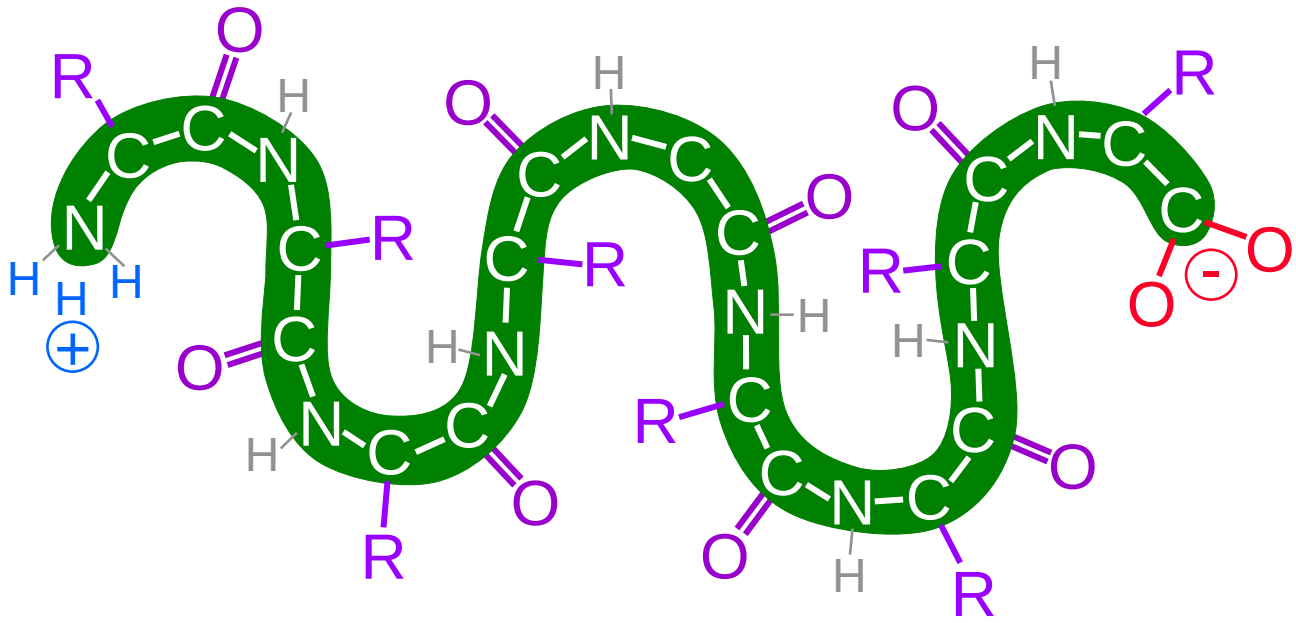
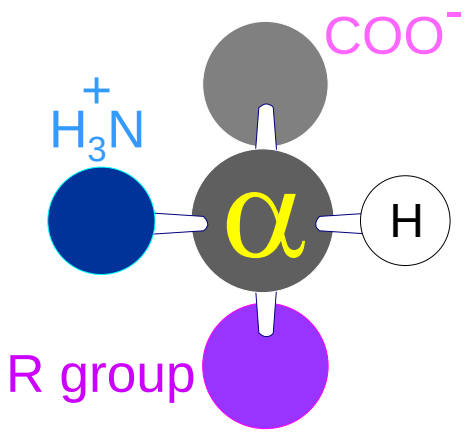
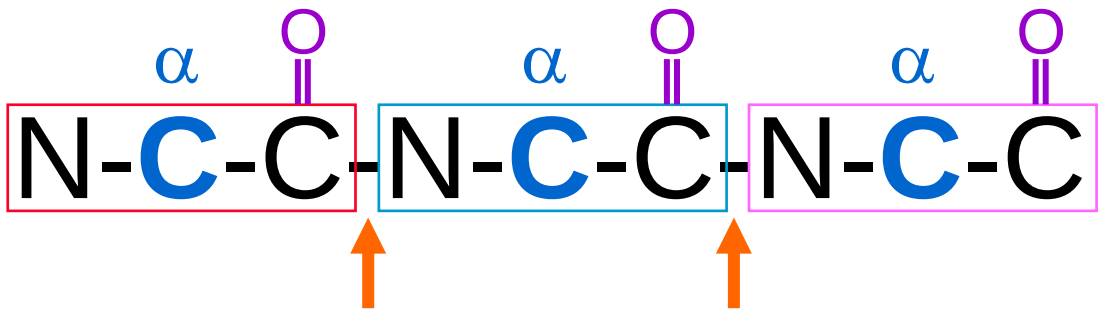
酵素分析方法

