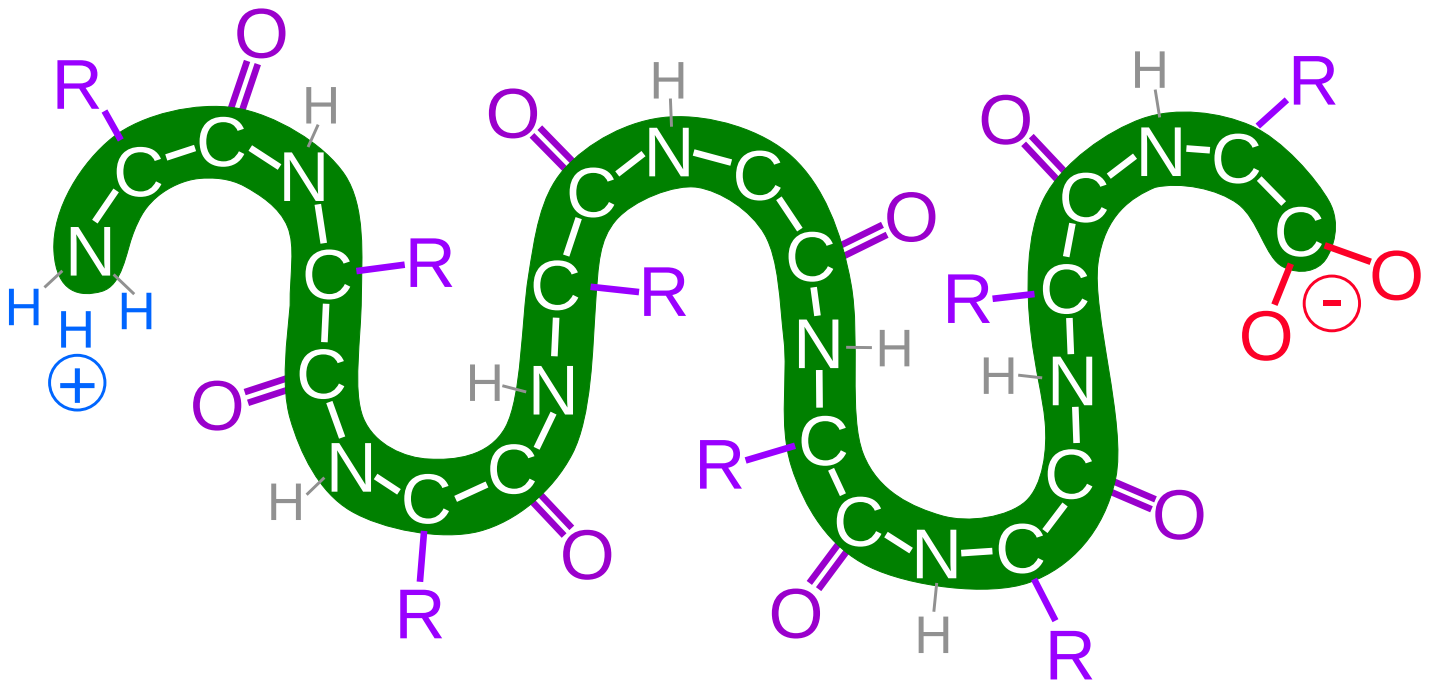
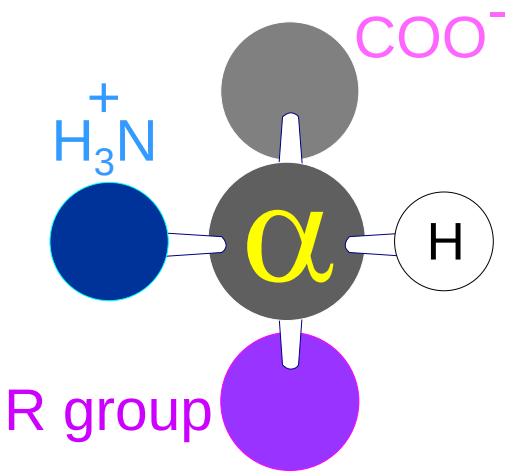
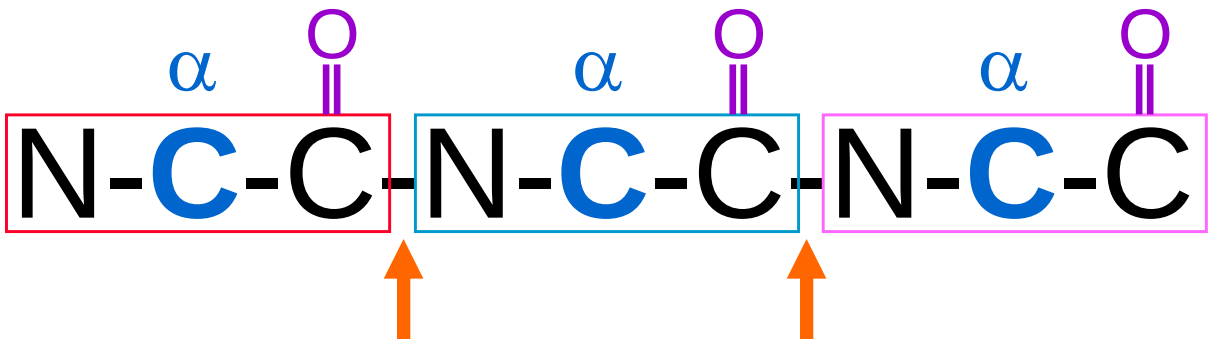
# 酵素分析方法

