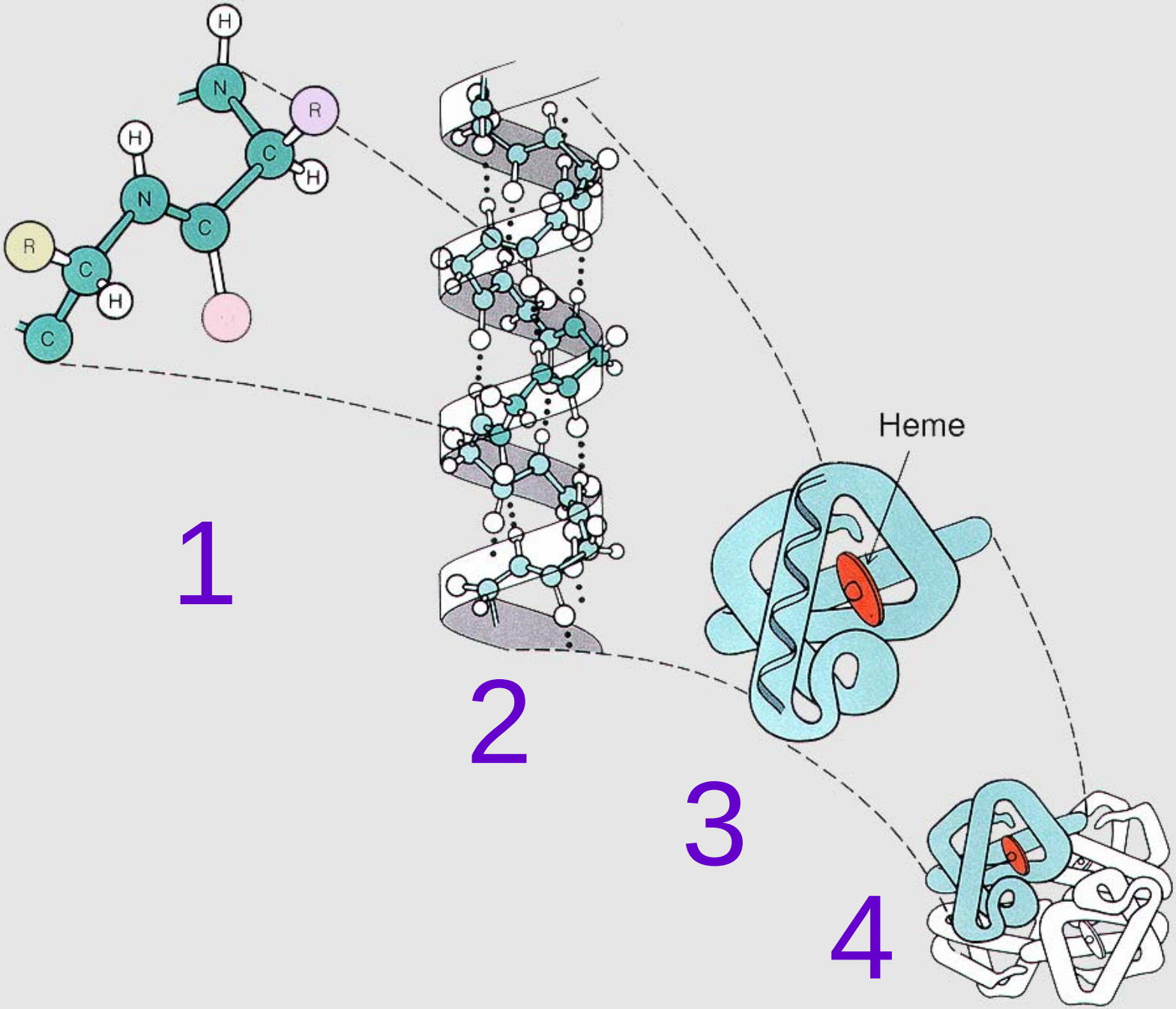
酵素純化方法

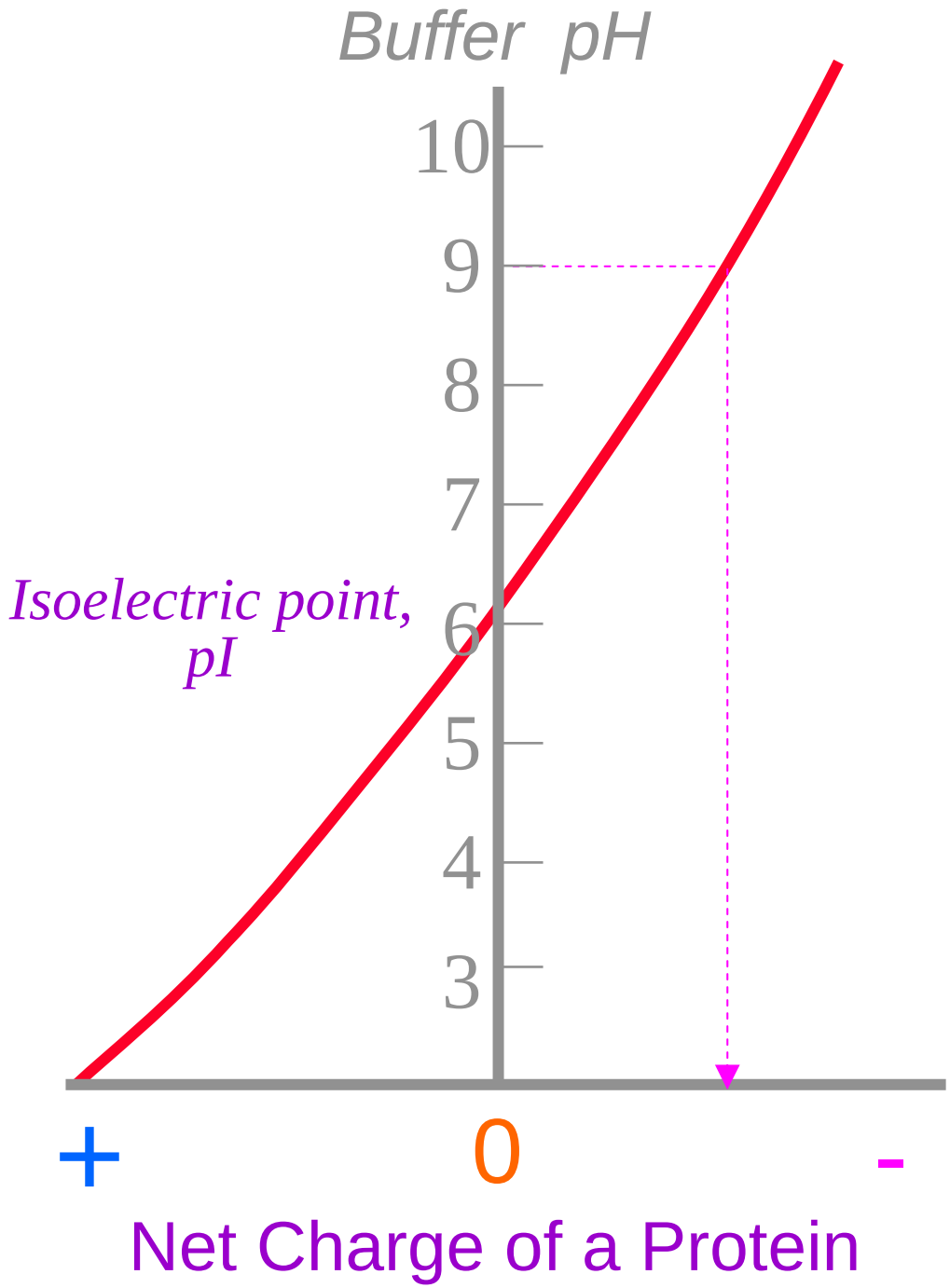
- 鹽溶鹽析　最經濟方便的純化
- 膠體過濾法　依照分子大小分離
- 離子交換法　利用分子電荷性質
- 親和層析法　最具專一性的吸著
- 純化策略　善用分子各種特性

蛋白質的四級構造

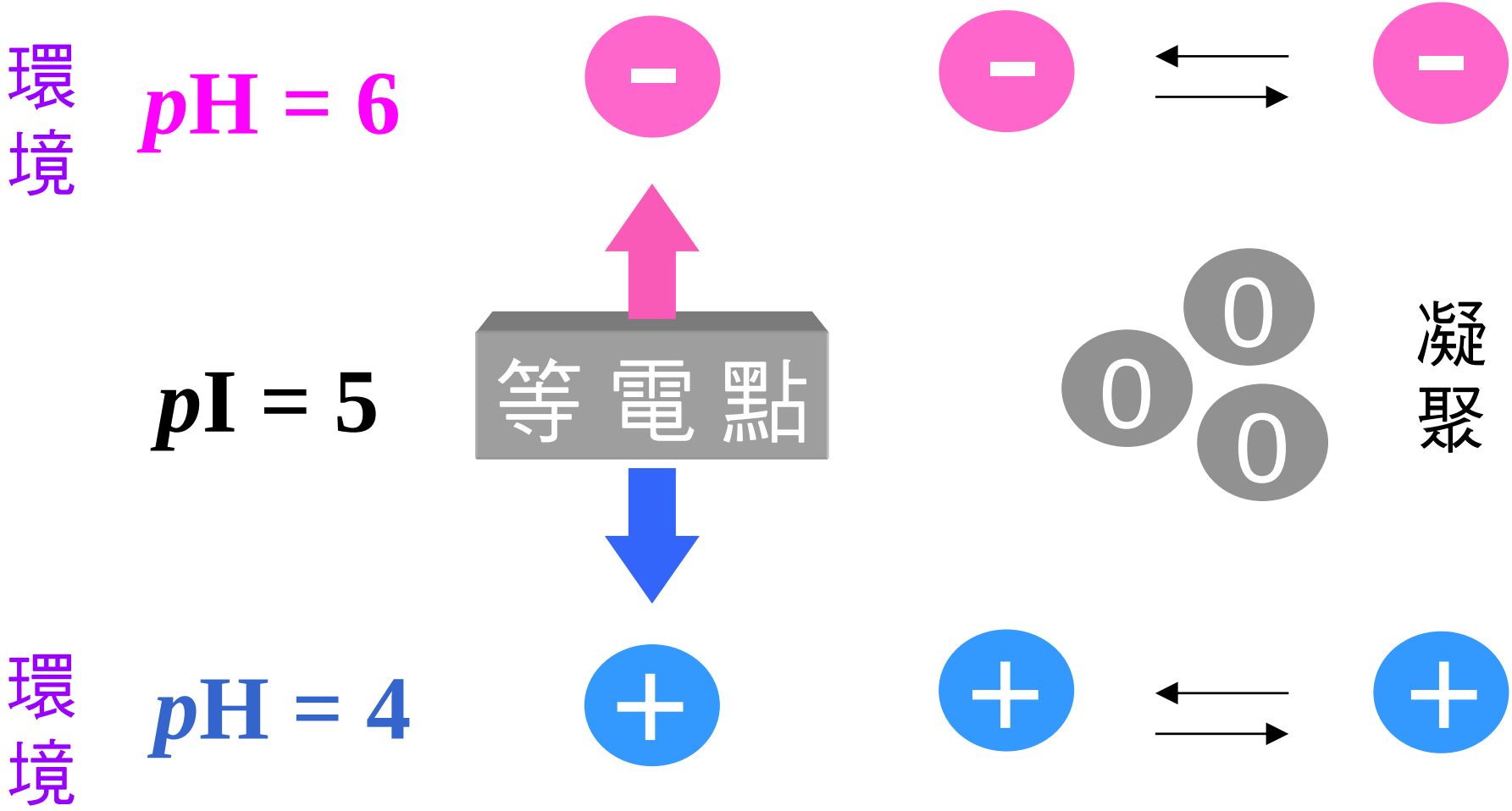


Mathews et al (2000) Biochemistry (3e) p.195

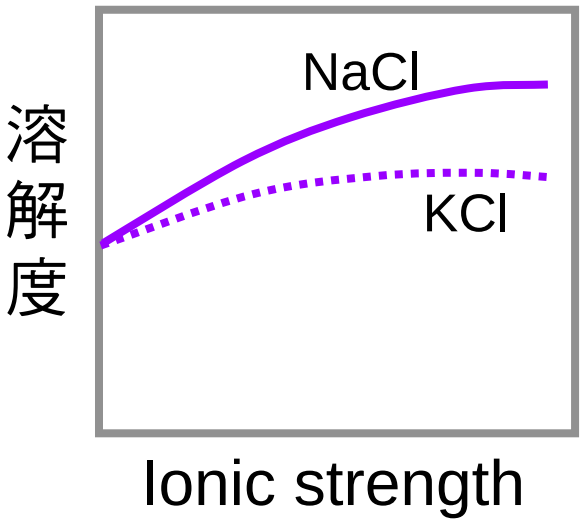
環境影響分子的帶電性質：

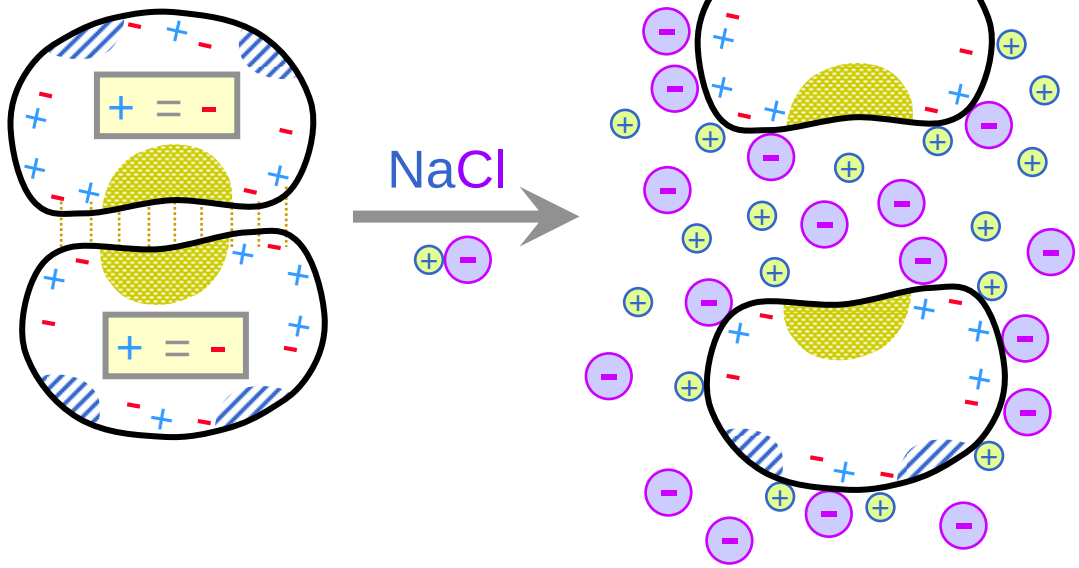


等電點與環境 *p*H 的關係：



鹽溶 Salting-in ：





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。

鹽對蛋白質溶解度的影響：

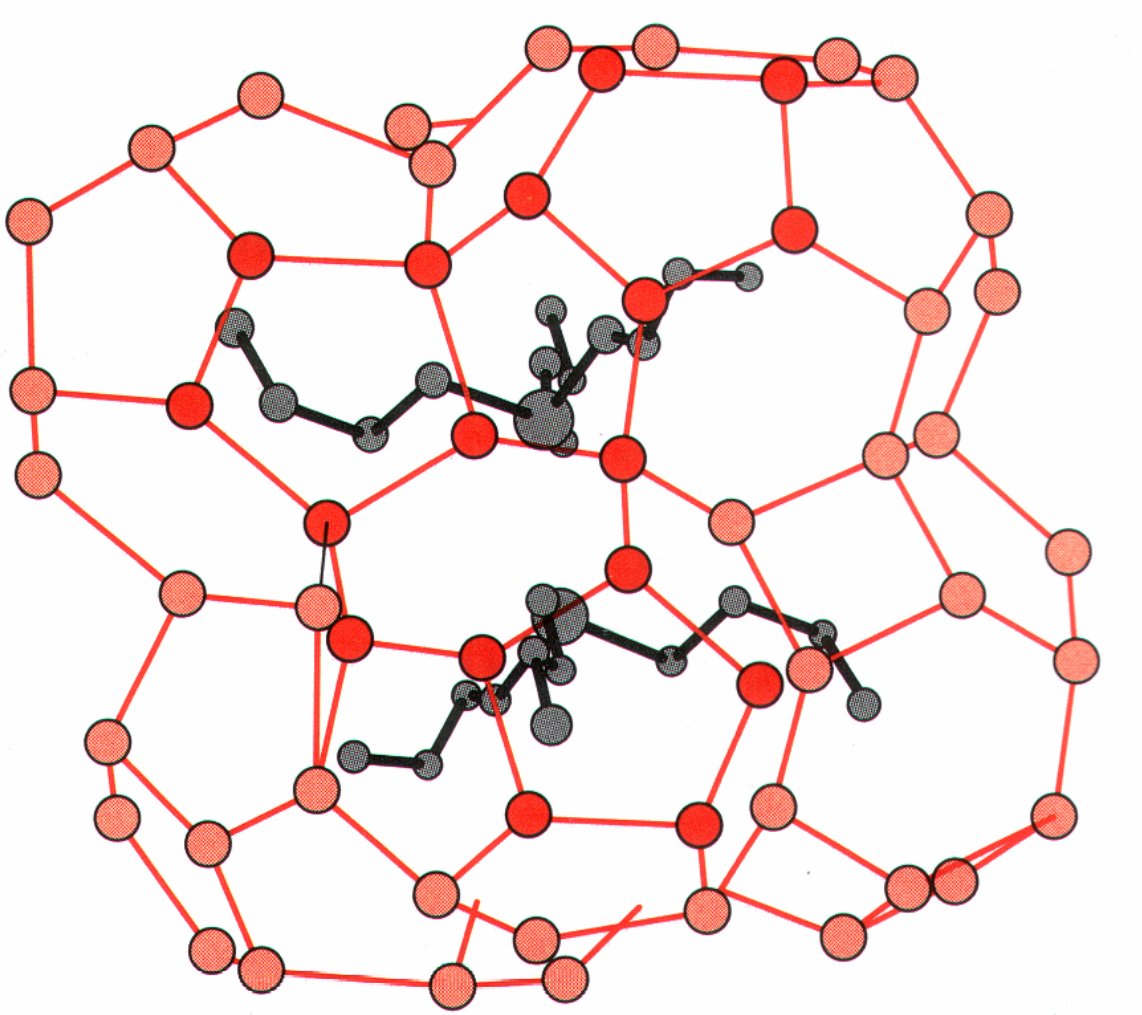
- 鹽溶 Salting-in:

  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

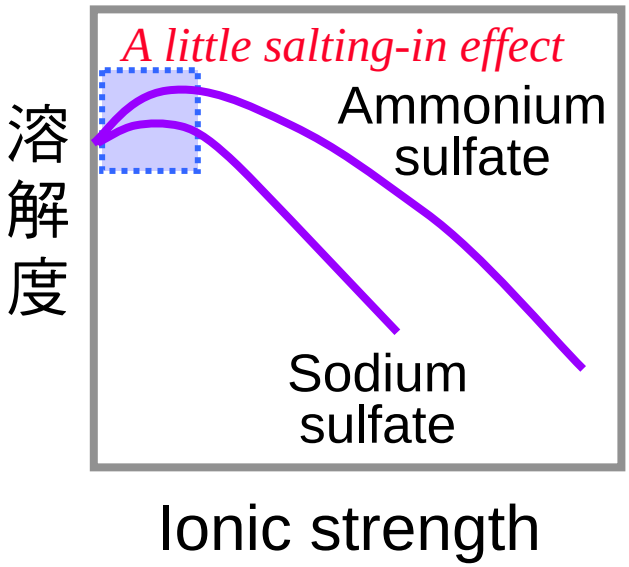
疏水性物質間的親和力： 水籠 Clathrate

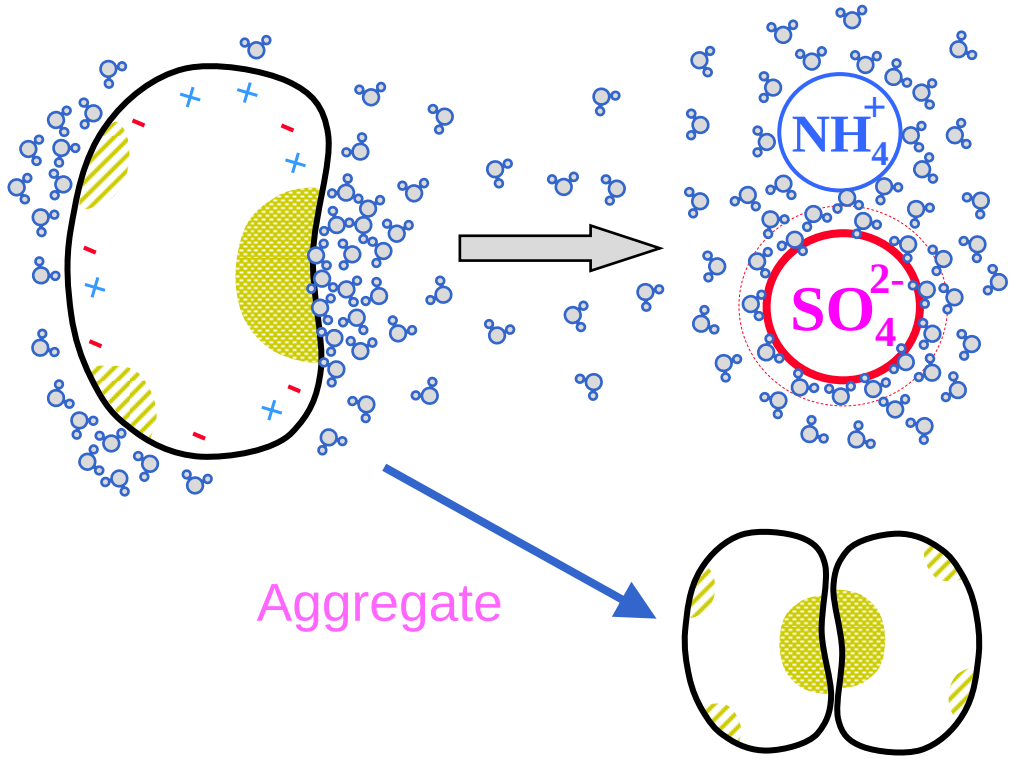


- 水分子會包圍在非極性分子四周，形成類似竹籠的構造，隔離非極性分子，水分子本身的流動性因此而降低。

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

鹽析 Salting-out ：





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。

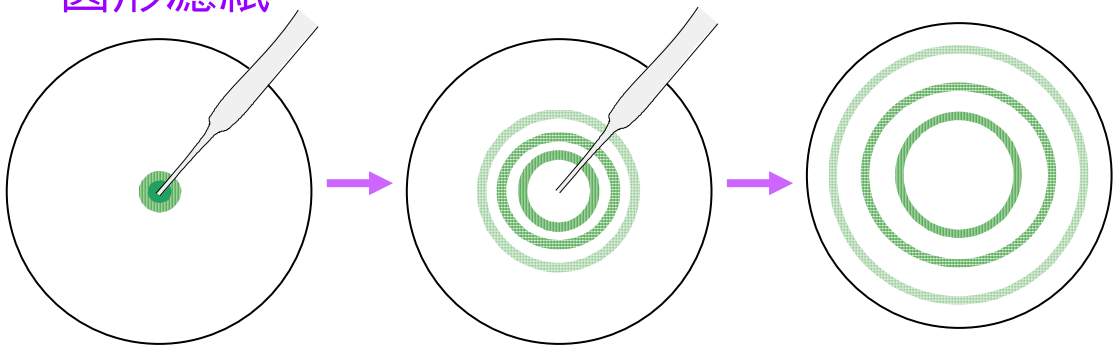
= hydrophobic

色析法的演進過程：

Martin, Synge (1952)