- 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

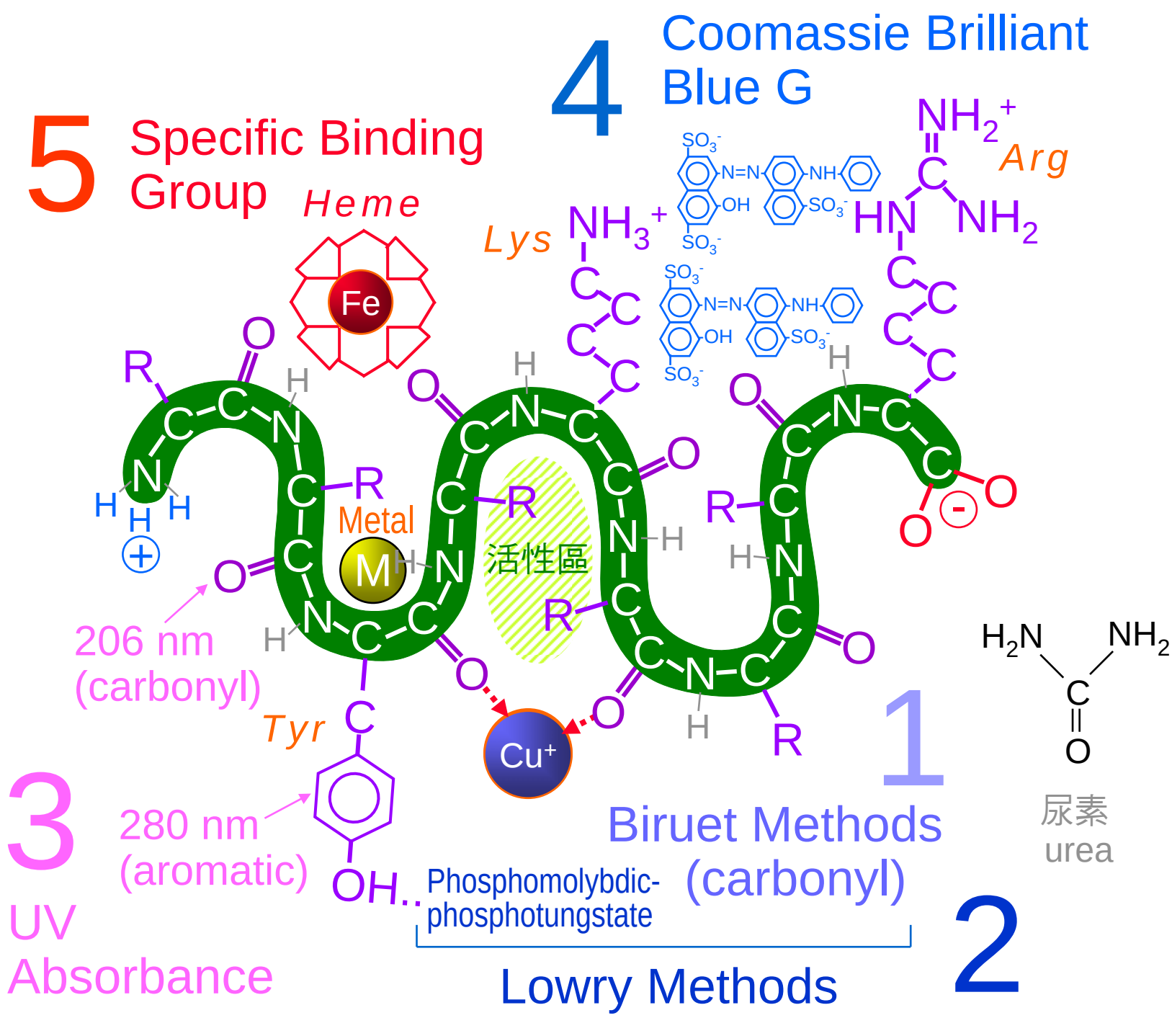
蛋白質構造的骨架：







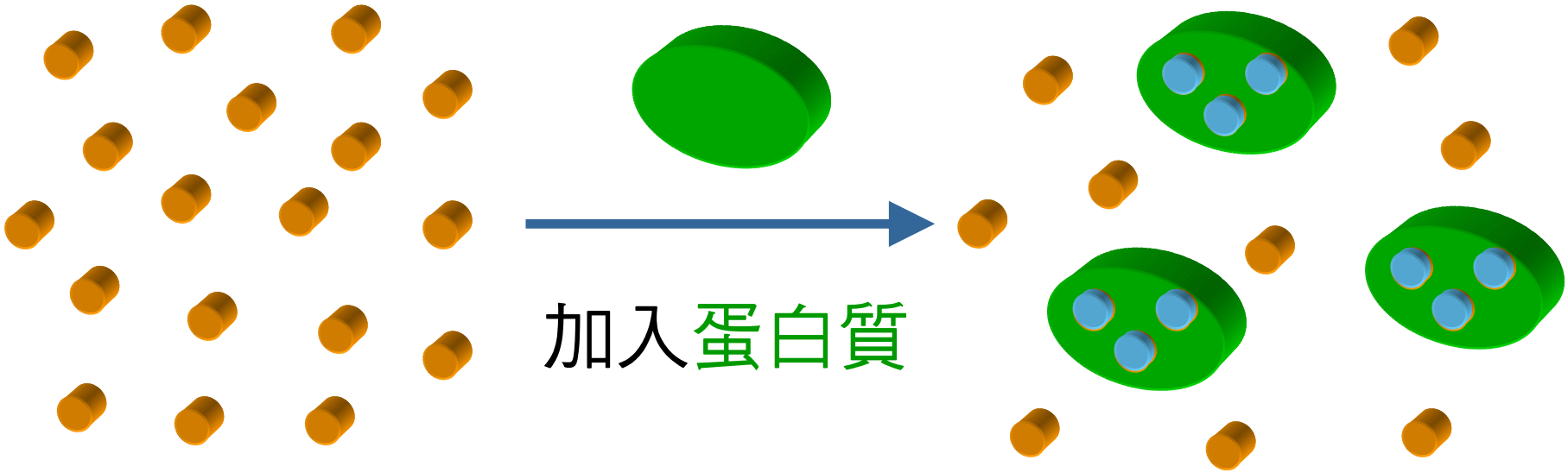
各種蛋白質定量法原理：


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

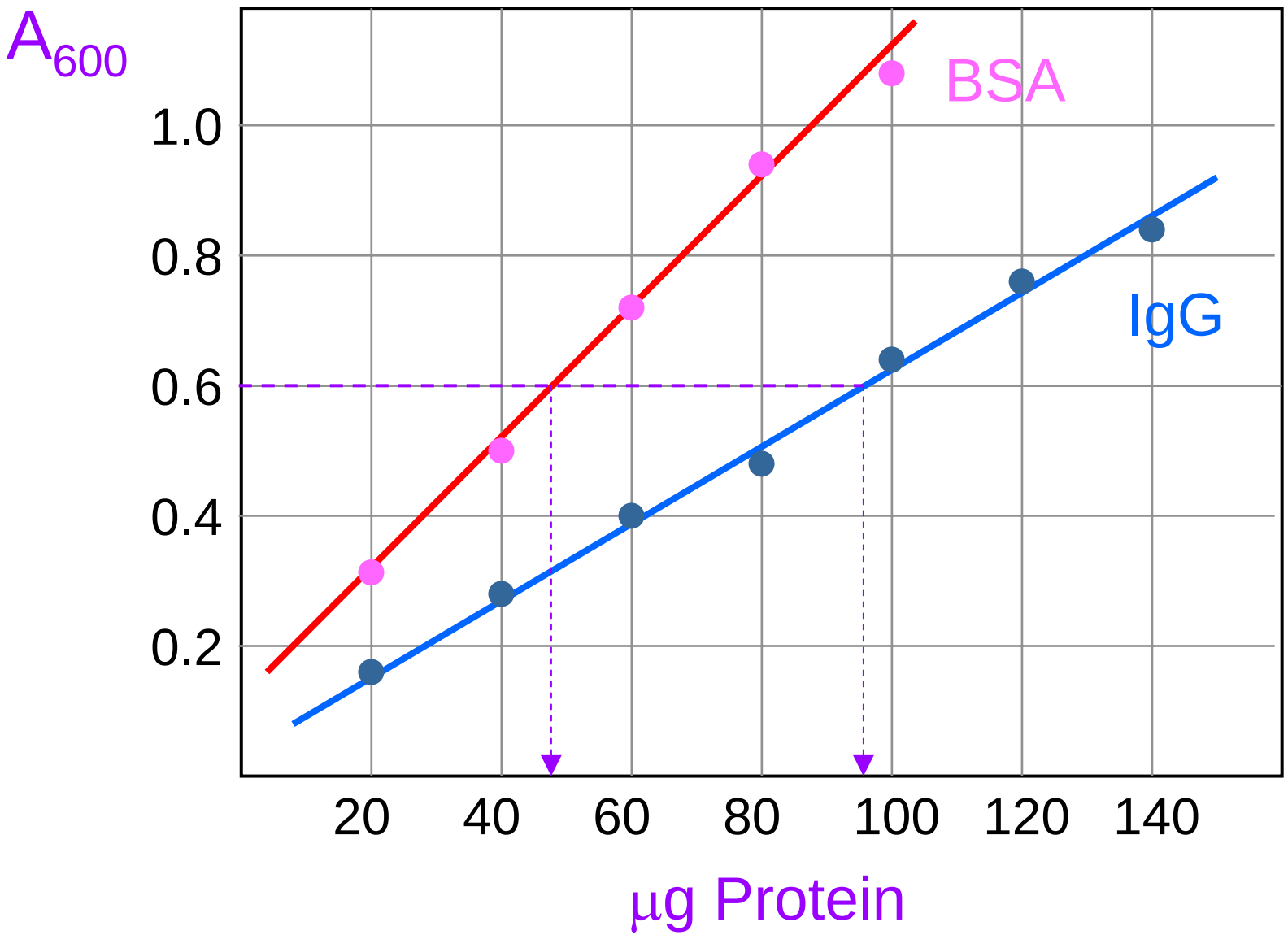
470 nm　　CBG 是一種指示劑　　595 nm



酸性環境下呈茶色

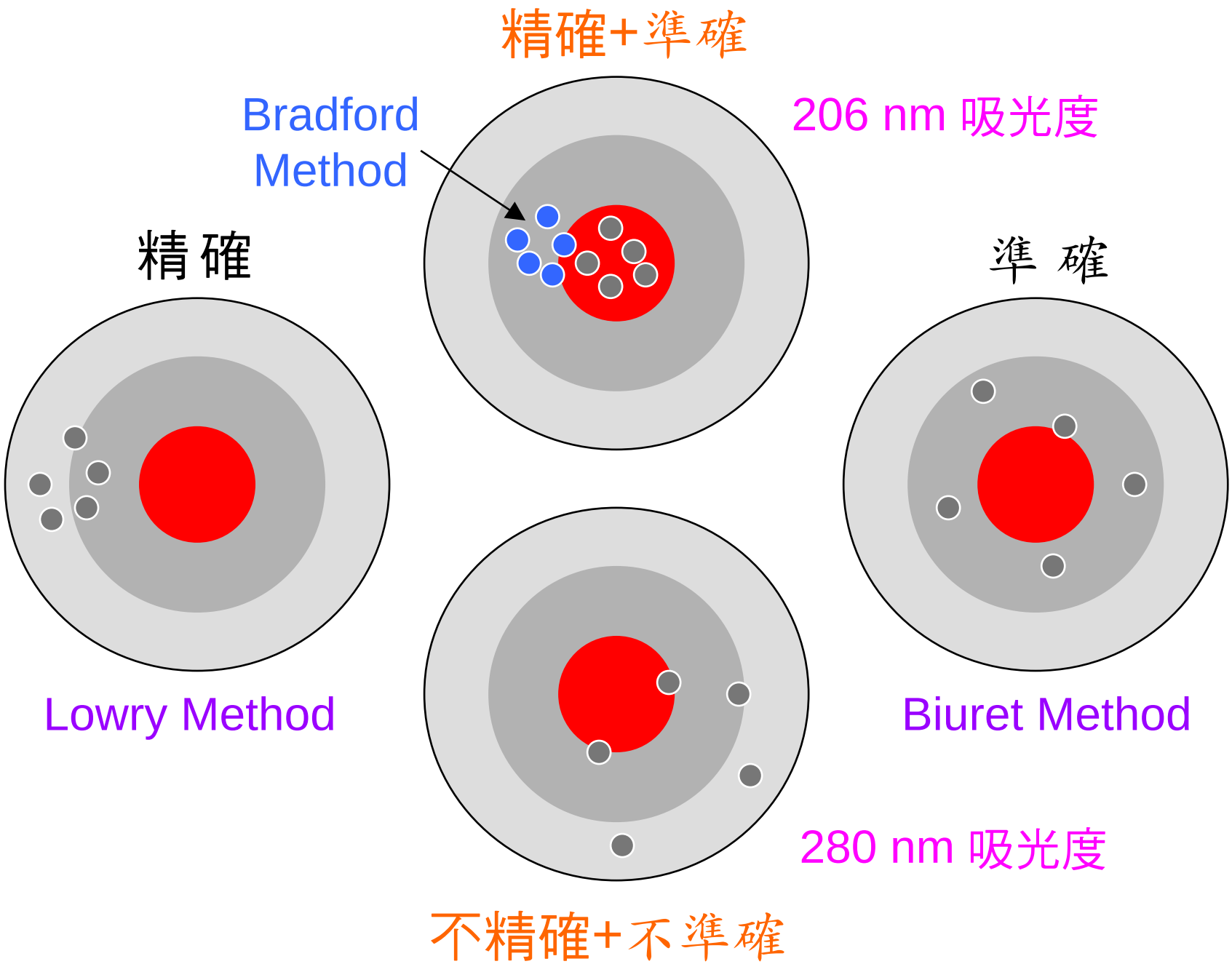
與蛋白質結合變藍色

不同蛋白質的定量差異：



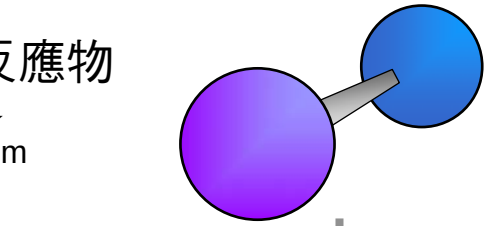
各種蛋白質定量法的比較：

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 銨離子干擾大
Lowry	0.05 - 0.5 mg	中	呈色較慢 多種離子有干擾
Absorbance 280 nm	0.05 - 2 mg	低	樣本可回收 其它物質干擾大
Absorbance 205 nm	0.01 - 0.05 mg	高	樣本可回收 氧分子干擾極大
Bradford Dye-binding	0.01 - 0.05 mg	中 高	呈色快 雜質干擾多 顏色不易洗除

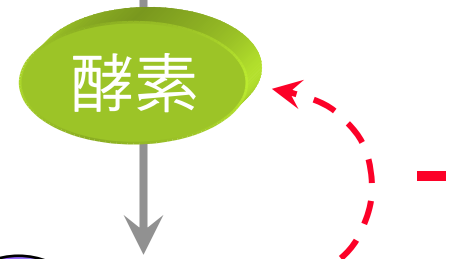

精確+準確
Bradford
Method
206 nm 吸光度
精 確
準 確
Lowry Method
Biuret Method
280 nm 吸光度
不精確+不準確

酵素反應及偵測方法：

使用大量反應物
10 x K_m



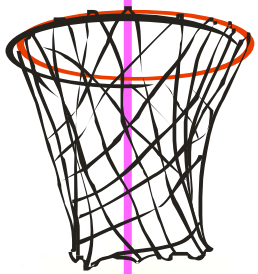
反應物



生成物可能有
回饋抑制

生成物

能否直接測量？

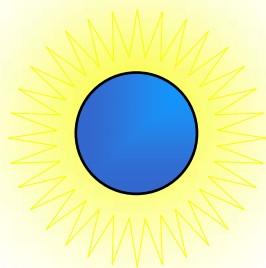


移除生成物並
轉成可測量者

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

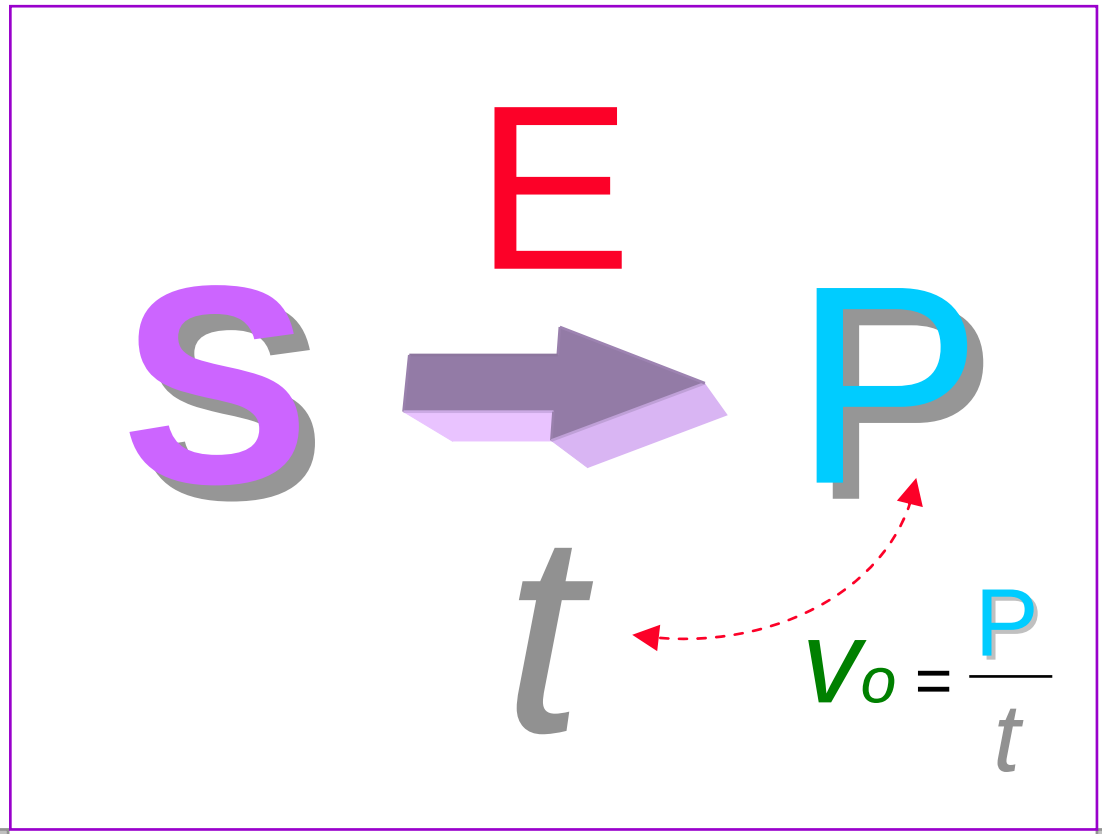


酵素活性的測定：

酵素量要適中

基質
要過量

10 x Km



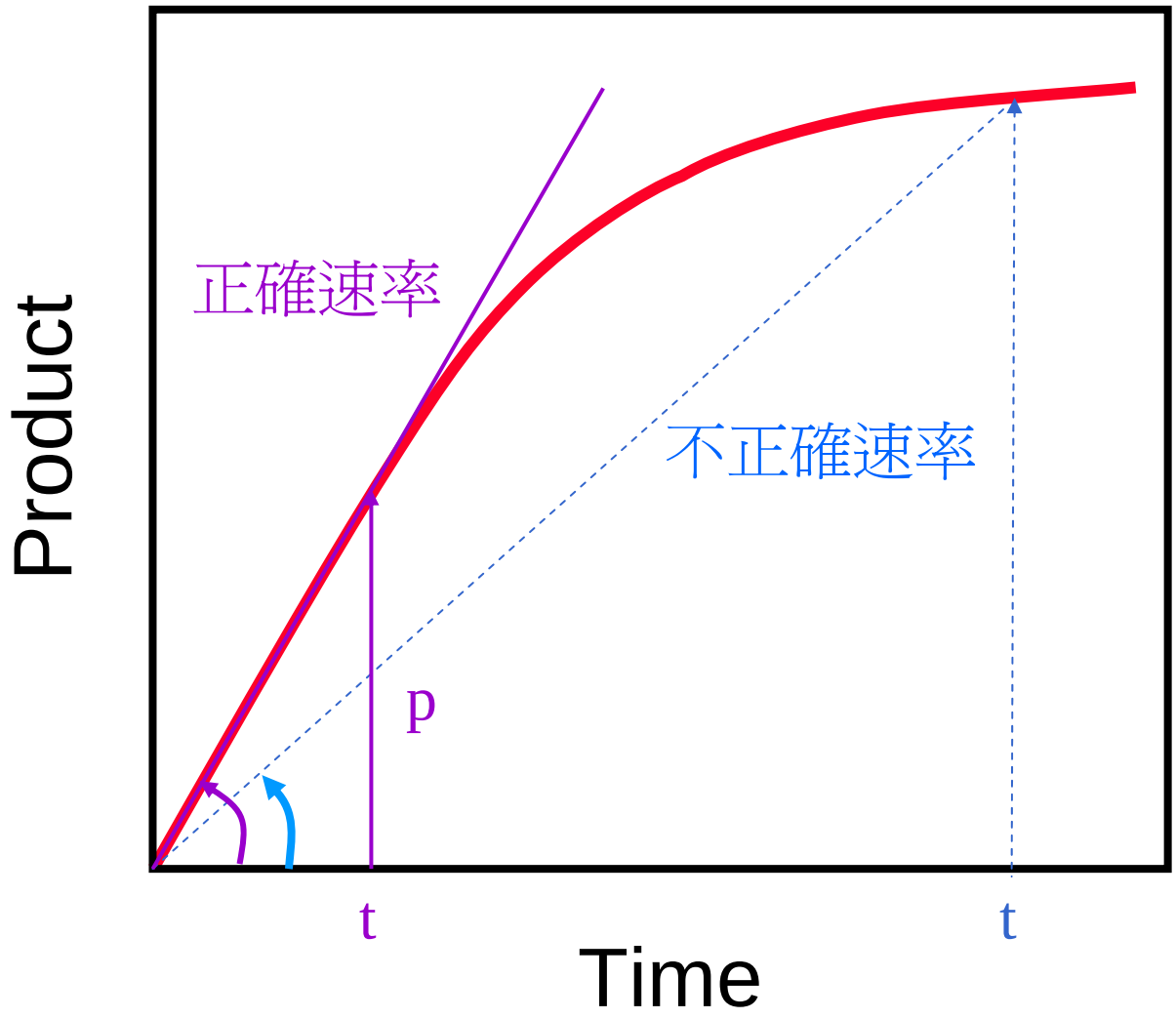
生成物
要能測得

酸鹼度

反應時間恰當

溫 度

測定時間內反應速率需成線性：

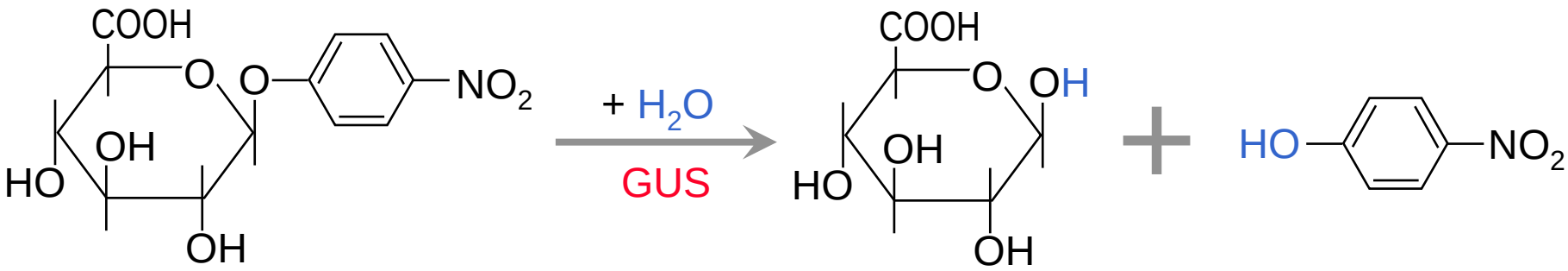


$$v = \frac{p}{t}$$

GUS 酵素活性的測定：

反應物

生成物



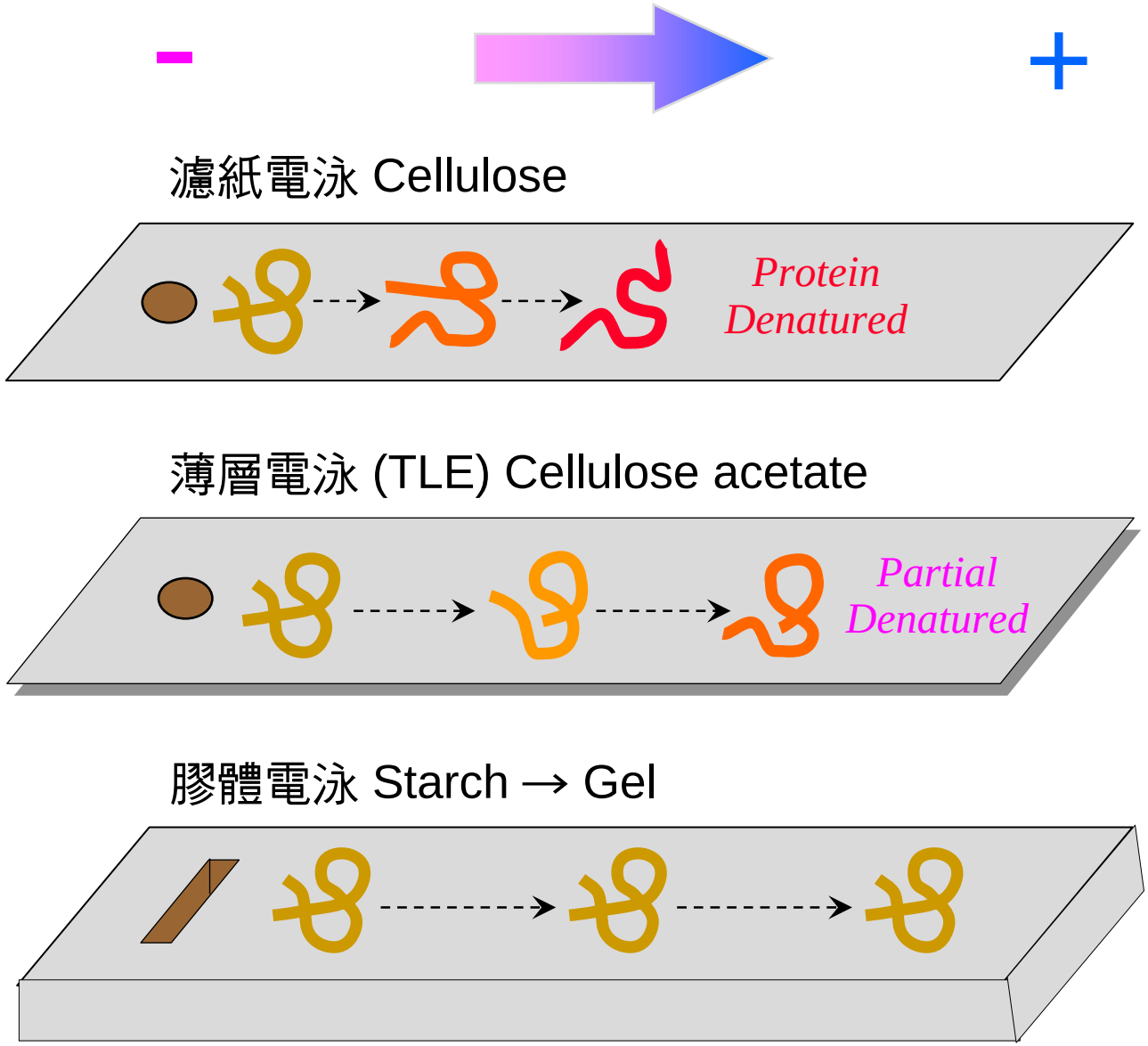
p-Nitrophenyl β-D-glucuronide (*p*NPG)

