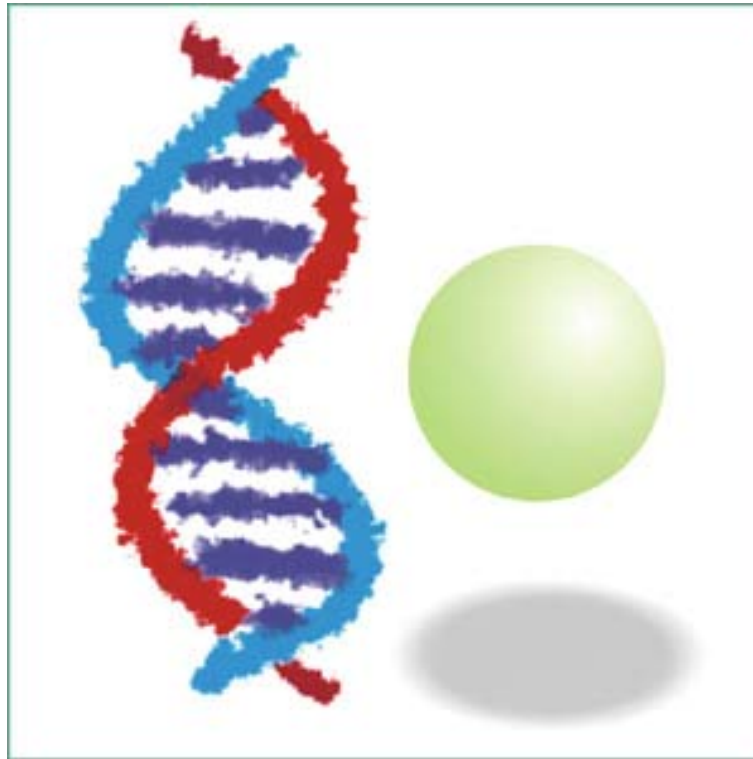


BST

生化科技系



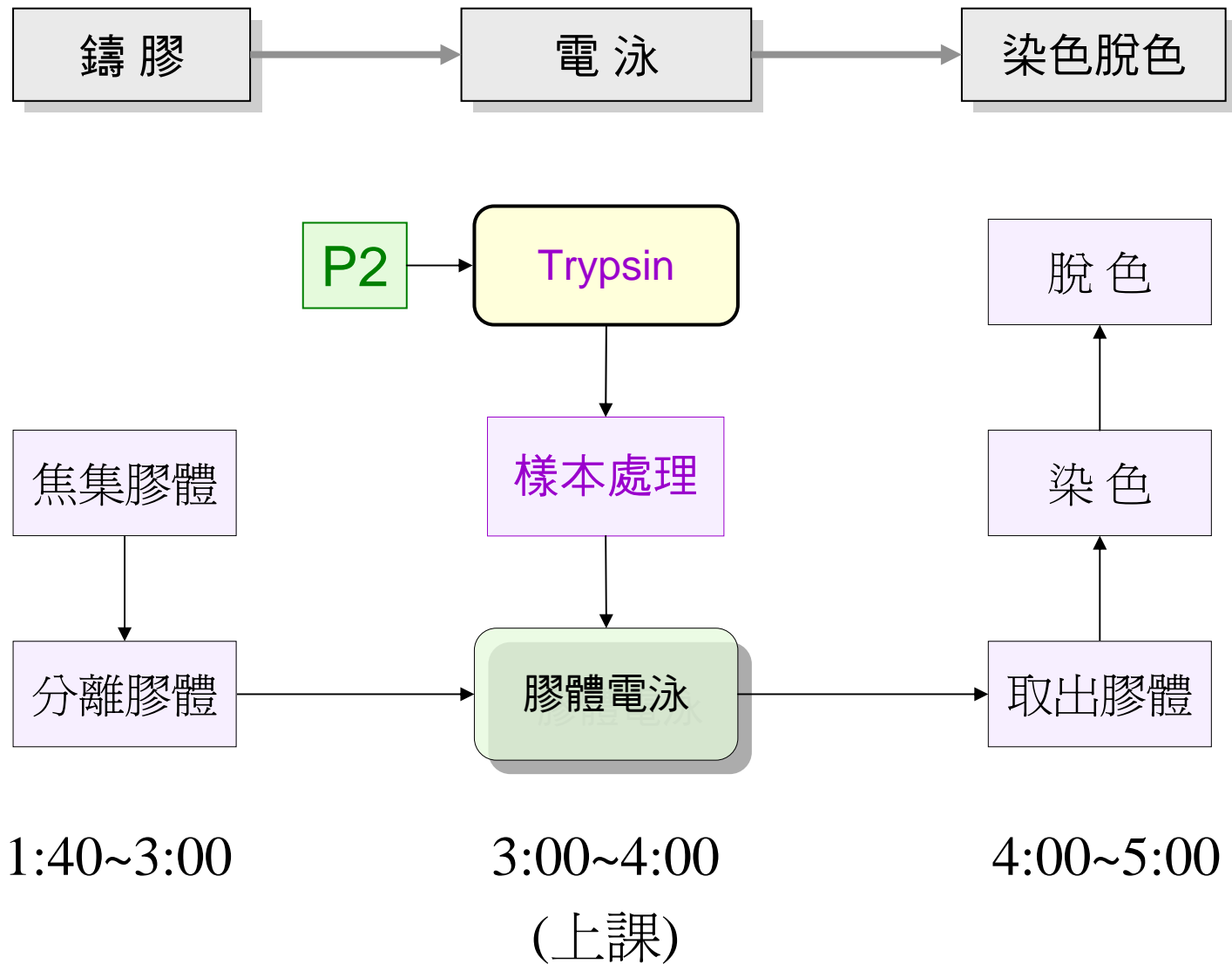
BCX

P3

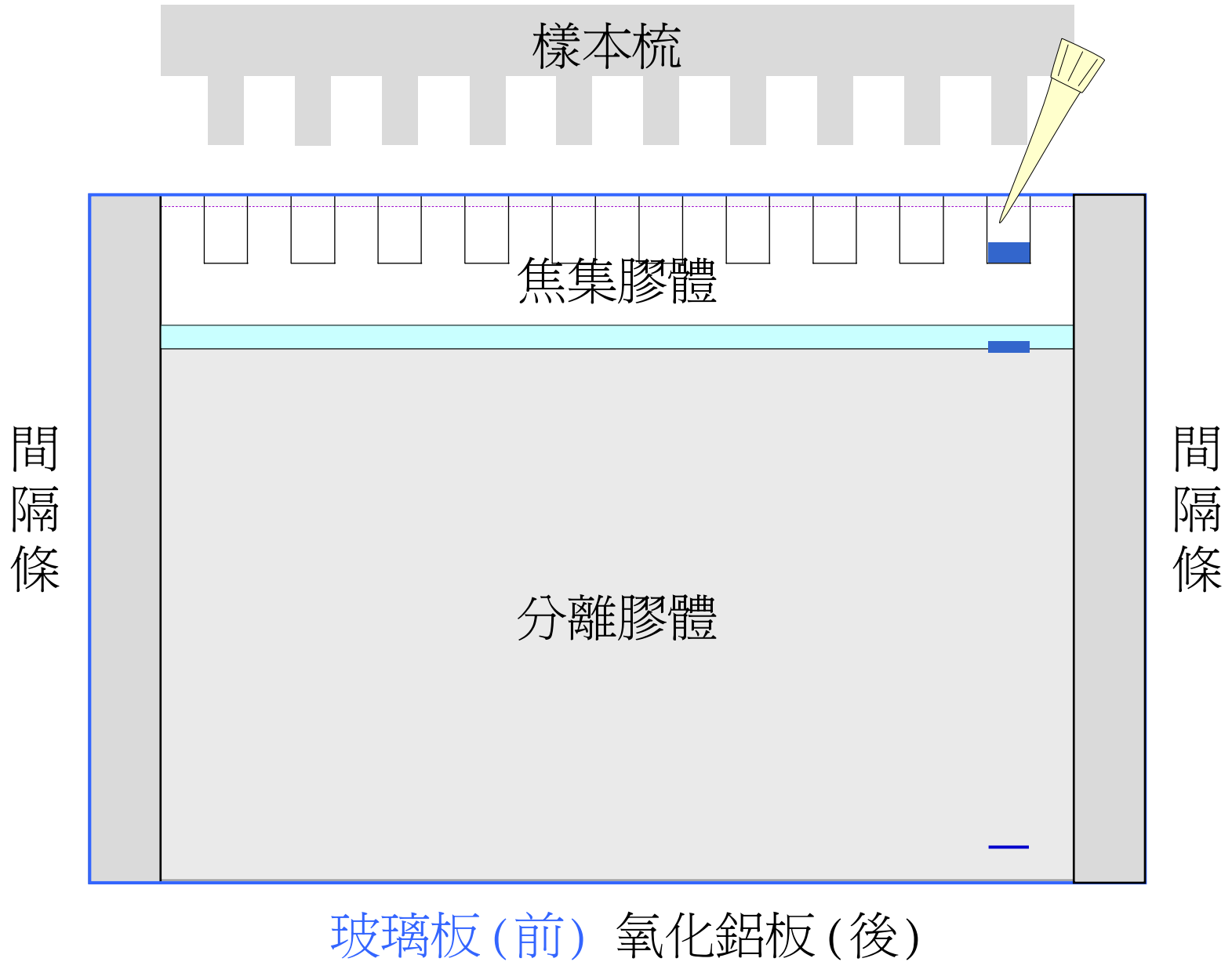
生物化學實驗

膠體電泳法

P3 膠體電泳法



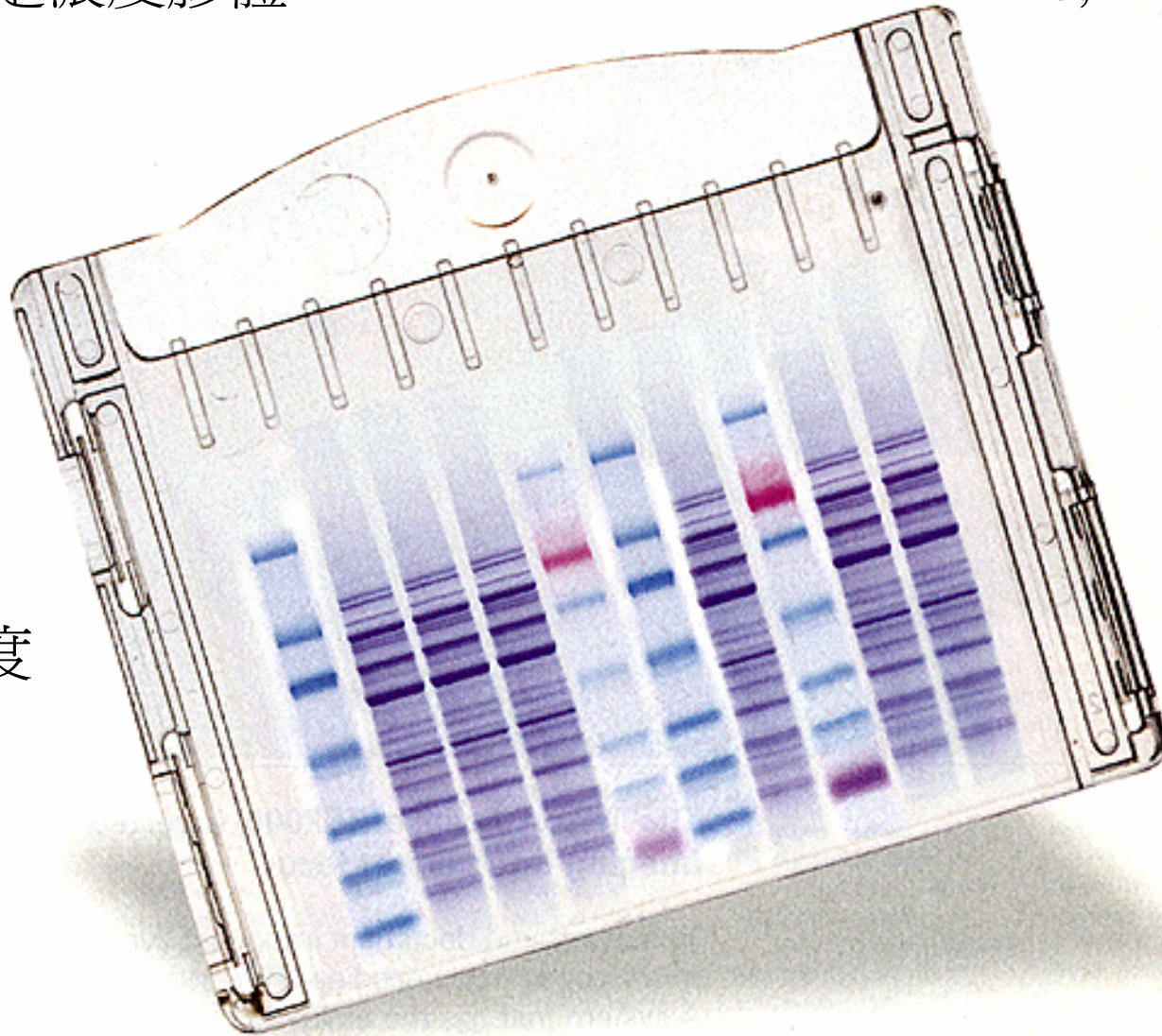
鑄膠過程與注入樣本



Pierce 商品供應預鑄膠片

梯度或固定濃度膠體

10, 12, 15 樣本槽