- 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

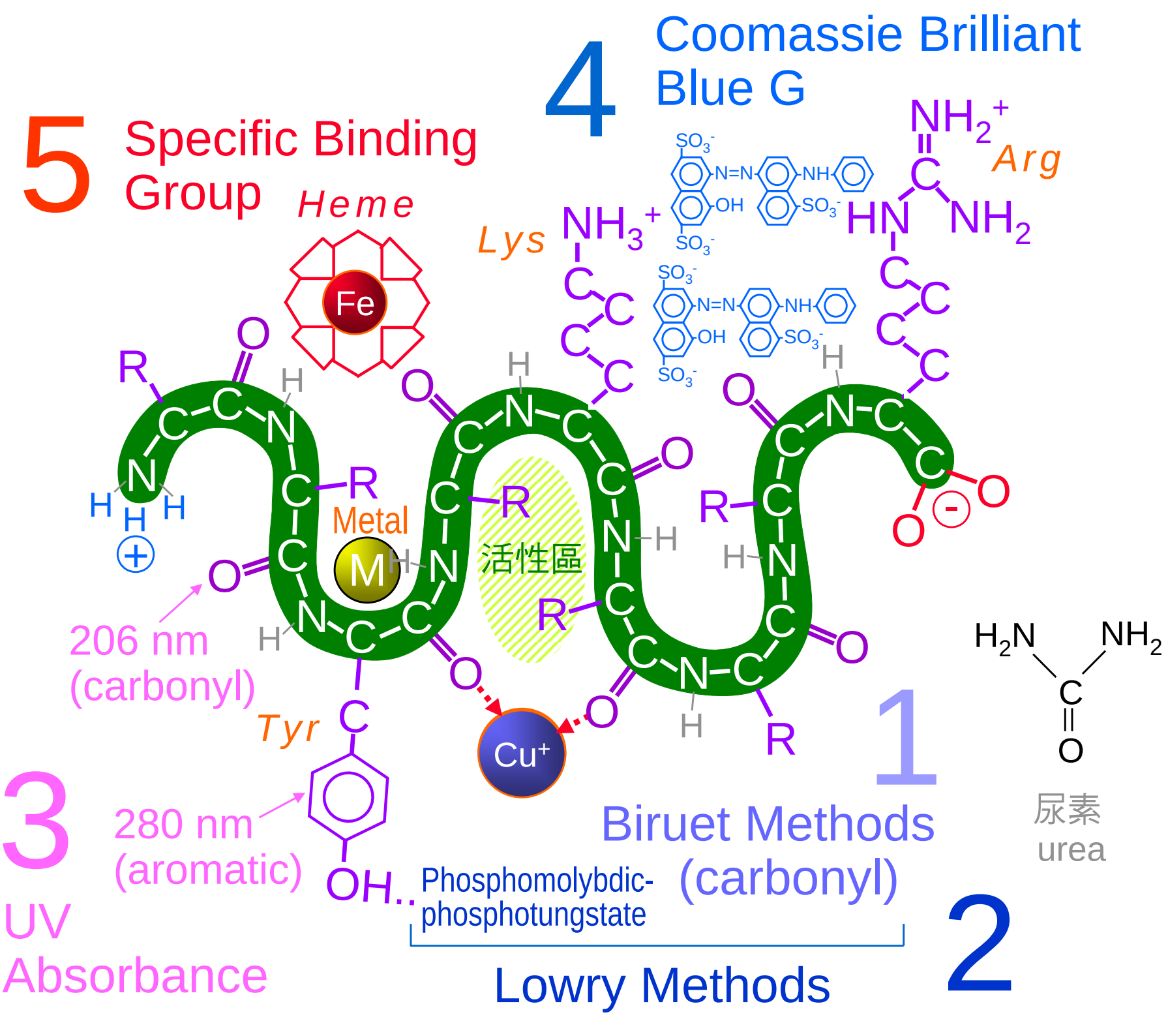
# 蛋白質構造的骨架





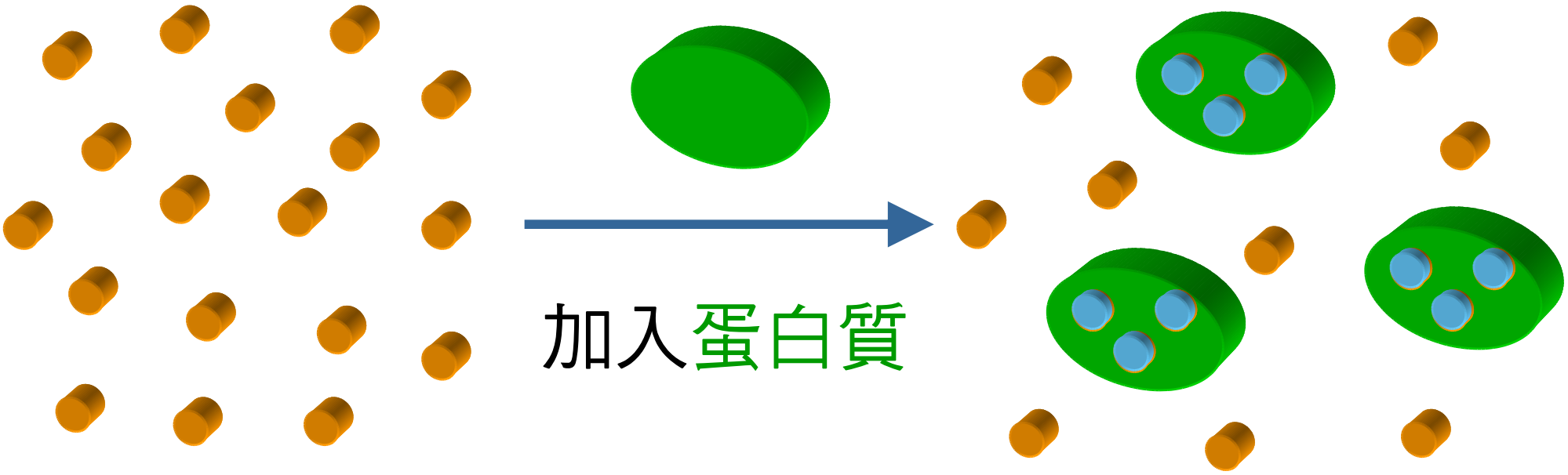
# 各種蛋白質定量法原理


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

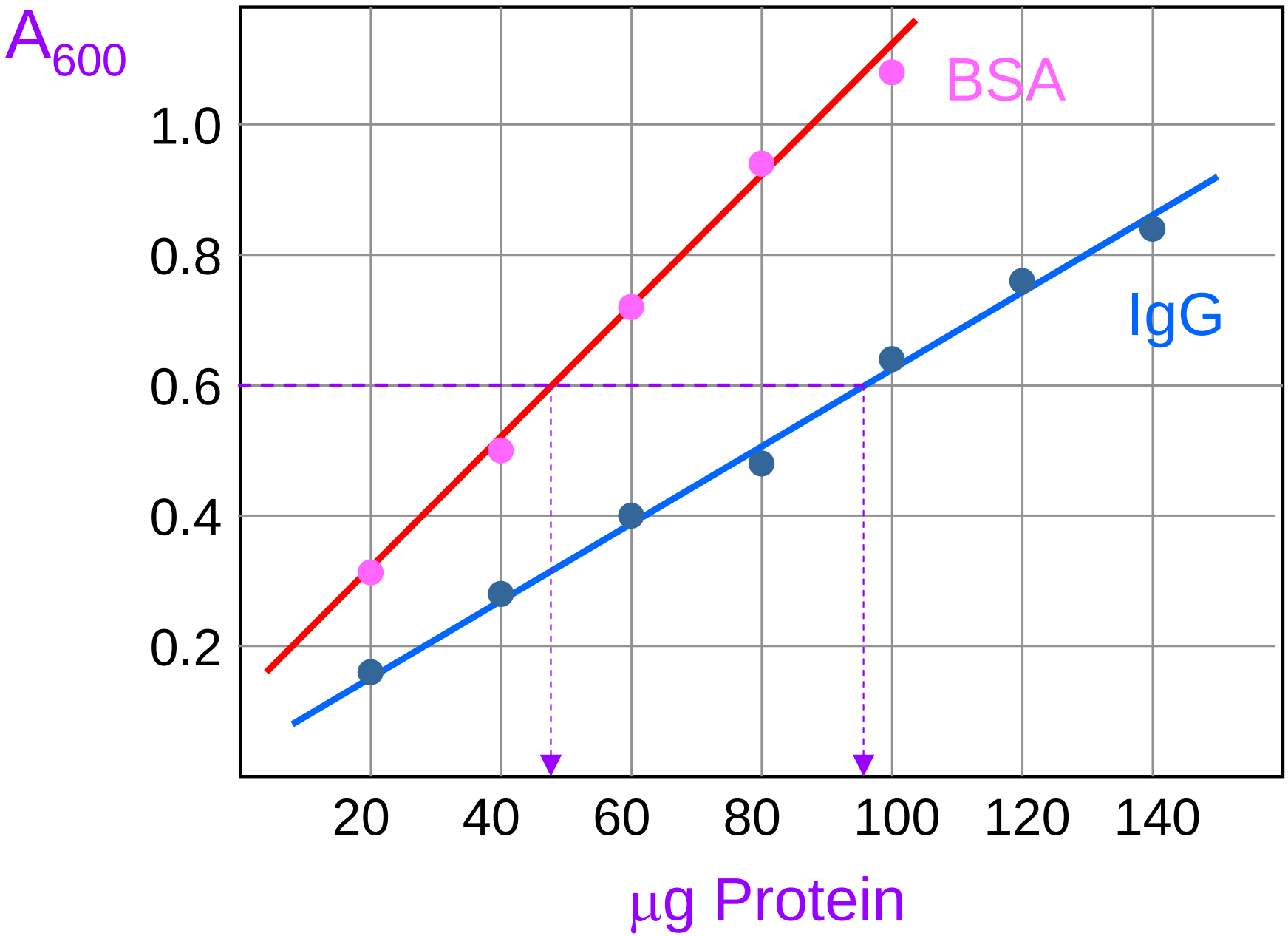
470 nm　　CBG 是一種指示劑　　595 nm




酸性環境下呈茶色

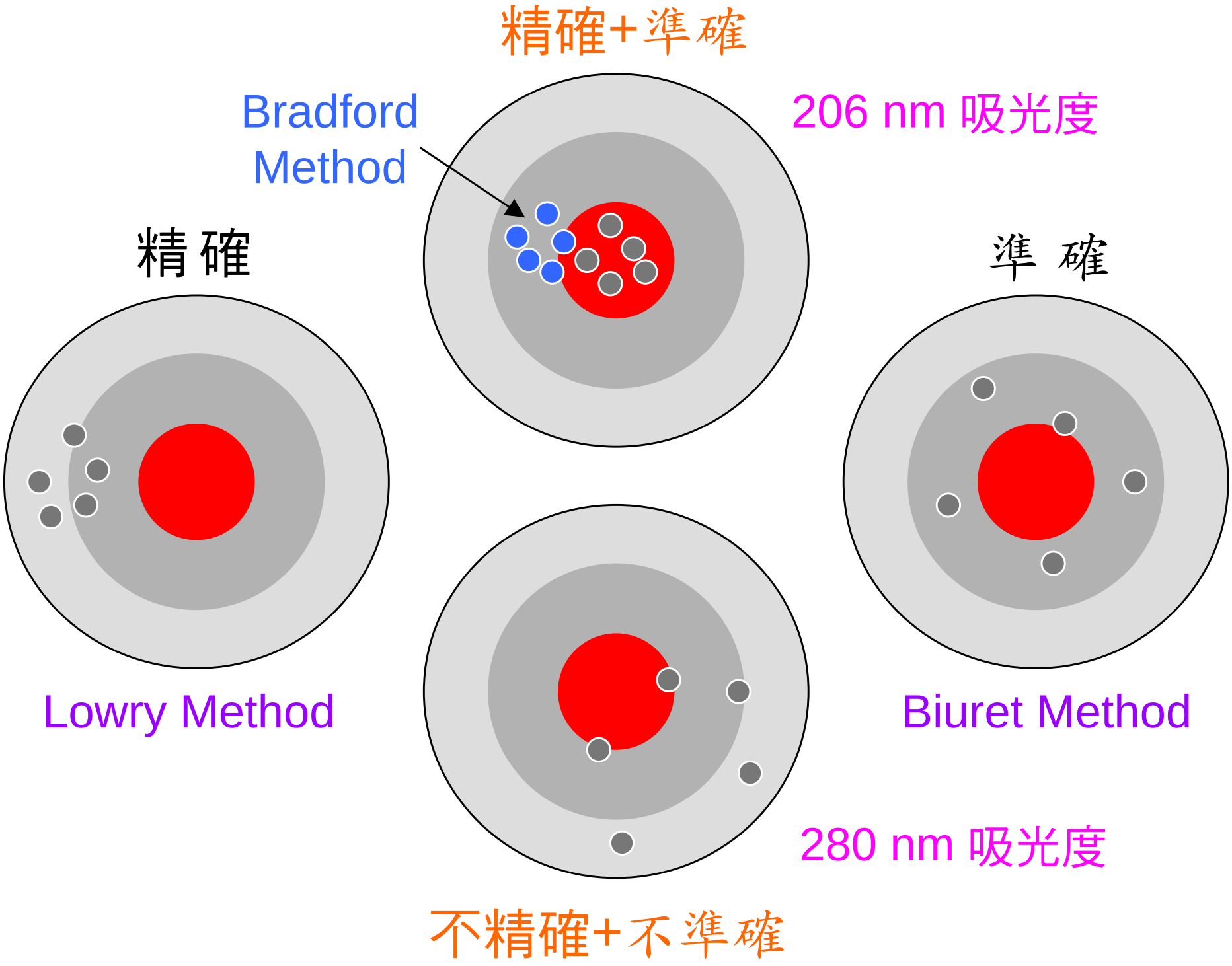
與蛋白質結合變藍色

# 不同蛋白質的定量差異

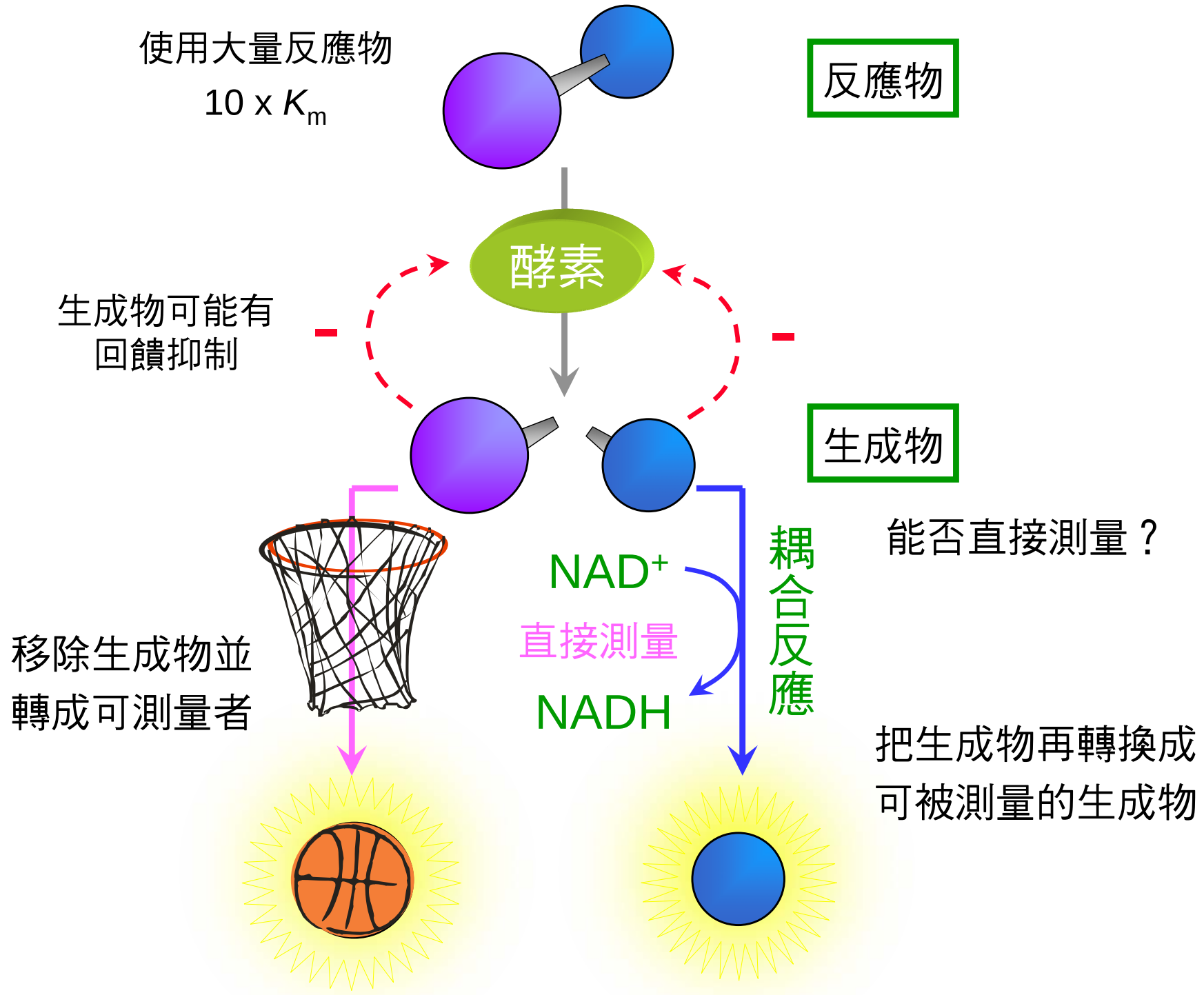


# 各種蛋白質定量法的比較

| 定量方法 | 靈敏度 | 準確度 | 其它說明 |
|---|---|---|---|
| Biuret | 0.05 - 5 mg | 高 | 呈色快速 有腐蝕性<br>銨離子干擾大 |
| Lowry | 0.05 - 0.5 mg | 中 | 呈色較慢<br>多種離子有干擾 |
| Absorbance<br>280 nm | 0.05 - 2 mg | 低 | 樣本可回收<br>其它物質干擾大 |
| Absorbance<br>205 nm | 0.01 - 0.05 mg | 高 | 樣本可回收<br>氧分子干擾極大 |
| Bradford<br>Dye-binding | 0.01 - 0.05 mg | 中 高 | 呈色快 雜質干擾多<br>顏色不易洗除 |


精確+準確
Bradford
Method
206 nm 吸光度
精 確
準 確
Lowry Method
Biuret Method
280 nm 吸光度
不精確+不準確

# 酵素反應及偵測方法

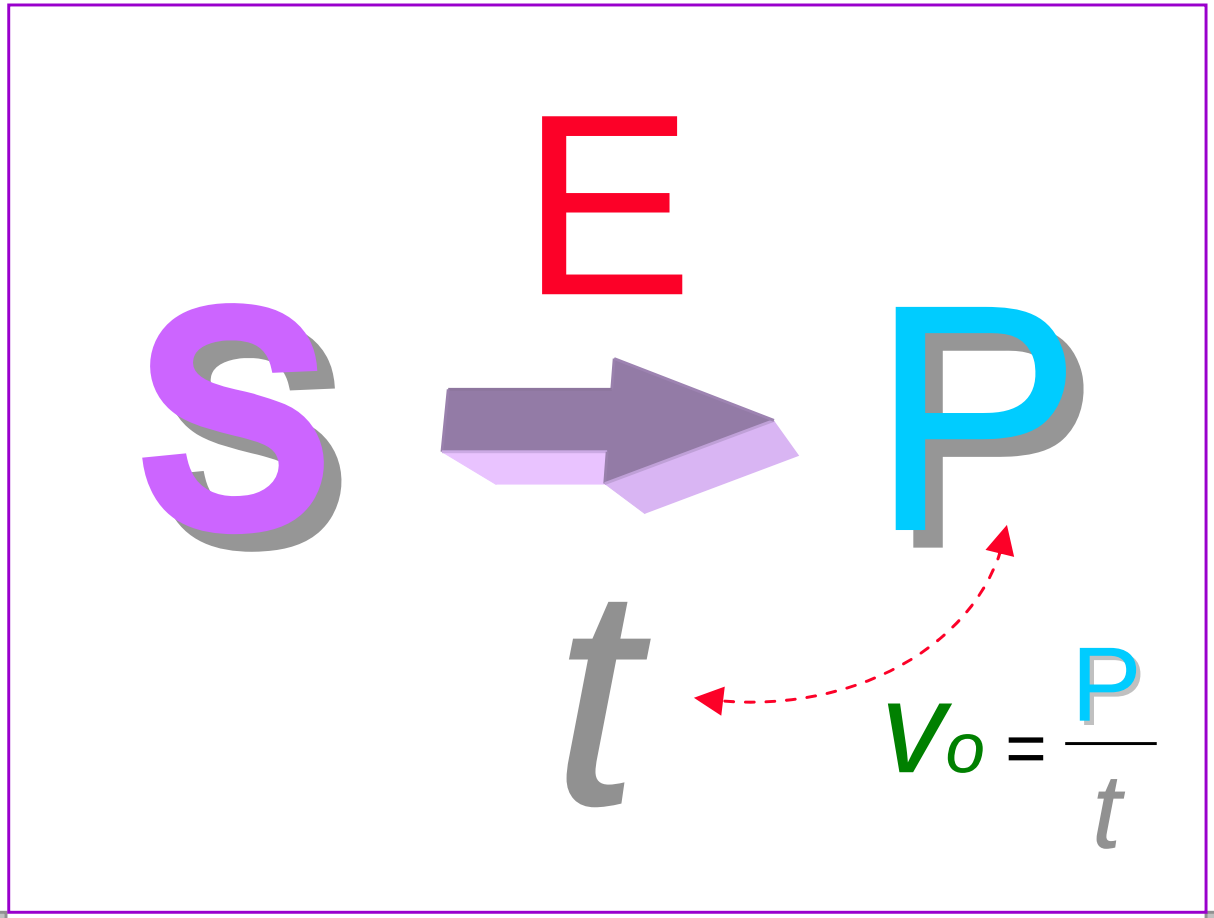

使用大量反應物
10 x $K_m$
反應物
酵素
生成物可能有
回饋抑制
生成物
能否直接測量？
NAD+
直接測量
NADH
耦合反應
移除生成物並
轉成可測量者
把生成物再轉換成
可被測量的生成物

# 酵素活性的測定

酵素量要適中

基質
要過量

10 x Km



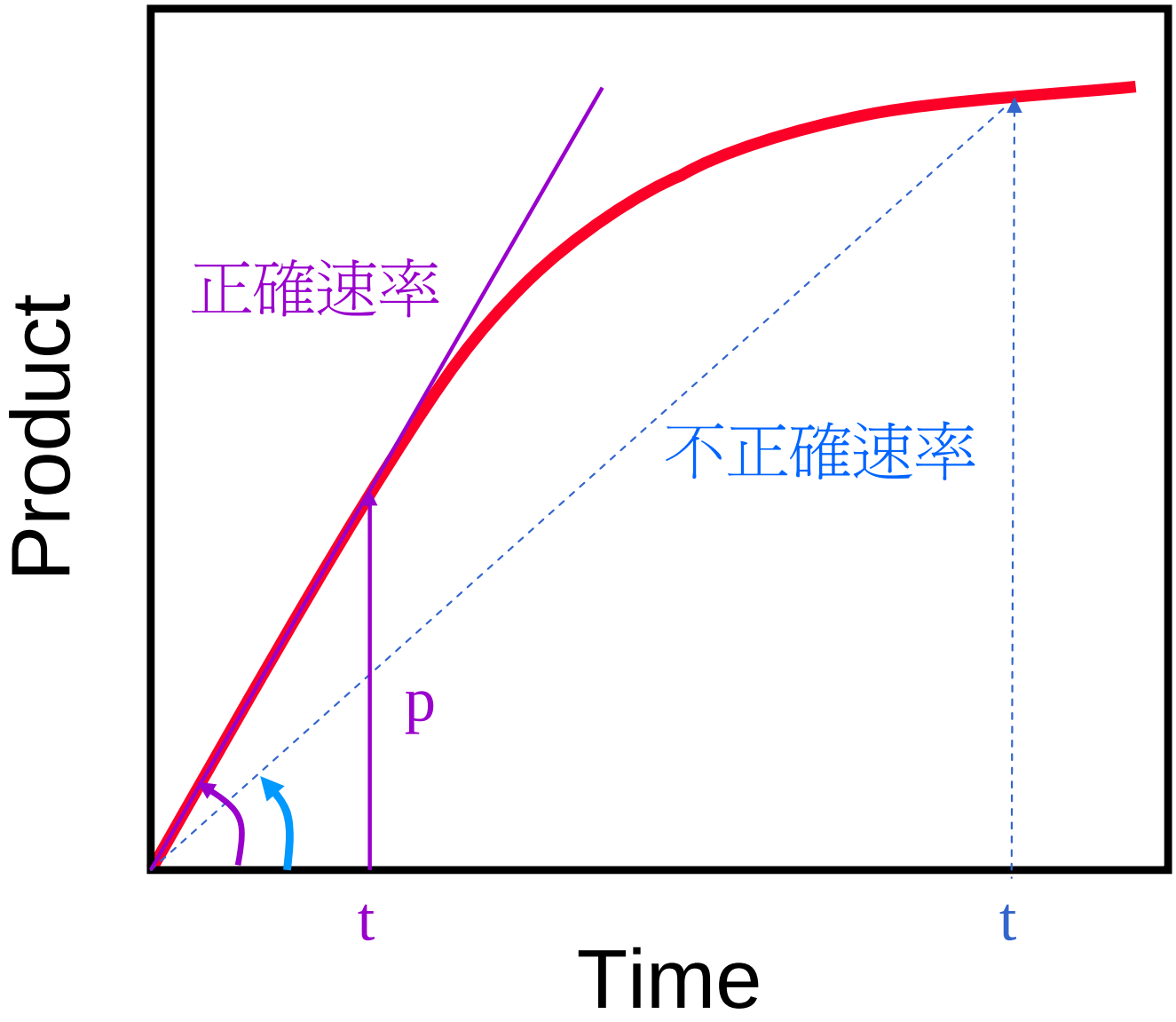

生成物
要能測得

酸鹼度

反應時間恰當

溫 度

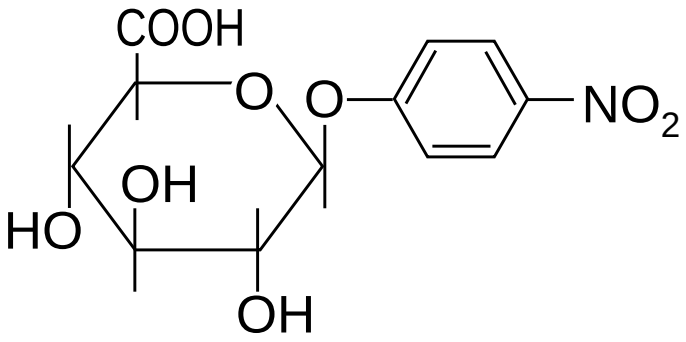
# 測定時間內反應速率需成線性



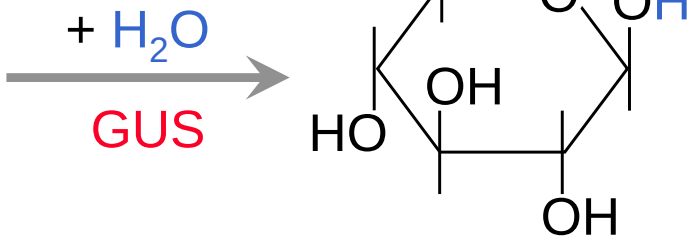

$$v = \frac{p}{t}$$

# GUS 酵素活性的測定

反應物



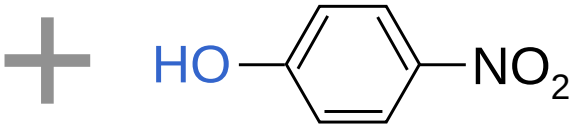

*p*-Nitrophenyl β-D-glucuronide
(*p*NPG)