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

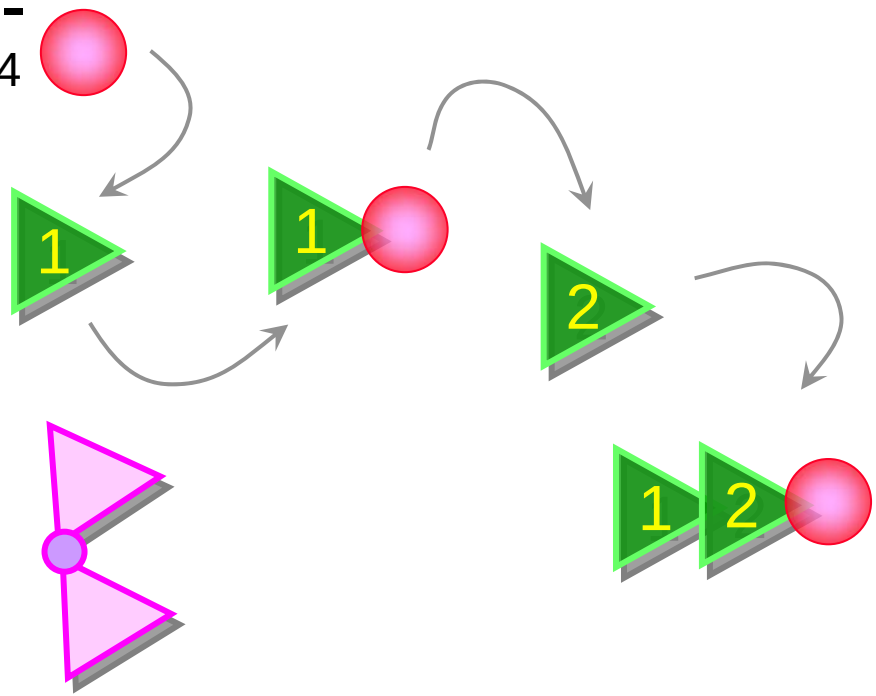
Bis(acrylamide) (bridge)

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)

