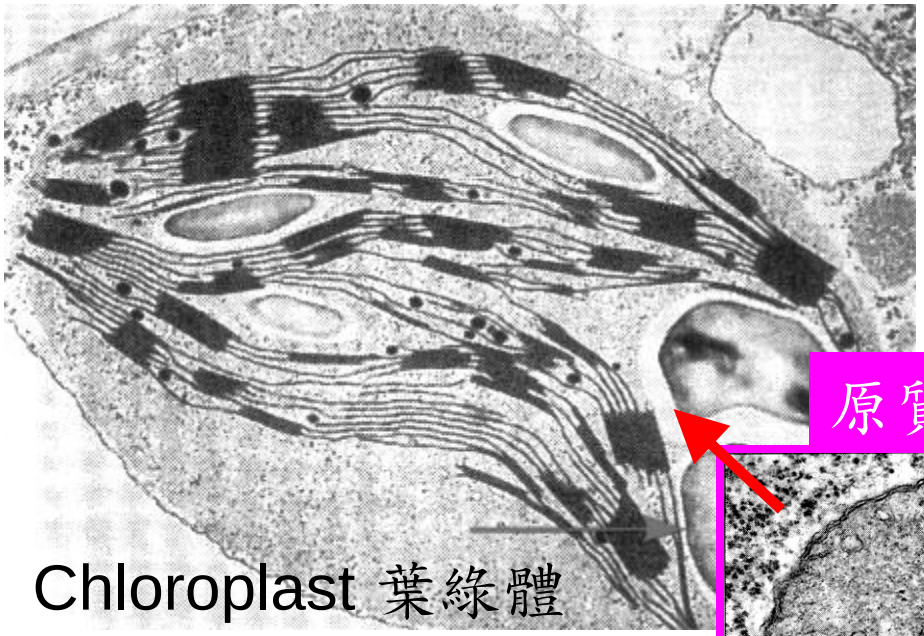
Old plant cell wall is very tough and hard to break

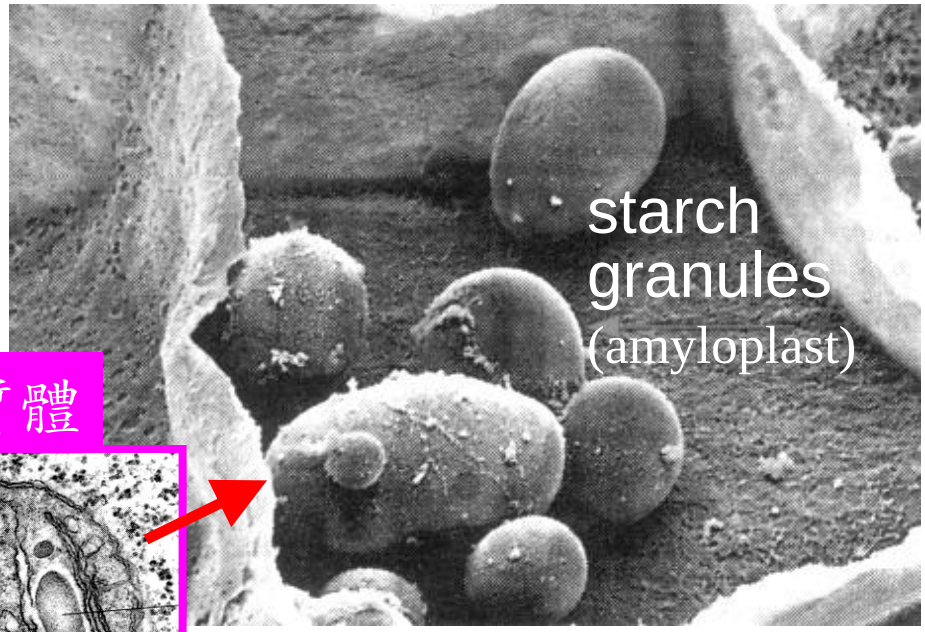


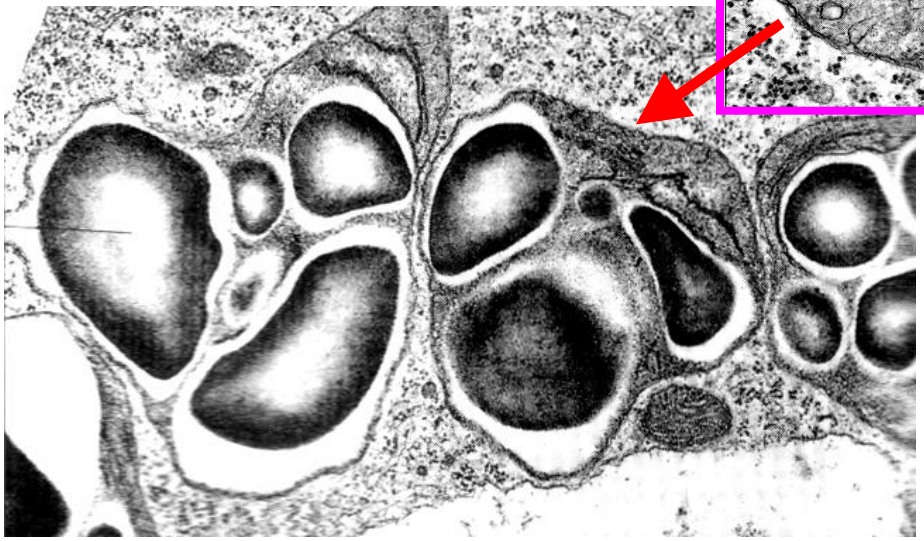


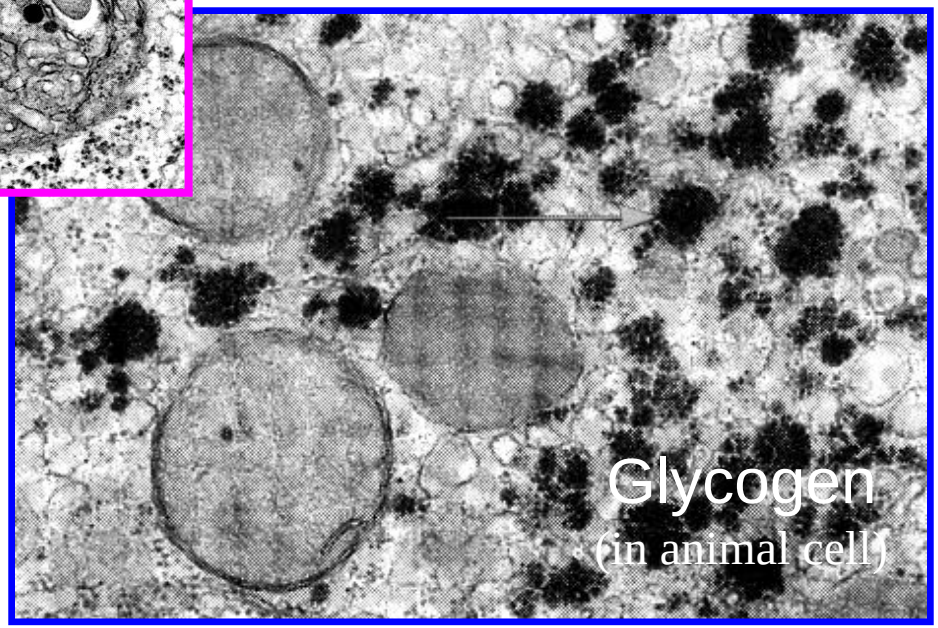
Buchanan et al (2000) Biochemistry and Molecular Biology of Plants p.54

Transformed from proplastid to plastid (amyloplast and chloroplast)









Alberts et al (2002) Molecular Biology of the Cell (4e) p.794

Mathews et al (1999) Biochemistry (3e) p.299

1.3 均質與抽取 Methods to break the cell

Dry method:

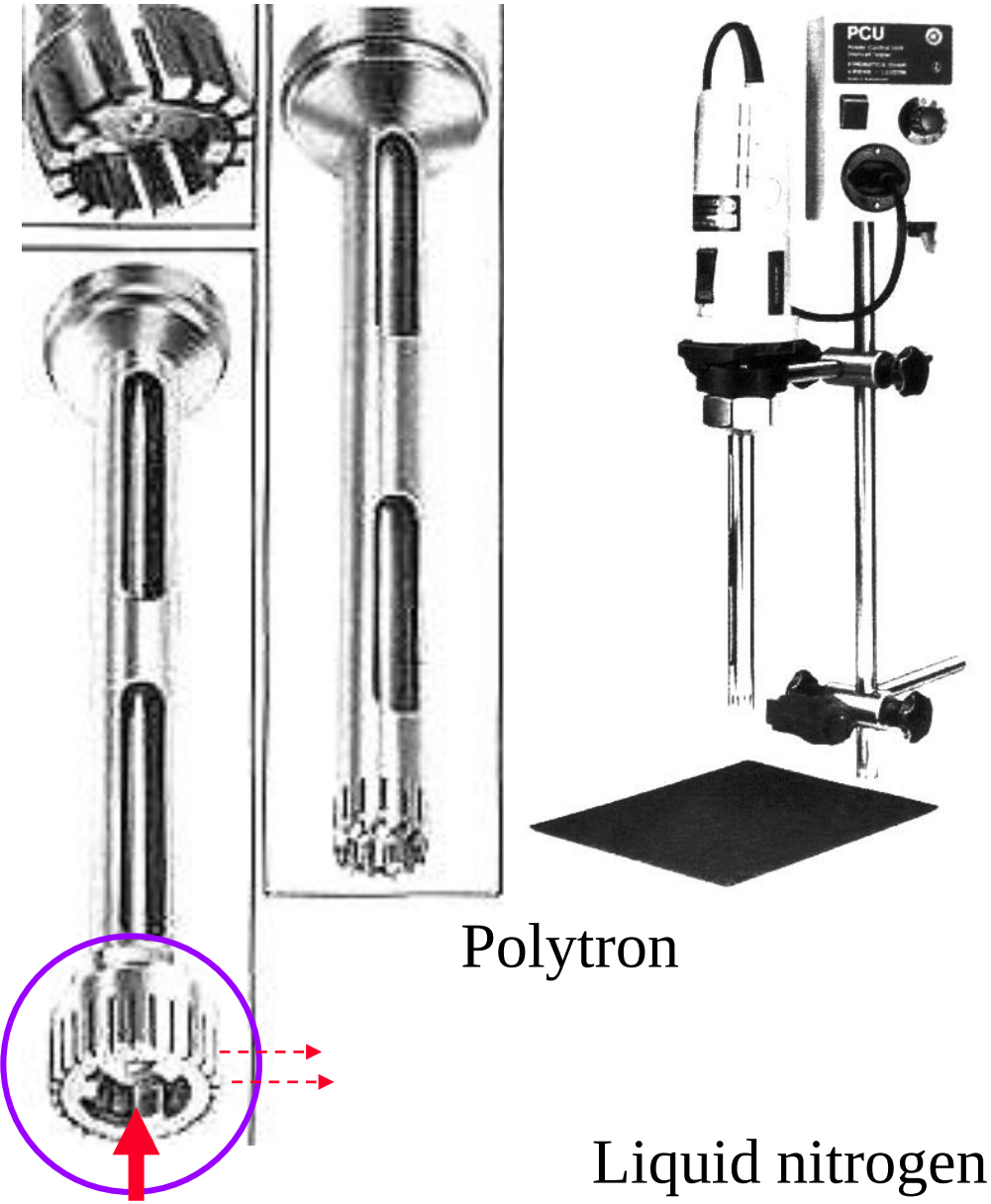
液態氮研磨 (grinding in liquid nitrogen), 磨粉機 (coffee grinder), 球磨機 (ball mill)

Wet method:

均質器 (homogenizer), 果汁機 (Waring blender), Polytron, 研砵 (mortar), 玻璃球 (glass bead mill), 超音波震盪 (ultrasonication), French press

Is your target protein released from the cell?

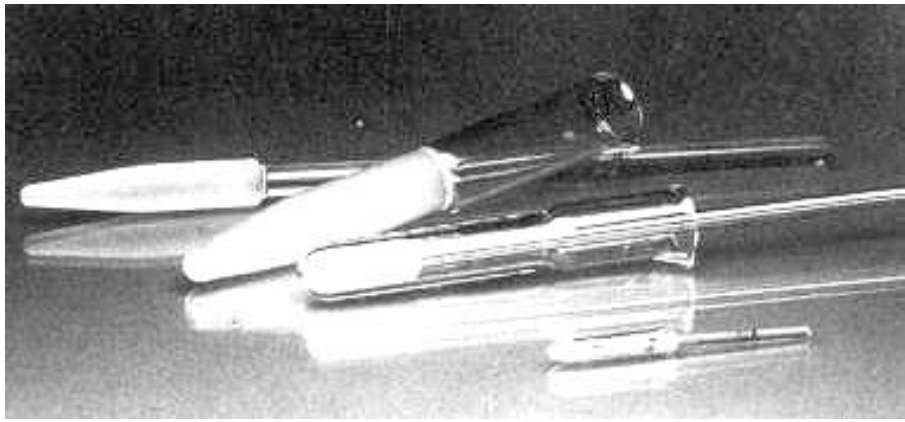
研磨樣本常用的方法 Popular methods



Polytron

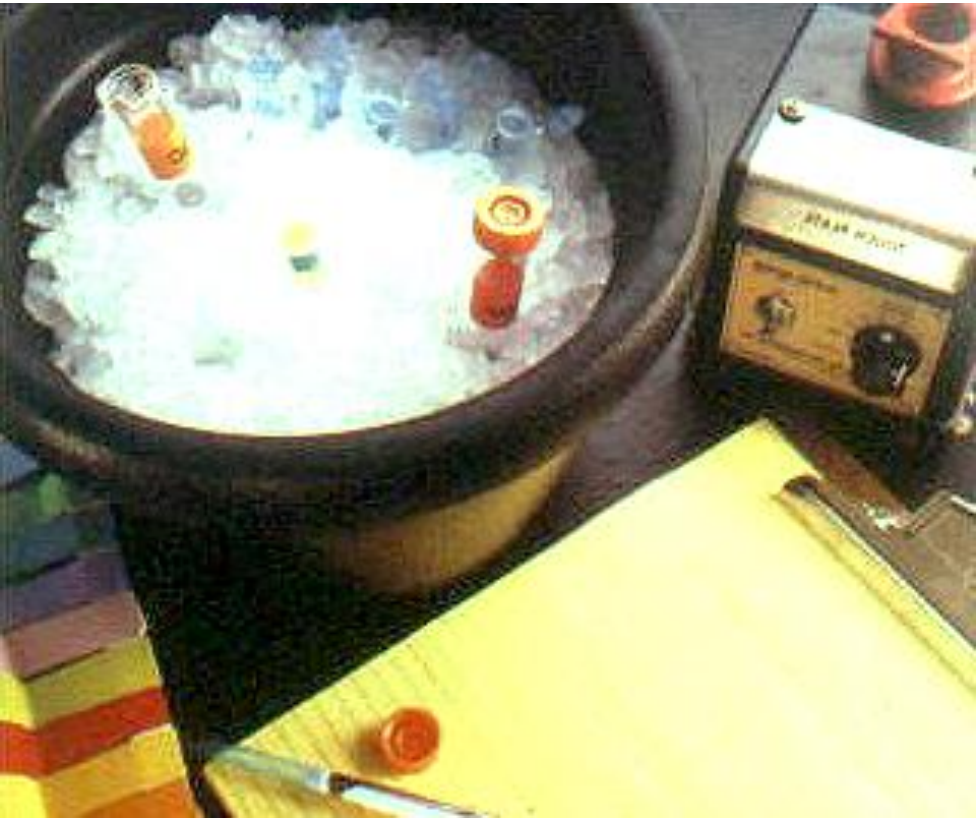
Liquid nitrogen

Glass homogenizer for breaking the cell but leave the organelles intact



■ 細胞打破之後 After breaking the cell…..