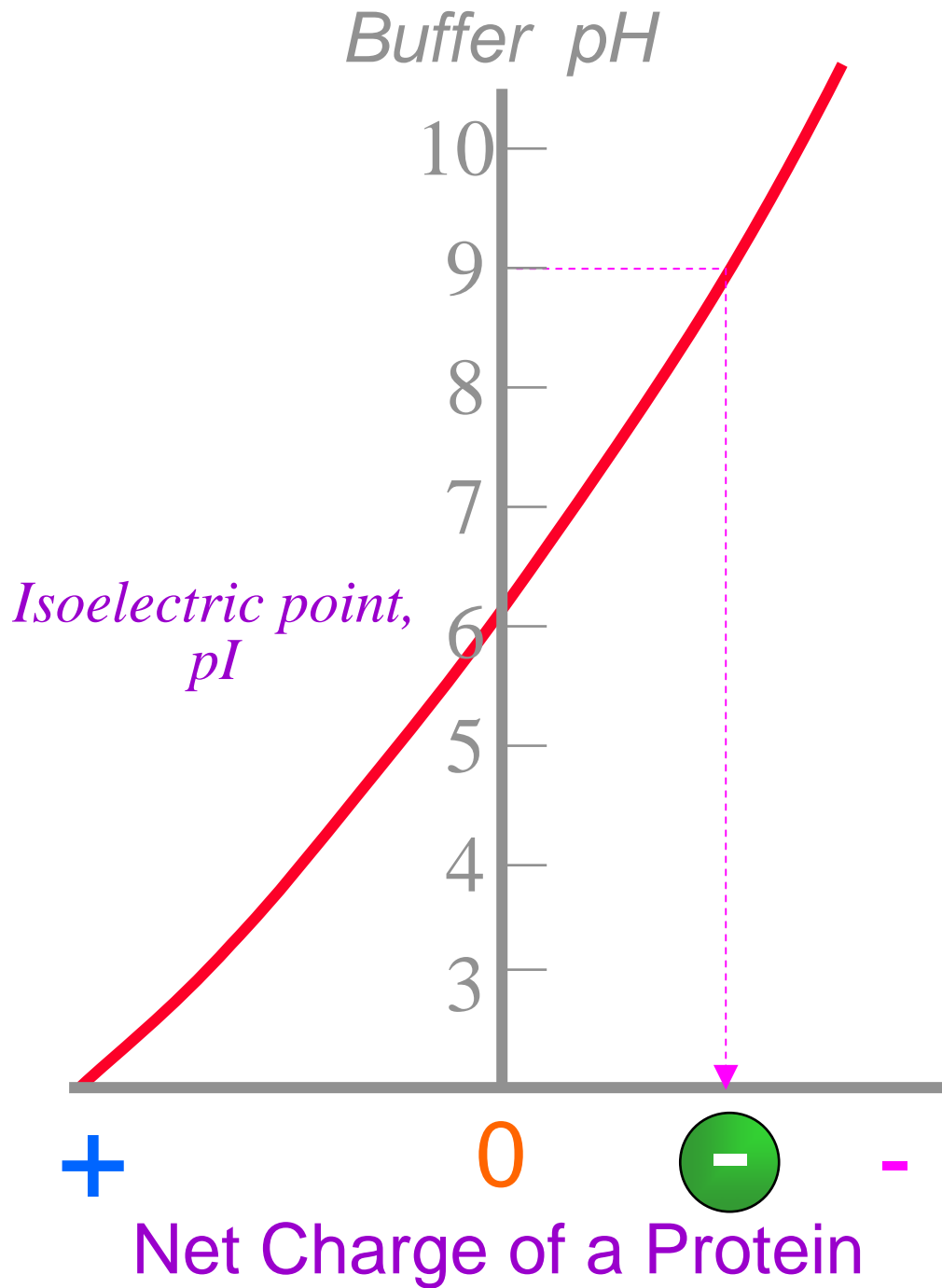
SDS 膠體

1 mm 厚度

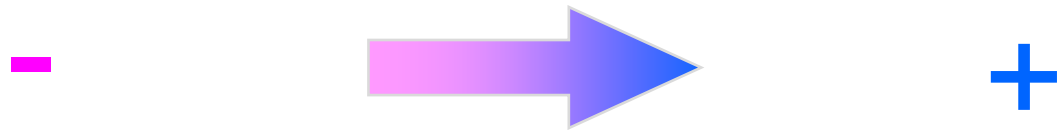
中性 pH 緩衝液

拋棄式塑膠外殼

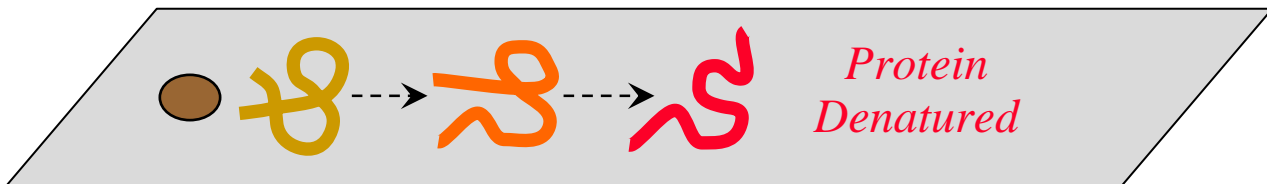
環境影響分子的帶電性質：



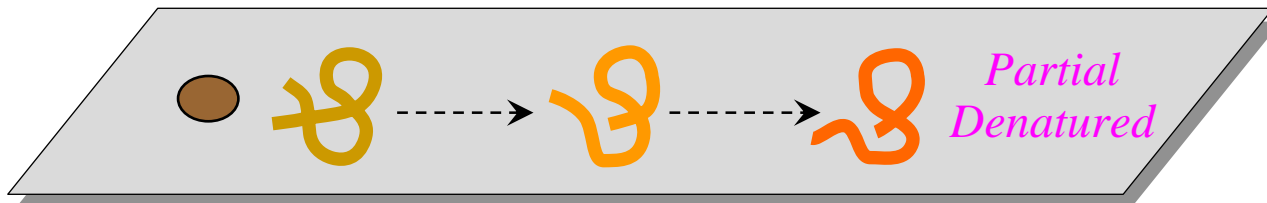
■ 電泳形式的演進：



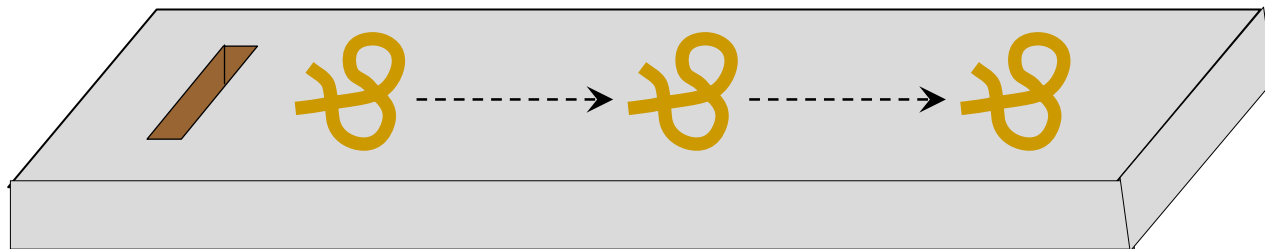