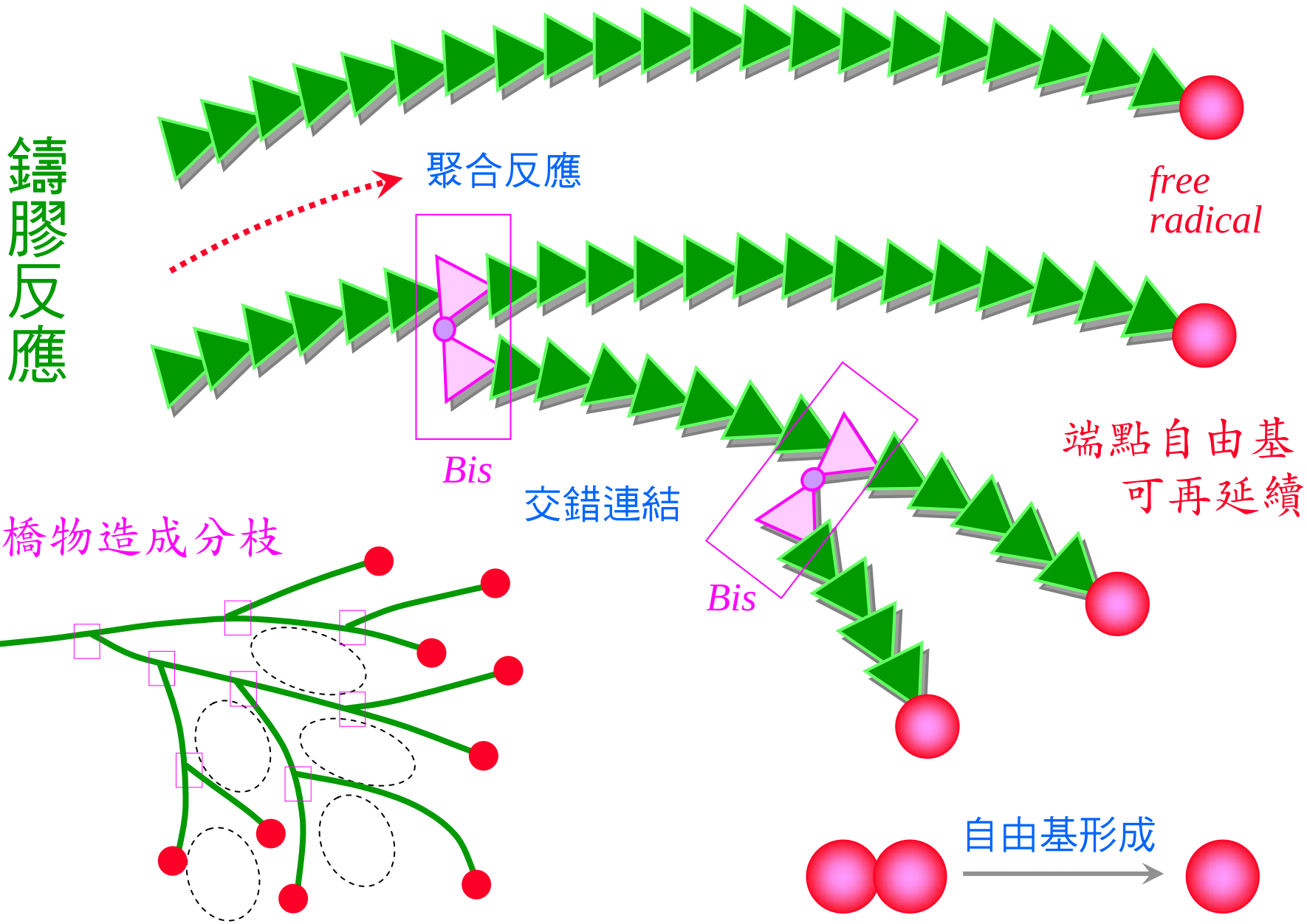


$CH_2=CH\text{-}CO\text{-}NH_2$

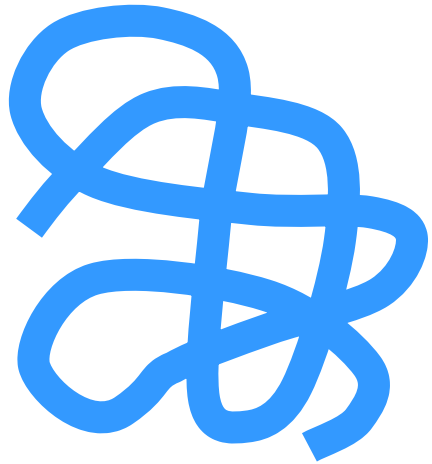
Acrylamide 有毒性！

澱粉加高熱會誘生？

單體聚合反應：

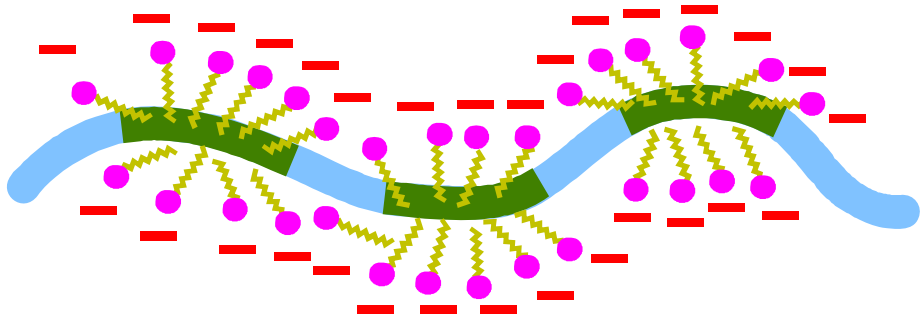

鑄膠反應
聚合反應
free radical
Bis
交錯連結
端點自由基
可再延續
架橋物造成分枝
Bis
自由基形成

SDS 在蛋白質表面 均勻敷上 一層負電：



SDS

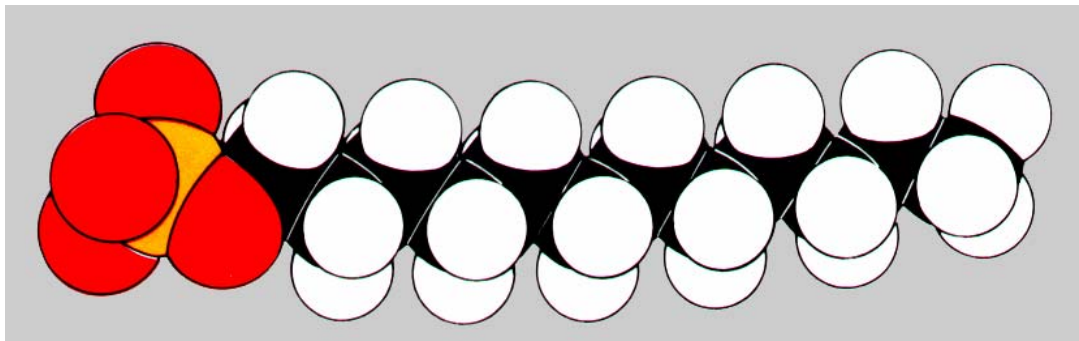
boiling



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



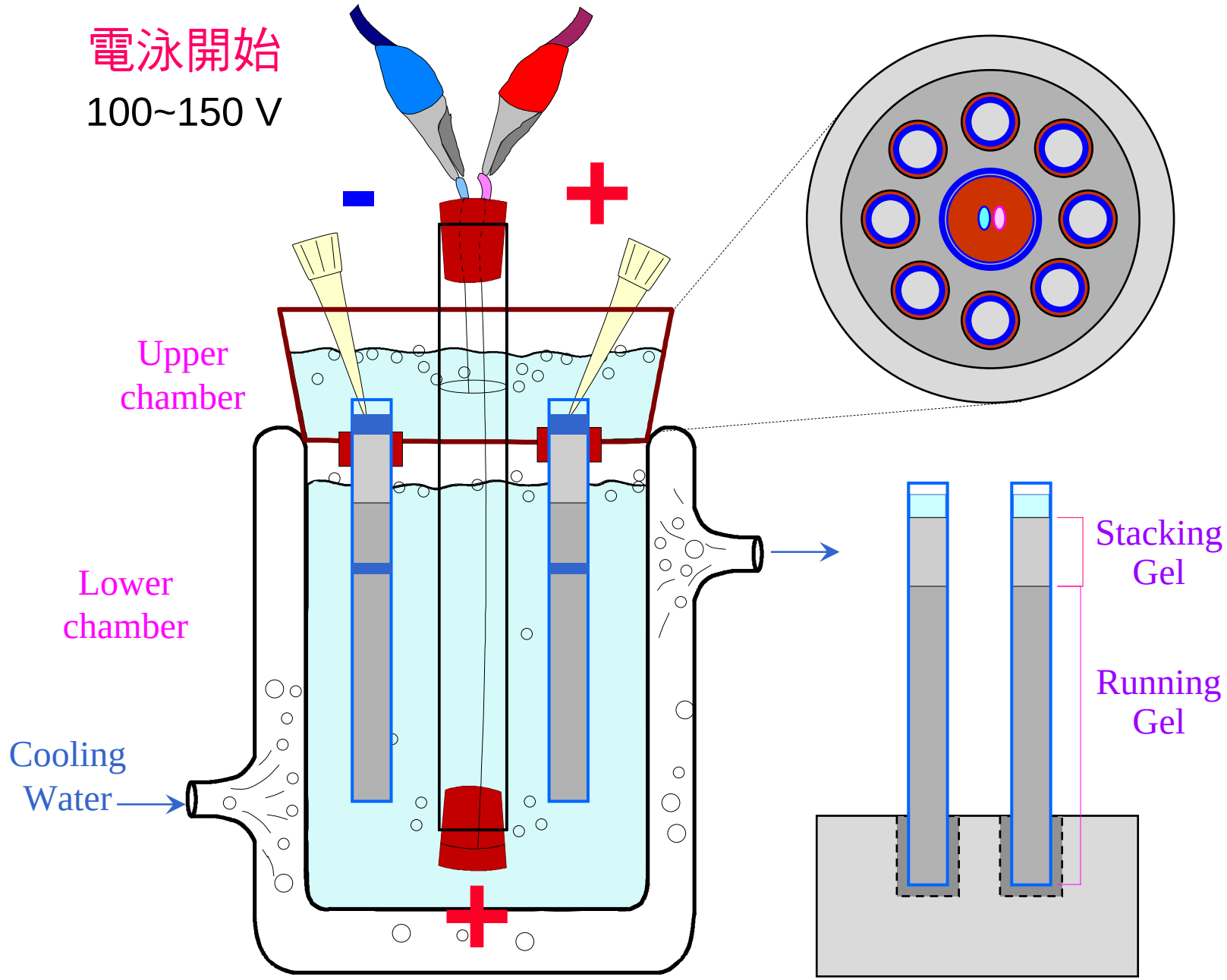
非極性尾部

電泳膠體系統的組成：

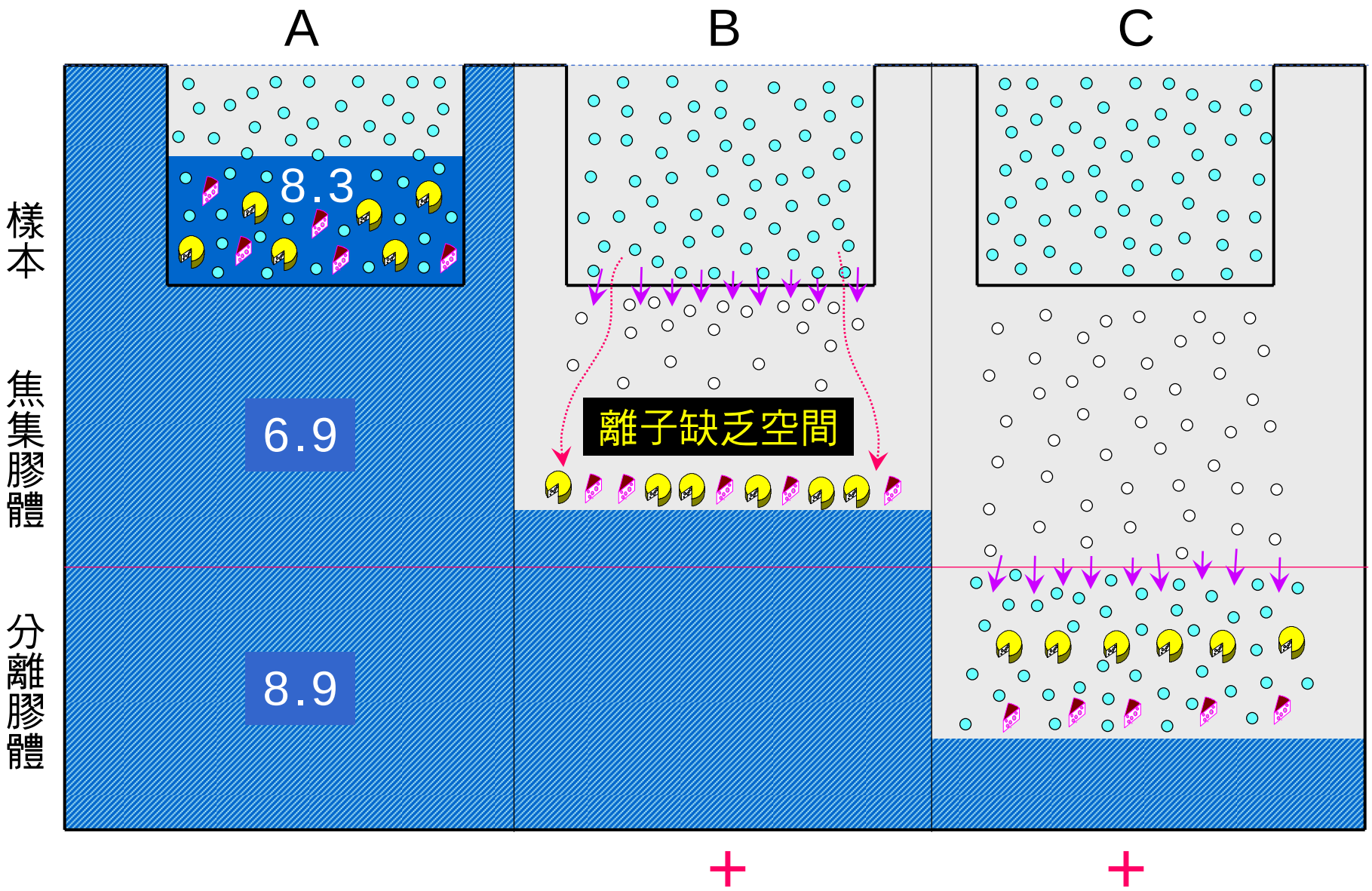
	電泳系統		緩衝液	pH	膠體濃度
1	上層 (負極) 緩衝液		Tris-glycine	8.3	-
2	樣本溶液		Tris-glycine	8.3	-
3	膠體	焦集膠體	Tris-HCl	6.9	5%
4	膠體	分離膠體	Tris-HCl	8.3	7.5~20%
5	下層 (正極) 緩衝液		Tris-glycine	8.3	-

- 膠體不連續性有焦聚樣本的作用

直立式電泳裝置：

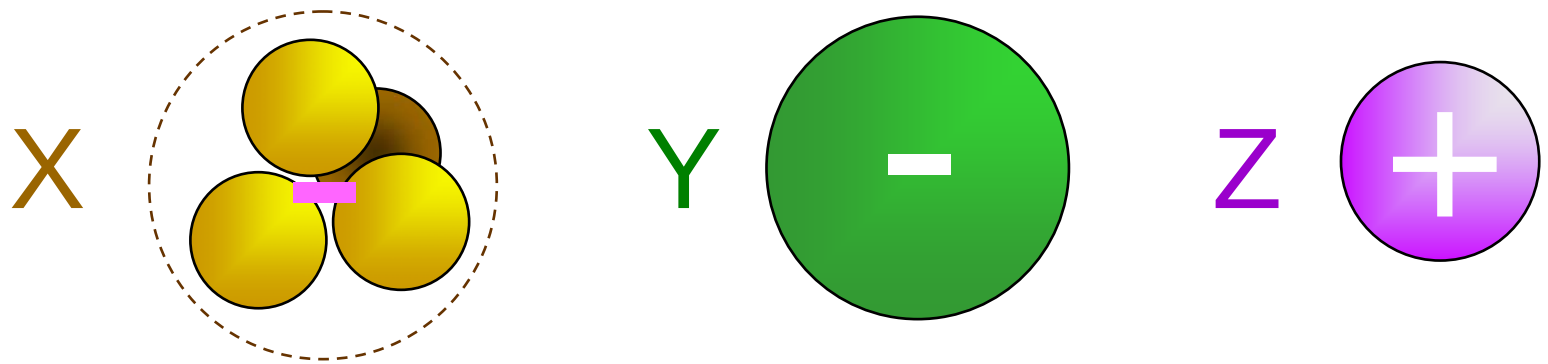

電泳開始
100~150 V
-
+
Upper chamber
Lower chamber
Cooling Water
Stacking Gel
Running Gel

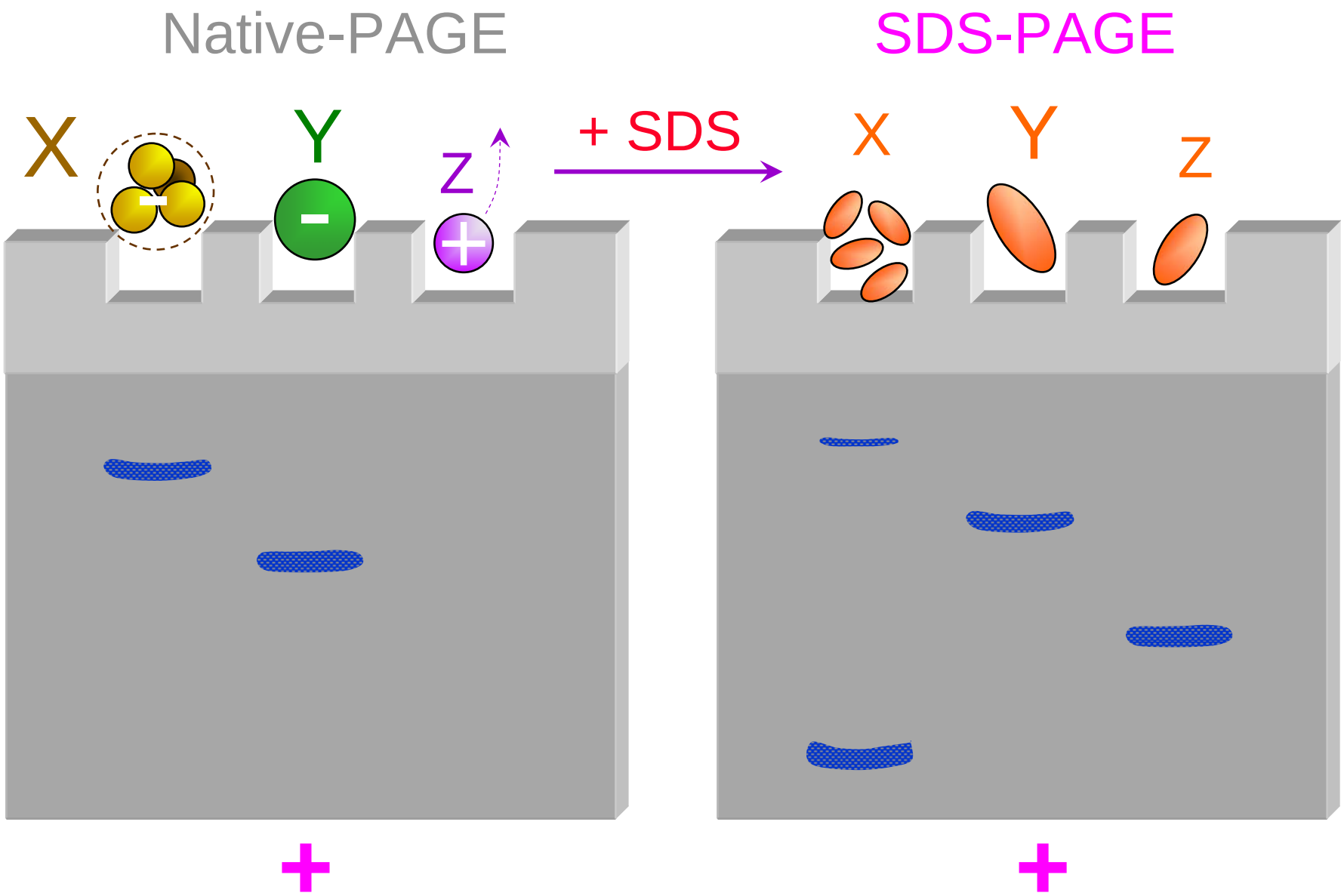
焦集膠體對蛋白質分子的焦集作用：



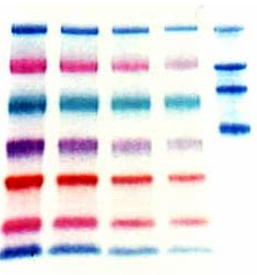
三種不同性質蛋白質的電泳比較：

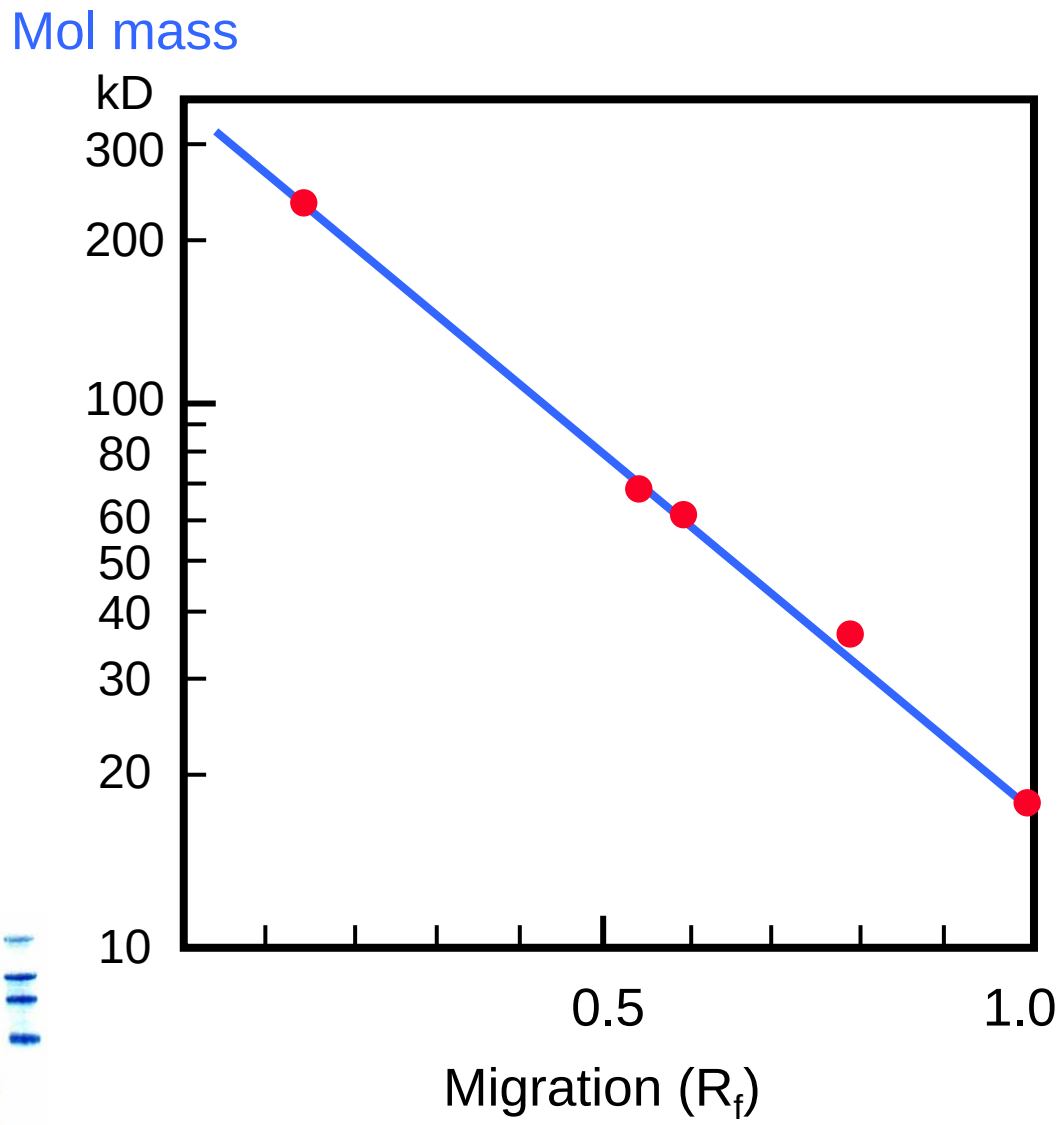
Protein	Quaternary Structure	Molecular Weight	pI	Mobility	
				Native PAGE	SDS-PAGE
X	Tetramer	(40,000)x4	5.8	Slow	Fast
Y	Monomer	88,000	5.2	Fast	Slow
Z	Monomer	60,000	9.3	Upward	Medium