每天到酵素實驗室
的第一個動作：
帶著冰桶取冰去！



(1) 降低溫度
Keep temperature low

(2) 儘速純化
Purify as soon as possible

(3) 避免氧化
Avoid oxidation

(4) 避免吸著
Avoid adsorption by flask

(5) 避免污染
Avoid contamination

植物色素之產生與去除 How to remove pigment

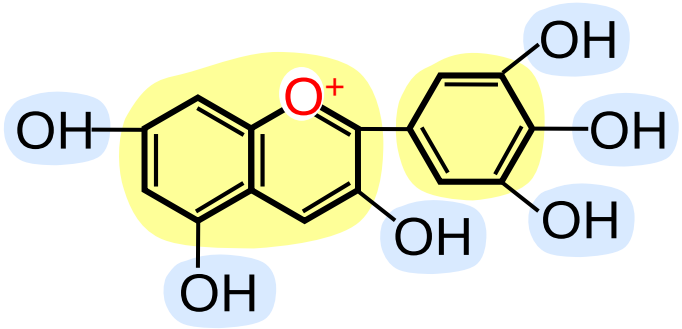
β-mercaptoethanol → inhibition ①

Phenol oxidase

② Decrease enzyme catalysis ← Low temperature

Oxidation

Phenolic compound →→→ **Pigment**



③ adsorption 吸 附

Polyvinylpolypyrrolidone

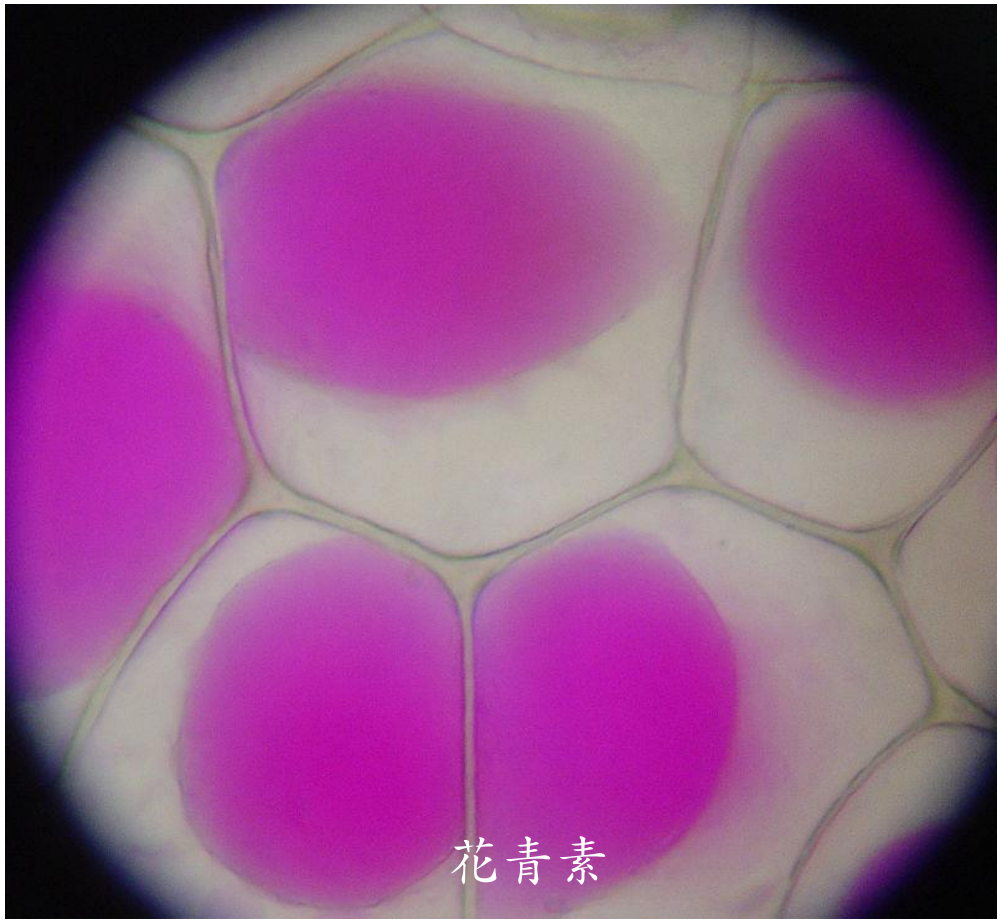
(PVPP)

圖 1.2

■ 植物色素干擾純化 Pigment is a problem

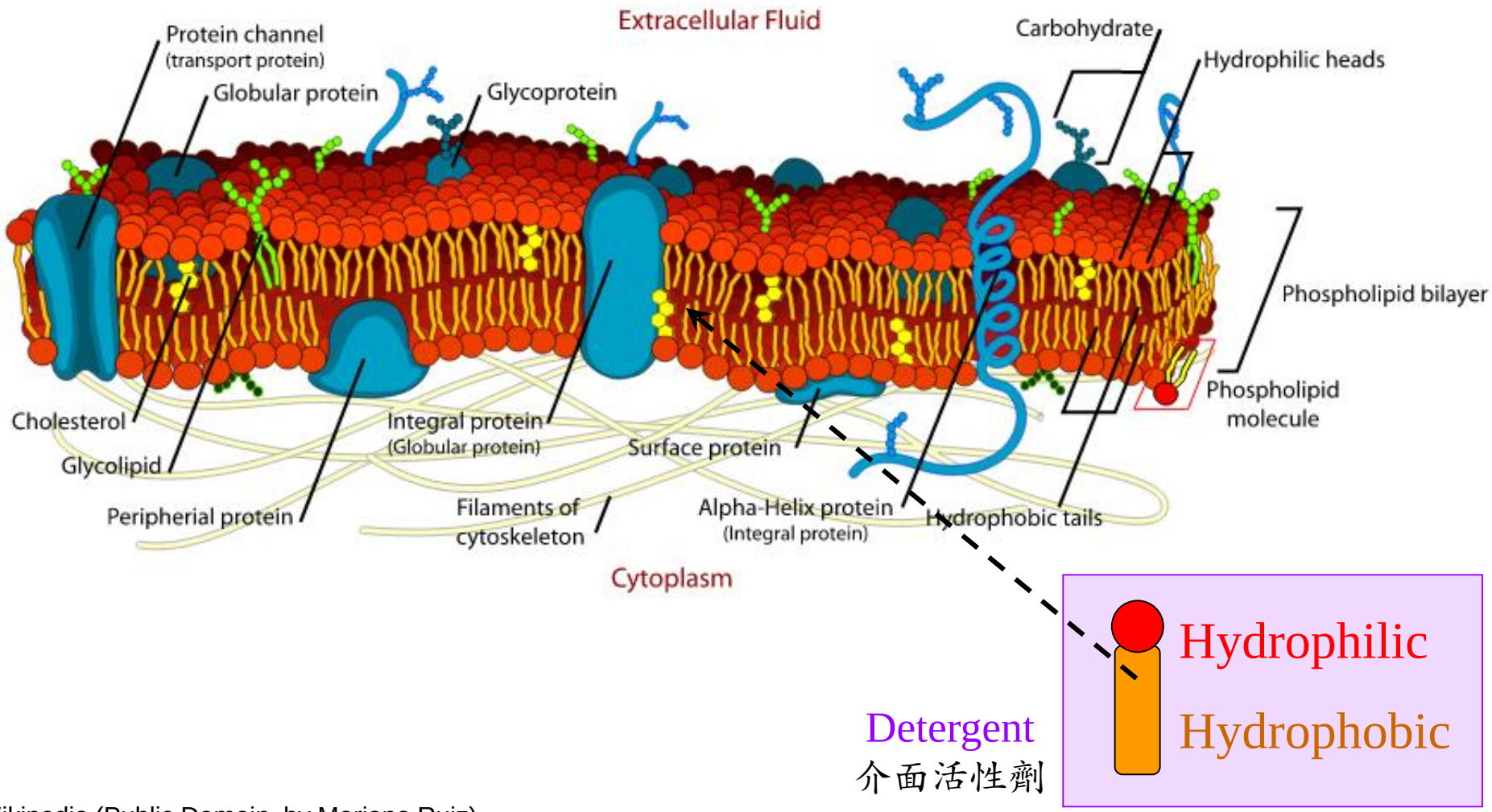
Pigment stains the gel during purification

The anthocyanin-storing vacuoles of *Rhoeo spathacea*, a spiderwort, in cells that have plasmolyzed (Wikipedia, CC by Mnolf)



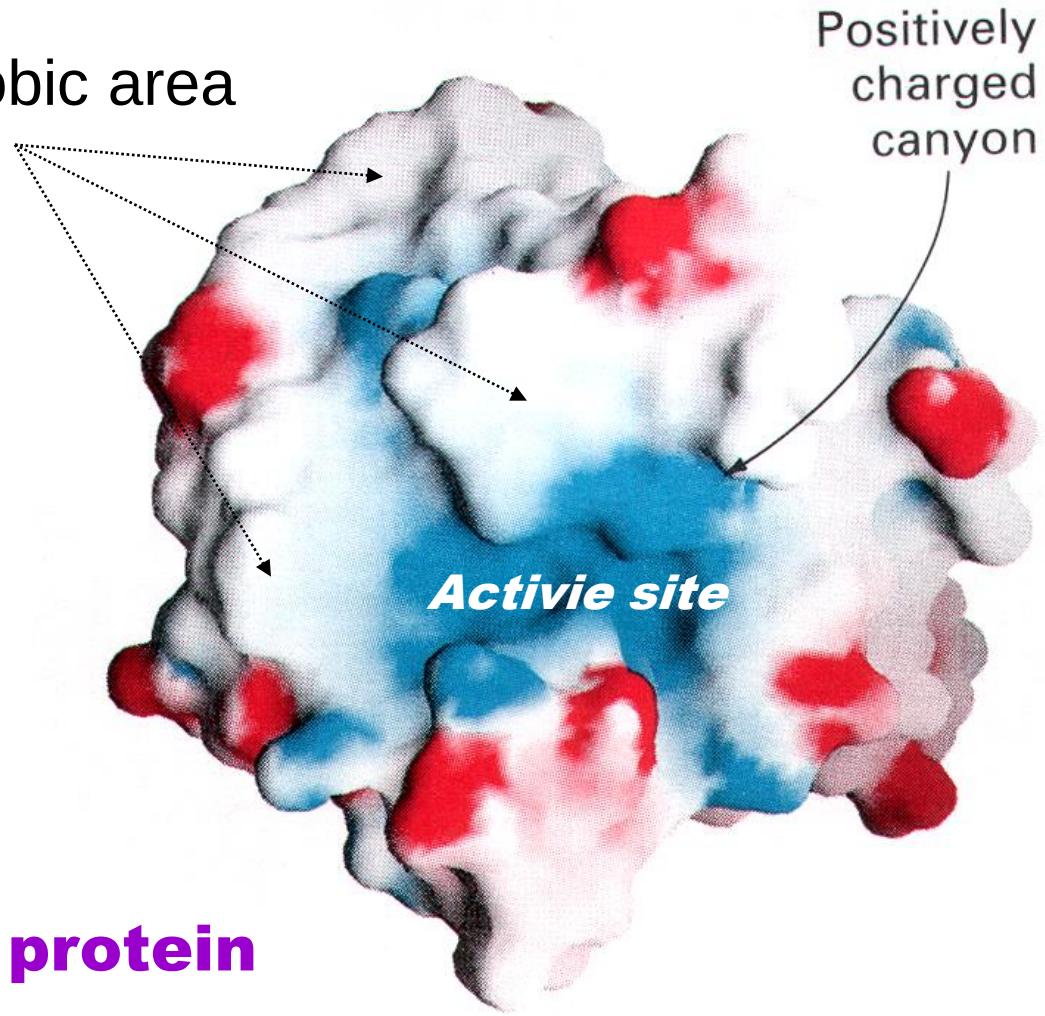
■ 細胞膜蛋白質抽取困難 Membrane proteins

Detergent (Triton X) is used to solubilize membrane proteins



Wikipedia (Public Domain, by Mariana Ruiz)