濾紙電泳 Cellulose



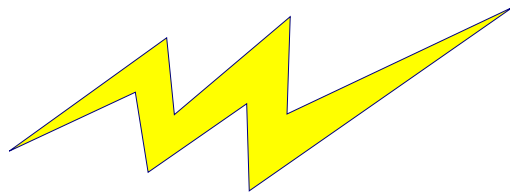
薄層電泳 (TLE) Cellulose acetate



膠體電泳 Starch → Gel



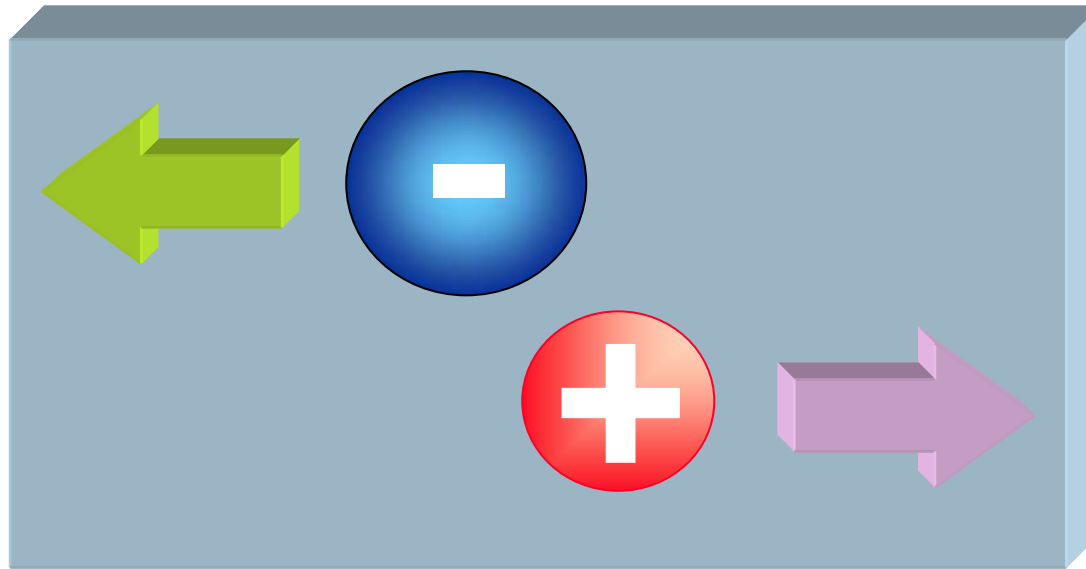
■ 影響電泳泳動率的因素：



外加電流電壓

Voltage

ANODE



CATHODE

Friction

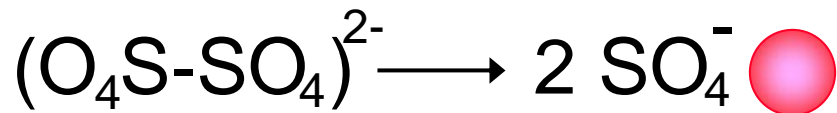
Charge

分子量 分子形狀

分子的等電點