生成物



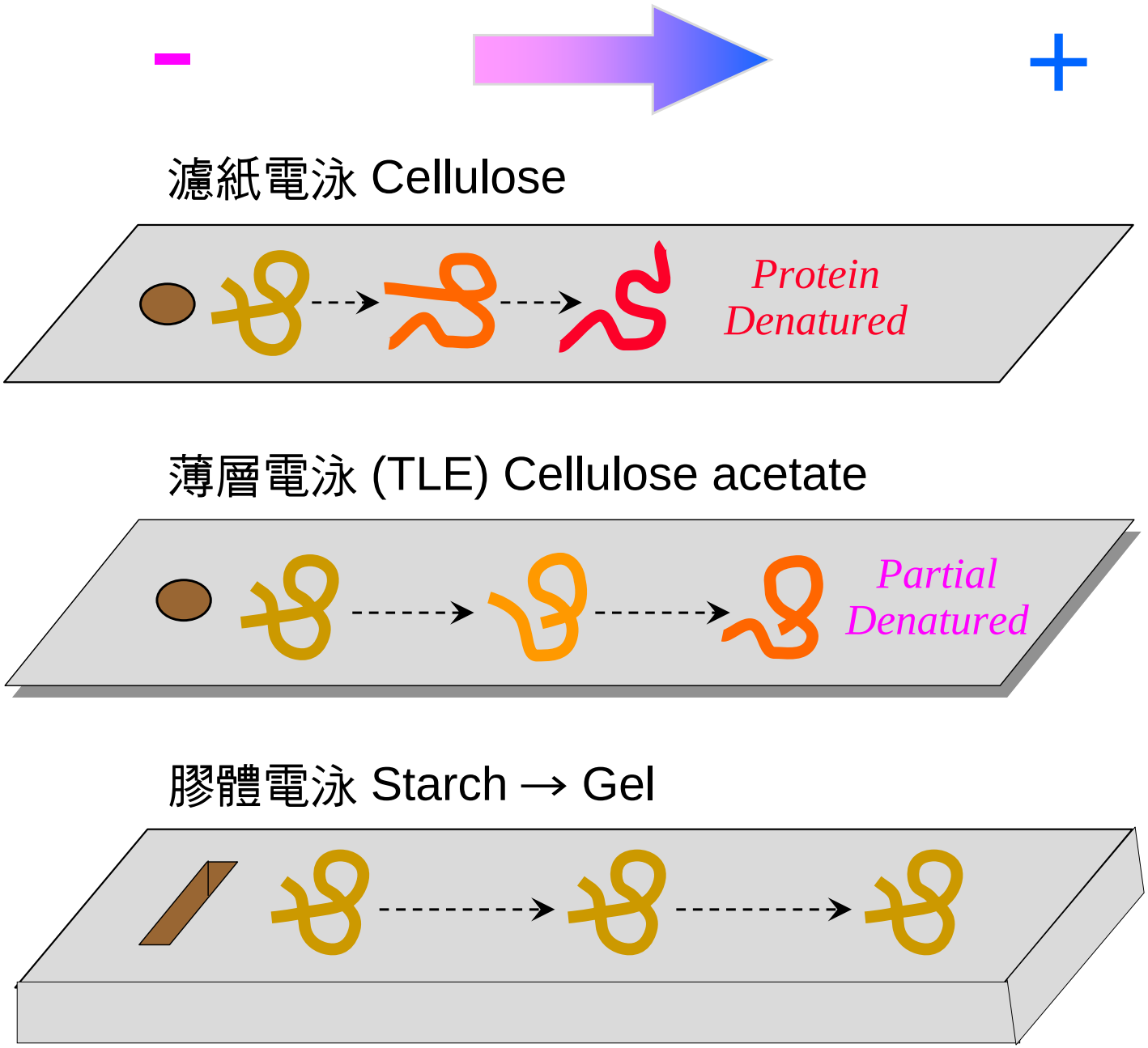

β-D-Glucuronic acid




*p*-Nitrophenol
(yellow)

415 nm

# 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

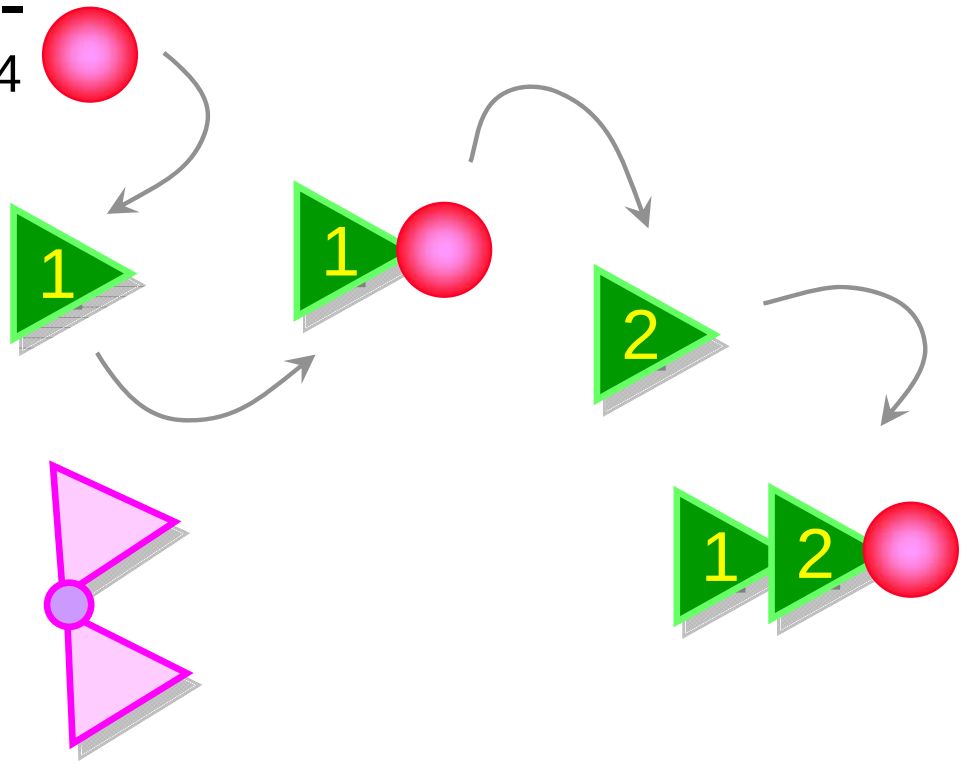
Bis(acrylamide) (bridge)

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)