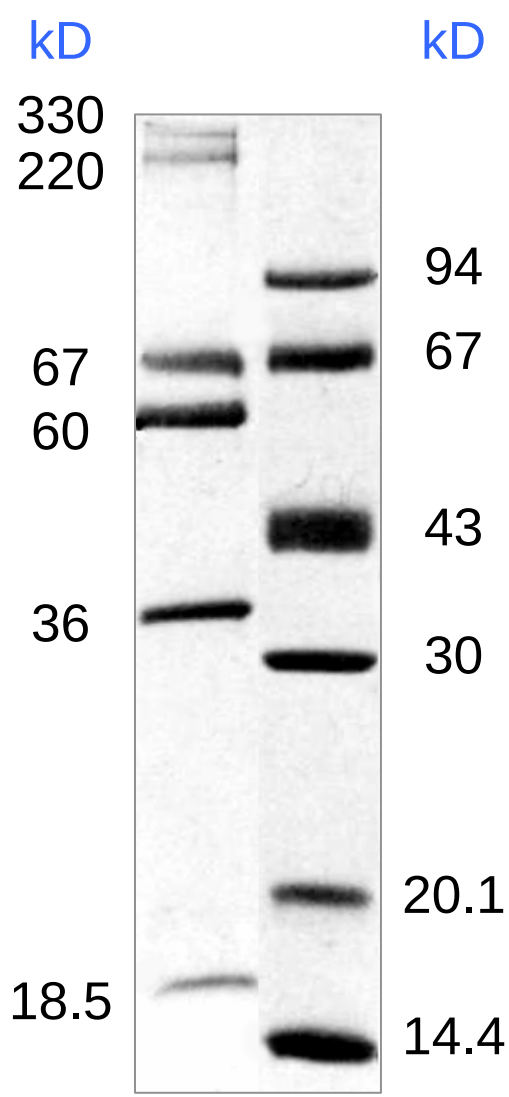



Native-PAGE
SDS-PAGE
X
Y
Z
+ SDS
X
Y
Z
+
+
分子量 及 淨電荷密度
均影響泳動率
只有 分子量 影響泳動率

單元體分子量的測定： SDS-PAGE

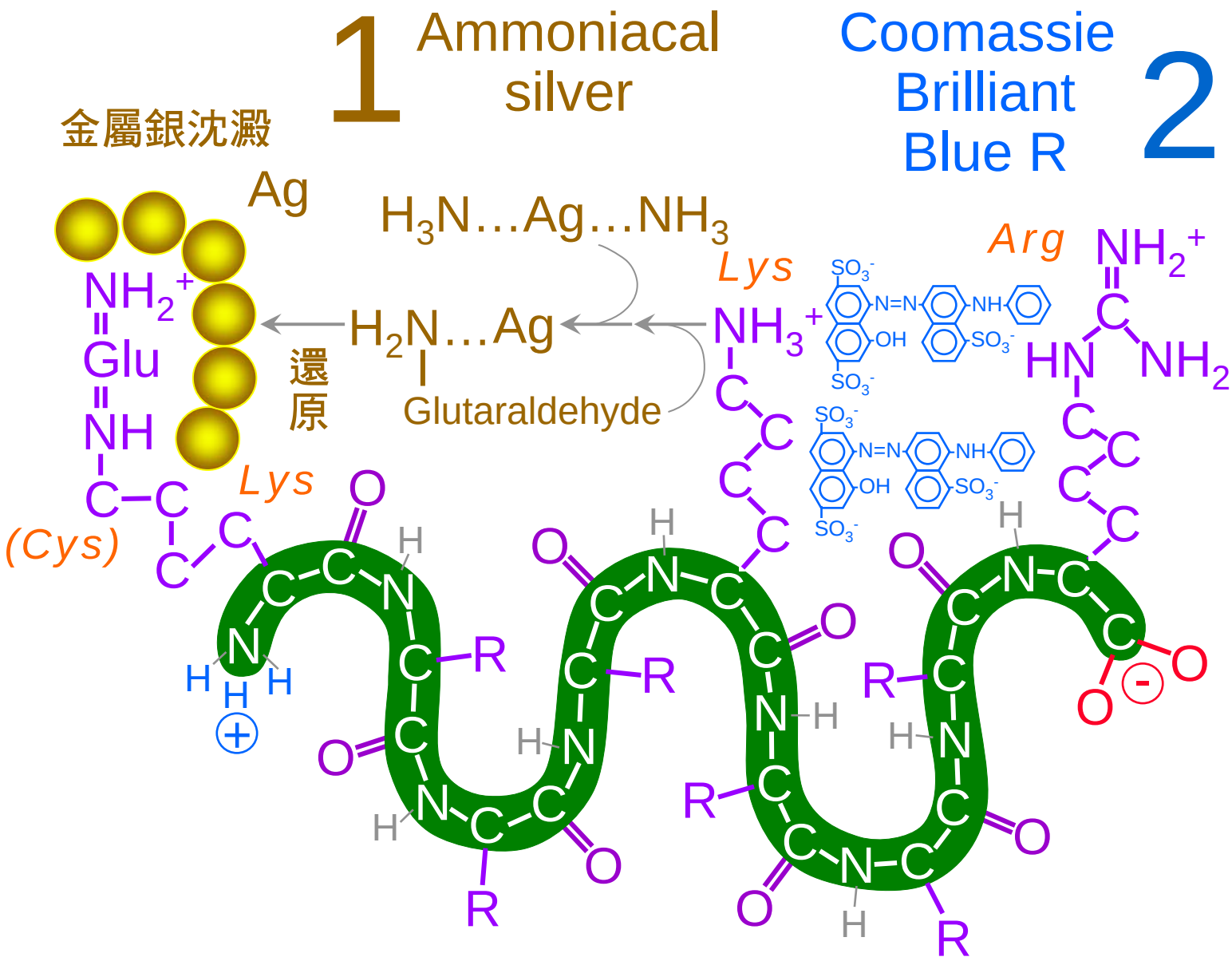






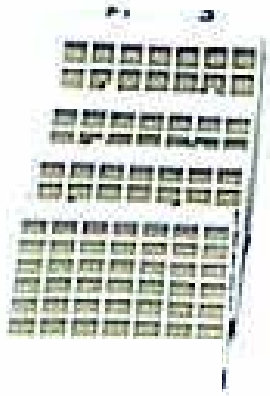
Pharmacia: Molecular Markers for electrophoresis

各種蛋白質染色法機制：

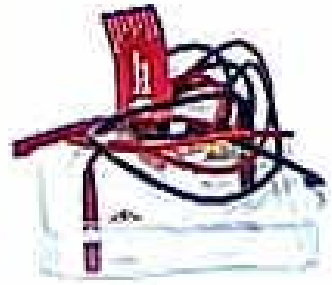

1
Ammoniacal silver
Coomassie Brilliant Blue R
2
金屬銀沈澱
Ag
$H_3N…Ag…NH_3$
$H_2N…Ag$
Glutaraldehyde
還原
Lys
Arg
(Cys)
Glu
NH
NH_2^+
NH_3^+
HN
NH_2
OH
SO_3^-
N=N
NH