■ 膠體的聚合反應：

Ammonium persulfate (free radical initiator)



自由基的生成者

Acrylamide (monomer)

成膠的基本單位

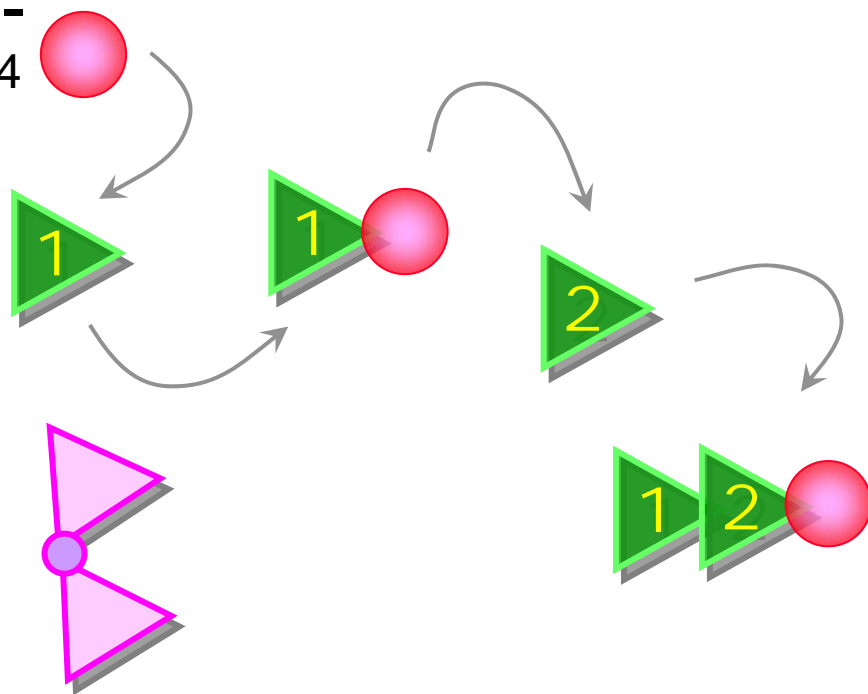
Bis(acrylamide) (bridge)

架橋使聚合產生分枝

TEMED (catalyst)

幫助傳遞自由基的催化劑

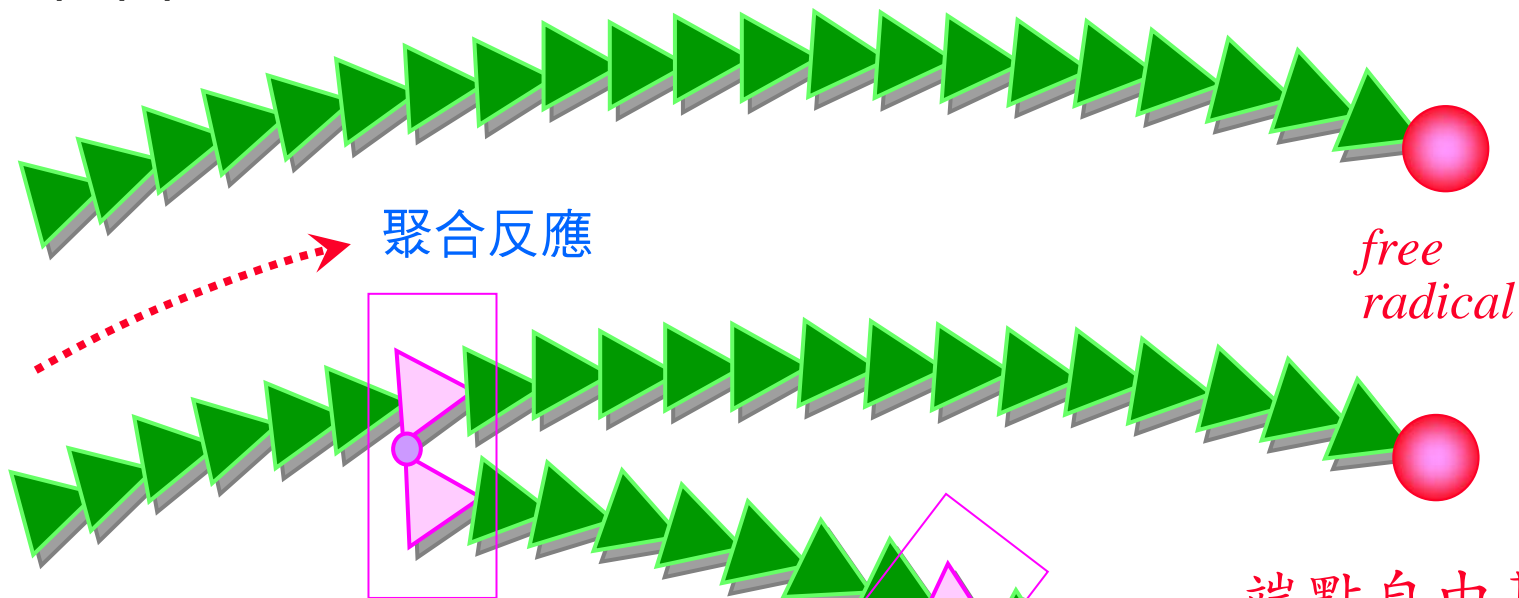
SDS (Sodium dodecyl sulfate)