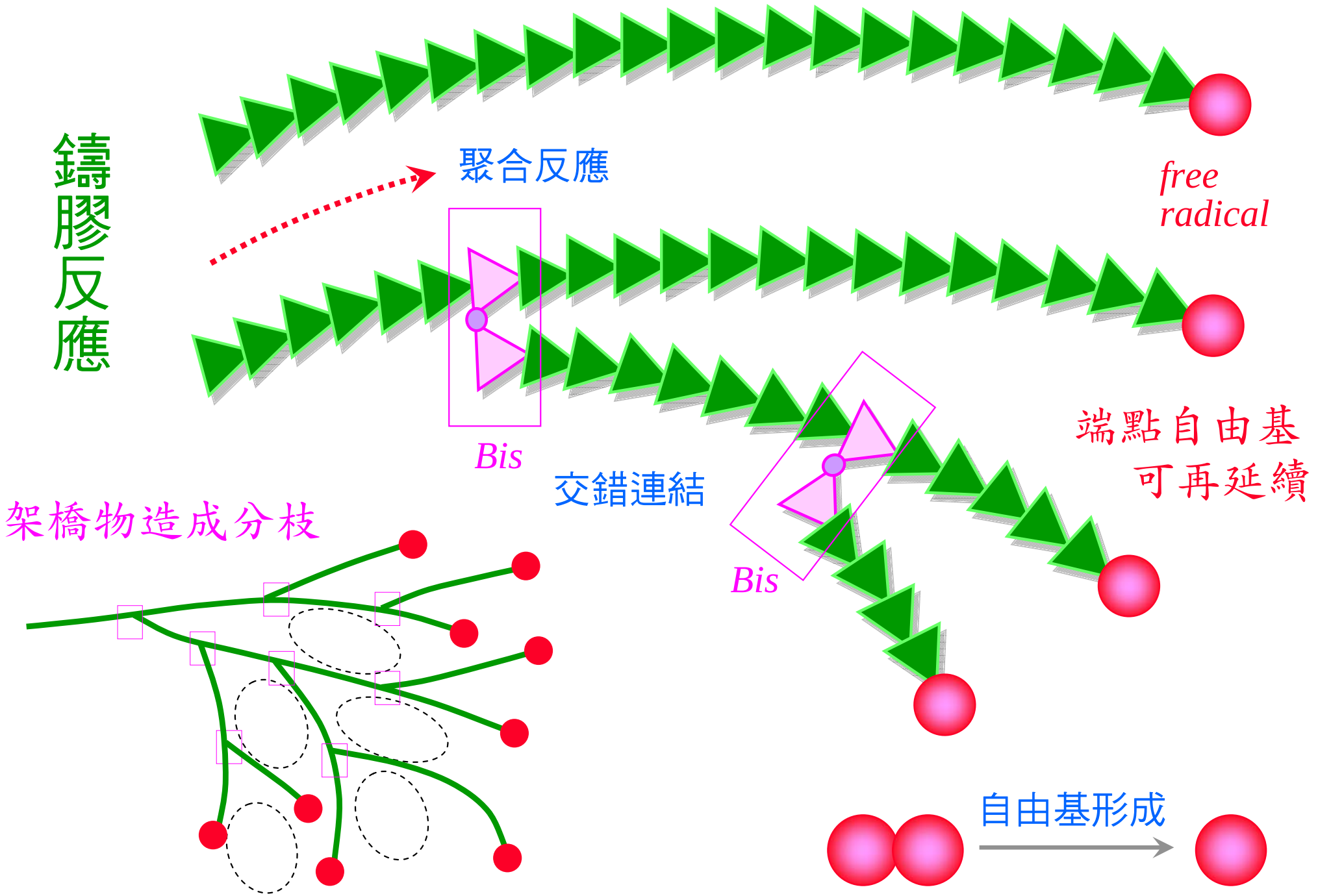



$CH_2=CH\text{-}CO\text{-}NH_2$

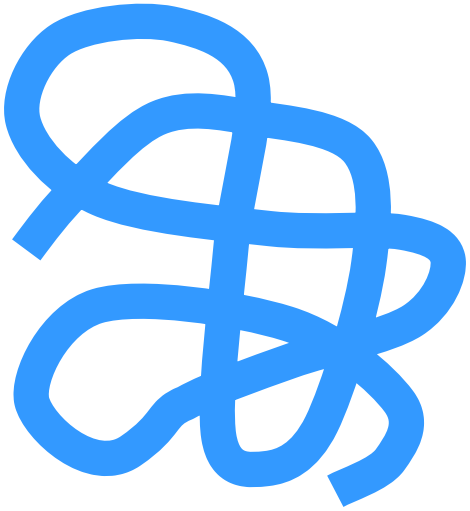
Acrylamide 有毒性！

澱粉加高熱會誘生？

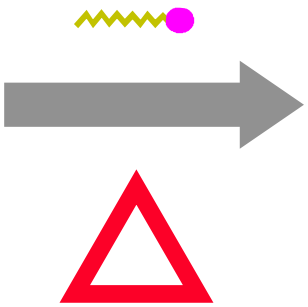
# 單體聚合反應


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

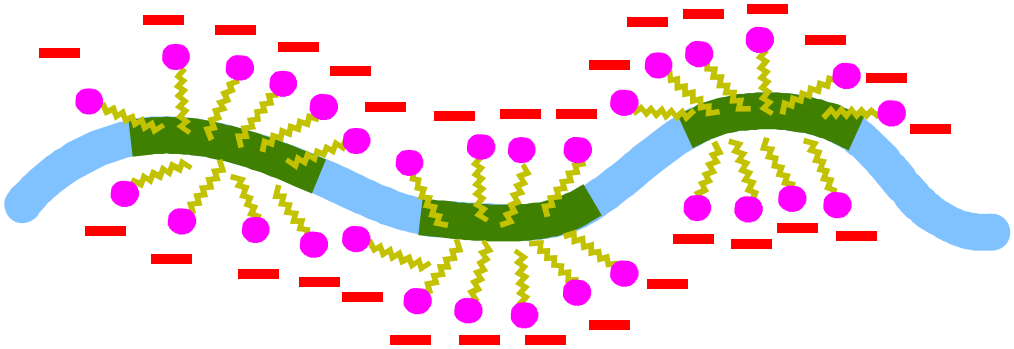
# SDS 在蛋白質表面 均勻敷上 一層負電



SDS



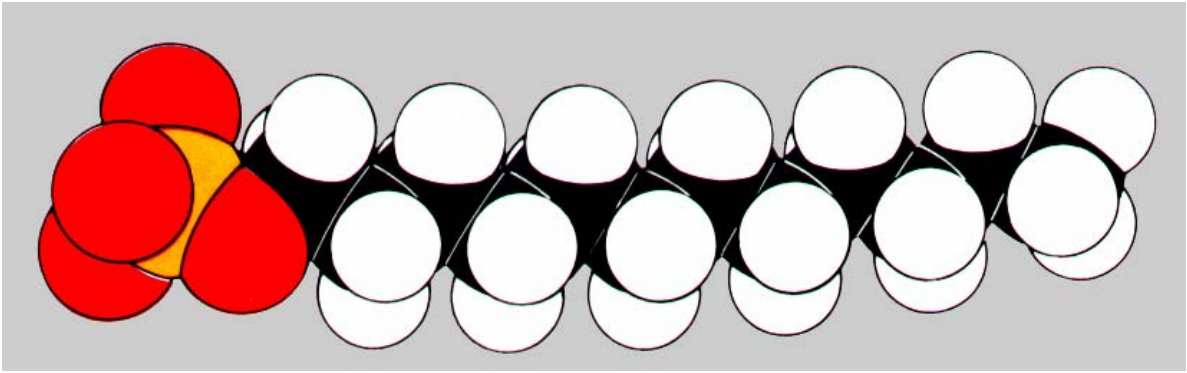
boiling



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



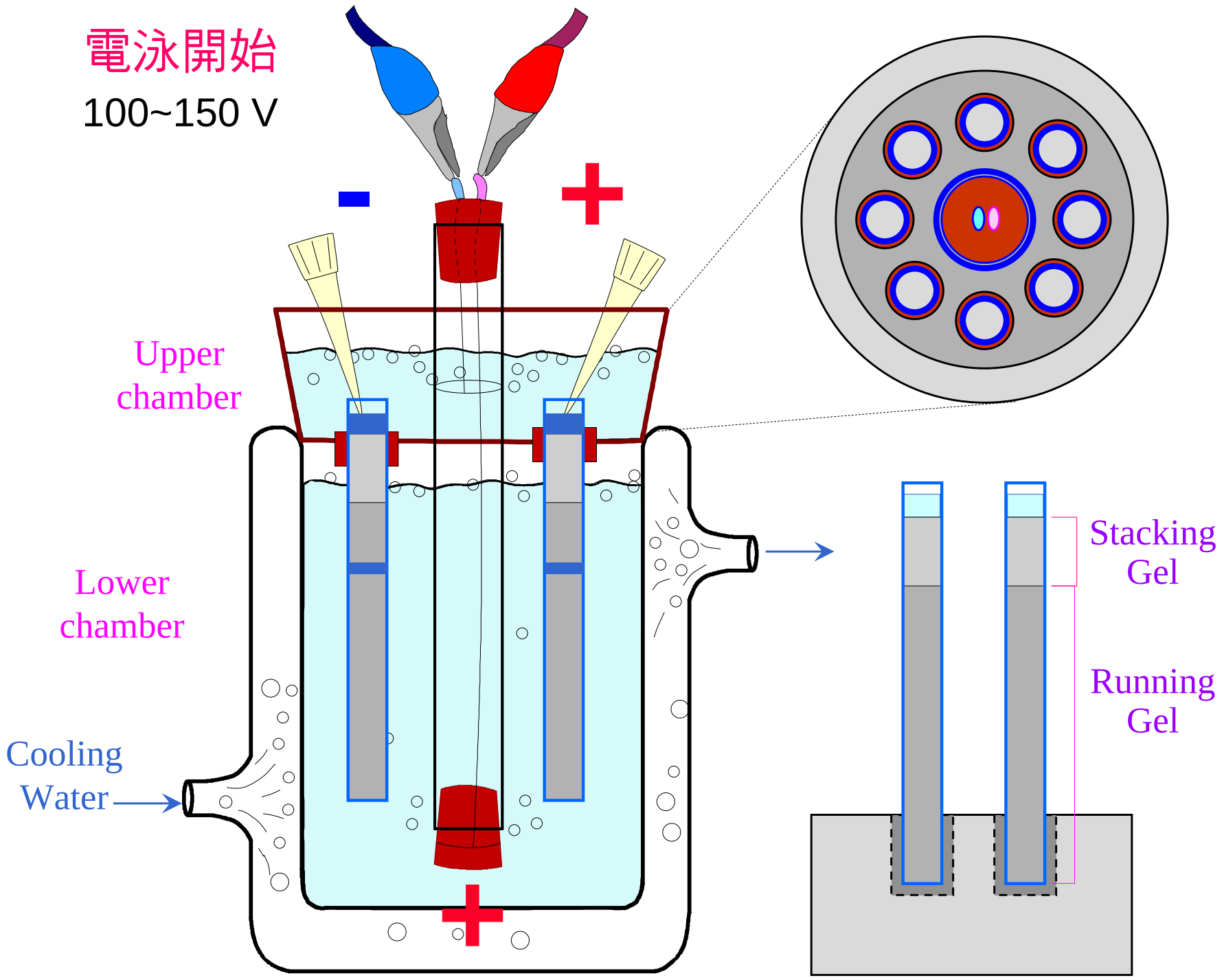
非極性尾部

# 電泳膠體系統的組成

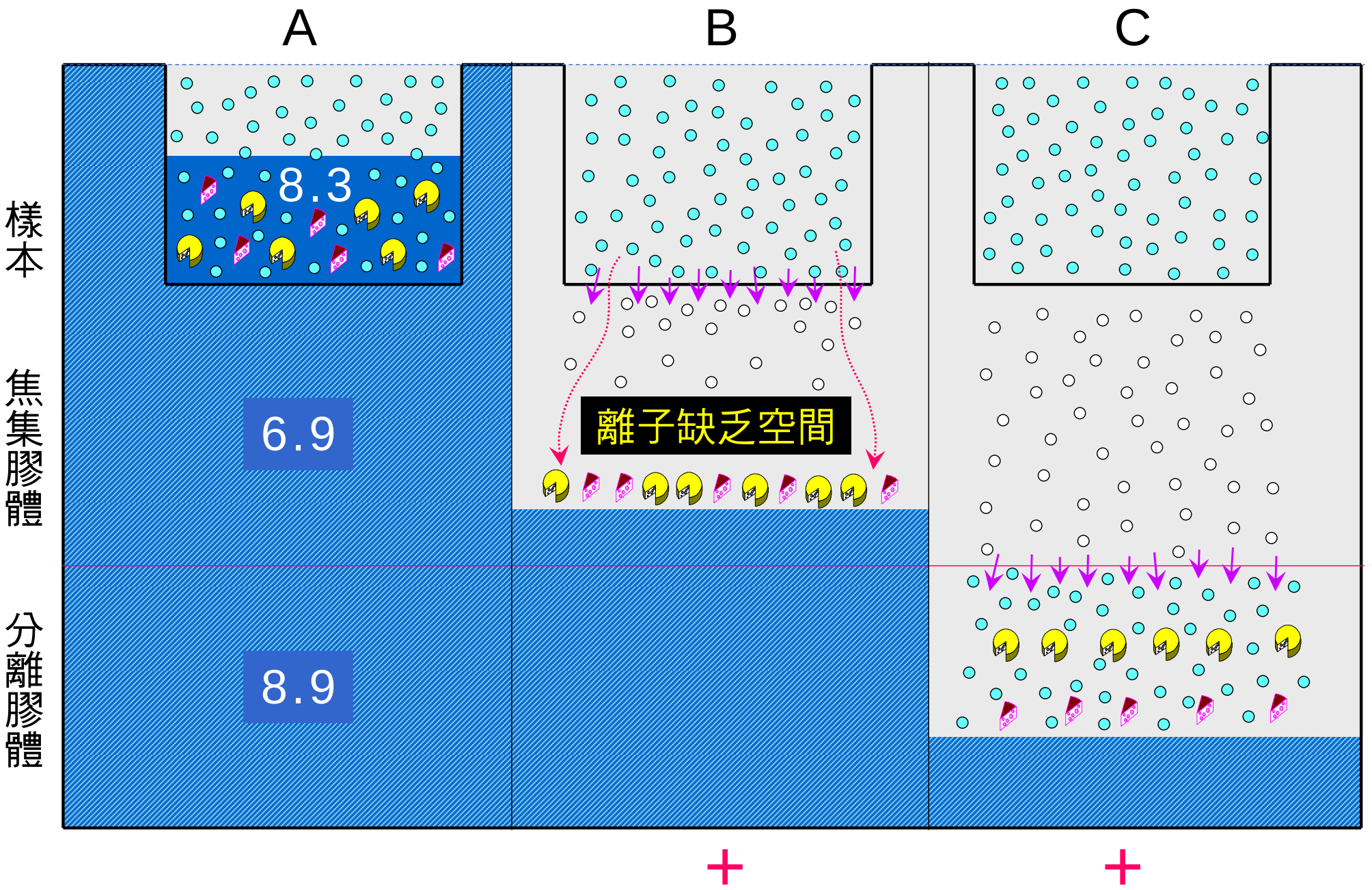
| 電泳系統 | | | 緩衝液 | pH | 膠體濃度 |
|---|---|---|---|---|---|
| 1 | 上層 (負極) 緩衝液 | | Tris-glycine | 8.3 | - |
| 2 | 樣本溶液 | | Tris-glycine | 8.3 | - |
| 3 | 膠體 | 焦集膠體 | Tris-HCl | 6.9 | 5% |
| 4 | 膠體 | 分離膠體 | Tris-HCl | 8.3 | 7.5~20% |
| 5 | 下層 (正極) 緩衝液 | | Tris-glycine | 8.3 | - |

- 膠體不連續性有焦聚樣本的作用

# 直立式電泳裝置

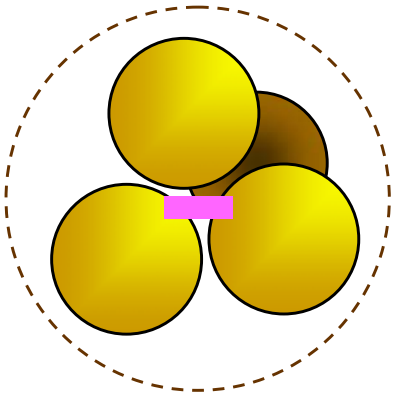

電泳開始
100~150 V
-
+
Upper chamber
Lower chamber
Cooling Water
Stacking Gel
Running Gel