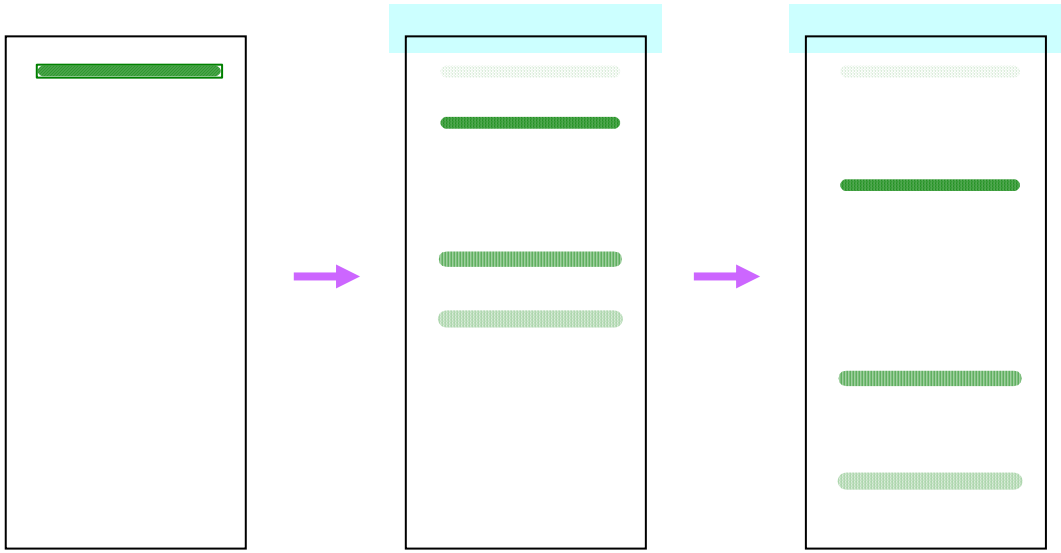
Paper partition chromatography (PPC)

圓形濾紙

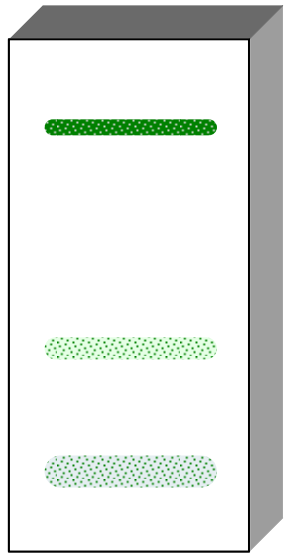


長條濾紙

加展開液

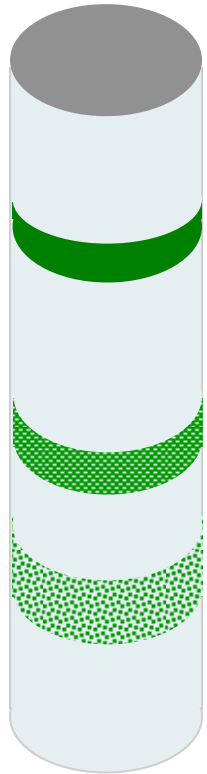


薄層層析
(TLC)



容量變大

管柱層析



容量更大

Adapted from Scope RK (1987) Protein Purification – Principles and Practice p.9

色層分析法的基本機制：

Like
Dissolves
Like

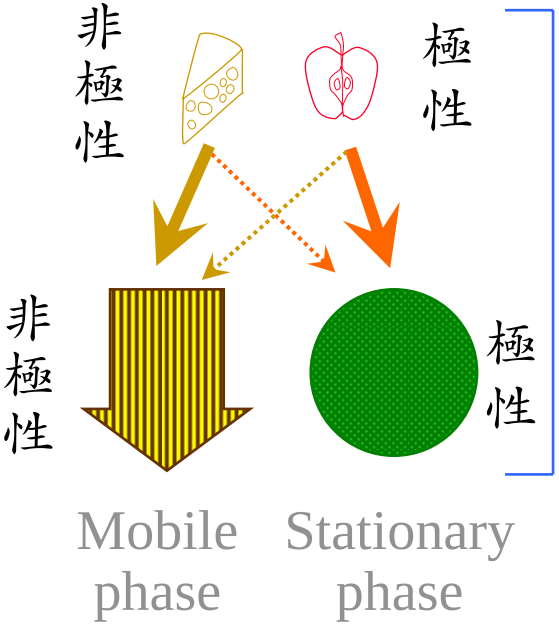
- 極性相似的兩分子間，其親和力較強。

極性 → 極性
非極性 → 非極性

色層分析法的基本原理：

A

兩相系統

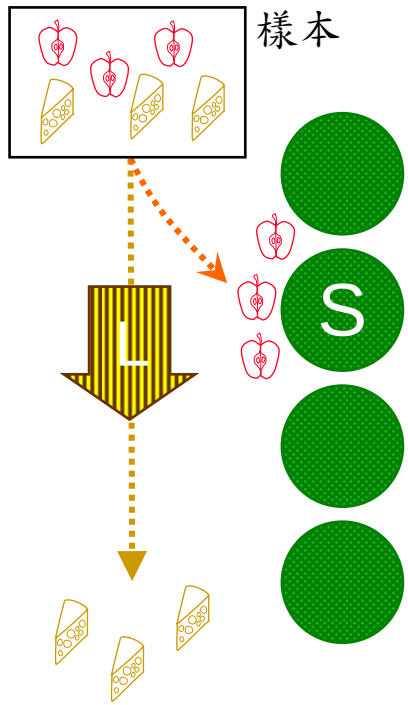


每次分離樣本
都要做一次選擇

One Plate of Separation

(理論板數)

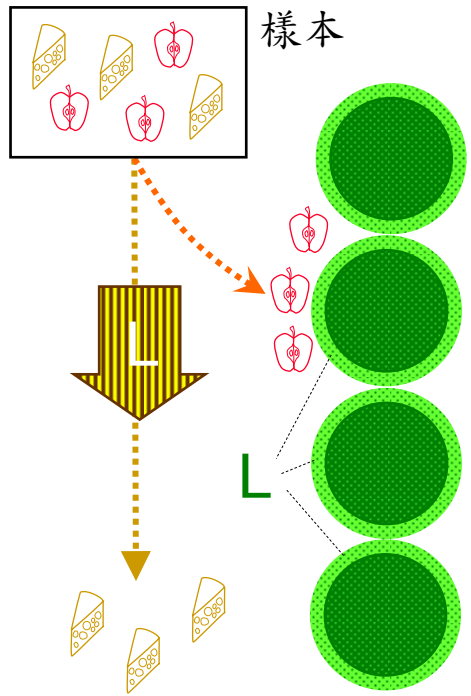
B



ADSORPTION

Liquid - Solid

C

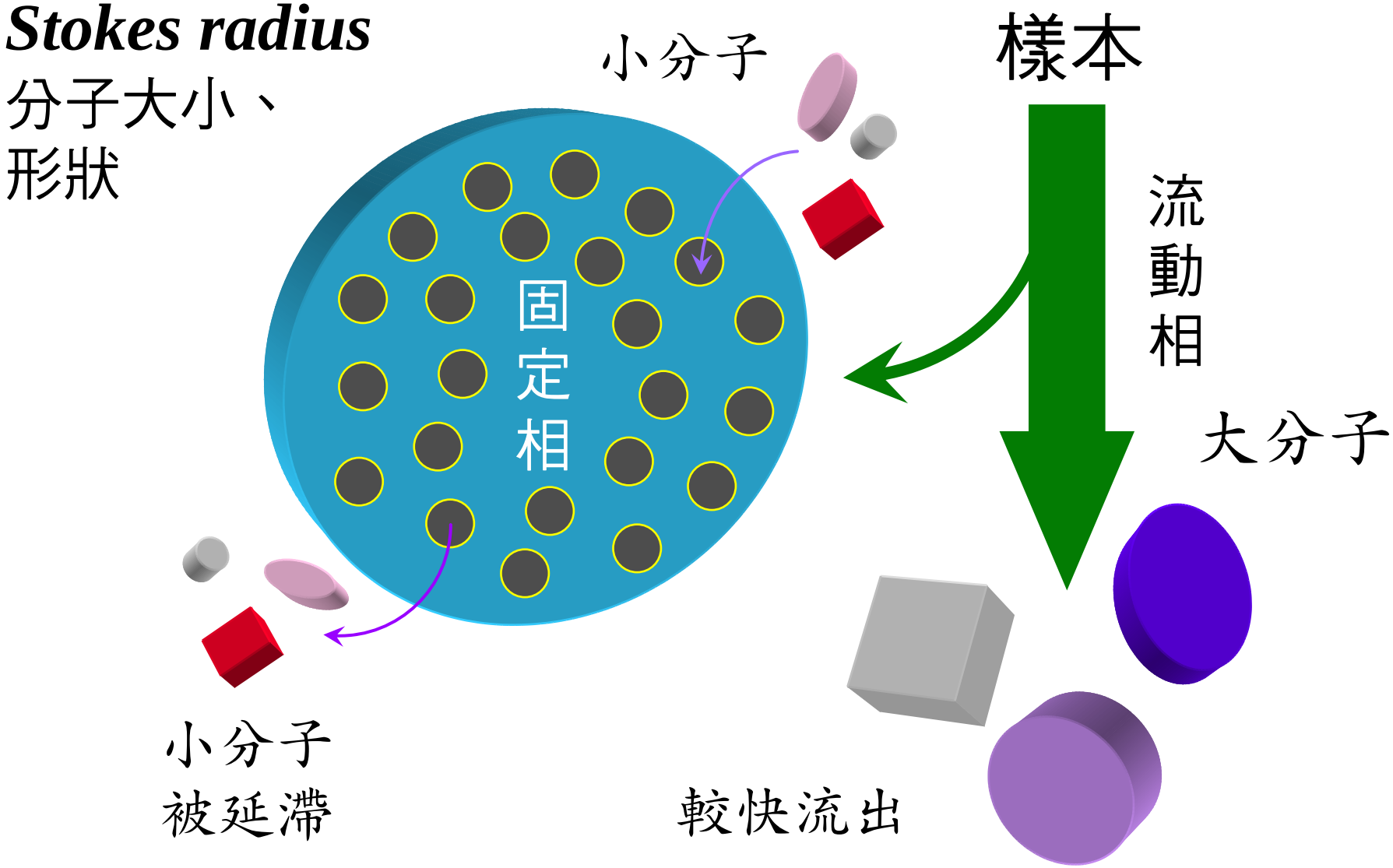


PARTITION

Liquid - Liquid

樣本分子依其分子極性
選擇親和兩相之一

膠体過濾法是一種 Partition 層析法：



各種色析膠體：

Pharmacia

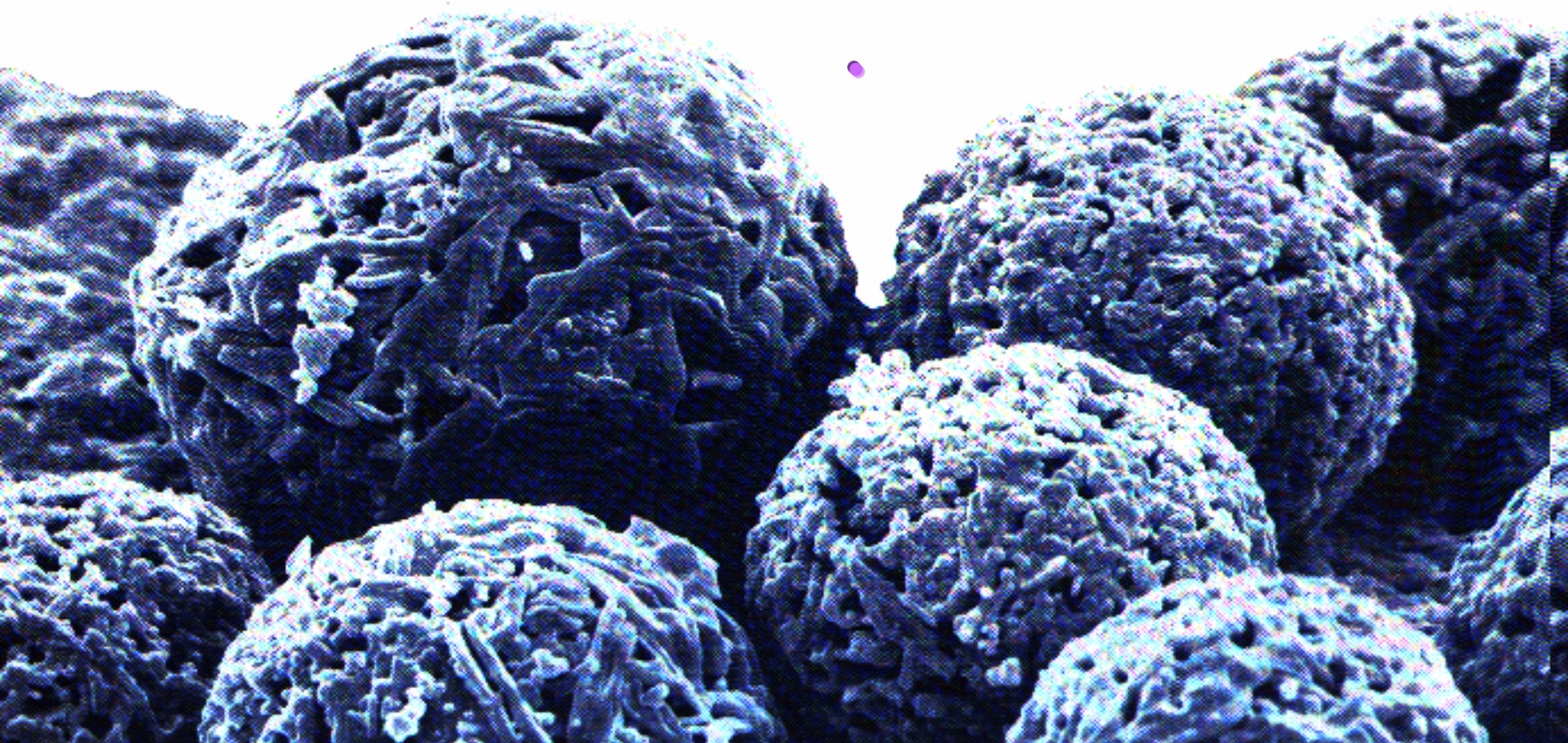
Sephadex	glucose (dextrose)
Sepharose	agarose
Sephacryl	glucose + acrylamide
Sephacel	cellulose