電泳槽及相關設備：

轉印三明治

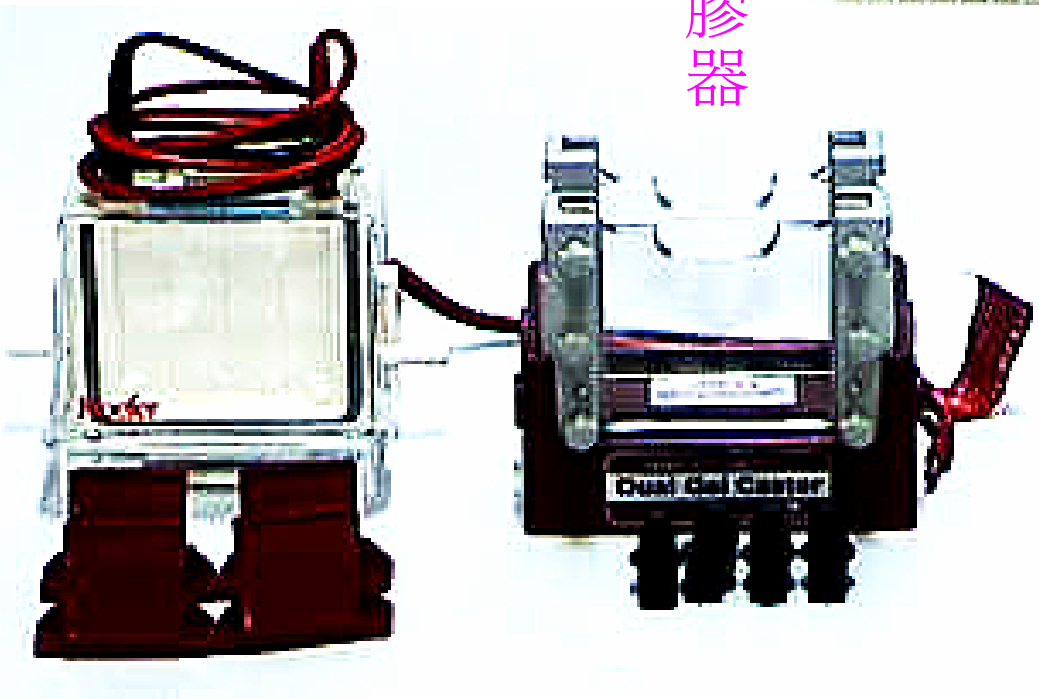


轉印槽



電泳槽

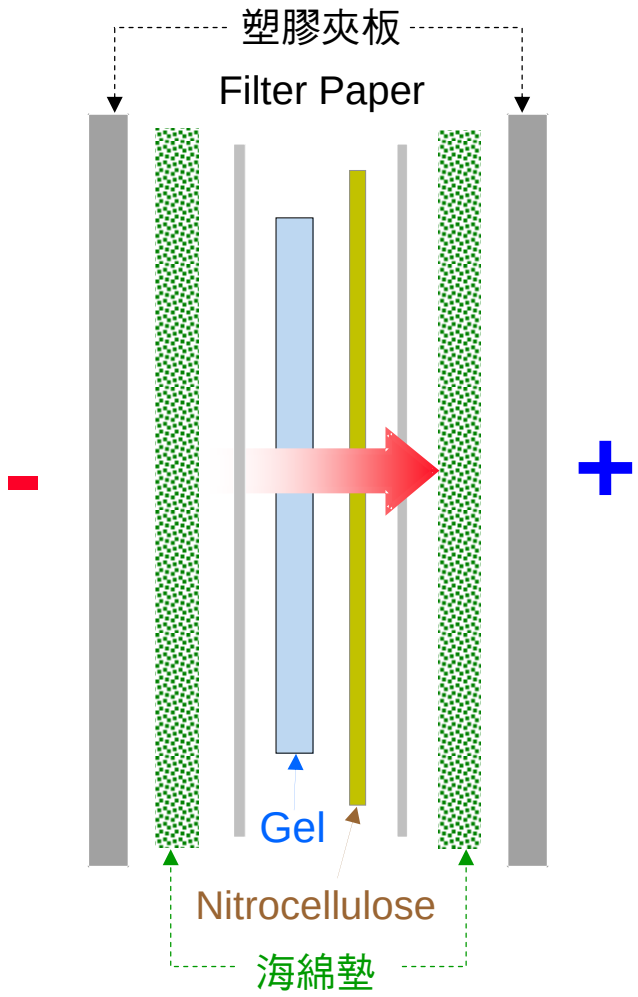
鑄膠器



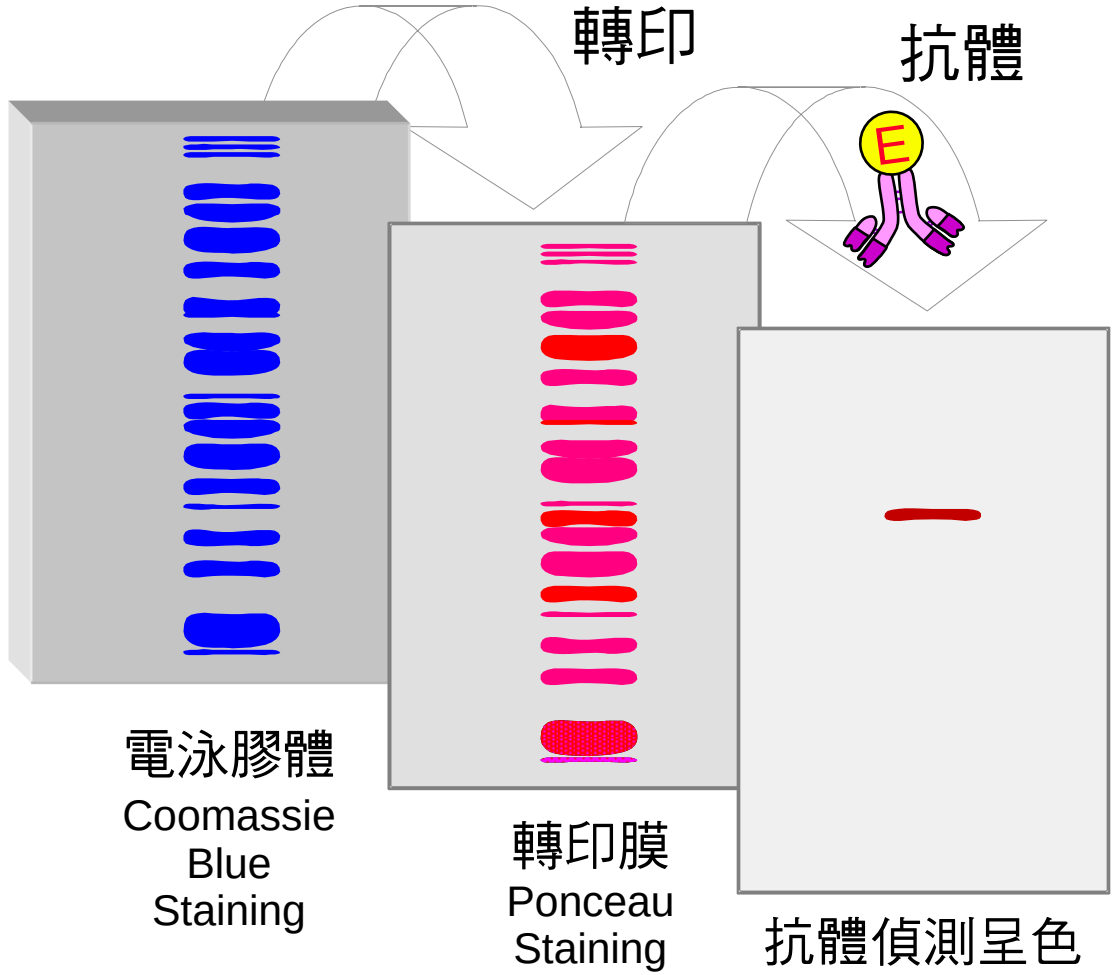
供電器

轉印及免疫染色流程：

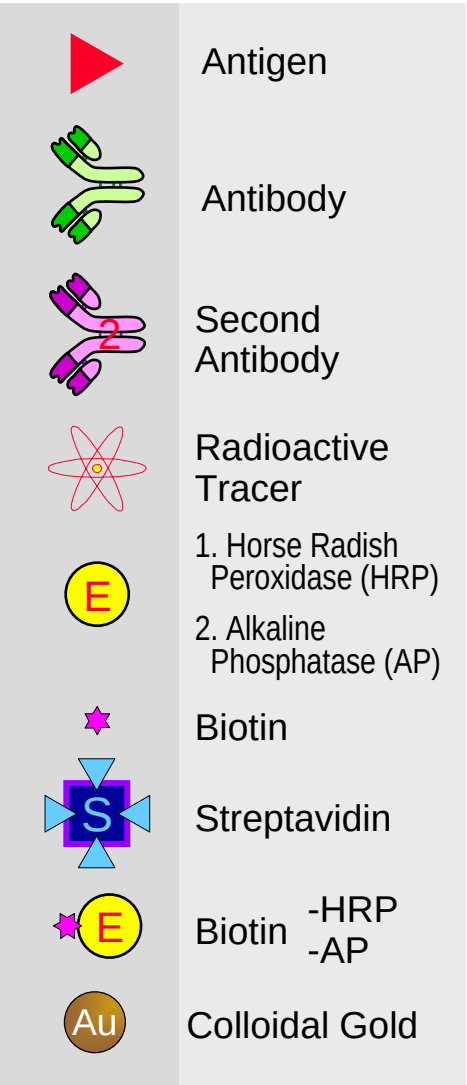
A 轉印三明治

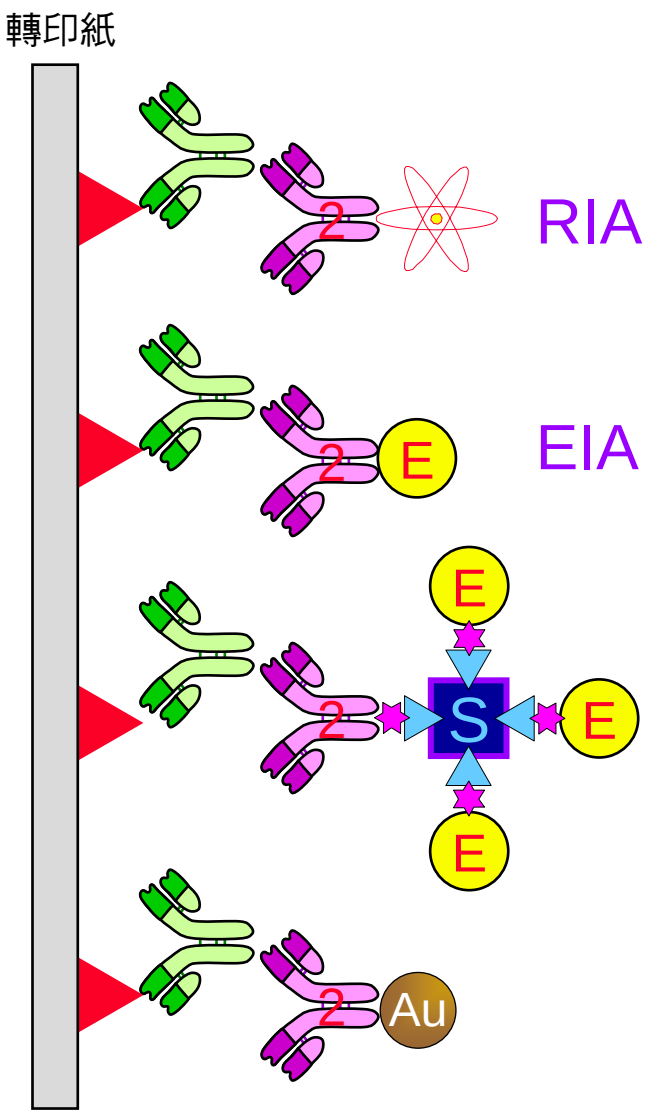


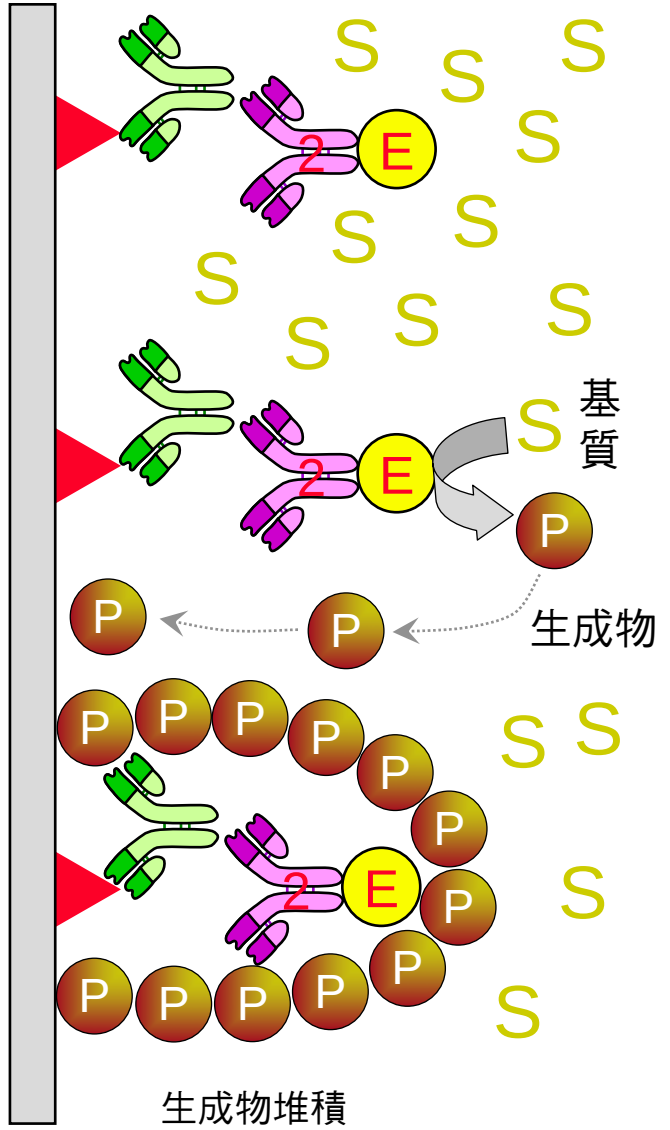
B 免疫染色流程及結果



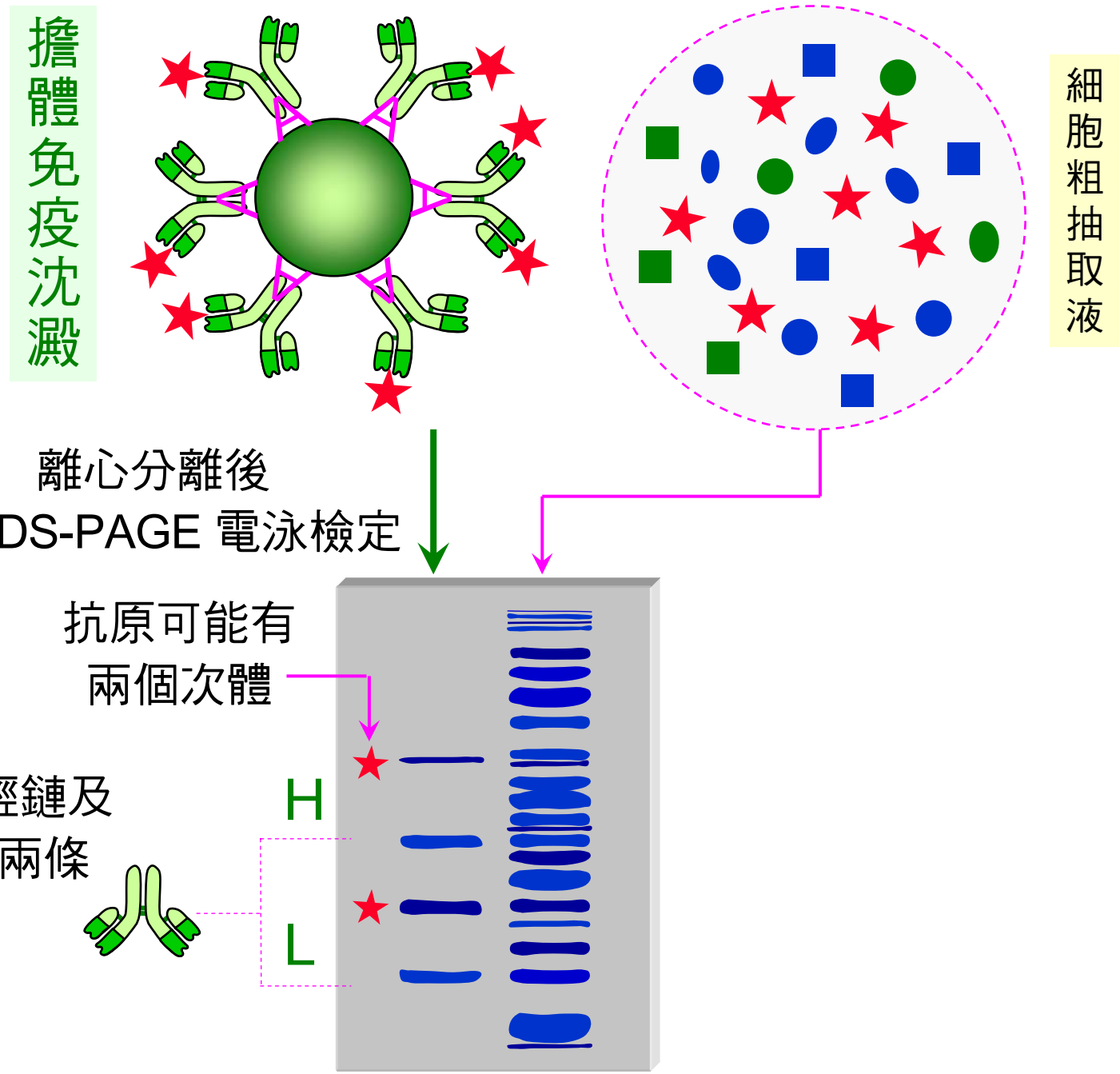
免疫轉印的種類與呈色機制：







擔體免疫沈澱的原理及應用：



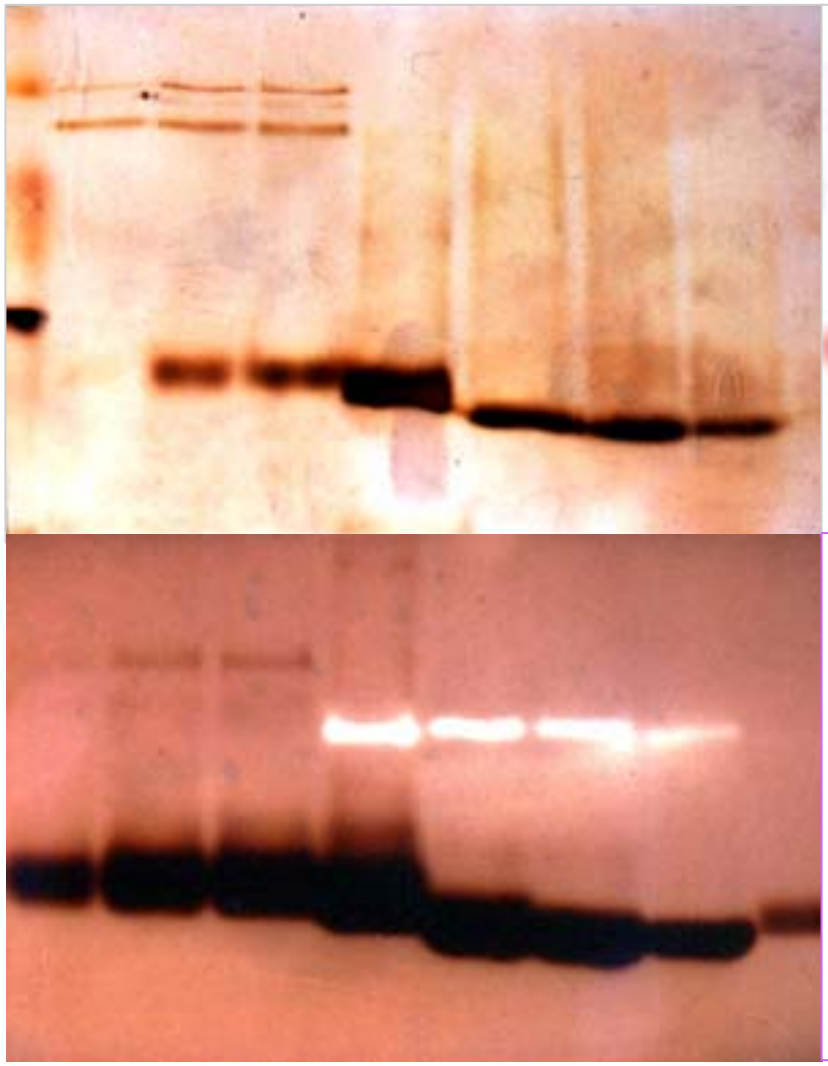