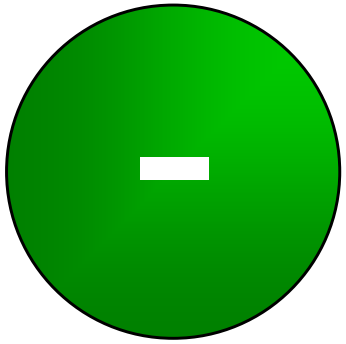
# 焦集膠體對蛋白質分子的焦集作用

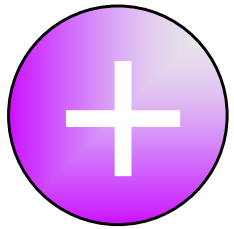


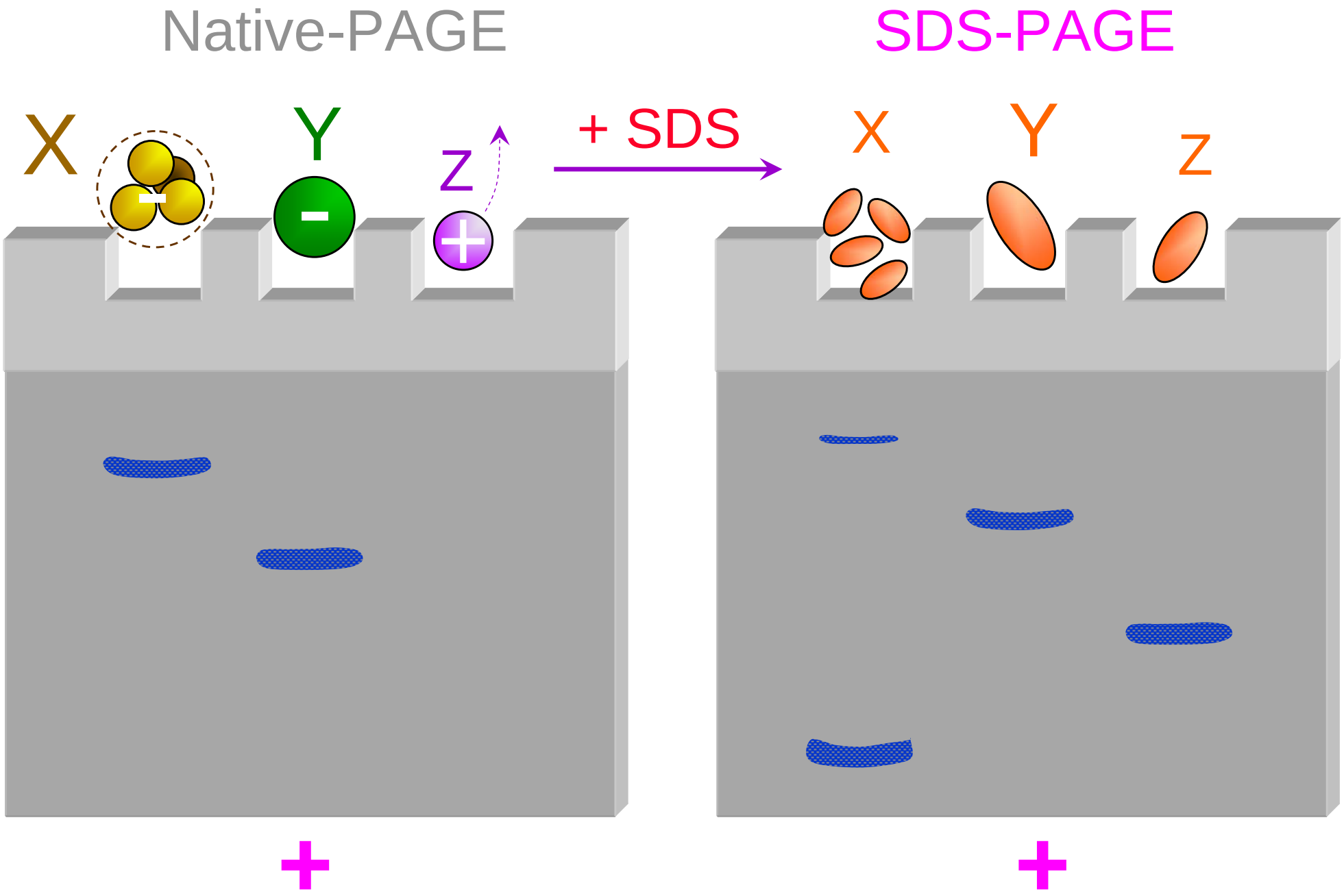
# 三種不同性質蛋白質的電泳比較

| 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 |

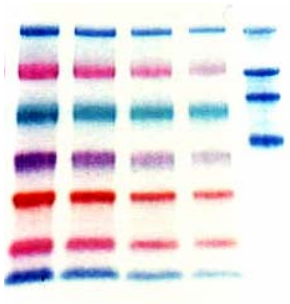
X 

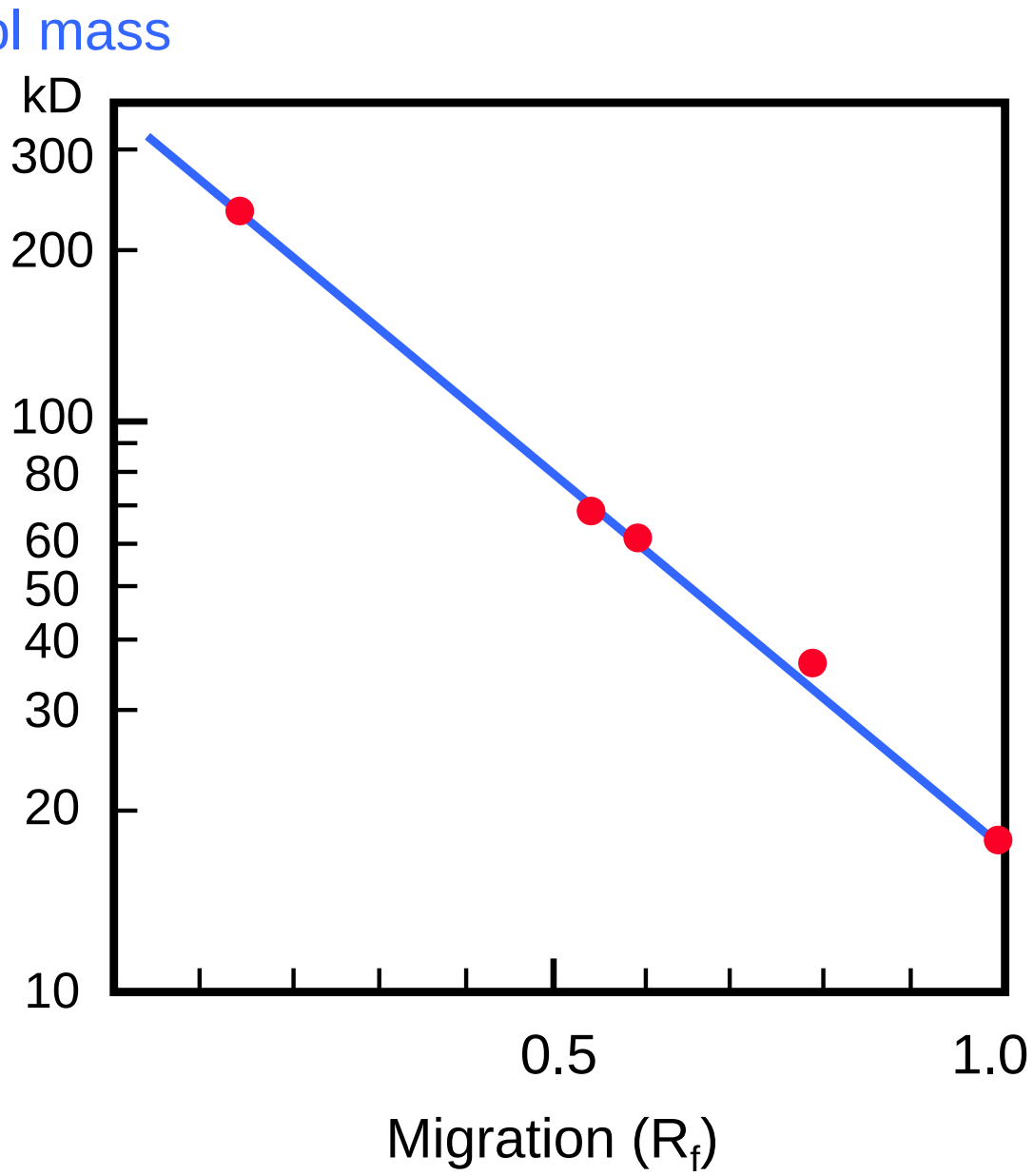
Y 

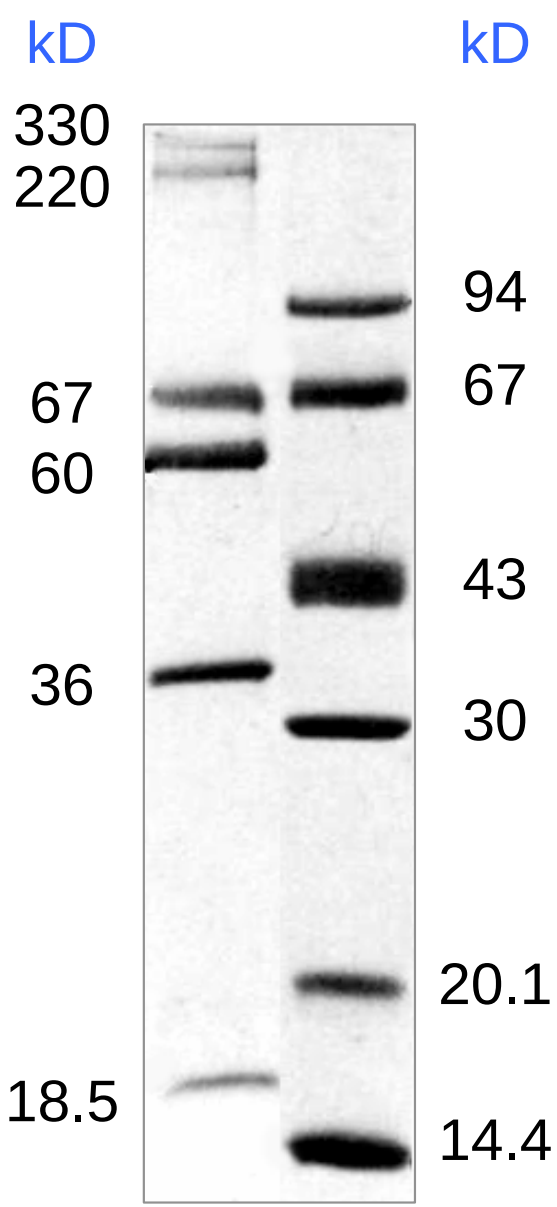

Z 


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

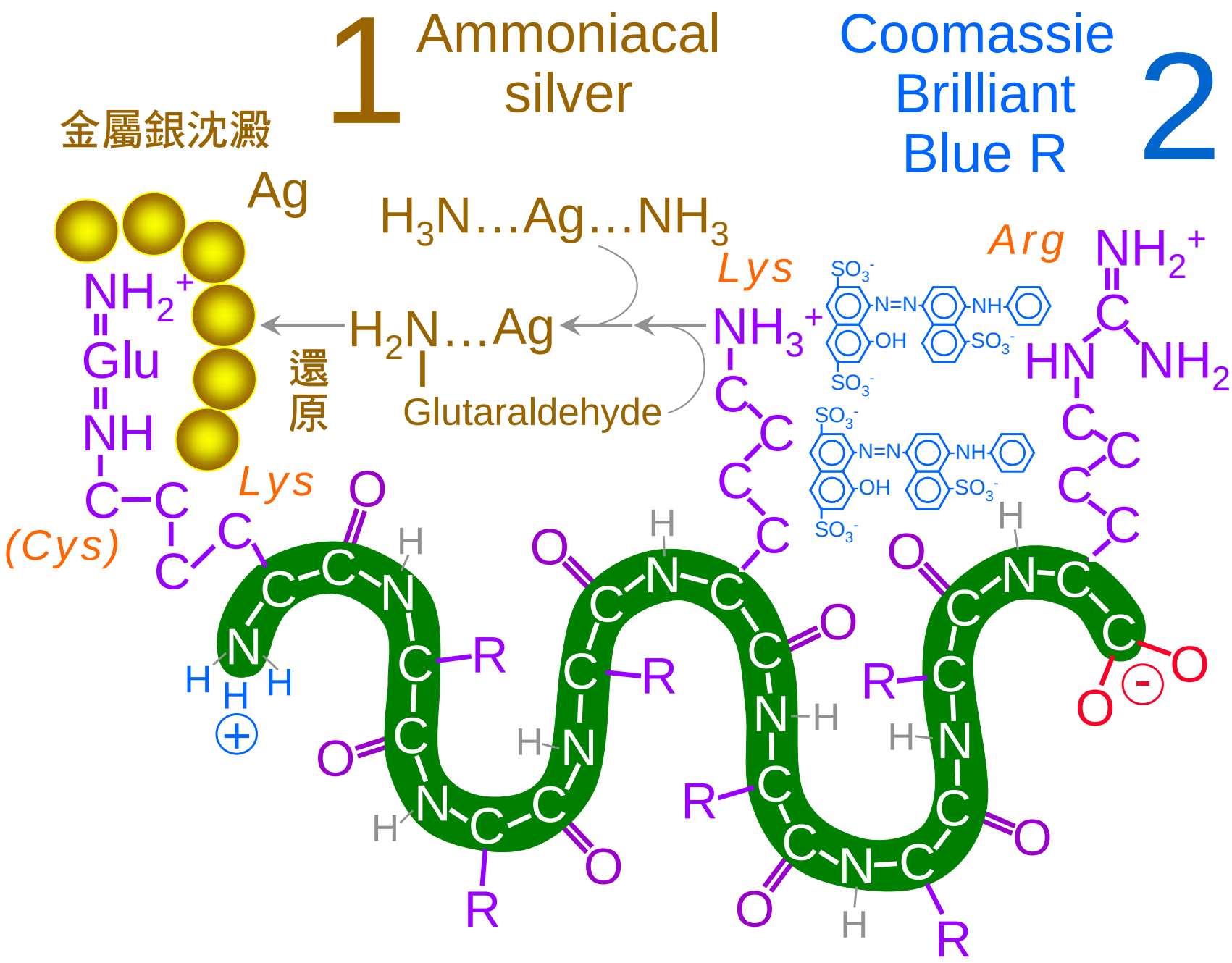




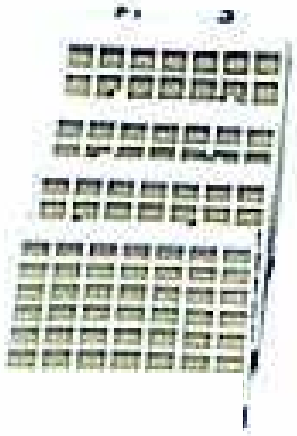

Pharmacia: Molecular Markers for electrophoresis

# 各種蛋白質染色法機制

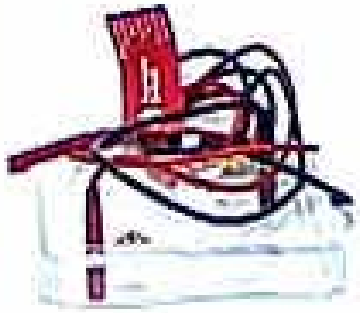

1
Ammoniacal silver
金屬銀沈澱
Ag
$H_3N…Ag…NH_3$
$H_2N…Ag$
Glutaraldehyde
還原
$NH_2^+$
Glu
NH
C–C
(Cys)
Lys
$NH_3^+$
Lys
Arg
$NH_2^+$
HN
$NH_2$
$SO_3^-$
N=N
NH
OH
2
Coomassie Brilliant Blue R