Acrylamide 有毒性！
澱粉加高熱會誘生？

單體聚合反應：

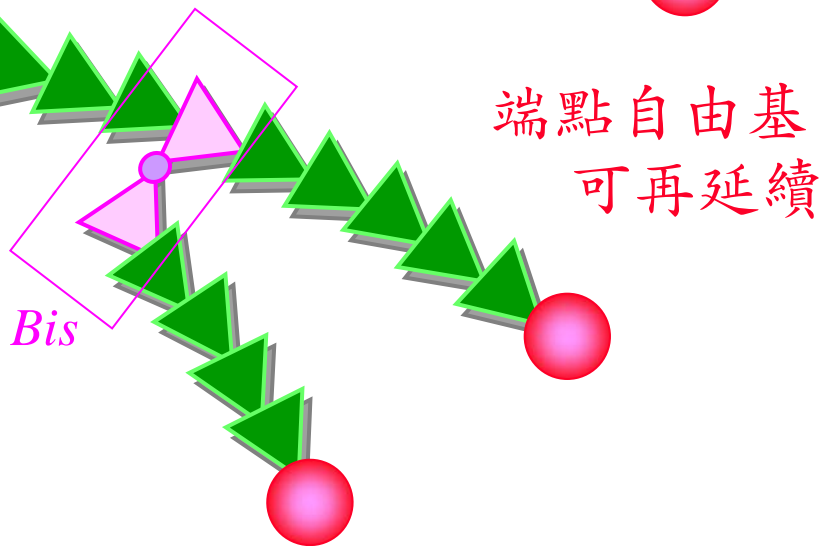
鑄膠反應



free radical

Bis

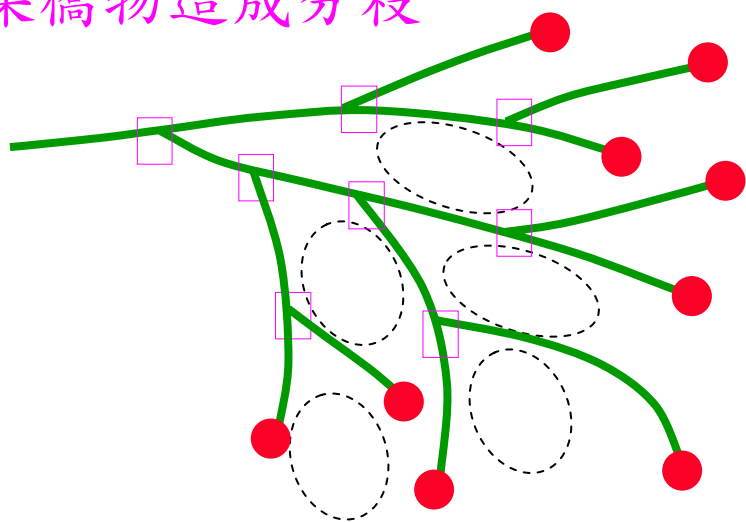
交錯連結



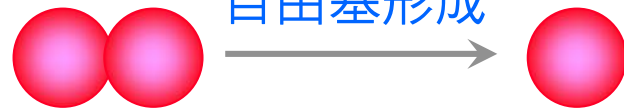
端點自由基
可再延續

Bis

架橋物造成分枝



自由基形成



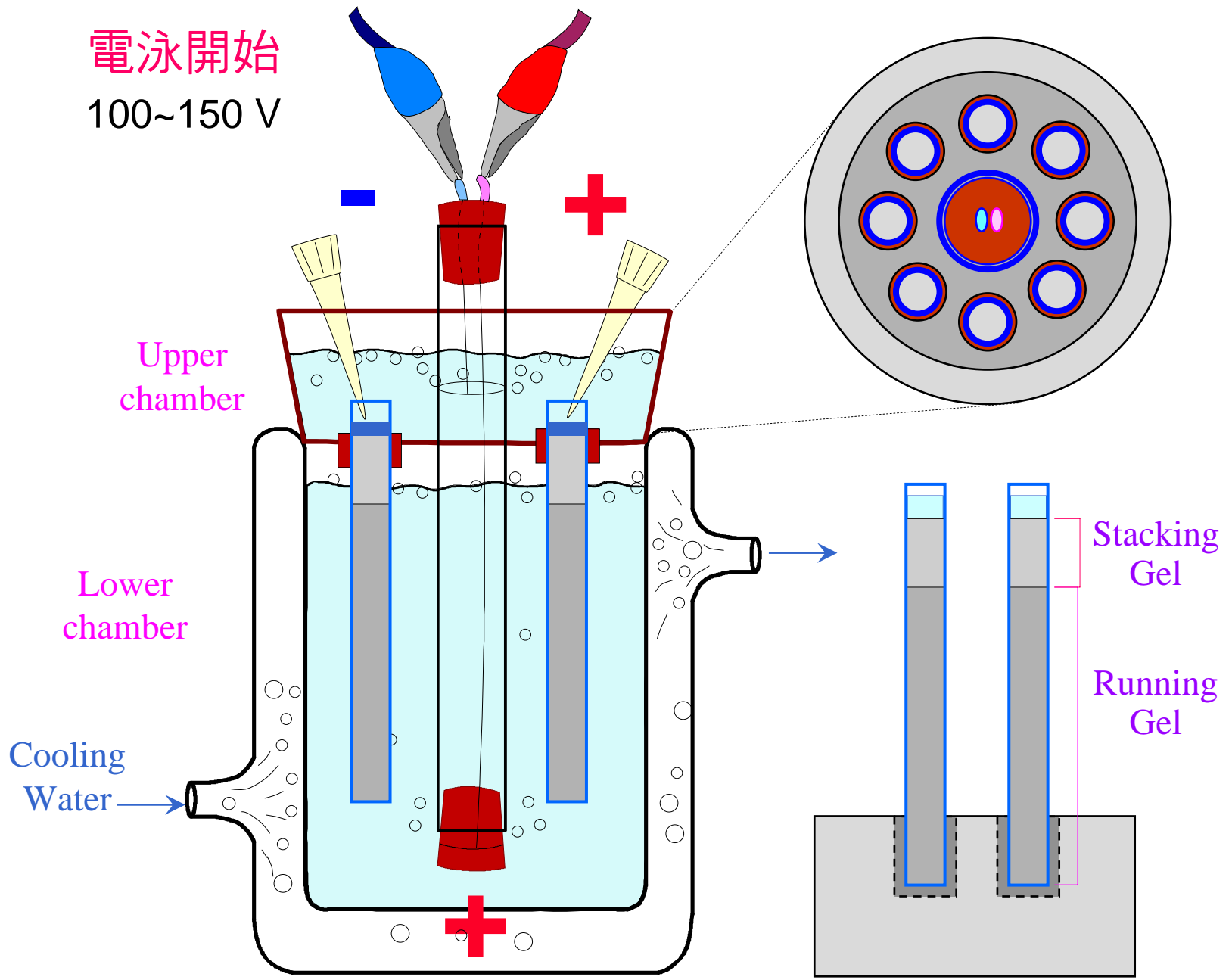
■ 電泳膠體系統的組成：

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 4	膠 體	聚焦膠體	6.9	5%
		分離膠體	8.3	7.5~20%
5	下層 (正極) 緩衝液	Tris-glycine	8.3	-