FPLC Superose, Superdex
Mono Q, Mono S

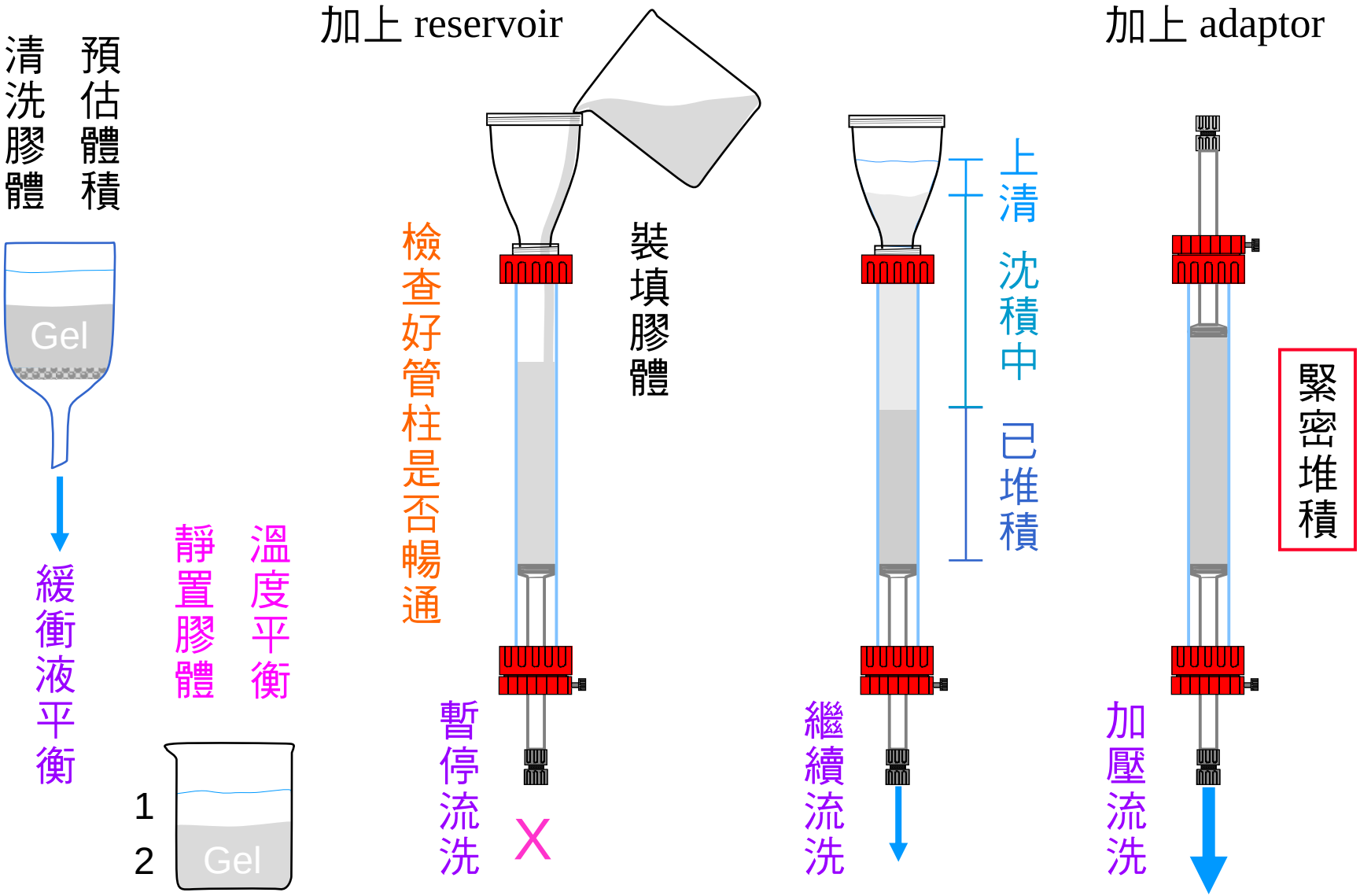
Bio-Rad

BioGel P	acrylamide
BioGel A	agarose

膠体過濾法的膠球：

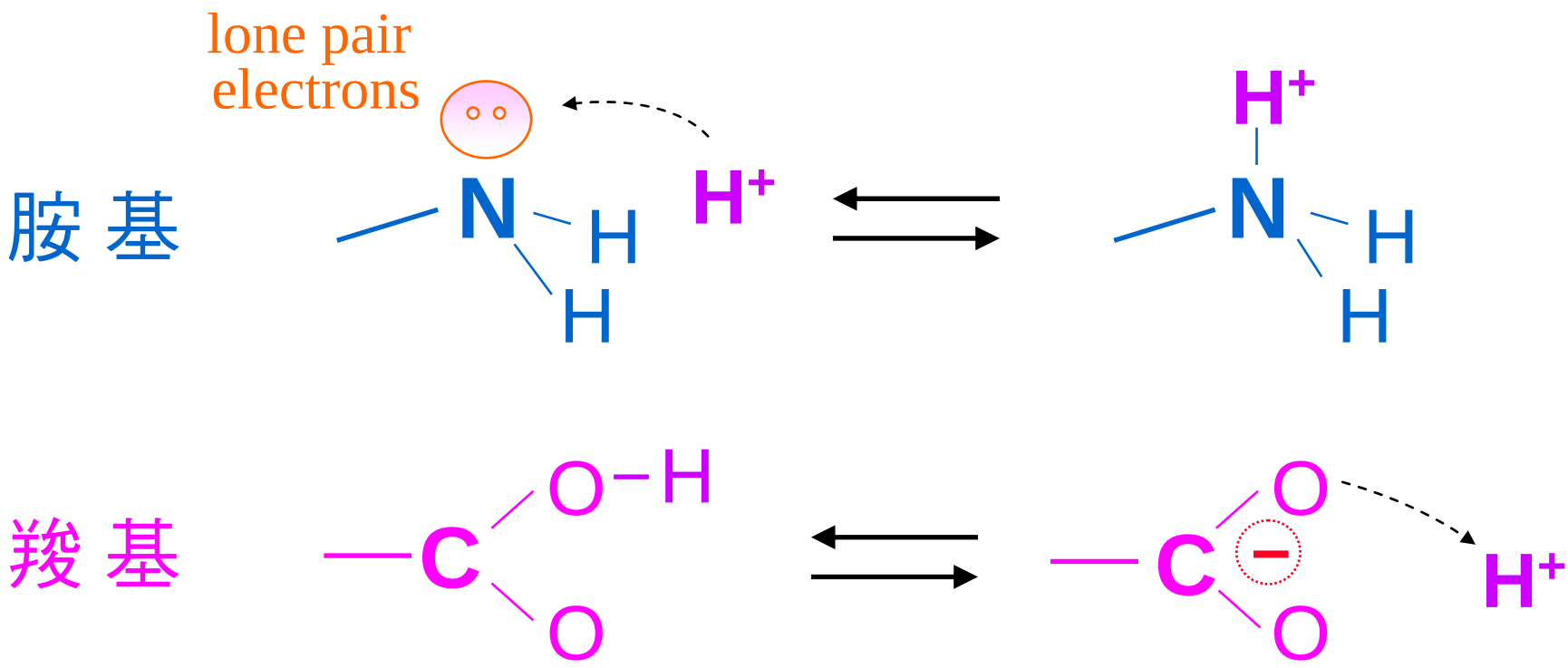


膠柱的裝填方法：

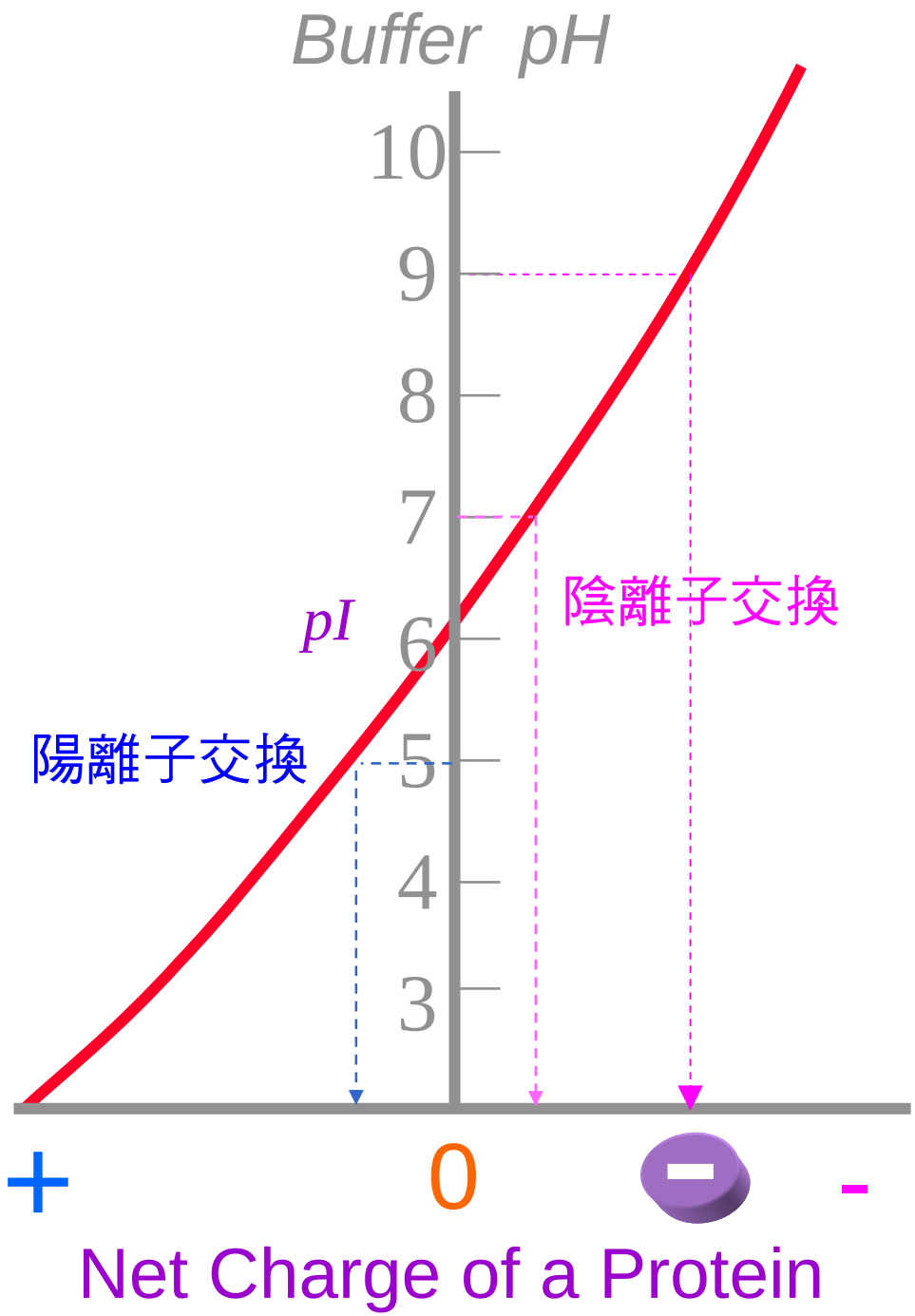

預估體積
清洗膠體
Gel
緩衝液平衡
溫度平衡
靜置膠體
1
2
Gel
加上 reservoir
檢查好管柱是否暢通
裝填膠體
暫停流洗
X
上清
沈積中
已堆積
繼續流洗
加上 adaptor
加壓流洗
緊密堆積

質子可以吸著或脫離一基團：

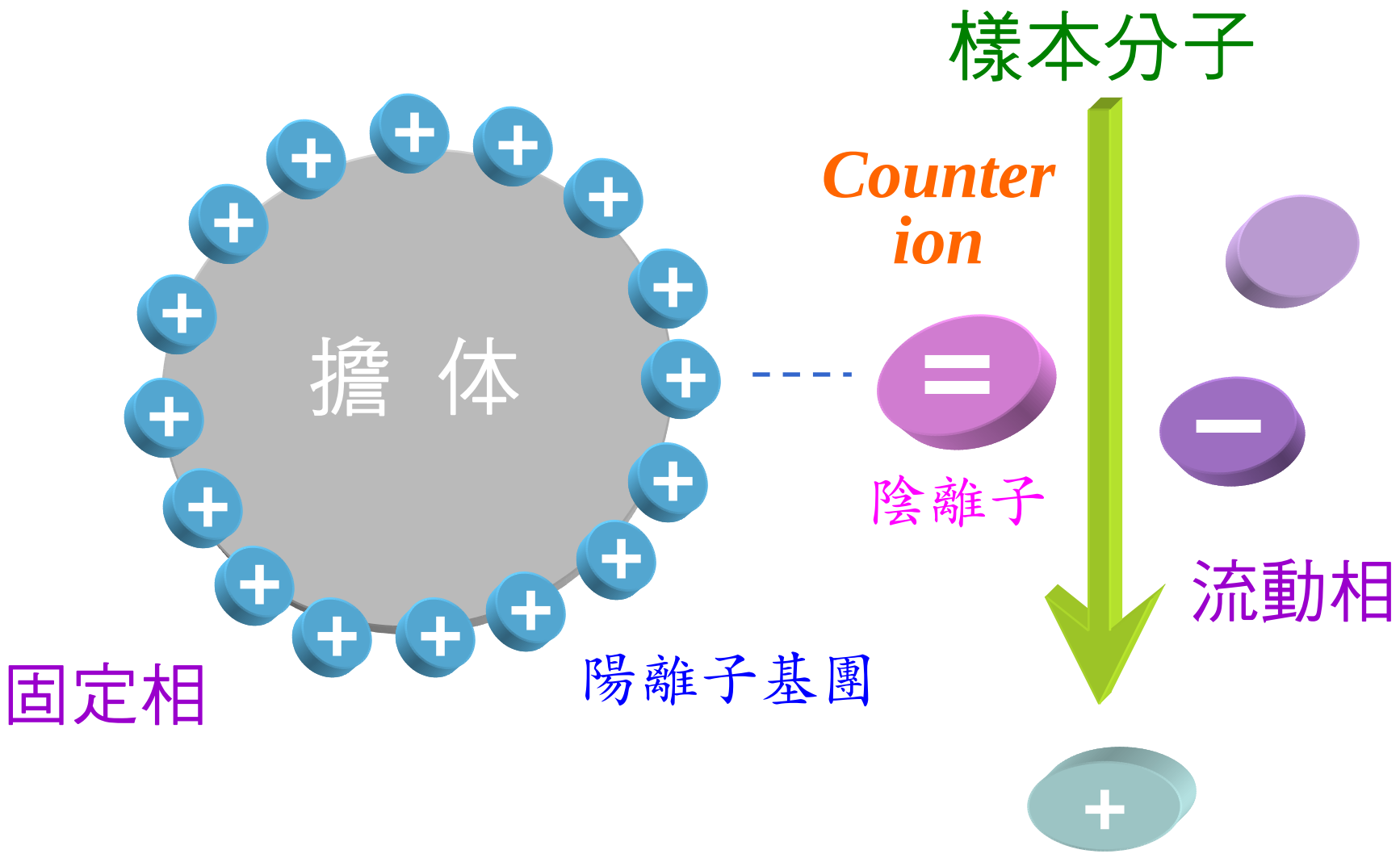
Proton： 最小且最多的生物粒子，影響酸鹼度及分子帶電性質



選擇離子交換介質：

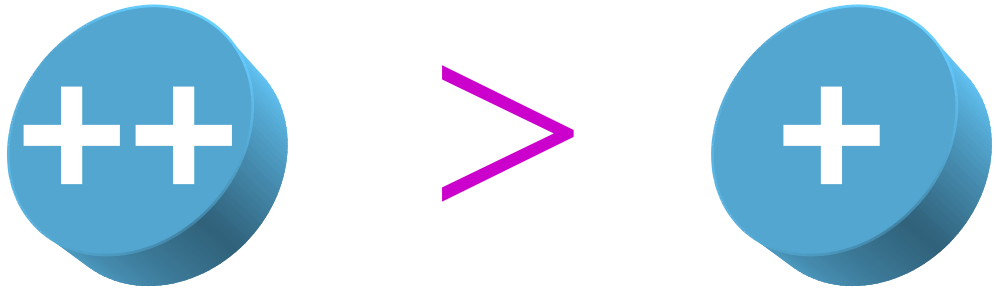


陰離子交換法 Anion Exchange：

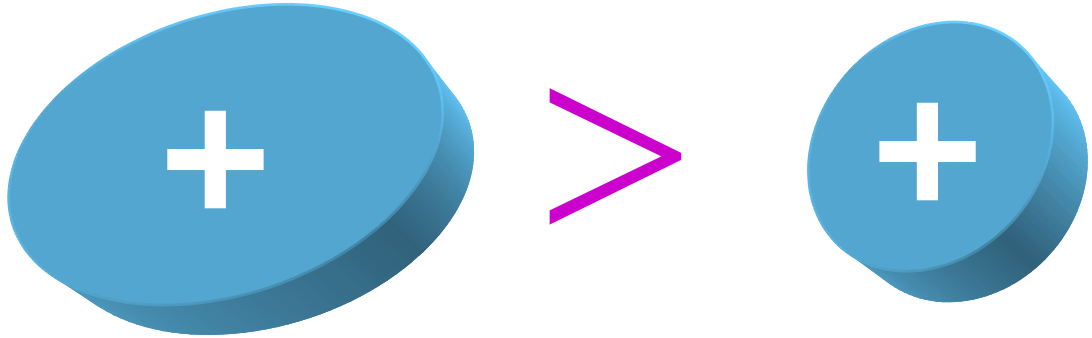


離子取代優先順序 Pecking Order：

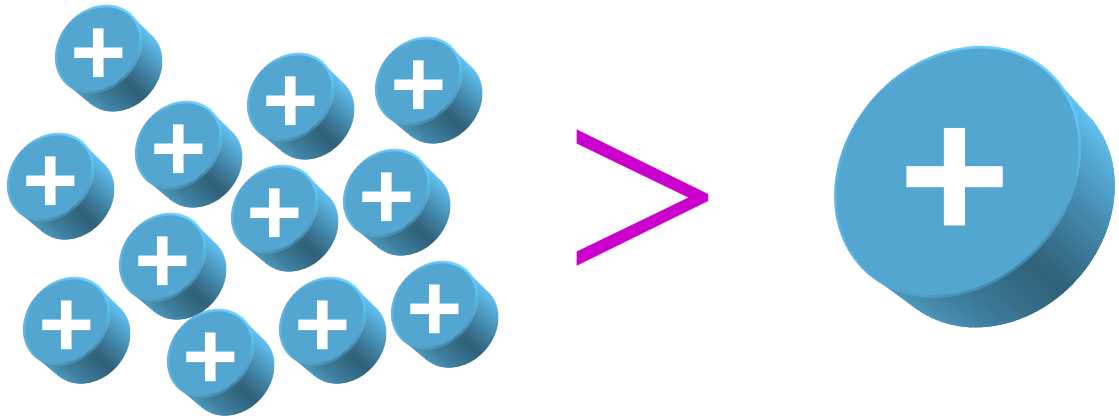
電荷高者



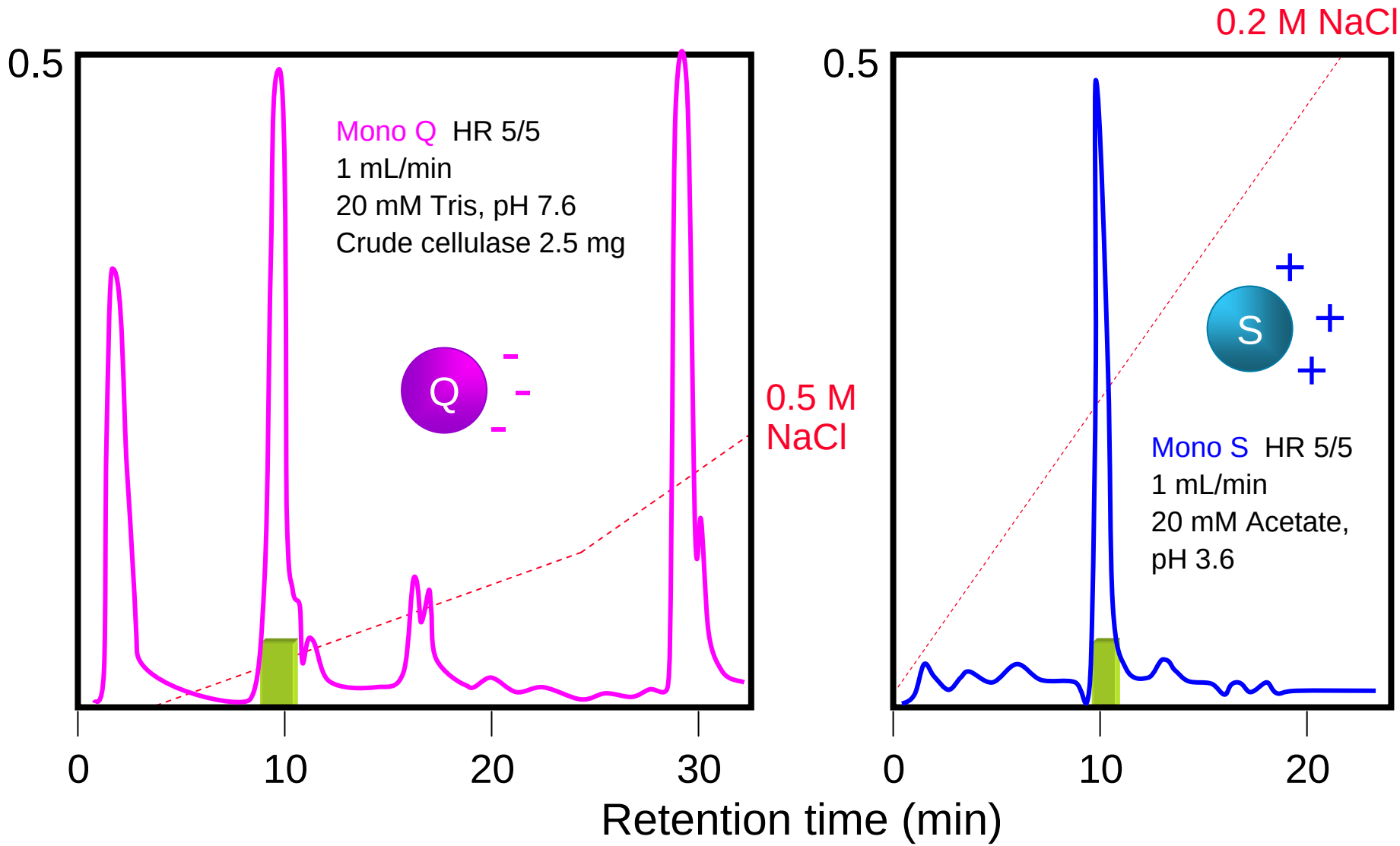
離子大者



濃度大者



離子交換法實例： Cellulase



Adapted from Pharmacia (1991) Ion Exchange Chromatography – Principles and Methods p.127

專一性結合力量的構成因素：

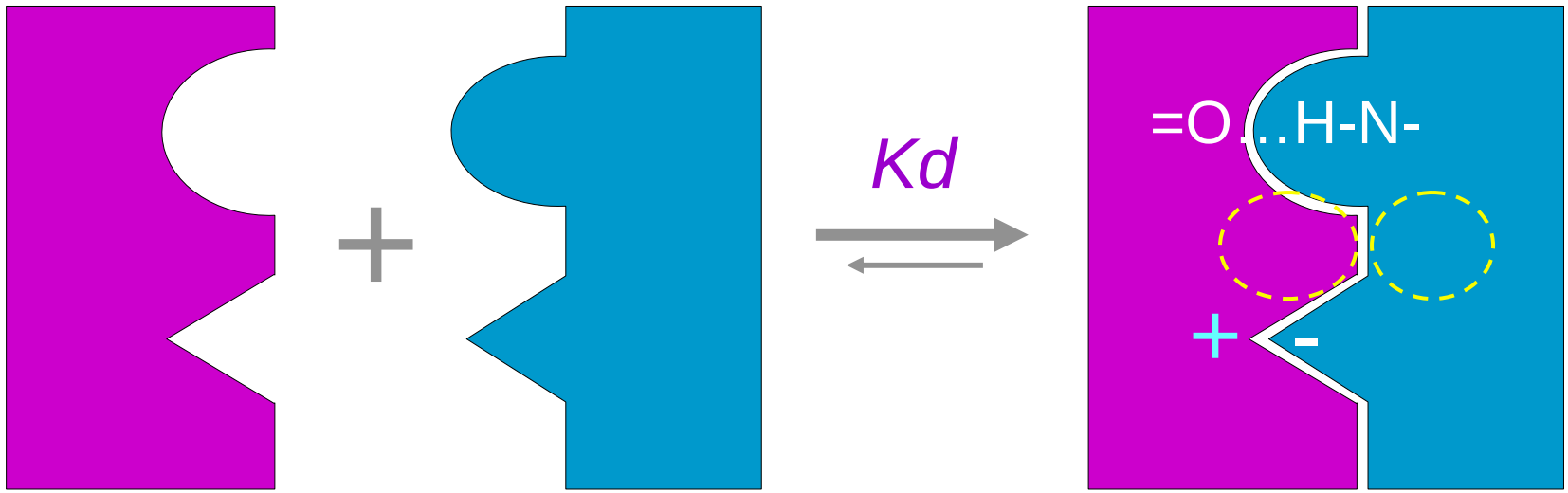
I. Conformational Match:

Van der waals interaction

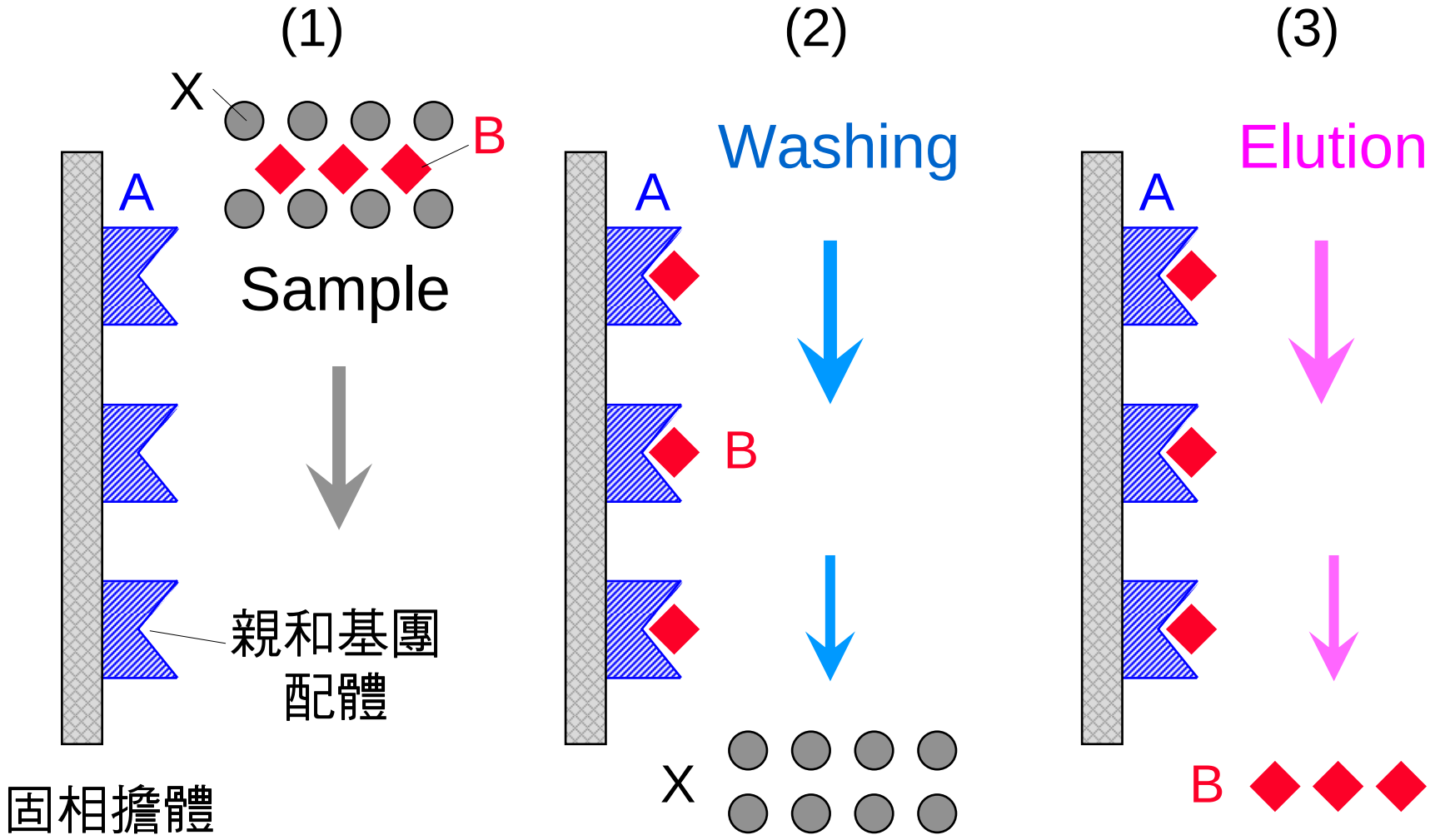
兩分子間因構形互補所造成的吸引力是由凡得瓦爾力所貢獻

II. Interaction Forces:

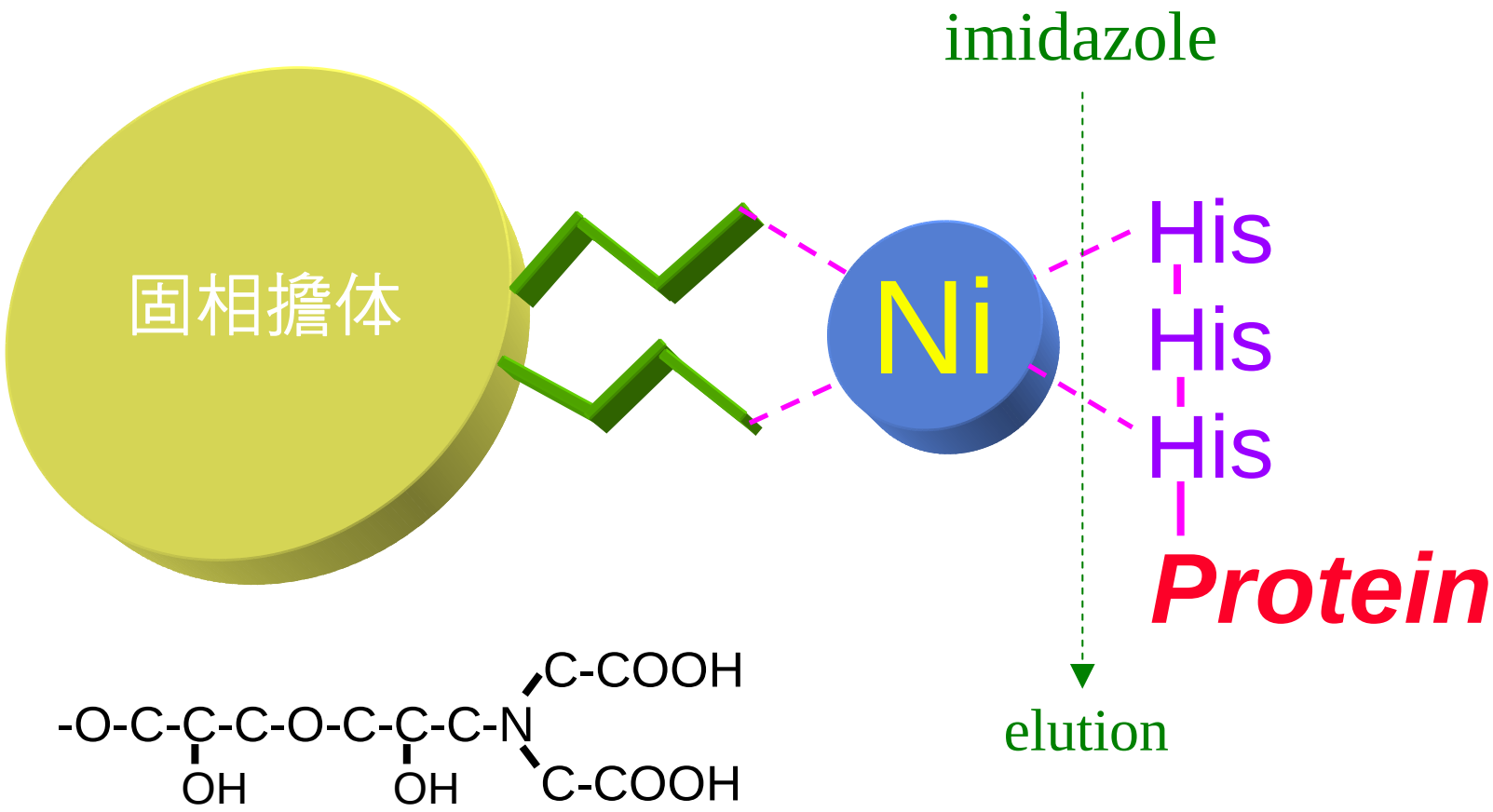
(1) Hydrogen bond
(2) Hydrophobic interaction
(3) Electrostatic interaction
(4) Van der waals interaction



親和層析法的作用機理：



金屬螯合層析法：



Metal **C**helate **A**ffinity **C**hromatography

組合純化步驟：

純化流程基本骨幹

硫酸銨分劃　　分子表面極性不同

↓

離子交換法　　分子淨電荷不同

↓

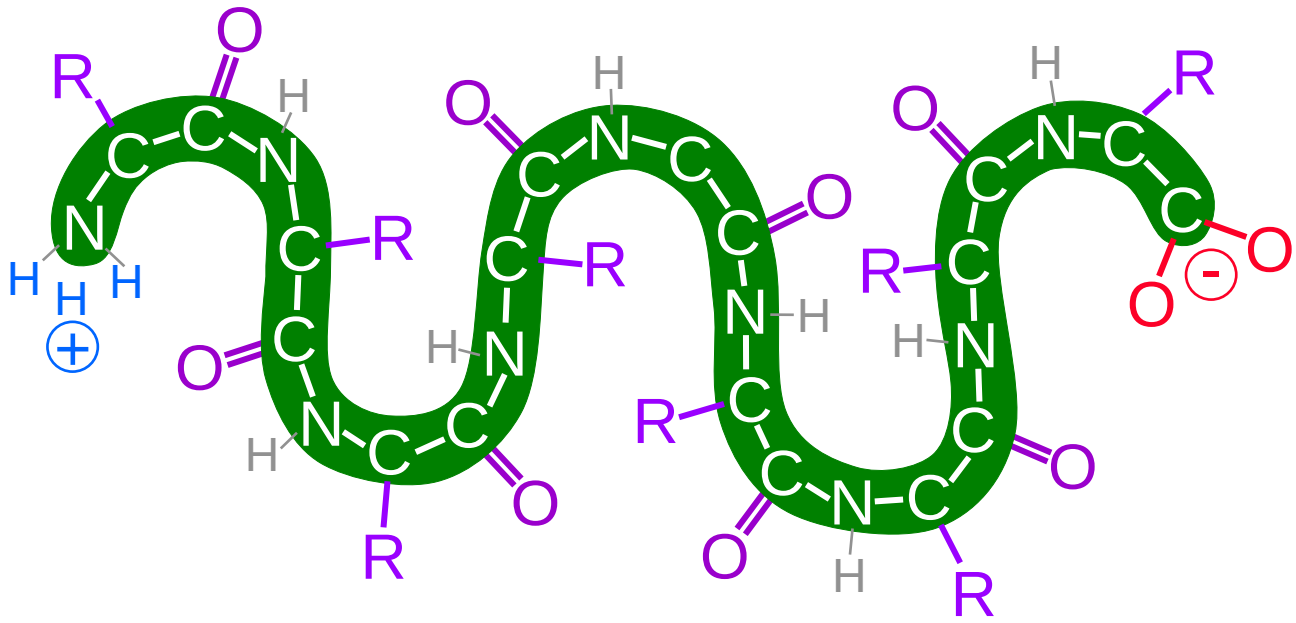
膠體過濾法　　分子量大小不同

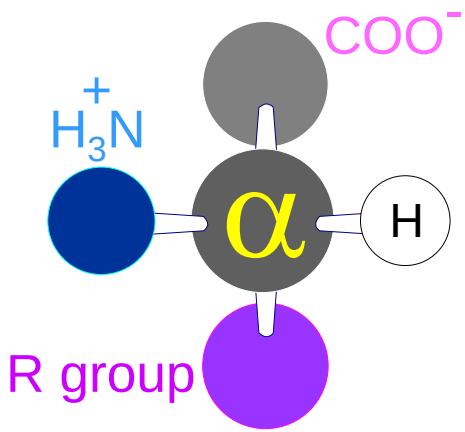
↓

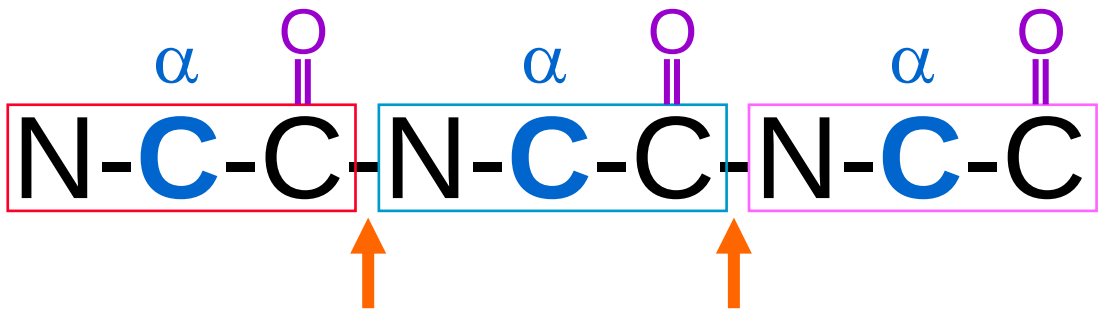
酵素分析方法

- 蛋白質定量　也是根基於分子構造
- 酵素活性分析　有催化活性的巨分子
- 膠體電泳法　SDS vs Native-PAGE
- 免疫轉印法　應用抗體的專一性
- Proteomics　解開基因序列之後

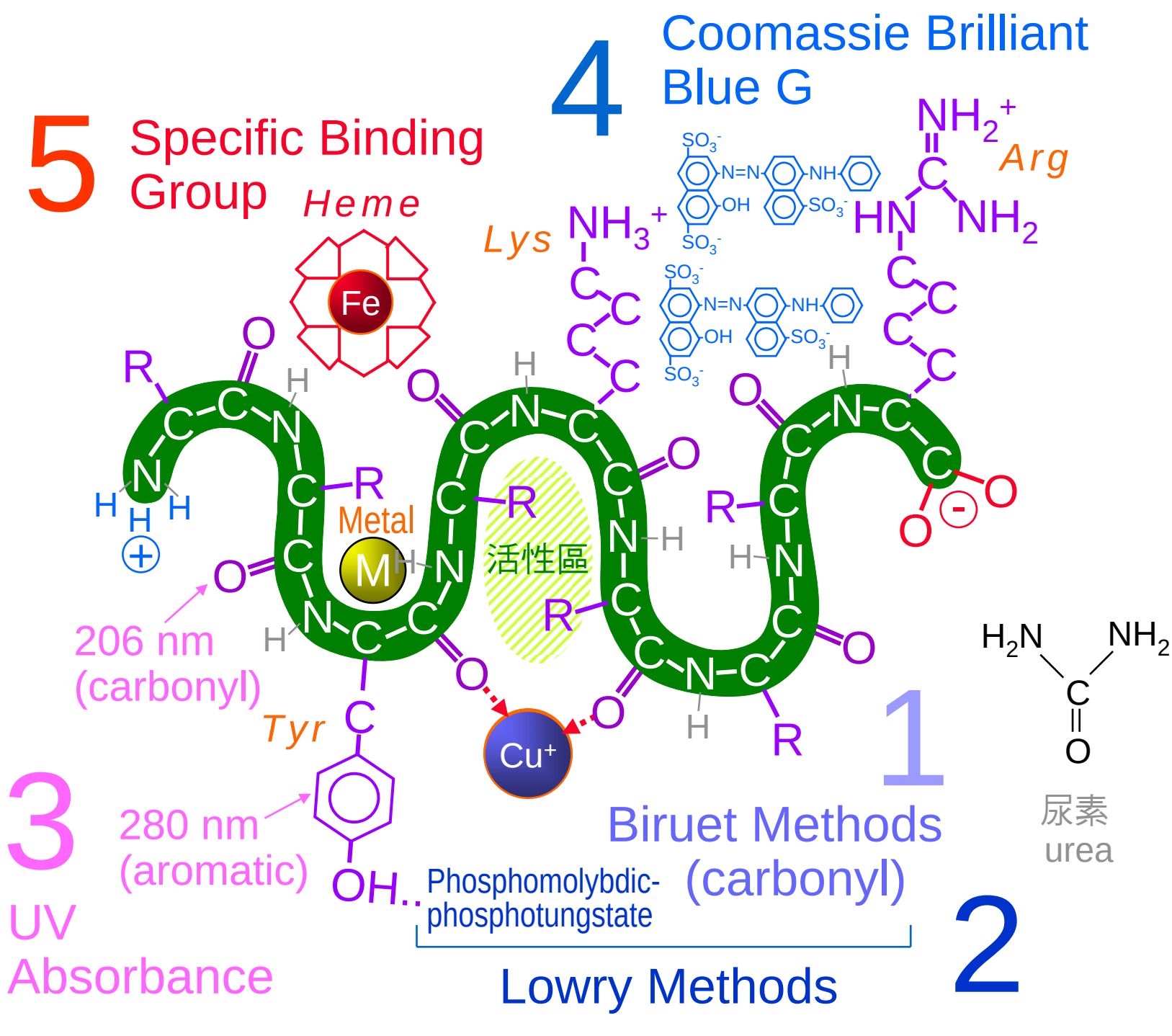
蛋白質構造的骨架：







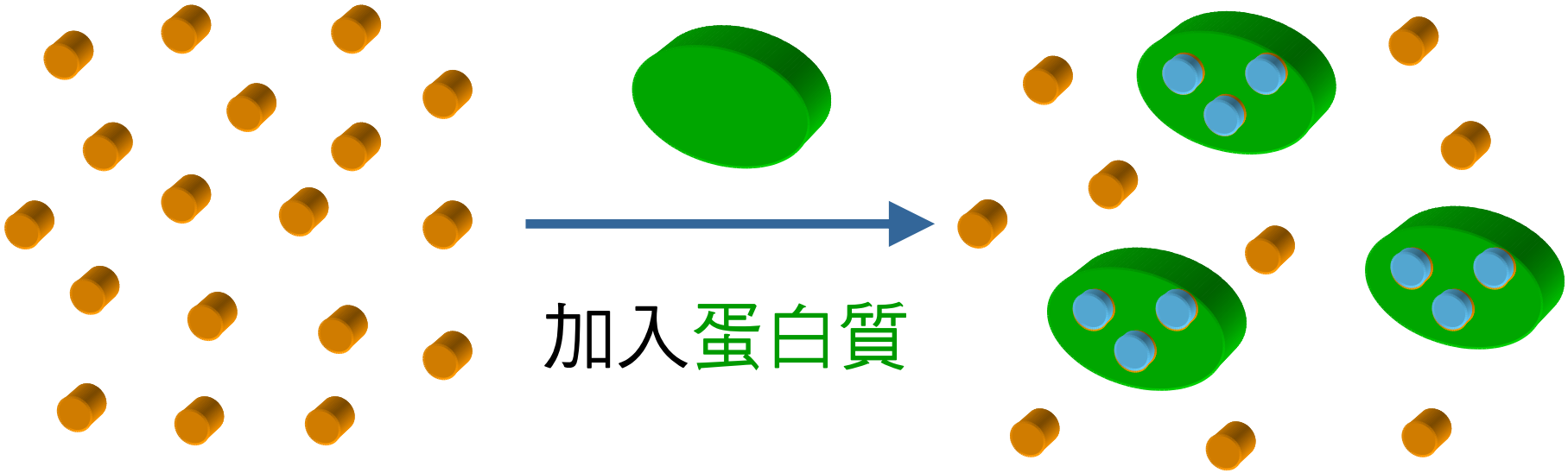
各種蛋白質定量法原理：


4
Coomassie Brilliant Blue G
5
Specific Binding Group
Heme
Fe
Lys
Arg
Metal
M
活性區
206 nm
(carbonyl)
Tyr
280 nm
(aromatic)
3
UV Absorbance
Cu+
1
Biuret Methods
(carbonyl)
尿素
urea
Phosphomolybdic-
phosphotungstate
2
Lowry Methods