澱粉磷解酶染色方法比較：

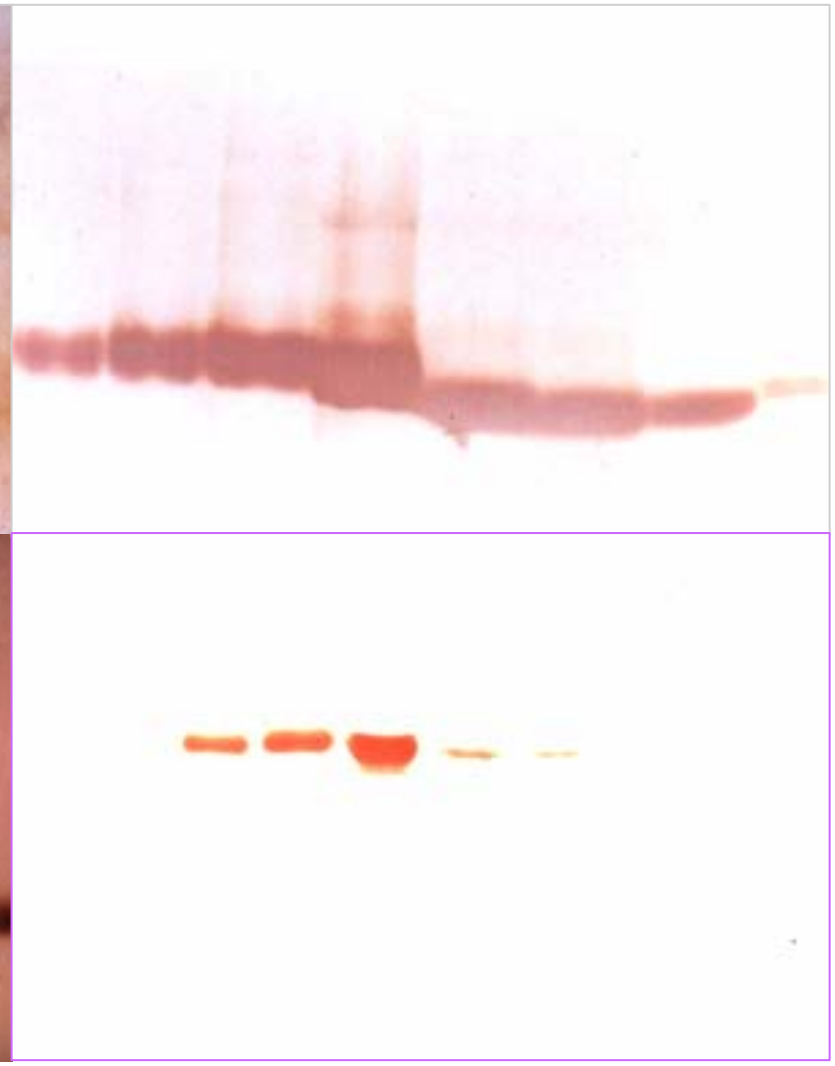
膠體過濾法各分劃

免疫轉印

硝酸銀

活性染色

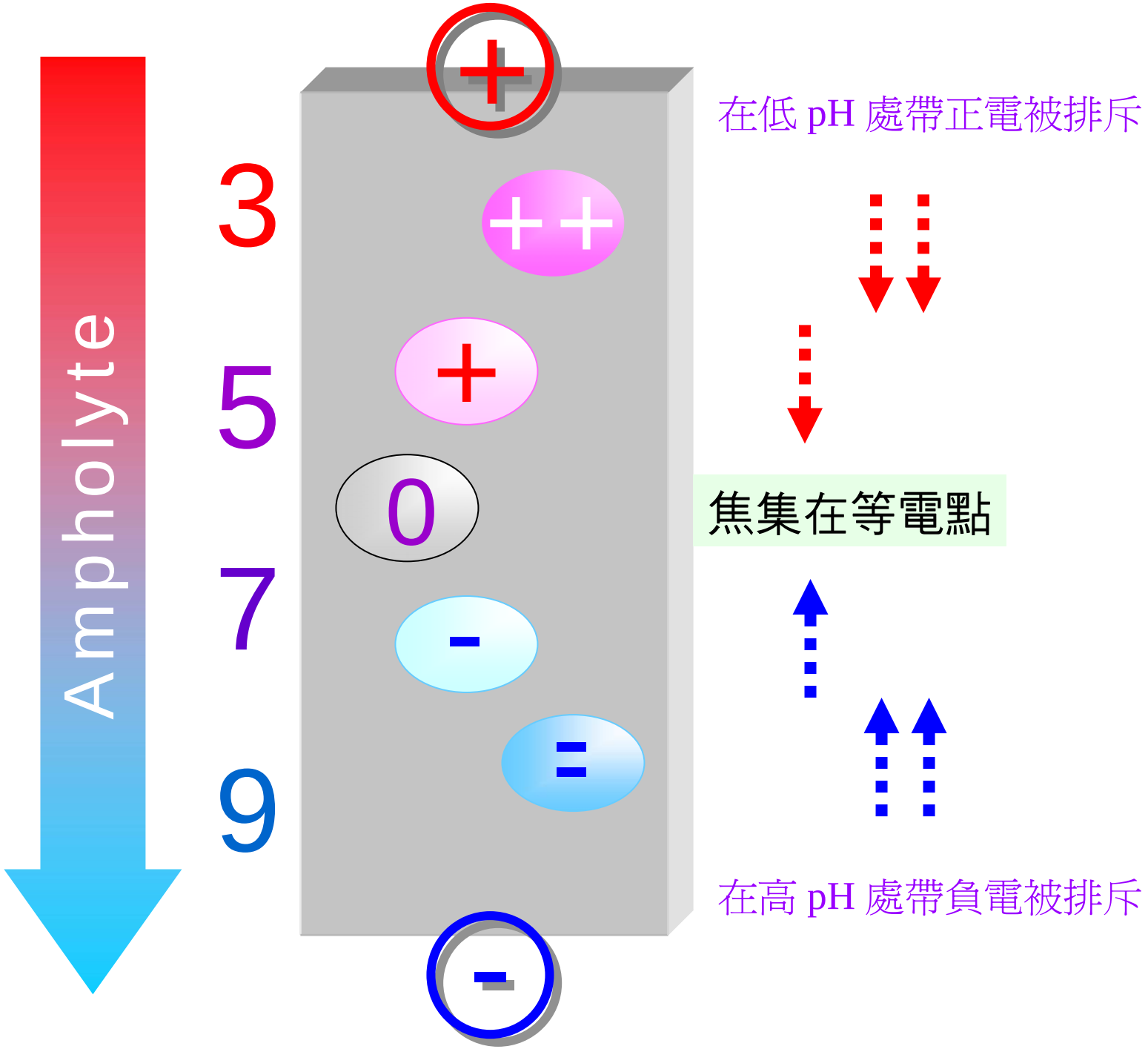




Disc-PAGE

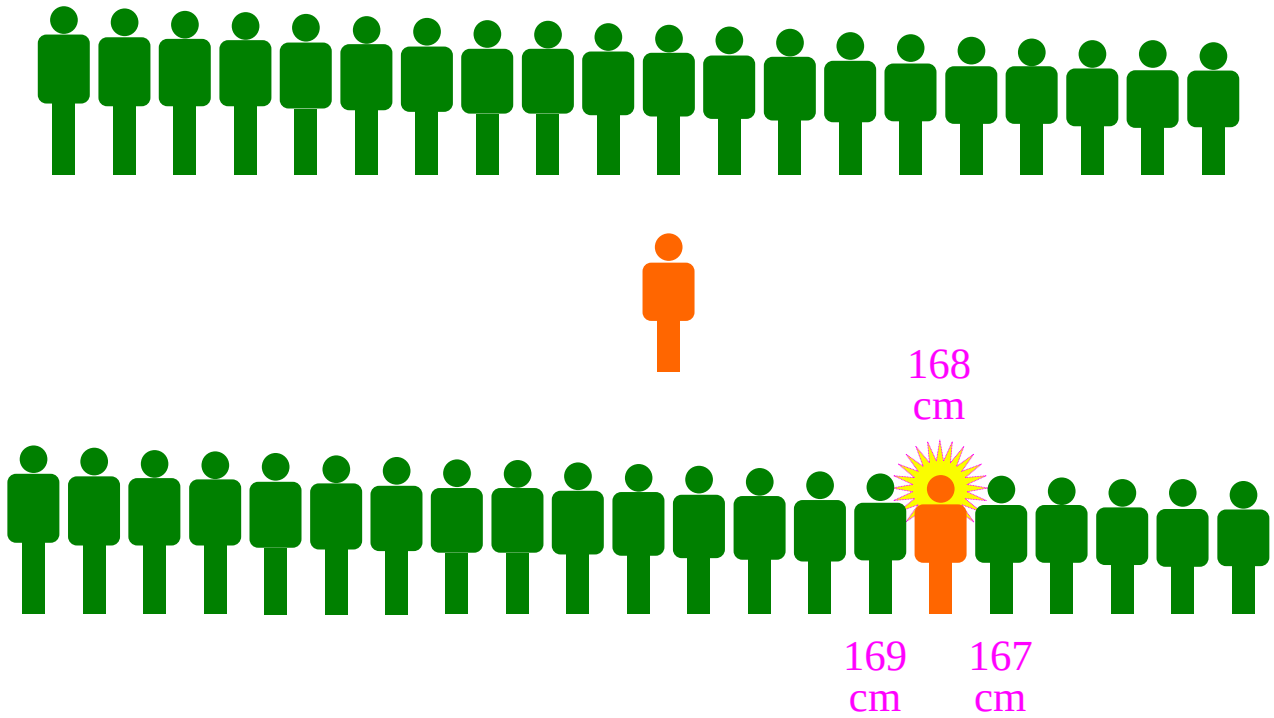
SDS-PAGE

等電焦集法的焦集方式：



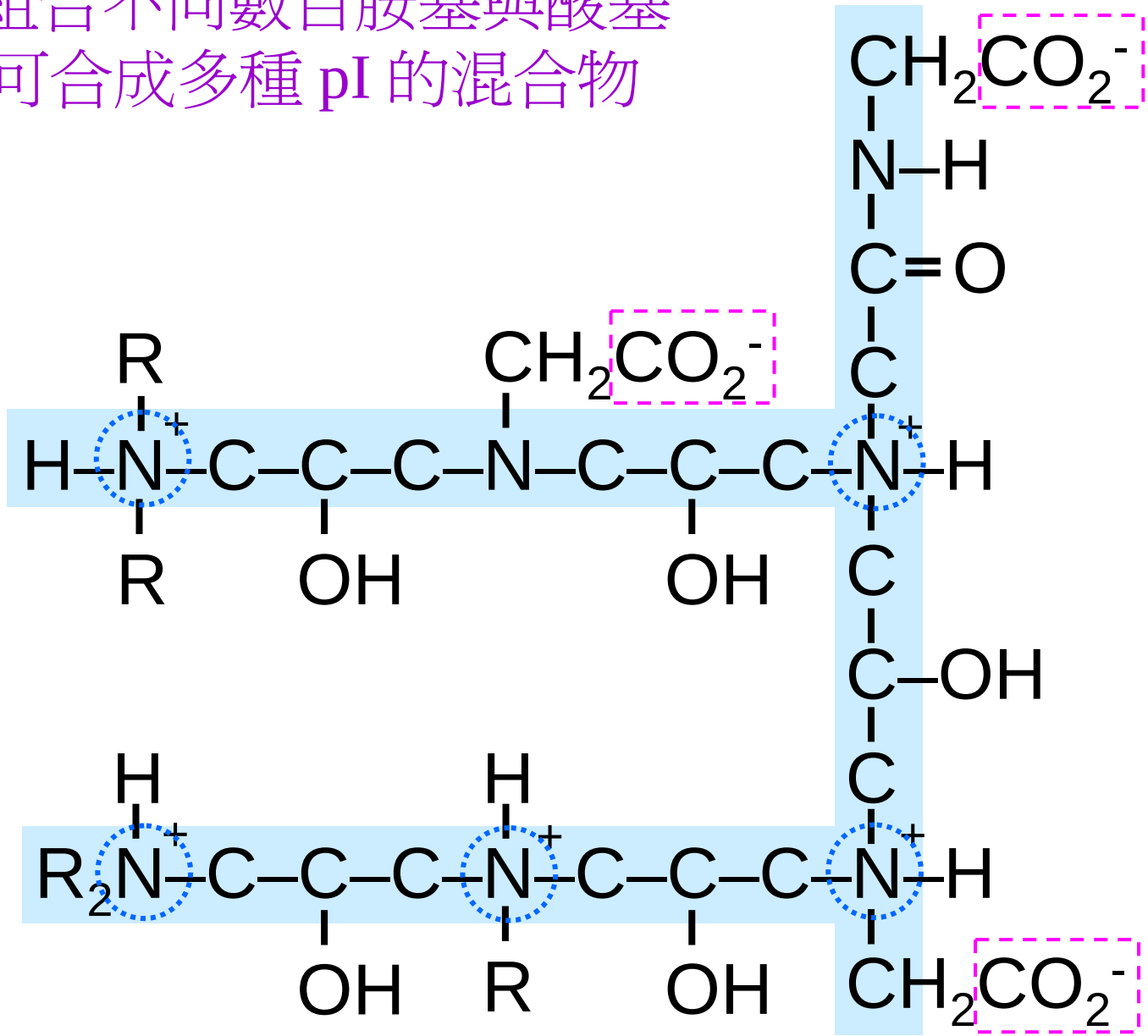
3.3.2 等電焦集法原理：

樣本分子 在一已知 pH 梯度 中焦集

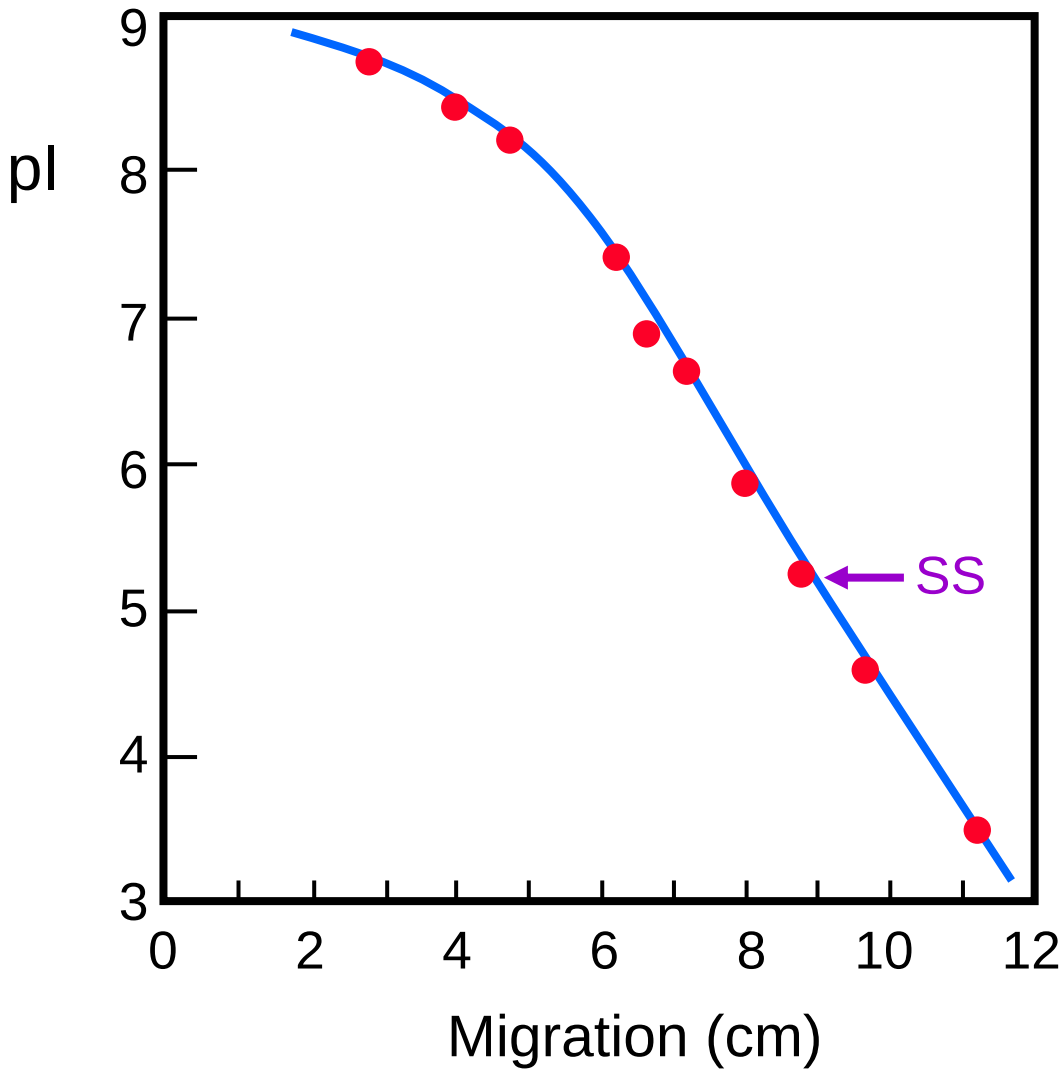


等電焦集的雙性離子構造：

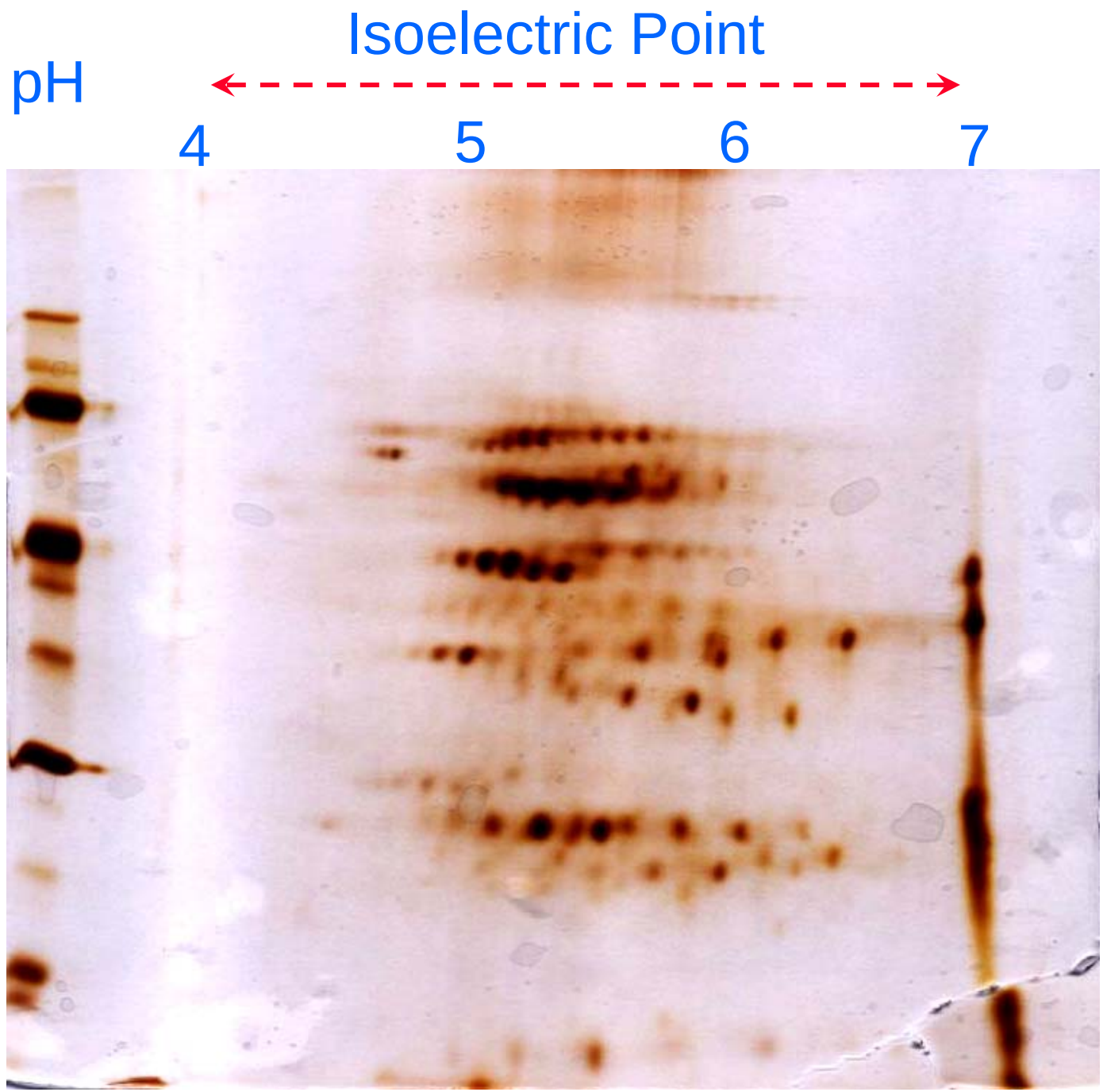
- 組合不同數目胺基與酸基可合成多種 pI 的混合物



等電點標準校正線： IEF