蛋白質表面的極性或非極性分布



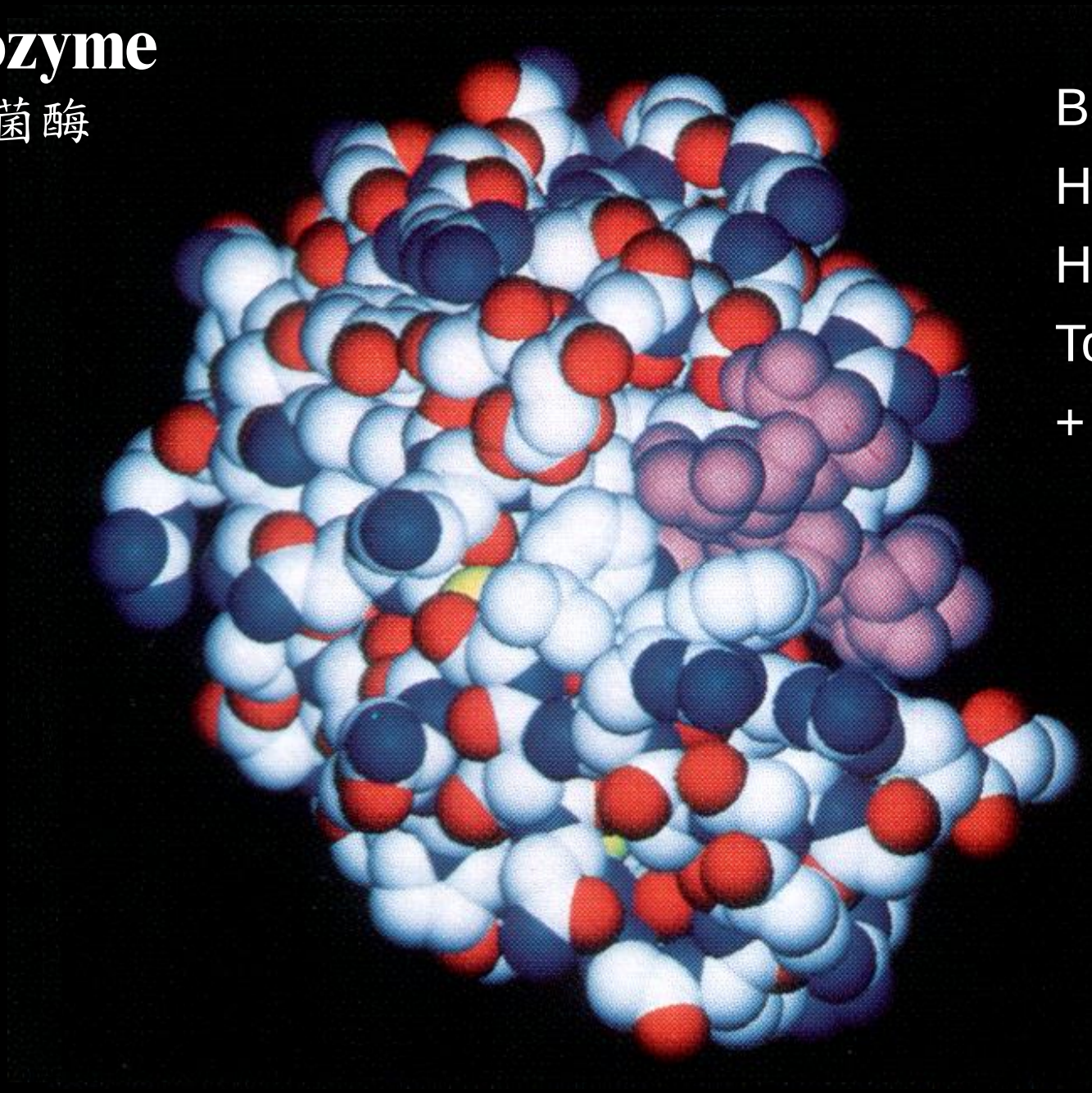
Superxoide dismutase (SOD)
超氧岐化酶

A typical protein

Protein surface has both polar and non-polar patches.