Bradford Method：

Coomassie Brilliant Blue G-250

470 nm　　CBG 是一種指示劑　　595 nm

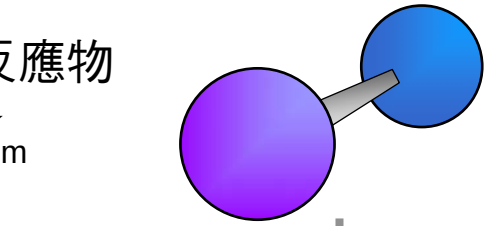


酸性環境下呈茶色

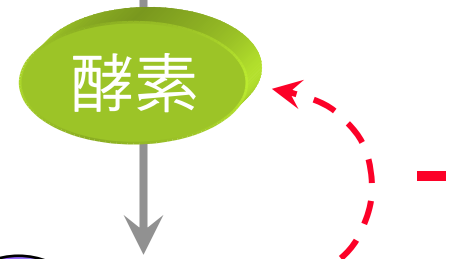
與蛋白質結合變藍色

酵素反應及偵測方法：

使用大量反應物
10 x K_m



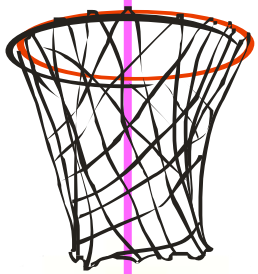
反應物



生成物可能有
回饋抑制

生成物

能否直接測量？

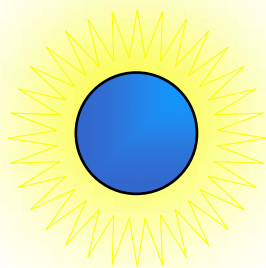


移除生成物並
轉成可測量者

NAD+
直接測量
NADH

耦合反應

把生成物再轉換成
可被測量的生成物

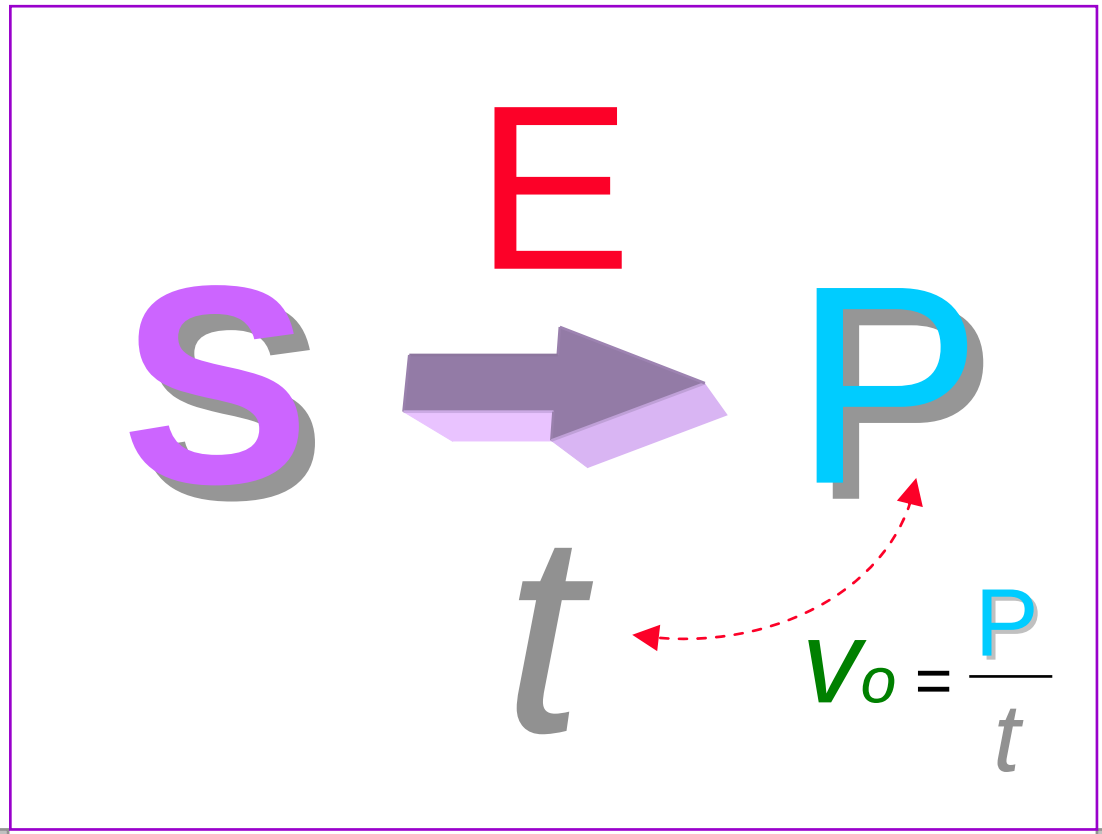


酵素活性的測定：

酵素量要適中

基質
要過量

10 x Km



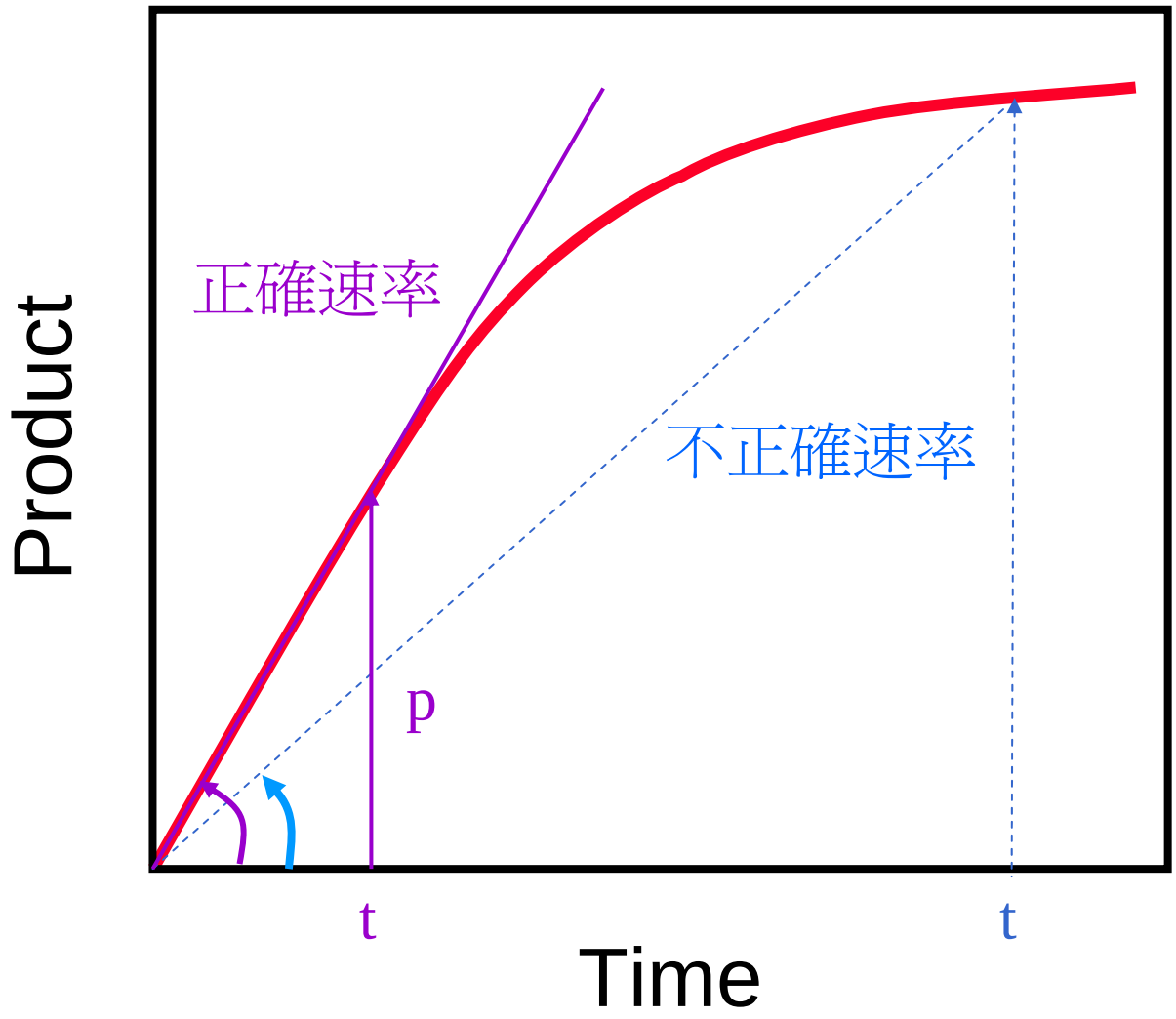
生成物
要能測得

酸鹼度

反應時間恰當

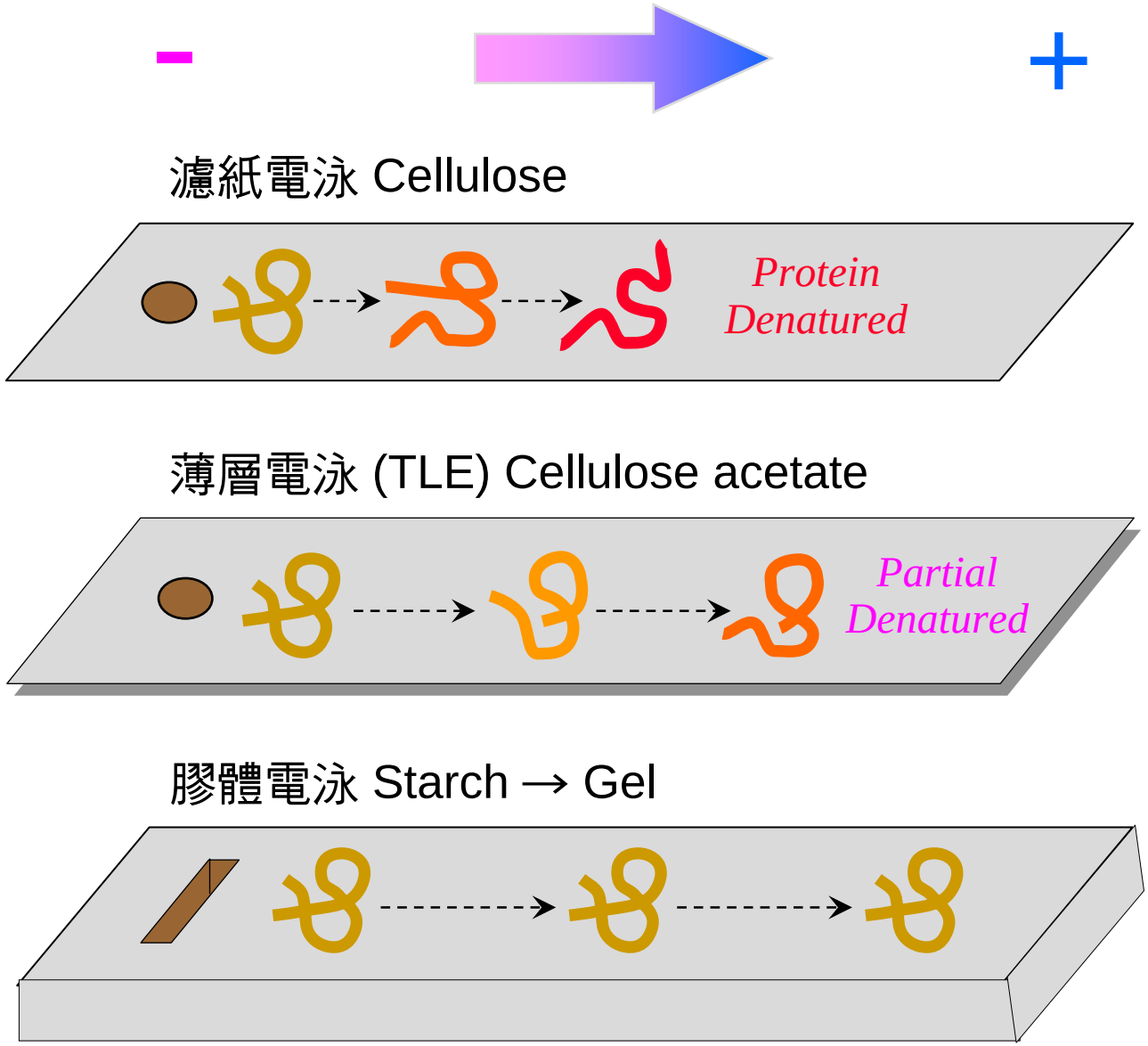
溫 度

測定時間內反應速率需成線性：



$$v = \frac{p}{t}$$

電泳形式的演進：


-
+
濾紙電泳 Cellulose
Protein
Denatured
薄層電泳 (TLE) Cellulose acetate
Partial
Denatured
膠體電泳 Starch → Gel

膠體的聚合反應：

Ammonium persulfate (free radical initiator)

$(O_4S\text{-}SO_4)^{2-} \longrightarrow 2\ SO_4^{-}$

自由基的生成者

Acrylamide (monomer)

成膠的基本單位

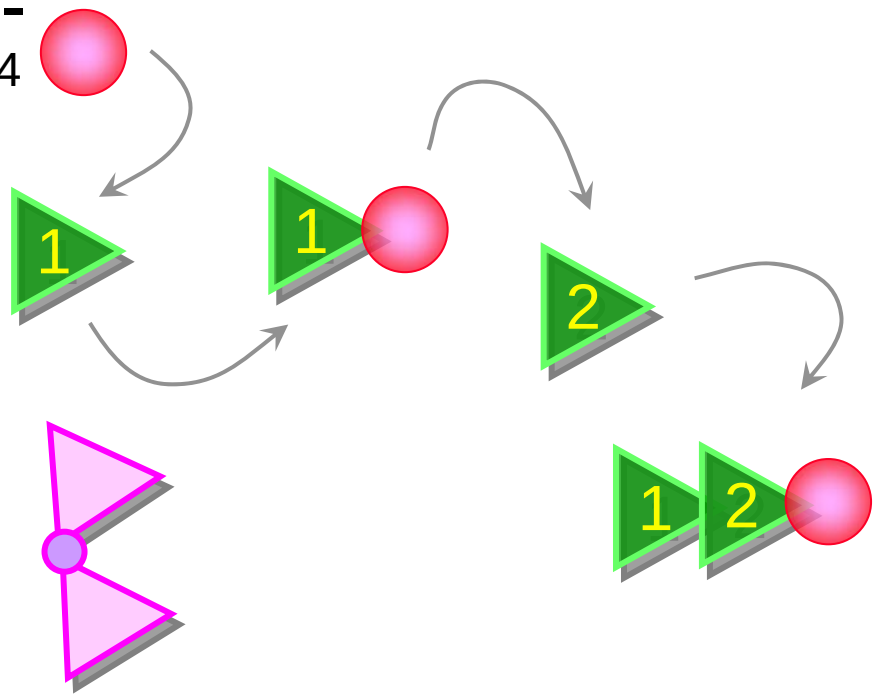
Bis(acrylamide) (bridge)

架橋使聚合產生分枝

TEMED (catalyst)

幫助傳遞自由基的催化劑

SDS (Sodium dodecyl sulfate)