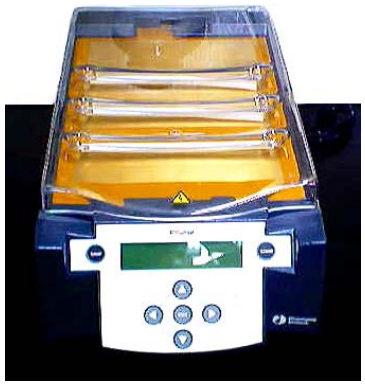


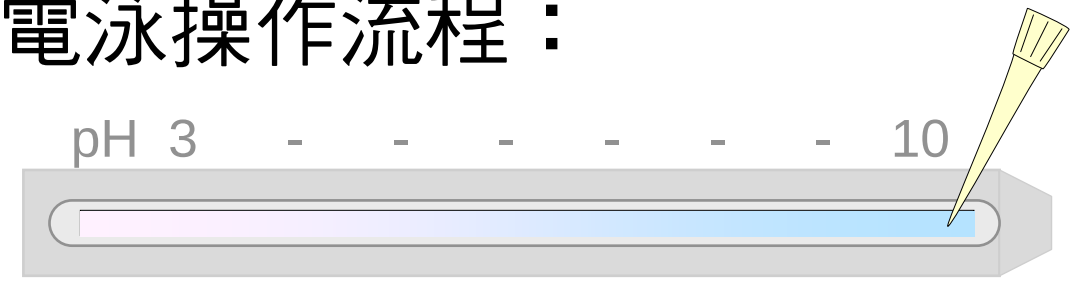
二次元電泳：

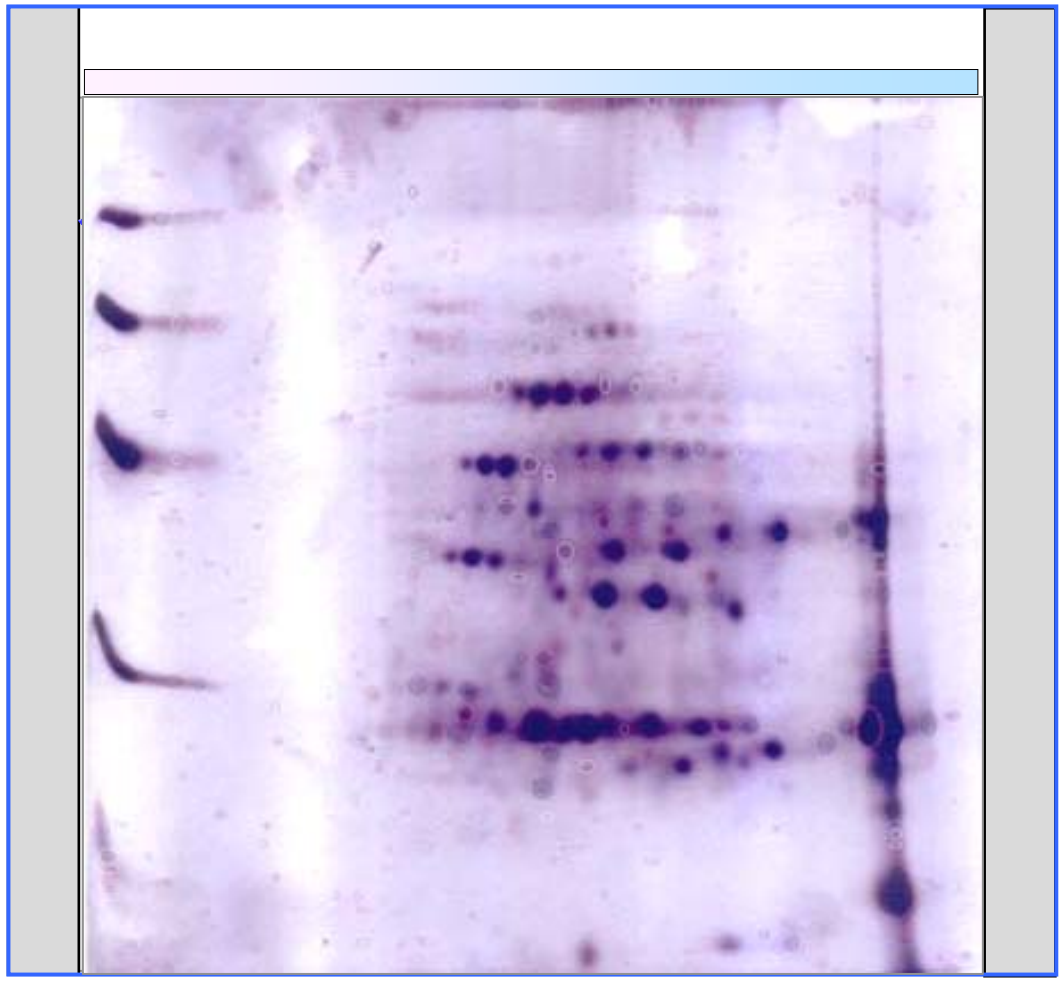


二次元電泳操作流程：

(1) IEF
等電焦集電泳





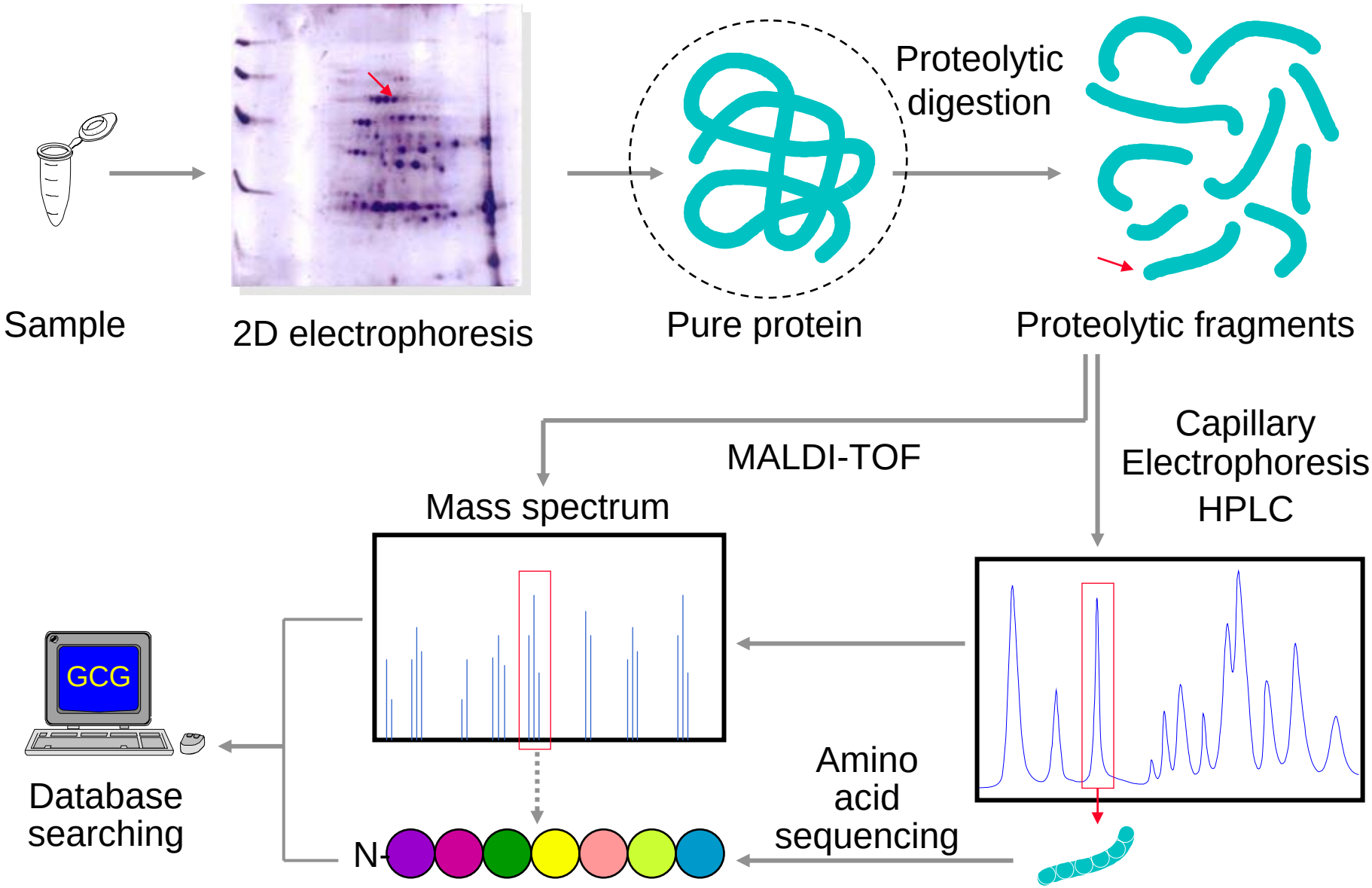


(2)
SDS-PAGE
分離膠體

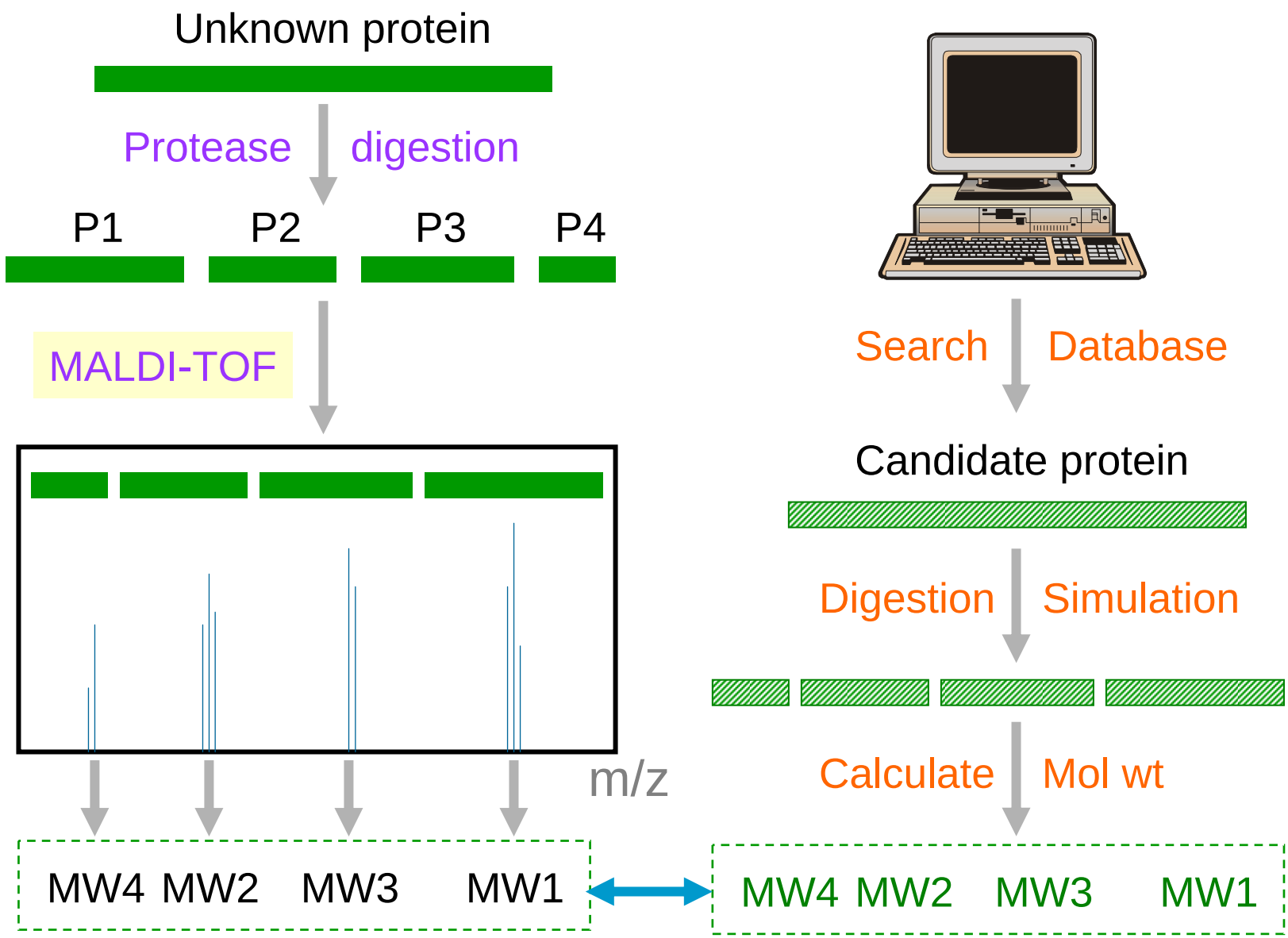
(3)
染色脫色

Juang RH (2004) Proteomics

蛋白質體可綜觀蛋白質的消長與身分：

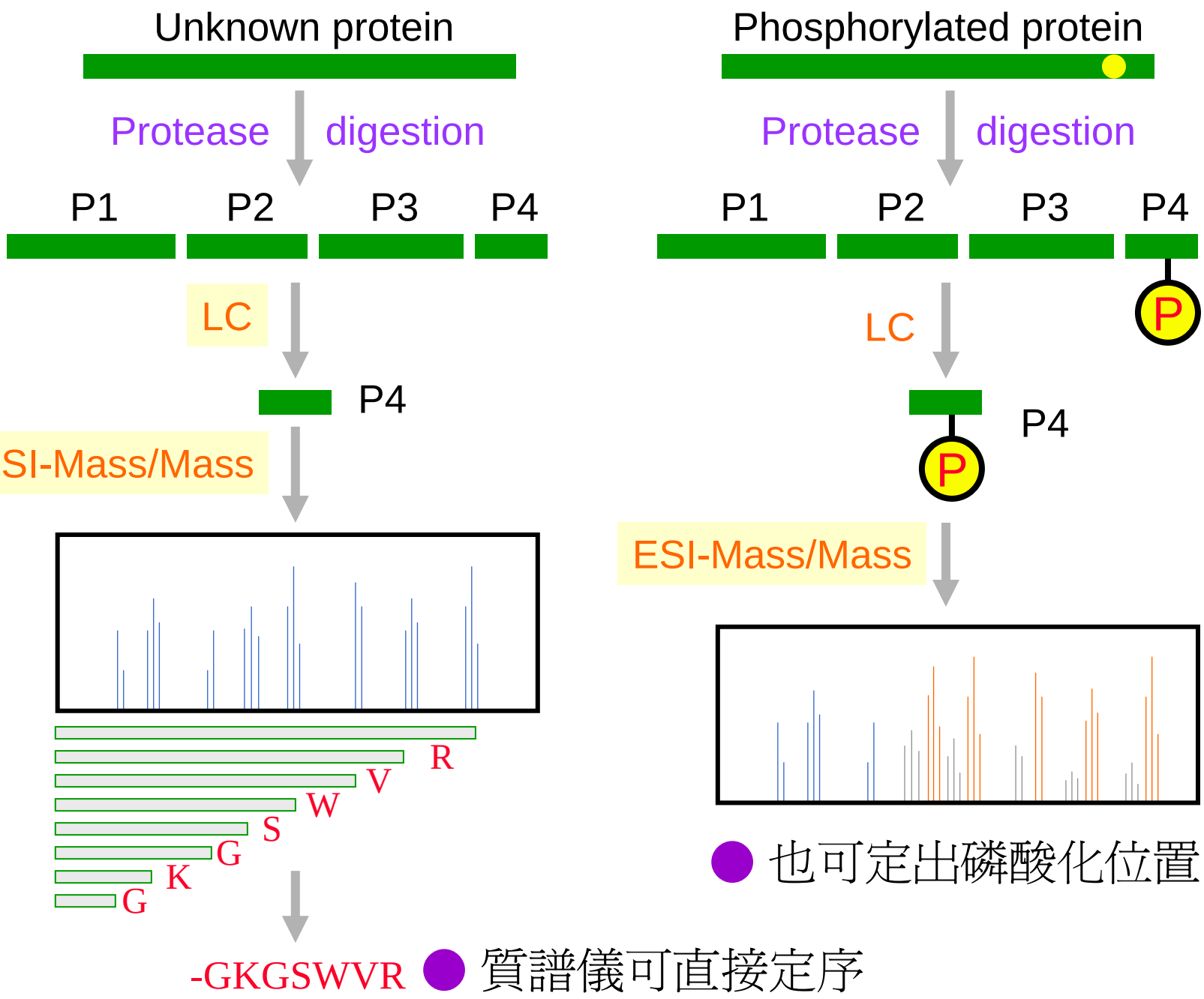


質譜儀可檢定蛋白質身分：



- 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