Stryer L (1995) Biochemistry 4/e Fig. 21-36

Lysozyme
溶菌酶



Backbone

Hydrophobic

Hydrophilic

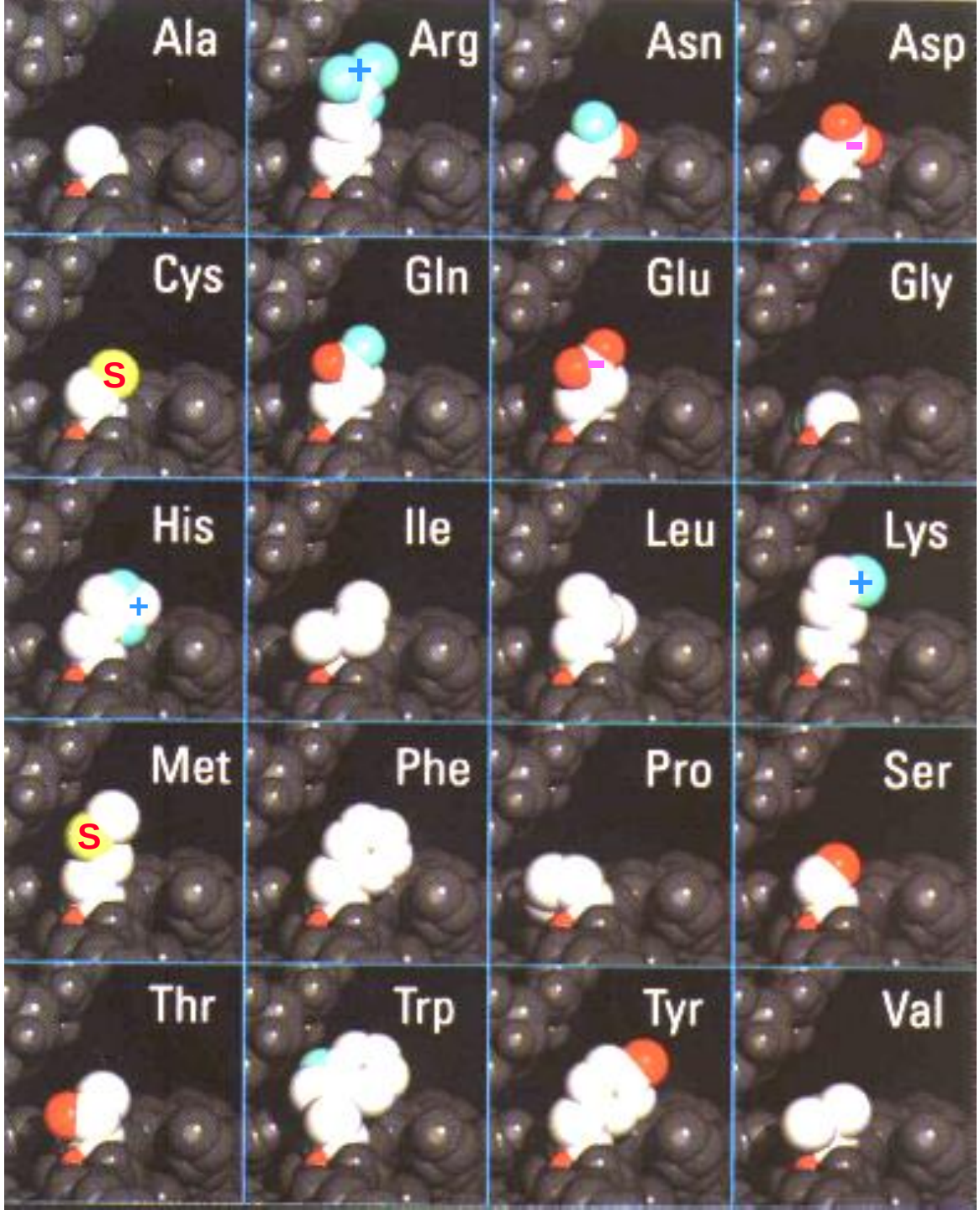
Total

+ substrate

Watson et al. (1987) Mol Biol Gene, Plate 3

二十種胺基酸特徵

Structural features of 20 amino acids



Side chains

有大有小
Large or small

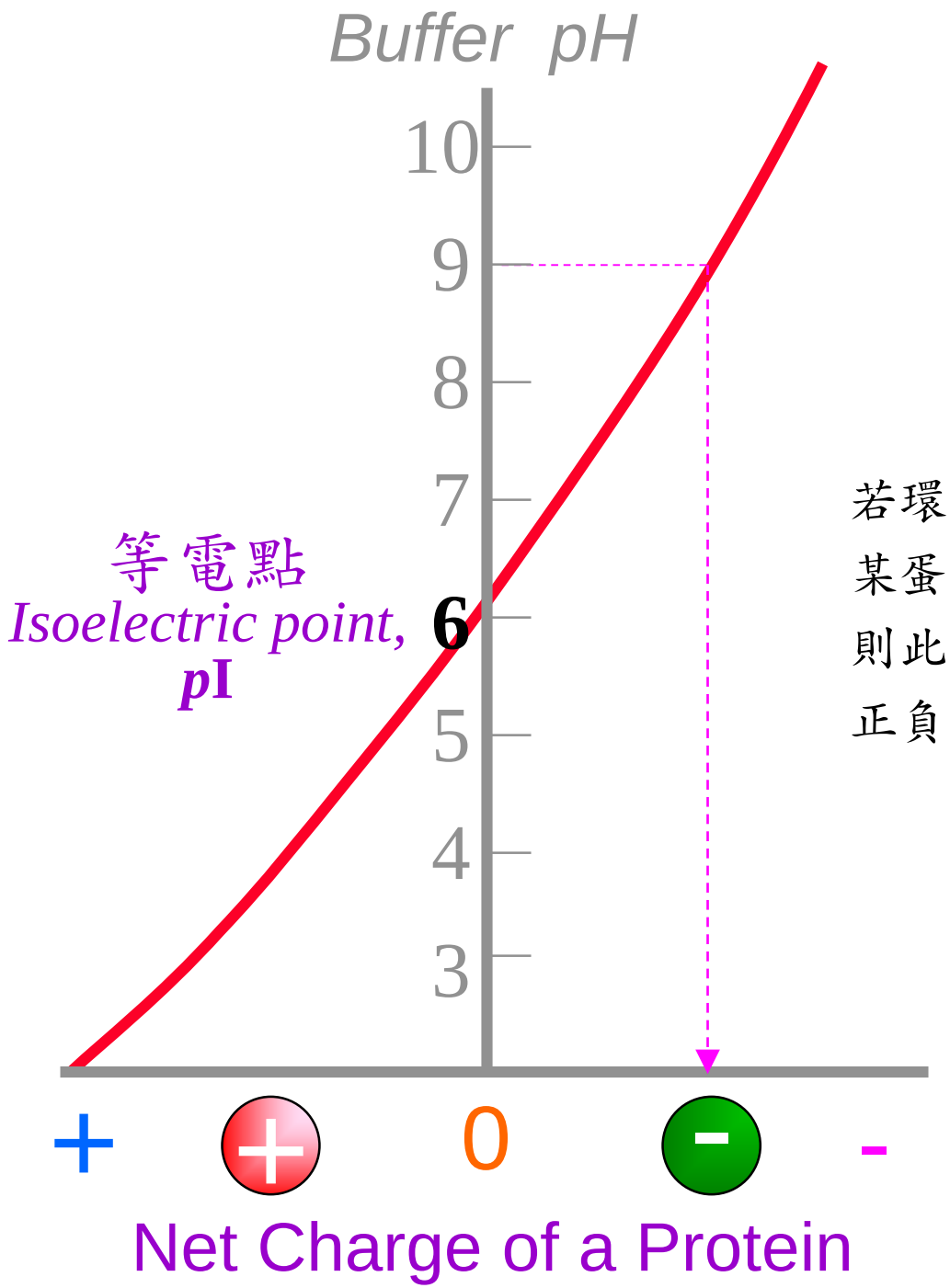
有正有負
Positively or negatively charged

有極性
非極性
Polar or non-polar

蛋白質構形與帶電性質均取決於其胺基酸序列

Bio-Lab 廣告 Cell 83 (4), 1995

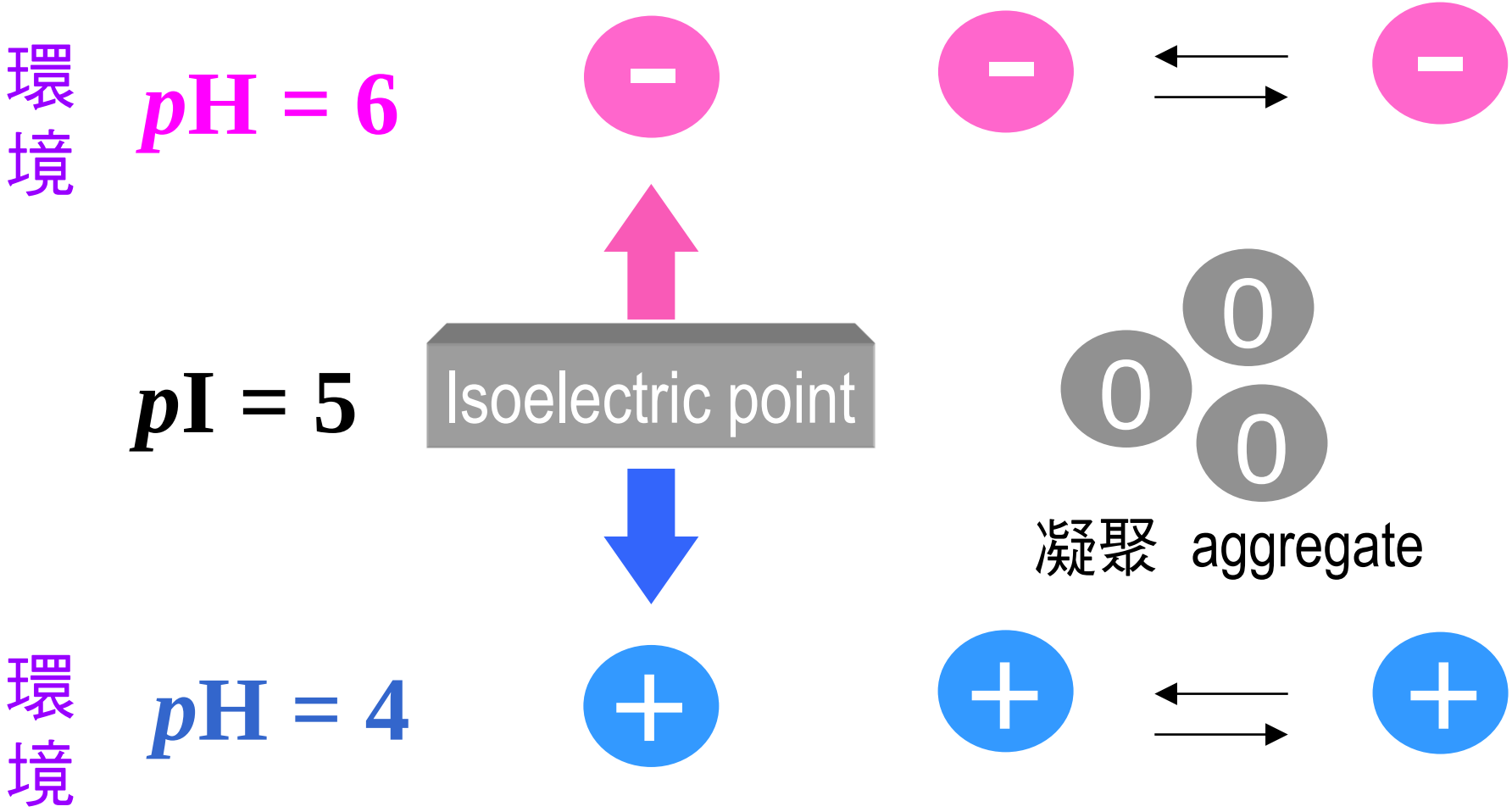
環境影響分子的帶電性質



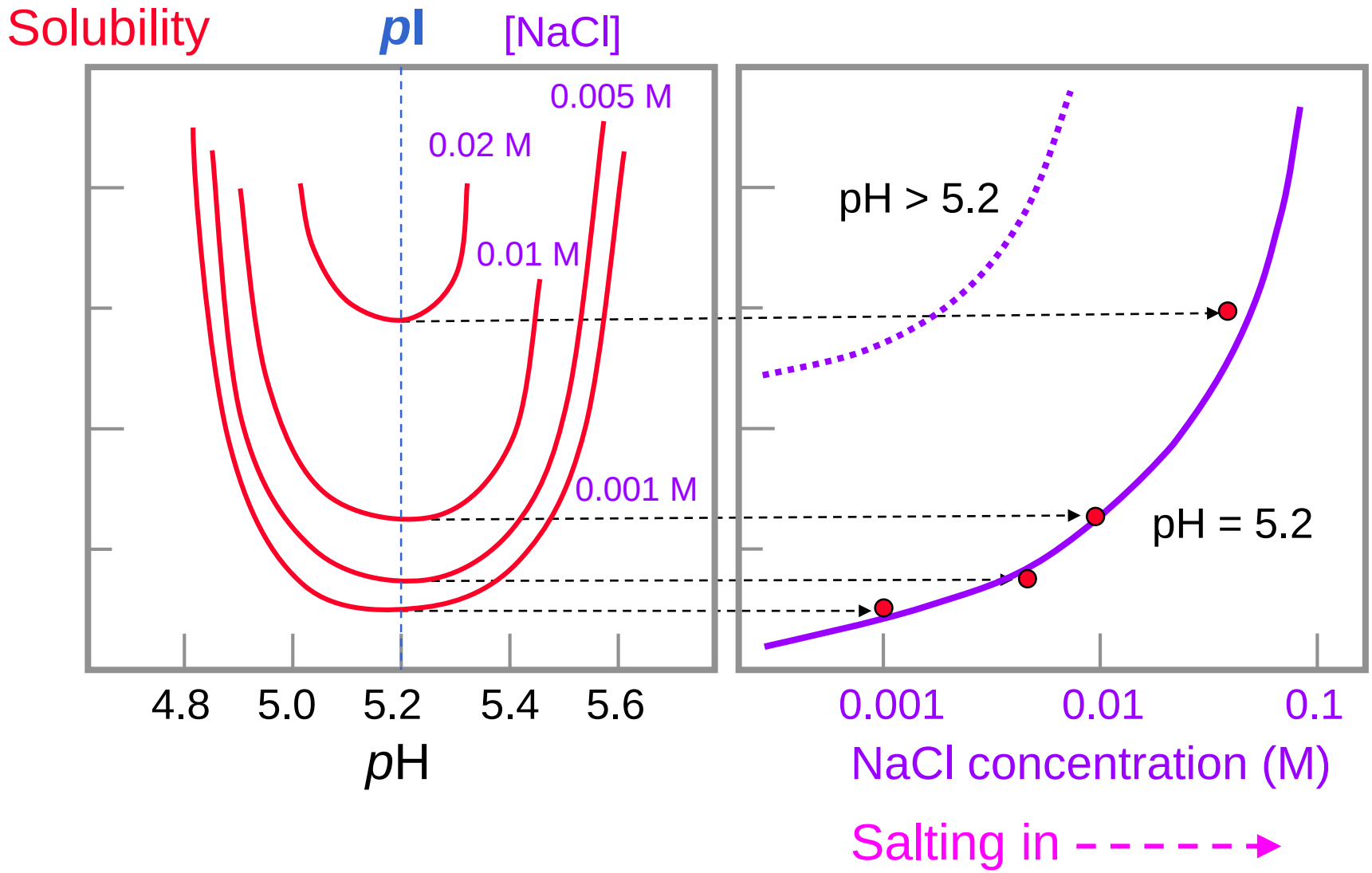