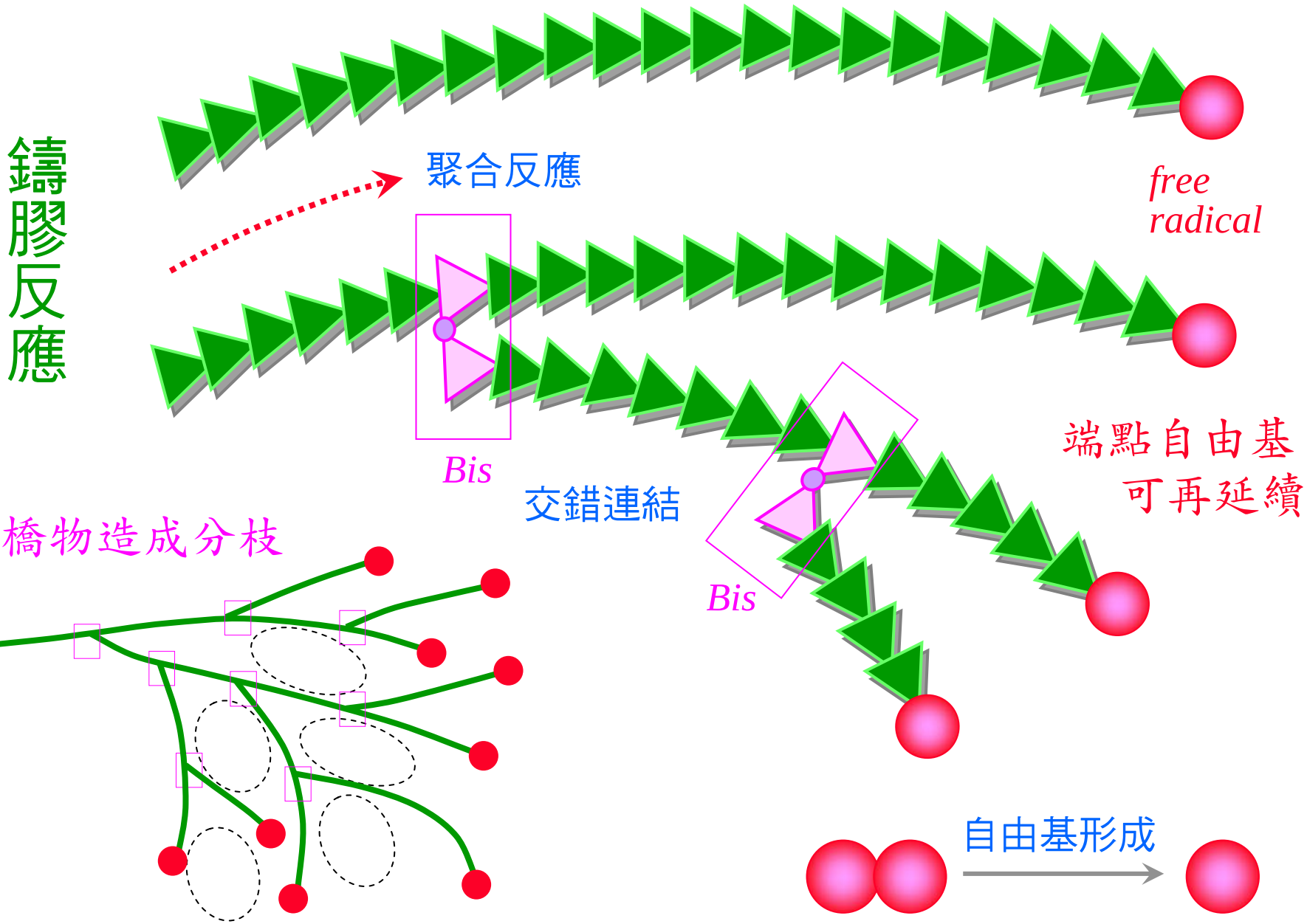


$CH_2=CH\text{-}CO\text{-}NH_2$

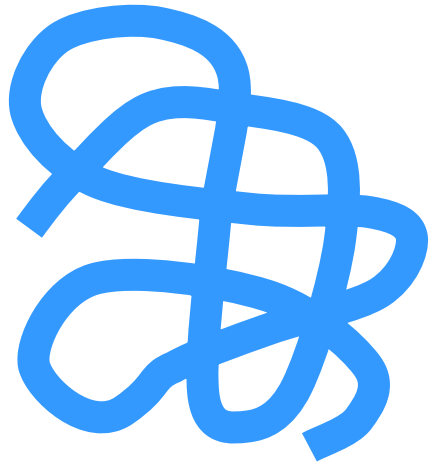
Acrylamide 有毒性！

澱粉加高熱會誘生？

單體聚合反應：

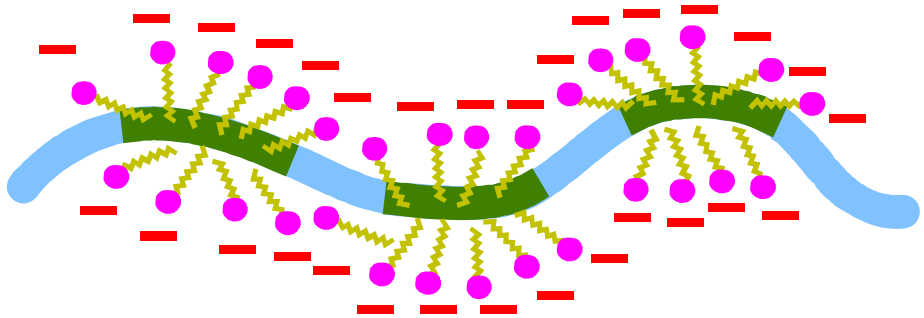

鑄膠反應
聚合反應
free radical
Bis
交錯連結
端點自由基
可再延續
架橋物造成分枝
Bis
自由基形成

SDS 在蛋白質表面 均勻敷上 一層負電：



SDS

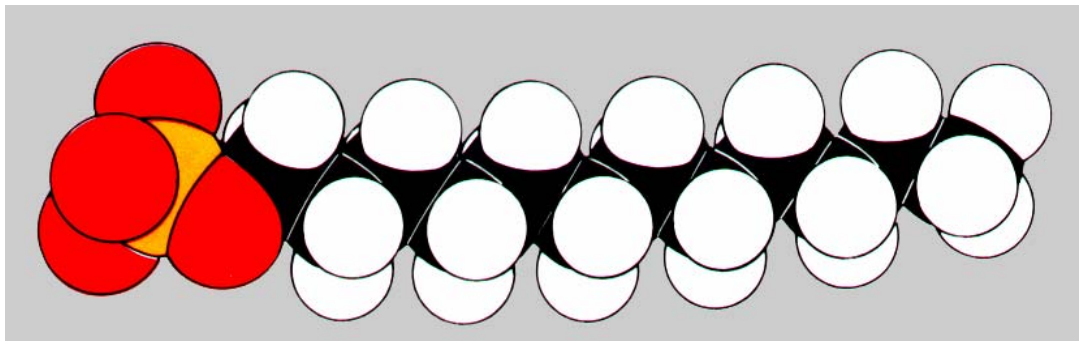
boiling



原態蛋白質

變性蛋白質成一線狀分子
並且均勻帶上一層負電荷

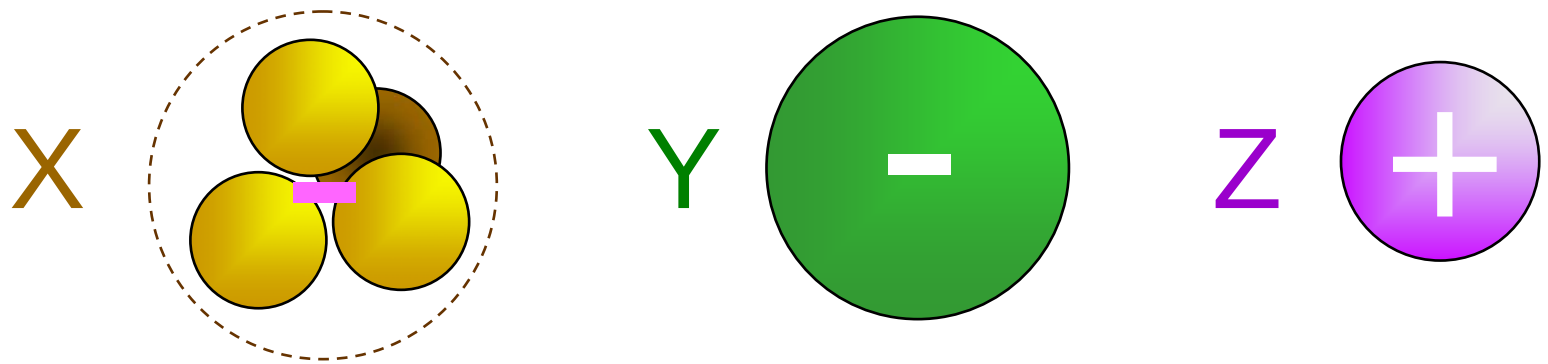
極性頭部

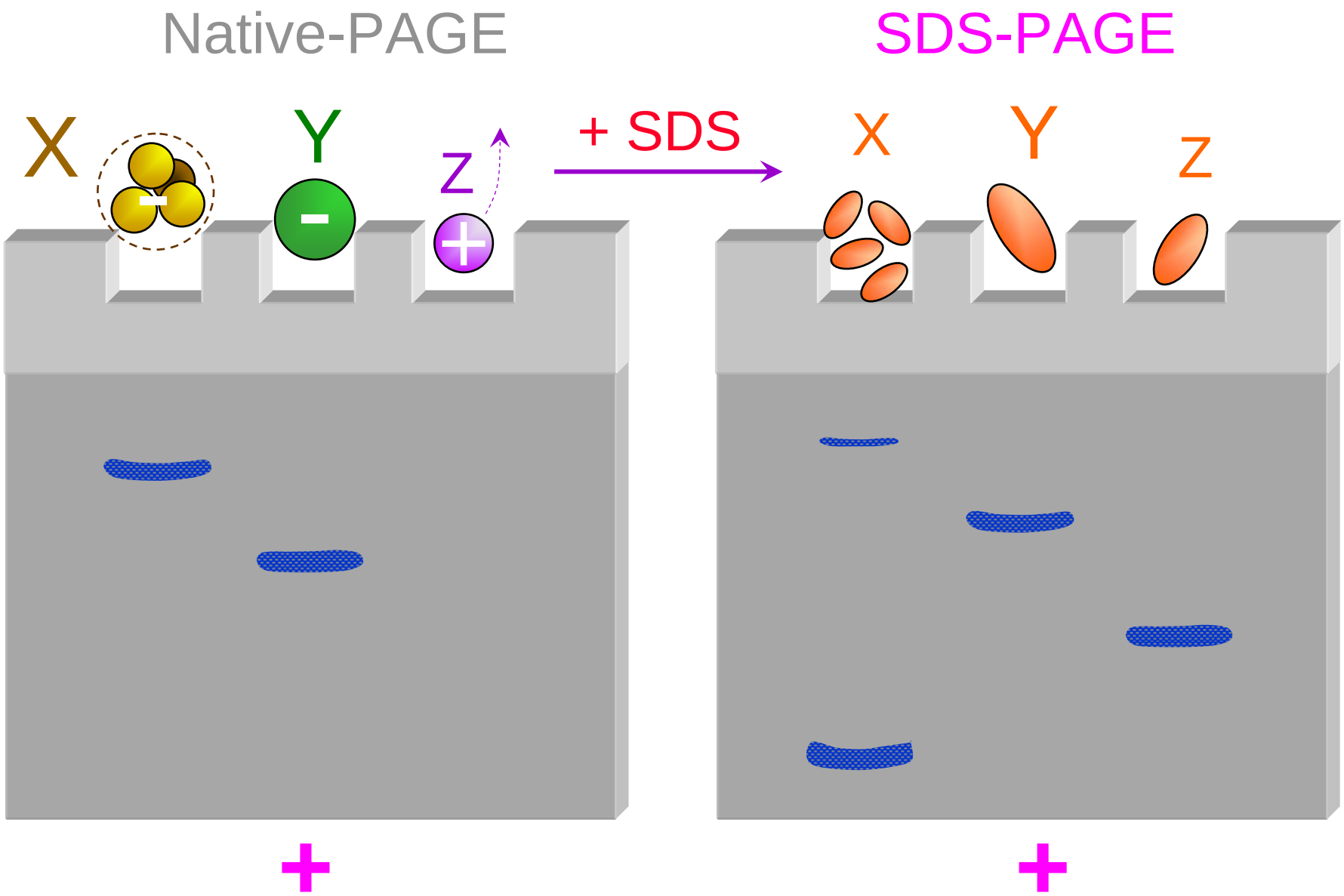


非極性尾部

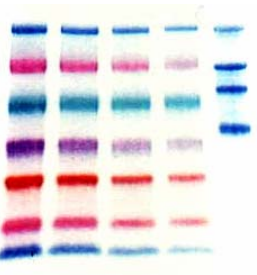
三種不同性質蛋白質的電泳比較：

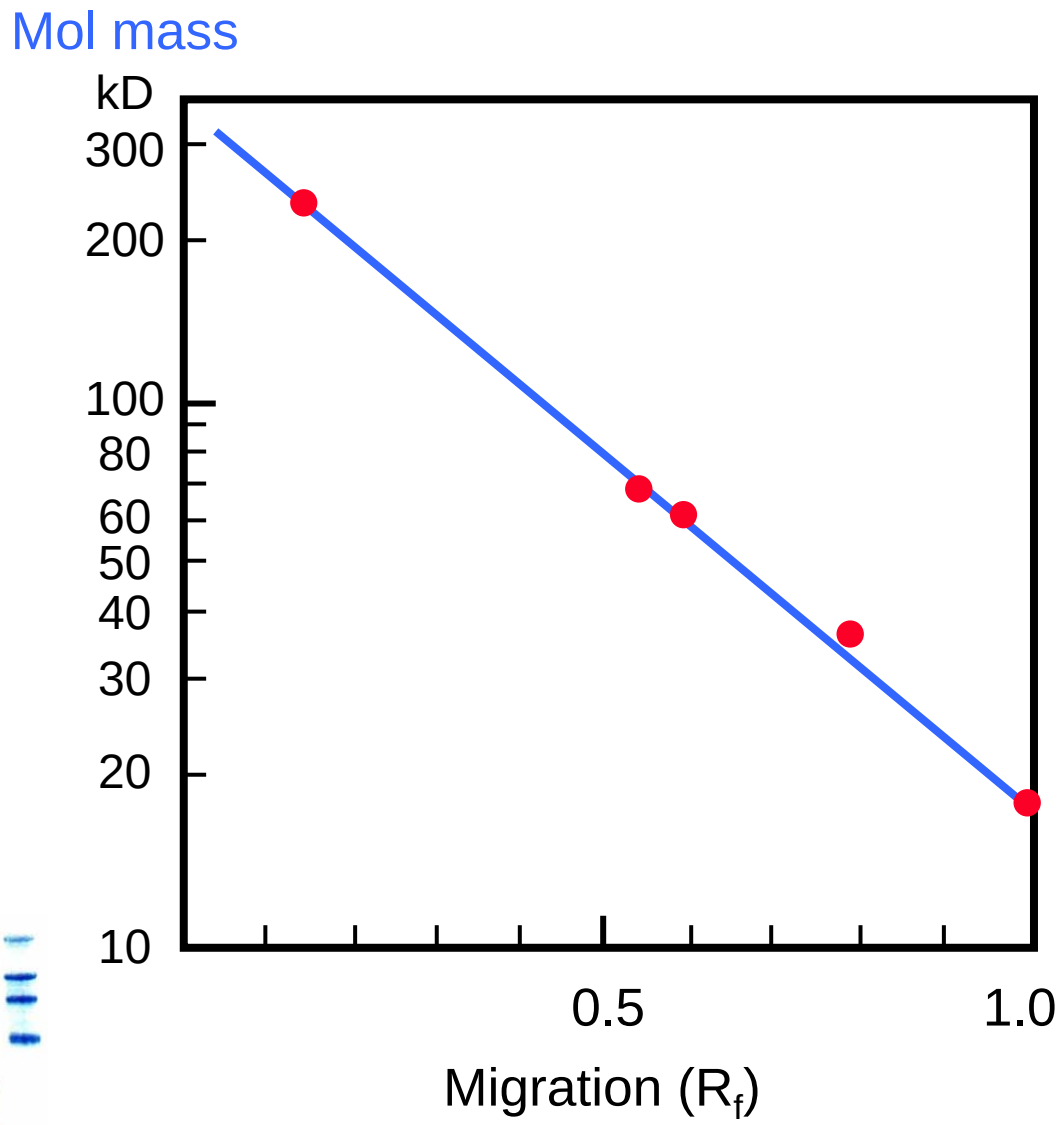
Protein	Quaternary Structure	Molecular Weight	pI	Mobility	
				Native PAGE	SDS-PAGE
X	Tetramer	(40,000)x4	5.8	Slow	Fast
Y	Monomer	88,000	5.2	Fast	Slow
Z	Monomer	60,000	9.3	Upward	Medium

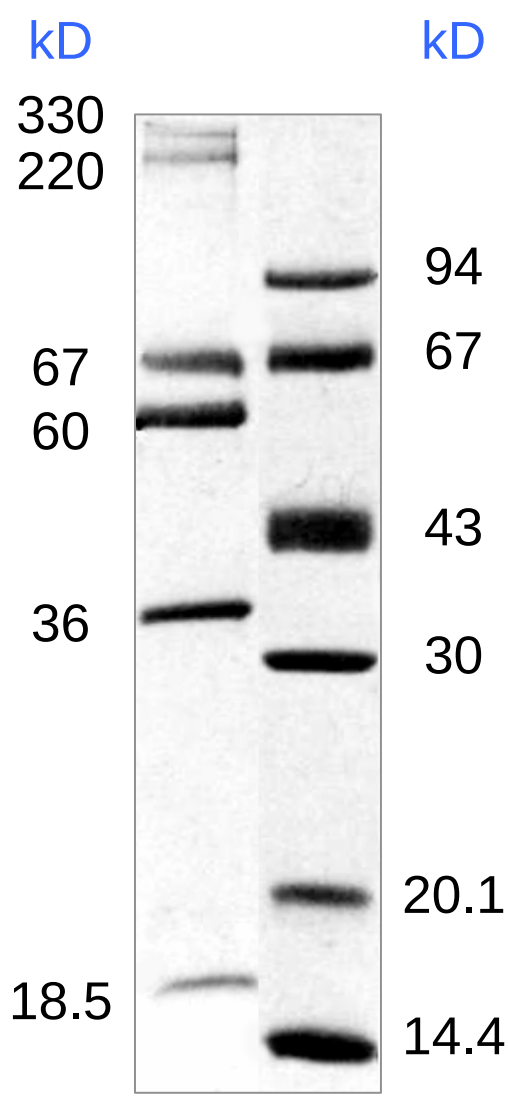



Native-PAGE
SDS-PAGE
X
Y
Z
+ SDS
X
Y
Z
+
+
分子量 及 淨電荷密度
均影響泳動率
只有 分子量 影響泳動率

單元體分子量的測定： SDS-PAGE

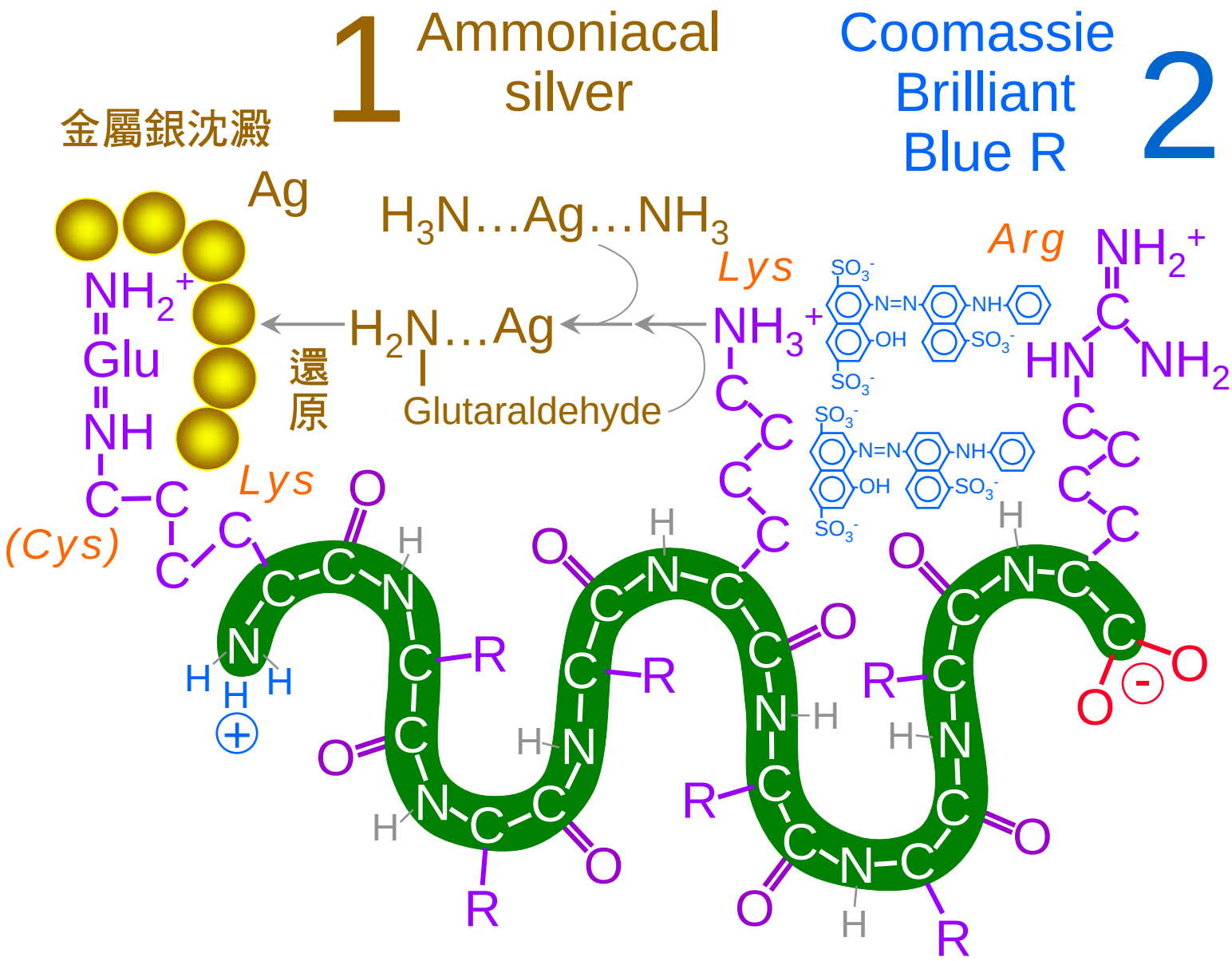






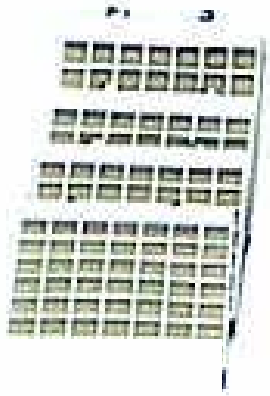
Pharmacia: Molecular Markers for electrophoresis

各種蛋白質染色法機制：

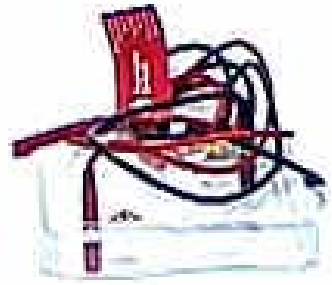

1
Ammoniacal silver
Coomassie Brilliant Blue R
2
金屬銀沈澱
Ag
$H_3N…Ag…NH_3$
$H_2N…Ag$
Glutaraldehyde
還原
Lys
Arg
(Cys)
Glu
NH
NH_2^+
NH_3^+
HN
NH_2
OH
SO_3^-
N=N
NH