● 膠體不連續性有聚焦樣本的作用

■ 直立式電泳裝置：

電泳開始
100~150 V



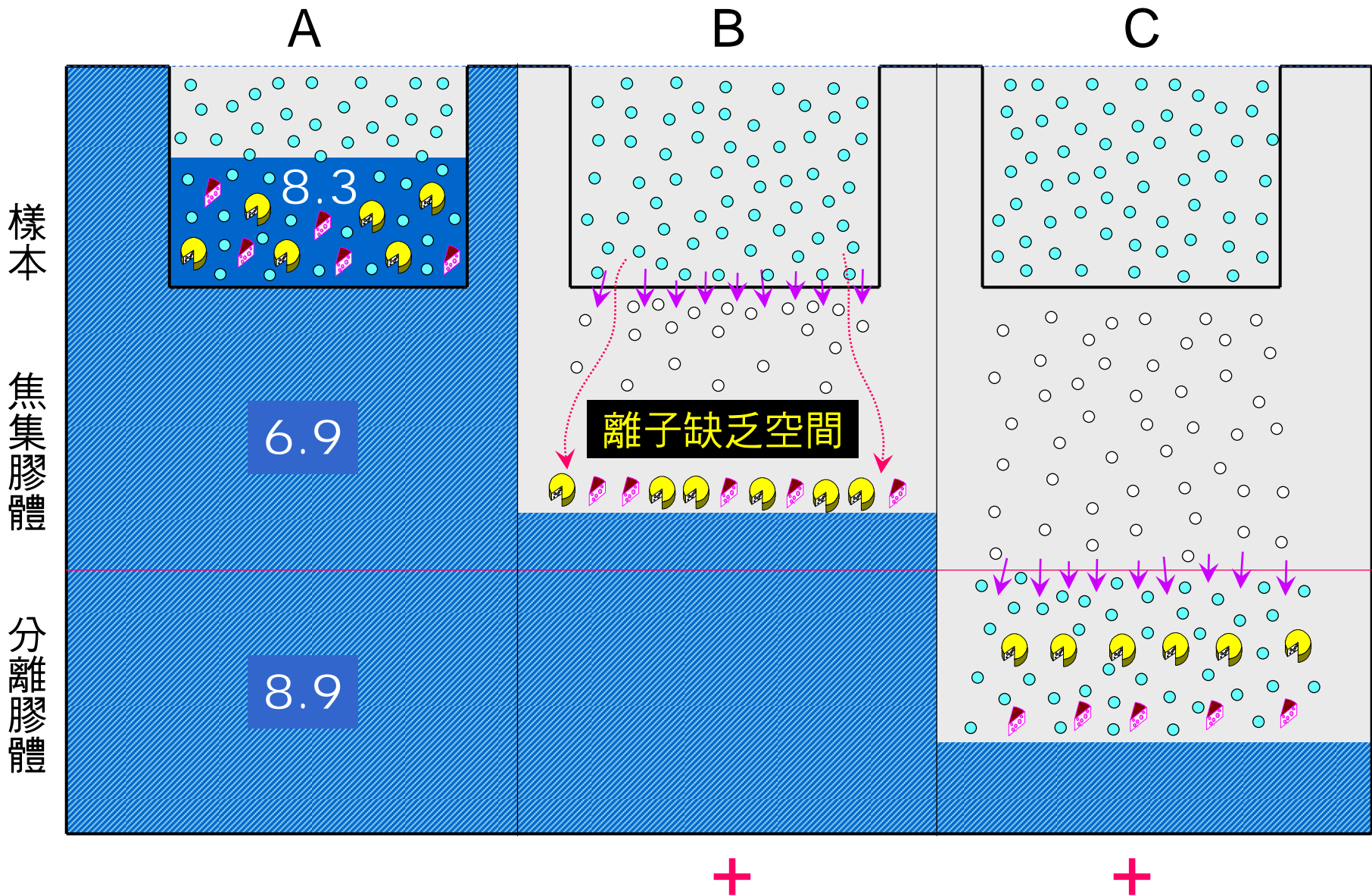
■ 在聚焦膠體中的三個主要作用角色：

Glycine: Negative charged 
No net charge 

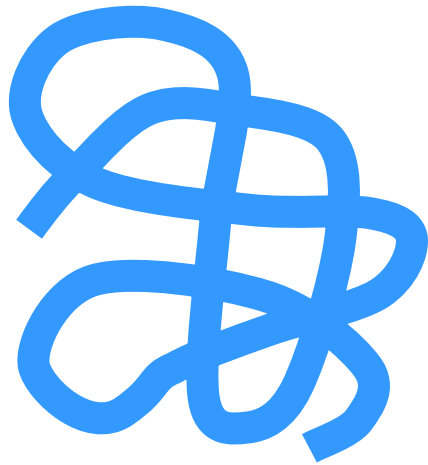
Chloride ion: 

Proteins:  小分子  大分子

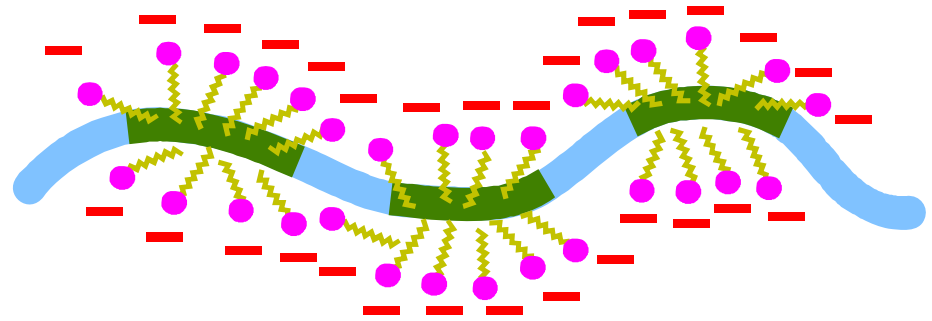
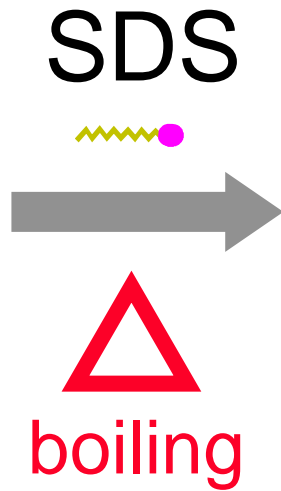
■ 焦集膠體對蛋白質分子的焦集作用：