# 電泳槽及相關設備

轉印三明治

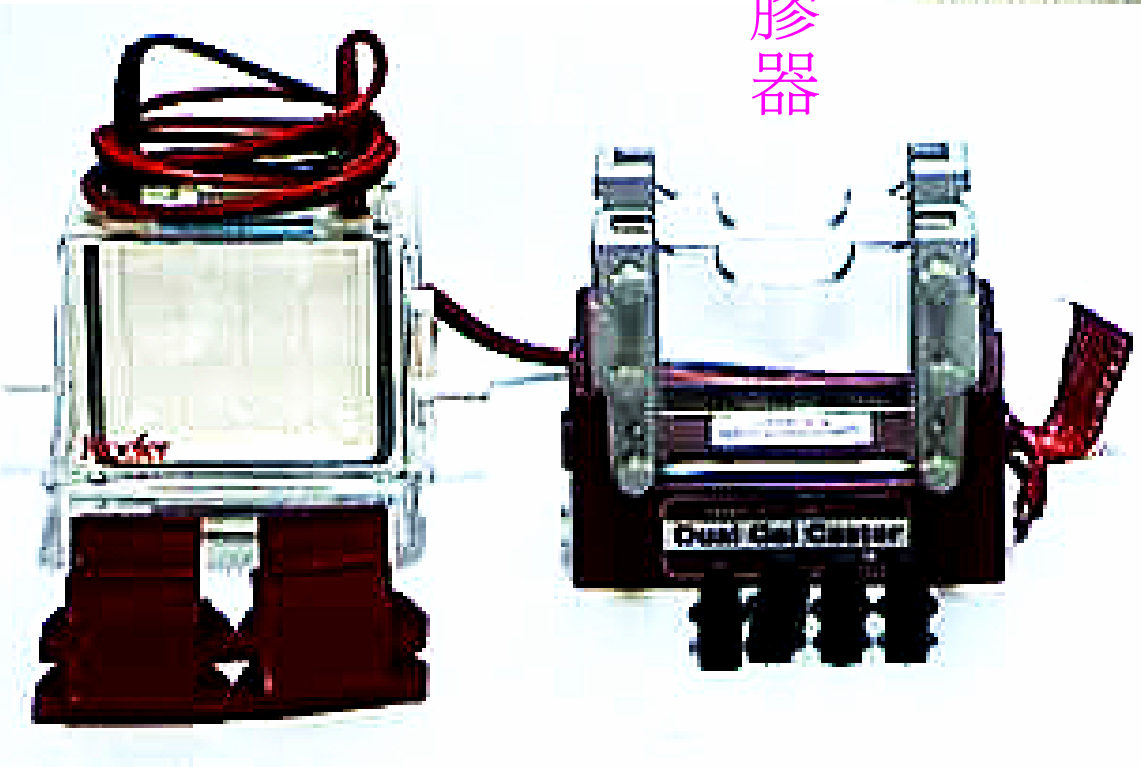



轉印槽



電泳槽

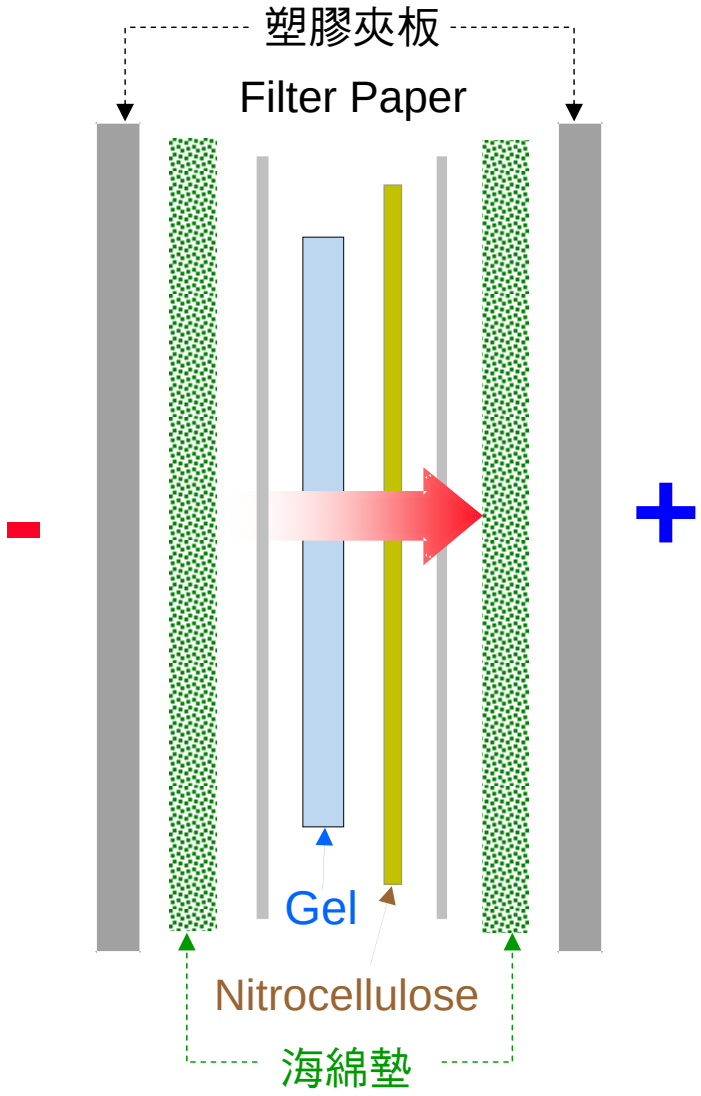
鑄膠器



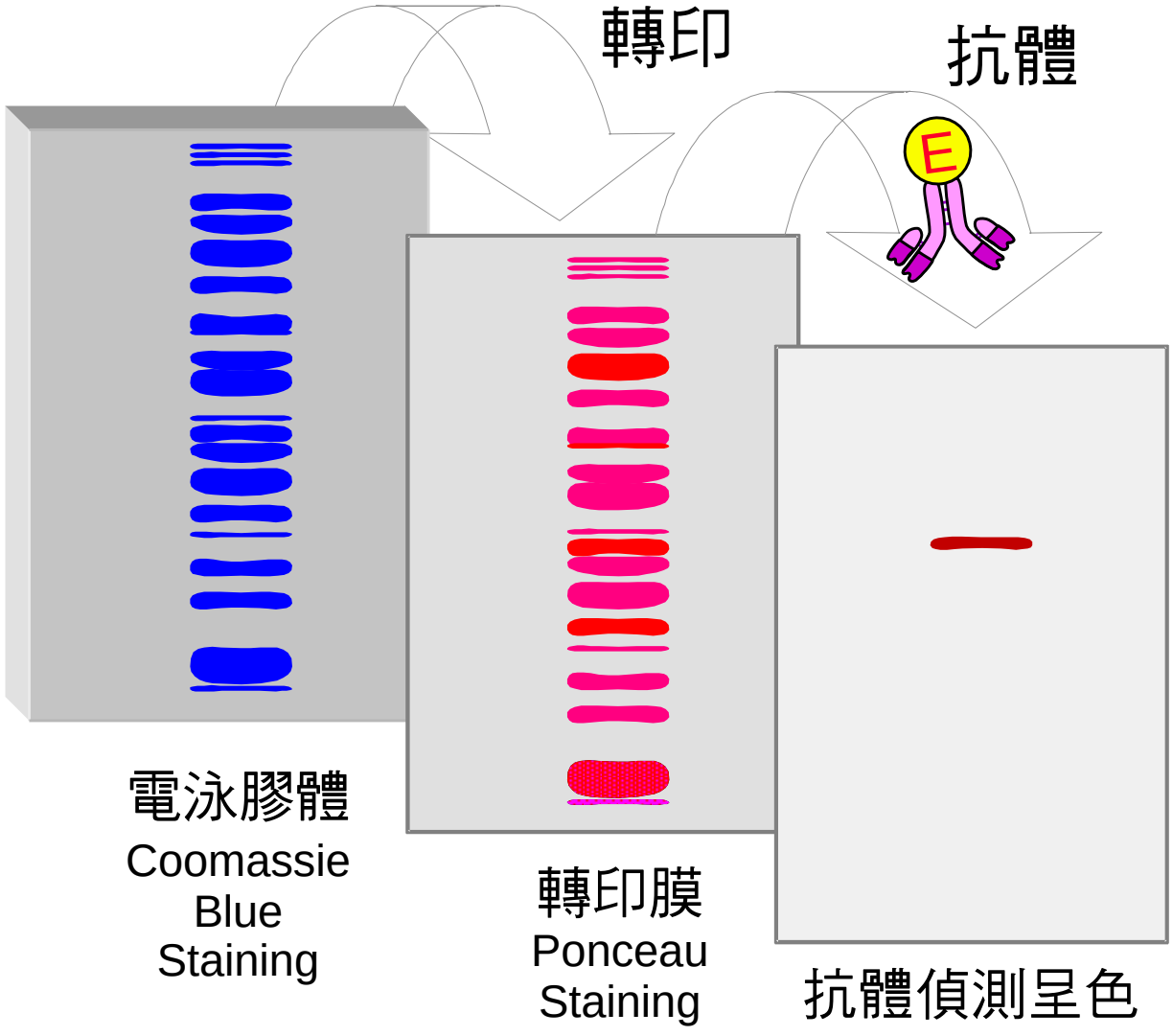

供電器

# 轉印及免疫染色流程

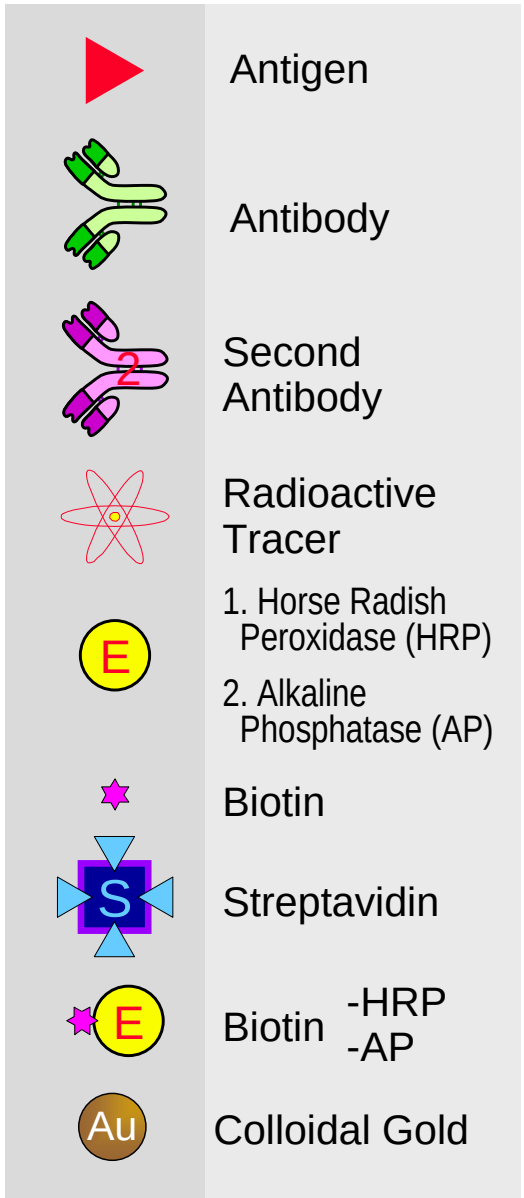
A 轉印三明治

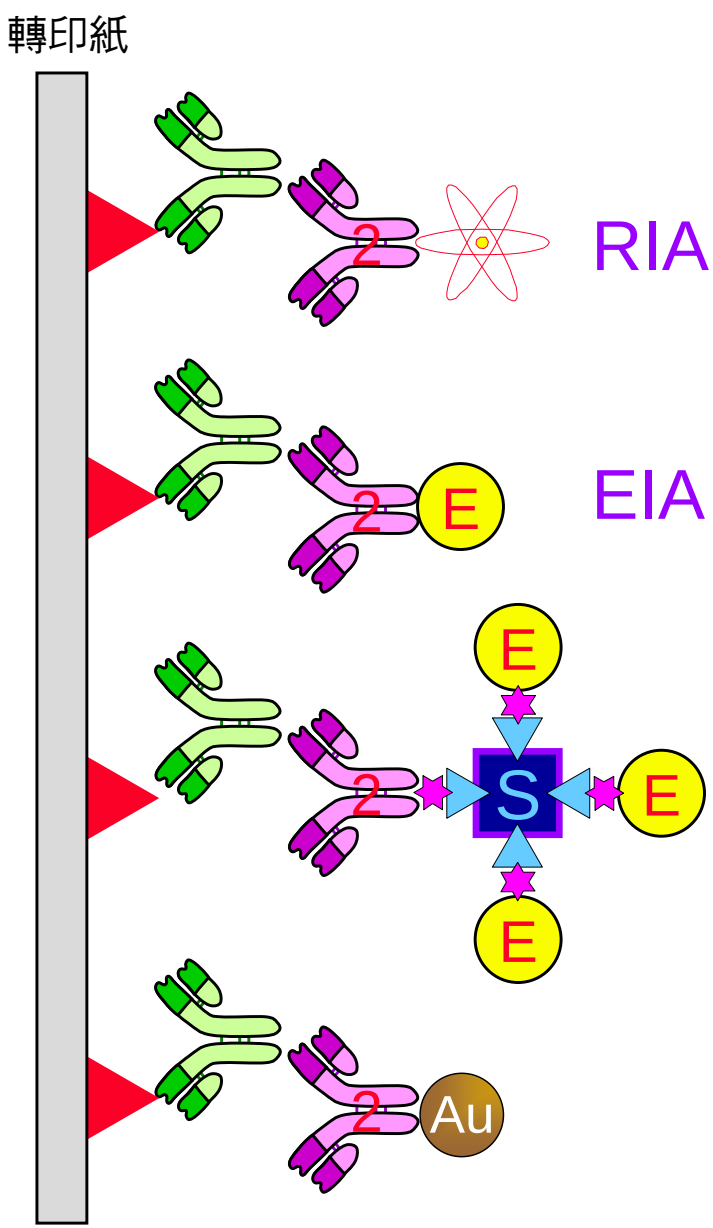


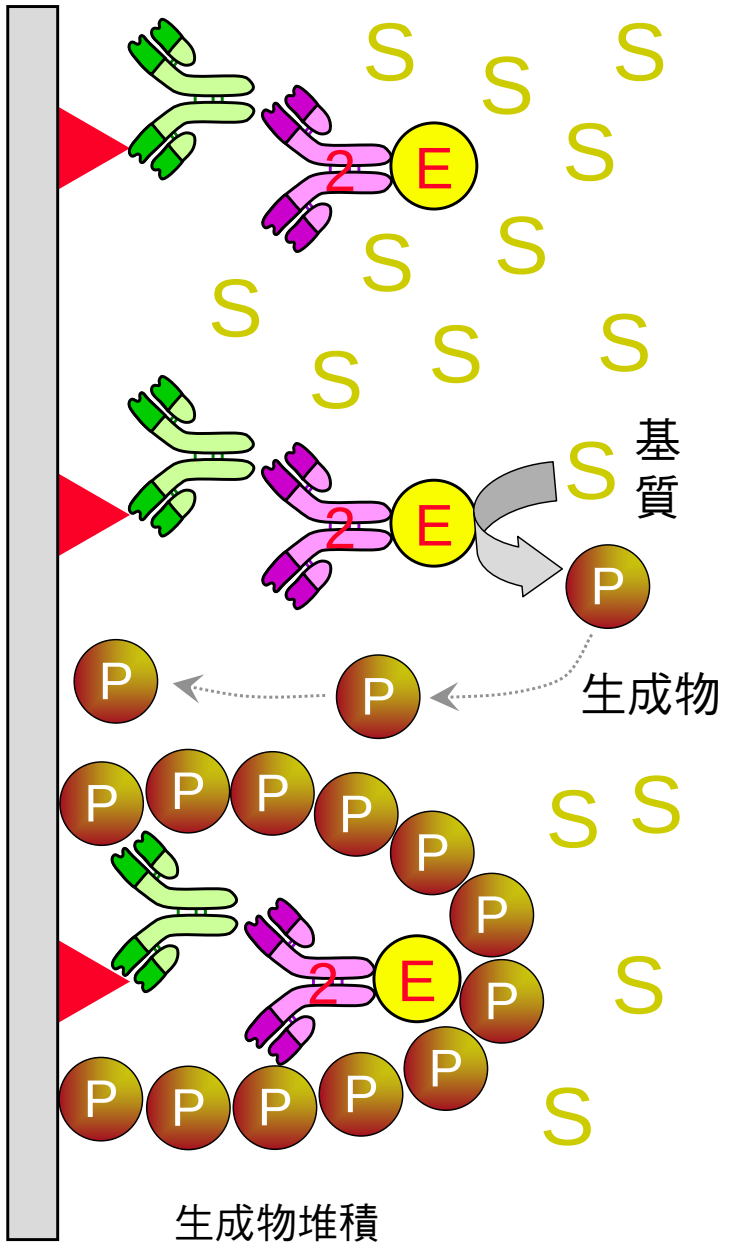

B 免疫染色流程及結果



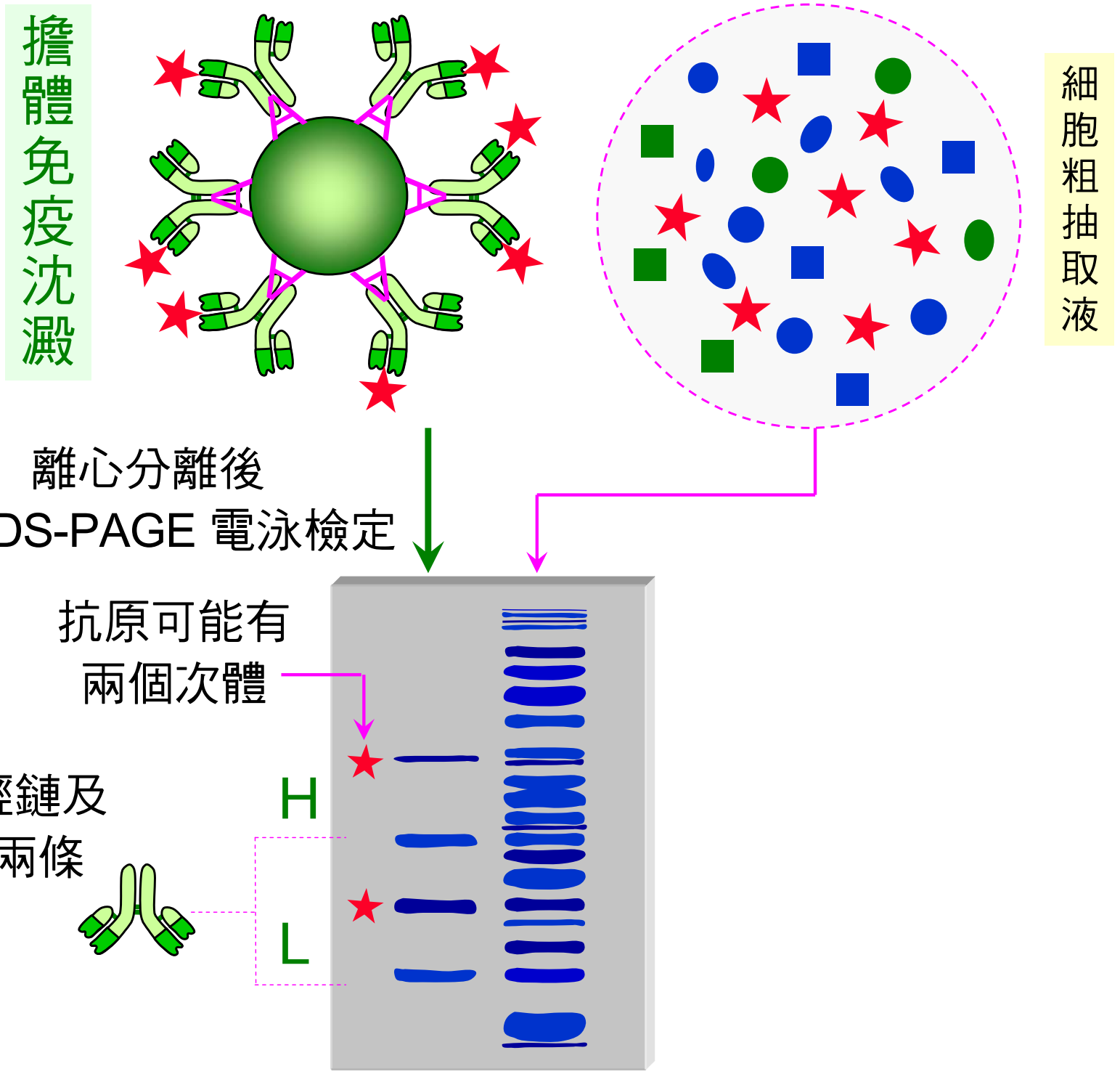
# 免疫轉印的種類與呈色機制



# 擔體免疫沈澱的原理及應用



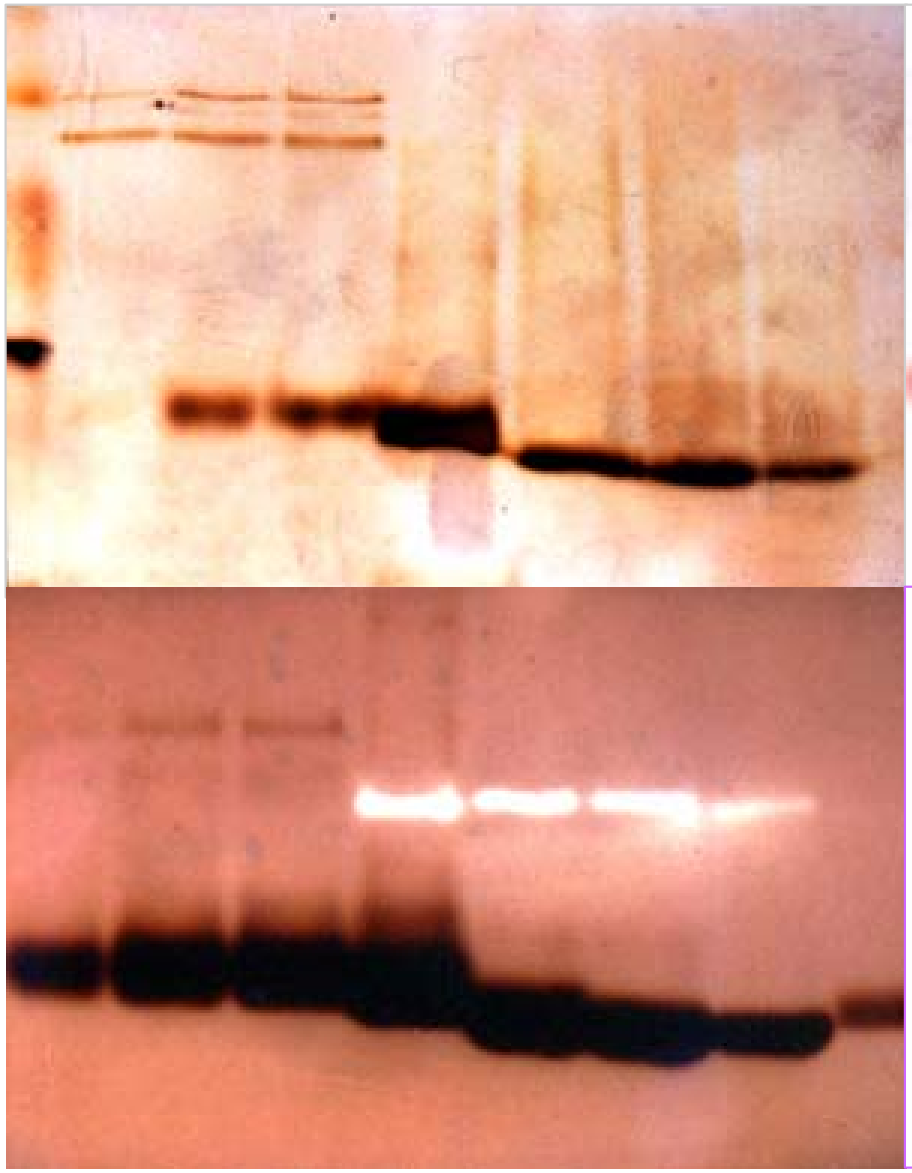