電泳槽及相關設備：

轉印三明治

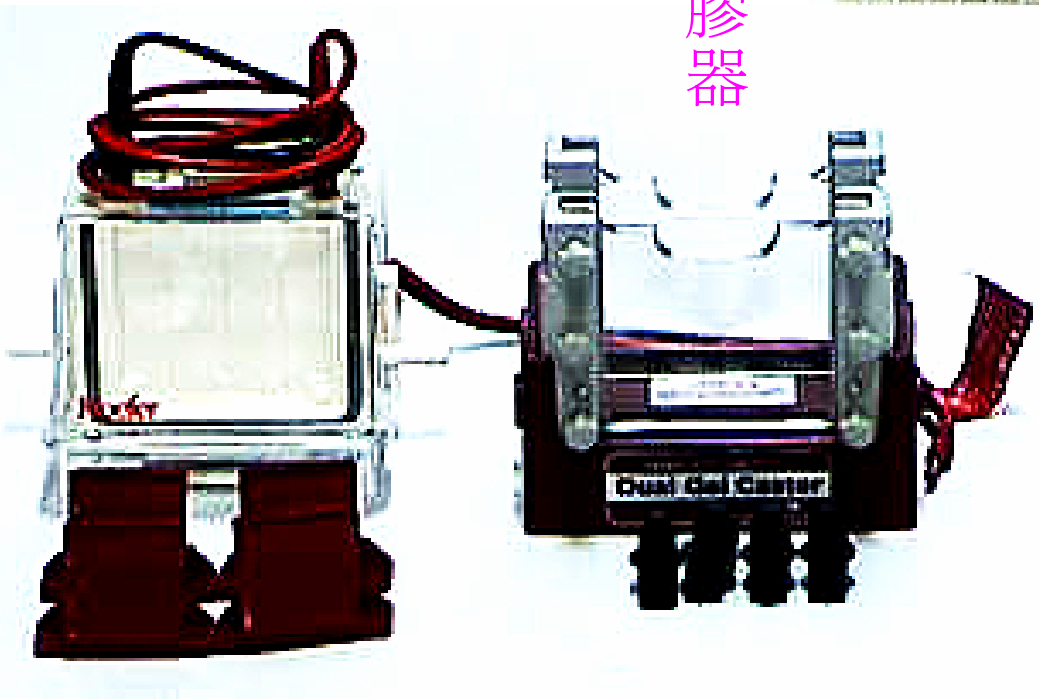


轉印槽



電泳槽

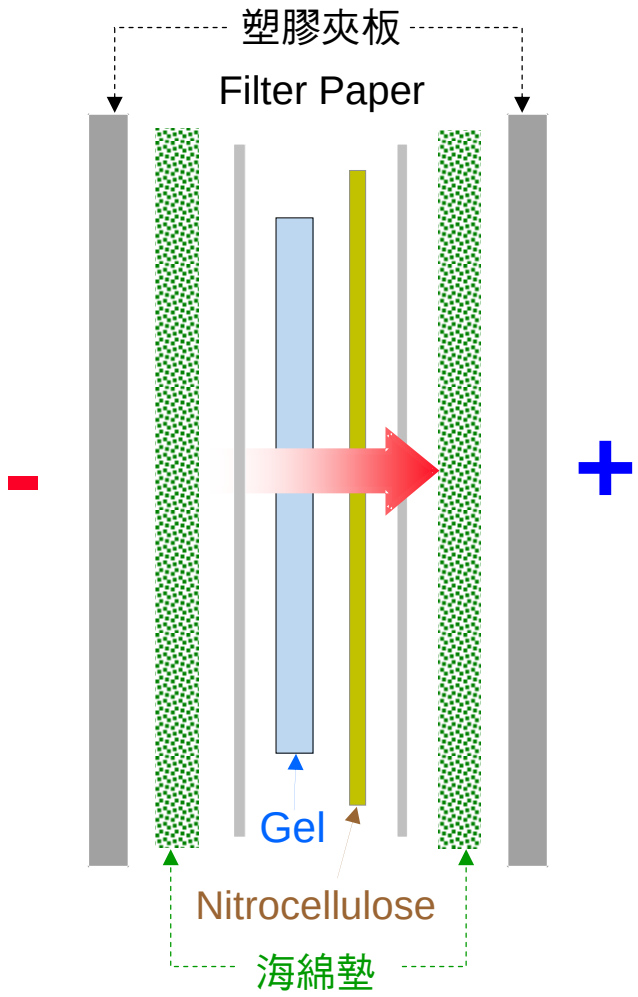
鑄膠器



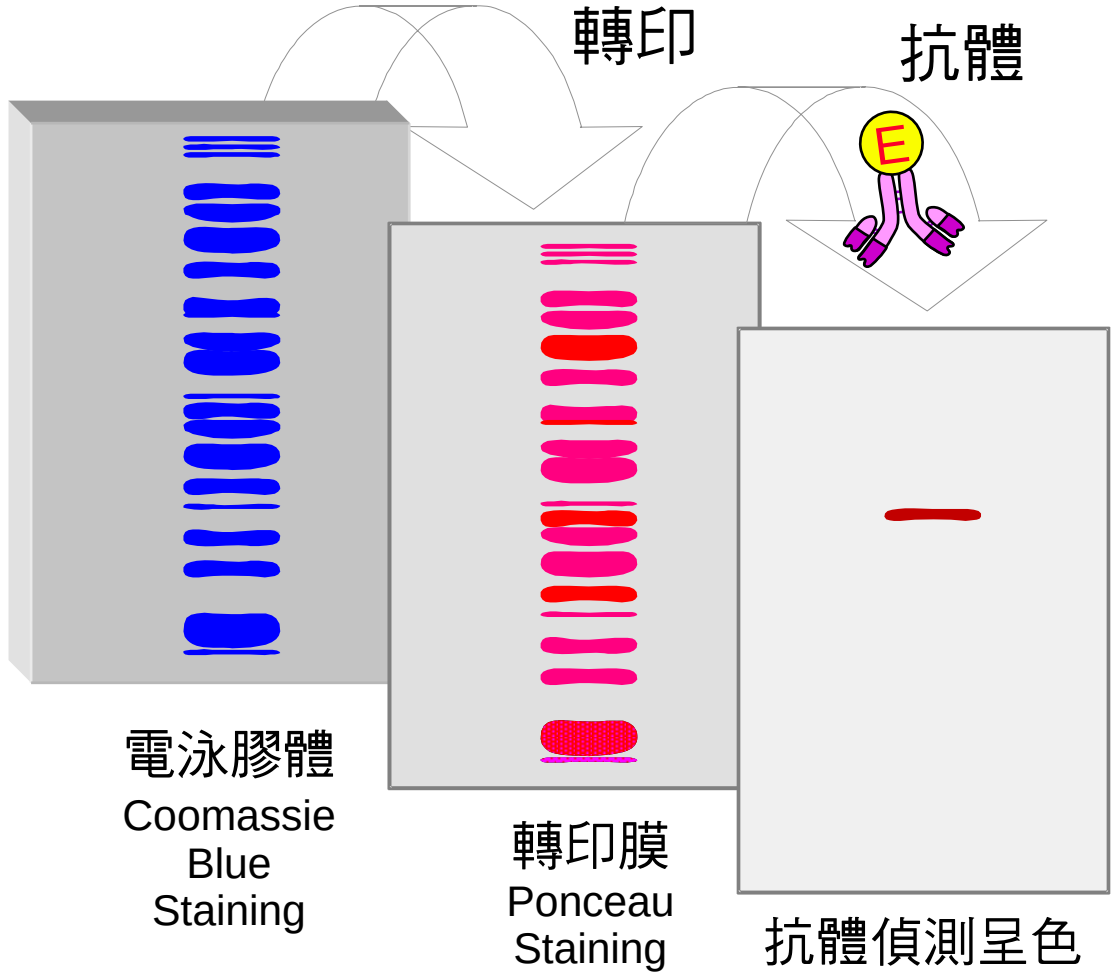
供電器

轉印及免疫染色流程：

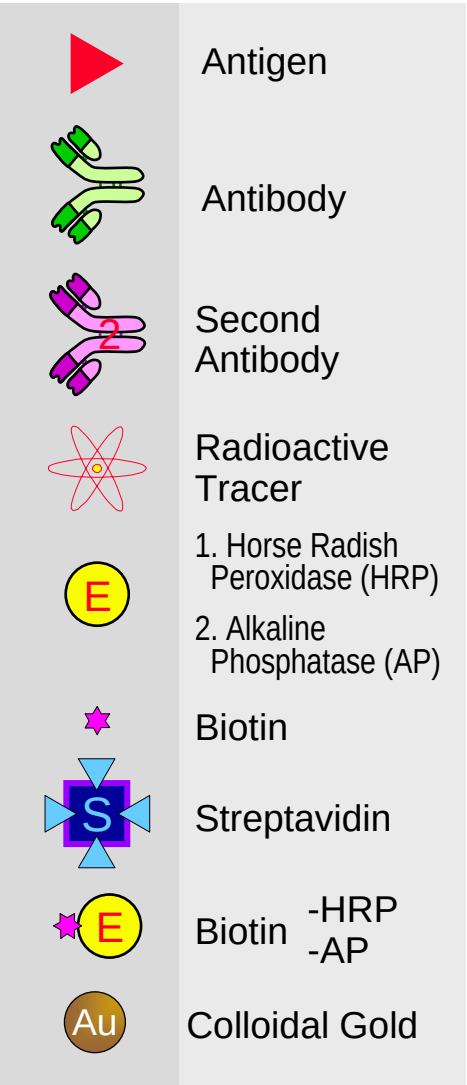
A 轉印三明治

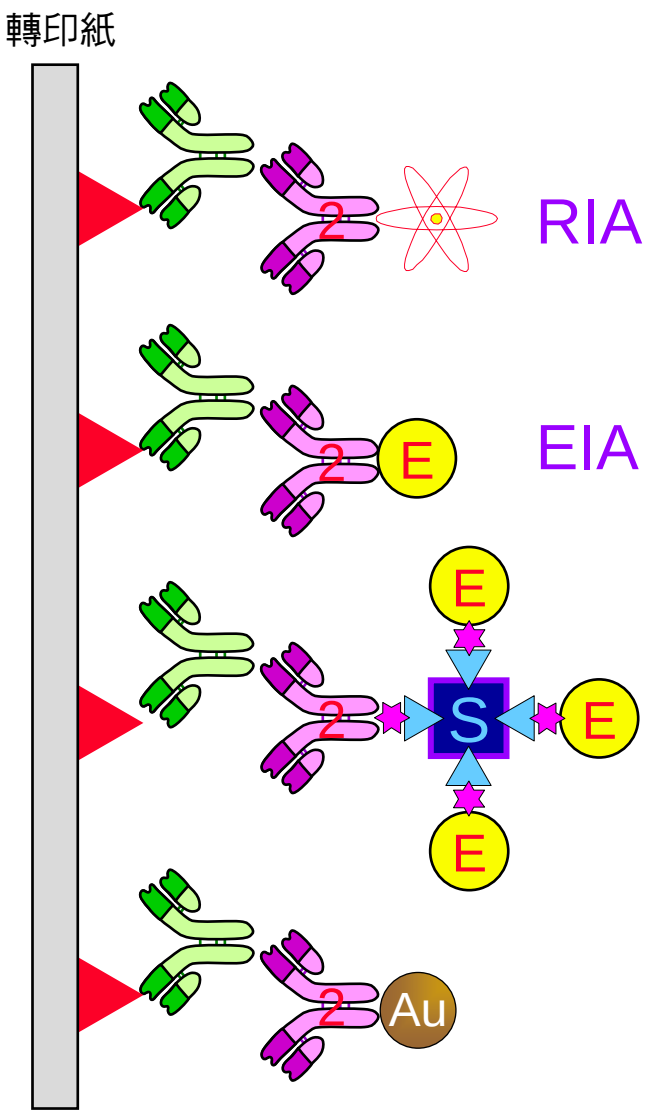


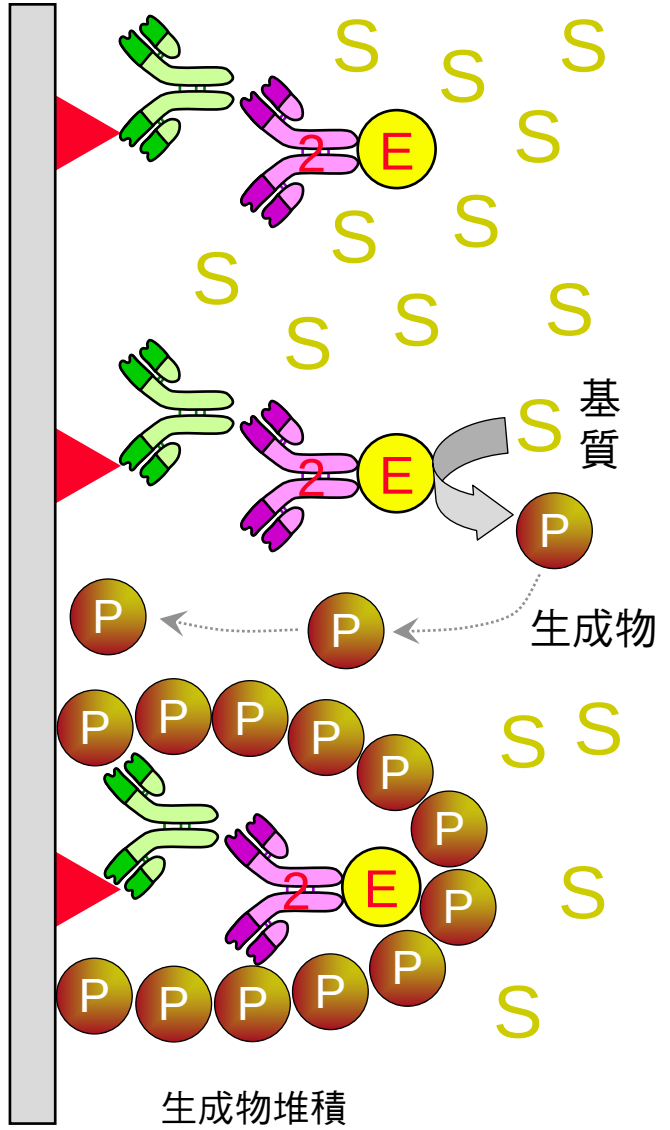
B 免疫染色流程及結果



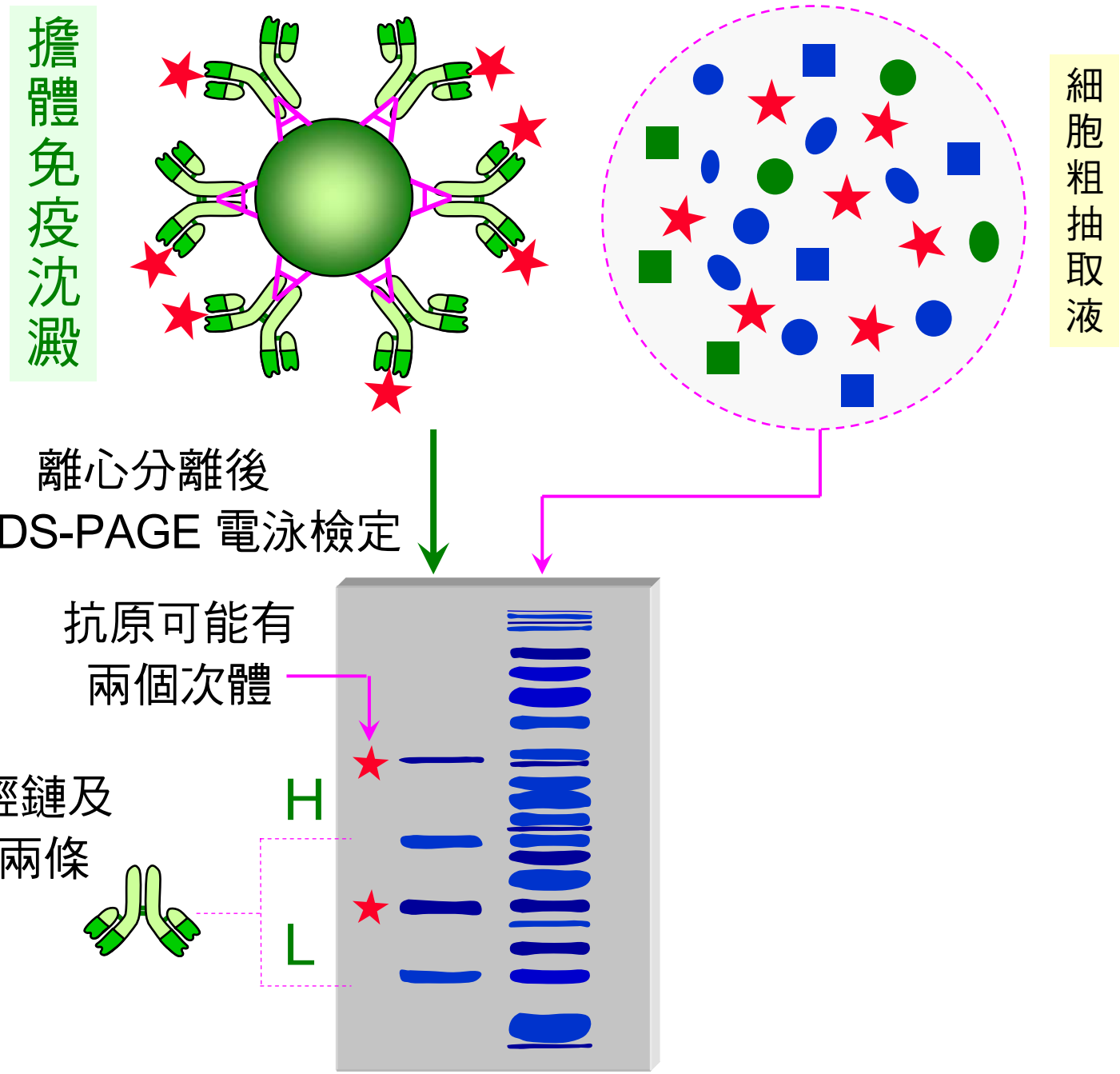
免疫轉印的種類與呈色機制：



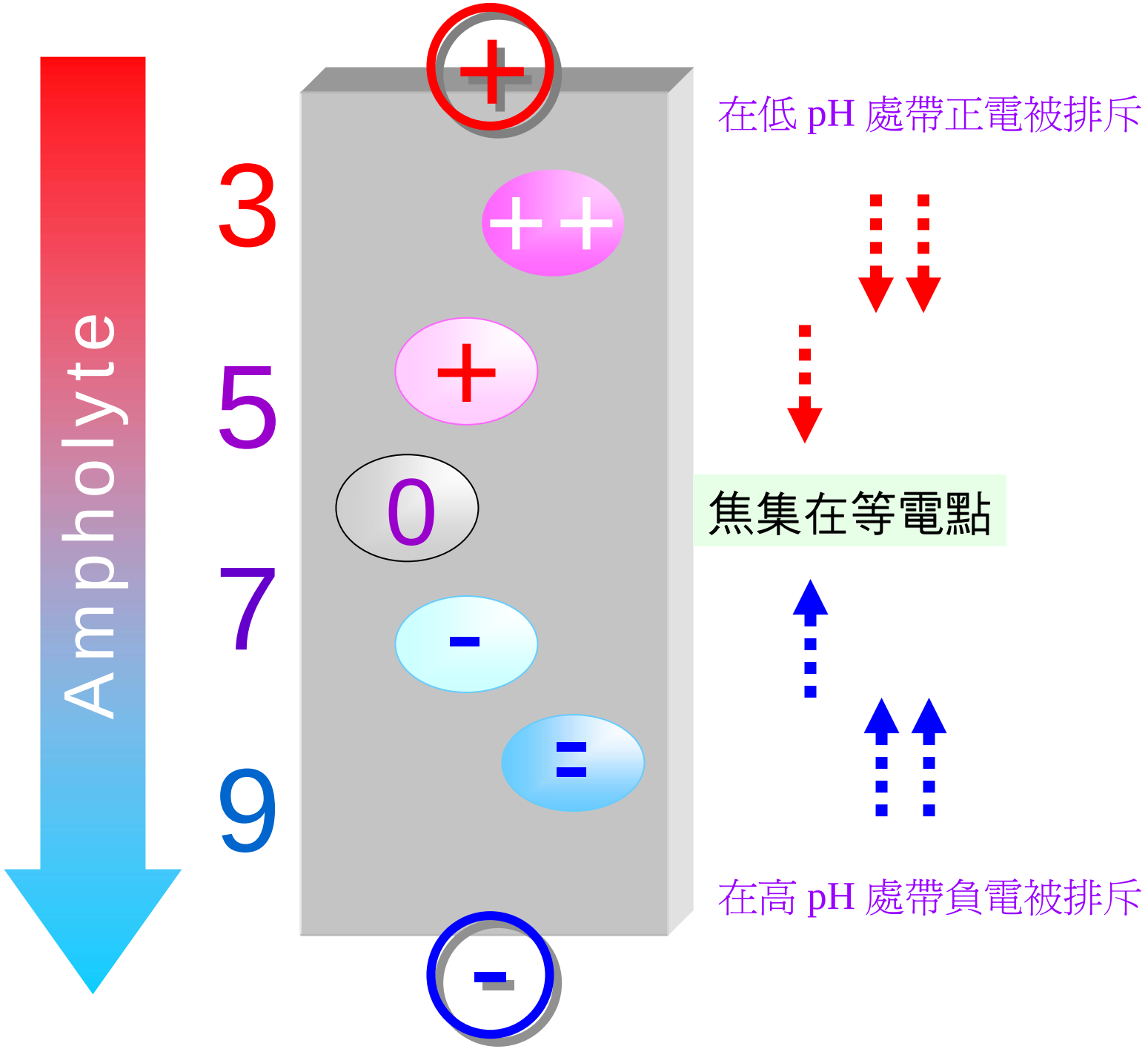




擔體免疫沈澱的原理及應用：

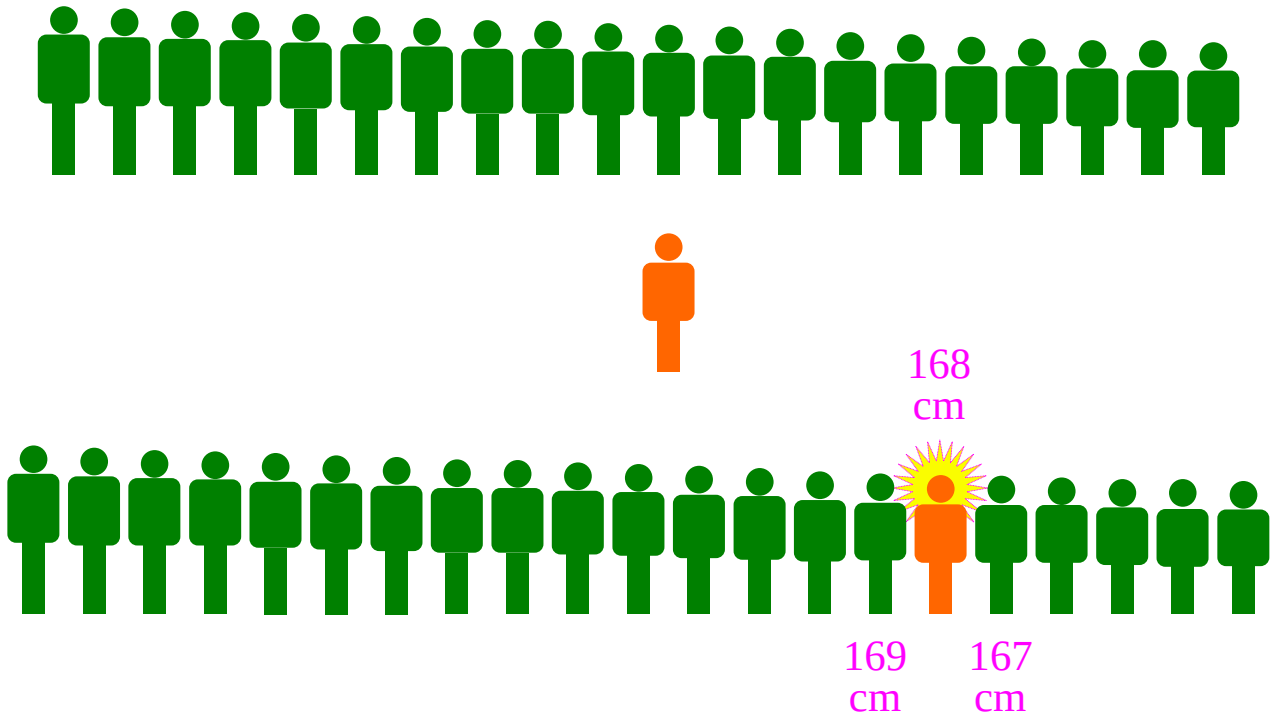


等電焦集法的焦集方式：



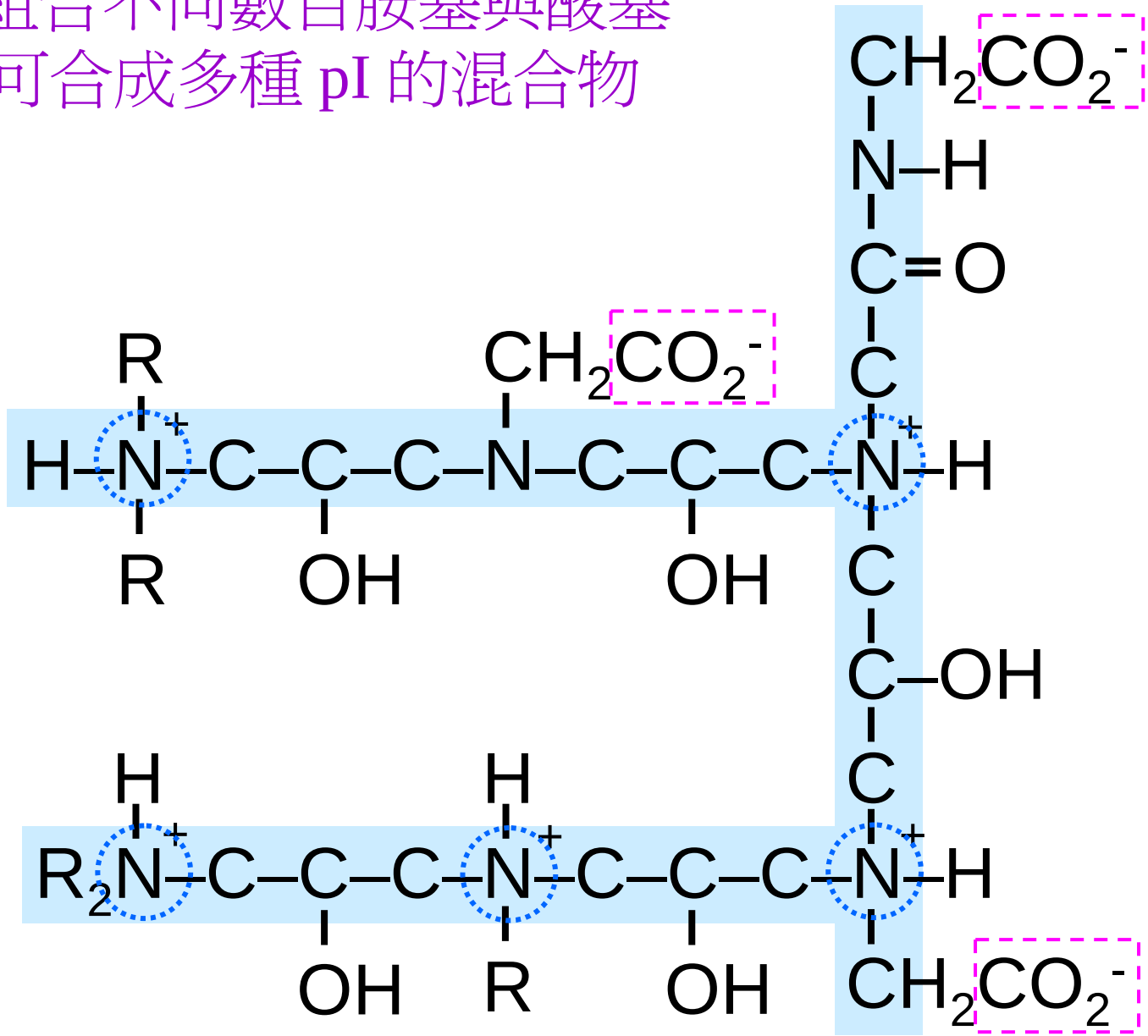
3.3.2 等電焦集法原理：

樣本分子 在一已知 pH 梯度 中焦集

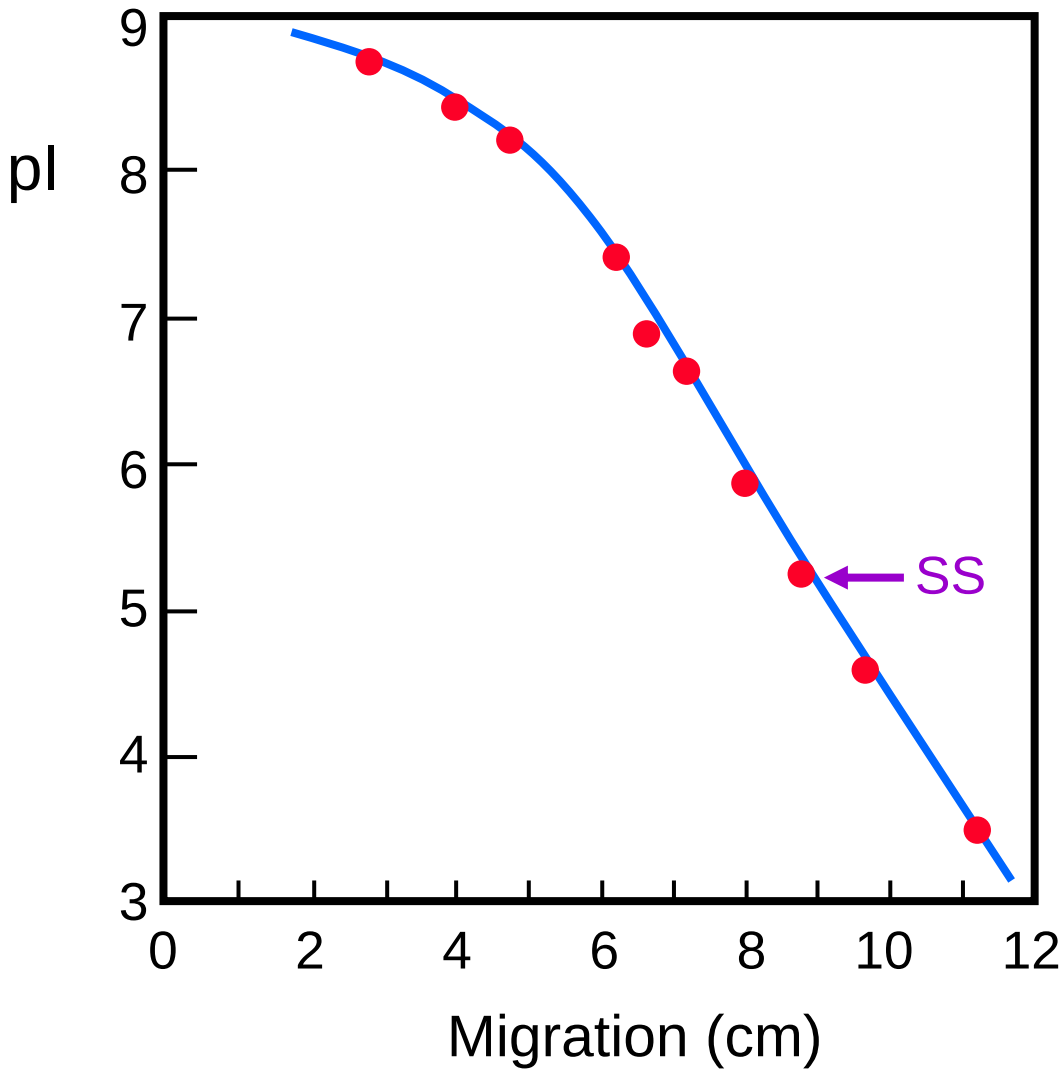


等電焦集的雙性離子構造：

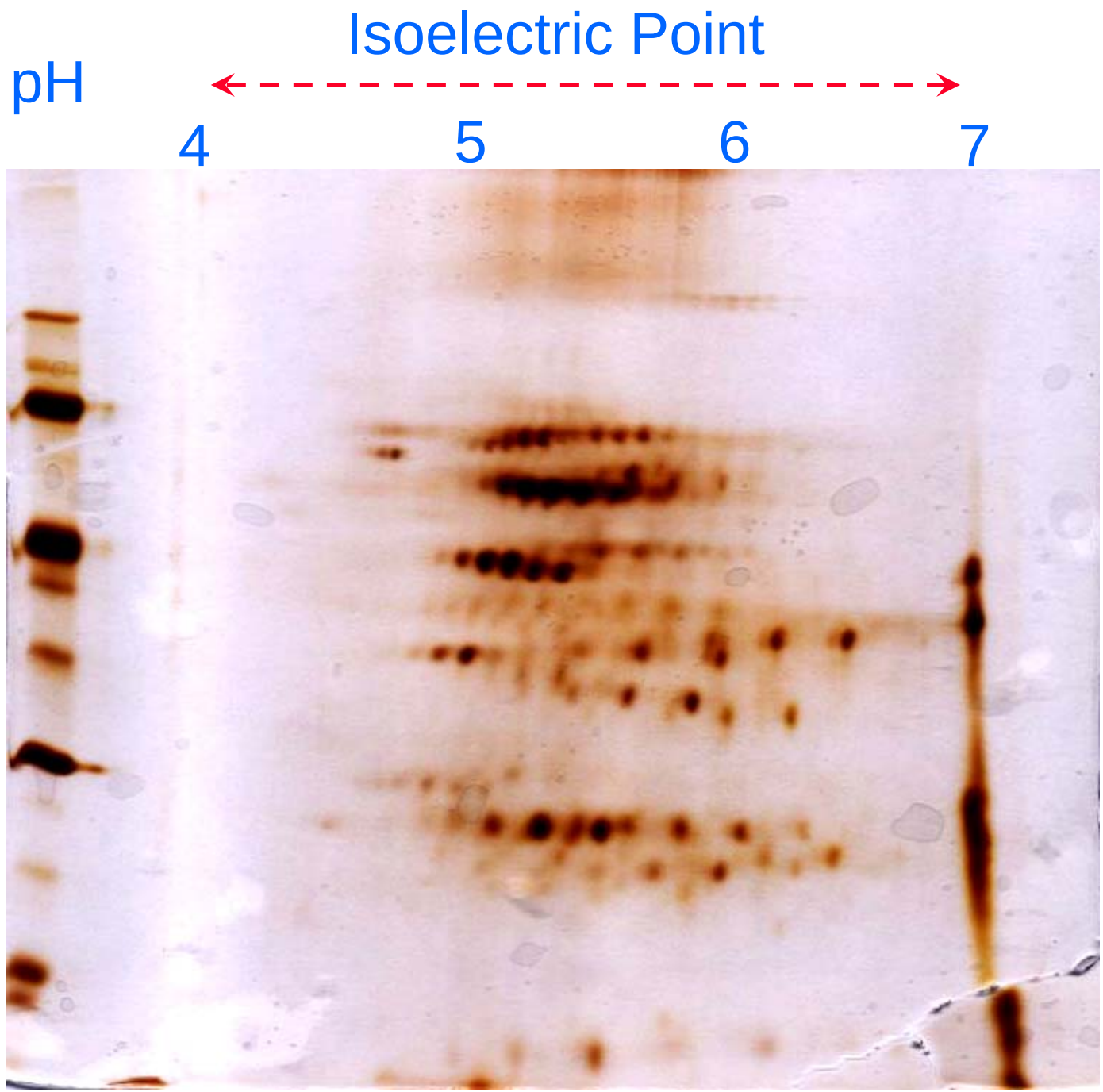
- 組合不同數目胺基與酸基可合成多種 pI 的混合物