■ SDS 在蛋白質表面 均勻敷上 一層負電：

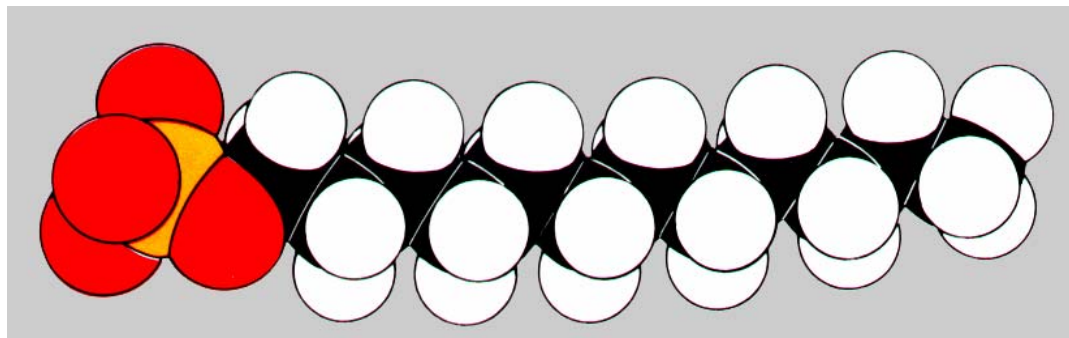


原態蛋白質



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

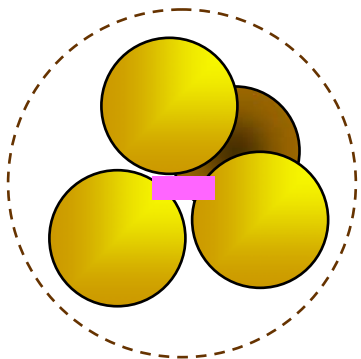


非極性尾部

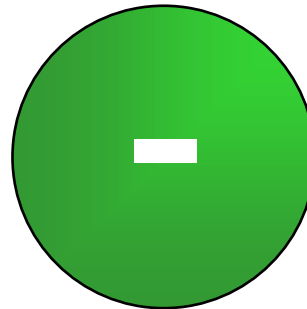
■ 三種不同性質蛋白質的電泳比較：

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

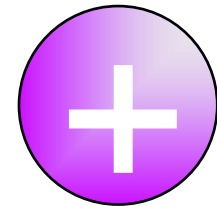
X



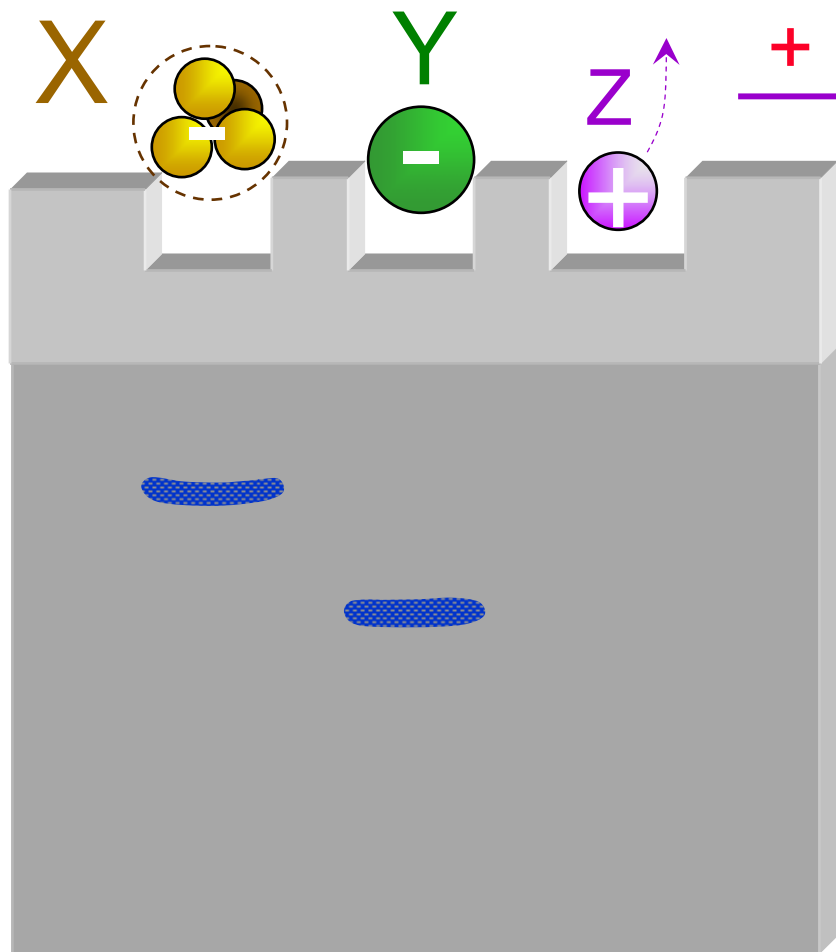
Y



Z



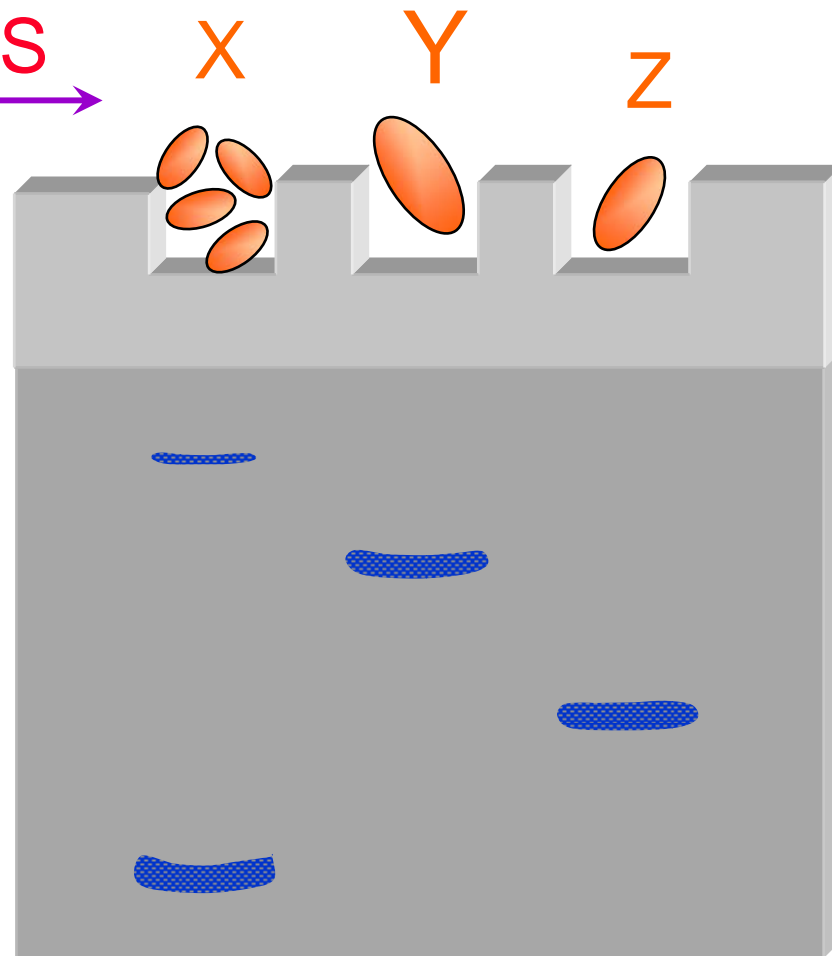