# 澱粉磷解酶染色方法比較

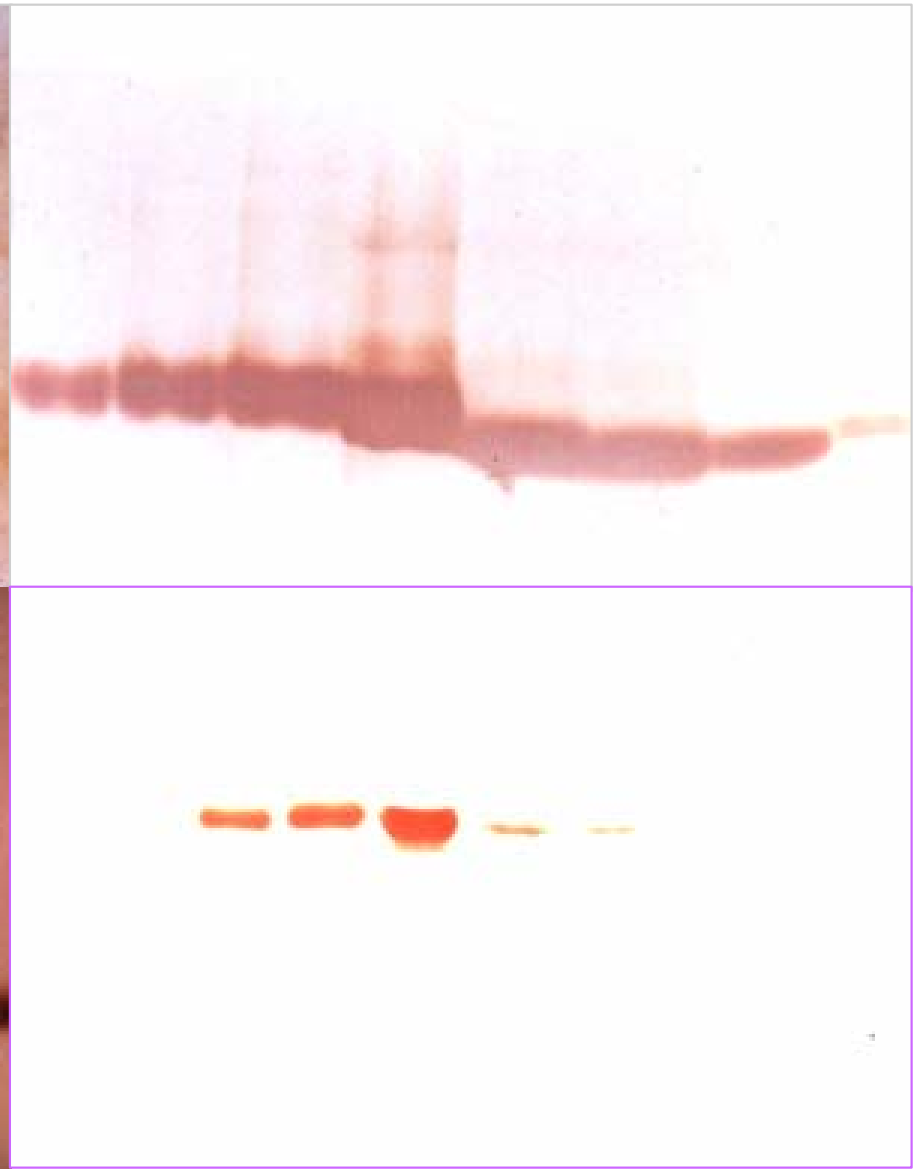
膠體過濾法各分劃

免疫轉印

硝酸銀

活性染色

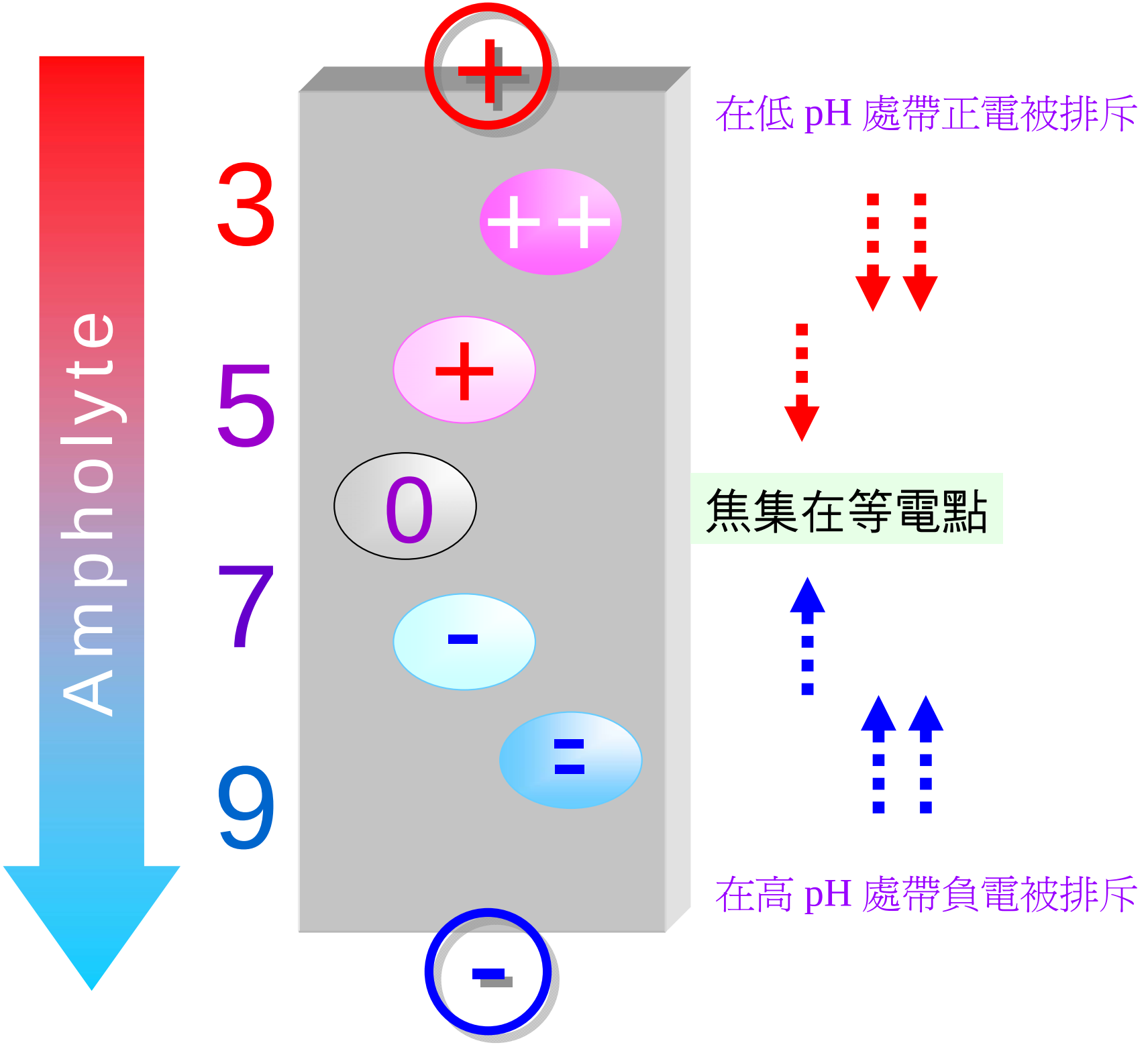




Disc-PAGE

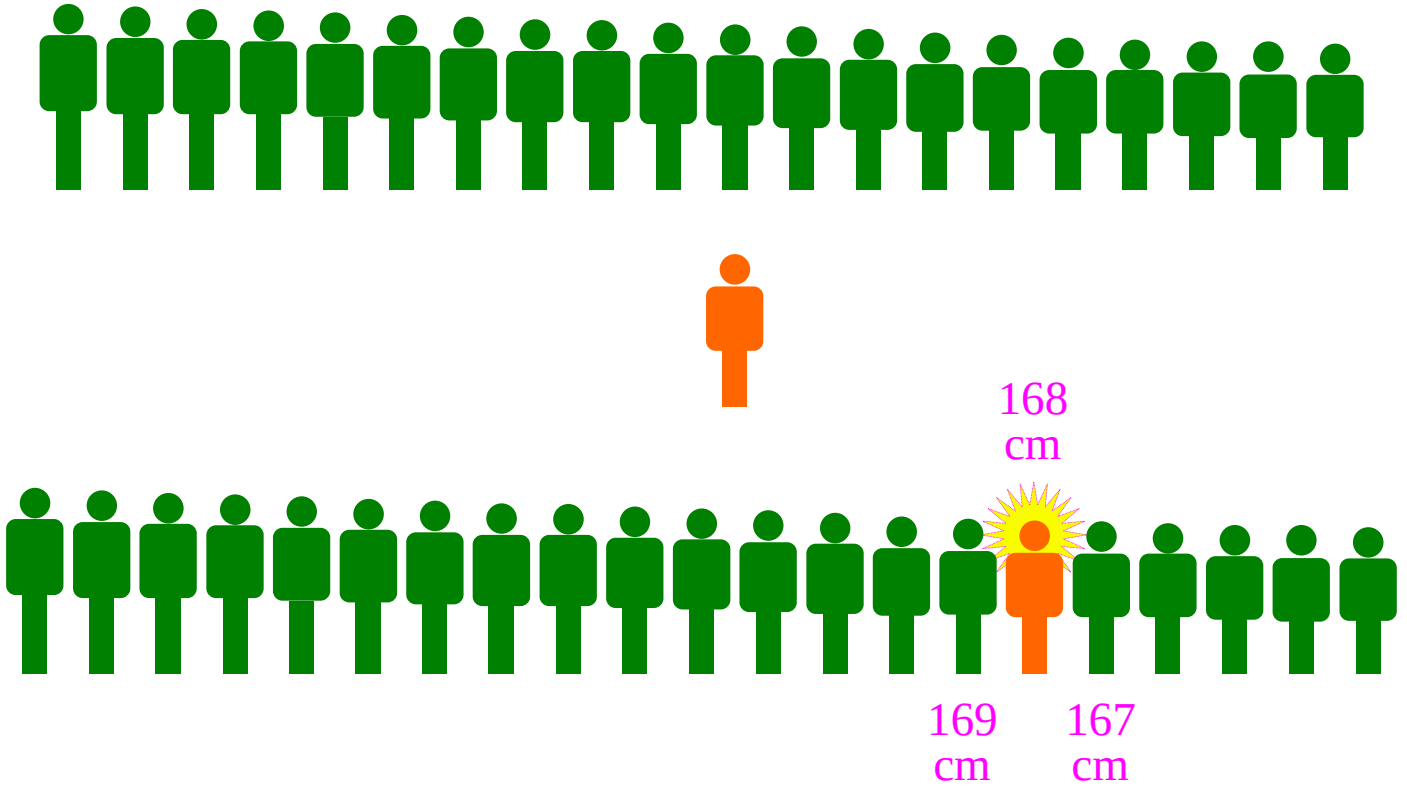
SDS-PAGE

# 等電焦集法的焦集方式



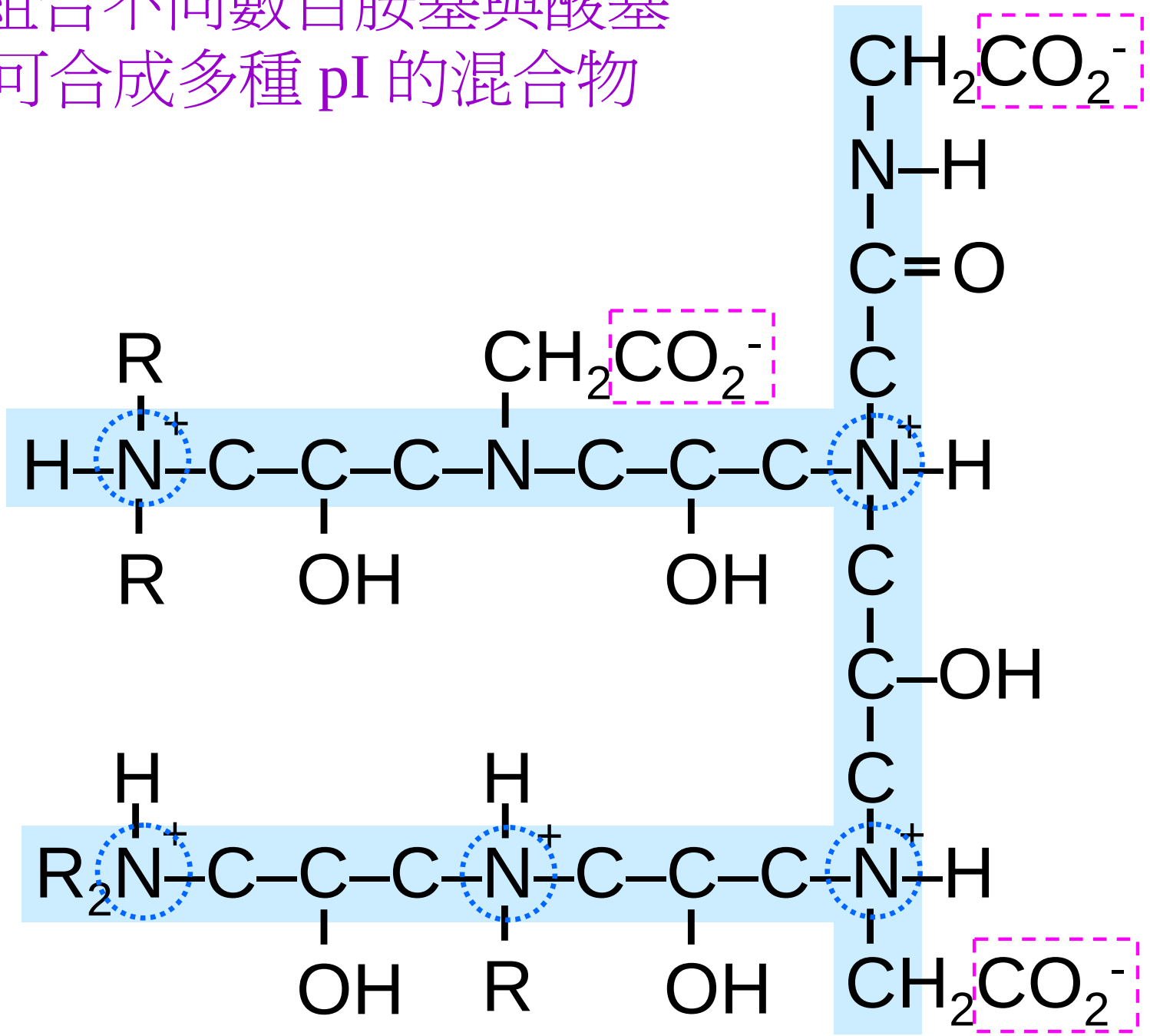
# 3.3.2 等電焦集法原理

樣本分子 在一已知 pH 梯度 中焦集

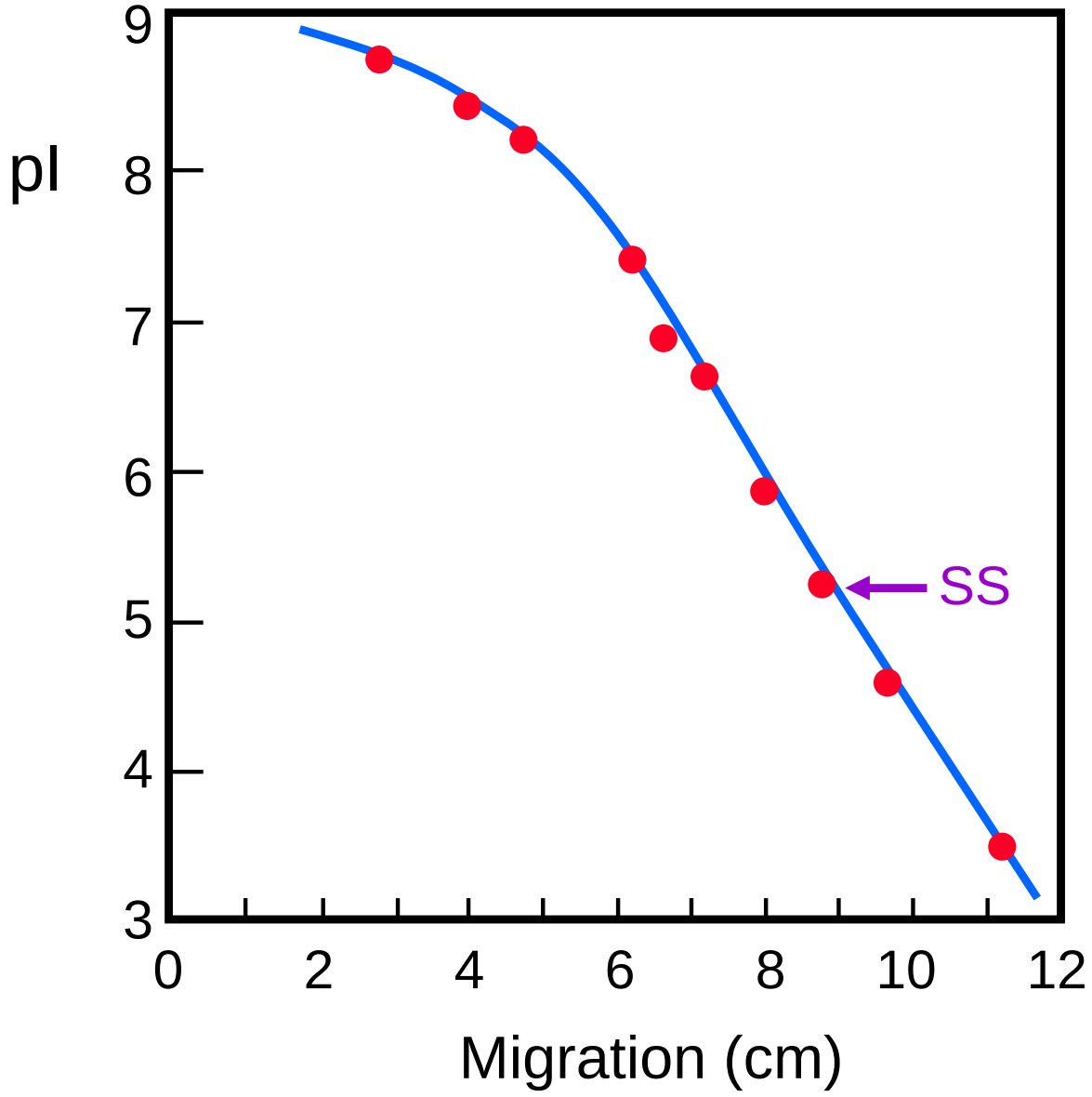


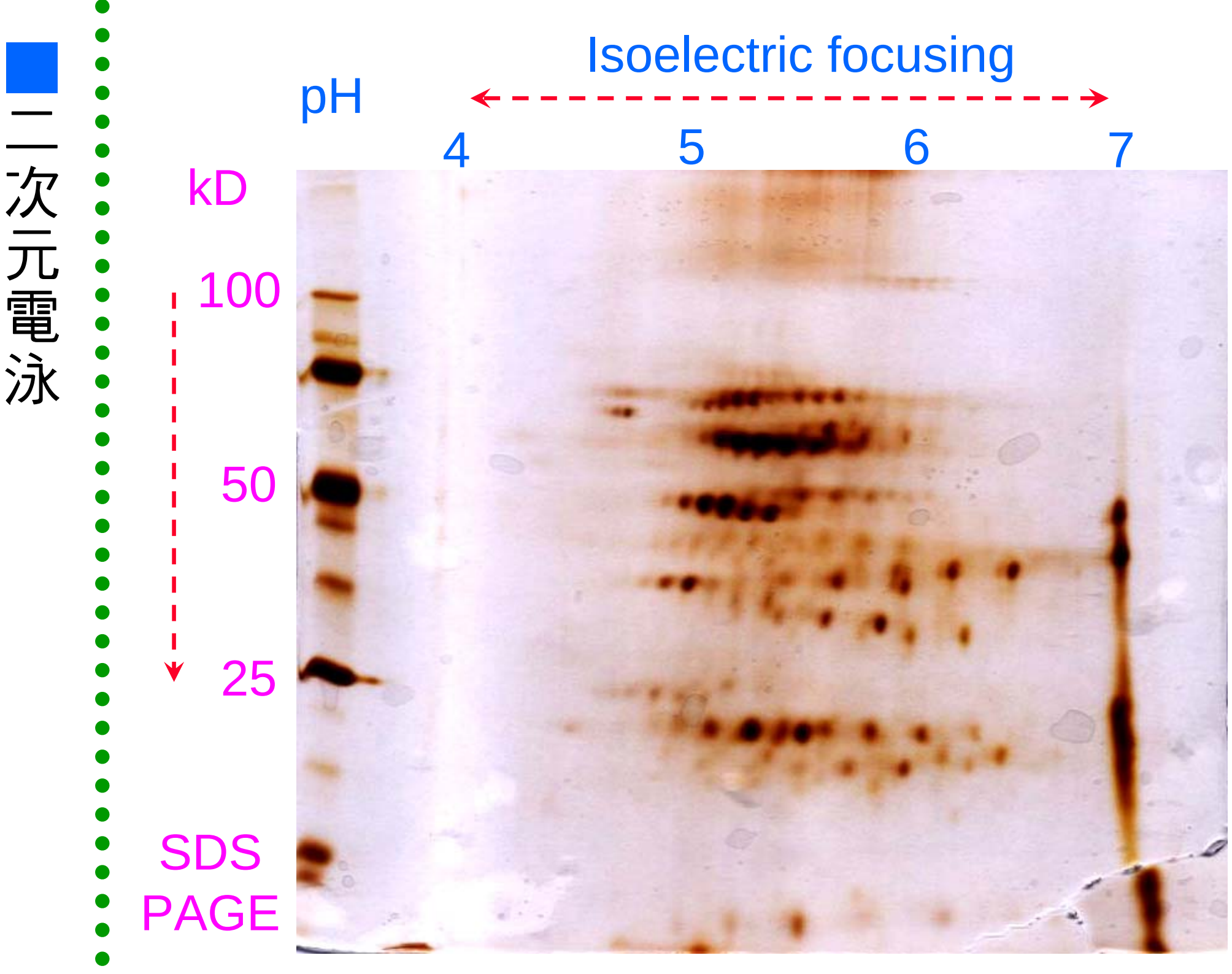
# 等電焦集的雙性離子構造

- 組合不同數目胺基與酸基可合成多種 pI 的混合物



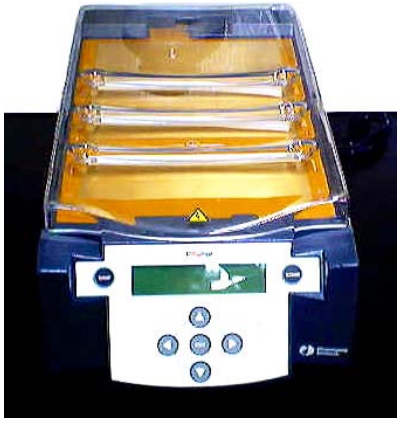
# 等電點標準校正線： IEF

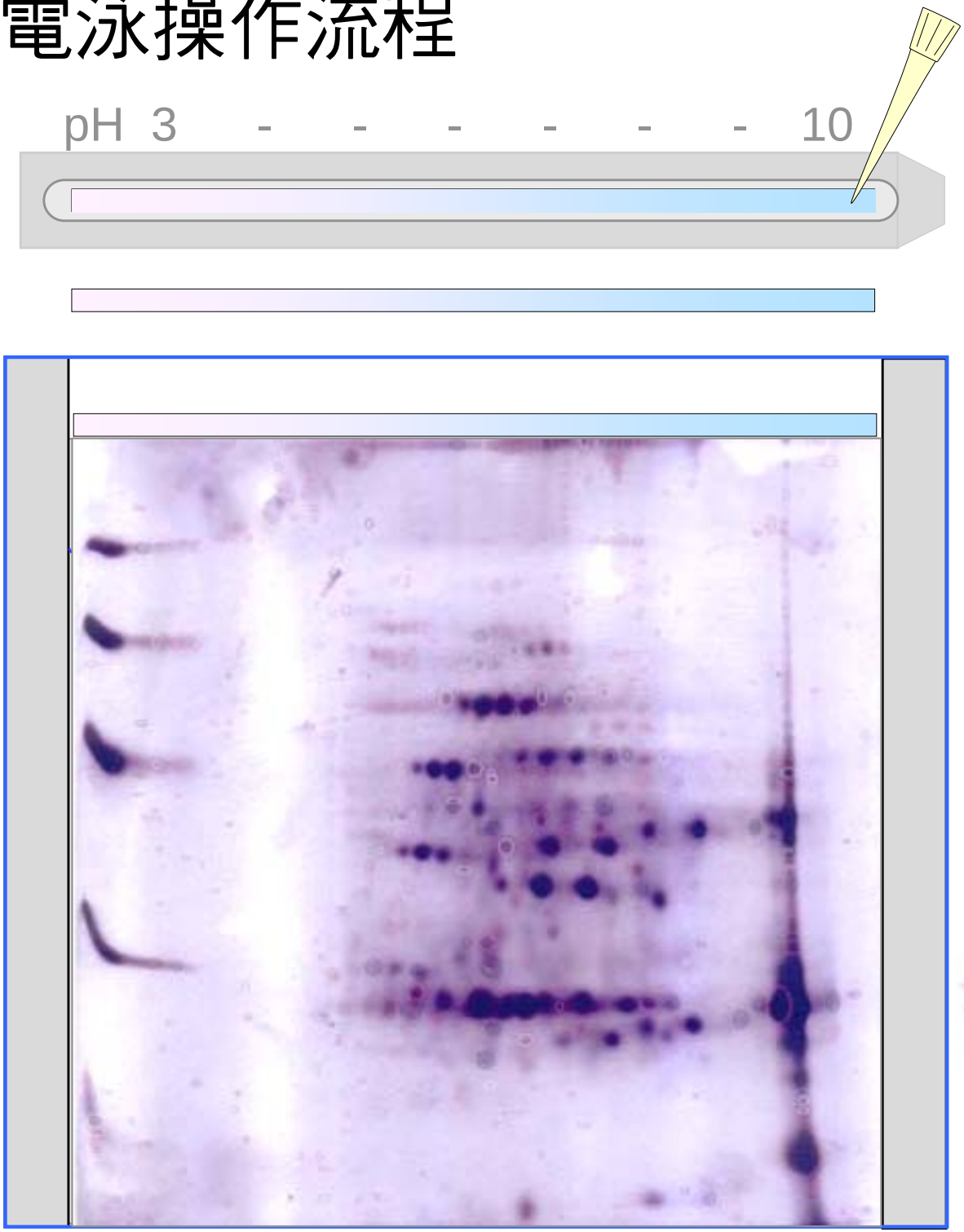