等電點標準校正線： IEF

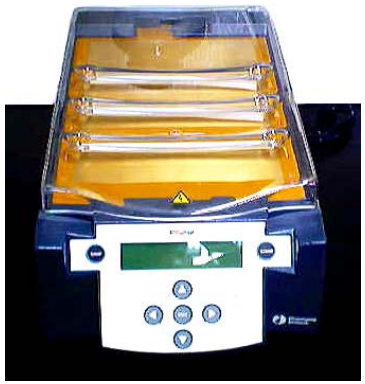


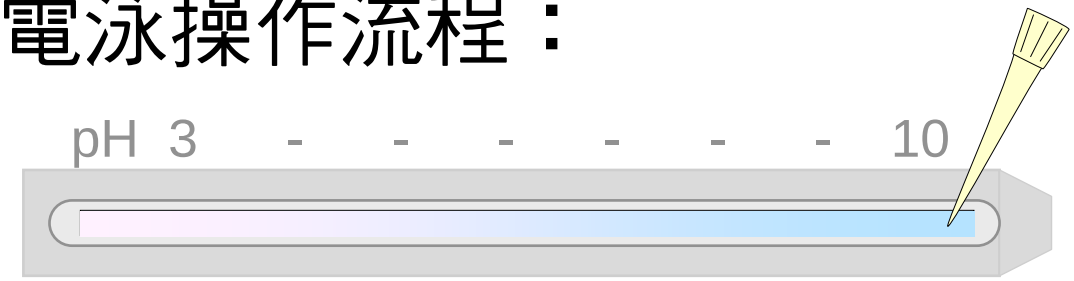
二次元電泳：

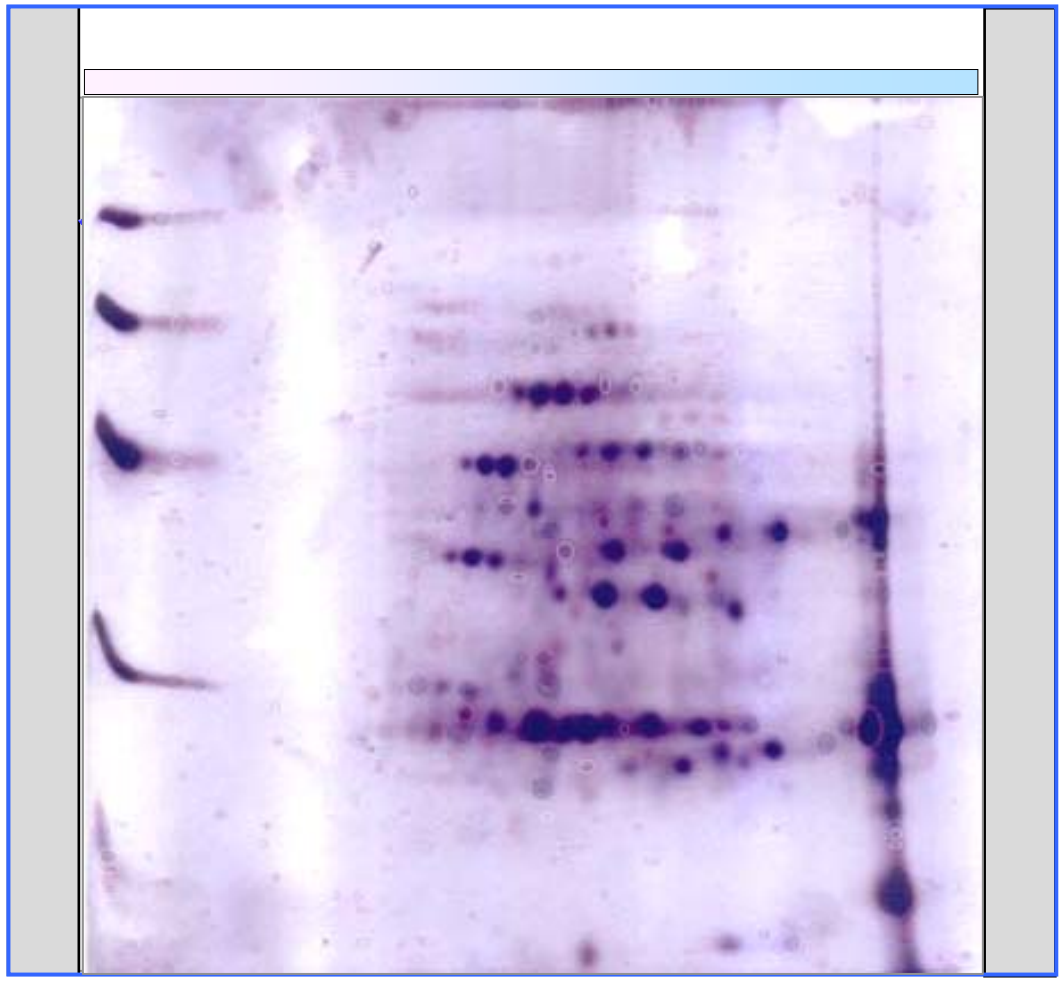


二次元電泳操作流程：

(1) IEF
等電焦集電泳





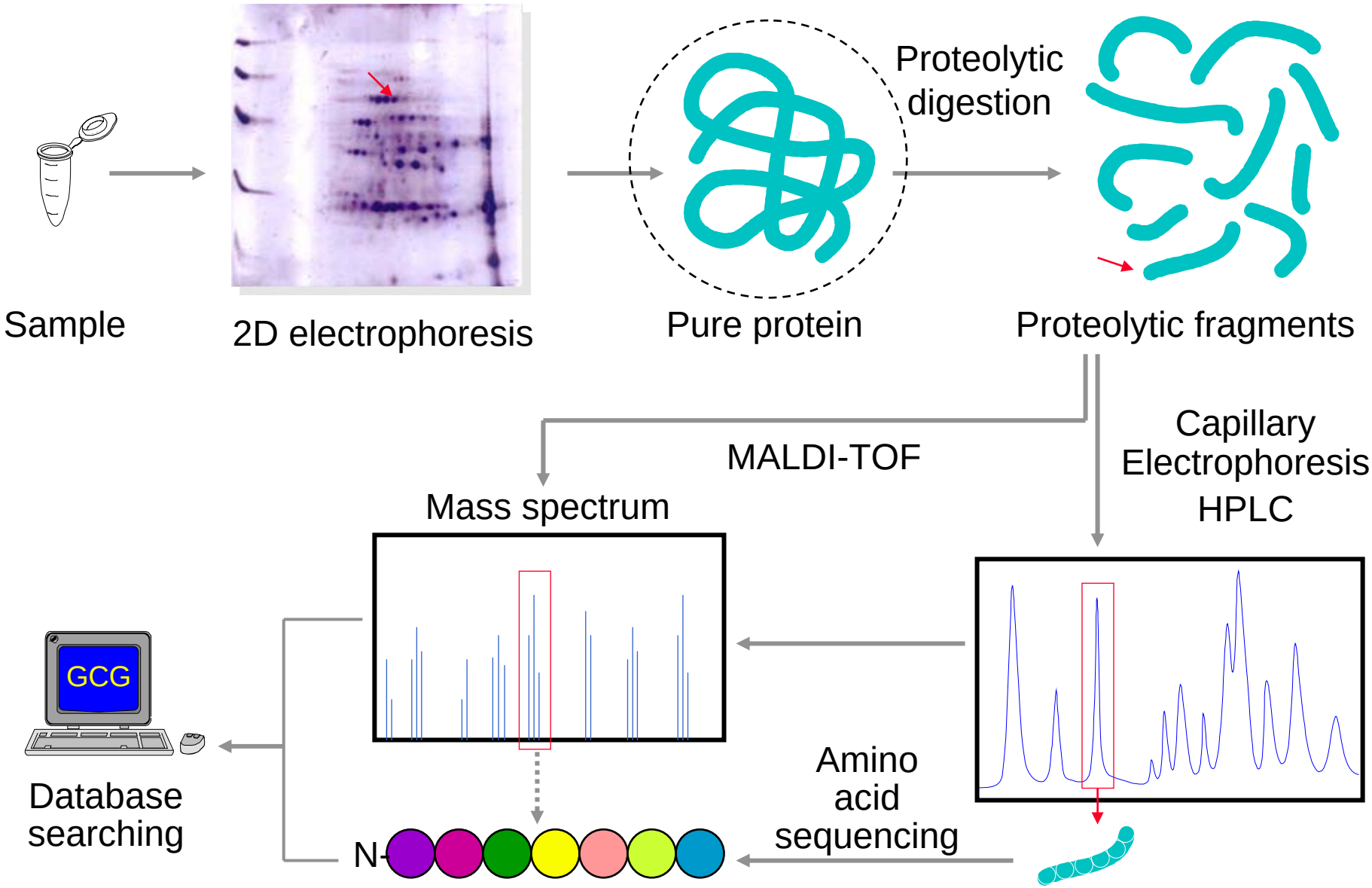


(2)
SDS-PAGE
分離膠體

(3)
染色脫色

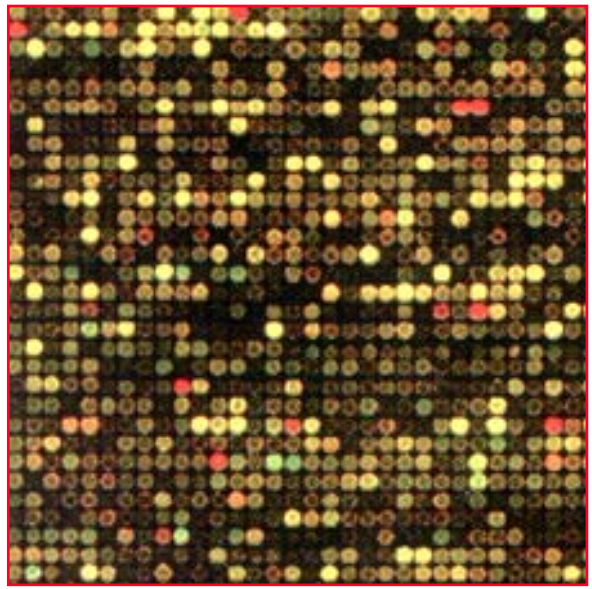
Juang RH (2004) Proteomics

蛋白質體可綜觀蛋白質的消長與身分：



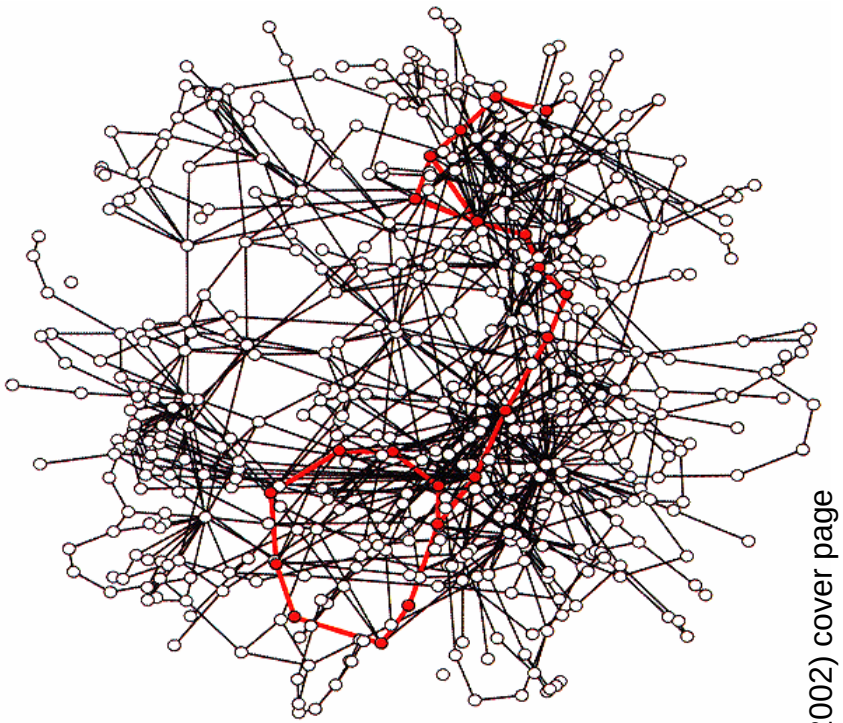
現代蛋白質科技的特點：

- 高產能 High-through put
- 快速 High-speed
- 微量 Micro-scaled



The Chemical Nature of Enzyme Catalysis

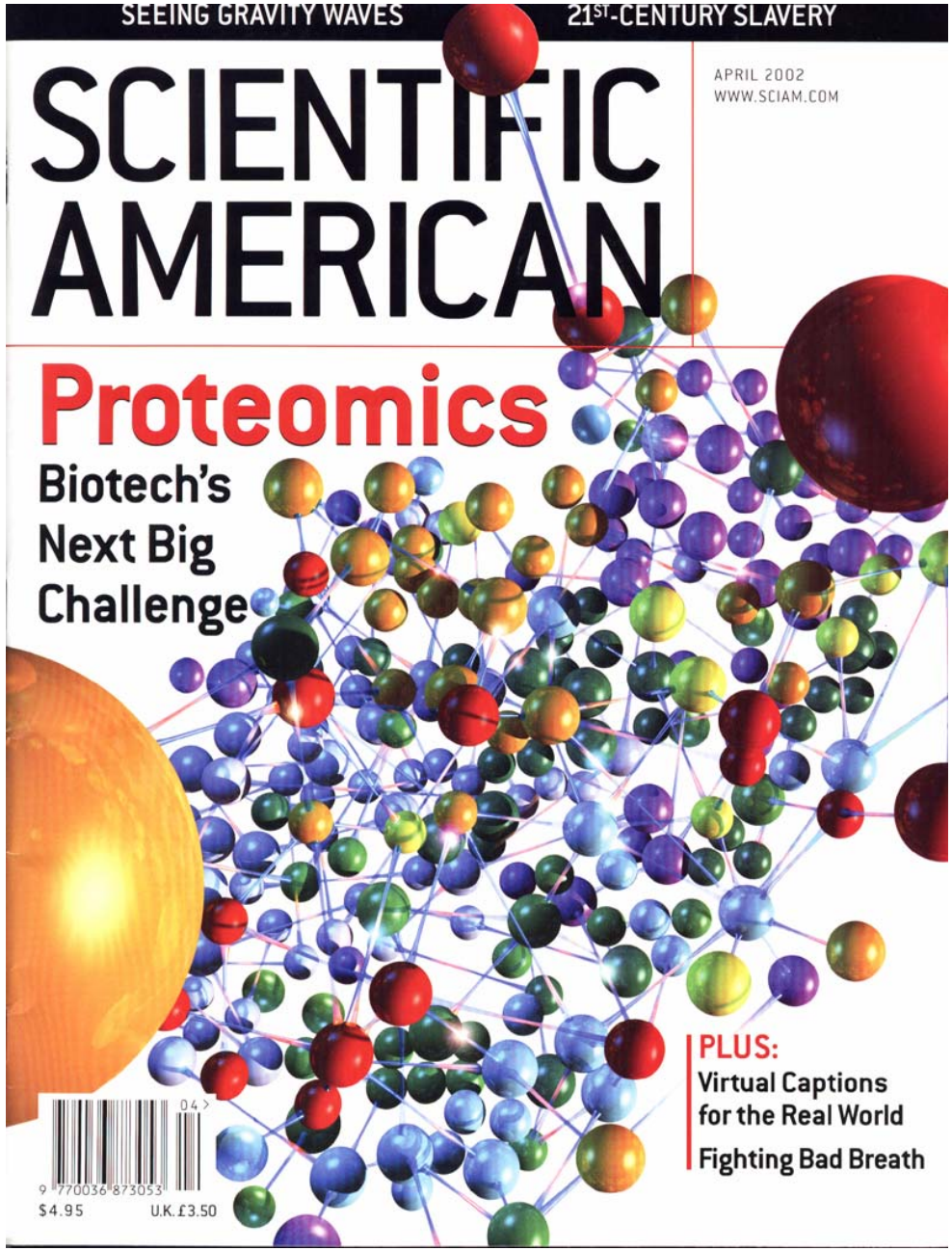
代謝路徑立體圖



Alberts et al (2002) Molecular Biology of the Cell (4e) p.107

Systems Biology

整體性的生物學觀念與工具



Scientific American (2002) cover page