若環境 pH 剛好是某蛋白質的等電點則此蛋白質表面的正負電荷數目相等

圖 7.1

等電點與環境 *p*H 的關係 pI vs pH



提高鹽濃度增加蛋白質溶解度



Higher salt concentration increases the solubility of a protein

1.4 鹽析與沈澱法 Salting-out and precipitation

鹽濃度影響蛋白質溶解度

Salt concentration effects protein solubility

- **鹽溶 Salting-in:**

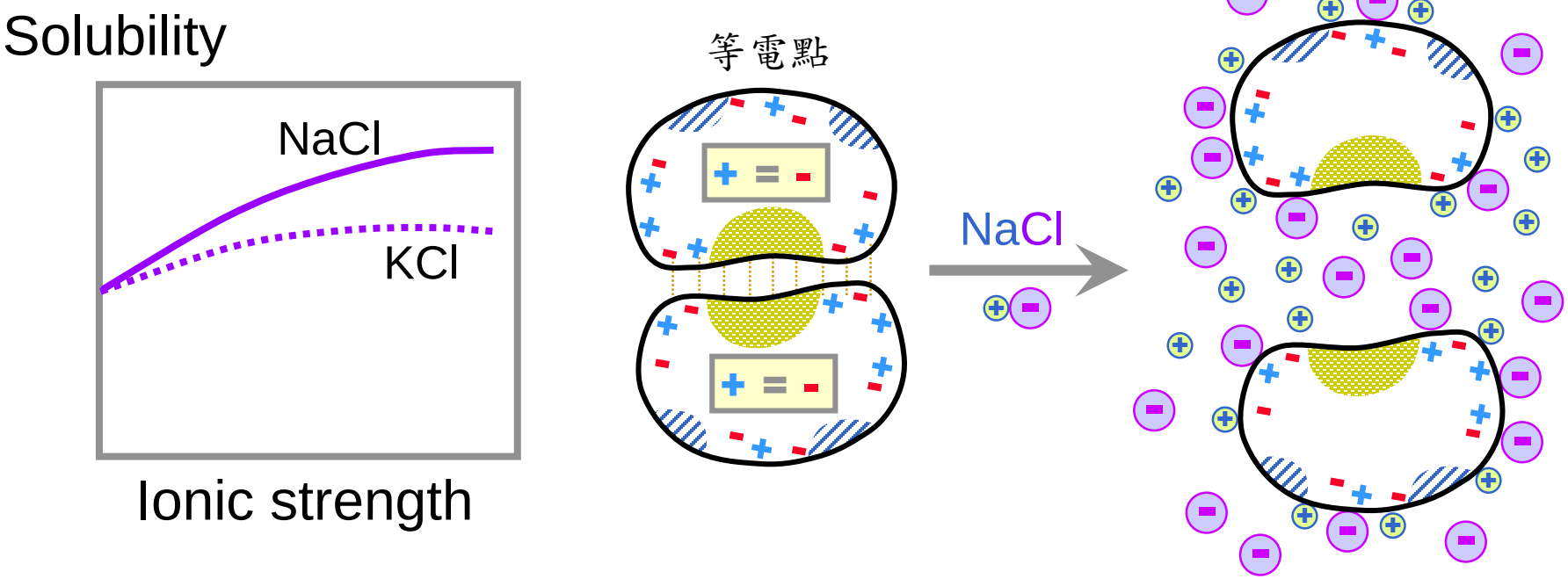
  加鹽使蛋白質溶入水溶液中

- **鹽析 Salting-out:**

  加鹽使蛋白質由水溶液中沉澱出來

所加入的鹽種類不同、性質不同

鹽溶 Salting-in effect



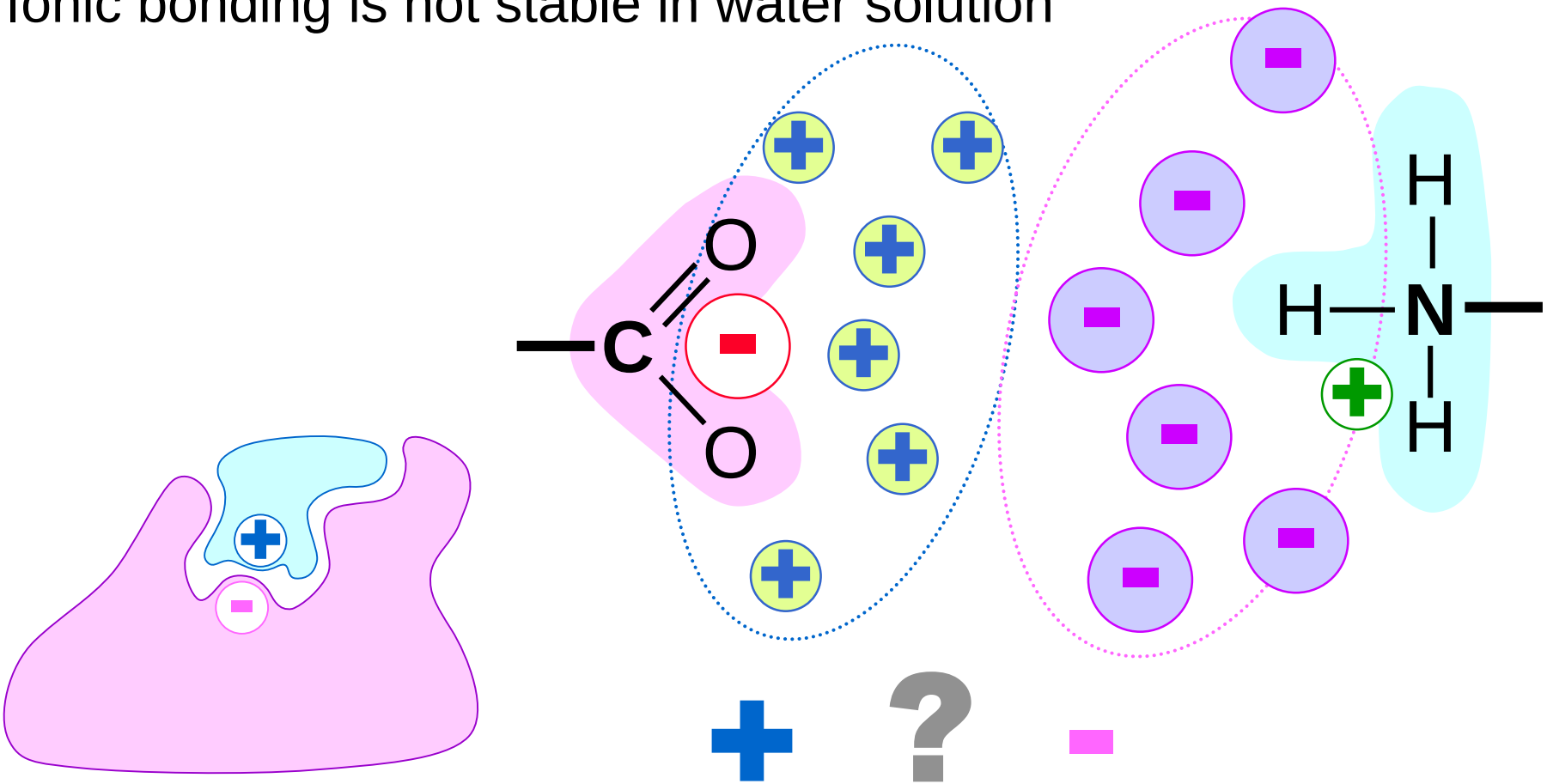
分子在其等電點時，容易互相吸引，聚合成沈澱；加入鹽離子會破壞這些吸引力，使分子散開，溶入水中。