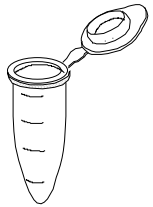

二次元電泳
Isoelectric focusing
pH
4
5
6
7
kD
100
50
25
SDS
PAGE

# 二次元電泳操作流程

(1) IEF
等電焦集電泳





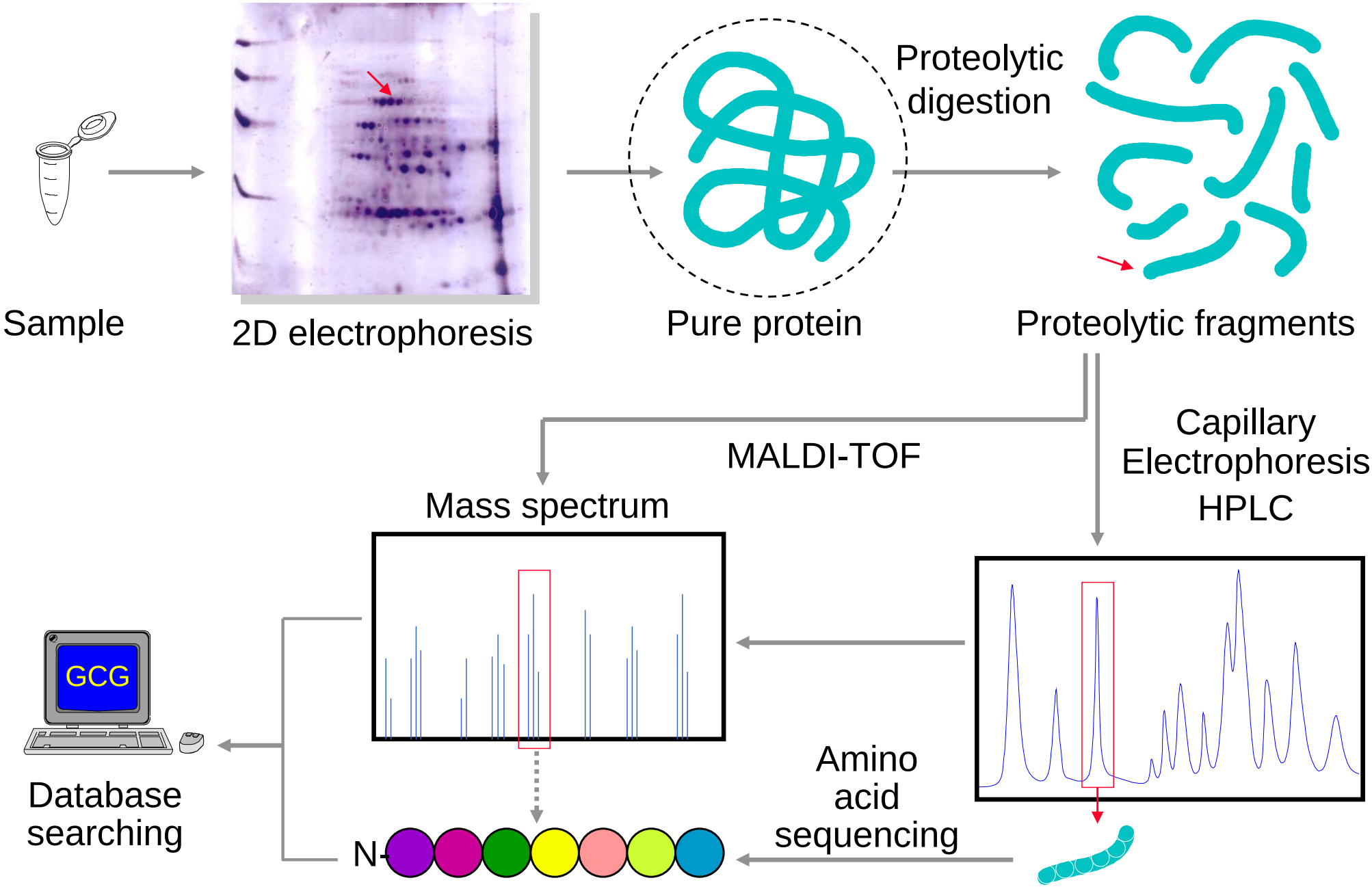



(2)
SDS-PAGE
分離膠體

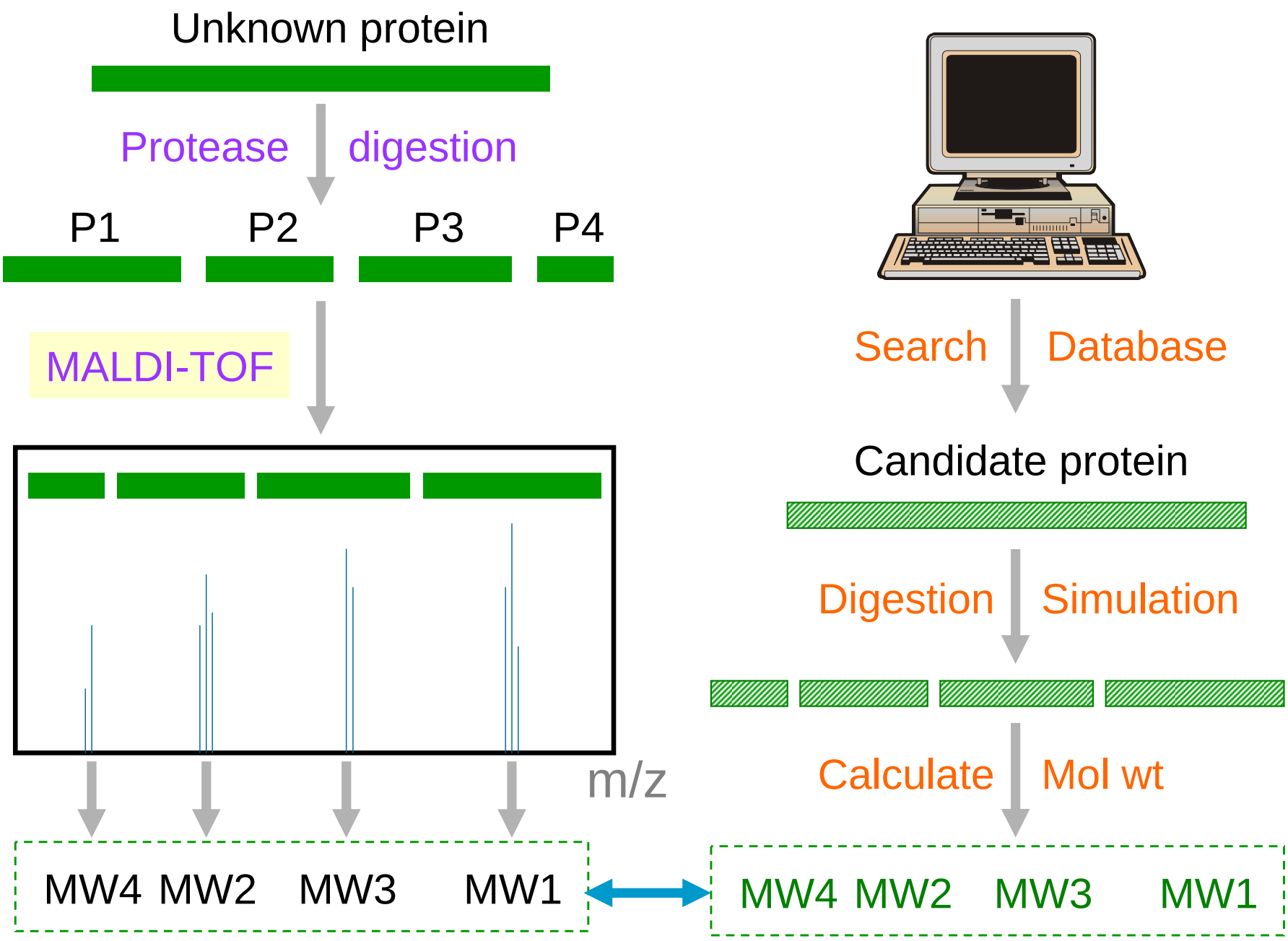
(3)
染色脫色

Juang RH (2004) Proteomics

# 蛋白質體可綜觀蛋白質的消長與身分

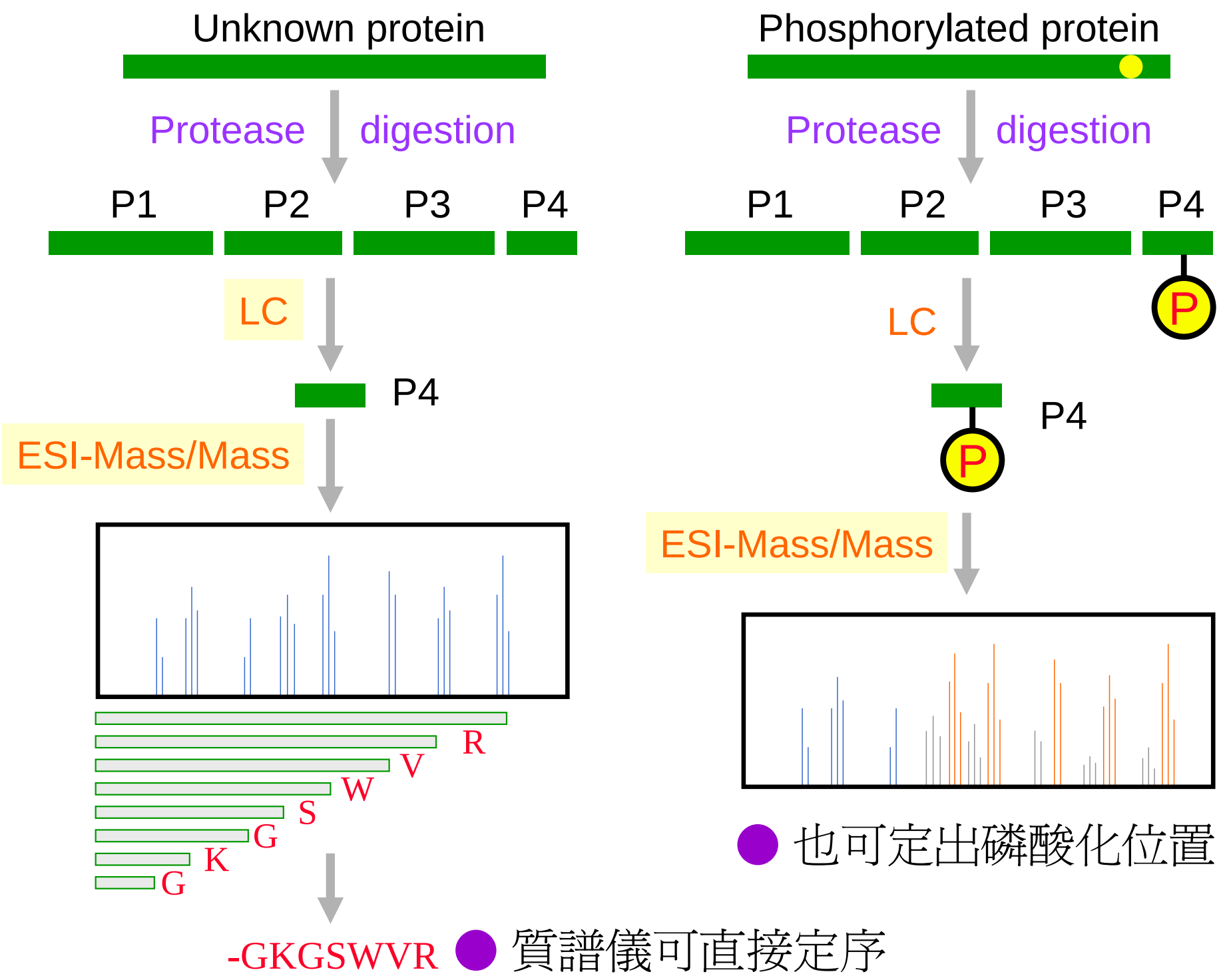


# 質譜儀可檢定蛋白質身分




- 比對各片段分子量可確定該蛋白質身分

# 質譜儀可進行蛋白質序列分析



# 現代蛋白質科技的特點

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