Native-PAGE



+

分子量及淨電荷密度
均影響泳動率

SDS-PAGE

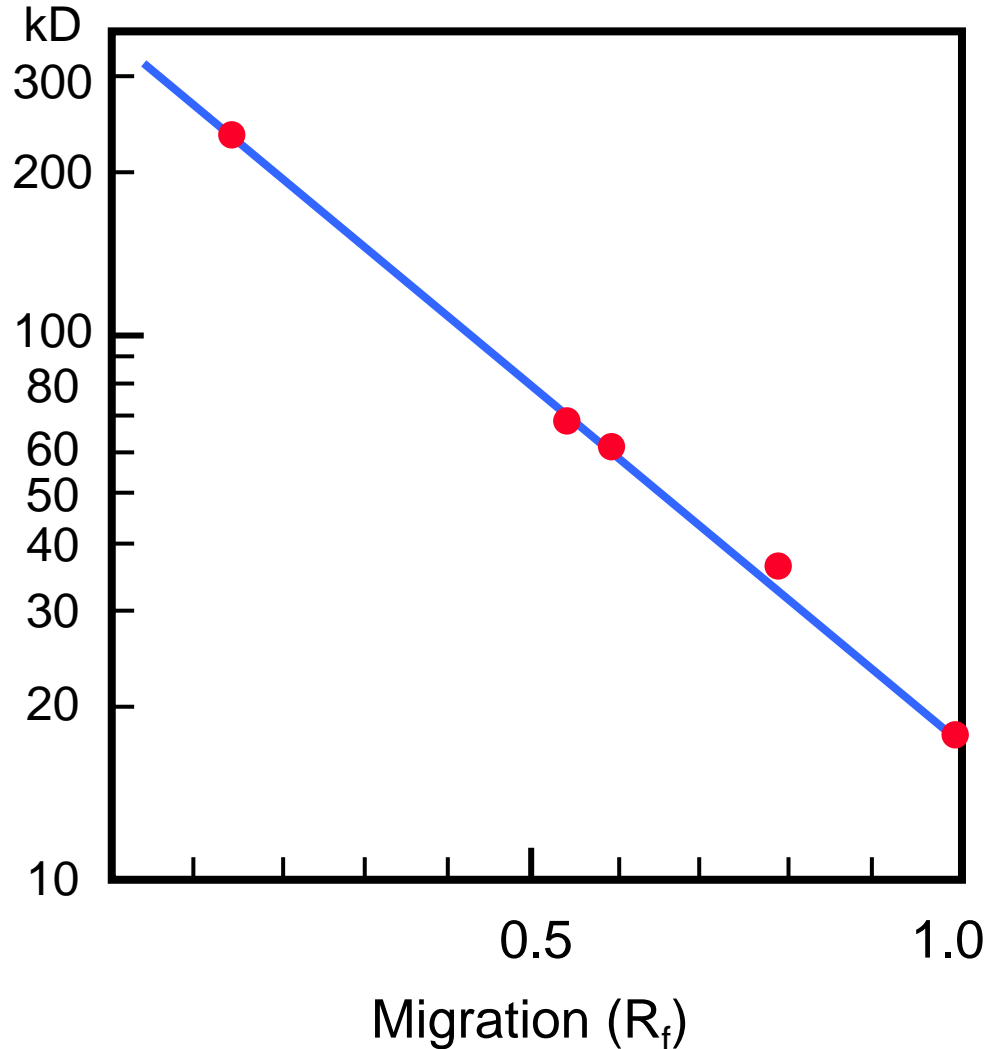


+

只有分子量影響泳動率

■ 單元體分子量的測定：SDS-PAGE

Mol mass



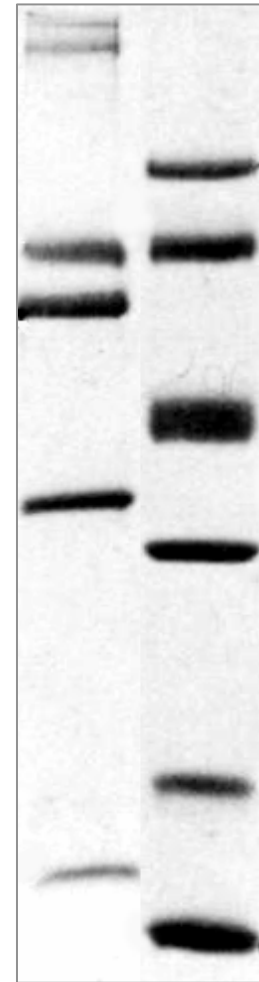
kD

330
220

67
60

36

18.5



kD

94

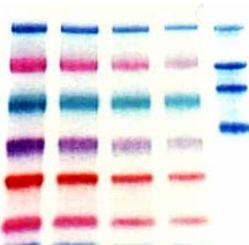
67

43

30

20.1

14.4



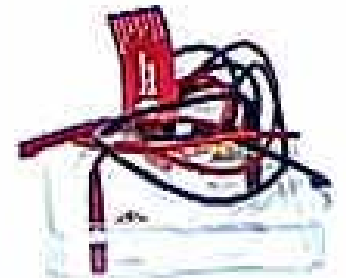
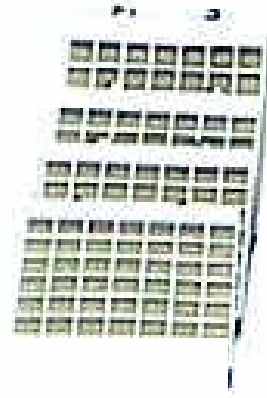
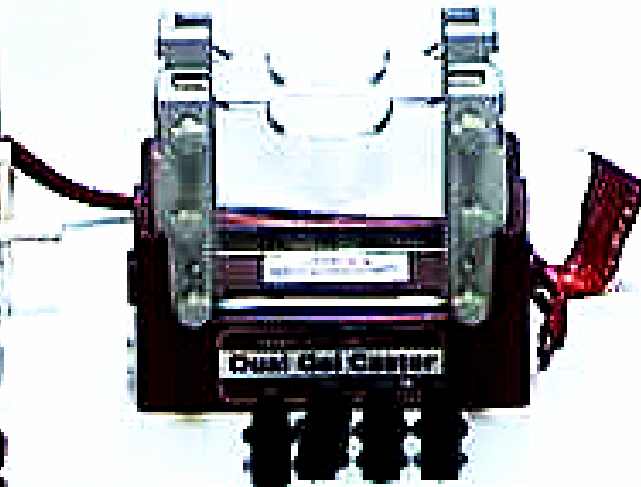
■ 電泳槽及相關設備：

電泳槽

鑄膠器

轉印三明治

轉印槽



供電器