圖 1.3

離子鍵在鹽溶液中不易形成

高中課本說離子鍵的強度與共價鍵差不多。正確嗎？

Ionic bonding is not stable in water solution

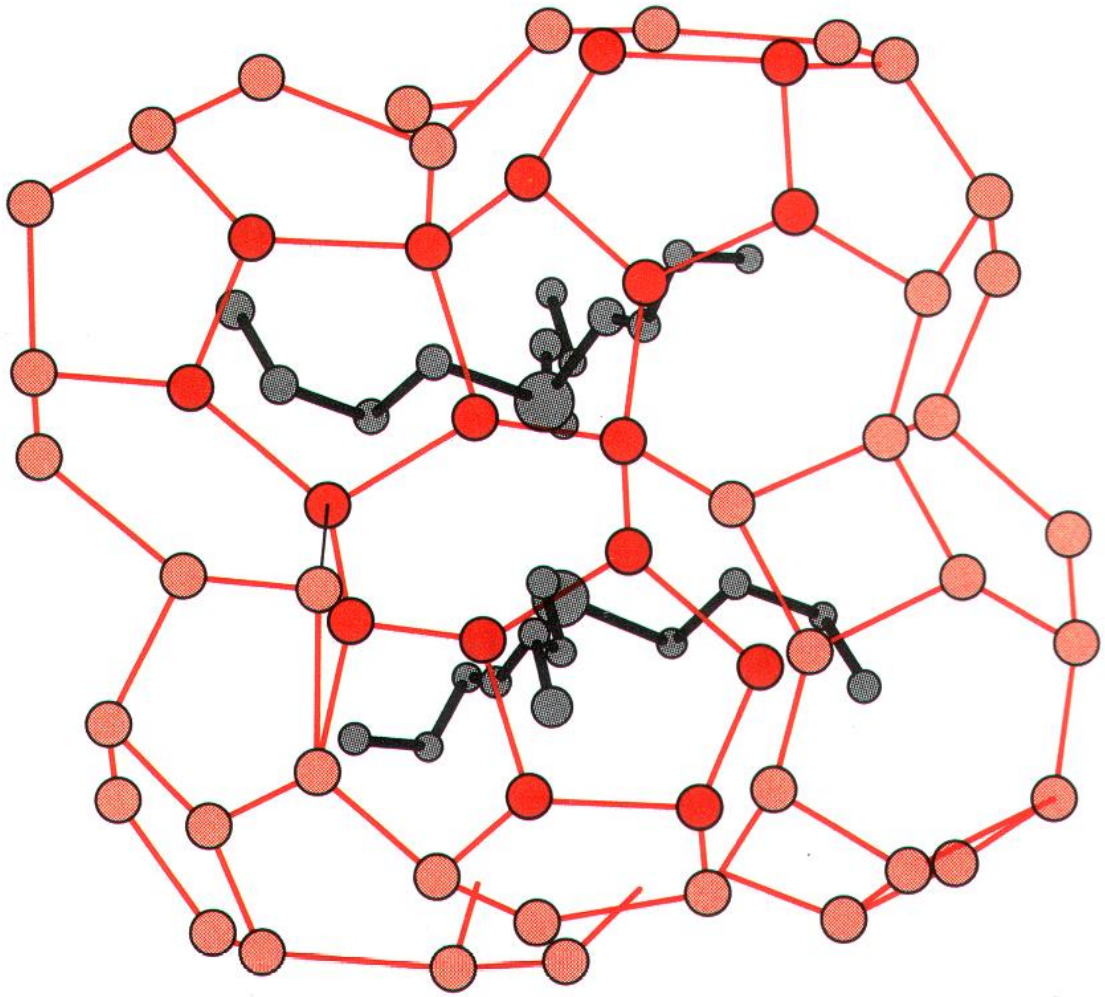


Adapted from Alberts et al (2002) Molecular Biology of the Cell (4e) p.115

● 為何水中的酵素與基質還是可以生成離子鍵？

But the enzyme-substrate pair could be bonded by ionic interactions in water (Why?)

疏水性物質間的親和力 水籠 Clathrate

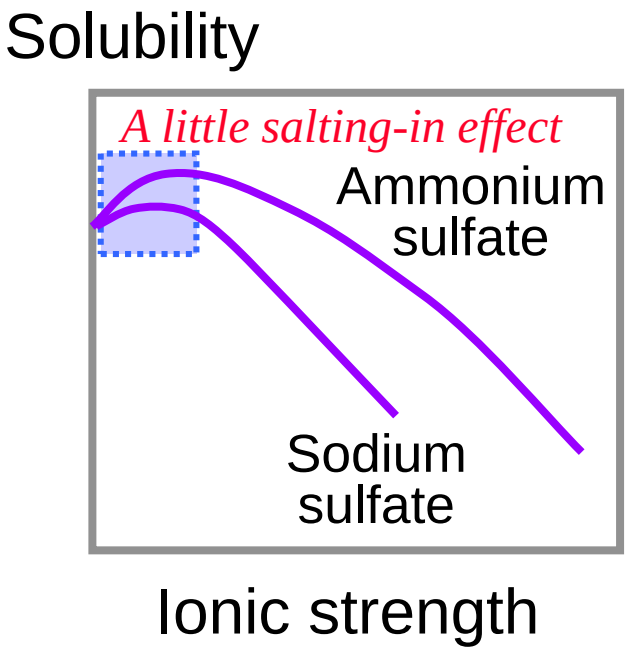


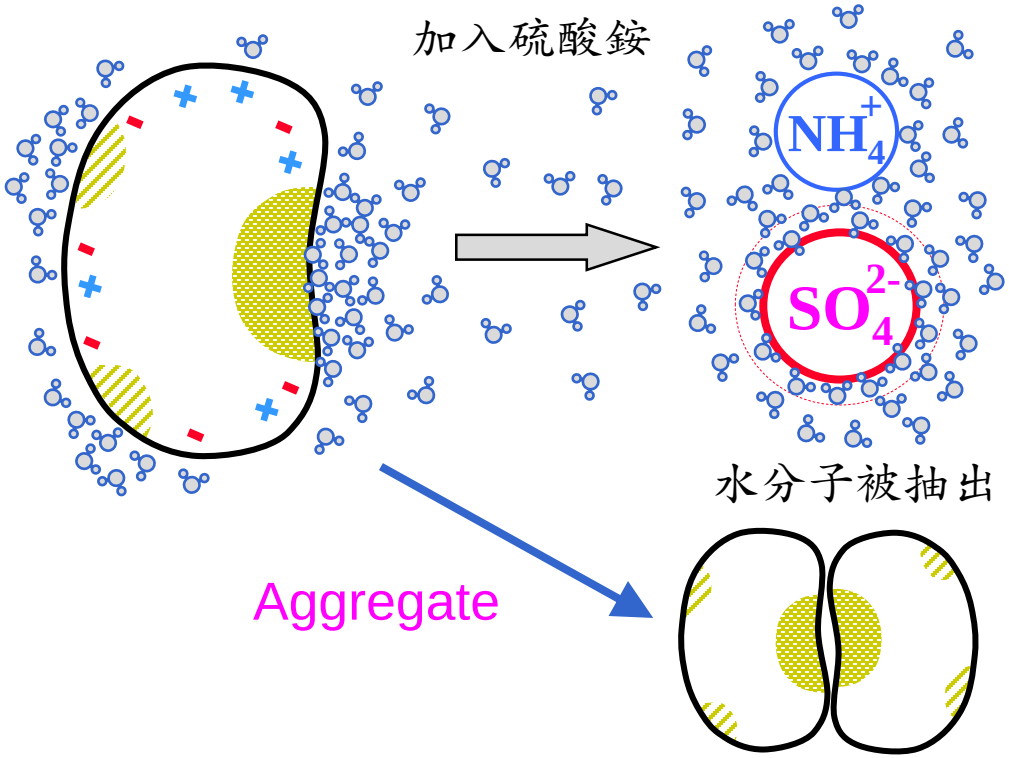
- 水分子會包圍在非極性分子四周，形成類似竹籠的構造，隔離非極性分子，水分子本身的流動性因此而降低。

Water molecules surrounding the outer surface of non-polar molecules are “immobilized” and form a “cage of water” to isolate these non-polar interface

Alberts et al (1994) Molecular Biology of the Cell (3e) p.49

鹽析 Salting-out effect





蛋白質分子表面的疏水性區域，都聚集許多水分子，當鹽類加入時，這些水分子被抽出，以便與鹽離子進行水合，暴露出來的疏水性區域互相結合，形成沈澱。

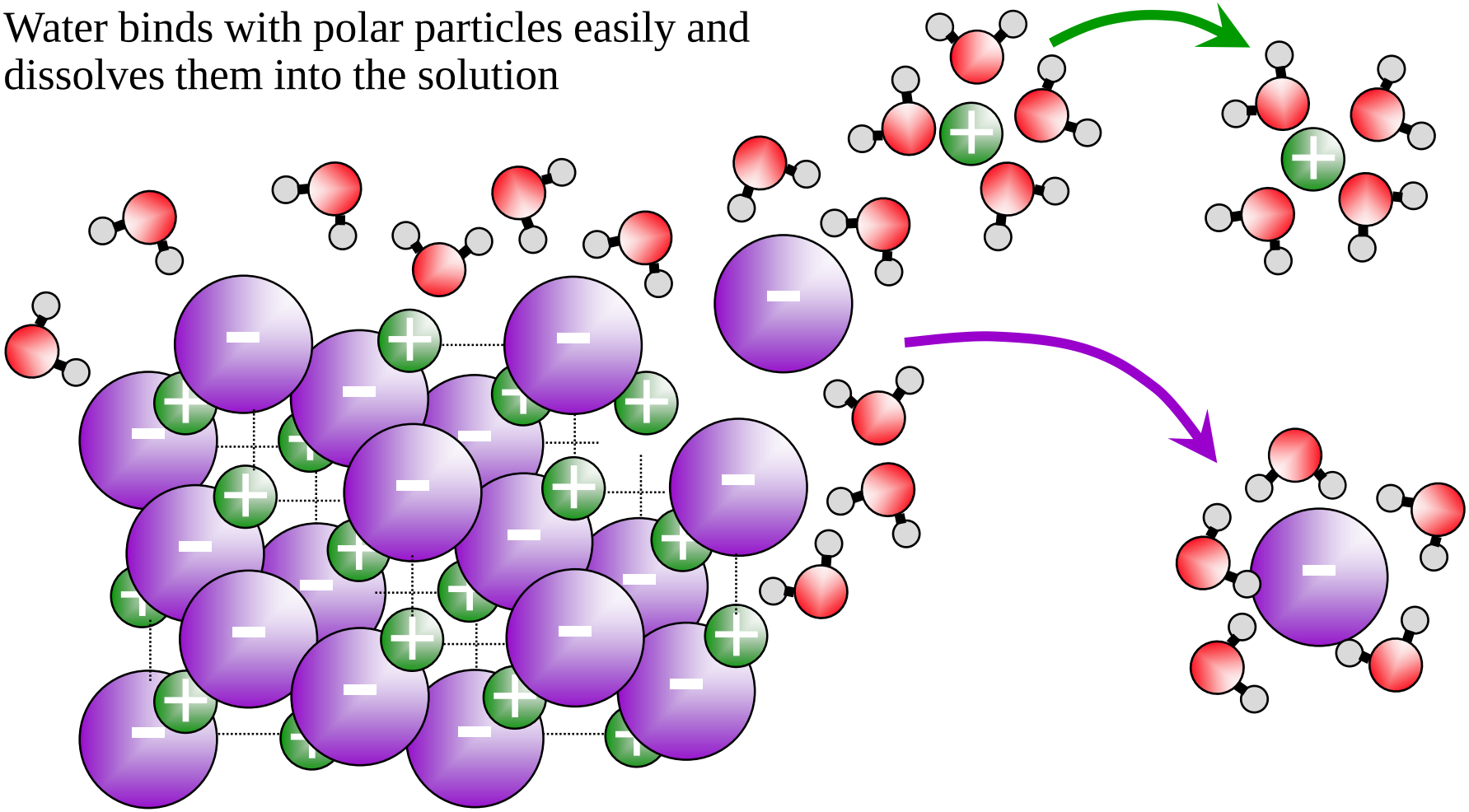
= hydrophobic area

圖 1.4

水合作用無所不在 Hydration is common

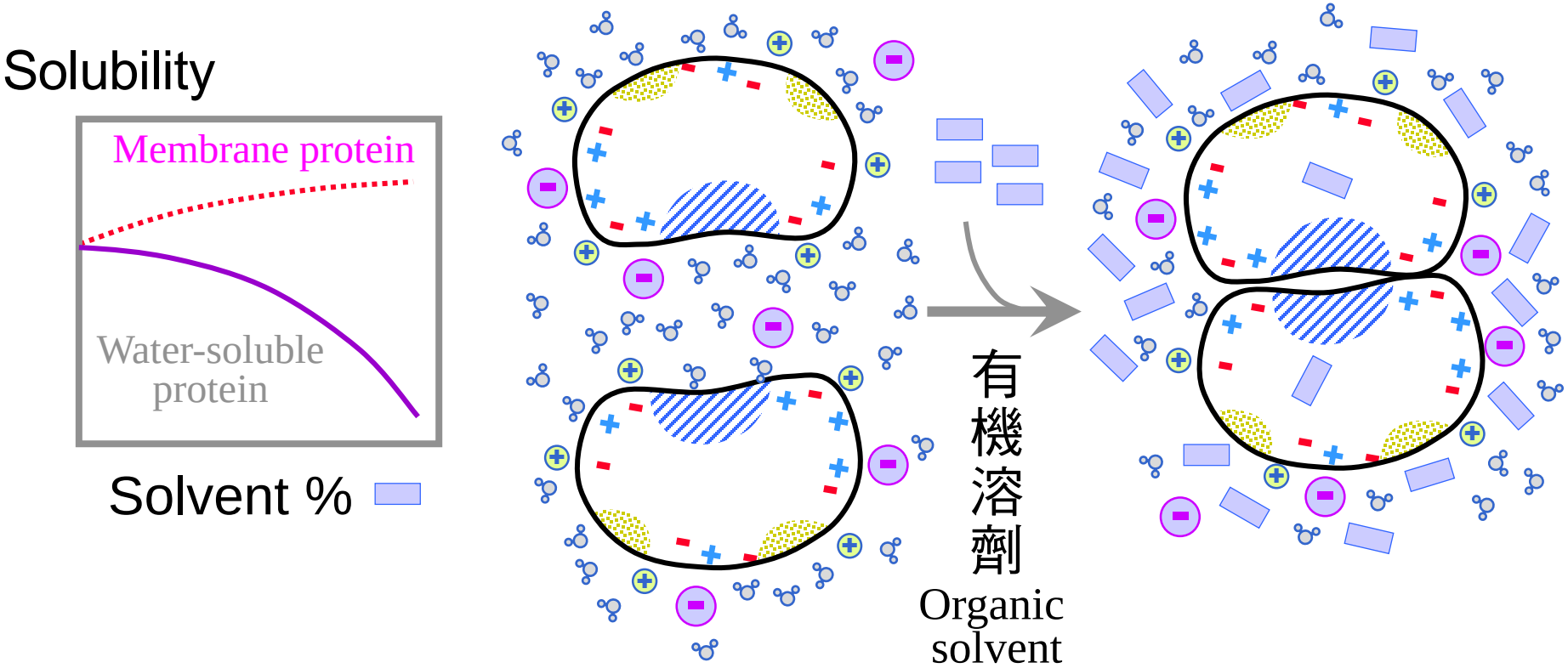
- 水分子易與極性粒子發生水合

Water binds with polar particles easily and dissolves them into the solution



Adapted from Nelson & Cox (2000) Lehninger Principles of Biochemistry (3e) p.87

有機溶劑沈澱法 Precipitation by organic solvent



降低水活性，使溶液的介電常數下降，增加蛋白質溶質分子之間的作用力，因而聚集在一起。

= hydrophilic

圖 1.5

各種鹽析沉澱法比較 Comparison of methods

	Salting-in 鹽溶	Salting-out 鹽析	有機溶劑沉澱
影響因素	蛋白質分子表面 帶電基團	蛋白質分子表面 非極性基團	除了 hydrophobic 以外的其他作用力
試劑	NaCl (單價離子)	硫酸銨 (兩價離子)	甲醇、丙酮等有機溶劑
發生機制	蛋白質在其 pI 下，因淨電荷為零而聚集沉澱；若加入鹽類，會阻礙其聚集而增加溶解度	硫酸銨的兩價離子，在溶液中搶走附在蛋白質表面 (非極性部分) 的水分子，使得非極性部分相互吸引而聚集沉澱	降低水活性，使溶液的介電常數下降，增加蛋白質溶質分子之間的作用力，而聚集在一起
圖	圖 1.3	圖 1.4	圖 1.5
其它說明	對較低濃度緩衝液進行透析是 salting-in 的反向過程	1) 非極性蛋白質較早沉澱下來 2) 在硫酸銨中蛋白質相當穩定	1)部分蛋白質會變性 2)有助沉澱的因素：蛋白質分子量大、緩衝液 pH 靠近 pI 3)脂溶性蛋白質的溶解度反而增加